LEARNING AND MEMORY

A COMPREHENSIVE REFERENCE

VOLUME EDITOR RANDOLF MENZEL
EDITOR-IN-CHIEF JOHN H. BYRNE

VOLUME 1

LEARNING THEORY AND BEHAVIOR

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Volume 1
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FOREWORD

A comprehensive reference work on learning and memory could not be better timed than this. During the second half of the twentieth century, the study of learning and memory moved from a descriptive science largely based on the pioneering behavioral analyses of Pavlov, Thorndike, Watson, Skinner, Kamin, Rescorla, and Wagner to a new mechanistic science of mind that combines these brilliant behavioral studies with an analysis of the underlying neural mechanisms, first in a regional manner by Milner, Tulving, Mishkin, Squire, Schachter, and Morris, then on the cellular level, and finally on the molecular level.

The challenges that now face the field are outlined by the five great pioneers in the study of memory – the editor-in-chief Jack Byrne and the editors of these four extraordinary volumes: *Learning Theory and Behavior*, edited by Randolf Menzel; *Cognitive Psychology of Memory*, edited by Henry Roediger; *Memory Systems*, edited by Howard Eichenbaum; and *Molecular Mechanisms of Memory*, edited by David Sweatt. The challenge faced by the contributors to these volumes was to combine the molecular mechanisms with the other three levels in order to provide a coherent, systematically and intellectually satisfying understanding of learning and memory. This is central to the new science of mind. Since memory is the glue that holds our mental life together, the topics covered by these four volumes are central to and paradigmatic for all aspects of the neurobiology of mental life, which has as its goal the understanding of all mental processes in neurobiological terms. Indeed, it is the plasticity of the brain that is the key to understanding the continuity of all mental function. The goal for each of these four volumes was to bridge the subdisciplines concerned with the various forms of memory into a coherent science. The chapters of each of these volumes succeed admirably in doing just that. As a result, this rich and rewarding reference work will serve as a superb framework for the decades ahead, a reference that will provide both the student and the working scientist with the intellectual background necessary to understand and function effectively in the study of learning and memory.

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PREFACE

Learning and Memory: A Comprehensive Reference is the most authoritative set of volumes ever produced on learning and memory and represents the state of the science in the early 21st century. The study of learning (the process of acquiring new information) and memory (retention of that information for future use) has intrigued philosophers and writers for centuries because our memories and plans for the future consolidate who we are, and disruption of these processes dramatically interferes with our daily lives. The fascination with learning and memory is not limited to the humanities, but has been the subject of intense scientific research. Psychologists are concerned with elucidating the features of learning and memory processes and systems, neurobiologists seek to determine the neuronal mechanisms of learning and memory, and neurologists and psychiatrists focus on research and treatment of failures or disruptions in learning and memory.

The study of learning and memory represents a scientific field that has matured at all levels – from the discovery of the protein chemistry and molecular biology of the cellular events underlying learning and memory, through the delineations of the properties and functions of neuronal networks, to formulating and testing the psychological and behavioral neuroscientific theories of learning and memory. In addition, many basic research findings have applied implications on such diverse fronts as education, legal issues hinging on eyewitness testimony, learning disorders in children, memory disorders following brain damage, and declines in memory in older adults.

The volumes in this *Comprehensive Reference* are the result of a meeting in London in July of 2005 where the editors planned the massive work of consolidating all facets of the study of learning and memory. We collected nearly all the topics (albeit from many different disciplines and directions) that we considered constituted scientific approaches to learning and memory and proceeded to parcel the topics into four volumes, resulting in *Learning Theory and Behavior* edited by Randolf Menzel; *Cognitive Psychology of Memory* edited by Henry Roediger III; *Memory Systems* edited by Howard Eichenbaum; and *Molecular Mechanisms of Memory* edited by David Sweatt. This was a formidable task, not only because of the richness and diversity of the subject matter, but also because we needed to logically place topics in the appropriate volume. Although some of the decisions may seem arbitrary, and indeed there is overlap both within and between volumes, each editor ended up with a set of coherent topics that they could organize and introduce in a logical manner.

With approximately 40 chapters per volume, it is no surprise that the editors cover an unusually wide range of intellectual territory or that there is a difference in interpretation by some authors. The organization is a significant editorial challenge and investment in and of itself. However, it is the editor's selection of authors, and the ensuing scholarship on learning and memory from different perspectives, that make this series unique. Authors were identified and invited based on their expertise on a particular topic, and their contributions represent a marvelous compendium of research in learning and memory. The chapters in this series not only represent scientific strength and breadth, but also range from learning at the synaptic level to a systems level approach, and include studies of remarkable learning capabilities in a variety of invertebrates and vertebrates, including human beings.

The first volume in the series, *Learning Theory and Behavior* edited by Randolf Menzel, consists of 38 chapters and sets the tone for the interdisciplinary and comparative approach to the study of learning and memory. He introduces the volume by emphasizing both the value and the limitation of the comparative approach in natural and laboratory settings, stressing that we need information from the behaving animal as well as the neuronal

structures in order to understand the processes involved in information storage and retrieval. Several chapters review progress from using animal models, including worms, molluscs, insects, rodents, birds, and nonhuman and human primates. In addition, concepts such as planning, decision-making, self-awareness and episodic-like memory, usually reserved for human beings, are discussed at several taxonomic levels. The final chapters take an engineering perspective and describe synthetic approaches, including modeling neuronal function and developing a concise theory of the brain.

The second volume, *Cognitive Psychology of Learning* edited by H. Roediger, is comprised of 48 chapters on various aspects of cognitive ability and the underlying neuroscience. The basics of attention, working memory, forgetting, false memories, remembering vs. knowing, the process of recognition, and episodic memory are covered. In addition, topics that are often not included in "memory" volumes deservedly receive attention here, e.g., learning of concepts and categories, learning of perceptual and motor skills, language learning, and implicit learning. This volume also covers memory processes throughout the human lifespan and includes chapters on individual differences in memory ability, both subnormal (learning disabilities) and supranormal (performance of mnemonists and experts in particular domains). Finally, chapters on applied aspects of memory research, dealing with such topics as eyewitness identification in the legal system and applications of research to educational issues, are included.

Volume 3, edited by H. Eichenbaum, consists of 29 chapters which represent a "progress report" on what we know about memory systems and their relationship to different parts of the brain. *Memory Systems* returns to a comparative approach of learning and memory. This volume introduces the concepts of multiple memory systems, and many chapters discuss in extensive detail the different features of declarative memory and their underlying brain structures. Procedural learning in humans and other animals is addressed, and a short section details the involvement of hormones and emotions on memory retention or loss. Finally, changes in memory systems associated with aging, disease processes, and drug use are addressed.

The final 42 chapters in Volume 4, *Molecular and Cellular Mechanisms of Memory* edited by J.D. Sweatt, represent a review of the state of the science of what we know at the systems, cell, and molecular levels on learning and memory formation, as well as providing a look at the emerging and future areas of investigation. Once again, this volume covers an impressive amount of information derived from studies at many taxonomic levels, from molecular associative learning mechanisms, through an array of studies on synaptic plasticity, to the cell level of fear conditioning.

The centrality of learning and memory to our daily lives has led to intense analysis by psychologists and neurobiologists for the past century, and it will undoubtedly remain at the forefront of research throughout this new century as well. It is our intention that this set of volumes will contribute significantly to the consolidation of this field, and it is meant as a resource for scientists and students interested in all facets of learning and memory. No other reference work covers so wide a territory and in so much depth.

Learning and Memory: A Comprehensive Reference would not have been possible without the tremendous work of the Editorial Board, who identified the topics and their authors, and reviewed each contribution. Special thanks also go to Johannes Menzel, Senior Acquisitions Editor at Elsevier, for supporting the project and Andrew Lowe and Laura Jackson, Production Project Managers, and Joanna De Souza, Developmental Editor, for ensuring that the production schedule was maintained.

John H. Byrne

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1.01 Introduction and Overview

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1.01.1 Introduction

The central question of behavioral neuroscience is: What is the source of information that creates and controls perception, reaction, and action in animals and humans?

Since Plato and Aristotle, Western philosophy has found two opposing answers that still guide our thinking today. Idealism proposes preexisting information unfolding as the organism develops, whereas empiricism states that all information is gathered by the interaction of the organism with the environment. As behavioral science developed into its modern form in the second half of the nineteenth century, these two philosophical approaches provided the epistemological framework for two different experimental approaches to animal behavior. Ethology emphasized the preexisting information inherited from the evolution of the species, whereas behaviorism, including Pavlov's physiology-driven approach, focused on collecting information through perception and action. Reference to idealism and empiricism gave these two opposing approaches a strong theoretical backbone, turning anthropomorphic descriptions of behavior and pure collections of observations into hypothesis-driven science. Ethology benefited from Darwin's theory of evolution that provided a conceptual framework for the accumulation and transmission of information, while behaviorism gained from the strength of laboratory-based experimentation and suitability for formal descriptions (*See* Chapters 1.03, 1.06). Although the history of the two behavioral science disciplines is a success story, we also know of their limitations and their failure to capture the breadth of behavior. The dualistic (and initially exclusive) conceptualization of the two forms of information that drive behavior is not adequate to explain brain functions. Regardless of the source of the information stored in the brain, it is expressed in properties of the brain, the wiring of neurons, and the communication between them. The two historical disciplines in behavioral science also failed to develop concepts that included brain functions. Ethology relied too heavily on simple-minded models of brain function, whereas behaviorism eliminated any reference to the brain.

In the end, behavior needs to be explained by underlying body functions, with the brain as the most important player in this game. Where are we now in such an attempt? This volume deals with behavior, theory, and system functions of the brain. Although cognitive neuroscience and the "emergent new science of mind" (Kandel, 2006) offer novel levels of integration between behavioral science and neuroscience, we still have a long way to go. The chapters in this volume should contribute to this exciting endeavor.

1.01.2 Biology of Learning and Memory: The Value of a Comparative Approach

Learning from experience is a property embedded into the survival strategies of all animals living in natural surroundings. Animal species live in different ecological niches, are equipped with different sensory and motor capacities, and communicate differently with other individuals of the same species and with other species (*See* Chapters 1.17, 1.20, 1.21, 1.22, 1.26, 1.31). They also come with different nervous systems, which can be large or small, and some are highly centralized, while others have several rather separate ganglia. There is no one model animal for this research

endeavor, and different animal species have different advantages. One species can be reared more easily in the lab, another has a better-worked-out toolbox of molecular genetics, another provides for large and identifiable neurons or allows recordings from multiple neurons simultaneously over long periods of time, while another has already been analyzed in a wide range of behavioral tests. The main goal of this research is to unravel the general rules and species-specific adaptations in selecting relevant information, adding it to existing knowledge, storing it such that passing time does not eliminate it, and making it available for better-adapted behavioral acts in the future. Comparative studies provide the tool for identifying generalities and specificities.

Observing animals in their natural habitat can suggest relevant research questions (*See* Chapters 1.17, 1.20, 1.21, 1.22, 1.23, 1.25, 1.26, 1.29). While we have ideas about what is worth observing and measuring, we need to be open-minded about unexpected outcomes, as these are often the discoveries that propel research. Food-storing behavior in birds and mammals (*See* Chapters 1.22, 1.23), communication via gestures and/or sound (*See* Chapters 1.16, 1.17) or by ritualized movements in bees (*See* Chapters 1.12, 1.25, 1.29), learning during courtship in *Drosophila* (*See* Chapter 1.28), and navigation (*See* Chapters 1.20, 1.21, 1.25, 1.26) are examples, and many more will be described in this volume. The animal species studied are varied: Worms (*Caenorhabditis elegans*; *See* Chapter 1.32), mollusks (*Aplysia, Limax, Lymnea, Hermissenda*; *See* Chapter 1.30), cephalopods (octopus, *Sepia*, *Loligo*; *See* Chapter 1.31), insects (*Drosophila*, *See* Chapter 1.28; the honeybee *Apis*, *See* Chapters 1.12, 1.25, 1.29), rodents (e.g., mice, rats, squirrels, chipmunks, deer mice, Merriam's kangaroo rats; *See* Chapter 1.22), birds (e.g., Clark's nutcrackers, Eurasian nutcracker, marsh tits, black-capped chickadees, pinyon jays, Mexican jays, Western scrub jays, willow tits, crested tits, many song-learning bird species, the oscines of the Passeriform birds; *See* Chapters 1.17, 1.22, 1.23), and primates (chimpanzees, orangutans, macaques, vervet monkeys, baboons, capuchin monkeys, and pygmy marmosets; *See* Chapter 1.16), including human beings. Each of these species provides us with the opportunity to discover novel ways of solving similar problems brought about by an ever-changing environment and to unravel general strategies by comparison.

The transition from the natural habitat to the laboratory is an essential step in hypothesis-driven behavioral research, but is by no means a simple step (*See* Chapter 1.11). We cannot expect to get full control over the animal, which is sometimes a misleading assumption in some of the behavioral studies (*See* Chapters 1.03, 1.06, 1.10, 1.18). However, the history of an individual's experience can be traced more accurately in the laboratory, and the proper control experiments can be established. The chapters in this volume provide ample evidence for successful transition from the natural environment to the laboratory.

Often an additional and more critical step is required. Animals need to be constrained for physiological measurements, or they are genetically manipulated to isolate cellular components of neural function. It is essential to remember that constrained animals or genetically manipulated animals are no longer the living creatures that we observed in their natural environment, and they are not even those seen in the laboratory behavioral tests. It might not matter so much whether a nutcracker caches a nut in the bark of a tree or in a plastic Lego building block, but it will make a great deal of difference whether a chimpanzee learns to move between tree branches or is sitting in a training chair connected to amplifiers while performing stereotypical arm movements. It is true that in most cases we do not yet have alternatives attempting to relate neural function with behavioral performance, but while presently there are no better experimental tools of neural recording, we must not forget the distance between the natural conditions and the experimental surroundings where we collect data.

Invertebrates such as the nematode *C. elegans* (*See* Chapter 1.32), the mollusks *Limax* or *Aplysia* (*See* Chapter 1.30), the fly *Drosophila* (*See* Chapter 1.28), and the bee *Apis* (*See* Chapters 1.25, 1.27, 1.29) are of particular value in laboratory settings since the transitions from natural to laboratory to experimentally interfering conditions presumably impact them less. Intermediate transition steps from natural to laboratory conditions can be made more easily, and the behavior of these invertebrates appears to be controlled more strongly by innate components. Nevertheless, transgenic nematodes and flies are not normal animals with just one isolated function that has been modified. It is, therefore, very advantageous that transgenes in *C. elegans* and *Drosophila* can be switched on and off rather quickly, and even more important, can be genetically rescued, which allows us to test the isolation of the targeted effect very carefully. Transgenic mice are by far more complicated to analyze, and special care must be taken in interpreting their behavioral alterations.

Many questions about learning and memory cannot be moved into the laboratory, and these may often be the particularly interesting questions (*See* Chapters 1.16, 1.22). This has two consequences: (1) The data are correlational in nature as control groups often cannot be studied or serve as partial controls and animal manipulations are very difficult or impossible, and (2) recording brain functions is difficult or impossible. These limitations should not reduce our efforts to collect data under natural conditions, as these data are essential for future laboratory studies and for comparative studies in humans.

A comparative approach should include human beings, and the motivation of many animal studies is to better understand humans. This is justified if appropriate caution is taken and the general limitations of a comparative approach are observed. Both ethology and behaviorism carry their historical burdens regarding inappropriate generalizations between animals and humans (*See* Chapters 1.03, 1.10), but cognitive neuroscience offers tools and strategies that help to guide such comparisons. If processes and mechanisms have been identified that apply across animal species, they are less likely to be species-specific adaptations and can safely be generalized to humans (e.g., cellular and molecular processes of neural plasticity as discussed elsewhere in this work, or basic rules of associative learning (*See* Chapters 1.05, 1.06, 1.09, 1.10, 1.11, 1.18)). The involvement of homolog brain structures for related forms of learning and/or memory formation are strong hints for homolog function. The hippocampus (e.g., in the case of spatial learning and episodic-like memory) and the amygdala (e.g., in fear learning) are two examples, and many more will be found in the chapters of this volume (*See* Chapters 1.14, 1.15, 1.21, 1.22, 1.23). Comparison between animals with very different brain structures (e.g., mammals and insects) is much more difficult, and often no more than analog functions can be assumed (e.g., navigation according to a geometric representation of space, different forms of memories according to their time course, and susceptibility to experimental interference).

One of the most important and controversial issues related to comparison between animals and humans relates to language and self-awareness (*See* Chapters 1.15, 1.16, 1.23, 1.37, 1.38). Although language acquisition has deep biological roots, the relation to nonverbal or acoustic communication in monkeys is not yet understood (*See* Chapter 1.16). The neural requirements of self-awareness exist in animals (*See* Chapters 1.37, 1.38), but it is not clear whether additional neural functions are required for the human form of self-awareness. The case of episodic memory, as discussed in several chapters (*See* Chapters 1.21, 1.22, 1.23), is a particularly interesting example because essential features of knowledge about what happened, when it happened, and where it happened exist at different degrees of complexity in many animal species (e.g., in the honeybee; *See* Chapters 1.12, 1.25, 1.29). Food-storing birds appear to relate these memories to themselves and appear to expect to find food at that location in the future (*See* Chapter 1.23), showing a capacity that is close to personal recollection in humans. Salwiczek et al. (*See* Chapter 1.23) call this memory episodic-like and see a gradual, rather than a principle, difference with the introspective experience of mental time travel in humans. This pragmatic approach might be exemplary in the sense that other human mental functions could also be broken down into additive features, which could then be tested for their existence in animals in various combinations and complexities. However, the demonstration of the existence of the components does not prove that the full function of a cognitive faculty as observed (or personally experienced) in humans exists in a particular animal species. Nevertheless, the strength of this approach lies in the assumption that there are no categorical differences between animals and humans, and gradual differences can be traced to different performances according to the complexity of the elements found. An example could be dance communication in honeybees (*See* Chapters 1.12, 1.25, 1.29). The bee communicates a location, and depending on the context, the dance might indicate a feeding place, a water or resin resource, or a new nest site. Although the communication process is symbolic and has a vocabulary (although a very reduced one) and a form of syntax (context-dependence), it does not qualify as a language because it lacks essential features, for example, semantics and grammar. One might call it language-like, as one might categorize other symbolic indexical forms of communications, but the point is that a research program can be set up by this decomposition strategy which allows scientists to search for the related neural processes of the components rather than the mental faculty as a whole. It appears to me that a similar research strategy is proposed by Changeux and Dehaene (*See* Chapter 1.38) in their attempt to decompose conscious processing and learning. They propose a unified or global workspace for the neural synthesis of past, present, and expected experiences. Such research approaches are promising because they

avoid the epistemological deadlock connected with the preoccupation of a categorical separation between animals and humans (MacPhail, 1998) or the assumption of equality between the animal mind and the human mind (Griffin, 1984).

1.01.3 Theories, Processes, and Mechanisms

Animal learning theory has been a rich research area over the last 60 years or so, and we may ask whether some of its concepts might join with physiological studies for a better understanding of the underlying processes. Theories derived from associative forms of learning have been elaborated the most (*See* Chapters 1.03, 1.06, 1.09, 1.10, 1.18), and it appears that three concepts are most useful in a search for functional implementations: associative strengths, associability, and prediction error (Dickinson, 2007).

1. Associative strength between two elements (stimulus or response) depends on the history of experience and the stimuli/responses involved and controls both acquisition and retrieval of memory. Although different behavioral theories compete for the best way of capturing the essence of associative strengths (e.g., Rescorla and Wagner, 1972; Pearce and Hall, 1980; Bouton, 1994; *See* Chapters 1.03, 1.06, 1.09), it is not yet clear whether unidirectional or bidirectional associations predict the relations between the elements. Neuroscientists are more than prepared to absorb this concept and translate it into processes of neural plasticity. Donald Hebb (1949) proposed such a neural implementation, and it is widely accepted that synaptic strength is closely related to associative strength (*See* Vol. 4). Long-term potentiation and long-term depression are processes that are based on the accurate timing of neural activity in the pre- and postsynaptic elements of neural nets (*See* Chapter 1.34).

The coincidence of spike activity as a means of modulating synaptic efficiency appears to play a role not only between pairs of pre- and postsynaptic neurons, but also in networks of many neurons. Singer (*See* Chapter 1.37) points out that coherence of spike activity is an essential feature of cortical nets in up- and downregulation of learning-related neural plasticity. It will be important to show that spike synchrony in biological networks is an emergent property similar to artificial networks (*See* Chapter 1.34) and to establish the causal relationship between these global network characteristics and learning. Since small networks composed of identified neurons do not depend on spike coherence in a global sense to establish associative changes in synaptic efficacy (e.g., in mollusks; *See* Chapter 1.30), it will be interesting to search for additional qualities of synchronizing neurons. Such additional qualities could lie in the fact that the three components of memory (formation, retrieval, and consolidation) are so tightly connected that only under conditions of synchronized activity are all three memory components activated. New contents can only be stored in distributed brain regions which jointly reorganize the network according to the new information.

2. Associability is another concept developed in behavioral learning theory that promises to be useful in neural studies. The concept captures the properties of the stimuli and/or outcomes that determine associative strengths as they are reflected in the salience of the stimulus, the predictability or surprise value of a stimulus, or the outcome. Cognitive dimensions of operant learning (*See* Chapters 1.06, 1.10) or perceptual learning (*See* Chapter 1.07) involve attention as a critical parameter of learning (*See* Chapter 1.13), a parameter that can be traced to particular structures (e.g., cholinergic projections from basal ganglia, amygdala, and the septohippocampal system).

3. Prediction error: Learning theories state that learning occurs as long as the outcome of a behavior is not fully predicted, and thus the deviation of the expected from the experienced outcome changes the current associative strengths. Behavioral theories differ with respect to their assumption of whether the error affects associative strength directly (e.g., Rescorla and Wagner, 1972; Mackintosh, 1975) or indirectly (Pearce and Hall, 1980). The implementation of the prediction error into machine learning (Sutton and Barto, 1990) has been very successful (*See* Chapters 1.34, 1.36), and strong neural correlates exist: for example, the neural properties of reward neurons (dopamine neurons of the mammalian ventral tegmentum (Schultz, 2006) and octopamine VUMmx1 neurons in the honeybee brain (Hammer, 1992; Menzel and Giurfa, 2001)).

When dealing with learning, several chapters in this volume draw their underlying concepts from both behavioral and neural data to document that the strongest expectations for an understanding of processes and mechanisms come from collecting experimental data from both behavioral and neural studies (Dickinson, 2007). How far will such a hybrid theory and understanding lead us?

Forty years ago, Kandel and Spencer wrote a seminal paper entitled "Cellular neurophysiological approaches in the study of learning," calling for a novel approach in translating basic psychological concepts of learning into strategies for the search for their neural implementations (Kandel and Spencer, 1968). Less than 20 years later, Hawkins and Kandel (1984) presented a first review on their finding on *Aplysia* associative and nonassociative learning and derived neural components comprising a cellular alphabet of learning (*See* Chapters 4.01, 4.02). This strategy has turned out to be most successful in localizing in space and time neural events induced by learning. It appears that the associative events are distributed, multifaceted, and dependent both on innate predispositions and earlier learning. *Drosophila* provides a particularly carefully studied case (*See* Chapter 4.07). Different neural structures are involved in learning the same odor by reward or punishment, and short- and long-term memories of the same content reside in different neural nets. Localizing the memory trace is an important step in a functional analysis.

A major unresolved issue in both behavioral and neural studies is the relationship between learning with and without external reinforcing or evaluating stimulus. As pointed out above, concise behavioral theories have been developed for Pavlovian and instrumental conditioning, but perceptual learning (*See* Chapter 1.07), navigational learning (*See* Chapters 1.12, 1.20, 1.25), and interval learning (*See* Chapter 1.19) provide cases in which no obvious external reinforcer may be present. Is associative learning a special case of a more general form of learning (the learning of temporal sequences; *See* Chapter 1.12), or is every kind of learning associative? Does an internal reinforcer provide the evaluating function in the latter forms of learning? Learning theory has not settled the debate, and it might well be that functional analysis will show that internal reinforcing circuits are active at the proper time when animals learn by observation. An important component in such forms of learning is attention (*See* Chapters 1.13, 1.36), as is assumed in a modeling study of navigational learning in the honeybee (Montague et al., 1995).

Only selectively attended stimuli are learned. Most importantly, modulatory circuits that appear to be involved in coding evaluating stimuli also participate in selective attention. It will be necessary to build conceptual bridges between the concept of associability as developed in theories of associative learning and the evaluating property of directed attention as described in observational learning. Further advances will only be made with the combination of behavioral and neural approaches.

1.01.4 What Is Memory and What Is a Memory Trace?

The many facets of memory are reflected in the many terms used to capture them (*See* Chapters 1.02, 1.04). Are there 256 different kinds of memory, as Tulving (1972) asked? Irrespective of whether we divide up memories according to time, cellular mechanisms, brain structures involved, categories of contents, type of learning, or type of retrieval, we always imply that memory directs behavior via the process of retrieving information. As pointed out at the beginning of this chapter, brains are equipped with information before, and independent of, acquired information. Thus the content of memory provides a knowledge base for behavioral guidance (including perception, planning, expecting, and thinking), and splitting it up may obscure the basic and unifying property of memory. One question that needs to be asked, then, is: How do we go about measuring the knowledge stored in memory? We do not know, and this ignorance might be one of the reasons why so much emphasis is placed on the need to define memory by retrieval processes (*See* Chapters 1.02, 1.04, 1.12, 1.14, 1.15, 1.24). As long as measurement of memory content is based only on retrieving it from memory, we will not be able to separate stored memory from used memory.

Nadel (*See* Chapter 1.04) quotes from Aristotle's *Ars Memoria*: "It has already been stated that those who have a good memory are not identical to those who are quick at recollecting. But the act of recollecting differs from that of remembering, not only chronologically, but also in this – that many of the other animals (as well as man) also have memory, but of all that we are acquainted with, none, we venture to say, except man, shares in the faculty of recollection." Indeed, the distinction between memory and recollection is multifaceted (*See* Chapters 1.02, 1.04, 1.05, 1.14, 1.15, 1.24), and one of the most important distinctions relates to memory formation versus memory retrieval. Behavioral measures, as well as human subjective introspection, reach memory via retrieval, but the postlearning reactivation process can work only if learning left traces in the form of an engram. Since the process of memory formation is

not directly accessible to behavioral studies, it has been seriously questioned from a behavioral analytical perspective, in terms of whether it makes sense to distinguish between memory as an entity independent of retrieval (*See* Chapters 1.04, 1.05). The notion of a physical memory trace, independent of its use, however, is a central presumption in neuroscience. Indeed, only when neurologically related interference procedures were introduced into memory research did a clear separation between memory formation and memory retrieval become possible. The key discovery in this context was the consolidation process.

Does a memory exist if it is not retrieved? This question is addressed in several chapters in this volume, and rather diverse opinions are expressed. If the knowledge stored in memory does not guide behavior, a behavioral biologist cannot know whether memory exists (and may thus define memory by its retrievability). But a neuroscientist cannot help but assume that the knowledge stored in memory continues to exist during time periods when it is not retrieved, because the physiological measures of memory are independent of whether the animal performs the corresponding behavior. The concept of memory consolidation is essential in this debate. Hermann Ebbinghaus (1964) described a fast and a slow component in forgetting, and William James (1890) proposed that these may be related to two forms of sequential memories: Primary and secondary memory. The concept of consolidation as a time-dependent process following learning was introduced by Müller and Pilzecker (1900) on the basis of their finding that new learning interfered with the formation of recently acquired memory for short, but not for long intervals. At this stage of analysis, a separation between an internal, time-dependent, and self-organizing process of memory formation and retrieval of memory was not possible, but when experimental interference was introduced and neurological cases of retrograde amnesia were analyzed, strong arguments in favor of an independent engram-building process could be presented (*See* Chapters 1.14, 1.15). However, the situation is not as simple as was believed (*See* Chapters 1.04, 1.05). For example, amnesia-inducing procedures could have led to competing learning processes. Irrespective of the unresolved questions in separating memory formation and memory retrieval processes, the body of evidence is overwhelming, proving that neural traces are indeed induced by the learning process independent of retrieval, and consolidation has a physical basis in the structuring and restructuring processes of neural net properties.

Procedures interfering with ordered neural activity or cellular metabolism during periods of consolidation induce retrograde amnesia. Memory gets better over time, even when it is not used. Sleep phases strengthen the consolidation process (Born et al., 2006) and are related to repetition of content-specific patterns of neural activity (Wilson and McNaughton, 1994). It appears to me that the debate about the nature of the memory trace (*See* Chapter 1.04) will continue as long as we cannot read the encoding processes and directly measure knowledge stored in neural nets. Once we can show these in suitable animals such as *Drosophila*, we will probably discover that, in addition to the constructive processes of reactivating memory and using its content, there is an essential component that exists independent of the reactivation process. Whether we like to call this lasting component memory is a question of definition.

Reactivation of memory leads to new learning and its subsequent consolidation processes (*See* Chapter 1.09). Only recently has neuroscience become interested in the mechanistic aspects of extinction learning and memory formation. The phenomena subsumed under the term reconsolidation provide case studies (*See* Chapters 1.24, 1.27). Reconsolidation refers to the effect that retrieving memory may lead to cue-dependent amnesia if the retrieval process is followed by treatment with an amnestic agent. What are these learning and reconsolidation processes? Does reactivation indeed make the old memory trace vulnerable to amnestic interference, indicating that new learning overwrites old memory, or do the learning processes involved in memory reactivation induce parallel consolidation processes that reflect the addition of a new memory trace to the existing one? The ongoing debate reflects the same dilemma addressed above. Our inability to measure knowledge as stored information directly restricts our mechanistic analysis to global and indirect arguments. Once again, behavioral analysis needs to be combined with fine-grained neural analysis addressing the critical question much more directly at the level of the neural elements of the engram.

What might be a suitable strategy toward a direct reading of knowledge? A first step should be to develop criteria that allow us to identify and localize a memory trace. Heisenberg and Gerber (*See* Chapter 1.28) address this question by defining four essential requirements of a memory trace:

1. Neuronal plasticity occurs in particular neurons that are localized and identified, and these neurons are essential for a particular kind of memory.
2. The neuronal plasticity in these neurons is necessary for this particular memory context.
3. Memory cannot be expressed if these neurons cannot contribute during retrieval.
4. Memory cannot be established if these neurons do not receive the required input for the memory content to be stored.

Note that this checklist of experimental procedures does not yet provide us with access to information stored in the memory trace, but we can hope that in a next step a localized and thus-characterized memory trace will be accessible to the really important question of how neural circuits encode and store particular pieces of information. So far only one organism, *Drosophila*, offers the opportunity to localize and characterize a memory trace at the level of cellular resolution, and, indeed, in applying this strategy to rather small neural circuits it was found that traces for short-term memory of an olfactory discrimination task and long-term forms of the same memory content appear to be localized in different, probably partially overlapping neural circuits. Furthermore, it was found that memory traces of appetitively or aversively evaluated stimuli of the same kind occupy different but partially overlapping neural circuits (*See* Chapter 1.28).

A whole battery of highly sophisticated molecular–genetic tools are available to measure the spatial–temporal patterns of memory traces in selected neurons and neural nets of the *Drosophila* brain (Keene and Waddell, 2007). Reading the dynamics of the neural elements during the learning process (i.e., consolidation and retrieval under conditions in which the animal tells us via its behavior whether it perceives, attends, and retrieves) will help us understand at least part of the knowledge stored in memory. How close are we then to direct knowledge reading?

Localizing and characterizing the memory trace by applying correlation analysis is the mainstream of the neuroscience approach today (*See* the volumes edited by Sweatt and Eichenbaum). Correlating elemental with system properties is an important step in any mechanistic analysis. The next step will be to establish closer, possibly causal, links following the strategy outlined by Heisenberg and Gerber (*See* Chapter 1.28). The tools also exist for the worm *C. elegans* (*See* Chapter 1.32) and are becoming available step by step for other species (e.g., the mouse). The hunt for direct knowledge reading will be embedded in a concerted approach to understanding the workings of neural nets and the brain as a whole.

1.01.5 The Engineer's Approach to Learning and Memory

Engineers compose and biologists de-compose, so a combination of these two strategies should be favorable to the study of a complex system such as the brain. Constructive thinking in theoretical neuroscientists is inspired by rules derived from behavioral studies (e.g., Hebb's rule), by the morphology of brains and the connectivity patterns of neurons (e.g., the matrix-like connectivity in the hippocampus), by the functional properties of neurons (e.g., synaptic plasticity), and by theoretical concepts developed independently from, but motivated by, thoughts about how the brain might work (e.g., autoassociative or attractor networks). Our volume contains chapters dealing with all of these aspects (*See* Chapters 1.33, 1.34, 1.35, 1.37, 1.38). Irrespective of the intellectual pleasure one experiences when thinking about theoretical neural nets, one might ask how the joint efforts propel our understanding. I see the following points that are also well-illustrated in the respective chapters:

1. Hypothesis-driven research like ours requires well-formulated concepts and hypotheses. Theories developed for neural nets shape these concepts and allow us to formulate predictions (*See* Chapters 1.33, 1.35).
2. The analysis of the vast amounts of data collected by anatomical, electrophysiological, optophysiological, and molecular studies requires the contribution of theoretical neuroscientists to extract relevant information and interpret it (*See* Chapters 1.34, 1.37).
3. There exists no concise theory of the brain. Global brain functions need to be constructed from elemental and network functions and implemented into a model (e.g., the neuronal workspace model of Changeux and Dehaene; *See* Chapter 1.38).

At any of these levels of a modeling approach, one has to decide what is considered an essential feature and which of the many characteristics of the neurons, their connectivity at the local and the global level, are implemented or not. Should one use simplified integrate-and-fire neurons or Hodgkin-Huxley-type neurons? Should the model care about the real gestalt of neurons or not? How seriously should one take the

neuroanatomical data on local and global connectivity? These and many other decisions are hard to make, and different choices produce serious debates about the suitability of these models. There are many measures of suitability: Are experiments stimulated, predictions offered, and interpretations of data supported or rejected? Five chapters (*See* Chapters 1.33, 1.34, 1.35, 1.37, 1.38) provide a range of examples where strong arguments can be presented for the suitability of the respective models and experimental approaches are suggested. Other examples are given in Vol. 4 that deal with small neural nets partially composed of identified neurons (*See* Chapter 1.30). Indeed, small biological neural nets (e.g., the stomatogastric ganglion in the lobster) have been successfully modeled, and the models in the electronic version were directly hooked up with the biological neural net to analyze the contribution of certain cellular properties (Golowasch et al., 1999). These approaches have been applied in the search for the neural implementation of operant learning in the buccal ganglion of *Aplysia* (Brembs et al., 2002; Vol. 4; *See* Chapter 4.10).

Given the technological advances with the expression of light-driven conductances in specified neurons (e.g., channel rhodopsin), similar analyses will be possible (e.g., in *Drosophila*; *See* Chapter 1.28), which emphasizes the need for theoretical concepts and models of the respective networks.

Ultimately, models of neural function should also predict behavioral outcomes. Singer (*See* Chapter 1.37) makes the case for the role of spike synchrony and oscillatory spike patterns in memory formation. Koene and Hasselmo (*See* Chapter 1.35) formulate predictions for the role of theta rhythm in the hippocampus for memory formation and retrieval, and Rolls (*See* Chapter 1.33) explicitly characterizes the properties of the autoassociative network of the hippocampus for behavioral phenomena such as completion and graceful degradation. It is to be expected that the success of the combined theoretical and experimental approach will make modeling an indispensable part of the search for the memory trace.

1.01.6 Conclusion

Curiosity-driven behavioral studies, theory-guided laboratory behavioral experiments, and modeling of neural functions define a unique workspace in the search for the engram. Joining forces will help, and the chapters presented here will hopefully facilitate communication between these disciplines. The task is indeed demanding, because the goal will not only be to localize and characterize the memory trace, but to measure the knowledge stored in the memory trace independent of and in addition to the behavioral read-out process.

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1.02 A Typology of Memory Terms

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1.02.1 Introduction

The English language provides us with the term memory to denote several interrelated ideas, such as 'the power of the mind to remember things' or 'something remembered from the past; a recollection' (both quotes are from the Oxford American Dictionary). These definitions of memory are fine for everyday conversation and communication, but scientists interested in studying the biochemical, neural, or psychological underpinnings of this topic have found the need to describe many distinctions about memory that laypeople do not use. Such a need is further underscored by a growing interest in interdisciplinary approaches to the study of human memory (*See* Chapters 3.05, 3.06, 3.14) and in adapting cognitive research paradigms to the study of nonhuman animal learning, an area that has developed largely in isolation of the human memory research tradition (e.g., Wright, 1998; Wright and Roediger, 2003; *See* Chapters 1.04, 1.13, 1.14, 1.15, 1.21, 1.22, 1.23). This chapter is intended to explain the meaning of some of the most popular terms that have been contributed to the literature.

We aim to paint with a fairly broad brush and not to get involved in matters such as whether one term (say, implicit memory) is to be preferred to another term (indirect memory), although buckets of ink have

been spilt on these matters. Rather, we intend to provide general definitions and meanings of terms without defending them as theoretically critical (or not). In this sense, the chapter is descriptive rather than theoretical, although we fully understand that by choosing one's terms and their definitions, one implicitly adopts a theory.

How many types of memory are there? In the early 1970s, Tulving wrote: "In a recent collection of essays edited by Norman (1970) one can count references to some 25 or so categories of memory, if one is willing to assume that any unique combination of an adjectival modifier with the main term refers to something other than any of the references of other such unique combinations" (Tulving, 1972: 382). Tulving added two more terms (episodic memory and semantic memory) in that chapter. Yet more important for present purposes, Tulving continued to keep a list of memory terms as he encountered them. Thirty-five years later, Tulving (2007) wrote another chapter entitled "Are there 256 different kinds of memory?" which was the number of combinations of the adjective + memory sort that he had collected by that time. The list goes from abnormal memory (at the beginning), through terms such as diencephalic memory and false memory, then on to rote memory and sensory memory, and finally, at the end of the list, to working memory. (There are 250 others.)

We hasten to add that we are not going to cover 256 kinds of memory in this chapter. We aim to provide a lexicon of some of the primary terms that readers will find in the four volumes of *Learning and Memory: A Comprehensive Reference*. We have tried to weave the terms together in a loose sort of story, so as not to provide just a glossary with a long list of terms and definitions. The story is one conception of the varieties of memory provided elsewhere (Roediger et al., 2002). We try to give a verbal definition of each type of memory we chose to include, as well as a practical example of how the type of memory might operate in a person or other animal, and we usually point to a paradigm by which this type of memory is studied, to provide kind of an operational definition.

The reader will notice that in some cases the same memory term (e.g., episodic memory) may refer to a process, entity (e.g., memory trace), system, mental state of awareness, or type of cognitive task, depending on the context. Such linguistic flexibility can easily lead to confusion, so we attempt to distinguish among different uses of each term where appropriate. The index of the book can be used to glean other uses of the term. In addition, semantic confusion can easily arise from the types of metaphors employed to describe a memory concept. Most cognitive psychologists use a spatial metaphor in which memories are conceived as physical entities stored in a mind space, and the act of remembering involves searching through the mind's space in order to retrieve the objects of memory (e.g., Roediger, 1980; Tulving, 2000; *See* Chapters 1.04, 1.05). In contrast, others have proposed nonspatial metaphors that make analogies to concepts such as strength – memories are comparable to muscles whose strengths are directly related to performance on memory tasks (e.g., Hull, 1943); construction – the act of remembering involves constructing memories from available information (e.g., Bartlett, 1932); depth of processing – memory is a by-product of the level of perceptual analysis (Craik and Tulving, 1975; *See* Chapters 1.07, 1.08); or auditory resonance – memories are like notes played on piano keys or individual tuning forks resonating (e.g., Wechsler, 1963; Ratcliff, 1978), to list but a few. To reiterate, we do not mean to provide exhaustive coverage, but rather to paint with broad strokes and to represent the way memory terms are used by cognitive psychologists and others.

We begin with consideration of some general distinctions made among types of memory. We then turn to the idea that it is useful to catalog memories by their time course in the system (from brief sensory memories, to short-term conscious memories, to various sorts of long-term memory). Most work has been devoted to the various types of long-term memory that have been described, so this is the focus of the next section of the chapter. Inevitably, given our organization, there is a bit of repetition because we needed to cover the same term (say, episodic memory) in more than one context.

1.02.2 Broad Distinctions

This section of the chapter is devoted to consideration of several broad distinctions among forms of memory. We consider the issue of explicit and implicit memory, conscious and unconscious forms of memory, voluntary and involuntary retention, intentional and incidental learning and retrieval, declarative and procedural memory, and retrospective and prospective memory.

1.02.2.1 Explicit and Implicit Memory

Explicit memory refers to cases of conscious recollection. When we remember our trip to Paris or

recognize that some words occurred in a recent list, these are instances of explicit memory. In cases of explicit retention, people respond to a direct request for information about their past, and such tests are called explicit memory tests. On the other hand, on tests of implicit memory, people are asked to perform some task, and the measure of interest is how some prior experience affects the task. For example, take the simple case of the word elephant appearing in a long list of words. If subjects are given a recognition test in which they are instructed to identify words studied in the list (and to reject nonstudied words), then their choice of elephant as a studied word would represent an instance of explicit retention. However, if a different group of subjects were given the same set of words to study and then were given a word stem completion test (with instructions to say the first word that comes to mind to the word stem ele_____), then this would constitute a test of implicit memory. The relevant measure on this test is priming, the greater probability of completing the stem with elephant rather than other plausible words (element, elegant, electricity, etc.) when the word has been studied than when it has not been studied. For example, the probability of producing elephant to the word stem might be 10% if the word had not been studied in the list and 40% when it had been studied, which would constitute a 30% priming effect. One reason for believing that these two measures represent different forms of memory is that they can be dissociated by many experimental (and subject) variables.

Graf and Schacter (1985) introduced the terms explicit and implicit memory to the field. Explicit retention refers to most typical measures of retention that psychologists have used over the years (recall, recognition, and their variations), whereas implicit memory refers to transfer measures when people may not be aware of using memory at all (Jacoby, 1984). Some writers prefer the terms direct and indirect memory for this contrast, because explicit tests measure memory directly, whereas implicit tests are indirect measures. Schacter (1987) offers a fine historical review of concepts related to implicit memory.

1.02.2.2 Conscious and Unconscious Forms of Memory

Conscious and unconscious forms of memory refer to the mental states of awareness associated with remembering the past. Attempts to describe human memory in relation to consciousness hearken back to the early introspective tradition of experimental psychology and the writings of Wilhelm Wundt, Edward Titchener, and William James (e.g., James, 1890/1950), as well as the psychoanalytic tradition and especially the well-known writings of Sigmund Freud (e.g., Freud, 1917/1982). Less well known is the fact that in the very first experiments on memory, Ebbinghaus (1885/1964) devised a relearning/savings technique for measuring memory that could detect unconscious knowledge. In fact, Ebbinghaus preferred savings measures over the merely introspective techniques of recall and recognition, because these latter tests cannot, almost by definition, measure memories that are not conscious (Slamecka, 1985).

In contemporary studies of memory, conscious recollection refers to the subjective awareness of remembering information encountered in the past, a process that is likened to the experience of mentally traveling back in time (Tulving, 1985). Tulving has also termed this state of awareness autonoetic (self-knowing) consciousness. In contrast, a noetic (knowing) state of consciousness is the type of awareness associated with retrieving previously learned information, such as a geographical, historical, or personal fact, without recollecting details about the place and time in which that information was originally acquired. For example, noetic consciousness might characterize the experience of a person being asked to name the capital of Canada and who, after thinking for a bit, responds "Ottawa" without remembering when he or she last encountered or originally learned this fact. Autonoetic consciousness, on the other hand, is reflected by the person's ability to think back to and re-experience an episode, such as a visit to Ottawa.

Conscious recollection may be intentional and effortful, or it may occur without the intent to explicitly remember information relevant to a given memory task, as is the case with involuntary conscious recollection (e.g., Richardson-Klavehn et al., 1996). This term refers to the fact that one may suddenly be remembering some event from the past without ever having tried to do so. In some cases of patients with damage to the frontal lobes, they may experience confabulation, or the experience of conscious recollection occurring for events that never occurred. The patients believe they are having memories, but in many cases the events are preposterous and could not have occurred. Such cases are extremely rare yet do occur.

Unconscious retention may be observed in performance on tests of implicit memory where individuals indirectly demonstrate their prior exposure to the test material under conditions in which they do not consciously recognize the material. Tulving has referred to the state of awareness associated with unconscious retention as anoetic consciousness. Unconscious retention also occurs when subjects show savings in retention without being able to recollect the experience that gave rise to the savings, as Ebbinghaus (1885/1964) first pointed out.

1.02.2.3 Voluntary and Involuntary Retention

Voluntary retention refers to deliberate, willful recollection, whereas involuntary or incidental retention refers to recollection that occurs without conscious effort. Involuntary retention, as the name implies, refers to memories that arise in consciousness unbidden, with no conscious effort to recollect. For example, in studies of autobiographical memory (memory for events in one's life), voluntary recollections may be assessed by asking individuals to remember personal events in response to queries (e.g., recall a memory from your past that is associated with an automobile). The naturalistic study of involuntary memories can be achieved by asking individuals to keep a diary and jotting down memories that seem to come out of the blue, as it were, wherever and whenever they occur (e.g., Berntsen and Rubin, 2002; Rubin and Berntsen, 2003).

It should be noted, though, that acts of voluntary or involuntary recollection may not be entirely pure, and one may influence the other. For instance, a person's attempt to remember the details of a baseball game that occurred years ago might be influenced by his inadvertently remembering details of a more recent game. Or when engaging in a test of implicit memory, such as completing word fragments, a person might become aware of the fact that some of the target words were encountered during the study phase and, therefore, might intentionally think back to the study phase to help complete the test word fragments (Jacoby, 1991). In addition, as previously mentioned, one might experience involuntary conscious recollection whereby thoughts of a past event come to mind automatically, and it might take further reflection to realize how the memory came to mind (Richardson-Klavehn et al., 1996).

1.02.2.4 Intentional and Incidental Learning and Retrieval

1.02.2.4.1 Intentional and incidental learning

Intentional and incidental learning refer to whether or not people intend to learn material to which they are exposed. Of course, as we go about the world watching TV, driving, or reading the paper, we rarely say to ourselves: I need to remember this commercial on TV. Educational systems provide the main form of relentless intentional learning, although of course we all sometimes try to remember the name of a new acquaintance or the name of a book or movie someone recommended. In the laboratory, intentional or incidental learning is manipulated by instructions to subjects. In an intentional learning situation, an individual studies certain materials with the express purpose of remembering them at some later point in time. In an incidental learning task, the same materials might be provided but with an orienting task to induce some sort of processing of the material but without any instructions concerning a later memory test. For example, in a standard levels-of-processing manipulation (e.g., Craik and Tulving, 1975), a person might be shown a list of words and asked to judge whether each word (e.g., BEAR) is presented in capital letters (graphemic or structural processing), whether it rhymes with a certain word like chair (phonemic processing), or whether it fits into a certain category such as animals (semantic processing). Subjects in incidental learning conditions would be told that the researchers are interested in studying the speed with which people can make such decisions. In the intentional learning conditions, they would be told the same rationale, but would also be told that their memory for the words will be tested later.

The natural expectation is that material studied under intentional learning conditions is better retained than under incidental learning conditions, and this outcome is sometimes obtained (Postman, 1964). However, at least when semantic orienting tasks are used, the differences between incidental and intentional learning conditions are surprisingly slight and often there is no difference at all (Craik and Tulving, 1975; Hyde and Jenkins, 1969).

1.02.2.4.2 Intentional and incidental retrieval

Just as intentionality can be manipulated during study of materials, so can it be manipulated during

testing. In fact, the distinction already drawn between explicit and implicit memory tests can be cast in this light. Explicit tests require intentional retrieval, but implicit tests reveal incidental retrieval (Jacoby, 1984). Under intentional retrieval conditions, a person is asked to engage in conscious, deliberate recollection of a past event (e.g., recalling a word from a previously studied list that completes a word stem). By contrast, incidental retrieval involves giving people the same word stem with the instruction to write the first word that comes to mind. Incidental retrieval is indexed by priming, the better performance in completing the stem with the target word relative to a control condition in which the word had not been studied.

As noted, the comparison between intentional and incidental retrieval is not necessarily a pure one, because performance on explicit memory tests may be affected by incidental retrieval just as performance on implicit memory tests can be influenced by intentional retrieval (Jacoby, 1991). Several solutions exist for attempting to gain leverage on this issue. Schacter and his colleagues (1989) proposed the retrieval intentionality criterion to test for the contamination of incidental retrieval measures by conscious recollection. The basic idea is to compare incidental and intentional recollection, holding all other study and test conditions constant. If performance differs markedly between the two tests when all conditions are held constant except for instructions just prior to the test, then one can have greater confidence that they measure intentional (conscious) and incidental (automatic or unconscious) retrieval. Roediger et al. (1992) crossed intentional and incidental study and test conditions with other variables and showed that incidental tests reflected quite different patterns of performance from the intentional tests. Jacoby (1991) proposed a different method, the process dissociation procedure, to separate conscious from unconscious influences during retrieval. Although providing the details of his ingenious method is outside the scope of this chapter, his method has proved extraordinarily useful in separating conscious from unconscious (or automatic) cognitive processes.

1.02.2.5 Declarative and Nondeclarative Memory

Declarative memory and nondeclarative memory (sometimes referred to as procedural memory) are terms that have gained prominence following their use by Squire (1982), although the original distinction was proposed by Ryle (1949). Ryle distinguished between declarative knowledge (knowing that) and procedural knowledge (knowing how). For example, we know that Washington, D.C., is the capital of the United States, but we know how to tie our shoes.

More recently, Squire has proposed declarative memory as an overarching category that includes episodic memory (remembering specific events of the past) as well as semantic memory (general knowledge). Declarative memory processes rely upon the hippocampus and related structures in the medial-temporal lobe including the perirhinal, entorhinal, and parahippocampal cortices. As it has been extended, the term declarative memory has become a bit of a misnomer, because the concept is often applied to infrahuman species that are not prone to making declarations. (Ryle tied his distinction specifically to linguistic usage so that people would know that such and such occurred.)

Procedural memory was originally intended to cover motor skills, such as tying shoes, riding a bicycle, or typing (Ryle, 1949), but it was broadened to cover mental as well physical procedures. For example, the mental processes involved in multiplying 24×16 are examples of mental procedures that can be studied. As Squire (1992) developed his theory, the term procedural memory became broader and covered such topics as priming on implicit memory tests, classical conditioning of responses, and habituation. Because of these and other uses, the broader term nondeclarative memory came into use. It refers both to traditional procedural tasks and to others such as priming and skill learning. The distinction between declarative and nondeclarative types of memory rests partly on evidence that different brain structures are involved in various forms of memory. The evidence supporting the differences between the different forms of memory has come both from studies of human amnesic patients with damage to the medial-temporal lobes and in animals where such alterations can be achieved experimentally (e.g., Squire, 1992).

As noted, the term declarative memory originally referred to memories that could be verbally stated (Ryle, 1949). This term has also been broadened so that it now includes many other kinds of memory, including spatial memory, some types of long-term visual memory, and any other form of memory subserved by the hippocampal complex. Nondeclarative memories include all other types, whether

they involve memories for physical movements and actions, priming, or skills.

Tulving (1985) has proposed a somewhat different schematic arrangement of episodic, semantic, and procedural memory systems. In Tulving's scheme, procedural memory is phylogenetically oldest and is shared among all organisms. Semantic memory grows out of (and depends upon) procedural memory. Episodic memory is evolutionarily most recent and, according to Tulving, only humans have this form of memory (see Tulving, 2005, for further elaboration). Different forms of consciousness are proposed for the three systems: anoetic (non-knowing) for procedural memory, noetic (knowing) for semantic memory, and autonoetic (self-knowing) for episodic memory. Tulving proposes that a critical function of autonoetic consciousness is planning for the future, which brings us to another critical distinction.

1.02.2.6 Retrospective and Prospective Memory

The vast majority of memory research deals with the ability to remember past events when given specific cues (as in explicit memory tests of recall or recognition) or with the effects of past experience on current behavior (priming on implicit memory tests). All tests that fall into these categories assess retrospective memory: memory for the past or effects of past experience on current behavior. In the past 2 decades, researchers have examined memory for intentions to be performed in the future, or prospective memory. Strictly speaking, prospective memory is retrospective in nature: it involves remembering a past intention. A prospective memory task differs from a retrospective memory tasks in that there is usually no explicit cue to elicit recall of the intention. Instead, a prospective memory task requires that subjects must use an environmental cue to know when to retrieve the intention, so it is a curious mix of incidental and intentional retrieval. We face prospective memory tasks all the time, whenever we need to remember to perform some act in the future. Prospective memory tasks can be classified as cue-based or event-based when some cue should remind us to perform an action (e.g., pass along a message to a friend when we see her) or time-based (e.g., remembering to take out the trash Tuesday evenings). Both cue-based and time-based prospective memory tasks have been investigated in naturalistic settings and in the laboratory. Retrieval of prospective memories may sometimes involve monitoring and may sometimes be spontaneous and effortless (Einstein and McDaniel, 2005).

We turn next to categorizations of memory based on (roughly) the time they persist, starting with varieties of short-term memory.

1.02.3 Types of Short-Term Memory

Information from the external world is believed to be represented in various storage systems that, roughly speaking, hold information for fractions of a second, seconds, or much longer. Atkinson and Shiffrin's (1969, 1971) influential theory, shown in **Figure 1**, provides one conceptualization. Our treatment here provides some amendments to their original theory.

1.02.3.1 Sensory Memories

The border between perceiving and remembering is blurred. There is no good answer to the question: When does perception end and memory begin? Even the operations in the two types of experiments are similar. When stimuli are presented rapidly to the visual or auditory system and some report or judgment is made quickly afterward, the experiment is referred to as one of perception. If the report or judgment is delayed after presentation, the study is usually called a memory experiment. Sensory memories are the brief holding systems for information presented to the various sensory systems; the information is thought to be held briefly in each system as it undergoes further processing.

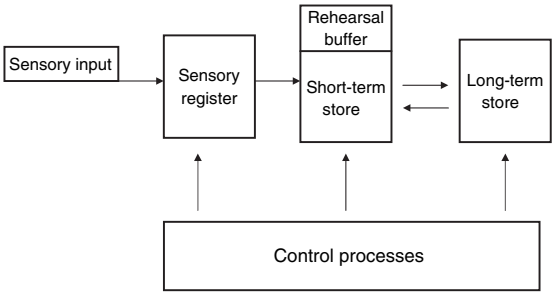

Figure 1 Simplified version of the original multi-store memory model of Shiffrin and Atkinson. Information is conceived as being transmitted through various memory stores. Adapted from Shiffrin RM and Atkinson RC (1969) Storage and retrieval processes in long-term memory. *Psychol. Rev.* 76: 179–193; used with permission of the American Psychological Association.

Sperling (1960) identified a rapidly fading store of visual information that he called precategorical visual storage. The term precategorical was in the title, because Sperling's evidence convinced him that the information was held in a relatively raw form, before linguistic categorizations had been applied. Somewhat later, other researchers proposed a system of precategorical acoustic storage, the auditory equivalent of Sperling's visual store (Crowder and Morton, 1969). These two sensory stores have been studied quite thoroughly by many researchers (*See* Chapter 2.03), but the names they are given in the literature today have been changed to iconic and echoic memory (following Neisser's (1967) suggested terminology). Iconic memory refers to the visual store, whereas echoic memory is used for auditory storage. Echoic storage seems to persist longer than iconic storage, although the decay characteristics of both systems have been debated and depend on such factors as stimulus intensity and the technique used to measure loss of information over time.

Researchers assume that similar storage systems exist for the other senses, but touch is the only sense that has been studied in this regard (and rather sporadically). The close association between smell and taste makes such studies difficult, although longer-term olfactory memory, in particular, has been well studied.

1.02.3.2 Short-Term Storage

Short-term memory (or short-term storage; the two are often used interchangeably) refers to retention of information in a system after information has been categorized and reached consciousness. In fact, contents of short-term memory are sometimes equated with the information of which a person is consciously aware. Information can be continually processed in short-term storage (e.g., via rehearsal or subvocal repetition). If a person is distracted, information is rapidly lost from this store.

Many different techniques have been developed to study aspects of short-term memory, but all have in common that subjects are given relatively brief numbers of items (often digits or words) and are asked to recall or recognize them later (often after some brief interfering task). Another term used for short-term memory is primary memory, owing to a distinction introduced by William James (1890/1950) between primary and secondary memory (reintroduced to the field much later by Waugh and Norman, 1965). Primary memory is what can be held in mind at once, whereas secondary memory referred to all other kinds of long-term memory. The terms short-term memory and long-term memory seem to have become accepted today.

1.02.3.3 Working Memory

Working memory is a term for the type of memory used to hold information for short periods of time while it is being manipulated (Baddeley, 2001). Working memory encompasses short-term memory, which in Baddeley's theory refers only to the short-term passive storage of information. Working memory also adds the concept of a central executive that functions to manipulate information in working memory and three separate storage components: the phonological loop, visuospatial sketchpad, and episodic buffer (see **Figure 2**). To test the short-term memory of humans, tasks that require short-term storage of information (such as digits in a digit span task) are used. On the other hand, working memory tests use tasks that require both short-term storage and manipulation of information (such as the operation span task, in which

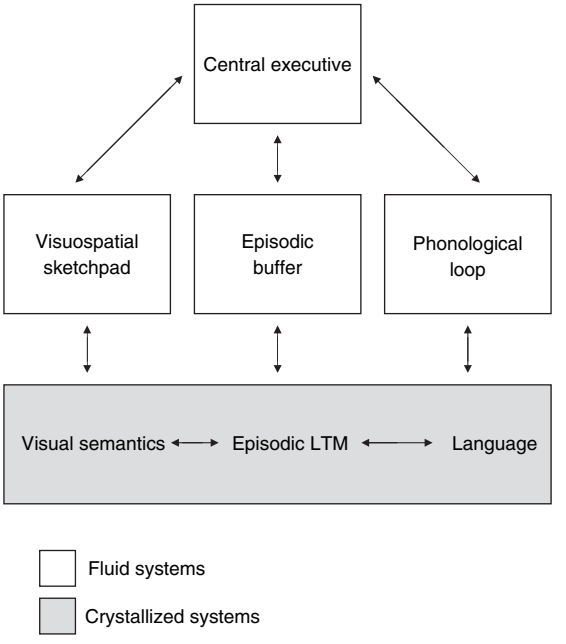

Figure 2 Baddeley's updated working memory model. Working memory is conceived as having separate storage components and a central executive process. LTM, long-term memory. Adapted from Baddeley A (2001) Is working memory still working? *Am. Psychol.* 56: 849–864; used with permission.

subjects solve simple arithmetic problems while also being given words to remember (*See* Chapter 2.04).

Three different storage systems are believed to constitute working memory. The phonological loop is involved in subvocal rehearsal and storage of auditory information (or written visual information) and is the most-studied component of working memory. The phonological loop is responsible for subvocal rehearsal and is used to account for many different empirical findings, such as the word length effect, or the finding that longer words are recalled less well than shorter words (because they take longer to rehearse).

The visuospatial sketchpad is similar to the phonological loop, except it maintains visual and spatial information, rather than acoustic information. The visual and spatial components of the sketchpad are at least partially separable, because one can observe dissociations between performance on visual working memory tasks and spatial working memory tasks (Baddeley, 2001). Most of the work on the visuospatial sketchpad up to now has focused on dissociating it from the other components of working memory.

The episodic buffer is the newest component of working memory, proposed only recently by Baddeley (2000) to explain several experimental findings. The episodic buffer is much like the phonological loop or the visuospatial sketchpad: It is a short-term store of information, although it is assumed to be able to store information of different modalities. Any information that is retrieved from long-term episodic memory (see section titled 'Episodic memory') is temporarily stored and manipulated in the episodic buffer.

The central executive component of working memory controls the subsystems. Some critics have complained that the concept is underspecified and that the concept is used to explain findings not well handled by the basic model. However, the executive component has much in common with other proposals of a central executive attention system that is used to explain how people can divide attention to different sources of information, switch attention among sources or tasks, or focus attention exclusively on one task.

1.02.3.4 Long-Term Working Memory

Long-term working memory extends the concept of working memory to account for a person's ability to readily access and utilize information stored in long-term memory. The concept of long-term working memory is particularly useful in explaining how skilled readers have the ability to easily read and comprehend texts. Indeed, the act of reading seems to require much more capacity and flexibility than the proposal for short-term or working memory can offer. A skilled reader must keep in mind words from previous sentences, paragraphs, or pages of text and readily access prior background knowledge in order to quickly and fluently process upcoming words and understand the text as a whole (e.g., Ericsson and Kintsch, 1995).

The concept of long-term working memory is also used to describe the superior mnemonic skills of experts functioning within their domain of expertise. For instance, chess masters demonstrate a remarkable ability to quickly encode and accurately remember the positions of every piece on a chess board sampled from the middle of a game, or to readily call to mind moves played in thousands of previous games in order to decide how to make the next move in a game. While researchers have attempted to explain the superior memory capacity of experts in their domain within the limits of short-term memory (Chase and Simon, 1973), evidence suggests that expert memory performance is mediated by long-term memory (e.g., Chase and Ericsson, 1982; Charness, 1991).

In contrast to the limited, fixed capacity of short-term working memory, the capacity of long-term term working memory is assumed to be flexible and may even be expanded through training. Thus, according to Ericsson and Kintsch (1995), long-term working memory is not a general cognitive ability, but rather a specialized ability that is acquired through the development of expertise for specific domains of knowledge. On the other hand, long-term working memory still depends upon the maintenance and utilization of a few retrieval cues in working memory that are, in turn, linked to retrieval structures stored in long-term memory.

1.02.4 Varieties of Long-Term Memory

Long-term memory is one of the most abused terms in psychology (and there is great competition for this honor). The reason is that the term is made to cover nearly every kind of memory not covered in the previous section. The term is used to refer to retention of words from the middle of a list presented 15 s previously to recollection of early childhood memories.

Not surprisingly, there exist various ways of carving up this huge subject. One is by type of material and mode of presentation, with the primary distinctions being among verbal memory, visual/spatial memory, and olfactory memory. Relatedly, learning of motor skills (sometimes called procedural memory, as discussed in the section 'Declarative and nondeclarative memory') is another critical and somewhat separate topic, sometimes called kinesthetic memory.

Another set of distinctions, which cut across those above, are among types of declarative (or perhaps explicit) memory: Episodic memory, autobiographical memory, semantic memory, and collective memory. We begin discussing specific codes thought to underlie long-term memory and then turn to the various types of explicit, declarative memory that have been proposed.

1.02.4.1 Code-Specific Forms of Retention

As humans possess multiple senses, there are multiple ways to sense new information and to encode that information. Raw sensory information comes in as visual, auditory, or olfactory information, as well as in other modalities. However, memories for tastes have not been much studied and because smell so greatly affects taste, separating these modalities would be difficult. Haptic memory, referring to memory for skin sensations, is also not much studied, although kinesthetic memory (for muscular movements) is a well-studied area. Studying memory for information presented in different sensory modalities has revealed both similarities and remarkable differences in how modality affects memory performance.

1.02.4.1.1 Visual–spatial memory

Memory for scenes and spatial relationships is often referred to as visual–spatial memory or just spatial memory (*See* Chapter 2.11). This type of memory is responsible for humans navigating around town in a car and for squirrels finding buried caches of acorns. Although spatial memory and episodic memory both rely on the hippocampus and surrounding areas, some theorists have argued that spatial memory is different from episodic memory and other relational (semantic) memory systems because it requires the formation of mental maps (O'Keefe and Nadel, 1978). On the other hand, Mackintosh (2002) argued that spatial learning is no different than other types of associative learning.

1.02.4.1.2 Imagery

Information presented either in events or pictures or words may be represented in the spatial system in imaginal form. One may see a butterfly and remember its appearance using this imaginal coding, or one may hear the word butterfly and be asked to form an image of the named insect. Converting verbal memories to images aids their memorability, either because the image is a deeply meaningful form (Nelson, 1979) or because coding information in verbal and imaginal codes provides additional retrieval routes to the information (Paivio, 1986).

1.02.4.1.3 Olfactory memory

Olfactory memory is more difficult to study than visual or auditory memory. Due to limitations of human olfaction, memory for odors has generally been tested with recognition tests, not with recall tests (see Herz and Engen, 1996, for a review). Olfactory memories seem to differ in some ways from other forms of memory, such as a tendency of smells to be particularly evocative of emotional memories. Indeed, the olfactory nerve is only two synapses away from the amygdala (responsible for certain types of emotions) and three synapses away from the hippocampus (which is critical for long-term memory). Olfactory memory is similar to auditory and visual memory in that performance on recognition tests decreases as the distracter set increases and as distracter similarity to targets increases. However, olfactory memory does differ from other kinds of memory in two respects. First, olfactory memory is highly resistant to forgetting: Multiple studies have shown that recognition performance for odors in a laboratory preparation is only about 5% less after 1 year than after a 30-s delay. Related to this remarkably flat forgetting curve is the finding that olfactory memory is highly resistant to retroactive interference. Proactive interference reduces olfactory memory performance greatly.

1.02.4.1.4 Skill learning

Perhaps the largest subset of different kinds of memory is the broad class of memories classified as types of kinesthetic memory or skill learning or procedural memory (the last of which was described earlier). Kinesthetic memories are those involved in motor skills: The swing of a baseball bat, how to keep a hula hoop going, and so on through hundreds of other examples (*See* Chapter 2.34). These are motor skills, the classic type of procedural memory. However, many other types of skill learning exist. There is verbal skill

learning, such as learning to read distorted or inverted text (Kolers and Roediger, 1984) – learning of grammars, both real ones and artificial ones (e.g., *See* Chapter 2.31) – and even the skillful learning of what items belong in what categories (*See* Chapter 2.47). Although a review of the various kinds of skill learning is beyond the scope of this article (see Gupta and Cohen, 2002), we can briefly point out one of the most consistent findings across the procedural learning literature: Skill learning is highly specific and transfer is often quite narrow, for example, learning to read inverted (upside down) text does not aid in learning to read backward text (Kolers and Roediger, 1984; Healy, 2007).

1.02.4.1.5 Verbal memory

Doubtless the greatest form of memory recoding and storage for human beings is based on language. People can remember events as verbal information even if they were originally presented in a different form (visual, auditory, or even olfactory or kinesthetic). Psychologists have long believed in the primacy of verbal coding, and Glanzer and Clark (1964) even proposed a verbal loop hypothesis, which theorized that all human experience is recoded into language. Subsequent research indicates that this hypothesis was a bit overstated and other forms of coding exist, but nonetheless verbal coding and verbal memories are critically important in human cognition. Verbal recoding can be impaired by instructing subjects to perform some irrelevant verbalization such as repeating nonsense words while being exposed to nonverbal information, a technique known as articulatory suppression.

1.02.4.2 Forms of Explicit Memory

We have covered these earlier in the 'Broad distinctions' section, but review them again here in more detail and provide more detailed examples of tasks used to study these forms of memory.

1.02.4.2.1 Episodic memory

Episodic memory refers to memory for particular events situated in space and time, as well as the underlying cognitive processes and neural mechanisms involved in remembering those events. A key ingredient of episodic memory that distinguishes it from other forms of memory is the retrieval of information regarding the spatial and/or temporal context in which the remembered event occurred. As previously mentioned, episodic memory is also associated with autonoetic consciousness, considered by some researchers to be an evolutionarily advanced, unique human capacity (e.g., Wheeler, 2000; Tulving, 2002, 2005).

One can point to a wide variety of examples of episodic memory, ranging from remembering what a friend wore at a party the night before to individual words studied in a list moments ago. In most contexts, episodic memory is synonymous with explicit memory, although the former term is usually used to represent a memory system and the latter term to designate types of tests that are used. Many tests have been designed to measure certain aspects of episodic memory in the lab, including free recall (recall of a set of material in any order), serial recall (recall of events in order), cued recall (recall of events given specific cues), recognition judgments (recognizing studied material intermixed with nonstudied material), source judgments (recognizing the source of presented material, such as whether it was presented auditorily or visually). Subjects may also be asked to make judgments of the recency of an event, its frequency of occurrence, or of some other quality. In addition, subjects can be asked to make metamemory judgments, or judgments about their memories. For example, a student might be asked to rate how confident he or she is in the accuracy of his/her recollections. Similarly, individuals might be asked to judge whether they can remember the moment an event occurred or the context in which it occurred or whether they only just know that they were previously encountered but cannot remember the context (Tulving, 1985). These remember/know judgments (with remember judgments reflecting episodic recollection) have been much studied (*See* Chapter 2.17).

1.02.4.2.2 Autobiographical memory

Autobiographical memory refers to memory for one's personal history (Robinson, 1976). Examples might include memories for experiences that occurred in childhood, the first time learning to drive a car, or even one's Social Security number or home address. Brewer (1986) divided autobiographical memories into categories of personal memories, autobiographical facts, and generic personal memories. Personal memories are memories for specific events in one's life that are accompanied by imagery. As such, personal autobiographical memories are thought by some to be the real-world analog to episodic memories as studied in the lab, because they are the episodes of one's life as dated in space and time. On the other hand, autobiographical facts are facts about the person that are devoid of personally experienced

temporal or spatial context information. For example, you know when and where you were born, but you cannot remember the event. Finally, generic personal memory refers to more abstract knowledge about oneself (what you are like) or to acquired procedural knowledge such as knowledge of how to ride a bicycle, ski, or play a musical instrument. Despite the conceptual overlap across classification schemes, a unique feature of autobiographical memory is that it must directly relate to oneself or one's sense of personal history.

A variety of techniques have been used to examine autobiographical memory. One approach is to simply ask people to report the most important personal events of their life (e.g., Fitzgerald, 1988; Berntsen and Rubin, 2002; Rubin and Berntsen, 2003) or to report self-defining memories (e.g., Conway et al., 2004). Another frequently used method is to ask people to describe for each of a given set of cue words the first personal memory that comes to mind, e.g., being given the word window and asked to retrieve a discrete event from your past involving a window. This task is known as the Galton-Crovitz cueing technique after its inventor (Galton, 1879) and its first modern proponent (Crovitz and Schiffman, 1974).

Many studies have plotted the temporal distribution of autobiographical memories across the life span, as described more fully by Conway (*See* Chapter 2.46). Briefly, such distributions usually exhibit three striking features (Rubin et al., 1986; Janssen et al., 2005). The first is that people tend to recall very little from the first few years of their life. This is referred to as childhood amnesia. Second, people tend to recall quite a few events from early adulthood, roughly the ages 15–25. This effect is called the reminiscence bump. Finally, most reported events are recalled from the last few years, which (like many other examples of good recall of recent information) is known as the recency effect.

Due to the personal nature of autobiographical memory, researchers have difficulty comparing what a person remembers to what actually occurred. Researchers overcome this challenge in one of several ways. One approach is to have subjects keep a diary for a length of time (e.g., days, months, years) and to record events that occurred to them at regular intervals or in response to specific cues. In addition to providing descriptions of the events that occurred, subjects might also record other accompanying details such as the exact time or location of the event and its emotional valence, salience, or distinctiveness. In turn, the diary entries are treated as the to-be-remembered stimuli in subsequent tests of memory. Moreover, as previously mentioned, the diary method is also used to capture involuntary recollections of personal events that are extremely difficult to elicit in laboratory settings. Such involuntary recollections tend to come out of the blue in response to environmental cues such as specific smells, words, or objects (e.g., Berntsen, 1996).

Another method is to assess individuals' recollections for specific historical events (e.g., the German occupation and liberation of Denmark during World War II) and then to compare the recollections with objective records of what occurred at the time, such as weather reports, newspapers, or radio broadcasts (Berntsen and Thomsen, 2005). Numerous studies of flashbulb memories (vivid recollections that surround a salient personal experience) focus on personal recollections surrounding unexpected, momentous, or emotionally charged events of public or personal significance, such as the assassination of President John F. Kennedy, the explosion of the space shuttle Challenger, or the more recent terrorist attacks on New York's World Trade Center (Brown and Kulik, 1977; Neisser and Harsh, 1993; Talarico and Rubin, 2003). However, it still remains unclear how reliable such memories are, what types of events induce flashbulb memories, and whether they really differ from memories for emotionally charged stimuli or circumstances (*See* Chapter 2.09).

1.02.4.2.3 Semantic memory

Semantic memory broadly refers to a person's general knowledge of the world. Of course, this is a vast store of information. Examples of semantic memory range from knowledge of words and their meanings, all kinds of concepts, general schemas or scripts that organize knowledge, and also specific facts about the world, such as the capital of France or famous battles in World War II.

It is reasonable to assume that when information is first learned, it is accompanied by information regarding the time and place of the learning episode. Over time and with repeated presentations of the same information, the accompanying episodic information may be lost or detached, and what remains is semantic memory. Still the distinction between episodic and semantic memory can easily blur. If someone asks about what you learned during a recent lecture, your response will likely reflect the influence of both episodic and semantic memory: Your reliance on temporal or contextual cues to remember particular points made during the lecture would reflect

episodic memory. In contrast, how you choose to reconstruct, organize, interpret, or paraphrase knowledge garnered from the lecture would reflect the influence of semantic memory.

In addition to tests of explicit and implicit memory, a variety of cognitive tests are designed to measure the contents and organization of semantic memory. These tasks might involve naming as many members of a category or words that start with a given letter that come to mind, providing word definitions, answering general knowledge questions. Other measures are designed to capture the psychological representations of word meanings by having individuals provide quantitative ratings of individual words along a variety of semantic dimensions (e.g., Osgood et al., 1957).

One of the most powerful tools for studying semantic memory is the word-priming technique in which individuals are asked to make lexical decisions (word–nonword decisions) for pairs of stimuli that might be semantically related or unrelated. For example, individuals are faster and more accurate at identifying a word (doctor) if it is was preceded by a related word (nurse) relative to an unrelated word (shoe). Indeed, comparisons in the response times for items that are semantically related versus unrelated to current or previously encountered stimuli have inspired and helped to distinguish among competing theories of how knowledge is mentally represented and accessed (e.g., Collins and Quillian, 1969; Meyer and Schvaneveldt, 1971; Collins and Loftus, 1975; Neely, 1977; *See* Chapter 2.28).

1.02.4.2.4 Collective memory

Collective memory is conceptualized in a variety of ways. In a literal sense, collective memory refers to remembering that occurs within any social context. When employees at a company meeting attempt to recall what was discussed during a previous meeting, they are engaging in a collaborative recall effort. In general, social situations can influence what individuals remember or choose to report of the past. A given social setting can dictate what sorts of recollections are most appropriate or commensurate with individual goals of communication. For instance, it is very tempting to highlight or embellish certain details of a remembered event in order tell a more entertaining story. And in turn, an individual can influence what other individuals of a group remember of the past. Studies of collaborative recall typically involve having a group of people study lists of words, pictures, or prose passages and then asking them to recall the previously studied materials either individually or in collaboration with the rest of the group (Weldon, 2000).

Work in this area has shown that collaborative recall can increase the amount of previously studied information recalled as compared to individual recall performance, but that collaborative recall tends to reduce or inhibit the amount of information recalled per individual within a group (e.g., Weldon and Bellinger, 1997). Furthermore, collaborative recall can induce recall errors, as erroneous information supplied by one member of a group is accepted and later remembered by other members of the group (e.g., Roediger et al., 2001; Meade and Roediger, 2002).

Collective memory also refers to a representation of the past that is shared by members of broader social groups defined by nationality, religion, ethnicity, or age cohort (*See* Chapter 2.48). Such a conception of collective memory is shared across the fields of psychology, anthropology, and sociology, as may be seen in the writings of Wilhelm Wundt (1910/1916), Sir Frederic Bartlett (1932), and the French sociologists Maurice Halbwachs (1950/1980) and Emile Durkheim (1915). Carl Jung (1953) used the notion of a collective unconscious in a similar sense. One commonly held assumption is that remembering is shaped by active participation within the life of a particular group. Thus, group characteristics may bias the recollections of individual group members. For instance, Russian and American high school students are likely to tell strikingly different versions of the history of World War II, with each group recalling and weaving together a different set of key events in their narratives (Wertsch, 2002). Despite the widespread use of the term collective memory, both in public discussions of how groups remember historical events such as the Vietnam War or the Holocaust and across academic disciplines, there is still little agreement as to its definition or methods of study (*See* Chapter 2.48).

In contemporary memory research, studies of collective memory bear resemblance to those of autobiographical memory in the sense that remembering one's personal history may be heavily influenced by one's cultural background. For instance, numerous studies of flashbulb memories have examined individual recollections for major historical events such as the assassinations of President John F. Kennedy, Martin Luther King, Jr., and the fall of the Berlin Wall in 1989. Some of these studies have shown striking differences in recollections across groups.

Berntsen and Thomsen (2005) examined Danes' memories for the German invasion of Denmark in 1940 and their liberation in 1945. Interestingly, they found that individuals who had ties to the Danish resistance had more vivid and accurate memories than those who did not. A key difference between autobiographical and collective memory might, therefore, lie in the impact of group identification on memory and the extent to which remembering in general is socially framed.

1.02.5 Conclusions

This chapter has surveyed some of the most common terms and distinctions among types of memory. Although we have considered only a fraction of the 256 types that Tulving (2007) identified in his (semi-serious) essay, we believe we have hit upon the great majority in contemporary use. Most of the terms used in this chapter were not used by researchers 50 years ago. We hazard the guess that someone examining the field in 50 more years might have an even greater variety of items to review, even if the serious contenders do not quite approach 256.

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1.03 History of Behavioral Learning Theories

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1.03.1 Instrumental Learning Historically and Today

One way to better understand a subject is to approach it historically – to contrast previous ideas, discarded or not, with contemporary ones. Another way is to examine current ideas in depth. The advantage of the historical method is that by contrasting older ideas with current ones, a better understanding is available of why one idea was rejected and another accepted. A disadvantage is that it requires time and effort and so may detract from an in-depth analysis of the evidence for and against contemporary ideas, which is the strength of the in-depth approach. So in examining some contemporary ideas, we would like to know if alternatives to it were raised in the past and, if so, the extent to which such alternatives are still seen as viable. Obviously a better understanding of a subject

matter requires both approaches described earlier. Accordingly, both are used here to better understand instrumental learning.

In an attempt to provide historical perspectives, brief thumbnail examinations of selected views of significant figures who have theorized about instrumental learning are provided. This treatment by no means provides a complete discussion of the theorizing of the historical figures, nor is it meant to. Rather, we selected for examination only those views of historical figures that can help to illuminate current approaches to instrumental learning. Some of these earlier views provide illumination because they are still accepted, and some because they have been replaced by other, more adequate views. In either case, contrasting earlier views with current ones is intended to provide a broader context for a better understanding of where, theoretically speaking, we are today.

The framework that encompasses all matters treated here is provided by two major issues. The two issues are relevant to any attempt to understand acquisition processes, e.g., instrumental learning or not. One issue is: What conditions are necessary for learning to occur? For example, if Event A precedes Event B, will Event A become a signal for Event B merely because the two occur together in close temporal relation (contiguity principle), or must Event B be a motivationally significant event such as food for a hungry animal (reinforcement principle)? Or must some other condition or conditions prevail for learning to occur? The second major issue is: When learning occurs, precisely what changes occur? Consider a hungry animal that traverses a runway (a tunnel of sorts) at the end of which is food. Does the animal learn an S-R association, an S-S association, an R-O association, or some combination of these, or is learning encoded in some more cognitive propositional form? According to the S-R view, a stimulus (S, runway stimuli), elicits a response (R, in the presence of these runway stimuli, run down the runway). According to the S-S view, the animal learns that the initial runway cues (S_1) are followed by terminal runway cues (S_2). In runway situations, the hungry animal would tend to use S_1 to approach S_2 when S_2 signals the availability of food. The S-S view, unlike the S-R view, does not suggest that a specific response is learned, only that the animal may perform any number of different responses to get to the second stimulus and food. The R-O view suggests that an association is formed between the response (run down the runway) and the food reward or outcome (O). Learning, in propositional form, may be expressed as follows: If in the presence of these stimuli I traverse this runway, I will obtain a satisfying morsel of food appropriate to my state of hunger.

In any instrumental situation, a response in the presence of a discriminative stimulus produces a reinforcer. For example, a person who enters a building (response) when the sky darkens (discriminative stimulus) to avoid being rained on (reinforcer) is exhibiting a form of instrumental learning. In the laboratory, a rat might cross a barrier when a tone sounds to avoid a shock. In this form of instrumental learning, called avoidance learning, as in various other forms to be discussed, a response (entering a building, crossing a barrier) in the presence of a discriminative stimulus (dark sky, tone) produces a reinforcer (avoiding rain, avoiding shock). Clearly we engage in instrumental behavior under many conditions: when we go to the supermarket, work for a paycheck, study for a test, or do myriad other things. In a word, instrumental behavior is ubiquitous.

The three components of instrumental behavior (a discriminative stimulus, a response, and a reinforcer) will each be examined in some depth, comparing contemporary views on these topics with selected views of major figures, such as Thorndike and Hull. This approach should provide a sound introduction to some of the major findings and theoretical issues of the day (*See* Chapters 1.06 and 1.10).

Instrumental learning cannot be fully understood by focusing on instrumental behavior alone. Two related matters that cannot be completely ignored when considering instrumental behavior are biology and Pavlovian conditioning. By biology we refer to the innate capacities an animal brings to the instrumental situation. Procedurally, Pavlovian learning differs from instrumental learning in not requiring a response to obtain a reinforcer. For example, in the laboratory, food may be presented following a tone of a given duration, regardless of whether or not a rat responds. Over the years a variety of differences between instrumental and Pavlovian learning have been suggested (*See* Chapters 1.06 and 1.10). Although these are interesting for considering the relation between the two, they do not constitute the single greatest reason why some considerations must be given to Pavlovian learning when considering instrumental learning. The major reason is the wide acceptance of the proposition that instrumental learning involves, and to some extent is influenced by, Pavlovian learning. By considering what has been called misbehavior, and some of the research and

theory it spawned, we can illustrate clearly why instrumental learning cannot be examined in any depth independent of biological factors and Pavlovian conditioning. Later we shall suggest that instrumental learning may be influenced by another form of learning in addition to Pavlovian learning.

1.03.1.1 Misbehavior

Keller and Marian Breland (Breland and Breland, 1961) trained a variety of animals ranging from birds to whales to make instrumental responses that might be amusing to people at zoos and other places. A very interesting finding in a variety of species was that the instrumental behavior, performed smoothly at first, began to deteriorate with further execution. For example, hungry pigs were required to deposit four or five coins in a piggy bank before food reinforcement was provided. Pigs learned this instrumental task rather easily. But with further execution, the pigs became slower and slower in depositing the coins. A major reason for the slowdown was that the pigs, instead of taking the coin to the bank straight away, would repeatedly drop it, root it, drop it again, toss it in the air, and so on. Of course these rooting behaviors with the coin are the sorts of behavior pigs perform to food itself. To explain this unexpected behavior in pigs (unexpected because it was thought at the time that with further execution instrumental responding would improve), which was called misbehavior, the Brelands suggested that the pigs were drifting back to the sort of instinctive behaviors they innately made to food, just as other species were exhibiting their own form of instinctive drift (Breland and Breland, 1961). In this view, innate responses an animal might have to, say, food were reinforced and strengthened and so came to interfere with "arbitrary" instrumental responses such as depositing a coin in a piggy bank. The idea that signals for food can cause animals to respond to those signals as they would, more or less, to the food itself has given rise to very surprising results under laboratory conditions. In a famous example, when a light signal for food was located some distance from the food itself, pigeons developed the tendency to peck the light before running back to the food hopper. Because food was available only for a rather short specified time this sign tracking behavior, as it is called, either reduced the quantity of reinforcement the animal received or caused the bird to miss reinforcement entirely. The 'rational' thing for the bird to do, of course, would have been to simply wait near the food hopper, ingesting food when it appeared over the entire time it was available. We see that innate behaviors of the animal can interfere with the smooth performance of instrumental behavior.

Sometime later Boakes et al. (1978) produced misbehavior under controlled laboratory conditions. Rats had to press a flap to obtain a ball bearing to deposit in a chute for food reward. As training progressed, the rats became more and more reluctant to deposit the ball bearings. They developed the tendency to mouth and paw the balls before letting them go. Boakes et al. (1978) suggested a rather different view of misbehavior than did the Brelands. The instinctive drift hypothesis as indicated suggested that food reward was strengthening instinctive food-related behavior. Boakes et al. (1978) suggested that the ball bearings were conditioned stimuli (CSs) eliciting unconditioned Pavlovian responses (URs). Essentially, the ball bearing CSs were eliciting innate food-related behaviors. Further evidence that misbehavior was due to Pavlovian learning interfering with instrumental learning was supplied shortly after by Timberlake et al. (1982).

In the case of misbehavior, instrumental behavior was disrupted. A little reflection serves to show that the interaction of Pavlovian responses with instrumental ones may not always disrupt instrumental performance. To elaborate on a familiar example, the Pavlovian relation between a dark cloud signaling rain and unpleasant wetness may energize the instrumental response of entering a building. One of the oldest, broadest, and most well-developed approaches to instrumental learning assumes that Pavlovian learning interacts with instrumental learning either to facilitate it or disrupt it, an approach generally called two-factor theory. The two factors, of course, are Pavlovian learning and instrumental learning. There are many examples of two-factor theory applied to a variety of instrumental learning phenomena. For now let us examine two-factor theory in relation to two different types of instrumental learning, avoidance conditioning and the acquisition and extinction of an appetitive response.

According to Mowrer (1960), a dark cloud signaling rain is classically conditioned (i.e., is Pavlovian). It supplies motivation for entering the building (i.e., fear of getting wet). Entering the building is an instrumental response, reinforced by a reduction of the fear of getting wet.

Amsel (1958) suggested that when an instrumental response is consistently reinforced with food, stimuli

that accompany the food, e.g., the stimuli in the feeding area, are classically conditioned to the feeding response that occurs. Once this Pavlovian conditioning occurs, stimuli prior to the feeding area may elicit a conditioned form of the eating response. This conditioned response itself produces stimuli that become conditioned to the instrumental response and energize it. This conditioning to the instrumental response of stimuli produced by classically conditioned eating responses is identified with expectancy of reward in the Hullian framework favored by Amsel. We shall hear more of Hull later. Amsel suggests that after expectancy of reward is established and the animal is subsequently nonrewarded, the discrepancy between the reward expected and the reward obtained will give rise to a frustrative response. This response will produce frustrative stimuli that, if introduced for the first time, will disrupt instrumental responding.

1.03.1.2 Guthrie

It will be helpful to begin with Guthrie's theory because his views, which are simple and easy to understand, provide a context for better illuminating a variety of other issues. Guthrie (1935) views instrumental learning as S-R associations established by contiguity. He suggests that a combination of stimuli that accompany some movement will on repetition tend to again produce that movement. A stimulus combination gains its full associative strength on its initial pairing with the response. One reason why repetition may be necessary to produce effective performance is that other stimuli may intervene between the movement made and a particular combination of stimuli. The effective association occurs only between simultaneous events.

In a famous experiment intended to demonstrate that behavior tends to repeat itself under highly similar conditions, Guthrie and Horton (1946) provided instead a good reason (like misbehavior much later) for being aware of an animal's natural behavior. In the Guthrie and Horton experiments, cats were photographed as they learned to escape from a small box. To escape, the cat had to make some response to a rod near the box. In responding to the rod, the cat's behavior was described as highly stereotyped behavior interpreted as providing evidence for S-R associations. The reader has probably had experience with hungry housecats brushing against his or her leg in a particular manner. If so, you have a pretty good idea of the response the cats made to the pole. They are instinctive greeting reactions made by a variety of cats, wild and domestic, known as flank rubbing. In a series of experiments Moore and Stuttard (1979) demonstrated that domestic cats would make the responses observed by Guthrie and Horton even when they are not hungry and are not rewarded for responding. They are greeting responses elicited by the sight of human observers. In the Guthrie and Horton study, the cats were aware of being observed by people. Keep this behavior in mind for later, when the behavior systems approach to instrumental learning is considered.

1.03.1.3 Estes Stimulus Sampling Theory

Stimulus sampling theory began as an attempt to formalize certain of Guthrie's ideas. Various versions of stimulus sample theory are in vogue today, as will be briefly considered later, so in this sense Guthrie lives today. In the Estes (1950) version of stimulus sampling theory, the stimulus is represented as a population of elements. On a trial, only a sample of elements from the entire population is effective (i.e., sampled). Two sources affecting which elements are sampled are random events (noises, etc.) and changes in the participant (variable behavior over trials, etc.). One view of stimulus sampling suggests that each element has a particular probability of being sampled. Another is that a number of elements is drawn without replacement from the population elements. Still another view suggests other possibilities. Estes assumed that each element is connected to one response. In this manner different S-R associations would be formed.

1.03.1.4 Thorndike

Thorndike's (1898) experiments in which hungry cats had to make some response to escape from a puzzle box to obtain food essentially introduced a new way to study learning and, for that reason alone, were justly famous. Learning was seen by Thorndike as forming connections. According to Thorndike, most learning could be explained in terms of animals making responses to situations as they were perceived. In an earlier formulation Thorndike identified three laws of learning: readiness, exercise, and effect. For learning to occur (i.e., for connections to be made), an animal had to be ready to learn (e.g., ready to read), practice or exercise was required, and the consequences of an act would determine whether

the connection would be strengthened or weakened, with satisfying consequences strengthening connections and annoying consequences weakening them.

Later on, the law of exercise was practically rejected, and the law of effect was modified. The famous finding that a blindfolded subject showed no improvement in drawing a line of a given length was among those that led to rejection of the law of exercise. The results of several experiments led Thorndike to believe that reward had a more powerful effect than punishment. For example, in certain choice experiments, Thorndike (1898) found that performance was much more affected by correct choice and reward than by incorrect choice and punishment. Punishment, according to Thorndike, caused animals to make other than the punished response. Something new introduced by Thorndike (1898) was the principle of belongingness. According to this principle, some stimuli are more easily associated with some responses than others. For example, taste cues are more easily associated with sickness than visual cues.

1.03.1.5 Hull

On considering Hull, we focus on his best-known single work, the *Principles of Behavior* (Hull, 1943). According to Hull, if in the presence of some stimuli a response was made, and the response was followed by a reinforcer, learning would occur in the form of strengthening a stimulus–response connection called a habit and symbolized as ${}_{S}H_{R}$. Reinforcement occurred when a need was reduced, as when a hungry animal obtained food. The bigger the need reduction, the greater the reinforcement. Greater reinforcement leads to stronger habits and more vigorous performance. But the expression of a habit in performance depends on several other factors, the most important for our purposes being drive (D), hunger drive produced by food deprivation, thirst drive being produced by water deprivation, and so on. Habit and drive interacted in multiplicative manner (i.e., ${}_{S}H_{R} \times D$). If D were zero, or near zero, as in the case of hunger drive in a recently fed animal, the product ${}_{S}H_{R} \times D$ would be near zero, and the animal would not respond, press a lever, run down a runway, and so on. Of course, if D and ${}_{S}H_{R}$ had high values, the product ${}_{S}H_{R} \times D$ would be large, and the animal would respond vigorously.

Responding produced inhibition, which opposed responding. The conditioned form of inhibition was symbolized as ${}_{S}I_{R}$. Reinforcement in this case was produced by the fading or diminution of inhibition with rest or not responding. Because in Hull's 1943 system ${}_{S}I_{R}$ was subtracted from ${}_{S}H_{R}$ (i.e., ${}_{S}H_{R} - {}_{S}I_{R}$), it may be seen that if the tendency to perform a response (${}_{S}H_{R}$) was opposed by an equally strong tendency not to perform the response (${}_{S}I_{R}$), responding would not occur. The failure of an acquired response to occur because of nonreinforcement is called extinction. It's important to note that extinction in Hull's 1943 system was due to competition between the tendency to respond (${}_{S}H_{R}$) and the tendency not to respond (${}_{S}I_{R}$). Although competing responses may play a role in extinction, as will become clear later, other mechanisms may play larger roles.

1.03.1.6 Skinner

Although few on the contemporary scene would claim to be, say, Hullians, many, it seems, would be happy to describe themselves as Skinnerians. Skinner's system may be classified as a descriptive one. Two examples may make clear what is meant by a descriptive system. A reinforcer is defined by its effects: Any stimulus that increases the probability of a response is a reinforcer. By drive Skinner (1938) means to point to a set of operations (say the number of hours since feeding) that have an effect on responding. Reinforcement does not arise from need reduction or a satisfying state of affairs. Drive does not arise from a state of need.

Thus Skinner is interested in functional relations between variables. He and his followers are not interested in explaining, for example, bar pressing as a habit arising from that response being followed by food that reduced a need, as Hull did. Sometimes Skinner's approach is classified as radical behaviorism. In our view it can be more correctly identified as a form of radical empiricism.

In Skinner's system the causes of behavior are not in the organism (its habits, drive, cognition, and so on) but are in the environment (reinforced behaviors are strengthened). This approach, among other things, has led Skinner to place much emphasis on schedules of reward – how, for example, ratio schedules (only some responses are reinforced), time schedules (reinforcement is available following a certain elapsed time), and other schedules influence behavior. So Skinner had little use for systems postulating hypothetic entities. He placed heavy emphasis on the practical implications of his system. In his book *Walden Two* (Skinner, 1948), he

essentially designed a society in terms of reinforcement principles. In other ways, too, Skinner went beyond the laboratory. For example, he applied reinforcement principles to verbal behavior.

It is important to note that Skinner did not necessarily deny the existence of nonobservable concepts such as cognitions. What he did deny is that such unobservable concepts, if they exist, cause behavior.

1.03.1.7 Tolman

Tolman (1932) suggested that behavior is molar and purposeful. Molar behavior, Tolman suggested, has properties all its own and cannot be identified with muscular movement, glandular secretion, or neural processes. By purpose, Tolman suggested, behavior occurs with some end in mind. Mailing a letter, buying a car, or going to a movie are purposeful behaviors executed with an end in mind. Signal learning is a distinctive feature of Tolman's system. For example, in traversing a complex maze, the learner is guided by internal and external stimuli rather than by executing a series of muscular movements. Put slightly differently, the participant follows signs to a goal, learns, in effect, a route rather than movements without meaning. This may be illustrated by an early experiment from Tolman's laboratory: Rats that learned to run through a complex maze still performed correctly when later the maze was flooded and entirely different movement patterns were required (swimming rather than running). This sort of reasoning led to what is without doubt Tolman's best-known idea on the contemporary scene, cognitive maps. Although there is some disagreement as to the definition of a cognitive map, Tolman (1948) suggested that it is a representation of the environment that includes paths, routes, and environmental relationships that a participant may use in deciding where to move.

Experiments by Blodgett (1929) and later Tolman and Honzik (1930) indicated that hungry animals could learn their way through a maze even when not given food reward. Animals performing more or less at chance prior to being given food immediately reduced errors substantially when given food reward. Acquired learning in the absence of appropriate performance was called latent learning. In order for learning that is latent to manifest itself in performance, appropriate conditions of motivation and reinforcement must be present.

1.03.2 Types of Instrumental Behavior

Various types of instrumental learning that have been used are briefly described next.

1. *Appetitive.* In an appetitive situation a response produces an appetitive stimulus such as food. Example: Pressing a bar produces food. In secondary reward training, pressing a bar might produce a stimulus, called a secondary or learned reinforcer, that was initially paired with a primary reinforcer such as food. Secondary reinforcers may be effective in increasing performance.
2. *Punishment training.* In punishment training a response produces an aversive effect such as shock. Animals make punished responses because some appetitive stimulus is provided, such as food. Evidence suggests that when nonreinforced trials are given along with reinforced ones, called partial reward, the nonreinforced trials are aversive (Brown and Wagner, 1964). Partial reward then may be a variety of punishment training.
3. *Extinction.* Following acquisition training, all trials may be nonreinforced. This is called extinction. Typically, behavior declines in extinction.
4. *Discrimination training.* Responses may receive appetitive training in the presence of one stimulus and nonreinforcement in the presence of another stimulus. Such discrimination training has many variations. For example, both stimuli may receive appetitive training, but appetitive outcomes may occur more frequently in one alternative than in the other.
5. *Escape training.* In escape training, a response terminates an aversive stimulus – usually shock. For example, an animal that is shocked may learn to run to a place in which shock does not occur.
6. *Avoidance training.* In a signaled avoidance situation, if an animal makes some response when some stimulus occurs, it can avoid an aversive event, usually shock; otherwise it is shocked. In a popular version of avoidance training, running or jumping to the other side of a box when a tone sounds can avoid shock. In Sidman or operant avoidance, the animal can avoid shock for a certain time (the response–shock interval) by making a response. Shock occurs at a shorter interval if a response is not made (the shock–shock interval). For example, the response–shock interval may be 20 s and the shock–shock interval 10 s. Thus, it pays the animal to respond.

7. *Passive avoidance.* In the passive avoidance situation, the animal is shocked for making a response. It differs from punishment training in that only the aversive shock event occurs, and not the appetitive event. As might be expected, behavior is quickly modified in the laboratory by passive avoidance training.

Sometimes the instrumental procedures just described are explicitly combined with Pavlovian ones. A popular example is as follows: After learning to press a bar for food, a rat may be shocked when a tone sounds. The tendency of the tone to reduce bar pressing is used to index fear of the tone.

1.03.3 What Makes a Stimulus a Reinforcer?

The three elements of an instrumental response are a discriminative stimulus, a response, and a reinforcer. In this section we consider various theoretical positions on reinforcers.

1.03.3.1 Contiguity

According to Guthrie (1935), reinforcement is not necessary for learning, temporal contiguity between stimulus and response being sufficient. Or one may assume, along with Skinner, that explaining why a reinforcer is a reinforcer is not necessary. All that is required is the description of a functional relationship between some response and some stimulus. The latent learning studies of Blodgett (1929) and Tolman and Honzik (1930) suggest that the effect of reinforcement is not on learning but on the motivation to engage in behavior learned by other means. Hull, who changed his position on reinforcement between his 1943 book and his 1952 book, suggested an important role for incentive motivation in determining behavior. He added to ${}_{S}H_{R} \times D$ a role for incentive motivation (V), so that in his later statement, we have ${}_{S}H_{R} \times D \times V$. Like D, if V is zero, behavior will not occur.

1.03.3.2 Satisfying Needs

As to the various explanations of what makes a reinforcer a reinforcer, Thorndike's idea that satisfaction and discomfort were key to understanding the effectiveness of reinforcers has several drawbacks: It is subjective, and like Skinner's view, it suggests that one can determine what is or is not a reinforcer only after the fact, after observing its effects on responding.

1.03.3.3 Need Reduction

Hull's idea of need reduction provides a means for determining whether a stimulus is or is not a reinforcer that is independent of its effects on the behavior it follows. For example, food deprivation will increase the need for food, and thus eating food will reduce the need and become reinforcing. Two problems with this formulation are that learning has been shown to occur when rats are given sucrose, which has no calories and thus does not reduce a need or drive (Sheffield and Roby, 1950), and when drive is increased rather than decreased, as with learning responses that increase sex drive (Sheffield, 1966). Hull's 1943 position was that greater need reduction increased the limit of asymptote to which learning could occur, a popular position today, as we shall see. In 1952, Hull assumed that reinforcement was necessary for learning but did not affect the asymptote of learning. More vigorous performance for larger reinforcers was now suggested to be due to V or incentive motivation, as indicated earlier. A major reason Hull modified his position was a set of findings provided by Crespi (1942). Crespi reported that after rats had been trained to respond, shifting reinforcement from large to small or vice versa produced a rapid change in behavior – too rapid, Hull thought, to be explained in terms of changes in learning or ${}_{S}H_{R}$. Rapid behavior change was due to a change in incentive motivation, or V.

Hull's 1952 position suggested that reinforcers can exert an effect on responding that is independent of its effects on the S-R association itself. More recent theories accept this view, as we will see in greater depth when we take up the issue of what is learned (i.e., what changes occur when learning occurs). For now it is enough to say the following: If a response is followed by a reinforcer, the execution of the response can be affected by devaluing the reinforcer in the absence of the response itself. That is, if the reinforcer is associated with lithium chloride, a poison that makes the animal sick, the animal will avoid that reinforcer, and its tendency to make the response that produced the reinforcer will be reduced (Rescorla, 2001). Along similar lines is the finding that merely placing the animal in an empty goal box, in effect showing the animal that food may no longer be available, reduces the vigor of responding,

an effect called latent extinction or nonresponse extinction (Young et al., 1960).

1.03.3.4 Premack Principle

Monkeys placed in a box by Butler and Harlow (1954) learned a response for the opportunity to look into a room. Rats will learn a maze simply by exploring it (Montgomery, 1951). These and other findings suggest that stimuli not necessary for physical survival can function as reinforcers. This view was forcefully put forward by David Premack (1965). According to Premack, activities are reinforcing to the extent that they are probable. For example, depriving a rat of food makes eating more probable, and thus reinforcing. Higher-probability responses reinforce lower-probability responses. Thus, higher-probability eating will reinforce lower-probability bar pressing. As another example, a teenager may be induced to clean his or her room, a low-probability behavior, for the opportunity to watch TV, a higher-probability behavior. This view, unlike the views of Skinner and Thorndike, allows us to determine, in advance of learning, the reinforcing capacity of particular behaviors. That is, before putting an animal in a learning situation, we can determine how probable it is that an animal would perform various behaviors under particular conditions. If the Premack principle is correct, it is possible to predict in advance which behavior will reinforce which.

1.03.3.5 The Response Deprivation Hypothesis

There is evidence that, contrary to the Premack principle, low-probability responses can reinforce higher-probability responses. It is a matter of depriving the animal of the less-probable response. For example, if ingesting X is less probable than ingesting Y, the tendency to ingest Y can be increased above its baseline by depriving the animal of X and making the availability of X contingent on ingesting Y (e.g., Timberlake and Allison, 1974; Allison, 1989). There are many interesting applications of the response deprivation hypothesis. For example, 3-year-old children who loved to run around and scream were induced in a fairly short time to sit still, a not highly probable activity for 3 year olds. Essentially, after the children sat quietly for a specified time period, they were allowed to run and scream for a few minutes (Homme et al., 1963).

The response deprivation hypothesis allows us, like the Premack principle, to determine in advance how reinforcing some activity might be. It also allows us to adjust reinforcers to particular animals or people. For example, a person who enjoys TV will perform other behaviors if those behaviors allow TV viewing, which is otherwise reduced. But if a person enjoys reading more than TV watching, let reading be contingent on TV watching.

A more subtle implication of the response deprivation hypothesis bears mention. Normally we think of instrumental behavior such as bar pressing as different from consummatory behavior such as eating. This distinction is violated by the response deprivation hypothesis. That is, any behavior can serve as a reinforcer if it is made to occur below its normal baseline. Animals deprived of food will run in order to eat, and an animal deprived of activity will eat in order to run.

1.03.3.6 Reinforcer Relativity

A popular view of reinforcement and its effects today is that it is determined by some state of the organism. For example, in Amsel's 1958 formulation of frustration theory, a given reward magnitude would tend to increase responding if it were larger than expected but would be frustrating and would tend to decrease responding it if were smaller than expected. The most recognizable form of this 'relativity' of reinforcement is contained perhaps in the Rescorla-Wagner model (Rescorla and Wagner, 1972). On a given trial, the learning actually accomplished might be lower or higher than that supported by the reinforcer employed. If it were lower, excitatory learning would occur, the change in excitation being greater the greater the difference between the learning accomplished and the learning supported by the reinforcer. However, if learning accomplished on a trial is greater than that supported by the reinforcer, inhibitory learning would occur, the inhibitory change being greater the greater the discrepancy between learning accomplished and learning supported by the reinforcer. It is probably the case that most learning psychologists accept some form of reinforcer relativity.

1.03.4 Discriminative Stimuli

Identifying the stimuli that give rise to instrumental responding is one of the most controversial types of

learning at present. We treat the topic under seven headings.

1.03.4.1 Common Understanding

In many theories, particularly earlier ones such as those of Thorndike, Hull, and Guthrie, the stimuli that controlled instrumental responding were described in commonsense terms. For example, if the animal ran down a black runway to obtain food, the situation might be described as the black runway elicits the instrumental response (I) of running. Symbolically this may be expressed as $S_B \rightarrow I$. In Hull's 1943 formulation, for example, this S-R formulation was considered adequate to explain instrumental responding. Both earlier in his journal publications (see Amsel, 1958, for Hull's collected journal papers) and later in his 1952 book, Hull would consider other important stimuli in controlling responding, as will be considered in due course. But in his 1943 statement, Hull considered instrumental responding to be controlled by exteroceptive stimuli such as "black runway" and certain interoceptive cues such as drive stimuli (stimuli produced by, e.g., hunger) and proprioceptive cues such as feedback stimuli from overt responses such as turning left or right.

1.03.4.2 Contextual Cues

Both in Pavlovian and instrumental learning, the idea arose that various contextual cues controlled responding in ways other than merely eliciting them as the S-R formulation considered earlier suggests. An important thing to note about contextual cues is that they could arise from any source: from the apparatus, from the passage of time, to just name a few. In one manifestation of this approach, the contextual cues affect responding by competing with or supporting the cues of interest to the experimenters. In the Pavlovian situation, for example, contextual apparatus cues occur along with the CS when the US is presented. To the extent that the contextual cues signal the US, they would, for one reason or another, weaken responding to the CS (see, e.g., Miller and Matzel, 1989; Rescorla and Wagner, 1972). In the instrumental situation, the contextual cues might also reduce instrumental responding. For example, animals that learned a discrimination under a so-called irrelevant cue (degree of floor tilt) had that discrimination disrupted when the floor was titled differently (Thomas, 1985). So one effect of considering context was to bring to our attention to the fact that the animal was responding to a greater range of stimuli than was previously recognized.

Another way of thinking about context is that it may function as a superordinate stimuli, informing the animal as to the specific relations that prevail under a given set of conditions. For example, in a wide variety of both instrumental and Pavlovian situations, it has been found that the extinction of responding in one context (e.g., white box) after acquisition in another context (e.g., black box) is 'renewed' when the animal is returned to the original black context. That is, responding extinguished in the black box reappears to some extent when the animal is returned to the original context, or even some novel context (see, e.g., Bouton, 1993, 2004).

Precisely how context regulates responding is a matter of some interest, which will be considered when reward produced memories are considered.

1.03.4.3 Configural Cues

When two or more cues are presented, they may be fused, so to speak, into a single representation that is perceived differently than the individual cues that comprise it. Such fused stimuli are called configural cues. In both the instrumental (e.g., Spence, 1952) and Pavlovian areas (e.g., Pearce, 1987), there are phenomena that, in the opinion of some, call for a configurational approach. We consider three examples of such phenomena. In a feature negative discrimination, the stimuli A and B are reinforced, while the combination of stimuli AB is nonreinforced (A+, B+, AB−). Animals will come to respond more strongly to the stimuli A and B than to the combination AB. It is perhaps clear that if the stimuli in a feature negative discrimination are conceptualized, as is commonly understood, then the feature negative discrimination could not be solved. That is, if the stimuli A and B are excitatory, then the compound AB must be even more excitatory. Other examples also difficult to understand are feature positive discriminations (A−, B−, AB+) and biconditional discriminations (e.g., AB+, CD+, AC−, BD−).

One way to deal with the learning of these complex discrimination problems is to assume that stimuli are configured. For example, in the negative patterning case, it may be assumed that the stimuli A and B differ from AB in that AB is a configuration. In this view, AB may be conceptualized as giving rise to a distinct stimulus X. In this way, excitation that generalizes from A and B will have less of an effect

on X than on AB. One configural approach is to assume that stimuli such as AB are configured under some circumstances but not others (e.g., Rescorla and Wagner, 1972). Another (Pearce, 1987) is to assume that all stimuli are configured whether presented alone (A, B) or in compound (AB). The former view suggests that in, say, the negative patterning case, generalization and discrimination occur between A and X and B and X. In the Pearce view, generalization and discrimination occur between the configurations A, B, and AB. Still another view, considered in the next section, rejects configuration, attempting to solve problems like negative patterning by considering stimuli to be composed of a number of discrete elements that interact.

1.03.4.4 Stimulus Sampling Theory Revisited

As we saw, Estes's version of stimulus sampling theory was derived from Guthrie's theory. Several different, newer versions of stimulus sampling theory are designed to explain phenomena such as negative pattern. One such that eschews any type of configural cue, proposing to postulate stimulus elements only, is that of McLaren and Mackintosh (2002). Prominent assumptions of this view are as follows: Some elements of a stimulus may be more weakly activated than others; however, elements weakly activated in individual stimuli may be strongly activated when two stimuli are presented in compound. This view explains a conditional discrimination such as the negative patterning problem, suggesting that certain elements weakly activated in stimulus A and in stimulus B are strongly activated in the AB compound. In this way the AB compound is made discriminative from A and from B using an element model and not a configural model. Thus, the animal can learn to respond strongly to the reinforced A and B stimuli and weakly to the nonreinforced AB compound.

1.03.4.5 Behavior Systems

According to the behavior systems approach (Timberlake et al., 1982), animals possess instinctive behavior systems such as feeding, predation, defense, mating, and so on. Certain stimuli are effective in activating a particular behavior system (see Timberlake, 2001). Stimuli employed in the laboratory are effective in activating a behavior system according to their similarity with natural stimuli. For example, a moving object will activate predator responses in a rat, which results in the responses such as chasing and biting.

1.03.4.6 Reinforcement-Produced Stimuli (Pavlovian Version)

Outcomes such as food reward, shock, nonreward, and so on, it is generally agreed, produce stimuli that may come to control instrumental responding. What mechanisms produce these stimuli? One approach, the Pavlovian one, is considered in this section. The other approach, the memory view, is considered later.

One version of the Pavlovian approach is associated with Hull and Spence and, in its most popular form perhaps, by Amsel (1958). Consider a rat that, having completed an instrumental response, is fed in the goalbox of the runway. Events in the goalbox can be seen as analogous to Pavlovian conditioning. Thus the goalbox cues (S_C) may be seen as the CS, the food as the US, and eating as the unconditioned response (R_G). The goalbox cues can be seen to elicit a conditioned eating response. This conditioned response is referred to as r_g, the conditioned form of the unconditioned eating response R_G. So we have $S_C \rightarrow r_g$. Runway cues prior to the goalbox (approach cues S_A) will elicit r_g to the extent that they are similar to S_C. Response in this formulation produces stimuli (i.e., $r_g \rightarrow s_g$). In the early portions of the runway, runway stimulus (S_A) elicits r_g, which produces s_g, and so s_g becomes conditioned to the instrumental response (I_R). This may be expressed as is shown in **Figure 1**.

This is a very powerful formulation. Earlier we indicated that merely placing the animal in the goalbox could lead to extinction. Such nonresponsive extinction is easily explained by the r_g mechanism. In the goalbox without food, the S_C-r_g association is extinguished as would any Pavlovian association. Thus, after goal placement, we would have $S_A \rightarrow I_R$. In the absence of the s_g-I_R association, instrumental responding would decline.

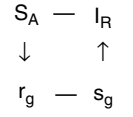

Figure 1 How stimuli produced by anticipatory responses come to control instrumental responses according to Hull, Spence, and Amsel.

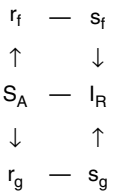

Figure 2 Amsel's formulation slowing how stimuli produced by anticipatory consumatory responses and anticipatory frustration responses come to control instrumental responses.

A powerful assumption introduced by Amsel is that if obtained reward was smaller than expected reward (r_g), the animal would be frustrated (R_F). As with R_g, a conditioned form of R_F will be elicited by goalbox cues (r_f). By the same means used in connection with r_g, r_f will become conditioned to runway cues and would produce s_g, which will become conditioned to I_R. We would have what is shown in Figure 2.

Among the many phenomena this view explains, the most prominent is the partial reinforcement extinction effect or PREE. The PREE consists of greater resistance to extinction when responses are both nonreinforced and reinforced (partial reinforcement or PRF) than when all responses are reinforced (consistent reinforcement or CRF). The PREE was a problem for all the major earlier theories (Thorndike, Hull, Guthrie, Skinner, and so on). Amsel's frustration view explains the PREE as follows. Extinction following CRF results in the introduction of considerable unconditioned frustration (R_F), which produces stimuli that elicit responses incompatible with the instrumental responses or I_R. Following PRF, frustration is conditioned to I_R, and so the animal is able to respond over a considerable number of nonrewarded trials.

What is sometimes called modern two-process theory, like the Hull-Spence-Amsel traditional version of two-process theory, assumes that classical conditioning is important in guiding and motivating instrumental behavior (Rescorla and Solomon, 1967). Modern two-process theory, unlike the traditional version, does not assume that some particular response is learned. What classical conditioning does, according to modern two-process theory, is to elicit a particular type of motivation, called a central emotional state. The emotional state elicited by the CS corresponds to that produced by the US. The emotional states do not result in a particular response. A frown may produce fear, anger, or indifference, for example – fear if the frowner is a stronger competitor, anger if the frowner is a weaker competitor, and indifference if the frowner is a stranger we likely will not encounter again. Instrumental behavior may be disrupted when, for example, a CS that is followed by shock elicits fear that disrupts bar pressing for food. A CS followed by food may, however, increase bar pressing for food by increasing hope. Traditional two-process theory with its emphasis on specific responses producing specific stimuli and modern two-process theory with its emphasis on motivational state affecting responding are not necessarily at odds with each other. For example, Rescorla, one of the authors of modern two-process theory, has recently found some of Amsel's assumptions useful for explaining extinction (Rescorla, 2001).

1.03.4.7 Stimulus Process Models

Earlier theories, on the whole, tended to assume a rather passive organism. For example, Spence (1936, 1937) suggested that all stimuli falling on the animal's receptors become associated with the instrumental response. Lashley (1951) put forward an attention view suggesting that animals actively and selectively attend to stimulus dimensions. Lashley's view did not gain general acceptance. At present a variety of proposals have been put forward suggesting that some stimuli may be more successful than others in gaining control over responding.

One suggestion, generally accepted, is that some stimuli are more salient than others and therefore will tend to be more successful in gaining control over responding. For example, brighter lights or louder tones may be more salient than less bright lights or less loud tones.

Another general type of suggestion is that over trials the organism may become more or less receptive to some stimuli than others. For example, Mackintosh (1975) assumed that stimuli will be attended to depending on the extent to which they predict reinforcement. Pearce and Hall (1980) made the rather different assumption that stimuli that predict reinforcement will not be attended to. Wagner (1978) assumed that stimuli will be processed to the extent that they are surprising. Each of these views can explain an important phenomenon that has been investigated in both instrumental and Pavlovian situations: blocking. In blocking, a given stimulus A is reinforced. Following this, stimulus A is presented along with stimulus B, and the compound is

reinforced. A typical finding is that responding to B is weak when B is presented in isolation. Mackintosh (1975) suggested that the animal is attending to A and not B, and thus blocking occurs. Pearce and Hall (1980) assumed that the compound AB predicts reward, and thus B is not attended to. Wagner (1978) assumed that the compound is not surprising and thus is not processed, and so blocking occurs.

1.03.4.8 Reward-Produced Memories

The stimuli produced by reinforcement events may be conceptualized as memories rather than the feedback stimuli of Pavlovian responses. In some cases (e.g., the PREE), the memory view and the r_g view successfully explain the same phenomena. In other cases some phenomena are better explained by one view than the other. For example, faster running sometimes occurs under PRF relative to CRF, an effect that the frustration model explains well. In another example, the sequence in which larger and smaller reinforcement occur may have profound effects on responding. The sequential model is better equipped to explain such findings (see, e.g., Capaldi, 1967, 1994).

The sequential model assumes that discriminable stimuli (memories) are associated with a large and small magnitude of reinforcement, with nonreinforcement, with different numbers of these events (e.g., the memory of two nonrewards in succession differs from that of a single nonreward), and with different combinations of reinforcement (e.g., the memory associated with nonreinforcement following reinforcement differs from that associated with the successive nonreinforcement or a single nonreinforcement).

The sequential model has been applied to a variety of phenomena. To best understand the implications of the view with a minimal amount of data, it is well to recognize that two PRF schedules equated along many dimensions (e.g., percentage of reinforcement, number of trials, magnitude of reinforcement) but differing in terms of the sequence in which the reinforced and nonreinforced trials are presented may produce quite different responses in acquisition and extinction, both when the interval between trials is short and when it is long (e.g., Capaldi, 1994). This is explained in terms of different sequences of reinforcement giving rise to different stimuli (memories) that then exercise control over responding. The details of this approach are too complex to be fully elaborated here, but some idea of the process may be gleaned from the following example: If a reinforced trial follows a nonreinforced trial, the memory of nonreward will tend to become a signal for reward. But if the nonreinforced trial follows the reinforced trials, then the memory of reward will tend to become a signal for nonreinforcement. In this example, quite different things are learned when the same number and percentage of rewarded and nonrewarded trials are presented in different sequences.

Perhaps the best intuitive understanding of how memories control instrumental responding is provided by a schedule in which reinforced and nonreinforced trials are given in single alternation fashion (SA schedule). Under the SA schedule, rats will come to show pattern responding and faster running on reinforced than on nonreinforced trials even at intervals between trials of 20 min and 24 h (Capaldi and Stanley, 1963).

1.03.5 Response

Two somewhat different but related issues are considered in this section. One is concerned with how to characterize the instrumental response. The other is concerned with the role the response might exercise in relation to reinforcement outcome (R-O association).

1.03.5.1 Characterizing the Response

In the simple T maze shown in **Figure 3**, the animal starts at S and can gain food by making the correct response. One of the most divisive issues of early theories was with how to characterize the correct response. Is the rat learning a movement pattern associated with left turning, as Guthrie would say, or is the animal learning something more general, such as going to a place? In an attempt to resolve this issue, a variety of procedures have been employed.

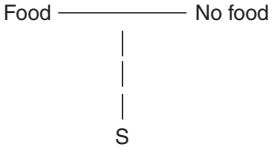

Figure 3 A simple T maze. The animal is placed in the maze at S and can respond by turning to the left or the right – a right turn produces food reward, a left turn no reward.

1.03.5.1.1 Extensive training

One procedure is to train the animal for a considerable number of trials to see whether the response becomes progressively more stereotyped with training. The idea here is that if the response is best characterized in terms of movements, it should show less and less variability as training progresses. But if the animal is learning something more general, such as obtaining food by approaching the windowed wall on the left, behavior could show considerable variability over trials. Muenzinger (1928) trained guinea pigs to approach a bar and to press it to obtain food. The animals were trained for 1000 trials; considerably more training was required for performance to improve. Muenzinger classified the animal's behavior into nine classes: press with left foot, press with right food, press with both feet, press with head, and so on. Behavior varied over trials, with one behavior rising and another falling. Tolman (1932) used results of this sort to support his idea that animals obtain goals and do not necessarily engage in movement patterns. For an alternative interpretation of these findings, see MacCorquodale and Meehl (1954).

1.03.5.1.2 Shift responses

Another approach is to allow an animal to obtain reinforcement by performing one response, then requiring another response to obtain the same reinforcement. For example, an animal might obtain reinforcement by running through a maze. Then after learning, as indicated, the maze might be flooded so that now a different series of movements (swimming) must be made to obtain food (Tolman, 1932). Lashley (1951) operated on animals so that a new response, rolling through the maze, rather than running, was required to obtain food. In both cases, considerable transfer was obtained from running to swimming and from running to rolling.

1.03.5.1.3 Blocked routes

A third way was to have animals obtain reinforcement by going to some particular place for food by a given route. After learning, the original path would be blocked and a variety of new alternative paths provided. The question was: Would the animal select an optimal path to the goal such as the shortest one? In a famous experiment of this sort, Tolman et al. (1946) obtained positive findings.

1.03.5.1.4 Cross maze

A related method involved the use of a cross maze of the sort shown in **Figure 4**. A trial would start by placing the animal at S1 or at S2. One group, the place group, was required to go to G1 to obtain food, both when started from S1 and when started from S2. The other group, the response group, was required to turn in a given direction, say left, for food. This means that when started from S1, the animal was required to go to G1, and when started from S2, the animal was required to go to G2. Better performance by the place group than by the response group would suggest that a more cognitive characterization of what was learned would be in order, according to Tolman et al. (1946). Better performance by the response group would indicate that more stereotyped responses were learned according to Tolman et al.

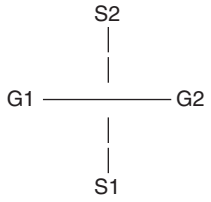

Figure 4 A cross maze. The animal may be placed at either S_1 or S_2. It may respond by turning either to G1 or to G2.

Considerable research was reported using the cross maze. The outcome of this research was that better place than response learning occurred under some conditions, the opposite being the case under other conditions (see Restle, 1957).

Restle suggested that animals may learn either stereotyped responses or broader responses, a conclusion that is not surprising, considering earlier research. For example, it was shown early on that rats that learned to run a certain distance for food would turn into an empty wall when the runway was lengthened, and they would fly out into empty space when the runway was shortened (Munn, 1950).

On the other hand, it is clear that animals can also obtain a reinforcer using other than the original response, such as swimming instead of running.

1.03.5.2 R-O Association

Classically, two sorts of associations have been posited by theorists such as Thorndike and Hull in instrumental learning, S-R associations and S-S associations (stimulus–outcome associations). In an S-S association, some stimulus (window on the right) elicits some response (turn left or approach the window). Recently several theorists have suggested that the reward outcome (O) may be anticipated on the

basis of the response, an R-O association. This makes common sense: A person might say "I am pressing the bar (R) in order to obtain food (O)."

A major procedure for investigation of R-O association involves reinforcer devaluation. We will now describe in general terms how this is done. The animal might be trained to make two different responses such as bar pressing (R1) or chain pulling (R2) to obtain two different reinforcers, say plain pellets (O1) and sucrose pellets (O2) (e.g., R1-O1, R2-O2). Following this training, one of the outcomes may be devalued by poisoning it following its ingestion. Without further training, the animal is given access to both R1 and R2. A critical finding is that the response followed by the poisoned reinforcer is reduced relative to the other (see, e.g., Colwill and Rescorla, 1985). This indicates that the animal is able to anticipate which O follows which R.

1.03.6 Extinction

Every theory of learning has to deal not only with acquisition of responding but with its extinction as well. The theories of extinction embraced by Thorndike, Hull, Spence, Estes, and others have not fared well. For example, Hull (1943) suggested a theory of extinction in which the tendency to respond and the tendency to not respond at the end of extinction would be in balance. Essentially, according to the Hullian position, at the end of extinction, reacquisition of responding would be impossible. At the other extreme, the Rescorla-Wagner view of extinction (Rescorla and Wagner, 1972) suggests that original learning would be erased by extinction, and reacquisition would have to start from scratch and be no different from original acquisition.

Numerous phenomena indicate that whatever was learned in the acquisition phase survives in extinction to a considerable extent. One is so-called spontaneous recovery, in which responding recovers following the elapse of time since extinction. Rapid reacquisition following extinction is another example. In rapid reacquisition, three phases may occur: CRF, extinction, and CRF again. In the third phase, responding that was lost in extinction quickly recovers.

Over the years three views of extinction have been suggested. One is the sort of competing response view suggested by Hull (1943) – that the tendency to respond in extinction is opposed by the growth in extinction of the tendency not to respond. Another is the unlearning view suggested, for example, by Rescorla and Wagner (1972) that whatever was learned in acquisition is unlearned in extinction. The third is that extinction is a variety of discrimination learning in which stimuli that are excitatory in acquisition are replaced in extinction by stimuli that are not excitatory (e.g., Capaldi, 1967, 1994). Some theories, Amsel (1958) for example, assumed all three. According to Amsel, in extinction r_g is extinguished, unconditioned frustration elicits responses incompatible with the instrumental reaction, and frustration may introduce entirely new nonexcitatory stimuli in extinction, particularly following CRF acquisition. Extinction, a highly popular area of investigation in the 1960s to about 1980, has recently become a fertile area of investigation again (see, e.g., Rescorla, 2001; Bouton, 2004).

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1.04 Multiple Memory Systems: A New View

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1.04.1 Introduction

> It has been already stated that those who have a good memory are not identical with those who are quick at recollecting. But the act of recollecting differs from that of remembering, not only chronologically, but also in this, that many also of the other animals (as well as man) have memory, but, of all that we are acquainted with, none, we venture to say, except man, shares in the faculty of recollection. (Aristotle, *Ars Memoria*)

While Aristotle clearly recognized that memory comes in different forms, and pointed out that remembering and recollecting seem different, he left many questions open. Is there a single memory that can be accessed in different ways – by remembering or recollecting? Are there multiple memories, one of which supports remembering, the other recollecting? If so, how do these relate to one another, if at all? Questions like these, asked in a variety of ways, have dominated the memory literature in recent years. While it is beyond the scope of this chapter to represent all these approaches (see Schacter and Tulving, 1994; and a collection of papers in a special recent issue of *Neurobiology of Learning and Memory* (Vol. 82) for a sampling of views; *See* Chapters 1.02, 1.05), I hope to illuminate some recent history and provide a new perspective on how one might think about memory and its various forms.

Memory is best conceived as a set of functions that serve specific adaptive purposes (cf., Klein et al., 2002). These functions all share the common property of enabling organisms to benefit from prior experience. Since these experiences are many and varied, and the needs of complex organisms are similarly diverse, it is hardly surprising that there would be many kinds of memory. This chapter considers the different types of memory and ask how they differ, how they are instantiated in the brain, and how they interact. It also speculates about the separate functions they serve and discusses implications of the fact that memory comes in multiple forms.

Before diving into this thicket, we need to clear some brush and ask what we mean by each of the terms in the phrase 'memory system'. What exactly is memory? What do we mean by a system? We'll start by briefly discussing what is meant by the term system and then address the nature of memory systems at some length. This will lead to the surprising proposal that we change the way we think about memory altogether.

1.04.2 What Is a System?

A standard definition of system goes something like this: A system is a group of interacting, interrelated, or interdependent elements forming a complex whole. There seem to be two critical parts to this definition. First, a system is composed of parts, and second, these parts interact in some way to form a whole whose properties are more than just the sum of the separate parts. As important as what the definition includes is what it does not. A system need not make up a coherent physical entity, and it need not always act in an interrelated fashion. The parts of a system can be distant from one another (an extended family for example), and they can sometimes act together and sometimes not. What is more, the parts of a system can be made up of similar things or of quite different things.

Complex biological systems come in a variety of forms, but one thing we can assume they all share is that they have achieved their present form through a process of selection, shaped by phylogeny, epigenetic needs, and their environmental niche. The brain is among the most complex of biological systems and is not one but many systems that must interact effectively to sustain life and maximize the potential for procreation. Memory, in this context, is but one of many neurobiological systems, the one that allows organisms to benefit from knowledge obtained during their individual lifetimes. Organisms also benefit from knowledge acquired in the course of phylogeny by virtue of the fact that fundamental aspects of the world we inhabit are built into the structure of the nervous system. Although this knowledge about the world may emerge over the course of individual development, it is in no sense learned or remembered: It is simply known. Basic facts about the physical world, such as the laws pertaining to gravity, space, time, and causality, are examples of this kind of knowledge. The associative rules that govern our understanding of how the things we experience relate to each other are another example (*See* Chapters 1.03, 1.06).

1.04.3 What Is a Memory, and a Memory System?

Memory is, by definition, a record created by an individual as a result of its past experience. But what is the nature of this record, and how long does it last? How is it accessed? It has long been understood that some memories are evanescent while others last a long time. A distinction between short-term and long-term memory has been part of the scholarly discussion of memory for more than a century, even though the boundaries between these two are not clearly worked out. In recent years, the notion of working memory has emerged to complicate matters. For some, short-term memory and working memory (which may or may not be the same thing) are memory systems, and given the generic definition of systems offered above, this seems indisputably true. However, these are not the systems of which memory researchers typically speak when they speak of multiple memory systems. The notion of multiple memory systems emerged well after the idea that memory could exist in different temporal forms was entrenched in the field.

For much of the past century, most memory researchers have adopted a particular framework that viewed memory as reflecting the presence of a coherent trace in the brain, an engram that could be identified if only we knew just what we were looking for. The difficulty inherent in this pursuit was forcefully expressed by Lashley, whose failed search for the engram led to the contrarian thought that:

> I sometimes feel, in reviewing the evidence on the localization of the memory trace, that the necessary conclusion is that learning just is not possible. It is difficult to conceive of a mechanism which can satisfy the conditions set for it. (Lashley, 1950: 501)

A possible solution to the problem raised by Lashley's work was provided by Hebb (1949) in his connectionist theory of synaptic change, cell assembly formation, and phase sequence activity. These postulated mechanisms presumed to show how an engram could be distributed within the brain and how the full pattern of the memory trace could be activated by many different paths, or cell assemblies (*See* Chapters 1.33, 1.34, 1.35). His theory showed how a memory trace could exist, could avoid total disruption when damage occurred, and could permit what we now call pattern completion. It seemed, in other words, that Lashley searched in vain because he was looking for the wrong thing, and that it still made sense to talk about a fixed memory trace.

In order to understand this debate, it is important to recognize that the term memory was at that time reserved for what we now call episodic memory (*See* Chapters 1.22, 1.23). The term was used to refer to the recollection, in Aristotle's terms, of specific

events in one's past, such as what happened yesterday, or the week/month/year before. This usage persisted at least until the 1960s. Other forms of knowledge that reflected prior experience were not labeled as memory *per se*. Instead, terms such as habit were used. One result of this usage was the long-standing resistance to attributing memories to non-human animals (again reflecting Aristotle).

A major shift in terminology resulted from the emergence of neuroscience and attempts to understand the neural bases of learning and memory. Since the relevant studies were being done in rodents and various invertebrates, the notion of what constituted a memory had to be broadened. The term memory came to be applied to just about any change in the nervous system that resulted from prior experience, becoming interchangeable with the notion of plasticity. Given this much broader definition of memory, it was only a matter of time before the idea that there were qualitatively different kinds of memory emerged. I have sketched out my understanding of some of the historical forces at play here in earlier papers (Nadel, 1992, 1994), so I will be relatively brief here.

When the patient HM was first described (Scoville and Milner, 1957), memory was thought of in the univocal way described above. Milner (1966) described the deficit created by medial temporal excision in HM and several other patients as follows:

> The pattern of amnesia which emerges from the clinical observations of the patients with bilateral hippocampal damage is one in which long-term memories survive, as does the ability to attend normally to on-going events. The essential difficulty appears to be in adding any new information to the long-term store. (Milner, 1966: 124)

HM could show some learning of motor skills (cf. Corkin, 1968), suggesting to Milner that "the pattern of amnesia demonstrated by these patients is incompatible with a unitary-process theory of memory" (Milner, 1966: 131). However, this did not lead immediately to the notion that there were multiple memory systems, since such motor learning, after all, did not actually involve memory by the definitions of the time. Instead, the data from amnesia were taken to support then current models of memory involving separate short-term and long-term systems, with a memory consolidation process responsible for the transition from one system to another. Milner suggested "It is possible that in normal learning, the hippocampal region acts to prime activity in cortical areas where storage is taking place" (Milner, 1966: 130). From this notion the idea that the hippocampus was critical for what is now called systems consolidation emerged, and it has played a central role in thinking about the hippocampal role in memory ever since (see Nadel, 2007, for a brief history and update of this idea). Although the concept of multiple memory systems is implicit in this notion, it remained implicit for some years.

Research attention turned instead to the issue of which brain areas were critical to the amnesic deficit, and the attempt to establish an animal model of the syndrome observed in HM. The initial failure to replicate HM's memory deficit with what were assumed to be comparable lesions in primates (Orbach et al., 1960) left researchers confused. Had significant changes in the function of the relevant structures emerged during evolution? Did the medial temporal lobe do something very different in primates and humans, not to mention rats? These kinds of questions were very much in the air in the 1960s.

In a seminal series of papers in the late 1960s, Warrington and Weiskrantz (1968, 1970) showed that amnesic patients could indeed benefit from some forms of prior experience in addition to showing motor learning. Their early demonstrations included the use of fragmented pictures and words – amnesic patients exposed to such materials took less and less time to identify the materials with repetition. They noted that there are "two types of task, motor learning and retention by partial information, which are relatively well preserved in amnesic subjects," and they wondered "Is there a common factor linking performance on these apparently dissimilar tasks?" (Warrington and Weiskrantz, 1970: 630). They pointed out that the presence of spared memory capacity might allow one to explain the apparent discrepancy in the data from the clinic and from animal studies, but this would necessitate dropping the idea that the defect caused by hippocampal damage was one of impaired memory consolidation.

From a historical perspective, it is intriguing that both research programs, in Canada and the United Kingdom, had uncovered the fact that in the amnesic syndrome some forms of learning and memory were spared while others were impaired, but neither jumped to the idea that there were multiple memory systems.

This advance came instead from the domain of animal research, in particular several programs focused on the functions of the rat and monkey

hippocampus. Three publications in 1974 proposed, in rather different ways, that there were multiple forms of memory and that the hippocampus was only responsible for one of them. Gaffan (1974) suggested that there were two forms of memory, one involving recognition, the other association, and that the hippocampal system was only critical for recognition. Hirsh (1974) suggested that memory could be either context bound or context free, and that the hippocampus was critical only for context-bound memory. Nadel and O'Keefe (1974), building on the discovery of place cells in the hippocampus of the rat (O'Keefe and Dostrovsky, 1971), suggested that the hippocampus was critical only for acquiring cognitive maps and that the place learning and episodic memories that depend upon them, whereas other forms of learning and memory depended on other brain circuits.

O'Keefe and Nadel (1978, 1979) laid out a comprehensive theory that rested squarely on the notion that there are multiple forms of memory.

> there are different types of memory . . . localized in many, possibly most, neural systems. . ..
>
> there is no such thing as the memory area . . . there are memory areas, each responsible for a different form of information storage. The hippocampus . . . both constructs and stores cognitive maps (O'Keefe and Nadel, 1978: 373–374).

The authors argued that the hippocampal system was concerned with knowing that, while other memory circuits were concerned with knowing how. This idea was taken up by Cohen and Squire (1980), who showed that amnesics could learn how to mirror read but could not remember that they had done so. Within a matter of a few years, the notion of multiple forms of memory was accepted in both the animal and human domains, and though there have been occasional attempts to argue against this view, the idea seems firmly rooted.

The answer to the question – what memory is – seems only to complicate matters, because memory is many things, not one. But how are these many things, these multiple forms of memory, to be distinguished from one another? Do they reflect the operation of different systems, in the sense described above? In order to answer this one question we need to ask, and answer, several others. Do the different forms of memory reflect different kinds of information? Do they differ in terms of the processes they instantiate? Do they differ in terms of the brain mechanisms and structures they involve? Do these separate learning and memory systems obey different rules of operation? Most researchers would agree that there are different memory systems only if the answer to most of these questions is yes.

Because of the central role played by the hippocampal system in thinking about memory, the early tendency was to talk about two kinds of memory, one dependent upon hippocampal circuits and the other not. Thus, O'Keefe and Nadel (1978, 1979) talked about locale and taxon memory, Cohen (1984) and Squire (1987) talked about declarative and procedural systems, and others talked about explicit and implicit systems, to name but a few. There are many commonalities among these various ways of handling the multiplicity of memory, but they all share the fate that they are too simplistic. Current approaches suggest as many as 5 to 10 kinds of memory. As the number of putatively separable memory systems increases, it becomes important to step back and ask the fundamental questions again. What exactly do we mean by memory? How is prior experience incorporated into current behavior?

1.04.4 What Is Memory, Redux

Given the expanded view of memory forced upon us by neuroscientific exploration of memory's biological bases, it seems time to rethink the notion of memory itself. If there are 5, 6, or 10 different kinds of memory, does it make sense to call them all memory? My answer is that it does not. Instead, I propose to go back to using the term as it had been used before recent developments widened its application. That is, I believe we should use the term memory solely to refer to what happens when an organism recollects the past. All else would best be called knowledge (Nadel and Wexler, 1984). As a function of experience we acquire knowledge, which is represented in various brain systems. Knowledge is used to generate both memories and behaviors. What we call a memory is constructed from this knowledge as required. The hippocampal system, serving as a contextual binding device, a creator of cognitive maps, plays a critical role in this construction process.

This way of thinking has its roots in debates more than 60 years ago between adherents of different views of learning and behavior. Following Tolman (1932), we assumed that organisms always act with purpose, trying out one or another strategy or hypothesis to deal with their current situation

(cf. O'Keefe and Nadel, 1978: chapter 2). We focused our attention on spatial behavior and spelled out a number of different hypotheses an animal could use to get around in the world, including place, guidance (or cue), and orientation (or response) hypotheses. Typically, hypotheses require complex interactions among different knowledge systems, only some of which reflect learning. That is, some of the knowledge an animal uses to act in the world comes from prewired circuits. For example, animals do not have to learn *de novo* that stimuli in close temporal contiguity are more likely to be causally linked to one another than stimuli widely separated in time.

By this analysis, we have multiple knowledge systems, which we use to generate various forms of behavior and to construct memories. Much the same set of questions applies to these multiple knowledge systems as applies to what have been called multiple memory systems. How do they differ, how do they interact, what rules of operation do they obey, how are they instantiated in the brain? If the reader prefers, she or he can continue to think about multiple memory systems as they read the words multiple knowledge systems.

One consequence of this proposed shift in terminology is that the idea of a brain system devoted purely to long-term memory no longer makes sense. This idea, stated perhaps most forcefully by Squire and his colleagues with reference to the medial temporal lobe (e.g., Squire, 1994), depended on the idea that damage to this part of the brain affected only memory but not short-term processing or perception. This idea has been criticized both in the past (Horel, 1978, 1994) and in recent writings (cf. Ranganath and Blumenfeld, 2005; Hannula et al., 2006), and in my view it should now be retired. It makes more sense to think about all neural systems as both processing and storing knowledge, with the differences between systems reflecting the nature of the knowledge being processed and stored, and the timescale of that storage.

1.04.5 Multiple Knowledge Systems

What kinds of knowledge does an organism need to acquire about its world in order to survive and even prosper? Bear in mind we are assuming that fundamental facts about how the world works are built into the organism's system by phylogeny and do not have to be relearned by each generation. What does have to be learned are those things that cannot have been acquired in the course of phylogeny. What are they?

Quite a lot it turns out: who your mother, father, sister, or brother are; who, what, and where you should approach or avoid; what you should eat and drink, and when; who you should try to copulate with, and when (and where); how to do various things such as explore, play, fight, hunt, escape predation, find your way home again, and more. A special form of acquired knowledge concerns those accidental conjunctions that we call events, when actors, actions, and worldly objects come together in combinations that could never have been predicted in advance. What sorts of knowledge/memory systems would provide the best way of learning all this and solving all the problems life confronts us with?

As a first cut, it is useful to note that organisms appear to need knowledge systems concerned with two very different kinds of information – first is the need for knowledge accumulated over many similar events, and second is the need for knowledge about unique occurrences, and the limits these might put on the application of cumulative knowledge (cf., Klein et al., 2002). Tulving's (1972) discussion of semantic and episodic memory captured this distinction. I have tried to make the case that the term memory should be reserved for recollections of unique episodes, but there has lately been considerable interest in cumulative knowledge, which has been viewed as allowing organisms to extract reliable information about the statistical structure of the environment. A significant complication in thinking about knowledge systems is that the systems engaged in processing these two types of information are in constant interaction. Indeed, how to think about the relations between episodic and semantic knowledge, and the brain systems underlying these forms of information, constitutes one of the major challenges for the future. We return to this issue later, after addressing in more detail the question of what kinds of knowledge systems the brain must contain.

Before turning to a discussion of types of knowledge systems, it is worth pointing out that thinking in terms of knowledge rather than memory forces us to put the function of knowledge acquisition front and center. What propels an organism to gather knowledge in the first place? This is a question that bedeviled early psychologists focused on biological categories and drives. The existence of curiosity and exploration were an embarrassment since the mere acquisition of knowledge could not be simply related to any particular biological drive. Within a

framework that emphasizes knowledge, curiosity and exploration become critical. Instead of being relegated to sidebar status, these behaviors deserve careful study in their own right. It remains a remarkable fact about modern cognitive neuroscience that so little attention has been paid to these core functions (cf. Avni et al., 2006; Whishaw et al., 2006; for two recent exceptions to this neglect).

1.04.5.1 Types of Knowledge Systems

One way to think about knowledge systems is in the following simplistic fashion – an organism needs knowledge about what it experiences, where and when things happened, who was involved, the value of the things experienced, and how to act in the future when confronted with similar experiences (*See* Chapters 1.21, 1.22, 1.23). One might imagine that organisms extract and store knowledge about each of these aspects of experience in separate systems, combining this knowledge when required to do so by the demands of a given situation, or an experimenter-defined laboratory task. As we consider each of these separately, it becomes clear that things are not as simple as they might seem. To start, these categories are not absolutely clear-cut – there is overlap between them in some instances, as we see later. Further, some kinds of knowledge are inferred rather than experienced. Perhaps the best example concerns why things happen. Organisms make inferences about causality, even though these are rarely backed up by direct experience, and these inferences become an important part of their knowledge base. Notwithstanding these problems, it still seems worthwhile thinking about knowledge systems along these lines. In what follows, I briefly discuss each of these kinds of knowledge. A serious analysis of this approach would require a substantial expansion of this discussion, one that is beyond the scope of this chapter.

1.04.5.1.1 Knowing what

It is best to start with the kind of knowledge that informs an organism about the stuff of which existence is made. All else is subservient to this kind of knowledge, since in the absence of what there is no where, when, why, or how. Or, as Kant put it, form in the absence of content is meaningless. What knowledge provides this content, the semantics of existence one might say.

The category of what knowledge is complex, comprising several different kinds of knowledge, including:

- What happened
- What entities were involved
- What properties these entities have

These three forms of knowledge are quite different in kind, and one might imagine that they are subserved by quite separate underlying neural and cognitive systems themselves.

Processing and storing information about what happened is central to memory for events, or episodes, and hence must incorporate the ability to capture sequence information. Every such sequence would consist of entities interacting in space and/or time. The entities themselves must be represented in a fashion that captures their properties, both structural and functional. The ventral visual stream (Ungerleider and Mishkin, 1982) is a well-known example of what I am here calling a what system, and indeed this is the name given to it by Ungerleider and Mishkin. It is reasonable to assume that similar what systems exist within each of the sensory/perceptual processing streams. These systems contain representations of entities in the world and their properties, representations that have been shaped by the experiences an organism has had with these entities in multiple event contexts. Each time an entity is encountered in the world, its "what" representation is activated, and as a function of this new experience, the representation altered. These representations are activated and hence mobilized in the act of memory retrieval.

1.04.5.1.2 Knowing where

This category is also complex, because animals need to know several things that could be referred to as where knowledge. They need to know where they are at any given moment. They need to know where important things, such as food, water, safety, and conspecifics, are located, both with respect to an environmental framework and with respect to where they are themselves located at any given moment. They need to know where events happened. They also need to know how to get from one place to another (*See* Chapters 1.20, 1.21, 1.25).

It seems clear that significant neural resources are devoted to processing and storing where knowledge. Extensive systems seem devoted to representing where things are relative to the current location of the organism. What is more, this information seems to be multiply represented, in that it is captured with respect to several frames of reference. That is, organisms simultaneously know where an object is with

respect to the head, the eyes, the hands, and the body. This is important because action depends upon such knowledge. In addition to these various ego-centered spatial systems, there is a system, centered on the hippocampal formation, that represents an organism's location in absolute, or allocentric, space. This system makes use of inputs from multiple sources, and includes elements such as place cells in the hippocampus (e.g., O'Keefe, 1976), head-direction cells in the thalamus and postsubiculum (e.g., Taube et al., 1990), and the recently discovered grid-cells in the entorhinal cortex (Hafting et al., 2005).

In addition to providing specific information about an animal's location in space, this system is also central to knowledge about context, that is, the spatial setting within which events happen (cf., Nadel and Willner, 1980; Nadel et al., 1985). It is this role, we have argued, that makes the hippocampal cognitive mapping system central to episodic memory, which by definition incorporates information about where an event transpired. It is certainly one of the main challenges for the future to discover why and how spatial mapping and episodic memory utilize the same circuits (*See* Chapter 1.33).

1.04.5.1.3 Knowing when

There are at least two kinds of when knowledge that organisms might need. First, they might need to know when, over a long span of time, an event occurred. Was it yesterday, last week, last year? This kind of knowledge is integral to episodic memory, and there remain debates about whether animals other than humans actually represent it. Second, organisms need to know when within a particular event the various parts of that event occurred. This might seem a trivial matter, but if one argues, as we are arguing, that the various parts of an event are processed and represented in separable brain regions, then being able to assemble them in appropriate temporal order, to know when each of them occurred with respect to the others, is critical. The simplest example will suffice to make this point: it makes all the difference whether A comes before or after B, because the attribution of causality depends upon knowing which of these two entities or events came first. An organism that gets temporal order wrong is going to make the wrong attributions and is not going to survive very long.

1.04.5.1.4 Knowing who

This is a somewhat simpler category, and one that might be of importance only in species with the capacity to recognize individuals, itself a function of the sophistication of its what systems. In humans, it plays a critical role, of course. George is smarter than Dick leads to a very different conclusion than Dick is smarter than George, although both statements employ the same words.

1.04.5.1.5 Knowing how

This is a big category, comprising all the knowledge referred to as procedural by many authors. There are some important distinctions to be drawn, however, between some of these forms of how knowledge. For example, knowledge of how to carry out some kind of motor act, such as brushing your teeth or driving your car or playing squash, is rather different than knowledge about how to get from one place to another (e.g., which route to take, not how to move). It is not within the scope of this chapter to go into much detail on this category of knowledge, but current work on the functions of the caudate nucleus in particular, and the basal ganglia in general, are shedding light on how we know about how.

1.04.5.1.6 Knowing valence

In addition to storing knowledge about the kinds of entities they confront in the world, organisms represent the value of these entities, whether they are good or bad, exciting or frightening, and so on. This kind of knowledge plays a critical role in determining not only how an organism deals with such entities in the future but also how strongly the knowledge itself is committed to storage. In general, the greater the valence, the more robust the storage, at least within most of the brain's knowledge systems. As with all kinds of knowledge, the use of value information is highly dependent upon the context within which an organism is acting, including its internal motivational context. Knowing that something tastes good is much more useful when one is hungry than when sated, for example. Considerable evidence in recent decades suggests that the amygdala plays a central role in representing value knowledge, but it is by no means the only structure so engaged. Extensive midbrain circuits are devoted to assessing and presumably storing information about the reinforcing value of various stimuli an organism comes into contact with. Portions of the cingulate cortex and frontal cortex also contribute to this system.

1.04.5.1.7 Implications of the existence of multiple systems

There are many implications of assuming that the brain is organized into multiple knowledge systems. Consider the fact that the knowledge about an episode in one's life is dispersed within the brain, across multiple systems. What happened, who was involved, where and when it happened – these various aspects of a memory are widely distributed, which means that retrieving and reporting an episode memory must be a constructive act, much as Bartlett (1932) and others have argued. And being constructed, memory for episodes must be open to error in a way that engrams were not supposed to be.

Different knowledge systems, utilizing separable neural substrates, could operate by distinct rules. O'Keefe and Nadel (1978), for example, proposed that there were two quite different brain systems engaged in learning and remembering, which they called the locale and taxon systems. The locale system was associated with the hippocampal formation, and the various taxon systems were associated with neocortical and subcortical structures. It was suggested that learning within these two kinds of systems reflected different operating principles. The taxon systems obeyed standard laws of reinforcement and followed associative principles. Learning within the locale system, by contrast, was assumed to proceed independent of reinforcements such as food, water, safety, or access to a mate – animals formed cognitive maps whether rewarded or not. Further, O'Keefe and Nadel asserted that learning within the locale system did not follow associative rules. Consistent with these speculations, Hardt and Nadel have shown that learning within the locale system differs from learning within taxon systems in that the latter reflect the operation of standard associative phenomena such as overshadowing and blocking, whereas the former does not. Instead, knowledge acquisition in the locale system is automatic, such that new information updates previous representations, whether or not reward contingencies have changed. Behind the assertion that these two learning systems obey different rules is the assumption that the underlying neural architectures in the systems subserving these two forms of knowledge differ in critical ways that allow different learning rules to be implemented in each.

In addition to these system-level implications, there are a number of others that reflect the fact that by being separate, knowledge systems can be affected differentially by all that life has to offer. In what follows, I discuss several examples, including development, aging, and the reaction of different systems to stress. In each case it will be seen that knowledge systems vary in how they are affected, and that these variations help us to understand a number of phenomena of considerable importance.

1.04.6 The Development of Knowledge Systems

It is reasonable to assume that each neural system develops at its own rate, and that there are differences among neural systems in this regard. To the extent to which a particular form of knowledge depends upon a specific neural substrate, it is then likely that the various knowledge systems have different developmental trajectories. The very capacity to know certain things depends upon the development of the underlying neural system that processes and represents that kind of knowledge. Until that happens, such knowing should be impossible. It is a reasonable further assumption that still-developing systems are susceptible to alteration, induced either by experience or the unfolding of some genetic program. Such factors are less likely to act on already-developed systems. This means that ways of knowing can be more or less influenced by early life experience as a function of when they develop.

Neural systems responsible for processing knowledge about objects in the world seem largely functional early in life, as must be the case if organisms are to respond appropriately to those entities and events that are of critical survival value. However, large differences are seen within this category. Systems responsible for knowing about the smell or taste of things seem in general to develop before systems responsible for knowing about the sight or sound of things. The generalization here might be that systems concerned with knowledge about internal states, and stimuli related to those states, develop before systems concerned with external states.

In most mammals, several neural systems, and the knowledge systems that depend upon them, show prolonged maturation, much of it postnatal. This has significant implications for understanding how both our memory and performance capabilities change during early life. A prominent example is the hippocampal formation, portions of which undergo substantial postnatal maturation (cf. Nadel and Hupbach, in press). Evidence about the development of the hippocampus

comes from studies of both structure and function. At present, the best evidence comes from studies with rodents, but we now know enough about primates and humans to state a general case. Across a wide range of species, it appears that the hippocampus first becomes functional at about the natural time of weaning. Unfortunately, we do not know when this is for humans, hence we must make guesses based on anatomical, physiological, and behavioral data to determine just when the hippocampus becomes functional in humans. Note that it is unlikely that this, or any other, brain structure suddenly becomes functional, as if by the flipping of a switch. It is more likely that hippocampal function emerges piecemeal, taking a considerable time to reach the adult state.

It has been known for several decades that the dentate gyrus of the rat is particularly subject to postnatal development (see Frotscher and Seress, 2006, for a recent review). Large numbers of dentate granule cells are created after birth in the rat in a special proliferative zone within the hippocampus itself. Initially it was thought that rodents were unique, and that postnatal maturation of hippocampus was either absent or less prominent in primates and humans. This, however, turns out not to be the case. Even in these species, the hippocampal system emerges into function after birth. Hippocampal pyramidal cells, unlike dentate granule cells, proliferate in the prenatal stage. But, two other critical components of any developed brain system – the integration of inhibitory neurons and the myelination of fibers – lag behind in hippocampus. Seress and his colleagues conclude that while cells are generally born early, further steps critical to normal function are quite prolonged.

In rodents, we can use the appearance of exploration and place learning as markers of the emergence of hippocampal function. Data from such studies in general support what was deduced from studies of structure – namely, that hippocampal function begins at about 3 weeks of life in the rat. Since the two major cognitive functions in humans that depend upon the hippocampus are episodic memory and memory for allocentric spatial location, delayed maturation should be reflected in the late emergence of these capacities.

Infants at quite a young age can learn about space as it relates to their body or its parts (eyes, hand, head). They can learn to crawl or walk to objects in space and readily solve simple spatial tasks such as 'go right' or 'go to the door and turn left.' These kinds of spatial learning do not, however, depend upon a functioning hippocampus. Instead they depend upon knowledge systems subserved by other, earlier-developing, neural systems. The capacity to know, and use, allocentric space, on the other hand, does depend upon the hippocampus, and data from a variety of studies suggest that this capacity emerges only between 18 and 24 months of age in humans (cf. Newcombe et al., 1998).

There are several major implications of the postnatal maturation of the hippocampus. The first concerns behavior. We assume adult behavior reflects the presence of both hippocampal and nonhippocampal systems, what they do and how they interact. Prior to hippocampal emergence, however, behavior reflects functions and behaviors dependent on brain systems operational at birth. The second major implication concerns development. A developing system is more susceptible to influence than an already developed one. This is presumably why environmental influences exert a particularly strong impact on the developing hippocampus.

1.04.6.1 The Delayed Emergence of Episodic Memory

In general, little evidence of episodic memory, as measured in standard recall and recognition tests, is observed until the age of 3 or 4 years. The absence of episode memories from the first 2 years of life resulting from the late maturation of the hippocampus can help us understand at least part of the syndrome of infantile amnesia (Nadel and Zola-Morgan, 1984; *See* Chapters 1.15, 2.37). It is a well-established fact that for most individuals, few if any early episode memories survive into adulthood. It is only after 2–3 years of age that significant numbers of episode memories appear to be formed and retained. Over the years, there has been considerable debate as to whether this syndrome, first discussed at length by Freud, reflects biological maturation or some other factors, such as the mismatch between the nonverbal coding of early memories and the verbal means used later in life to retrieve and report memories or the emergence of a sense of self at around 2–3 years of age. Arguments against the biological case depend on assertions that the hippocampus develops early in life, as noted above. These assertions, as we have seen, rested either on the use of inappropriate tasks or on incorrect interpretations of the nature of contextual coding and the hippocampal role in it. Now that a consensus has emerged to the effect that hippocampus is most likely to become functional

between 18 and 24 months of age in children (Newcombe et al., 2007; Nadel and Hupbach, in press), we can conclude that a significant part of infantile amnesia reflects biological maturation.

Further support for this view comes from the study of the unique population of developmental amnesics, individuals with damage to the hippocampus caused, typically, by an early anoxic or ischemic event (cf. Vargha-Khadem et al., 1997). These individuals, mentioned earlier, went unrecognized for quite a while because they did reasonably well in educational settings. Only careful testing brought out the fact that they suffered from quite severe losses in the domain of episodic and spatial memory. Developmental amnesics have general difficulties orienting in space and time, remembering events, finding their way through any but the most familiar environments, and remembering where they placed objects. However, they are usually not impaired in their social and language development and score low to average on standard tests of intelligence. They have a relative preservation of semantic memory and often show normal scores on immediate or short-term episodic memory tests, but they are unable to retain episodic information over longer periods of time. Studies using structural magnetic resonance imaging suggest that the described symptoms of developmental amnesia are caused by bilateral hippocampal volume reduction of at least 20%–30%.

The increased susceptibility to influence following from postnatal development of the hippocampus manifests itself in two rather different ways. First, the hippocampus seems to be very sensitive to environmental perturbations. Careful studies of the neuropsychological impact of early exposure to lead, for example, suggest that impairment of hippocampal function contributes to the resulting cognitive deficit (e.g., Finkelstein et al., 1998). Second, genetic conditions that influence development in a general way seem to have their greatest impact on late-developing parts of the nervous system (and other organ systems as well). There is a kind of selection bias inherent here: Genetic conditions that affect structures formed early in development might have such devastating effects that they are inevitably lethal. Influences on late-developing structures might be prevalent simply because they are the only ones that can be survived.

Down syndrome presents such a case. This condition, resulting from an error in very early embryonic life, almost always reflects the existence of an extra copy of chromosome 21 (cf. Nadel, 2003). As a consequence, extra gene product results, and this in turn leads to a variety of problems in a host of biological systems. In almost all cases, these problems seem to impact the later-developing parts of the relevant system. Thus, in the nervous system, the hippocampus, cerebellum, and prefrontal cortex, all of which mature late, are disproportionately affected. How these effects translate into the mental retardation observed in Down syndrome remains to be determined, and the creation of appropriate mouse models is moving toward that goal. Williams syndrome might present another such case, as it has recently been shown that children with this syndrome, caused by deletion of a subset of the genes on chromosome 7, have significant abnormalities in hippocampal structure and function (Meyer-Lindenberg et al., 2005).

It seems clear from these various examples that some knowledge systems, and in particular those critical for what we are here calling memory, develop later than other knowledge systems critical for such things as what and how much knowledge. It is worth pointing out that at the other end of the age scale, aging also has an uneven impact on knowledge systems. Evidence suggests that as in development, it is the hippocampal system, central to episodic memory, that is most at risk (cf. Burke and Barnes, 2006).

1.04.6.2 The Impact of Stress

Another way in which the existence of multiple knowledge systems matters is that these systems can be differentially affected by experience. One very important example is offered by how knowledge systems are affected by arousal and stress. The literature in this area has been confusing, since evidence existed that arousal facilitates memory formation (Reisberg and Heuer, 2004), while at the same time acute stress, which is undoubtedly arousing, has been shown to impair memory in several studies (Jackson et al. 2006; Payne et al., 2006). The best way to understand this discrepancy is in terms of multiple knowledge systems and how they are differentially affected by stress. Payne et al., for example, showed that memory for neutral information is impaired by stress at the same time that memory for emotional information is facilitated. This result is best understood by assuming that emotional information (value knowledge) is handled by one system in the brain, the amygdala, while neutral detail, typically of the background context (where knowledge), is handled by another brain system, the hippocampus. It has been established that within much of the range of physiological stress, amygdala function is enhanced while

hippocampal function is impaired. Thus, the same stress manipulation can simultaneously increase acquisition of one kind of knowledge while decreasing acquisition of another. The implications of this simple fact for various legal issues, such as the viability of eyewitness testimony, and the veracity of recovered memories, are immense (see Jacobs and Nadel, 1998; Payne et al., 2003, for some discussion of these matters; *See* Chapters 2.14, 2.44).

1.04.7 Conclusions

That there are multiple systems engaged in acquiring and deploying knowledge gained from experience seems clear. I have argued that it is better to think about these as knowledge systems rather than as memory systems. This difference is not a mere semantic quibble, since a number of consequences flow from this change in terminology. However one does refer to these systems, the fact that they exist as separable entities has a variety of implications that I have tried to briefly explore in this chapter.

One final implication that I have not explored concerns the fact that the existence of multiple, separate, systems opens up the possibility of competition between systems for control of behavioral output. Within the domain of spatial behavior there is strong evidence that such competition exists between, for example, strategies that depend upon the egocentric information subserved by caudate and other structures and the allocentric information subserved by hippocampus and related structures (cf. Nadel and Hardt, 2004). Similar findings have been reported recently for a nonspatial task involving probabilistic classification (Foerde et al., 2006). It remains for future research to explicate these competitive relations in greater detail, as they will ultimately turn out to be extremely important in understanding how organisms deploy optimal knowledge in various circumstances. Looked at in this way, all forms of behavior involve decision-making at some level, creating linkages between previously disconnected literatures on memory and choice.

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1.05 Retrieval from Memory

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1.05.1 Retrieval from Memory

The field of learning and memory has traditionally emphasized acquisition and storage as the critical determinants of learned behavior (*See* Chapters 1.02, 1.04). In the field of associative learning, this orientation is clearly evident in Pavlov's work, which suggested that spreading activation between nodes was necessary for memory formation (Pavlov, 1927). Similarly, Hebb (1949) proposed that experiencing a learning event temporarily activates certain neural circuits that, while active, strengthen the synaptic connections that constitute the basis for that experience becoming permanently stored in memory. This emphasis on acquisition and storage can be seen at many different levels of analysis. For instance, in the human memory literature, Craik and Lockhart (1972) proposed that the acquisition and storage of new information would create a more durable memory trace if processing during the acquisition (i.e., study) phase occurred at a relatively deep level (i.e., more integrative semantic than superficial phonetic). As another example, the Rescorla–Wagner rule for the formation of associations assumes that competition between stimuli trained together in a Pavlovian conditioning preparation occurs exclusively during the training phase (Rescorla and Wagner, 1972). All these views share the common assumption that any disruptive manipulation that occurs during training will inevitably result in that learned information being permanently lost and unavailable in future encounters within a similar situations. The influence of this view is so pervasive that the term *learning* itself for some researchers represents the totality of information processing underlying stimulus-specific changes in behavior resulting from prior experience. This use of the term discourages consideration of any of the post-acquisition processing that may also be necessary to see learned behavior. By definition, learning is the process by which experience is encoded and results in stimulus-specific changes in behavior that can be observed later. By memory, we are referring to any stimulus-specific permanent change in the brain (structural and chemical) resulting from past experience that allows usage of that previous experience on future occasions. Finally, retrieval is the process of reactivating an established memory so it can influence ongoing behavior, and thus we will argue that retrieval is a key component of memory performance.

In the early 1960s, however, a number of studies showed that information thought to be lost or not encoded was still available, provided that the appropriate retrieval cues were presented at the time of testing (see Tulving and Thomson, 1973, for a review). In other words, these studies suggested that memory deficits typically thought to result from processing limitations during training could be recovered at the time of testing. These experiments, conducted primarily with human subjects, provided a strong rationale for investigating a critical stage in

the information processing stream: retrieval from memory. In most of these experiments, subjects were presented with material to be learned (encoding) and subsequently evaluated to determine the conditions under which that information could be retrieved and brought to bear on response generation. As a result of this research, the emphasis on encoding that dominated the early stages of human memory research shifted to also encompass retrieval mechanisms. Interestingly, these observations with human subjects promoted changes in the study of animal memory. A vast amount of data concerning retrieval processes has been gathered from nonhumans since the late 1960s, and ever since, the notion of retrieval has proven to be a useful heuristic for the study of memory phenomena.

This chapter will focus on memory research with nonhuman animals. In the first section, we will review the empirical evidence suggesting that deficits in memory tasks do not necessarily reflect a deficit in acquisition or storage. Specifically, we will summarize representative experiments in which memory deficits are observed after changes in the internal state of the organism, or the administration of amnesic treatments such as electroconvulsive shock or protein synthesis inhibition. Moreover, we will review recent evidence suggesting that already consolidated memories, when reactivated, undergo a new period of vulnerability, so-called reconsolidation. Importantly, memory deficits can also be obtained by manipulating the amount of information presented either during training of the target memory (cue competition) or by presenting additional information at other points in the study (interference). Cue competition and memory interference experiments have also contributed evidence concerning the role of encoding and retrieval of memory and therefore will also be analyzed in this section. In the second part, we will summarize how these observations led to the development of memory models that account for a wide range of phenomena. As is the case in most scientific disciplines, there is no single approach that accounts for all the empirical evidence available. Therefore we will review three models that address the phenomena described in the first section. The first general framework is that proposed by Spear in the late 1970s that explains memory deficits induced by changes in the context or insufficient retrieval cues at the time of test. The second retrieval framework we will review is focused on associative phenomena originally thought to reflect learning deficits. As we will see, these deficits are easily anticipated in a framework that emphasizes retrieval mechanisms. The third framework is that proposed by Bouton to explain several characteristics of extinction that are important to understand some anxiety disorders in human populations (*See* Chapter 1.09). Lastly, we will briefly review neurobehavioral studies in which the physiological substrates of memory retrieval have been investigated. The importance of these studies lies in the fact that inquiries into the neurobiological basis of memory are inspired by behavioral models, such as the ones we describe in the section titled 'Theories of memory retrieval.'

1.05.2 Empirical Evidence

Several key empirical observations suggest that numerous deficits in acquired behavior result from information processing that occurs when subjects are tested rather than when they acquire or store information. Critical here is the fact that, theoretically speaking, if information is inadequately acquired or is not retained, then that information will not be available to influence the animal's behavior in any test situation. In contrast, the following observations suggest that the target information has often been sufficiently encoded and stored, but a processing deficit occurs when the information is evoked, which translates into decreased performance at the time of testing. We will review decrements in retrieval that arise from natural changes in the organism's internal state and from changes in the state of the external environment between training and testing, as well as those that arise in the laboratory as a result of programmed invasive manipulations (i.e., experimentally induced amnesia). Moreover, we will review recent evidence suggesting that memories that are retrieved from an inactive state could be subject to new consolidation processes, so-called reconsolidation (*See* Chapter 1.24, 1.27). Finally, we will review demonstrations of recovery from performance deficits arising in situations in which multiple cues are simultaneously present during training (cue competition) and from exposing subjects to select nontarget information removed from target training (stimulus interference).

1.05.2.1 Changes in the Organism's Internal State

One of the earlier observations that suggested a deficit in retrieval was state-dependent learning. State-dependent learning refers to the observation that

when the internal state of the organism is different at testing than it was at training, acquired performance is impaired (Overton, 1964). Operationally, state-dependent learning is observed when subjects experience training under one of two internal states (a state induced by the presence or absence of a drug in Overton's case) and tested under the opposite internal state. The common finding is that when subjects are trained in a nondrug state and tested in a nondrug state, behavior consistent with training is observed. Conversely, if subjects are trained while in a drug-induced state (e.g., amphetamine) and tested while not in a drug state, a decrement in performance is typically observed. This decrement could easily be accounted for by a deficit in learning, perhaps due to the drug-altering perceptual processes or the encoding of information. Similar decrements in performance are observed when subjects are trained while undrugged and tested while drugged. These decrements can be accounted for by perceptual processes at the time of testing. However, subjects trained under a drugged state and tested under the same drugged state do not always show a decrement in memory performance. This suggests that the observed performance decrements often result from retrieval deficits due to a change in the internal state of the organism (Spear, 1978). If the internal state of the subject during testing is the same as during training, regardless of whether it is a state induced by administration of a drug, performance consistent with training is observed. State-dependent learning has been observed in a wide range of memory tasks and with states induced by several different drugs, such as amphetamine, alcohol, and morphine (Overton, 1972, 1985), and also emotional states induced by various manipulations (for a review, see Overton, 1985). Obviously *state-dependent learning* is a misnomer; the phenomenon would more accurately be called *state-dependent retrieval*.

Another example of state-dependent learning that can be understood as a retrieval failure is the Kamin effect (Kamin, 1957, 1963). This effect typically has been observed in avoidance tasks in which subjects show poor retention of avoidance training when they are tested at intervals ranging from 1 to 6 h after training. The interesting observation is that such poor retention is not observed if subjects are tested a half hour after training or more than 24 h after training. In other words, subjects show a U-shaped retention function, with retention being good immediately after training and at later intervals, but not between 1 and 6 h after training. This U-shaped function has been viewed as the result of memory retrieval being dependent on the internal state of the organism. A more specific hypothesis was based on the fact that most of these demonstrations of the Kamin effect used aversively motivated tasks, which are known for their capacity to induce a stressful internal state. Moreover, the release of a hormone closely correlated with stress, adrenocorticotropic hormone (ACTH), is inhibited relatively soon after a stressful experience (McEwen and Weiss, 1970). Researchers reasoned that, because of ACTH inhibition, the internal state of the subjects from 1 to 6 h after training was different from the internal state during training, leading to a failure to retrieve the appropriate information required to perform in the task. Consistent with this interpretation, exposing subjects to foot shocks immediately before testing overcame the deficient retention observed at intermediate test intervals (Klein and Spear, 1970). Moreover, exposure to other stressors, such as immersion in cold water for 2 min, also alleviated the intermediate interval deficit in retention (Klein, 1972). Note that this stressor was unrelated to the training situation in terms of external, sensory attributes of the memory (other than being stressful), but it apparently restored the internal state that was temporarily inhibited after the original stressful experience. Importantly, the cold water bath had no effect at other retention intervals, suggesting that the effects are not related to overall activation or motivational effects of the stressor (Klein, 1972). Presumably, exposure to either foot shock or cold water provided subjects with an internal state (ACTH release) that corresponded to that of training and consequently alleviated the deficit observed at intermediate intervals. Moreover, this deficit in retention was also alleviated if subjects were infused with ACTH into the lateral anterior hypothalamus or if the same structure was electrically stimulated (Klein, 1972). More recently, Gisquet-Verrier and colleagues observed an alleviation of the Kamin effect when they exposed subjects simultaneously to the conditioned stimulus (CS) and the training context in a brightness-discrimination avoidance task 7.5 min before an intermediate interval test (Gisquet-Verrier et al., 1989; Gisquet-Verrier and Alexinsky, 1990). Overall, all of these demonstrations suggest that the Kamin effect can be alleviated if (a) the subject's internal state is restored (either by administration of ACTH or exposure to a stressor), or (b) the appropriate retrieval cues (such as the CS and the training context) are presented before testing.

Similar recovery effects have been found when retention deficits were induced with hypothermia. Specifically, subjects showed impaired retention if they were immersed in cold water immediately after training (Vardaris et al., 1973). However, if they were recooled before testing, no impaired retention was observed (Hinderliter et al., 1976; Mactutus and Riccio, 1978). These results have been interpreted as arising from a retrieval failure if there is a mismatch between training and testing in the subject's internal state. Consistent with this explanation, administering the recooling treatment immediately before testing presumably alleviates the effects of the hypothermic treatment by providing contextual cues that were present close to the time of training.

1.05.2.2 Experimentally Induced Amnesias

The notion that deficits in acquired behavior result from a processing deficit at the time of retrieval has received additional support from experimental manipulations known to induce amnesia (*See* Chapter 1.14). It is important to note that the three examples from the previous section resulted from changes in the internal state of the subject. In contrast to state-dependent learning and the Kamin effect, in the studies reviewed later, a performance deficit was induced by the administration of an amnestic agent. For example, if soon after training subjects are administered an electroconvulsive shock (hereafter ECS; Duncan, 1949), hypothermia (described earlier; Vardaris et al., 1973), or protein synthesis inhibitors (e.g., Barraco and Stettner, 1976), little behavior indicative of retention is observed. These observations have been taken by many as evidence that amnestic treatments work by disrupting memory consolidation (Gold and King, 1974; McGaugh, 1966, 2000). Memory consolidation is defined as a time-dependent process by which recent learned experiences are transformed into long-term memory, presumably by structural and chemical changes in the nervous system (e.g., the strengthening of synaptic connections between neurons). Support for this explanation comes from the fact that the effects of amnestic treatments are retrograde in nature, with recent memories being more vulnerable than earlier memories to the amnestic treatment (e.g., Duncan, 1949). The temporally graded nature of amnesia is explained by the consolidation view in the following way: After a given learning experience, memories undergo a consolidation process that leaves the memory trace more stable as time passes. Following this logic, the shorter the interval between training and the administration of the amnestic treatment, the larger the impact of the amnestic agent on the formation of the memory trace.

Since the discovery of these amnestic treatments, however, a number of observations have suggested that, instead of disrupting the consolidation process, these treatments might alter the memory's retrievability, thereby rendering it inaccessible at the time of retrieval (*See* Chapter 1.14). Evidence supporting this notion comes from studies that reminded subjects of the original episode. For example, Lewis et al. (1968) trained rats in a passive avoidance task and immediately administered amnesia-inducing ECS. They tested their subjects 20 h later and observed that subjects that received the amnestic treatment showed no behavior indicative of the passive avoidance event; that is, the amnestic treatment was effective in inducing amnesia. However, 4 h after training, they placed other rats given the amnesic treatment immediately following training in a separate compartment (different from that of training) and administered a foot shock (unconditioned stimulus – US), similar to the reinforcer used in training. When these subjects were tested 20 h later in the training compartment, they showed recovery from the amnestic treatment, as evidenced by longer avoidance latencies. In other words, when they exposed rats to a reminder of the initial training experience, subjects' performance indicated that the memory trace was not altered by the amnestic treatment. Subjects lacking original training showed no effect of such reminder shocks. Because this recovery effect could have been specific to the avoidance tasks involving stressful situations, Miller et al. (1974) conducted a similar study, but instead of using an aversive preparation, they used an appetitively motivated task with sucrose as a reinforcer. Interestingly, they reported that, after the amnestic treatment, a foot shock did not affect a recovery of memory. Moreover, they showed that exposure to the sucrose solution following the amnestic treatment reversed the retention deficit induced by the amnestic treatment. Further experiments demonstrated that exposure to the training apparatus also recovered the memory rendered silent by the amnestic treatment. Thus, exposure to any of several elements from the training task restored access to the target memory, whereas exposure to task-irrelevant stimuli, even if they were of strong affective value, did not restore memory.

1.05.2.3 Reconsolidation

As part of the effort to understand and contrast approaches to memory, studies of experimentally induced amnesias gained popularity during the late 1960s and 1970s. This popularity was recently reinvigorated when new findings suggested that old memories when reactivated need new (*de novo*) protein synthesis to become once again stable and permanently stored in the brain (Przybyslawski and Sara, 1997; Nader et al., 2000; *See* Chapter 1.24). This phenomenon has been called reconsolidation. For example, a day after Nader et al. exposed their rats to simple CS → US pairings, they presented the CS alone (which presumably reactivated the memory trace for that association) and immediately infused anisomycin (a protein synthesis inhibitor) into the lateral and basal amygdala. When they tested subjects 1 day later, they observed decreased conditioned freezing to the CS relative to anisomycin-treated rats lacking the CS exposure on day 2, suggesting that the memory trace that was activated needed new protein synthesis to become stable again. These findings brought back the question extensively debated in the 1970s: Are memories destroyed or simply made inaccessible after a retrieval manipulation followed by an amnestic agent (e.g., Gold and King, 1974; Miller and Springer, 1973, 1974)? Several recent reports suggest an answer to this question. For example, Lattal and Abel (2004) observed that after reactivating a memory of contextual fear and administrating systemic anisomycin, subjects showed decreased conditioned freezing when tested 1 day later in the training context. However, if the test was delayed by 21 days (a standard retention interval used for spontaneous recovery from extinction of fear memories), no effect of protein synthesis inhibition was observed. In fact, Lattal and Abel observed that 21 days after anisomycin treatment, response to the context was larger than that observed during immediate retrieval (1 day after training). Other recent studies also cast doubt on the generality of the reconsolidation account because similar recovery from retrieval-induced reconsolidation has been observed (Anokhin et al., 2002; Fisher et al., 2004; Power et al., 2006; but see Debiec et al., 2002; Duvarci and Nader, 2004). Moreover, some studies did not observe any immediate effect of anisomycin after retrieval-induced reconsolidation (Lattal and Abel, 2001; Vianna et al., 2001; Biedenkapp and Rudy, 2004; Cammarota et al., 2004; Hernandez and Kelley, 2004). Overall, these contradictory findings, together with demonstrations of recovery from retrieval-induced amnesia after inhibition of protein synthesis, show that further research is needed to determine whether anisomycin erases the reactivated memory or simply constrains future retrieval of the memory. More generally, it is still unclear if amnestic agents following initial training impair consolidation (storage) or subsequent retrieval (e.g., Gold and King, 1974; Miller and Matzel, 2006).

Two points deserve further discussion here. First, as recently pointed out by Rudy et al. (2006), anisomycin also has other effects beyond inhibition of protein synthesis, such as genetically programmed cell death (apoptosis). Moreover, the apoptotic cascade occurs at lower doses than those that are necessary for the inhibition of protein synthesis. Whether this is the main cause of the observed amnesia remains to be determined, but it is noteworthy that tests have not yet been conducted to determine the role of apoptosis in experimental amnesia. Second, a recent report from the same laboratory that sparked early interest in reconsolidation showed that memories activated indirectly by an associate of the target cue do not undergo reconsolidation (Debiec et al., 2006). They trained rats in a second-order conditioning preparation in which one cue was paired with the US in a first phase ($CS_1 \rightarrow US$), and in a second phase another cue was paired with the cue trained in phase 1 ($CS_2 \rightarrow CS_1$). This procedure ordinarily results in conditioned responding to CS_2, presumably because when presented at test it retrieves a neural representation of the US through an associative chain mediated by CS_1 (or a direct $CS_2 \rightarrow UR$ link; see Rescorla, 1980, for a discussion). Notably, Debiec et al. found that responding to CS_1 was not impaired after retrieval through presentation of CS_2 and subsequent administration of anisomycin. This suggests that the reactivation treatment, at most, leaves only part of the memory in a labile state, but alternatively the entire content of the memory might not be substantially altered.

In contrast to reconsolidation accounts of the reconsolidation phenomenon, there are retrieval-focused accounts of reconsolidation such as that proposed by Millin et al. (2001). In line with Spear's (1973, 1978; see following discussion) views concerning retrieval, Riccio and his collaborators have proposed that when previously stored memories are reactivated, reprocessing of the attributes of these memories will take place for some time after the reactivation episode. As a result, the internal

context provided by an amnestic treatment becomes associated with the target memory. At test, the context provided by the amnestic treatment is not ordinarily present, and thus retrieval failure occurs. Consistent with this account, amnesia induced by the administration of anisomycin has been observed to be alleviated if subjects are administered anisomycin just prior to testing (Bradley and Galal, 1988). Another explanation of this phenomenon has been advanced by Miller and Matzel (2000; also see Nadel and Land, 2000), who proposed that the amnestic treatment produces a change in the memory representation that interferes with retrieval itself, leaving the memory trace silent after the administration of the amnestic treatment. Regardless of the specific version of the retrieval explanation for the reconsolidation phenomenon put forth, these alternative views can be contrasted with reconsolidation accounts by determining the extent to which amnestic treatments really erase the memory trace or simply render it inaccessible for future use. Based on the recovery data presented earlier, the latter alternative seems to be the more plausible at this time (Prado-Alcalá et al., 2006).

Four points are important to keep in mind. (1) Recovery from amnestic treatment is often observed when subjects are exposed to a portion of the event that had been presented during training. Such reminder treatments are most effective soon before testing, but sometimes have enduring effects even when presented 24 h before testing. (2) Control groups have demonstrated that the recovery is not purely the result of altering stress levels in the subject. (3) Recovery can be obtained by reminding subjects about the training situation not only with the US, but also with other cues (such as contextual cues or sometimes even the CS) that are part of the target memory. (4) The effect of the reminder does not result from new learning concerning the cue-outcome relationship, as long as learning is defined as receiving relevant new information from events in the environment. Subjects exposed to the reminder without any prior learning experience did not show any evidence of relevant learning after the reminder experience.

So far, we have argued that most impairments in memory retention result at least in part from a retrieval failure rather than an acquisition or storage failure. We have based our assertion on studies involving changes in the state of the organism (state-dependent learning and the Kamin effect), experimentally induced amnesia (ECS, hypothermia, and antimetabolites), or reconsolidation phenomena. Next, we review evidence suggesting that decrements in learning and memory that are thought to result from processing limitations (competition) at the time of training, or from deleterious effects on learning or retention due to learning additional information (interference), could instead result from a retrieval deficit at the time of testing.

1.05.2.4 Cue Competition and Outcome Competition

Cue competition refers to a decrement in behavioral control by a target CS (X), which results from the addition of a nontarget stimulus to a simple CS–US learning situation (*See* Chapters 1.03, 1.07, 1.08). This can be observed in a number of different circumstances. For example, one can add a second CS to training and train a compound of the target cue and the added CS instead of the target cue alone (i.e., AX → US as opposed to X → US) and see less behavioral control to the target cue X (overshadowing; Pavlov, 1927). Alternatively, one can train a nontarget cue in a first phase and in a second phase train a compound of cues that contains the target cue as well as the previously trained nontarget cue (A → US in phase 1; AX → US in phase 2). This results in less behavioral control by X, the target cue, than in subjects lacking phase 1 (blocking; Kamin, 1969). A second rather different form of stimulus competition can be seen when, instead of adding a second CS, a second outcome is added. For example, one can train a cue followed by an outcome in a first phase and that same cue followed by the same outcome plus a new outcome in a second phase, and then later assess performance governed by the association between the cue and the second outcome ($X \rightarrow O_1$ in phase 1, $X \rightarrow O_1O_2$ in phase 2, test $X \rightarrow O_2$; Rescorla 1980; Esmoris-Arranz et al., 1997). This is called blocking between outcomes. Taking these examples together, one can see that the addition of nontarget stimuli (a cue or an outcome) attenuates behavioral control by the target cue X.

Phenomena like blocking between cues gave rise to a family of models of Pavlovian conditioning that emphasized critical differences in information processing during acquisition. These models assumed that limitations in processing (e.g., attention to the CS or US) during training impeded the normal establishment of associations and consequently resulted in the observed decrements in behavioral control (e.g., Rescorla and Wagner, 1972; Mackintosh, 1975;

Pearce and Hall, 1980). Similar to consolidation theory (e.g., McGaugh, 1966, 2000), these behavioral models predict that what has not been stored due to processing constraints during learning will not be reflected in behavior simply because the information was not initially acquired.

As previously mentioned, overshadowing is observed as a response decrement that results from training a target cue (X) in the presence of another, usually more salient, cue (A). Interestingly, Kauffman and Bolles (1981; see also Matzel et al., 1985) conducted overshadowing training ($AX \rightarrow US$) and subsequently extinguished the overshadowing cue (A) by presenting it in the absence of reinforcement. After extinguishing the overshadowing cue, they observed a recovery from overshadowing, that is, strong behavioral control by the overshadowed cue X at test. Similarly, after blocking treatment ($A \rightarrow US$ followed by $AX \rightarrow US$), extinguishing the blocking cue (A alone presentations) can result in recovery from blocking (Blaisdell et al., 1999). However, extinction of the competing cue is not the only manipulation known to affect overshadowing and blocking. Kraemer et al. (1988) found a recovery from overshadowing training when they interposed a long retention interval between overshadowing treatment and testing, which suggests that the association between the overshadowed cue and the outcome had been established during training but was not reflected in behavior soon after training. A similar recovery from blocking has been observed after interposing a long retention interval between training and testing (Batsell, 1997; Piñeno et al., 2005).

In the same way that recovery from experimentally induced amnesia has been observed after exposing subjects to some portions of the events presented during training (CS, US, or the training context), stimulus competition phenomena have been observed to be attenuated as a result of these manipulations. For example, Kasprow et al. (1982) observed a recovery from overshadowing after they exposed subjects to two brief presentations of the overshadowed cue (the target CS). In another series of experiments, a similar recovery from blocking was observed (Balaz et al., 1982). Specifically, Balaz et al. observed a recovery from blocking after reminding subjects of the training experience either by presenting the blocked CS, the US, or the context in which subjects were trained. Additional control groups demonstrated that this recovery was specific to the blocked association and was not due to nonspecific increases in responding. All these demonstrations of recovery from cue competition are problematic for models that emphasize impaired acquisition (see earlier discussion) because, if the association between the overshadowed or blocked cue and the outcome was not learned during training, any manipulation that does not involve further training with that cue should not alter behavioral control by that cue.

Although little attention has been given to competition between outcomes, some recent studies have not only observed similar competition phenomena as those observed between cues (e.g., blocking between outcomes; Emoris-Arranz et al., 1998), but also observed that these deficits in behavioral control can be alleviated with the appropriate manipulations. For example, Wheeler and Miller (2005) observed reliable blocking between outcomes ($X \rightarrow O_1$ during phase 1; $X \rightarrow O_1O_2$ during phase 2), similar to the blocking between cues trained together originally observed by Kamin (1969). That is, responding based on the $X \rightarrow O_2$ association was weaker than in subjects that had received $X \rightarrow O_3$ in phase 1. Wheeler and Miller went on to extinguish the blocking outcome (O_1) and observed recovery from blocking between outcomes (i.e., behavioral consistent with the $X \rightarrow O_2$ association). Moreover, they observed a similar recovery when they interposed a retention interval between training and testing, and when they briefly presented the blocked outcome before testing (i.e., a reminder treatment). Overall, the results of these experiments demonstrated that blocking between outcomes can be attenuated by several manipulations similar to those that often yield recovery from blocking between cues.

1.05.2.5 Interference between Cues and Outcomes Trained Apart

Earlier we distinguished between impairments in acquired behavior that arise from training stimuli together (competition) and from experiencing additional training apart from training of the target stimuli (provided that the additional training includes one of the target associates). The latter deficit is called interference. We have already described how impaired performance that arises from stimulus competition can be recovered, thus suggesting that retrieval mechanisms play a fundamental role in behavioral control influenced by stimulus competition. Next we will review the basic conditions under which interference is observed and the different manipulations that often result in

recovery from interference. Interference in Pavlovian conditioning is evidenced as impaired behavioral control by a target cue when it is paired with a given outcome in one phase of training and paired with another outcome in an earlier or later phase of training (e.g., $X \rightarrow O_1$ in phase 1 and $X \rightarrow O_2$ in phase 2). But this is not the only situation in which interference is observed. Interference is also observed when a target cue (X) is trained with an outcome in one phase of training and in a separate phase of training another cue is trained with the same outcome (e.g., $X \rightarrow O$ in phase 1 and $Y \rightarrow O$ in phase 2). A common characteristic of these two forms of interference is that there is always a common element between the two phases of training (the cue X in the case of interference between outcomes (*See* Chapter 1.09), and the outcome O in the case of interference between cues). The critical feature of these decrements in otherwise anticipated behavioral control by X is that the interfering cues (or outcomes) are not trained together with the target cue (or outcome).

Extinction, latent inhibition, and counterconditioning (Pavlov, 1927; Lubow, 1973; Bouton and Peck, 1992; Brooks and Bouton, 1993) are three treatments that can be viewed as forms of interference between outcomes. In simple extinction, a cue is first paired with an outcome ($X \rightarrow US$), and in a second phase it is trained in the absence of the outcome (X-alone presentations; i.e., $X \rightarrow$ No US). As a result of these nonreinforced presentations, the cue loses behavioral control, which used to be taken as evidence of unlearning the original $X \rightarrow US$ association (e.g., Rescorla and Wagner, 1972). However, overwhelming evidence has shown that the original $X \rightarrow US$ association is not destroyed during the second phase, but rather, new learning during phase 2 interferes with the expression of the phase 1 learning. Pavlov (1927) was the first to find a [partial] recovery effect from extinction. His observation (widely replicated since then; e.g., Brooks and Bouton, 1993; Rescorla and Cunningham, 1978) was that conditioned responding soon after extinction was minimal, but if a retention interval was interposed between extinction and the test, a recovery (which Pavlov termed *spontaneous recovery*) from extinction was observed. In other words, behavior after a retention interval was relatively similar in subjects who received extinction training and those who did not receive extinction training. What was puzzling at that time (and might have encouraged Pavlov to name the effect 'spontaneous recovery') was the fact that the extinguished response was recovered despite the fact that the subjects did not undergo any treatment other than interpolation of a long retention interval. Another manipulation that also leads to recovery from extinction is a shift in context between extinction and testing. This phenomenon is called renewal. For example, one might train subjects in a distinctive context A and conduct extinction training in a different context (B). The critical determinant of renewal is that testing be conducted outside the extinction context. If subjects are tested in a different context from the one used during extinction (ABC or ABA, where the first letter denotes the training context, the second the extinction context, and the third the test context), recovery from extinction is observed (Bouton and Bolles, 1979; Bouton and King, 1983; Bouton and Swartzentruber, 1989). If subjects are tested in the same context in which extinction took place (AAA or ABB), no such recovery is observed. A similar finding is observed when the context is defined to include the internal state of the organism, which can typically be altered by administering a drug that would change the internal state. For example, renewal has been observed when the extinction context is characterized by alcohol intoxication, and subjects are tested in a sober state, which creates a different internal context (Cunningham, 1979).

Latent inhibition is observed as a retarded emergence of behavioral control due to nonreinforced presentations of the target CS prior to conditioning (X-alone presentations in phase 1; $X \rightarrow US$ in phase 2), in comparison with subjects that experience the same phase 2 training without phase 1 treatment (Lubow, 1973). Similar to extinction, in a latent inhibition treatment subjects experience the target CS alone, but before the reinforced trials rather than after. One characteristic of latent inhibition that is suggestive of the response deficit being a retrieval effect is its context specificity. Specifically, it has been observed that if subjects experience phase 1 and phase 2 training in different contexts, latent inhibition is abolished (Channell and Hall, 1983). Presumably, during phase 1 nonreinforced presentations, the CS becomes associated with the context in which it is being presented, and these associations interfere with subsequent behavioral control after reinforcement in the same context. Thus, a context switch or massive extinction of the context (Grahame et al., 1993) between phases 1 and 2 attenuates the latent inhibition effect. A recent observation that has captured researchers' attention is the super latent inhibition effect (De la Casa and Lubow, 2000,

2002; Wheeler et al., 2004). This effect is typically observed when a retention interval is interposed between phase 2 reinforced training and testing. One critical condition necessary for this effect to be observed is that subjects have to spend the retention interval in a context different from that of conditioning. Thus, the superlatent inhibition effect might be seen as a shift from behavior based on recency (phase 2 training) to behavior based on primacy (phase 1 training) after interposing a long retention interval between phase 2 training and testing.

Counterconditioning is a phenomenon similar to extinction, but during the second phase of training, the target cue is associated with a qualitatively different outcome instead of being associated with the absence of an outcome, as is the case with extinction. The phase 2 treatment radically attenuates conditioned responding based on phase 1 training (Pavlov, 1927). What distinguishes extinction from counterconditioning is the motivational nature of the outcomes, in that in counterconditioning, the outcomes of phases 1 and 2 engage different motivational systems. For example, in a counterconditioning experiment, subjects might experience a CS followed by food in the first phase (X → food) and the same CS followed by foot shock in the second phase (X → shock; this is called appetitive-aversive transfer, but counterconditioning is also observed when the two phases are reversed, which is called aversive-appetitive transfer). After this training, the CS → shock association is thought to retroactively interfere with the CS → food associations. The question of interest is whether phase 2 learning destroys the memory of phase 1 training or if it simply interferes with the expression of that association. To address this question, Peck and Bouton (1990) trained phases 1 and 2 in two physically distinct contexts and tested subjects in the phase 1 context. Consistent with an explanation in terms of retrieval disruption rather than impaired retention, subjects tested in the phase 1 context showed responding appropriate to phase 1 training, indicating that the information had not been erased (or unlearned) but rather that behavior appropriate to each phase could be observed depending on the contextual cues present at the time of testing. Similarly, Brooks et al. (1995) trained rats with X → shock pairings in phase 1 and X → food pairings in phase 2. Before testing, they exposed the critical group to six unsignaled shocks and at test (relative to appropriate control groups) found that subjects froze to the CS, consistent with the shock US, rather than approached the food hopper, which demonstrated reinstatement of original training after counterconditioning. Moreover, this reinstatement effect was dependent on the shocks being presented in the context in which testing would occur, because no reinstatement was observed when the shocks were presented in a context other than the test context.

As we mentioned earlier, interference is observed not only when a cue is associated with two outcomes (extinction and counterconditioning), but also when two cues are associated with the same outcome. For example, Escobar et al. (2001) paired a cue with an outcome (X → O) in a first phase and subsequently paired a second cue with the same outcome (A → O) and observed impaired responding to X (relative to subjects that received A and O explicitly unpaired in phase 2), thereby providing a demonstration of retroactive interference. In subsequent experiments, they showed that the retroactive interference effect could be alleviated if subjects experienced phase 1 (X → O) and phase 2 (A → O) training in different contexts and subsequently were tested in the context in which the X → O association was trained. That is, interference was affected by the context in which testing took place. Similarly, in another experiment, they presented priming stimuli (stimuli presented during phase 1 or during phase 2 sessions but far removed from presentations of the target cue or interfering cue) that distinctively signaled phase 1 or 2 of their procedure and observed retroactive interference to be dependent on which priming cue was presented immediately before the critical test. When they primed phase 2, robust retroactive interference was observed, but when they primed phase 1, an alleviation of retroactive interference was observed, relative to subjects that did not receive any priming treatment at the time of test. Similar findings were reported by Amundson et al. (2003) but in a proactive interference preparation (i.e., A → US training followed by X → US training). Specifically, they observed an attenuation of proactive interference with responding to X when they primed the second phase of their training procedure, presumably because this impaired retrieval of the competing (phase 1) association and facilitated retrieval of the second association. Moreover, they observed recovery from proactive interference when they extinguished the phase 1 association. In summary, based on these and similar findings, it is reasonable to conclude that interference is highly dependent on the cues provided at the time of testing, thus suggesting a strong role for retrieval mechanisms. Moreover, there

are now several demonstrations of parallels in interference between cues and between outcomes in that both of these effects can be alleviated if the appropriate conditions prevail at the time of testing.

1.05.3 Theories of Memory Retrieval

There are several approaches to memory retrieval, each having been designed to explain different phenomena. However, the frameworks that we describe next point to several shared principles that have proven to be important and reliable tools for the study of retrieval from memory following learning. No single approach accounts for all the deficits in retention we have reviewed earlier. But each framework has provided an explanation of a number of observations and therefore deserves discussion.

1.05.3.1 Matching of Information as Critical for the Retrieval from Memory

Tulving and Thomson (1973) proposed the encoding specificity principle of retrieval that at the time accounted for many of the retrieval effects that were found with human participants. The principle states that subjects form a representation of events that encompasses not only the target events themselves but also many of the events surrounding the target event. Additionally, these episodic memories encode not only which events occurred, but where and when each event occurred relative to neighboring events. Moreover, the encoding specificity principle asserts that items (words, in the framework of the proposal) presented to aid retrieval will be effective only if they (or very similar items) were presented during training, consistent with the view that subjects encode a global representation of an item, its semantic meaning, and its content during training. Critical to this proposal is the notion that what enters into a memory representation (what it is encoded) is determined by the perceived functional meaning of an item, and this in turn determines which retrieval cues will be effective for memory retrieval.

Along similar lines, Spear (1973, 1978) has emphasized that similarity between retrieval cues and cues presented during training is critical for memory expression. He proposed the following principles as a conceptual framework for effective memory processing.

1. Attributes of memory function independently. In other words, an animal encodes a collection of separate but associated attributes that correspond to the events that form a memory episode. A constellation of attributes will be activated every time the subject experiences an event that has some similarity in attributes to the target memory. Moreover, an associated attribute might be activated when an event memory is activated. That is, some attributes might activate an event memory that in turn will activate attributes that need not have been presented but rather are associatively linked to retrieval cues presented. Additionally, attributes might activate memories of other attributes, regardless of whether they activate the full event memory representation.
2. The process of retrieval is determined by the number of retrieval cues that correspond to attributes of that memory. A memory failure is observed when an insufficient number of retrieval cues correspond at test to the memory of a given event, other things being equal. Moreover, events that are presented during testing but were not presented during training will lead to retrieval of nontarget memories that will interfere with retrieval of target memories (similar to the phenomenon of external inhibition that Pavlov, 1927, described).
3. A contextual cue is any event noticed by the organism, with the exclusion of the target stimuli that form the learning experience (e.g., the CS and US in a Pavlovian conditioning situation). Forgetting is caused in large part by a change between the context of acquisition and the context of retrieval. Context changes can result from different sources, and the differences in contextual information are more likely to increase as time passes (i.e., as the retention interval is increased; Bouton, 1993, views this as resulting from time actually being part of the context). Thus, in this framework sensory contexts from training will dissipate rapidly (soon after the stimuli are terminated), and neurochemical and hormonal states will dissipate somewhat more slowly. Importantly, contextual information here can be composed of internal stimuli (or states) and external stimuli, such as the physical attributes of the context where training or testing takes place. The internal context can also change due to preprogrammed factors such as aging or cyclical changes

in hormonal states (e.g., estrus). Contextual attributes learned during new experiences also might interfere with retrieval of the target memory if they share attributes in common.

This general framework has been one of the foundations for studying retrieval mechanisms in memory retention. For example, it easily explains state-dependent learning and the Kamin effect by assuming that the conditions at test are different than those during training. Consistent with this framework, several manipulations that recreate the training conditions at the time of testing have proven effective in restoring the deficient behavior observed due to changes in the organism's internal states or changes in the environment.

1.05.3.2 The Comparator Hypothesis: A Retrieval-Focused View of Cue Competition

As was mentioned in the introduction, models that emphasize processing during encoding and storage have been prevalent in the study of learned behavior in animals. One such example is the well-known Rescorla and Wagner (1972; also see Wagner and Rescorla, 1972) model. This model asserts that error correction at the time of training governs what information is acquired; that is, acquisition should be greatest when the discrepancy between the US that occurs and the US that is expected based on all cues present is largest. The model has been widely applied to Pavlovian conditioning situations in which one or more cues are concurrently paired with a given outcome. After repeated pairings, subjects emit a conditioned response when one (or more) of these cues are presented. One of the strengths of the model is that it elegantly anticipates the occurrence of cue competition (overshadowing, blocking, etc). In fact, the model was conceived in part to account for such phenomena after Kamin's (1969) demonstration of blocking. However, one of the weaknesses of the model is that it only emphasizes processing during training, with the additional vague assumption that associative strengths map monotonically onto behavior. Given all the aforementioned examples of recovery from cue competition (see prior section on empirical evidence), it is attractive to detail here a model that also explains cue-competition phenomena but appeals centrally to a retrieval mechanism.

One such model is the comparator hypothesis of conditioned responding (Miller and Matzel, 1988). The model assumes that all pairs of stimuli, including cues, that are presented together gain associative strength with each of the other stimuli present, independent of the associative status of other cues present during training. In other words, in this model there is no competition between cues for associative strength during training – all information is stored. Associative strength between stimuli A and B depends only on their spatiotemporal contiguity and saliencies. The training context also gains associative strength, although at a much slower rate due to its lower salience than punctate stimuli. Thus, following Bush and Mosteller's (1951) error correction rule, the comparator hypothesis assumes noncompetitive learning of associations between cues and outcomes, of within-compound associations between cues trained together, and of associations between cues and the training context. What is critical in the comparator hypothesis is the process of retrieval of memories. According to this framework, responding to the presentation of a CS will be determined by a comparison between the representation of the US that is directly activated by the target CS → US association and the representation of the US that is indirectly activated conjointly by the associations between the target CS and any other cues presented during training (punctate cues or the training context) and the association between those cues and the US. In **Figure 1**, a depiction of the comparator hypothesis is provided. The

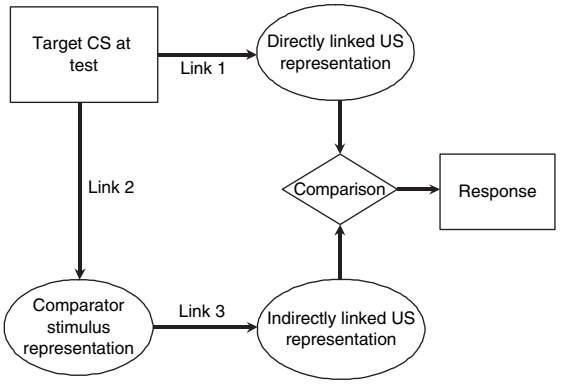


Figure 1 The original comparator hypothesis (after Miller RR and Matzel LD (1988) The comparator hypothesis: A response rule for the expression of associations. In: Bower GH (ed.) *The Psychology of Learning and Motivation, Vol. 22*, pp. 51–92. San Diego, CA: Academic Press). Rectangles represent physical events, and ovals correspond to internal representations of events that were previously associated with those physical events. The diamond represents the comparator process. Responding is directly related to the strength of link 1 and negatively related to the product of links 2 and 3.

boxes represent the external events observable in the test situation, that is, the target stimulus and the conditioned response. Ovals represent internal representations of events that were presented during training, and the diamond represents the comparator process (also internal). Direct activation of a US representation due to presentation of the target cue at test depends on the strength of the association between the target cue and the outcome, represented by link 1. Importantly, the target cue also activates a US representation mediated through other cues that were presented in the training situation, and this is represented by links 2 and 3. Responding is determined by a comparison between the directly activated representation of the US (the strength of which is determined by the target CS-US association, i.e., link 1) and the indirectly activated representation of the US (the strength of which is determined by the product of links 2 and 3). This comparison process is represented by the diamond. In an overshadowing situation (AX → US; with X being the target cue to be tested), the comparator hypothesis states that during training, subjects associate the target cue with the US (link 1 in **Figure 1**), the target cue with the overshadowing cue A (which is X's comparator cue; link 2), and A with the US (link 3). These associations are acquired independently of each other (i.e., cues do not compete during training for associative strength with the US). Critical for the comparator hypothesis is what happens when the target cue X is presented during testing. When X is presented, it directly activates a representation of the US, but it also indirectly activates a representation of the US, mediated by the overshadowing cue (which was associated during training with both the target cue and the US). This indirectly activated (competing) US representation is said to decrease (downmodulate) responding to the target cue, and thus overshadowing is observed relative to a group that experienced elemental training (X → US alone).

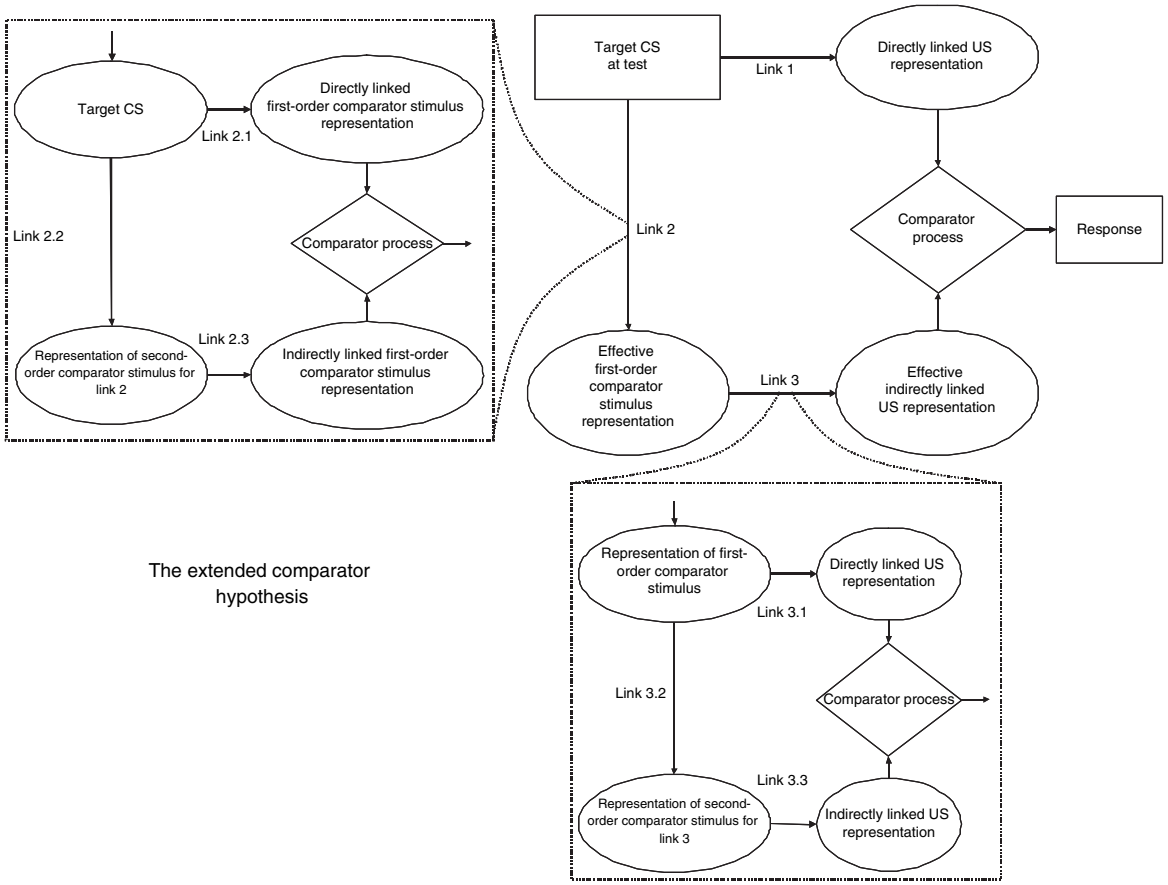
What differentiates the comparator hypothesis from acquisition-focused models (i.e., those that focus on limitations in processing during training) is not only the mechanism through which it explains cue competition phenomena, but also several novel predictions that have received empirical support in recent years. One such prediction is that after overshadowing training, extinguishing the overshadowing cue (that is, presenting A alone after training) should result in recovery from overshadowing. Specifically, by presenting A alone after overshadowing training, the links that mediate the indirectly activated representation of the US decrease in associative strength, and consequently the overshadowing cue should no longer interfere with the representation of the US that is directly activated by X. Importantly, here we have an example of a change in behavioral control by X as a result of conducting a posttraining manipulation that does not involve further training of X (that is, subjects have no additional experience with X after the overshadowing treatment), and still a recovery from cue competition is observed. Most models that emphasize processing during acquisition (e.g., Rescorla and Wagner, 1972; Wagner 1981) cannot account for these results for two reasons: (1) they do not have a mechanism that allows learning about absent cues, and (2) these models state that overshadowing results from a deficit in the establishment of the X → US association during overshadowing training, so any manipulation that does not involve the presentation of X should not affect its behavioral control. In fact, recovery from overshadowing after extinguishing the overshadowing cue has been observed repeatedly (e.g., Kaufman and Bolles, 1981; Matzel et al., 1985; Urcelay and Miller, 2006), thereby lending support to a retrieval-failure account of cue competition.

A related example is recovery from blocking. After blocking treatment (A → US followed by AX → US), responding to X is usually diminished relative to a group that experienced an irrelevant cue during training of phase 1 (B → US; AX → US). According to the comparator hypothesis, acquisition of the X → US association proceeds without any competition between A and X. At the time of testing, the blocking cue is thought to decrease responding to the blocked cue through its associations with X and the US (links 2 and 3). Similar to overshadowing, the comparator hypothesis predicts that extinguishing the blocking cue should result in recovery from blocking, as has been empirically observed (e.g., Blaisdell et al., 1999).

For several years, the comparator hypothesis was unique in predicting these recovery effects that could not be explained by traditional models that emphasized acquisition processes (e.g., Rescorla and Wagner, 1972; Wagner, 1981). However, recent revision of the Rescorla–Wagner (1972) model by Van Hamme and Wasserman (1994) and of Wagner's (1981) SOP model by Dickinson and Burke (1996) introduced mechanisms that allow for learning about an absent cue provided an associate of the absent cue is present. Posttraining extinction of a companion cue (i.e., an overshadowing or blocking cue) constitutes a

situation in which these models anticipate new learning about a target cue. Consequently, these two models are able to account for phenomena such as recovery from overshadowing and blocking as a result of extinction of an overshadowing or blocking cue. This prompted a revision of the comparator hypothesis, in part to differentiate this model from the revised versions of acquisition-focused models and also to account for data that were problematic for the original comparator hypothesis (Williams, 1996; Rauhut et al., 1999).

The extended comparator hypothesis (Denniston et al., 2001; also see Stout and Miller, in press, for a mathematical implementation of this model) carries the same assumptions as the original comparator hypothesis, but it further assumes that the links mediating the indirectly activated representation of the US (links 2 and 3) are also subject to a comparator process in which nontarget cues present during training can compete for roles as comparator stimuli for the target cue (see **Figure 2**). In other words, the extended version of the model is similar to the original version but allows more than one cue (comparator) to modulate conditioned responding to the target cue. If there's more than one comparator stimulus for the target, these comparator stimuli can in select situations cancel each other with respect to their capacity to modulate responding to the target cue. Thus, the extended comparator hypothesis makes a number of new predictions that allow for differentiating this retrieval-based account from models that emphasize processing during acquisition. For example, it makes the counterintuitive prediction that combining select pairs of treatments that



The extended comparator hypothesis

Figure 2 The extended comparator hypothesis (after Denniston JC, Savastano HI, and Miller RR (2001) The extended comparator hypothesis: Learning by contiguity, responding by relative strength. In: Mowrer RR and Klein SB (eds.) *Handbook of Contemporary Learning Theories*, pp. 65–117. Hillsdale, NJ: Erlbaum). Note principles similar to the original comparator hypothesis but with the inclusion of second-order comparator processes that operate over links 2 and 3. The magnitude of second-order comparator processes directly affects conditioned responding to the target CS, by decreasing the effectiveness of first-order comparator stimuli.

alone lead to a decrement in conditioned responding (e.g., overshadowing and the deficit seen in responding when trials are massed; see Barela, 1999, for a review of the trial massing effect) under certain circumstances can result in less (rather than more) of a decrement in behavioral control. In other words, the model predicts that the two response-degrading treatments can counteract each other, and thus less of a decrement in behavioral control should be observed during testing than with either treatment alone. How does the model anticipate this result? Because the extended comparator hypothesis allows for cues (other than the target cue) to compete with each other for comparator status provided they share a within-compound association, any treatment that establishes two comparator stimuli as potential competitors for comparator status with respect to the target cue can lead to a mutual reduction in the comparator roles of each of these stimuli and thus a reduced decrement in conditioned responding to the target cue.

As a test of this prediction, Stout et al. (2003) manipulated overshadowing and trial spacing, so that one group received elemental training with spaced trials (X → US spaced), one group received overshadowing training with spaced trials (AX → US spaced), and two more groups received identical training but with massed trials (X → US and AX → US massed). In this last group, presumably both the overshadowing cue (because of the compound training) and the training context (because of massed trials) should have been effective comparators for the target cue X, and they also should have served as comparators for each other (because A and the context should have been strongly associated). Interestingly, Stout et al. observed strong behavioral control by X in the group that experienced elemental training with spaced trials, less responding in the groups that experienced only one of the response-degrading treatments (either overshadowing or massed trials), and a recovery from these treatments (more responding) in the group that experienced both overshadowing treatment and massed trials. Similar counteractive effects have been observed when combining overshadowing with several other treatments that presumably establish the training context as a strong comparator for the target cue. These treatments include pretraining exposure to the CS alone (i.e., latent inhibition, Blaisdell et al., 1998), long CS duration during conditioning (Urushihara et al., 2004), unsignaled outcomes interspersed among the CS-outcome trials (i.e., degraded contingency, Urcelay and Miller, 2006), and unsignaled outcome alone before or after the CS → US trials (Urushihara and Miller, 2006).

In summary, the comparator hypothesis (Miller and Matzel, 1988) and its extension (Denniston et al., 2001) have proven to be powerful alternatives to associative models that emphasize acquisition processes, as evidenced by their explanatory and predictive power. However, as we shall see next, there are several effects for which the comparator model cannot account. In the next section, we detail a model that accounts for interference effects outside the domain of models of associative learning designed to explain cue competition phenomena.

1.05.3.3 Bouton's Retrieval Model of Outcome Interference

Perhaps one of the most intriguing findings that Pavlov (1927) documented was the occurrence of a recovery in conditioned responding after a retention interval is interposed between extinction treatment and testing. Since Pavlov's time, there have been many theoretical frameworks proposed to explain extinction and its recovery under different circumstances. One old view of extinction is that nonreinforced presentations of an already trained CS will result in a loss of the CS's associative strength with the US, leading to an irreversible loss of behavioral control. For example, the Rescorla and Wagner (1972) model explains extinction in this manner. However, as we have mentioned before, if the associative strength of an extinguished CS is reduced, there is no reason to expect that responding to the CS will ever recover without further training with the CS, which is opposite to the spontaneous recovery effect observed when a long retention interval is interposed between extinction and testing.

After conducting extensive work on extinction, Bouton (1993) proposed an alternative to the models that view extinction as unlearning of the original association between the CS and US (*See* Chapter 1.09). This model emphasizes retrieval mechanisms that apply to a wide range of phenomena. Bouton's model has four principles.

1. Contextual stimuli influence memory retrieval. That is, analogous to Tulving and Thomson's (1973), Spear's (1978), and Riccio's (Riccio et al., 2002; Riccio et al., 2003) emphasis on the importance of similarity between the conditions of training and testing, Bouton proposed that

retrieval of a representation depends on the similarity between the conditions present at the time of testing with those that were present during training. Thus, as changes between the context of training and the context of testing are introduced, retrieval failure (i.e., forgetting) is more prone to occur. Furthermore, and perhaps in disagreement with Spear's elemental view, Bouton has proposed that CSs and contextual information are stored as interactive units containing information about the cues, the context, and the US. These interactive units function as an AND gate that requires activation of both the cue and the context for the activation of the representation of the US.

2. Time is part of the training context. As time elapses following training, the temporal component of the test context that is provided by external and internal cues is likely to change. In other words, the passage of time by itself will progressively result in a context change. Therefore, forgetting as a result of the passage of time is an instance of retrieval failure due to a change in the temporal context between training and testing.
3. Different memories depend differentially on the contextual information. In his 1993 seminal article, Bouton proposed that excitatory memories are relatively stable over time and do not depend on contextual information as much as do inhibitory memories (including those that represent extinction experience). Additionally he stated that a memory's sensitivity to contextual information depended on whether the training procedures promote subjects to encode and integrate contextual information with other features of the memory representation. One such procedure is when a CS takes a new meaning different from that of earlier training, as is the case when a CS is consistently followed by the US in one phase and it is no longer followed by the US (operationally, extinction) in a second phase. In this case, the second phase makes the CS an ambiguous signal for the US, and thus the model anticipates that the second-learned association will depend more on contextual information. In fact, Bouton (1997) later clarified this issue by stating that regardless of whether a memory is excitatory or inhibitory, each instance in which there is ambiguity with regard to the content of a memory (i.e., training in two sequential phases with opposing outcomes in the different phases), the second-learned meaning will be more context dependent. This later assertion has received empirical support from Nelson (2002; also see De la Casa and Lubow, 2000, 2002).
4. Interference occurs at the time of testing rather than at the time of training. This principle captures all of the previously mentioned principles by stating that interference occurs at retrieval rather than during learning. With ambiguity in the meaning of the CS, the activation of an outcome representation occurs in direct relationship to the similarity between the context at test and those during the different phases of training. Additionally, information that is similar to, but incompatible with, the target memory will compete for a limited available space in working memory (i.e., currently active memory). That is, activation of a conflicting memory could reduce activation of the target memory. Thus, forgetting can result from two sources: retrieval failure (because of a change in context) and interference due to activation of conflicting information.

Bouton's model explains spontaneous recovery from extinction by assuming that the second-learned meaning of the CS (that is, CS → no outcome or an inhibitory association between the CS and the outcome) is context dependent, based on the principle of context dependency of the second-learned meaning of a cue. Moreover, the model states that a change in temporal context is analogous to a change in spatial context. As a result, when the temporal context of the second meaning of a cue is changed between phase 2 of treatment and testing (i.e., interposing a long retention interval after extinction), the memory representation of extinction treatment cannot be as readily retrieved. Thus, a recovery from extinction is observed with a long retention interval. A similar explanation is put forth by this model to explain all forms of renewal, spatial as well as temporal. In renewal, the second-learned meaning of the CS (CS → no US) should be context dependent, so any change in the spatial attributes of the context between extinction treatment and testing will result in interference in retrieving that memory. As a result of such a context shift, a recovery from extinction should be observed.

Another finding that is problematic for models that emphasize processing during acquisition is reinstatement, which is a recovery from extinction observed when subjects experience outcome-alone

presentations in the test context prior to testing. In this case, Bouton's model assumes that the test context becomes associated with the outcome so that at test it biases retrieval in favor of the memory of reinforcement. Thus, a recovery from extinction is observed. Another way by which this model has been tested is by associating a neutral cue (a priming stimulus) with the phase of treatment in which extinction occurs. If the test is conducted in a context different from that of extinction treatment, renewal typically occurs. However, if during testing subjects are presented with this neutral cue from the extinction phase soon before being tested with the target cue, renewal is attenuated, presumably because this cue retrieves the memory of extinction treatment (Brooks and Bouton, 1994). A parallel attenuation of spontaneous recovery from extinction has been observed when the temporal context, as opposed to the spatial context, is altered between extinction training and testing (i.e., interpolation of a long retention interval). That is, reduced spontaneous recovery has been observed when a retrieval cue for the memory of extinction treatment is presented before testing following a long retention interval (Brooks and Bouton, 1993).

As we previously discussed, counterconditioning is another example of the context dependency of memory. If counterconditioning training is conducted with an appetitive reinforcer given in one context and an aversive reinforcer given in a different context, conditioned responding to the CS is guided by the context in which subjects are tested (Peck and Bouton, 1990). The retrieval model outlined earlier simply states that whichever memory representation is facilitated by contextual cues will be more prone to guide behavior.

In summary, we have reviewed general models of memory that emphasize retrieval mechanisms as critical for behavioral control. In general, Spear's (1978) model emphasizes the similarity between the total information presented during training and that presented at test and by this simple principle explains memory phenomena such as state-dependent learning and the Kamin effect. The comparator hypothesis (Miller and Matzel, 1988) is an associative model that emphasizes competition between representations and explains cue-competition phenomena and recovery from cue competition that does not involve further training with the target cue. Bouton's model (1993, 1997) emphasizes the role of retrieval cues in situations in which one cue has more than one meaning, as in the cases of extinction, latent inhibition, and counterconditioning. One obvious conclusion is that each model has been designed to account for a family of phenomena at the expense of explaining other phenomena. For example, the comparator hypothesis (Miller and Matzel, 1988) accounts for cue competition phenomena and several other effects in classical conditioning, but it does not explain the recovery from extinction or counterconditioning effects that are consistently observed. Moreover, this model does not explain state-dependent learning. In contrast, Bouton's retrieval model (Bouton, 1993, 1997) accounts elegantly for interference effects, but it does not incorporate any mechanism that accounts for cue competition phenomena. Perhaps the biggest challenge these models face is to explain phenomena outside their current domain without necessarily increasing their complexity and thus losing predictive power. As we shall see in the next section, a few efforts have been made to integrate these behavioral models with the neurobiological evidence concerning the role of retrieval in memory performance.

1.05.4 Neurobiology of Retrieval

Recent technological advances have widened the possibilities of understanding learning and memory phenomena by studying their underlying neurophysiological basis. Interestingly, the focus on acquisition processes (e.g., Waelti et al., 2001) and memory consolidation (e.g., McGaugh, 2000) has dominated the field, perhaps because of the discovery of potential molecular mechanisms underlying long-term potentiation (Bliss and Lomo, 1973) that are thought to be the basis for the formation of memories (but see Shors and Matzel, 1997, for an alternative view). However, the neurobiological basis of retrieval mechanisms has also received some attention. But before we review some experiments that studied the role of different brain regions in retrieval, it is important that we clarify the general strategy underlying these studies. In any memory experiment, there are at least three identifiable phases amenable to study, namely acquisition, consolidation, and retrieval. As pointed out by Abel and Lattal (2001), one of the problems associated with different manipulations (pharmacological, genetic, and lesions) is that, with the exception of recently developed inactivation techniques that allow researchers to temporarily inactivate a specific anatomical area, they can affect more than one of the three stages. For example, a lesion soon after acquisition might impair not only consolidation but also

retrieval. A lesion before any training might alter training, consolidation, and/or retrieval. Moreover, pharmacological manipulations might be temporary, but the effects of the mere exposure to the drug are not always completely known, as reflected earlier when we discussed the apoptotic effects of anisomycin (Rudy et al., 2006). Clearly all these considerations have to be taken into account to obtain valid information regarding the neurobiological underpinnings of memory.

Next, we briefly summarize the main findings regarding contextual determinants of memory retrieval. Notably, because of space limitations, we will only summarize a few studies that have the merit of integrating behavioral theories with neurobiological data. Studies such as these are few in number (but see, for example, Fanselow, 1999; Waelti et al., 2001; McNally and Westbrook, 2006; for notable exceptions focused on acquisition).

The hippocampus is one of the most extensively studied brain regions with regard to retrieval mechanisms (as well as acquisition processes). In general, the hippocampus has been implicated in both the coding and retrieval of spatial information and also in relations between events in the environment (e.g., Maren, 2001; but see Wiltgen et al., 2006). In fear conditioning, the hippocampus is thought to assemble contextual representations before they reach the amygdala, which is the site in which fear-motivated information is mainly processed. As we previously mentioned, contextual information is critical for the retrieval of associations. One of the questions researchers have recently asked concerns the role of the hippocampus in the retrieval as opposed to acquisition of contextual information. For example, behavior indicative of extinction is observed when contextual cues facilitate the retrieval of the extinction memory as opposed to the acquisition memory. Based on this finding, Wilson et al. (1995) investigated the effect on context-dependent extinction of fornix (one of the two primary inputs into the dorsal hippocampus) lesions made prior to training. It is important to recall that two of these context-dependent effects are renewal and reinstatement, and in terms of Bouton's theory of retrieval, they are mediated by different mechanisms. In the case of renewal, the context seems to disambiguate the two meanings a CS has after acquisition and extinction training. In the case of reinstatement, the test context-US association facilitates retrieval of the original CS → US association. Wilson et al. (1995) observed that fornix lesions attenuated reinstatement, which depends on context-US associations, but not renewal nor spontaneous recovery which depend more on the properties of the context to disambiguate information. This outcome is surprising because it leaves no role for the hippocampus on the retrieval of ambiguous information. However, other studies using temporary reversible lesions have found that the hippocampus does in fact participate in the retrieval of information needed to disambiguate the meaning of an extinguished CS. Specifically, Corcoran and Maren (2001) used muscimol (a gamma-aminobutyric acid$_A$ (GABA$_A$) receptor agonist) infusions into the dorsal hippocampus just prior to testing (i.e., retrieval) to investigate the effect of the hippocampus on the retrieval of ambiguous memories (renewal). They found that the renewal effect was attenuated when they deactivated the dorsal hippocampus. Similar findings have been observed by Corcoran and Maren (2004; although the effect was not seen in ABA renewal). Overall, these findings raise several interesting points: (1) These results show that the hippocampus, a brain region known for its role in the encoding and retrieval of contextual information, is critical for the expression of extinction memories, which are context dependent. (2) Reversible lesions have the advantage of allowing dissection of the different processes (such as retrieval) involved in memory performance. (3) Behavioral theories (e.g., Bouton, 1993) can provide fertile grounds for research in the neurobiology of learning and memory and vice versa. Clearly, the key is to combine information from both approaches as a starting point for conducting further research.

Another recent study exemplifies context-dependent memories and the role of the hippocampus in such learning. As previously stated, latent inhibition refers to retarded emergence of behavioral control that results from CS-alone exposures prior to CS → US pairings (Lubow, 1973). A retrieval-based account such as the comparator hypothesis (Miller and Matzel, 1988) explains latent inhibition by positing that, during CS preexposure, subjects associate the CS with the context, which interferes during testing with the retrieval of the CS → US association. Consistent with this explanation, extinction of the training context abolished the latent inhibition effect (e.g., Grahame et al., 1994; Westbrook et al., 2000). Moreover, if CS preexposure is conducted in one context and reinforced training in a second context, latent inhibition is not observed (Channell and Hall, 1993). Consistent with these predictions, Talk et al. (2005) found that context extinction following the

CS-US pairings increased neural firing to the preexposed CS in the posterior cingulate cortex, a structure hypothesized to have a role in retrieval of learned behavior. Presumably, the hippocampal formation sends (through the fornix) contextual information to the posterior cingulate cortex. When the contextual information is decreased as a result of the context extinction treatment, an increase in neural responses to the CS is observed, and this is reflected in the diminished latent inhibition.

These examples have the merit of integrating retrieval-focused behavioral theories of memory and neurobiological evidence concerning the underlying mechanisms of retrieval. We believe that further understanding of the neurophysiology of learning and memory will be most fruitful when it has some relationship to behavioral models. As these examples demonstrate, research guided by knowledge obtained in behavioral experiments (and the theoretical developments that follow those results) seems to be a reliable foundation for investigation of the neural foundations of retrieval.

1.05.5 Concluding Remarks

In this review, we started with the premise that a vast majority of the research concerning mechanisms of learning and memory has been guided by the notion that memory depends uniquely on mechanisms of acquisition and storage. Although we should not underestimate the contribution of these processes, we pointed out numerous phenomena suggesting that retrieval mechanisms also play an important role in determining stimulus control of behavior. We reviewed various examples, ranging from forgetting due to natural changes in the environment or in the organism's internal state, through amnesia experimentally induced in the laboratory, to situations in which additional (sometimes conflicting) information decreases target behavior. In all of these examples, there was evidence that the information was not lost, but rather was present but not expressed at the time of testing. Providing conditions during testing similar to those during training strongly facilitates retrieval and, as a consequence, stimulus control of behavior. In a similar vein, facilitating retrieval by the aid of reminder cues has also proven effective for memory performance. These demonstrations imply that information was stored but not expressed at the time of testing, which suggests a strong role of retrieval mechanisms.

In the second section, we described several models that emphasize processing at the time of retrieval as being critical for memory expression. Perhaps the unsatisfying conclusion from this section is that there is not a single approach that accounts for all of the data available. Some models seem to fare well in accounting for some phenomena, but fail to explain fundamental aspects of other phenomena. As an example, we pointed out how well the comparator model accounts for cue competition phenomena, but also recognized its failure in addressing important aspects of extinction and competition between cues trained apart. Obviously, the ultimate goal of any science of behavior is not only to explain behavior but also to predict it. At least the models we reviewed provide strong foundations for future theoretical developments, and in some ways each proposal has proven its heuristic value as a tool to guide new research. Current behavioral models have stimulated research into the neurobiological underpinnings of memory. We see this avenue as perhaps the most fruitful in the future because bridging the gap that exists between behavior and its underlying neurobiological basis is an important step toward societal application.

Retrieval, the act of making stored information available for use, is as important as acquisition in terms of adaptive value. Consider, for example, what would happen if an organism faces a dangerous situation and is able to survive. If the animal does not retain that information by virtue of either acquisition failure or storage failure, memory of that experience will not be available for future use. On the contrary, if the animal stores the information, it will be available for later encounters with the dangerous situation. This brings us to the question: why does retrieval failure occur? Perhaps retrieval failures, as we have seen, arise from processing limitations. But more important, it seems plausible that retrieval failures arise from a strategy for organizing information based on how relevant this information is in the test situation. That is, leaving some information less accessible enables organisms to have access to other information that could be more important, depending on the demands imposed by the immediate environment.

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1.06 Operant Behavior

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1.06.1 Introduction

1.06.1.1 Behavior as a Function of Its Consequences

In a famous experiment, Edward Thorndike (1898) trained cats to escape from puzzle boxes by activating various mechanisms. The time taken by the animals to escape the box on successive trials tended to decrease. During early trials, the cat tried various ineffective behaviors (pawing at the door, scratching the wall of the box, etc.) until by accident, it triggered the mechanism unlocking the latch. Starting from this point, trial times improved because ineffective acts gradually dropped out. This led Thorndike to formulate his famous law of effect according to which behaviors followed by positive consequences are strengthened, while behaviors followed by negative consequences are weakened. Later, the American psychologist B. F. Skinner (1938) coined the term operant behavior for activities that follow the law of effect.

Operant behaviors are behaviors guided by their consequences. In most cases, this requires that the animal first solve an assignment-of-credit problem by deciding which events are a consequence of a specific behavior. This is operant learning. Once this is done, the behavior and its consequence are linked in a feedback loop, and depending on the motivational properties of the consequence, the emission of operant behavior will be regulated by it.

In this chapter, we will first deal with operant learning and the question of how animals are able to detect the connection between a behavior and its consequences. Then we will deal with the maintenance and regulation of operant behavior by consequences. But first, we must say a word about the procedures used to study operant behavior in psychology.

1.06.1.2 Operant Conditioning

The main procedure for the study of operant behavior in psychology is operant conditioning, a term proposed by Skinner in the early 1930s (Skinner, 1938; *See* Chapters 1.03, 1.10). In operant conditioning, delivery of a biologically significant stimulus or a neutral stimulus signaling such a stimulus is made contingent on an easily repeatable response with low energy cost. The main dependent

variable is the response rate (number of responses emitted per unit of time). The standard version uses either hungry rats or pigeons in an experimental cage called a Skinner box (**Figure 1**), which allows for automated delivery of stimuli and recording of responses without any intervention by the experimenter. Rats are usually trained to press a lever for food pellets, whereas pigeons peck at illuminated response keys to gain access to grain. Electric shocks are used if negatively valued consequences are required by the experiment.

Operant conditioning procedures are classified according to their effect on responding and whether the consequence is the presentation or removal of a stimulus. If the procedure leads to an increase in the response rate, it is a reinforcement procedure and the consequence a reinforcer; otherwise, if it leads to a decrease in responding, it is a punishment procedure, and the consequence is a punisher. A reinforcer can be either the delivery of an appetitive stimulus (positive reinforcement, such as a hungry rat pressing a lever for food) or the withdrawal of an aversive one (negative reinforcement, for example, a rat pressing a lever to stop electric shock). Similarly, a punisher can either be the delivery of an aversive stimulus (positive punishment, such as a rat pressing a lever that produces electric shock) or the withdrawal of an appetitive stimulus (negative punishment, for example, food withdrawn from a cage if a hungry rat presses a lever). As a practical matter, research has focused on positive-reinforcement procedures (to the point that the word positive is usually omitted).

Figure 1 Pigeon in a Skinner box. By pecking on the illuminated disks (response keys), the pigeon occasionally gains access to food delivered in the feeder (opening below the response keys).

The rule that describes how reinforcement depends on responding, whether number-based, time-based, or according to some other rule, is called the schedule of reinforcement (Ferster and Skinner, 1957). Simple schedules arrange reinforcement for only one response, whereas concurrent schedules arrange reinforcement for two or more simultaneously.

The basic schedules of reinforcement are ratio and interval schedules. In ratio schedules, a certain number of responses (the ratio of the schedule) must be emitted for reinforcement to occur. The ratio is fixed in fixed-ratio (FR) schedules while it varies after each reinforcement around a mean in variable-ratio (VR) schedules. The ideal case of a VR is a random-ratio (RR) schedule where each response has a constant probability of reinforcement equal to the inverse of the ratio. In interval schedules, a certain amount of time (the interval of the schedule) must have elapsed before a reinforcement is made available. The interval is fixed in fixed-interval (FI) schedule but varies randomly around a mean after each reinforcement in variable-interval (VI) schedules. The ideal case of a VI is a random-interval (RI) schedule where the probability of a reinforcer being scheduled each time step is equal to the inverse of the interval. VI schedules usually generate a steady rate of responding, whereas in FI schedules, animals typically pause after trial onset (reinforcement) before starting to respond (Ferster and Skinner, 1957).

Traditionally, when describing an operant procedure using schedules of reinforcement, the acronym of the schedule (i.e., FR, VI) is given followed by its relevant parameter (i.e., its ratio for a ratio schedule or its interval for an interval schedule). So, instead of saying that a rat had to press a lever 50 times before a reinforcer was delivered, we would say that lever pressing was reinforced according to an FR 50. In the same way, instead of saying that a peck on a response key was reinforced only if 15 s have elapsed since the last reinforcement, we would say that key pecking was reinforced according to an FI 15 s. Instead of saying that a rat had the choice between two levers (a concurrent schedule), one delivering reinforcement according to a VI 15 s, the other according to a VI 30 s, we would say that the rat was exposed to a concurrent VI 15 s VI 30 s.

Finally, schedules of reinforcement can deliver either primary reinforcers – stimuli having biological significance for the organism (e.g., food for an hungry animal) – or conditioned reinforcers – stimuli that are correlated with the delivery of a primary

reinforcer. For instance, pecking on a red key for a pigeon could be reinforced according to a VI 15 s, but when reinforcement occurs, instead of food being delivered, the key turns green, and pecking it is reinforced with food according to an FR 15. The green key is considered a conditioned reinforcer because it signals the future delivery of food. Such a procedure is also an example of a chain schedule (a chain VI 15 s FR 15 in this case) where reinforcement for one schedule is access to another schedule, and the transition from one to the other is signaled by a change in some stimulus. Tandem schedules resemble chain schedules, except that the transition from one schedule to the other is unsignaled.

Concurrent chain schedules (**Figure 2**) combine chain schedules with concurrent schedules. They comprise two phases: the initial link and the terminal link. During the initial link, the animal has a choice between two schedules, usually VI or RI with the same interval. When a response is reinforced on one of the two schedules, this leads to the terminal link: The two schedules used during the initial link are deactivated, and the animal is left with only one response opportunity, reinforced with food according to its own schedule of reinforcement. Each initial link leads to a different terminal link schedule: for instance, one VI initial link could lead to an FI 15 s terminal link, whereas the other leads to an FI 30 s terminal link. The allocation of behavior on the two schedules during the initial link is used as a measure of the relative preference of the animal for the two terminal-link schedules. In this example, the animal would respond more often on the (initial-link) VI leading to the FI 15 s than on the one leading to the FI 30 s.

1.06.2 Operant Learning

1.06.2.1 Nonoperant Effects in Operant Conditioning

Before trying to understand the condition in which operant learning takes place, it is important to realize that not all the effects observed in operant conditioning are due to the operant aspect (i.e., to the response-reinforcer contingency *per se*). Reinforcers are highly motivating stimuli, and their mere delivery has an effect on the organism: It arouses it, increasing its general level of activity. Killeen et al. (1978) showed that the arousing effect of a single reinforcer delivery (measured as general activity) decreases exponentially with the time elapsed since the reinforcer delivery; when reinforcements are repeated, arousal builds up to an asymptotic level. According to Killeen (1998), this model implies that response rate should be proportional to reinforcement rate.

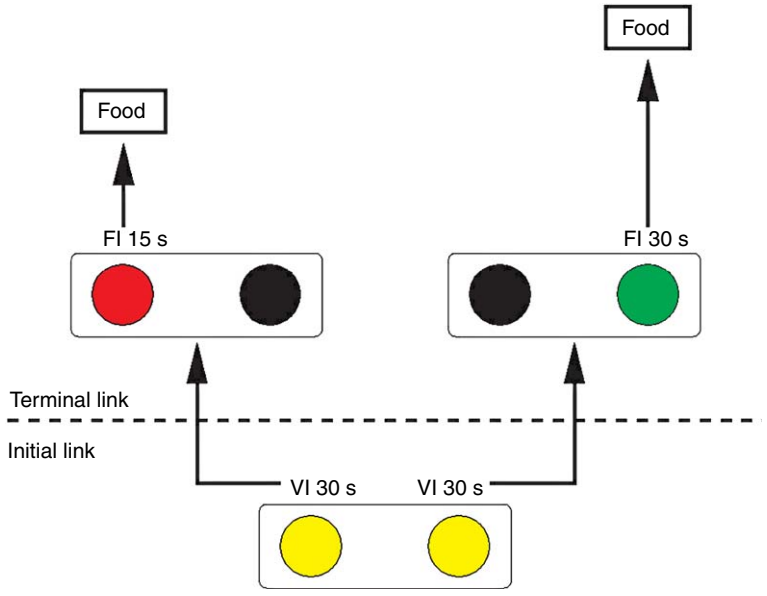

Figure 2 Example of a concurrent chain schedule: One initial link leads to an FI 15 s (red key; other key dark), whereas the other leads to an FI 30 s (green key, other key dark). The rate of entry in the initial link is determined by independent VI 30-s schedules (two yellow keys).

Functions relating response rate to reinforcement rate in VI are actually negatively accelerated (Catania and Reynolds, 1968; Herrnstein, 1970; Shull, 2005; **Figure 3**), but because rats are much more sensitive to reinforcement rate than pigeons, a linear approximation is reasonable for them. By contrast, pigeon functions quickly reach an asymptote (Shull, 2005; **Figure 3**). In this view, response rate in simple schedules is more determined by arousal than by the response-reinforcer contingency, although this might be masked by the fact that different response topographies might be selected by different schedules. For example, VR schedules lead to a higher rate of responding than VI schedules, even when matched for obtained reinforcement rates (i.e., Baum, 1993), presumably because VR schedules selectively reinforce shorter interresponse times than VI (Peele et al., 1984; Dawson and Dickinson, 1990).

Arousal is reinforcer-specific: Animals are more likely to engage in behavior related to the kind of reinforcers delivered. For instance, golden hamsters engage in activities directed at the environment (locomotion, active contact with the environment such as wall-scratching) when food deprived, more rarely in self-care activities such as grooming or scent-marking (Shettleworth, 1975). Similarly, rats receiving electric shocks will most likely freeze or flee (Bolles and Riley, 1973; Karpicke et al., 1977).

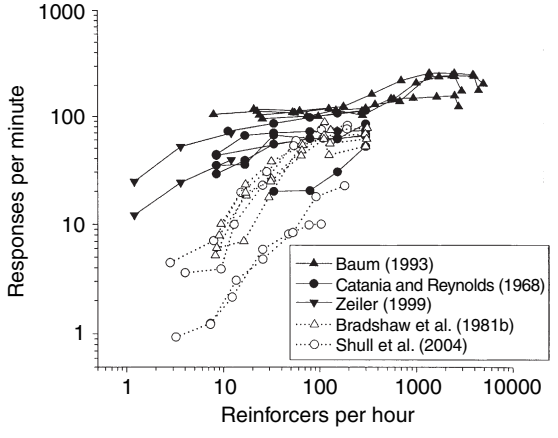

Figure 3 The relation between response rate and (food) reinforcement rate in simple VI schedules collected from various studies. Overall, it is hyperbolic, but rats (empty symbols) are much more sensitive to variations in the reinforcement rate than pigeons (filled symbols). From Shull RL (2005) The sensitivity of response rate to the rate of variable-interval reinforcement for pigeons and rats: A review. *J. Exp. Anal. Behav.* 84: 99–110, with permission from the Society for the Experimental Analysis of Behavior, Inc.

This provides the necessary behavioral variation from which the reinforcement contingency will select the appropriate response.

As a consequence, a behavior cannot be reinforced by a reinforcer if it is not naturally linked to that reinforcer in the repertoire of the animal (*See* Chapters 1.06, 1.18, 1.22). Hence, environment-related activities can be reinforced by food in hamsters but not self-care responses (Shettleworth, 1975), and rats will learn to press a lever for food (food consumption in rats involves manipulation) but not to avoid an electric shock, although they will quickly learn to freeze, run to another box, or jump over a barrier (all parts of the rat defensive repertoire) to achieve such a result (e.g., Biederman et al., 1964; Hineline and Rachlin, 1969). Although pigeons can learn to peck a key for both food and water, the topography of their responses differs depending on the kind of reinforcer used. They peck at the key the way they peck at food if food is used as the reinforcer (beak open until contact with the target), and the way they peck at water (beak closed until contact with the target) if water is used as the reinforcer (Jenkins and Moore, 1973).

The most spectacular example of the principle according to which only behaviors related to a specific reinforcer can be reinforced by it is instinctive drift (Breland and Breland, 1961), where, in procedures using food reinforcement, a species-specific food-related behavior will replace an operant response not naturally linked to food situations in the repertoire of the organism. For instance, a raccoon reinforced to put coins in a slot machine will learn the task, but then suddenly start washing the coins, while a pig trained in the same procedure will bury them instead (Breland and Breland, 1961).

Besides the reinforcement contingency, other variables contribute to the selection of the response, notably the Pavlovian stimulus–stimulus contingency that any operant procedure creates between the experimental context and the reinforcer. In some cases, this effect is so strong that there is nothing left for the response-reinforcer contingency to select for. This is notably the case of pigeon key pecking; simply illuminating the key briefly before intermittently delivered food is sufficient to trigger key pecking, a procedure known as autoshaping (Brown and Jenkins, 1968). This happens even if key pecking actually postpones food delivery (Williams and Williams, 1969). Other responses in other species, including lever pressing in rats, can be autoshaped. The exact nature of the autoshaped response depends on the

relation between the reinforcer-predicting stimulus and the reinforcer-elicited behaviors. Pigeons peck at small circular objects (like grain), and this is probably why they will peck at a key, a small circular object, when that stimulus becomes a predictor of food: This behavior is compatible both with the kind of reinforcer delivered and with the stimuli present in the environment. Indeed, if a small dark dot is added to the center of the key, hence making it even more similar to the stimulus naturally supporting pecking, autoshaping is faster (Jenkins et al., 1981). In rats, if the stimulus predicting food is another rat instead of a lever, autoshaped responding will take the form of social behaviors directed at the stimulus rat (Timberlake and Grant, 1975).

Another aspect of the performance that seems to be determined by Pavlovian relations between the context and the reinforcer, at least in simple schedules, is the temporal regulation of behavior. If food is delivered at a specific time relative to trial onset (in an FI, for instance), animals adjust to this regularity, and their response rate is a function of time-to-reinforcement (Skinner, 1938), a phenomenon known as interval timing (more on this later). Wynne and Staddon (1988) exposed pigeons to a response-initiated schedule where the first peck on the key after trial onset started a fixed delay-to-food and measured the waiting time (the time between trial onset and the first response). This should have reinforced short waiting time, but instead, the pigeons waited an amount of time equal to a fixed proportion of the last time-to-food interval. On the same issue, Baum (1993) showed that if the reinforcement rate (which is the inverse of the average time of reinforcement) is equalized, wait times in VI and VR are identical (see also Jozefowiez et al., 2005, for additional experimental evidence). Note that, on the other hand, interval timing also occurs in concurrent schedules. For instance, Jozefowiez et al. (2005) exposed pigeons to a concurrent FI 20 s FI 60 s and observed temporally regulated choice: The pigeons started responding on the FI 20 s, then as time in a trial increased, switched to the FI 60 s. This is obviously operant timing, as it requires the animal to know with which response a specific time of reinforcement is associated. This argument has been used by Jozefowiez et al. (2005, 2006) to argue that different processes control responding in simple schedules versus concurrent schedules.

A corollary of interval timing is that if the animal is more likely to engage in reinforcer-related activity as the time of reinforcement grows nearer, it is less likely to engage in those activities when the time of reinforcement is far away and so more likely to engage in other activities at those times (*See* Chapters 1.12, 1.19). Staddon (1977) has divided the activities taking place during an interreinforcement interval into three classes. On one end of the interval, close to the time of reinforcement, is the terminal response – reinforcer-related activities such as key-pecking in pigeons or lever-pressing in rats. At the other end of the interreinforcement interval, close to trial onset, are interim activities (also termed adjunctive behavior). Interim activities, although unrelated to the reinforcer in the repertoire of the animal, are affected by the experimental parameters, their rate of emission being an inverted U-shaped function of the reinforcement rate (Staddon, 1977; Reid and Staddon, 1990). The classic example is polydipsia in rats (Falk, 1961): In any procedure where food is delivered at fixed intervals, rats will drink to excess just after food delivery – up to several times their daily water requirement – if given the opportunity. The excessive nature of polydipsia clearly indicates that it cannot be merely explained by efficient time allocation or homeostasis.

The type of adjunctive behavior observed depends on species-specific interaction between motivational systems and the connections existing between these systems and the stimuli present in the environment: If presented with another rat instead of water, rats will attack it (Thompson and Bloom, 1966; Gentry and Schaeffer, 1969), but on the other hand, if given access to both water and another member of their species, they will display schedule-induced polydipsia rather than schedule-induced aggression (Knutson and Schrader, 1975). In the same situation, pigeons only display schedule-induced aggression (Yoburn and Cohen, 1979). The motivation to engage in adjunctive behaviors is so strong that they can be used as reinforcers for other activities. For instance, a pigeon will learn to peck a key to be able to attack another pigeon (Cherek et al., 1973).

Finally, the time not filled by interim or terminal behavior is filled with facultative activities. In contrast to adjunctive behavior, they are not affected by the reinforcement rate and tend to be suppressed if more time is required by interim and terminal behavior (Staddon and Ayres, 1975).

So there is much more going in operant conditioning than simply operant learning. Yet there is clear evidence that the response-reinforcer contingency also plays a role. This is obviously the case in concurrent schedules, but it is also true for simple

schedules. Even though pigeons will peck at a key and rats will press a lever anyway if these stimuli are signals for food, they are still more likely to do so if there is also a response-reinforcer contingency (Rescorla and Skucy, 1969; Woodruff et al., 1977), especially for rats (Lowe and Harzem, 1977). An omission procedure, where emission of the response postpones the delivery of the reinforcer, also has an effect on the behavior – on rats' approach behavior (Holland, 1977) or pigeons' key pecking (Schwartz and Williams, 1972).

There is also good evidence that animals can discriminate when a stimulus is a consequence of their behavior or not. In an experiment by Killeen (1978), pigeons were pecking on a key that turned black from time to time. If this change was the consequence of peck, reinforcement was scheduled on a second key; but if the change was spontaneously caused by a scheduling computer, they were reinforced for a peck on a third key. Pigeons had no problem mastering this task.

1.06.2.2 Determinants of Operant Learning

The operational distinction between Pavlovian and operant learning is clear: The animal learns a relation between a response and a stimulus in operant learning but a relation between two stimuli (the conditioned stimulus, CS, and the unconditioned stimulus, US) in Pavlovian learning. But are these two different forms of associative learning or does the same mechanism underlie both? The data favor the second hypothesis because the conditions leading to the learning of a CS–US relation in Pavlovian conditioning also lead to the reinforcement of a response in operant conditioning.

One important variable known to affect Pavlovian learning is the temporal contiguity between the CS and the US (Rescorla, 1988; *See* Chapter 1.03). Although testing to see if contiguity is also important in operant learning is a bit more complicated than it appears (for instance, if a response is emitted and scheduled for reinforcement D seconds later, do we allow more responses during the interval? If yes, the delay will be less than D. If no, we might artificially reduce responding), all studies that have looked at it have found that response rate is a decreasing function of the delay (Sizemore and Lattal, 1977; Lattal and Gleeson, 1990; Dickinson et al., 1992; Bruner et al., 1998; Stuphin et al., 1998). Also, even though it is commonly acknowledged that small delays have catastrophic consequences on the acquisition of operant responding, recent studies have shown that after appropriate training, a substantial response rate can be sustained even with 30-s delays in rats, pigeons, and even Siamese fighting fish (Lattal and Gleeson, 1990; Lattal and Metzger, 1994; Bruner et al., 1998; Stuphin et al., 1998).

The other important determinant of Pavlovian learning is that the CS must be a good predictor of the US (Rescorla and Wagner, 1972). Once again, this seems also to be the case for operant learning, where the response has to be a reliable predictor of reinforcement. Responding is suppressed when the probabilities of response-dependent and response-independent reinforcement are equal, and it actually seems that the amount of responding is proportional to these two probabilities (Hammond, 1980; see also Lattal, 1974. In similar fashion, Pavlovian learning depends on the difference between the probability that the US is presented in the presence vs. absence of the CS; Rescorla and Wagner, 1972). This effect is reinforcer-specific: Response-independent food delivery reduces the rate of responding of a food-reinforced response but has no effect on a water-reinforced one (Dickinson and Mulatero, 1989). Moreover, response-independent food delivery has no effect if signaled by a stimulus, a manipulation that preserves the predictive value of the response (Hammond and Weinberg, 1984; Dickinson and Charnock, 1985).

Another line of evidence comes from a study by Williams (1999) that showed that operant learning does not occur if the reinforcer delivery is predicted by a stimulus. Naive rats were placed in a Skinner box, and lever-pressing led to food delivery 30 s later. There were three conditions. In the control condition, the delay of reinforcement was unsignaled. Consistent with earlier results of Lattal and Gleeson (1990), lever pressing increased in frequency despite the 30-s delay. In another condition, pressing of the lever turned on a house light that stayed on until food delivery. This kind of procedural arrangement is known to considerably aid delay conditioning because of the supposed conditioned-reinforcement properties of the food-predicting stimulus. Indeed, Williams (1999) found a higher response rate in that condition than in the unsignaled-delay-of-reinforcement one. But if the house light was turned on 5 s before reinforcement instead of just after response emission, responding was totally suppressed. According to Williams (1999), the higher reinforcement-predicting value of the light, which could not be compensated in this case by its

conditioned reinforcement properties, blocked the learning of the response-reinforcer relation (see Williams, 1999, for additional experimental support for this hypothesis).

1.06.2.3 The Content of Operant Learning

Operant behaviors are emitted because of their consequences: A pigeon in a Skinner box is pecking at the key because it gives access to food. But does the pigeon 'know' that? In the framework of associative theory, this question is about whether operant learning leads to the formation of stimulus-response associations between the experimental context and the response or response-outcome associations between the response and its consequence.

Data seem to support the latter view. They come mainly from the reinforcer devaluation procedure where, after an operant behavior has been established, the value of the reinforcer to which it gives access is modified to see if this has any impact on the performance. For example, Colwill and Rescorla (1985) first trained a rat to press a lever for food. The rat was then given free access to food and made sick by injection of lithium, a procedure that creates a strong aversion for the food consumed prior to the poisoning (taste aversion learning; Garcia et al., 1966). When reintroduced into the Skinner box after that manipulation, response rate was massively depressed compared to baseline performance. At the same time, another operant response (chain pulling), which had been reinforced with water, was unaffected by the devaluation of the food reinforcer. The experiment by Dickinson and Charnock (1985) that we discussed previously, showing that response-independent food delivery had no effect on a water-reinforced response, points to the same conclusion. On the other hand, responding is not totally suppressed following reinforcer devaluation. That could be a clue that stimulus-response associations are also formed and partly control operant performance along with response-outcome associations (see Hall, 2004, for further discussion).

1.06.3 Interval Timing

Now that we have considered the conditions in which operant learning takes place, we can turn to the second issue regarding operant behavior – how it is regulated by its consequences. This is mainly the study of schedules of reinforcement, which has been heavily skewed toward interval schedules and concurrent schedules, the former because they provide a way to study interval timing, whose influence seems pervasive in operant conditioning, and the latter because they are an experimental model of choice and decision making. We deal with interval timing in this section and with concurrent schedules and choice in the section titled 'Operant choice.'

1.06.3.1 Basic Facts

Interval timing is the ability of animals to perceive temporal relations between two events, ranging from seconds to several minutes (*See* Chapter 1.19) (based on neurological evidence, it seems that timing in the milliseconds range and circadian timing are different processes (e.g., Lewis and Miall, 2003; Lewis et al., 2003). In interval timing, the first event is termed the time marker because it starts the to-be-timed interval. On FI, where the time marker is trial onset and the interval is ended by reinforcement, interval timing leads to some temporal regulation of behavior. In most species, the animal pauses after trial onset and then, as time in a trial increases, starts responding at an increasing rate, reaching its maximum shortly before the time of reinforcement (Ferster and Skinner, 1957; Lejeune and Wearden, 1991). Some have argued (e.g., Gibbon, 1991; Cheng and Westwood, 1993) that this pattern of responding, called a scallop, is an artifact caused by averaging many trials together and that performance in individual trials actually follows a break-and-run pattern, with the animal moving abruptly, at a point varying from trial to trial (the break point), from a state of low responding to a state of high responding. But scallops were initially described based on the real-time performance of individual animals (Ferster and Skinner, 1957). Data from Schneider (1969), usually cited in support of the break-and-run hypothesis, actually show that although the break-and-run pattern dominates performance for short fixed intervals, scalloping is characteristic of longer intervals. Staddon (2001, Box 13.1) showed that this is what should be expected if the probability of responding increases monotonically during a trial.

For this reason, the pattern of local response rate across a trial, an estimate of the pattern of probability of responding across a trial, seems a better dependent variable than partial measures such as pause or break point. In FI, response rate increases during a trial, following a sigmoid, Gaussian function (Killeen et al., 1978; Lejeune and Wearden, 1991; **Figure 4**). What would happen if reinforcement was not delivered?

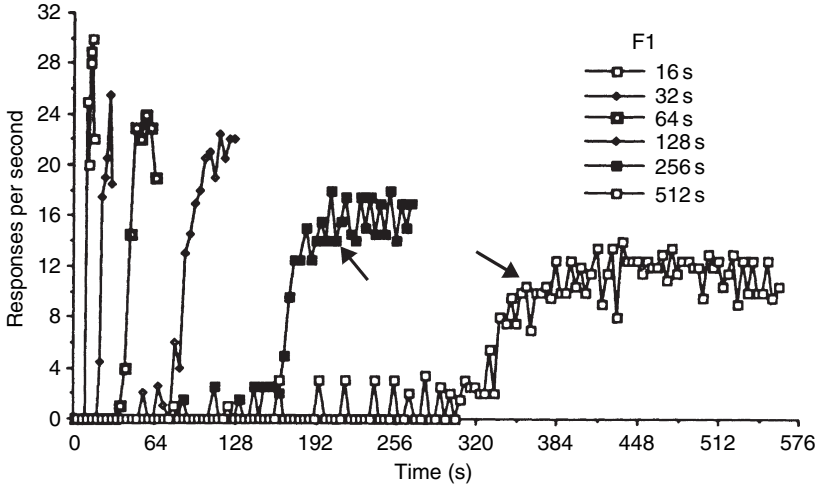

Figure 4 Proportional timing in FI: Response rate in FI is a sigmoid function of the time-to-reinforcement. Plotted in relative time, curves from FI with different intervals would superimpose. From Gibbon J (1991) Origin of scalar timing. *Learn. Mot.* 22: 3–38, with permission from Elsevier.

The peak procedure where, along with standard FI trials, empty trials lasting three or more times longer than the FI trials and ending without reinforcement are thrown in has been designed to answer this question (Catania, 1970; Roberts, 1981). After sufficient training with this procedure, response rate increases according to a sigmoid function up to the time of reinforcement and then decreases from that point, still according to a sigmoid function (**Figure 5**). The time of maximal responding (peak time) corresponds more or less to the time of reinforcement. It is not much affected by the number of peak trials, which mostly affect the absolute response rate, partly through their effect on arousal, because adding peak trials reduces the overall reinforcement rate.

Timed behavior, as observed in FI and the peak procedure, has two fundamental characteristics. The first is proportional timing: Performance is tuned to the time of reinforcement (**Figures 4** and **5**). Although this temporal adaptation of behavior was once believed to emerge only after extensive training with the procedure, recent research (Innis and Staddon, 1971; Wynne and Staddon, 1988; Higa et al., 1991; Higa and Staddon, 1997) has now shown that pigeons and rats can modify their behavior immediately following a change in the FI interval. Second is the scalar or timescale invariance property: Response curves superimpose when plotted in relative time (Dews, 1970; **Figure 5**). These properties have skewed theoretical thinking about interval timing toward a psychophysical framework.

In a psychophysical task, the subject is presented with two stimuli and asked if they are of similar intensity (or frequency, or color, etc.). The subject's responses are usually variable, assumed to be because of noise in sensory systems, but the probability that the two stimuli are judged identical is a function of their difference in intensity. If one stimulus is held constant, the curve showing how the probability that the subject judges the two stimuli identical as a function of the intensity of the second stimulus is called a psychometric function. A general property of many sensory systems is that they respect Weber's law: The higher the intensity of a stimulus, the more difficult it is for the subject to perceive differences when it is varied, an outcome that could result from a logarithmic encoding of stimulus intensity, a conjecture known as Fechner's law. One interesting consequence of Weber's law is that if psychometric functions are plotted on a relative stimulus scale, they superimpose (Falmagne, 1985).

This has led researchers to consider that the response rate curves obtained from animals in FI and peak procedures are indeed psychometric functions: The subject is comparing a representation of the current time in a trial to a representation of the time of reinforcement; the closer they are, the higher the probability of responding. Timescale invariance is observed because time perception follows Weber's law.

1.06.3.2 Scalar Expectancy Theory

Most theories of timing agree on this framework (Staddon, 2001) but differ about what is the basis for the representation of time. For many years, the most

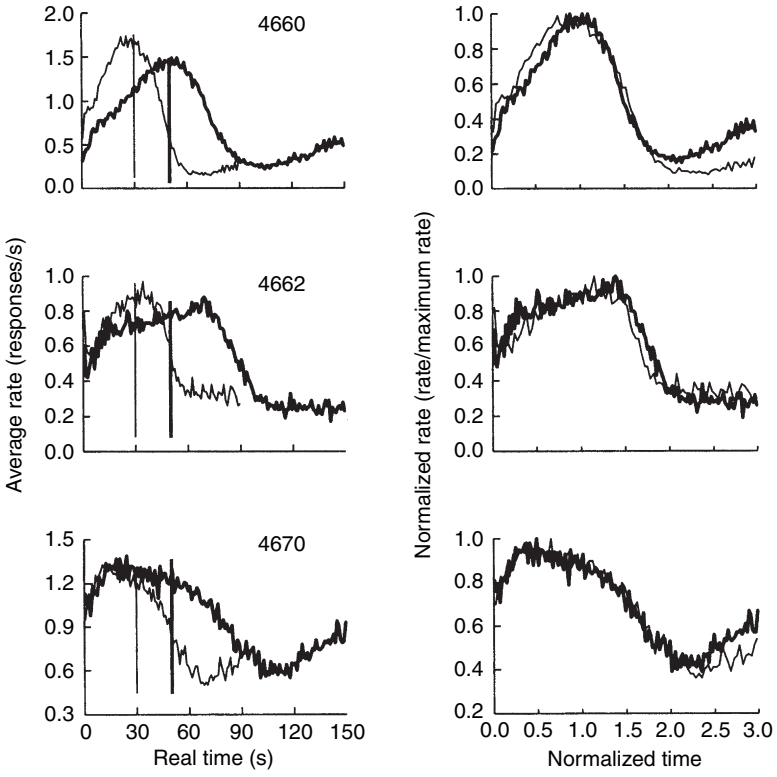

Figure 5 Proportional timing and timescale invariance in the peak procedure: Response rate in the peak procedure increases up to the time of reinforcement and then decreases, in both cases in a sigmoid fashion. Plotted in relative time, curves from peak procedures with different intervals superimpose. From Gallistel CR and Gibbon J (2000) Time, rate and conditioning. *Psych. Rev.* 107: 289–344. Copyright American Psychological Association. Reprinted with permission.

influential model was scalar expectancy theory (SET, Gibbon, 1977; Gibbon et al., 1984; **Figure 6**). SET assumes an internal pacemaker generating pulses at a more or less constant rate as soon as the time marker is presented (like most theories of timing, SET does not say how the animal identifies a time marker). These pulses accumulate in short-term memory, providing the basis for the representation of time which, as a consequence, is linear. When a reinforcer is delivered, the number of pulses currently accumulated is stored in long-term memory, but error is supposed to occur during the encoding process, leading to a Gaussian long-term memory distribution of time of reinforcement with a standard deviation proportional to the mean: This ensures that Weber's law is respected (Falmagne, 1985), even though Fechner's law is not (time is supposed to be encoded linearly, not logarithmically). At the beginning of a trial, the animal is supposed to sample one value from this distribution, which it will constantly compare to its representation of the elapsed time-in-trial to decide if it responds or not.

The empirical basis for SET is its ability to account for the basic data in timing procedures. But alternative theories of timing (i.e., the behavioral theory of timing, Killeen and Fetterman, 1988, and its neural network version, the learning-to-time model, Machado, 1997; the multiple timescale theory, Staddon, 2001, 2005; the packet theory of timing, Kirkpatrick, 2002) do just as well. Moreover, SET's achievement comes at the cost of parsimony. In adding to an already substantial number of free parameters, details of the models are usually modified to fit results from different procedures. For instance, the comparison rule between the representation of the current time in trial and the representation of the time of reinforcement is not the same depending on whether the data to be accounted for have been collected in an FI, a peak procedure, a bisection procedure, or a time-left procedure (Wearden, 1999; see the following paragraphs for a description of the latter two timing procedures).

In any event, it seems that the basic data about timing can be simulated by any model respecting

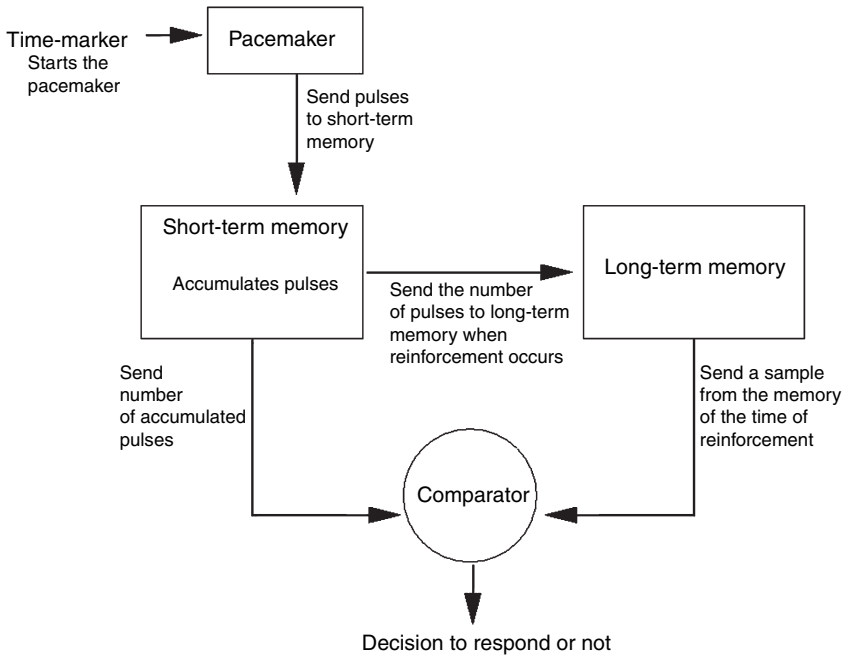

Figure 6 Scalar expectancy theory. When a time-marker is presented, the pacemaker starts emitting pulses at a more or less constant rate into short-term memory, where they are accumulated. When reinforcement occurs, the number of pulses currently in short-term memory is transferred to long-term memory. During a trial, the current number of pulses in short-term memory is continuously compared to a sample of the time of reinforcement retrieved from long-term memory to decide if a response should or should not be emitted.

Weber's law, and so it is not sufficient for a timing model to account just for those data. It must have additional empirical support for its assumptions. The behavioral theory of timing, for instance, is rooted in the data about adjunctive behavior we reviewed in the previous sections, considering that the sequence of behaviors an animal goes through a trial (from interim to facultative to terminal behavior) is the basis for interval timing instead of its consequence, as we have proposed. The multiple timescale theory is based on a model of memory developed to account for habituation data. What data support the existence of SET's internal pacemaker?

The best evidence is from the study of drug effects on timing performance. Meck (1996) proposed that dopamine agonists, such as amphetamine, accelerate the rate of the pacemaker. (Dopamine antagonists are supposed to have the reverse effect. Meck's (1996) model also has predictions concerning the effect of cholinergic drugs, but we will focus on dopamine antagonists in this review.) Then, in the peak procedure, chronic injection of a dopamine agonist should (1) immediately move the peak time to the left because the criterion number of pulses at which the animal starts responding is reached faster, but (2) the peak time should gradually return to its original value as a new criterion is learned due to the fact that, with each reinforcement, the number of pulses in memory is transferred to long-term memory and a new representation of the time of reinforcement, generated by the drug-accelerated pacemaker, progressively overrides the previous one generated by the slower pacemaker. Once the dopamine agonist injection is discontinued, the same process takes place in reverse: The peak time shifts to the right and then progressively comes back to its original value. Similar effects should be observed for other measures of temporal control, such as the bisection point in the bisection procedure (see following paragraphs for a description of that procedure).

Although some studies have found the predicted effect of dopamine agonist on temporal dependent variables (i.e., Maricq et al., 1981; Maricq and Church, 1983; Spetch and Treit, 1984, with a bisection procedure; Maricq et al., 1981; Eckerman et al., 1987; Frederick and Allen, 1996; Kraemer et al., 1997, with a peak procedure), at least as many have failed to find such an effect (e.g., Lejeune et al., 1995; Chiang et al., 2000; Odum and Schaal, 2000, with a bisection procedure; Bayley et al., 1998; Knealing and

Schaal, 2002, with a peak procedure; see Odum et al., 2002, for a complete review). Moreover, studies using the peak procedure usually report only the peak time, whereas SET predictions are much broader and implicate the whole response function. It should be shifted to the left, and because of the scalar property, its variance should decrease. Saulsgiver et al. (2006) found that this is not what happens: Low responding early in a trial increases, while high responding before the time of reinforcement decreases; response rate after the time of reinforcement is mainly unaffected. As a consequence, the peak time shifts to the left, but the variance of the response-rate function increases, a result opposite to the SET interpretation.

In a critical review of the literature, Odum et al. (2002) argued that rate dependency provides a unifying account of drug effects in timing procedures, explaining results from experiments showing an effect and from those failing to find one. Rate dependency is a well-established and general empirical effect of drugs on operant behavior (Dews, 1958, 1981): High response rates are reduced by drug administration, whereas low response rates are increased. In an FI, for instance, this leads to a flattening of the whole response function. Odum et al.'s (2002) own studies, using a procedure borrowed from Catania and Reynolds (1968) that allowed them to differentiate timing from rate dependency (see Odum, 2002; Odum et al., 2002, for further details), seem to confirm that the dopamine-agonist effect in timing procedures is mainly due to rate dependency. (On the other hand, Odum (2002) found more support for an effect on timing of cholinergic drugs such as atropine or physostigmine, although the effect was small for physostigmine and the reverse of what is predicted by SET (Meck, 1996) for atropine.)

The results are just as problematic for other predictions of SET, like the progressive tolerance effect predicted if the dopamine agonist is injected chronically (Maricq et al., 1981, reported such an effect, but Frederick and Allen, 1996; Chiang et al., 2000; McClure et al., 2005; and Saulsgiver et al., 2006, failed to find it) or the rebound in peak time predicted after chronic injections of amphetamine cease (i.e., Saulsgiver et al., 2006). All in all, drug effects on timing performance provide no support for a pacemaker account of interval timing. The effects that are observed seem to be more a consequence of rate dependency than of a perturbation by the drug of an isolable temporal-information-processing mechanism.

An indirect way to prove the existence of an internal pacemaker would be to show that animals represent time linearly. Two experimental procedures have been designed to tackle this issue. The older one is the bisection procedure (Stubbs, 1968; Church and Deluty, 1977). It is a trial-based procedure where, following the presentation of one of two temporal stimuli, short or long, the animal is given a choice between two response alternatives. The animal is trained to make one response if the stimulus was short and another one if the stimulus was long. Then, on unreinforced probe trials, the animal is presented with stimuli having intermediate durations between the short and long stimuli used during training.

The closer the duration of the test stimulus to the duration of one of the stimuli used in training, the higher the probability the subject picks the response associated with that stimulus. Of special interest is the point of bisection, that is to say, the stimulus duration for which the animal picks each response with the same probability. On the animal's subjective timescale, the representation of that stimulus duration is supposed to be halfway between the representations of the duration of the two stimuli used during training. It usually falls around the geometric mean between these two durations. On a straightforward psychophysical interpretation, this result implies that time is represented logarithmically, rather than linearly, as the pacemaker approach implies. However, the linear assumption of SET can be made consistent with geometric-mean bisection by suitable modifications to other parts of the model (Gibbon, 1981).

In an attempt to resolve this issue, a more complex procedure, the time-left procedure (Gibbon and Church, 1981), was devised (**Figure 7**). Basically, it is a concurrent-chain procedure with VI initial links. At a random time T varying from trial to trial, the animal's response will commit it either to the time-left side, where food will be delivered C-T s later, or to the standard side, where food will be delivered S s later. According to Gibbon and Church (1981), the performance in this task is based on a series of mental computations. If we denote by $f(x)$ the animal's representation of an interval of x s, and by t the time elapsed since trial onset, then at each time step, the animal is supposed to compute the difference $f(C) - f(t)$ (time left to reinforcement on the time-left side) and compare it to $f(S)$ (time to reinforcement on the standard side): if $f(C) - f(t) > f(S)$ (which is more likely early in a trial since $S < T$), the animal

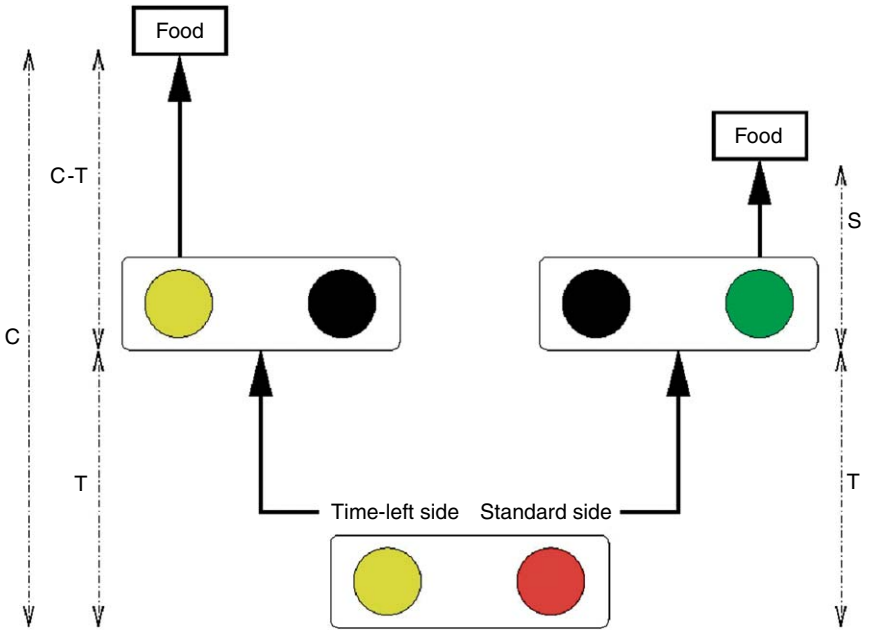

Figure 7 The time-left procedure for pigeons. The two keys are available to the pigeon for T s with T varying randomly from trial to trial. If, at time T s in a trial, the pigeon pecks on the time-left side, then (a) the standard side becomes unavailable and (b) reinforcement is delivered on the time-left side C-T s later so that time-to-reinforcement from trial onset on the time-left side is always fixed at C s. On the other hand, if at time T s in a trial, the pigeon pecks on the standard side, then (a) the time-left side becomes unavailable, (b) the standard side key color changes (by contrast, the time-left side key color remains the same), and (c) reinforcement is delivered on the standard side S s later.

picks the standard side; if $f(C) - f(t) < f(S)$ (more likely later in a trial when t is large enough), the animal should pick the standard side.

Because of noise, the animal's actual choice will be, of course, not as clear cut, but the preference of the animal for the time-left side should increase as a function of time in a trial, which is indeed what is observed (**Figure 8**). Moreover, when $f(C) - f(t) = f(S)$, the animal should be indifferent between the time-left and the standard side. $f(C) - f(t)$. Different hypotheses about the representation of time (linear vs. logarithmic) predict different locations of this point of subjective equality. Additionally, according to Gibbon and Church (1981), if the representation is logarithmic, the location of the point of subjective equality should be determined by the C/S ratio: if $C = 30$ s and $S = 20$ s, the point of subjective equality will be the same as if $C = 15$ s and $S = 10$ s. This highly counterintuitive prediction is, of course, not observed (Gibbon and Church, 1981), and although the point of subjective equality is not located precisely where it should be if time representation is linear (the animal switches to the time-left side well before it should), it is more compatible with this hypothesis than with Gibbon and Church's version of the logarithmic one (**Figure 8**).

There are both philosophical and empirical problems with the Gibbon–Church analysis (Staddon and Higa, 1999). Their argument relies on the postulate that the animal's behavior is based on mental subtraction and comparison between the various durations involved in the procedure, but this assumption has no empirical basis. If different assumptions are made, notably concerning the way subjective time maps onto preference (see Cerutti and Staddon, 2004a, and Dehaene, 2001, for two examples), results from the time-left procedure can readily be reconciled with a logarithmic (or other monotonic) representation of time.

At an empirical level, puzzling results obtained in some other explorations of the time-left procedure (e.g., Preston, 1994; Cerutti and Staddon, 2004a; Machado and Vasconcelos, 2006) have shown that the processes involved in it are far from clear, a conclusion that should not surprise given its complexity. As **Figure 7** shows, in a standard time-left with pigeons, the color of the time-left side key is constant, whereas the standard side key color changes

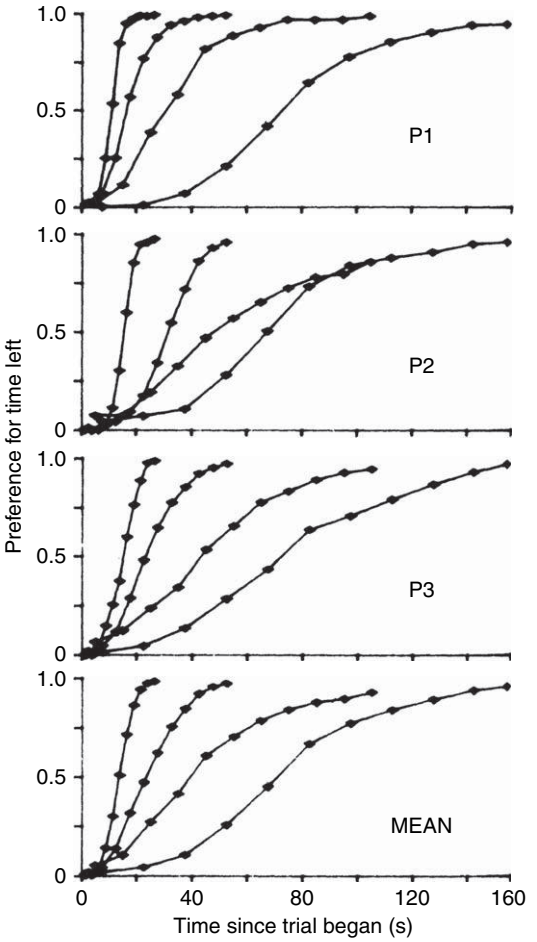

Figure 8 Results from Gibbon and Church's (1981) time-left procedure. The three top panels show individual subjects, whereas the bottom panel shows the group average. For each graph, the overall time-to-reinforcement on the time-left side (C) was (from the most leftward curves to the most rightward) 30, 60, 120, and 180 s. The duration of the standard side (S) was always half of the duration on the time-left side. Preference for the time-left side increases with time in a trial. From Gibbon J and Church R (1981) Time left: Linear versus logarithmic subjective time. *J. Exp. Psychol. Anim. Behav. Process* 22: 3–38. Copyright American Psychological Association. Reprinted with permission.

when the animal becomes committed to that side. Cerutti and Staddon (2004a) showed that this is the only configuration leading to an increase in the preference for the time-left side as time in a trial increases; every other variant (e.g., the color of the key changes on both sides or on the time-left side but not on the standard side or on neither side) leads to an exclusive preference for the time-left side – despite the fact that the temporal relations (the only ingredient in the Gibbon–Church analysis) are the same for all arrangements.

Machado and Vasconcelos (2006) found that if the animals are trained separately with the time-left and standard sides, their performance changes when the two schedules are put together in a concurrent schedule, a result not expected if the animal was basing its behavior on the comparison of the temporal intervals involved in the experiment (the performance should be perfect from the start in this case).

Finally, in a simplified variant of the time-left procedure, Cerutti and Staddon (2004a) found that pigeon performance was strongly influenced by when the animal could be committed to a schedule. In their study, $C = 60$ s and $S = 30$ s. The pigeon could be committed to a schedule either anytime between 5 s and 55 s in a trial, anytime between 5 s and 30 s in a trial, or anytime between 30 s and 55 s in a trial. The pigeon's response allocation differed in each condition, a result that cannot be reconciled with the idea that the animal's behavior is controlled by a set of mental comparisons between the various intervals used in the experiment. Before we know more about the actual controlling variables in the time-left procedure, no definitive conclusion can be drawn from it regarding the shape of the representation of time.

1.06.3.3 Beyond Psychophysics

There is currently no proof that interval timing relies on an internal pacemaker. Actually, the whole framework for the study of interval timing may need some revision. First, the emphasis on Weber's law needs to be reevaluated, as many studies have now reported what look like violations of it (e.g., Dreyfus et al., 1988; Stubbs et al., 1994; Zeiler and Powell, 1994; Crystal, 1999; Bizo et al., 2006), although this needs to be considered carefully. A performance might appear to violate Weber's law, not because the underlying timing mechanism does not follow it but because processes other than timing influence the performance (e.g., Jozefowiez et al., 2006).

But beyond Weber's law, the whole psychophysical approach, the idea that interval timing relies on some kind of 'knowledge' the animal has of the intervals involved in the experiment, may need to be abandoned. In the peak procedure, the animal is usually first trained on an FI for several sessions before peak trials are thrown in. As we saw, during those peak trials, their response-rate pattern evolves according to a kind of bell-shaped function that peaks at the time of reinforcement. But this behavior

is observed only after several sessions where the animal is exposed to a mix of reinforced FI trials and unreinforced peak trials. The first time a peak trial is introduced, the animal just keeps on responding until the end of the trial, apparently never noticing that the time of reinforcement has passed (an example of something studied many years ago as the reinforcement-omission effect, Staddon and Innis, 1969; Kello, 1972). This is a surprising result if we assume, as most theories of timing do, that the time of reinforcement is precisely what the animal learns when exposed to an FI.

Even more damaging evidence comes from a recent series of experiments by Machado and collaborators (Machado and Keen, 1999; Machado and Pata, 2005; Machado and Arantes, 2006). They trained pigeons in two bisection tasks simultaneously. In one task, response R1 (i.e., pecking on a red key) is reinforced after a 1-s stimulus while response R2 is reinforced after a 4-s one. In the other task, response R3 is reinforced after 4 s, whereas response R4 is reinforced after a 16-s one. Hence, both R2 and R3 are associated with a 4-s stimulus, so according to the psychophysical approach, they should be equivalent. But they are not. If given the choice between R2 and R3 after a test stimulus is presented, the pigeons are not indifferent as they should be according to the cognitive view. Instead, they switch their preference from R2 to R3 as the duration of the stimulus is increased (Machado and Keen, 1999; Machado and Pata, 2005). If these same animals are exposed to a new bisection procedure, performance is stable from the start if R3 is reinforced after a 1-s stimulus and R3 after a 16-s stimulus but is greatly disturbed in the reverse case (Machado and Arantes, 2006; **Figure 9**).

These data cannot be reconciled with the traditional psychophysical/cognitive account of interval timing and suggest the involvement of a simpler associative process, such as the one described in Machado's (1997) Learning-to-Time model. Instead of the time associated with each response, pigeons in a bisection task would learn through reinforcement and extinction to (1) approach the key associated with the short-duration stimulus and avoid the one associated with the longer-duration stimulus early in a trial and (2) do the reverse (i.e., avoid the key associated with the short-duration stimulus and approach the stimulus associated with the longer-duration stimulus) late in a trial (Machado and Arantes, 2006). Hence, when given the choice between R2 and R3 in Machado and Keen (1999) and Machado and Pata's (2005) studies, the pigeons would behave as they have learned previously, approaching R3 early in a trial while avoiding R2 and doing the reverse later. This pattern of approach/avoidance behavior is perfectly compatible

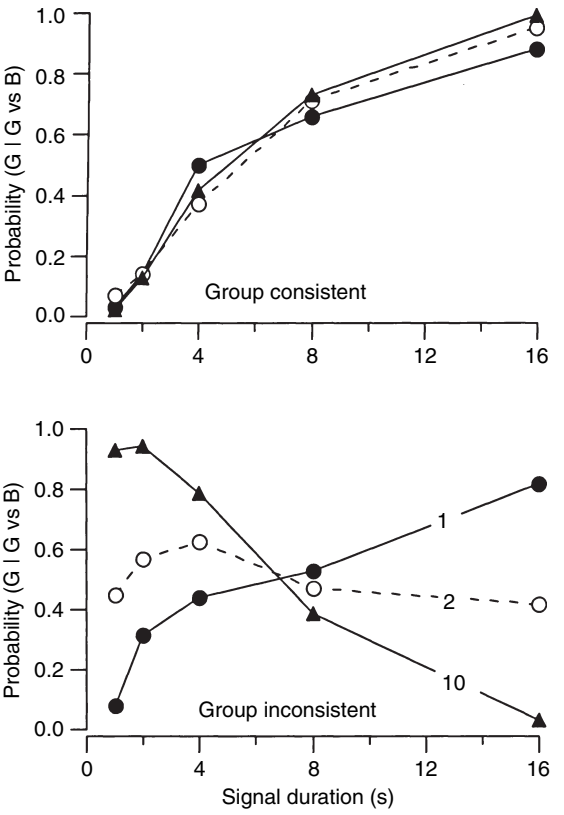

Figure 9 Group data from Machado and Arantes (2006). Pigeons were initially trained in two bisection procedures, one pitting a 1-s stimulus versus a 4-s one, the other pitting a 4-s stimulus versus a 16-s one. They were then trained in a third bisection task using the same two responses reinforced after a 4-s stimuli in the previous tasks. For the group whose data are shown in the top panel, the response previously reinforced after the 4-s stimulus pitted against a 1-s stimulus was now reinforced after a 16-s stimulus, whereas the response previously reinforced after the 4-s stimulus previously pitted against a 16-s stimulus was now reinforced after a 1-s duration. The y-axis of each graph shows the probability of emitting the response previously associated with a 4-s duration. For the second group, whose data are shown in the bottom panel, the reverse arrangement was used. Each curve in the graph represents the performance of the pigeons during the first (filled circles), second (empty circles), and tenth (filled triangles) sessions of the new bisection task. As can be seen, the pigeons' performance from the first group is stable from the first session. On the other hand, for the second group, performance takes several sessions to settle down. From Machado A and Arantes J (2006) Further tests of the Scalar Expectancy Theory (SET) and the Learning-to-Time model (LeT) in a temporal bisection task. *Behav. Processes* 72: 195–206, with permission from Elsevier.

with a new bisection procedure associating a 1-s stimulus with R3 and a 4-s stimulus with R2, and hence, the performance in this case is stable from the beginning. Otherwise, the pigeon has to relearn a new pattern of approach/avoidance behavior.

1.06.4 Operant Choice

1.06.4.1 The Matching Law

In a seminal experiment, Richard Herrnstein (1961) studied pigeons in concurrent VI VI schedules. He found that once behavior had stabilized, the ratio of response rates matched the ratio of reinforcement rates:

$$\frac{x}{y} = \frac{R(x)}{R(y)} \quad [1]$$

where x and y are response rates on the two keys, and $R(x)$ and $R(y)$ are the reinforcement rates obtained.

The matching law is a fairly general phenomenon. It has been found with several species, including, of course, rats but also humans (e.g., Bradshaw et al., 1976). Instead of having a schedule associated with each response key, an alternative, called a Findley procedure (Findley, 1958), is to have both associated with the same key but signaled by different colors that are under the animal's control. The animal switches between the two schedules by pecking a second key, called a change-over key. Matching is still observed in this case. When different feeding schedules are associated with different places (e.g., the two ends of a long box), animals allocate the time spent in the two places to the relative rate of associated reinforcement (Baum and Rachlin, 1969). Matching is also observed in trial-based procedures where the animal is only allowed one response per trial, which leads either to reinforcement or to a blackout (Sugrue et al., 2004; Lau and Glimcher, 2005).

Matching behavior is consistent with reinforcement-rate maximization in concurrent VI schedules (Staddon and Motheral, 1978; Baum, 1981), but large deviations from matching lead to only a small reduction in the obtained reinforcement rate, so it is unlikely that matching is the result of any kind of explicit maximizing process. Moreover, Equation (1) rarely fits experimental data exactly, which has led to the proposal of a popular alternative termed the generalized matching law (Baum, 1974; see also Staddon, 1968):

$$\frac{x}{y} = b\left[\frac{R(x)}{R(y)}\right]^a \quad [2]$$

where a and b are free parameters. Equation (2) can also be converted to logarithmic form, which has the advantage that the response-rate ratio remains a linear function of the reinforcement-rate ratio, allowing parameter b to be interpreted as bias and a as a measure of the sensitivity to the reinforcement rate ratio.

Violations of matching indicate that rather then being some kind of primary behavioral process (Herrnstein, 1970), matching is the outcome of more basic behavioral mechanisms. Unfortunately, determining these mechanisms can be difficult, as it seems that almost any model incorporating some form or the other of the law of effect predicts matching (Hinson and Staddon, 1983). Hence particular attention should be devoted to violations of the matching law as a way to limit the range of possible models.

Parameter b in the generalized matching law is a measure of any bias the animal might have toward one schedule. For instance, it can be systematically varied by using different kind of reinforcers for each schedule (e.g., wheat vs. buckwheat for pigeons; see Miller, 1976). The controlling variables for parameter a, on the other hand, remain more mysterious. Parameter a measures sensitivity to the reinforcement-rate ratio: When a is lower than 1, the animal is less sensitive to the reinforcement rate ratio than it should be according to the matching law (undermatching); when a is larger than 1, the animal is more sensitive to that ratio than it should be (overmatching). Reviews of the literature (e.g., Baum, 1979; Wearden and Burgess, 1982) have concluded that undermatching is more the rule than the exception, parameter a usually taking values between 0.8 and 0.9. Overmatching is seldom observed, except in very special situations (like concurrent VI FI schedules, Nevin, 1971; Trevett et al., 1972).

The causes of undermatching are not clear. The way the VI is scheduled (RI schedules lead to less undermatching, Taylor and Davison, 1983), the number of trials in a session (fewer trials lead to less undermatching; Todorov et al., 1983), and the number of conditions to which the subject has been exposed (more undermatching is obtained if the

subject has been exposed to more conditions; Keller and Gollub, 1977) are a few of the variables that have an effect. The last two variables seem to indicate an effect of interference in memory. Animals seem also to be more sensitive to reinforcement-rate ratio when the overall reinforcement rate is high (Alsop and Elliffe, 1988; MacDonall, 2006), a problem for the matching law, which implies that animals should be sensitive only to the ratio of reinforcement rates, not to their absolute values.

The variable most often cited as having an effect on the sensitivity to the reinforcement rate ratio is the presence or absence of a procedural arrangement known as a changeover delay (COD). COD works like this: When the animal switches from one schedule to the other (called a changeover), there is a small period of time (the COD, typically between 0.5 and 2 s) during which no reinforcement can be delivered. The rationale for a COD is to avoid reinforcement for a third class of behavior, switching between schedules. In the limit, switching could predominate over 'stay' choice responses, leading to apparent indifference between the two choices – extreme undermatching. According to this analysis, the main cause of undermatching would be the occasional reinforcement of switching behavior; indeed, adding a COD generally reduces the amount of undermatching (Shull and Pliskoff, 1967). On the other hand, a COD is not essential to obtain matching (e.g., Shull and Pliskoff, 1967; Heyman, 1979; Hinson and Staddon, 1983).

1.06.4.2 The Structure of Choice

Moreover, other studies have shown that some reinforcement of switching behavior is necessary to obtain matching in concurrent schedules. If pigeons are trained separately on two simple VIs and then allowed to choose between them in the usual concurrent procedure, they will respond exclusively on the VI associated with the higher reinforcement rate (extreme overmatching; Crowley and Donahoe, 2004; Gallistel and Gibbon, 2000).

This result has potentially far-reaching implications for theories of operant choice. It suggests that switching is reinforced when a switch to a schedule is followed by reinforcement (switch reinforcers), whereas staying on a schedule is reinforced by reinforcers collected while responding on that schedule (stay reinforcers; Skinner, 1950; Houston and McNamara, 1981; MacDonall, 1999). The final performance is the outcome of both stay and switch reinforcement. Consider, for instance, a concurrent VI 40 s VI 60 s. In this view, responding on the VI 40 s is reinforced according to a VI 40 s, but switching over from the VI 40 s is reinforced according to a VI 60 s.

This view is all the more plausible given that the tendency for an animal to switch between schedules is a function both of the probability that a switch is followed by reinforcement and the duration of the COD (e.g., Shull and Pliskoff, 1967; Pliskoff, 1971; Shull et al., 1981; Shahan and Lattal, 1998). Shull et al. (1981) also showed that a reinforcer delivered just after a changeover response has a massive impact on the rate of switching back and forth between the schedules. Also, in contrast to concurrent VI VI, concurrent VR VR schedules do not generate matching but exclusive preference for the schedule with the higher ratio (Herrnstein and Loveland, 1975); a reinforcer will not be scheduled on a VR until the animal works on it and hence, switching behavior will never be reinforced. If responses on a VR are counted not only for the schedule the animal is currently working on but also for the other one (hence leading to the reinforcement of switching behavior), matching is observed (MacDonall, 1988).

Another study by MacDonall (2005), in which he explicitly manipulated the reinforcement contingency for switching behaviors, provides additional support. Rats were exposed (for example) to a concurrent VI 36 s VI 360 s. In one condition, the schedule worked exactly as a traditional concurrent VI VI: Reinforcement for staying on the VI 36 s (respectively on the VI 360 s) was collected according to a VI 36 s (respectively VI 360 s), whereas reinforcement for switching away from the VI 36 s (respectively VI 360 s) was collected according to a VI 360 s (respectively VI 36 s). In the second condition, reinforcements for both staying and switching from the VI 36 s (respectively VI 360 s) were scheduled according to a VI 36 s (respectively 360 s). MacDonall (2005) reported that the latter condition yielded strong deviation from matching – unexpected by traditional views of operant choice but perfectly understandable in terms of the stay/switch framework.

From this view, the important empirical question is to understand how run length (number of responses emitted on a key between a changeover to that schedule and a changeover to the other schedule) and dwell time (time elapsing between a changeover to a schedule and a changeover to the other schedule) are determined by schedule parameters. The analysis is made easier by the fact that, in concurrent VI

schedules, both of these dependent variables are distributed exponentially, indicating a constant probability through time of terminating a visit to a schedule (i.e., Heyman, 1979; Gibbon, 1995). Two contradictory accounts have been proposed.

The first one is by MacDonall (1998, 1999, 2000, 2005), who found that run length and dwell time on a schedule were power functions of the ratio of switch and stay reinforcers plus a bias, a kind of molecular generalized matching law. MacDonall (2006) also found some modulating effect of the overall reinforcement rate.

The second one can be inferred from an influential experiment on paradoxical choice by Belke (1992) in which pigeons were exposed to a concurrent VI 20 s VI 40 s and to a concurrent VI 40 s VI 80 s. Their behavior allocation conformed to matching in both conditions. On unreinforced probe trials, the pigeons were given the choice between the two VI 40 s, the one associated with the VI 20 s and the one associated with the VI 80 s. Even though both schedules signaled the same reinforcement rate, the pigeons preferred the VI 40 s associated with the VI 80 s in a proportion of 4 to 1. Even more surprising, Gibbon (1995) showed that, if given the choice between the VI 20 s and the VI 40 s usually presented along with the VI 80 s, they preferred the VI 40 s to the VI 20 s in a proportion of 2 to 1.

These results have a surprisingly simple explanation (Gallistel and Gibbon, 2000): They can be predicted if we simply assume that the probability to leave a schedule is a linear function of the reinforcement rate on the other schedule, that is to say, the rate of switch reinforcement. Indeed, in Gibbon's (1995) experiment, the dwell-time distribution on the VI 20 s is identical to the dwell-time distribution on the VI 80 s (Gallistel and Gibbon, 2000); both were pitted against a VI 40 s.

Further research is necessary to decide between MacDonall's account and the one that incorporates Belke's results. MacDonall did not directly measure run length and visit duration but deduced them from the number of responses made on a schedule and the number of changeovers to that schedule, which might not provide accurate estimates. On the other hand, it is surprising that stay reinforcers would have no effect on performance, although that could be explained by the fact that most of the reinforcers in concurrent VI schedules are collected just after a changeover (i.e., for switching) (Dreyfus et al., 1982). We also note the existence of a few other puzzling results (i.e., Williams and Bell, 1999; McDevitt and Williams, 2003), which suggest that the determinants of performance in procedures such as Belke's (1992) are still not completely understood.

1.06.4.3 Local and Global Control of Behavior in Concurrent Schedules

Experiments on the matching law are typical of most studies of operant choice where the focus is on stable, asymptotic behavior obtained after many sessions in which the animal is continually exposed to the same condition. But recently, there has been more interest in the study of choice in transition.

Davison and Baum (2000) exposed pigeons to a concurrent VI schedule where, during a single session, the reinforcement-rate ratio changed after a fixed number of reinforcers (usually 10). The change was signaled by a 10-s blackout. Averaging across conditions and subjects, they were able to show that each reinforcer had an effect on choice, pushing the animal's preference toward the reinforced side. The effect of successive reinforcers on the same side had a diminishing effect on performance, whereas a 'disconfirming' reinforcer, for a switch response, on the other side had a stronger effect in pushing preference toward its side (**Figure 10**). This result has now been replicated in several studies, some using rats (Aparicio and Baum, 2006) or more traditional concurrent-schedule procedures where the reinforcement rate ratio does not change several times during the same session (Landon et al., 2002). This suggests some kind of surprise-driven learning mechanism similar to the one described in the Rescorla and Wagner (1972) model of Pavlovian conditioning: Behavioral change occurs when unexpected events take place in the environment.

Another interesting finding is that this local effect of reinforcement on behavior is modulated by more global variables. For instance, Davison and Baum (2000) reported that the change in behavior following reinforcement was larger when the overall reinforcement rate was high, leading to a more extreme preference for the schedule associated with the higher reinforcement rate (**Figure 10**). This is similar to Alsop and Elliffe's (1988) finding that sensitivity to the reinforcement rate ratio is higher when the overall reinforcement rate is higher. In other words, control of performance was more local with the higher reinforcement rate, with the last reinforcement delivered to the animal having a more important influence on performance in this case.

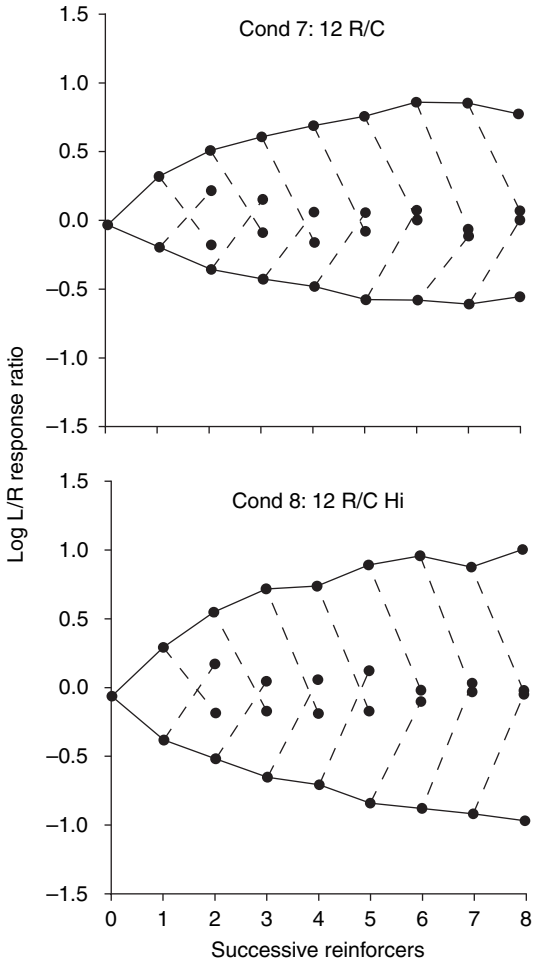

Figure 10 Effect of individual reinforcers on preference in concurrent VI schedules. Each reinforcer drives preference toward its schedule, although each successive reinforcer has a diminishing effect on behavior. The overall reinforcement rate was higher for the data presented in the bottom panel: The effect of individual reinforcers is more important in that condition than in the other. From Davison M and Baum WM (2000) Choice in a variable environment: Every reinforcer counts. *J. Exp. Anal. Behav.* 74: 1–24, with permission from the Society for the Quantitative Analysis of Behavior, Inc.

The best-documented global influence on the local control by reinforcement is the effect of overall environmental variability: When animals are submitted to the same experimental condition for many sessions, their behavior tends to change slowly when this condition is changed, whereas, on the other hand, when the environment is highly unstable, with experimental conditions changing often and unpredictably, they show more immediate behavioral change in response to a modification of the environment, with less carryover effect from past conditions (e.g., Davis et al., 1993; Mazur, 1997; Schofield and Davison, 1997; Gallistel et al., 2001; see Staddon and Frank, 1974, for similar effects in multiple schedules). Other variables that have an influence on the local effect of reinforcement are the range of variation in the reinforcement-rate ratio (Landon and Davison, 2001; the more distinct the two schedules, the more local the control of behavior) and in the location of reinforcement (Krageloh et al., 2005). If reinforcement is alternating between the two schedules, behavioral control is more local than if several reinforcers are collected in a row on the same schedule.

1.06.5 Reinforcement Theory

Through operant learning, animals are able to learn about the consequences of their behavior. If these consequences have reinforcing value, they will be able to modulate the emission of the behavior that caused them. But not every event is a reinforcer. What, then, makes a reinforcer reinforcing? The answer depends on whether we are considering primary reinforcers (whose effect on behavior seems to be largely innate) or conditioned reinforcers (whose effect on behavior is due to the fact that they signal the delivery of a primary reinforcer; *See also* Chapter 1.03).

1.06.5.1 Primary Reinforcement

An accurate summary of our current knowledge of why primary reinforcers are reinforcing is still Skinner's (1953) definition of a reinforcer, which all in all boils down to "a reinforcer is something that reinforces behavior." In other words, we recognize a primary reinforcer through its effect on behavior, but we are hardly able to explain why it has such an effect. This is fine if your main interest is how a reinforcer modulates behavior, which indeed is the main topic of operant research, but is more problematic if you want to know why a reinforcer modulates behavior, a legitimate question, especially in an applied setting. The elaborate theories of primary reinforcement developed during the heyday of general learning theory in the 1950s (such as Hull's 1943 drive-reduction theory) have not stood the test of time but have not been replaced. Little more than commonsense generalities can be said about primary reinforcers. Primary reinforcers affect behavior presumably because they contribute to the Darwinian

fitness of the organism (Skinner, 1966), but this is little help in identifying them. The fact that food, water, and sex are powerful primary reinforcers seems to indicate that they are linked to basic biological needs. But this is not very helpful either, as this kind of explanation becomes quickly circular. Wheel running is reinforcing in rats (i.e., Belke and Heyman, 1994; Belke, 1997) just like the opportunity to observe complex stimuli is in monkeys (Butler and Harlow, 1954; Butler and Alexander, 1955). Do we have to postulate a wheel-running drive in rats and an observation drive in monkeys? Does this add anything to our understanding of the reinforcing effect of wheel running or observation?

The most creative attempt to address the problem of primary reinforcement derives from Premack's (1962, 1965) proposal that behavior with a high probability of emission can be used as a reinforcer for behavior with a lower probability. Hence, feeding, a behavior with a high probability of emission in a hungry animal, can be used to reinforce lever pressing or key pecking, behaviors with a low probability of emission. Although the theory does not tell how the probability of emission of a behavior is set, it is an improvement over explanations of primary reinforcement in terms of biological needs because probabilities can be measured prior to conditioning.

In one experiment, Premack (1965) measured the preference of children for playing pinball versus eating candy. He then showed that making eating candy contingent on playing pinball reinforced this activity only in children preferring candy over playing pinball, whereas it had a punishing effect for the other children, and vice versa. In another study (Premack, 1962), rats had a restricted access to wheel running but unlimited access to water in one condition, whereas the reverse was true in another condition. Access to wheel running was an effective reinforcer for water drinking in the first condition, whereas water drinking was an effective reinforcer for wheel running in the second condition. As a last example, Chalop et al. (1990) found that echolalia and perseverative behavior, activities with a high probability of responding in autistic children, were very efficient reinforcers for these subjects, whereas food reinforcement had no effect.

Premack's view has been developed into behavioral-regulation models of operant performance (Timberlake and Allison, 1974; Rachlin, 1974; Allison, 1993; Staddon, 1979, 2001, 2003/1983). In these views, the animal has a preferred allocation of behavior, which works as a behavioral bliss point. By making access to one behavior contingent on the omission of another, the animal is forced away from the bliss point. Behavior then adjusts to restore the system as close to the bliss point as possible. For instance, a hungry rat would prefer to spend most of its time eating and only a small portion of its time pressing a lever. By creating a contingency between pressing a lever and food delivery, an operant procedure does not allow the rat to settle on this preferred distribution of activity and forces it to increase its rate of lever-pressing to increase its rate of eating so that it is closer to the preferred allocation of behavior than it would be if it did not press the lever.

In contrast to Premack's theory, behavioral regulation makes the counterintuitive prediction that in some circumstances, a behavior with a low probability of responding may reinforce a behavior with a high probability of responding (e.g., Eisenberg et al., 1967; Mazur, 1975). Staddon (1979, 2003/1983) showed how basic data for simple schedules could be accounted by the bliss-point model. It can also be shown that this approach leads to a view of operant behavior equivalent to the classical microeconomic theory of consumer demand (i.e., Staddon, 2001, 2004/1983) and has favored the development of an economic approach to operant performance (Hursh, 1984).

Behavioral regulation is an original and elegant approach to the problem of primary reinforcement. It is radical in the sense that it denies what is implicit in most treatments of operant behavior – that there are special events (i.e. reinforcers and punishers) that have the power to modify behavior. This is actually a problem because it is hard to see how to integrate a behavioral regulation account of operant performance with the causal analysis we presented in the previous part of this chapter. How can we, for instance, interpret the effect of the delay of reinforcement and of the predictive value of the response, data that make a lot of sense within a more traditional associative account of operant learning, within the framework of behavioral regulation? The Irish mathematician Hamilton showed that the laws of Newtonian physics, the very model of causation, could be expressed as an optimality model. It is possible that this is the case also for operant behavior. Even though behavioral regulation looks like a model of the causality of operant behavior, the real mechanism would be the more traditional strengthening and weakening of behavior by reinforcement and punishment postulated since Thorndike's original formulation of the law of effect, even though the effects of this mechanism can often

be predicted by an optimality model such as behavioral regulation. In this case, we should expect behavioral regulation to fail when animals are exposed to very unusual reinforcement schedules, which is exactly what was observed in a study by Ettinger et al. (1987), for instance.

1.06.5.2 Conditioned Reinforcement

In contrast to primary reinforcers, the question of why conditioned reinforcers affect behavior is less problematic: It is because they signal primary reinforcement. Learning with delayed reinforcement is much aided if a conditioned reinforcer is used to bridge the delay, as in the experiment by Williams (1999) discussed earlier. The question, then, is how do parameters of primary reinforcement determine the effectiveness of a conditioned reinforcer? The procedure usually used to address this question is the concurrent-chain schedule we described previously (**Figure 2**). The basic assumption is that response allocation during the initial link follows the matching law (i.e., the response-rate ratio during the initial link matches the conditioned reinforcement rate ratio). Hence, the response-rate ratio during the initial link is supposed to be a measure of the preference of the animal for one terminal link over the other.

When terminal links are both variable schedules (VI or VR), the response-rate ratio during the initial link sometimes matches the rate of primary reinforcement during the terminal links (Herrnstein, 1964b), suggesting that the value of a conditioned reinforcer could simply be equal to the rate of primary reinforcement in its presence. But, when given the choice between a variable schedule (VI or VR) or a fixed schedule (FI or FR), animals are strongly biased toward the variable alternative, even when they arrange identical reinforcement rates (Herrnstein, 1964a; Killeen, 1968; Davison, 1969, 1972; Mazur, 1986). They are indifferent between the two only when the reinforcement rate is computed using the harmonic mean of the various intervals used in the schedule instead of the arithmetic mean, as it is usually done. This is consistent with data collected in behavioral ecology showing preference for variable delay to food over fixed delay to food and relative indifference between variable and fixed amount of food (Staddon and Innis, 1966; Bateson and Kacelnik, 1995; Kacelnik and Bateson, 1996). This preference for VI over FI could be explained by assuming that the value of a conditioned reinforcer is an amount-dependent hyperbolic function of the delay to primary reinforcement it signals (Mazur, 2001).

This would also account for data from self-control procedures where animals are given the choice between a small immediate reward and a larger delayed one. The classical result (Ainslie, 1974; Green et al., 1981; Laibson, 1997; Green and Estle, 2003 for data in rats, pigeons, and humans) is that, for short delays, the animal will pick the smaller reward (impulsive behavior) while, for long delays, it will pick the larger reward (self-controlled behavior). This choice pattern emerges automatically if we assume that choice between delayed reinforcers is based on their value and if these values are an amount-dependent hyperbolic function of the delay of reinforcement. As **Figure 11** shows, for long delays, the discounted value of the larger reward is higher than the value of the smaller reward, hence the animal displays self-control; on the other hand, for short delay of reinforcement, the value of the smaller reward is larger than the value of a larger reward, and the animal is impulsive (Rachlin, 1974; Ainslie, 1975; Herrnstein, 1981).

The best evidences for hyperbolic discounting come from studies using an adjusting procedure. The initial link is an FR 1 instead of an RI, so that the first response moves the animal to the terminal link. The delay and amount of reinforcement are held constant at one terminal link. If the subject chooses this link, either the delay (in the adjusting-delay procedure) or the amount (in the adjusting-amount procedure) of reinforcement available at the

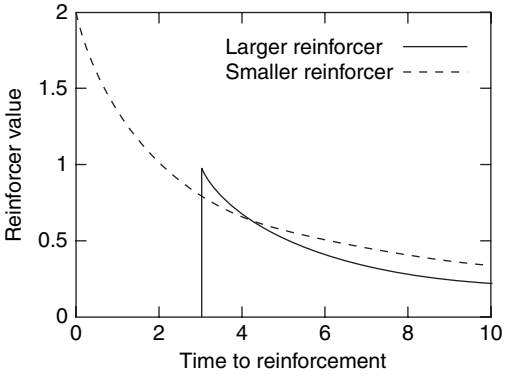

Figure 11 If a reinforcer value is an amount-dependent hyperbolic function of the time to reinforcement, the animal's preference for a large delayed reward over a smaller more immediate one will decrease and will eventually reverse as the time of the smaller reward delivery approaches.

other initial link is reduced (in the case of the delay) or increased (in the case of the amount). The reverse takes place if the subject chooses this terminal link. The goal is to determine a point of subjective equality where the animal is indifferent between the two terminal links. At this point, the values of the two conditioned reinforcers are supposed to be equal. If delay is manipulated, hyperbolic discounting predicts that, at indifference, the delay on the adjusting side will be a linear function of the delay on the constant side. The same way, if the amount is manipulated, hyperbolic discounting predicts that the amount on the adjusting side will be a hyperbolic function of the amount on the constant side. Both predictions have been confirmed empirically (Mazur, 1987, 2000; Rodriguez and Logue, 1988; Richards et al., 1997; Green et al., 2004).

All this research relies on the assumption that choice during the initial link is a function only of the values of the terminal links. But it is not; the longer the initial links, the less extreme the preference for the terminal links, reaching almost indifference with extremely long initial links (Fantino, 1969; Squire and Fantino, 1971; Fantino and Davison, 1983). This indicates that either choice is influenced by variables besides the values of the terminal links and/or that the value of a terminal link is context-dependent. Both kinds of influences have been incorporated in modern theories of conditioned reinforcement (i.e., delay reduction theory, Fantino, 1969; Squire and Fantino, 1971; hyperbolic value-added model, Mazur, 2001; contextual model of choice, Grace, 1994, 1996). All those models are equivalent in terms of their ability to fit the data.

Another interpretation of the surprising fact that a conditioned reinforcer value seems to depend on the context is to question the very notion of conditioned reinforcement and the usual assumption made about the control of behavior in concurrent chain schedules. This is exactly what Staddon and collaborators (Staddon and Ettinger, 1989; Staddon and Cerutti, 2003; Cerutti and Staddon, 2004a,b; Staddon, 2003/1983) have done by proposing that interval timing underlies most effects attributed to conditioned reinforcement. Their analysis is based on some results in simple chain schedules that are puzzling for a conditioned-reinforcement account. For example, no more than six FI or FR links can be linked together in a chained schedule without extreme pausing developing in the early links – leading to a dramatic drop in the obtained reinforcement rate (Kelleher and Gollub, 1962). Weak responding during the early links is sometimes observed with as few as three schedules chained together (Catania et al., 1980; Davison, 1974), and extreme pausing can develop even in a three-link chain schedule if the time from trial onset to primary reinforcement is long enough (Kelleher and Fry, 1962). On the other hand, if the change from one link to the other is not signaled by a stimulus change (tandem schedule) or if the stimuli used to signal such transitions change randomly after each trial (scrambled chain schedule), the animals have no difficulty mastering these tasks and achieve close to the maximum possible reinforcement rate (Kelleher and Gollub, 1962).

These results are surprising because stimulus changes in a chain schedule are supposed to act as conditioned reinforcers, so should aid performance, not disrupt it. Another surprising result is that it does not make any difference if the stimulus change from one link to the other is response-dependent or not (Catania et al., 1980), whereas we saw previously that an actual response-reinforcer contingency (as opposed to mere contiguity between the emission of the response and reinforcement) is critical to operant learning.

Based on these data, Staddon and collaborators proposed that stimuli in chain schedules work not as conditioned reinforcers but as time-markers. Hence, they lead the animal to pause an amount of time proportional to the time between the onset of the stimulus and primary reinforcement. This accounts for the excessive pausing observed in chain schedules (Staddon and Ettinger, 1989; Staddon and Cerutti, 2003). Supporting this view, Staddon and Cerutti (2003) showed that pause duration in the first link of a two-chain schedule was a linear function of time to primary reinforcement in data from Davison (1974). Innis et al. (1993) studied two-link chain schedules with one link of fixed duration and the other varying from reinforcer to reinforcer according to an ascending then descending sequence. Pauses in each link tracked their respective time to primary reinforcement with a lag of one interval.

Applied to concurrent chain schedules, this view considers preference for terminal links during the initial link to be an artifact of interval timing. Consider, for instance, a concurrent chain schedule with FI 15-s and FI 30-s terminal links (**Figure 12**). The animal will pause longer on the initial link leading to the FI 30 s because the time-to-reinforcement is longer on that side. Hence, the animal will respond more on the initial link leading to the FI 15 s,

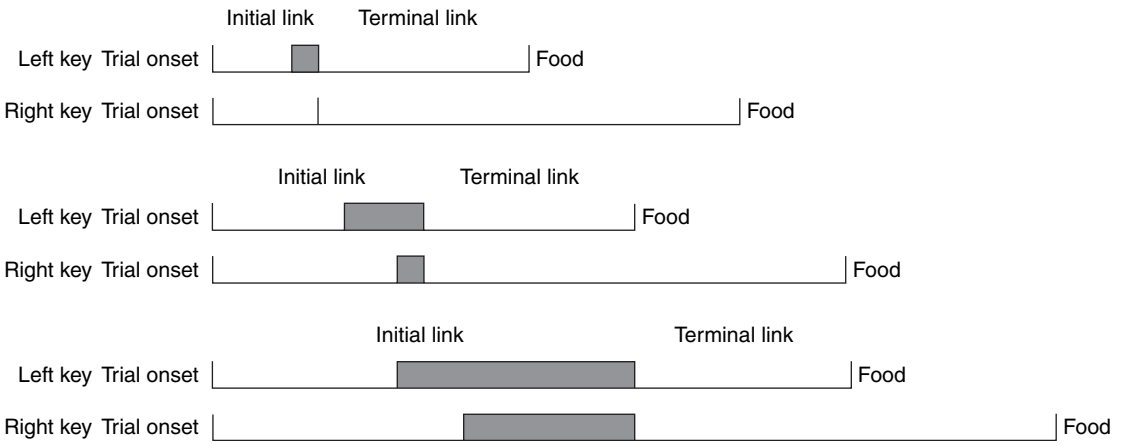

Figure 12 Interval timing account of concurrent chain performance. The animal pauses on an initial link for an amount of time proportional to the time between the onset and the initial link and primary reinforcement. As a consequence, it looks as if it prefers the initial link leading to the terminal link with the higher reinforcement rate. As the initial link duration is increased, the difference in pausing makes less and less of a contribution to the reinforcement rate ratio, and preference converges on indifference.

which will look like a preference for the FI 15-s initial link. Note that as the initial link duration is increased (while the terminal-link duration remains the same), pausing takes up a smaller fraction of the total first-link time so that the time available for responding becomes more equal on the two sides (**Figure 12**), eventually reaching indifference – as the data show. This analysis also explains the preference for variable over fixed terminal-link schedules because variable time-to-reinforcement leads to shorter pausing than fixed. Finally, Staddon and Ettinger (1989) and Staddon and Cerutti (2003) showed how this timing view of concurrent performance can account for hyperbolic discounting and data on self-control (the equations derived from the timing view turn out to be identical to those derivable from hyperbolic discounting).

More research will be needed to conclude if the timing approach of Staddon and collaborators is a better account of concurrent-chain performance than more traditional explanations in terms of conditioned reinforcement, but strong support for it can be found in a study by McDevitt and Williams (2001). They used terminal links associated with a 5-s and a 15-s delay-to-reinforcement. In one condition, each initial link was correlated with a different stimulus (as in standard concurrent-chain schedules), whereas the same stimulus was associated with both of them in another condition. A conditioned reinforcement approach would lead one to expect no differential responding during the initial link in the single-stimulus terminal-link condition because the consequences of responding are identical for both choices. But McDevitt and Williams (2003) actually found the same strong preference for the 5-s terminal link in both conditions, concluding that time-to-reinforcement, not conditioned reinforcement, was the main controlling variable.

1.06.6 Conclusion

Operant behavior involves adaptation to the consequences of responding; it is the prototype of adaptive behavior during the life of the individual – the ontogenetic equivalent of Darwinian natural selection in phylogeny. Techniques for exploring operant behavior exploded with the invention of the Skinner box and the discovery of the orderly and powerful effects of schedules of reinforcement. In the 1950s and 1960s, much research addressed the limits of operant conditioning and in particular the intimate relationship between the processes underlying operant and classical conditioning. In recent decades, two research areas, interval timing and choice, have dominated the field. We have reviewed these topics and, in addition, discussed post-Skinner developments in the economics of operant behavior. We hope, with this chapter, to have aroused the interest of the reader for this fascinating and lively field of behavioral science.

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1.07 Perceptual Learning

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1.07.1 Introduction

It is not the aim of this chapter to provide a review of all the work that has been published in recent years under the heading of perceptual learning. The aim is more specific and is guided in part by theoretical considerations; it is to set perceptual learning in the context of what we know about learning more generally – to assess the extent to which the phenomena of perceptual learning can be explained in terms of known learning processes, to identify aspects of perceptual learning that cannot be explained in this way, and to attempt to outline the nature of any new learning process that may be required.

Nonetheless, the first major section of the chapter will offer a selective review of what may be considered to be the most important recent (and some not-so-recent) experimental findings. This is necessary not only to provide the grist for the explanatory mill of later sections, but also to allow us to define the field. As we shall see, the range of phenomena that have been studied under the heading of perceptual learning is exceedingly broad. However, it is possible to discern a set of important issues that is common to most of them and that constitute the core features of perceptual learning. The attempt to explain – to understand the psychological mechanisms responsible for – these core features is dealt with in the next two major sections of the chapter. The first of these deals with the application of the principles of associative learning theory to perceptual learning effects; the second is concerned with the role of nonassociative (principally attentional) learning processes.

1.07.2 Phenomena

All the studies to be described in this section have been regarded, by their authors or by later commentators, as involving perceptual learning. The variety is impressive. All major sensory systems have been subject to study; vision perhaps predominates, but there are also many studies of hearing, touch, olfaction, and taste. The stimuli used have varied, from the apparently simple (e.g., a touch with a pointer), to the undeniably complex (e.g., pictures of human faces). The stimuli used have often been difficult to discriminate from one another – for simple stimuli because they are chosen to lie close together on the dimension of difference; for complex stimuli, largely because of the presence of a host of irrelevant, nondistinguishing features in each of the displays. But perceptual learning has also been investigated in

experiments using stimuli that, on the face of things, seem readily discriminable one from another – one of the earliest and most influential studies in nonhuman animals (Gibson and Walk, 1959) looked at the effects of training with two simple geometrical shapes, a triangle and a circle. This example serves to make the further point that relevant phenomena have been studied in a range of species – our own species is best fitted for psychophysics, but experiments on laboratory animals have some advantages when it comes to investigating basic mechanisms of learning. Finally, although all the work to be considered concerns the effect of experience with stimuli on the subject's subsequent response to them, the exact form of the experience given has been varied – notably some experimenters have been concerned with the effects produced by mere exposure to stimuli, whereas others have given explicit training with feedback (also called knowledge of results, or reinforcement).

In an attempt to impose some order on this apparent chaos, the sample of experimental work reviewed next is organized under a set of convenient headings. It will be evident, however, that these do not form exclusive categories and that many of the experiments described could legitimately be placed under more than one of the headings (*See* Chapters 1.08, 1.13).

1.07.2.1 Simple Sensory Thresholds

The study of perceptual learning appeared early in the history of experimental psychology. No sooner had psychophysics been established as a coherent enterprise than its proponents began to study the effects of experience on the sensory threshold measures that were one of its primary concerns. In 1859 A. W. Volkmann (Fechner's brother-in-law; Woodworth and Schlosberg, 1954) published a study of the effects of practice on the two-point tactile threshold (Volkmann, 1859). He found that with practice, the ability of a subject to make the discrimination – two points or one – was dramatically increased; that after a hundred or so trials, the minimum distance required for a judgment of two points was reduced by about half. The effect was limited to the general area of skin on which the stimuli had been applied, except that positive transfer was observed when the test was carried out on the equivalent region of the other hand or arm.

We can say straightaway, on the basis of these results, that the conventional title for studies of this sort is potentially misleading. Although the stimulus may be simple, the mechanism responsible for the change in threshold is not. The fact that training failed to transfer to an adjacent patch of skin on the same arm indicates that the improvement is not a consequence of some general learning process (such as might result simply from familiarization with the procedure). But the fact that it did transfer to the other limb indicates that it is not a consequence of some change in the particular receptors that were stimulated in training – a more central mechanism must be involved.

Modern studies of difference thresholds have confirmed, for a range of stimuli, the essence of Volkmann's (1858) findings. Practice at the task (usually some version of a two-alternative forced-choice task in which the subject is presented briefly with two events, one after the other, and has to say whether they are the same or different) will produce a reduction in the magnitude of the difference that can be reliably detected. This is true for auditory frequency (e.g., Demany, 1985), for the orientation of visually presented lines (Shiu and Pashler, 1992), for hyperacuity (the ability to judge whether or not two line segments are colinear, e.g., McKee and Westheimer, 1978), for complex sinusoidal gratings (Fiorentini and Berardi, 1980), for the direction of motion of an array of moving dots (Ball and Sekuler, 1982; Liu and Weinshull, 2000), for the discrimination of visual texture (Karni and Sagi, 1991), and for many other tasks (see Fahle and Poggio, 2002). In most of these studies, the subjects were given feedback during training, but its role in producing the effect is unclear. Shiu and Pashler (1992) included subjects given no feedback, and these showed no within-session improvement, but this procedure was still capable of producing learning, as the subjects showed an improvement from one training session to the next.

As for Volkmann, the extent to which the effects of training transfer to other stimuli has been a focus of interest for modern experimenters. The intention has been to identify the stage in the perceptual system at which the training has had its effects – for instance, training that produces effects specific to the area of the visual field to which the stimuli were presented points to processes occurring at an early stage in the visual system, where retinotopic organization is still maintained. And, as was true for Volkmann's study, the pattern of transfer has turned out to be far from simple. For most of the visual tasks described, a degree of retinal specificity has been obtained, with performance falling to the starting

level when the stimuli were presented in a different retinal location. But this does not mean that no transfer occurred – both Liu and Weinshull (2000) and Shiu and Pashler (1992) found that the new task was learned more rapidly than the original. And Ahissar and Hochstein (1997), using a version of the texture discrimination task of Karni and Sagi (1991), found transfer to a new retinal location when the initial training had been given on an easy version of the task (Liu and Weinshull, 2000, obtained a similar result for direction of motion). In other cases, the effect of training has proved specific to the particular stimuli used, but not to the receptors stimulated. Demany and Semal (2002) found that the effects of auditory training transferred readily to the other ear but not to a new discrimination involving a different frequency range (see also Amitay et al., 2005). And to return to the topic of tactile discrimination with which we began, Sathian and Zangaladze (1997) have demonstrated that the effects of training on a discrimination of the roughness of a pattern of ridges will transfer to a finger other than that used in training, but not to a new task in which the orientation of the pattern must be judged. The only conclusion justified by these observations at this stage is that although practice can facilitate performance on these simple discriminations, it would be foolish to conclude that these examples of a perceptual learning effect are to be explained solely in terms of processes occurring very early in the sensory/perceptual processing system.

1.07.2.2 More Complex Stimuli

In a study directly inspired by those described in the previous section, Furmanski and Engel (2000) looked for a perceptual learning effect with more complex visual stimuli. These were degraded pictures of everyday objects (e.g., a telephone, a pencil sharpener; see **Figure 1**) presented very briefly. The subjects had to name the object and were told if they were right or wrong. Initially performance was poor, but training (800 trials a day over several days) produced a sizable reduction in threshold (in the exposure duration necessary for correct identification). This effect was specific to the pictures presented in training, but not to retinal location – transfer was good when the size of the image was changed. Enhanced discrimination when the stimulus set is familiar appears to be a quite general phenomenon. An example for a very different procedure (and species) is provided by Todd and Mackintosh (1990). Their subjects, pigeons, were presented with 20 pictorial slides in a training session, each being presented twice, in random order. They were rewarded for pecking at the first but not at the second presentation. The pigeons learned this discrimination, but did so less well when a new set of pictures was used each session than when the same set was used throughout. This result – better performance on a judgment of relative recency than of absolute novelty – may seem surprising, but it makes sense if we accept that prolonged experience with a given set of pictures will enhance the subject's ability to discriminate among them.

Figure 1 Two of the pictures of objects used by Furmanski and Engel (2000). To render discrimination difficult, the contrast was reduced to 12.5%, and each presentation was followed by the mask shown at the bottom of the figure. Used with permission.

Intriguing as the examples just cited may be, popular interest in the phenomenon of perceptual learning is most readily evoked by description of the special skills shown by experts in dealing with even more complex stimuli – the experienced radiographer who can detect a tumor on an x-ray where the rest of us see only a meaningless blur (Myles-Worsley et al., 1988) or the chicken sexer who can make a determination after inspecting the pinhead-sized genital eminence of the day-old chick for less than 0.5 s (Biederman and Shiffrar, 1987). These are abilities acquired through experience. Knowledge of results may play a role in this (if only because the chicken sexer will soon hear about it if a large proportion of the hens turn out to be cocks), and often there will be explicit training in which an established expert instructs the novice in what to look for (see Biederman and Shiffrar, 1987). And however exotic these specialized skills may seem, there are certain areas in which, even in the absence of explicit

instruction, almost all of us have acquired the status of experts – we are all of us experts in dealing with the visual cues responsible for face recognition and the auditory cues underlying our native language.

Despite occasional embarrassing mistakes, our ability to discriminate among people, largely on the basis of their facial characteristics, is impressive. That this ability depends on experience is indicated by the fact that children are less good at it than adults (see Chung and Thomson, 1995). Further evidence comes from the so-called own-race effect – superior discrimination when the faces belong to individuals from our own racial group (Malpass and Kravitz, 1969). This effect depends on the fact that we have (usually) had much more experience of such faces – the magnitude of the effect is much reduced in people who have had extensive interaction with races other than their own (Chiroro and Valentine, 1995). What might be thought as a parallel, own-language effect describes our ability to discriminate the speech sounds of our native language. Native English speakers readily distinguish (on the basis of differences in the third formant) between the phonemes /r/ and /l/, a task that native speakers of Japanese find exceedingly difficult (e.g., Goto, 1971). Japanese speakers, on the other hand, can make distinctions (according to changes in the second formant) within the category of sounds that English speakers regard as all being examples of /r/ (Iverson et al., 2003). Explicit training, in which Japanese subjects were given feedback after being required to distinguish between word pairs such as *lock* and *rock*, has been shown to enhance their discriminative ability (Iverson et al., 2005).

The examples discussed so far in this section have involved training in which feedback has been given; that is, the subjects have been told that their identification of a stimulus has been right or wrong. (This is explicitly arranged in most of the experimental studies, but something equivalent will occur in the natural environment as we learn the discriminations necessary for language or for the recognition of faces.) But perceptual learning effects can be obtained without such feedback, as a result of mere exposure to the stimuli (when this is arranged appropriately). Lavis and Mitchell (2006) required people to discriminate between pairs of checkerboards of the sort shown in **Figure 2**. When they are first presented with the one checkerboard followed, after a short interval, by another, people are poor at answering the question: Same or different? But performance was much enhanced by mere preexposure to the displays when this was organized such that the different stimuli were presented in alternation. Interestingly, preexposure, consisting of a block of trials with one stimulus followed by a block of trials with the other, was much less effective in enhancing subsequent discrimination. This outcome (to be discussed in detail later) is of interest as it accords with the influential analysis of perceptual learning offered by Gibson (1969), who emphasized the role of stimulus comparison in producing the effect (we may assume alternating preexposure is likely to foster the processes involved in comparison).

1.07.2.3 Categorization

The auditory discrimination described earlier is one that involves categorization; that is, faced with a range of different stimuli, the native English speaker learns to put instances of one set (which will differ

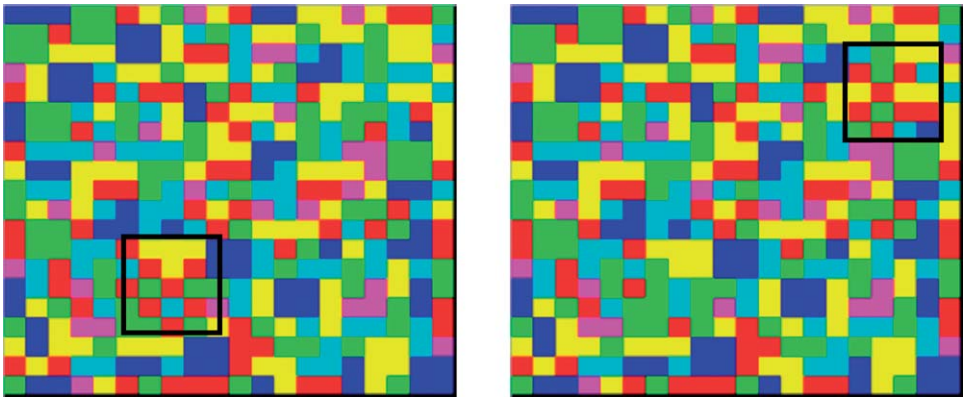

Figure 2 Examples of the colored checkerboard stimuli used by Lavis and Mitchell (2006). The unique elements are indicated (for the purposes of illustration only) by the black squares. Images courtesy of Y. Lavis.

among themselves in some respects) in one category (meriting /r/) and instances of another set in a different category (meriting /l/). The same is true of some of the visual tasks – one male chick will differ from another, but the experienced chicken sexer puts them all in the same category. An experimental demonstration of how such an ability can be acquired through experience comes from a study of face recognition by Quinn et al. (1999). In this experiment, the faces were pictures not of people, but of cats, and the participant's task was to sort them into two categories, male and female. The discrimination was difficult – initial performance was at chance level – but reinforced training with a subset of the pictures, those most easily identified as male or female, produced positive transfer to the ability to categorize other pictures.

Successful performance on a categorization task such as that used by Quinn et al. (1999) may seem to involve a reversal of the sort of perceptual learning effect that we have been concerned with so far – although discrimination between male and female is enhanced, the subjects appear to be less sensitive to differences among individuals that fall into a given category. But whether this is really the case requires explicit investigation. It is quite possible that the within-category discrimination was also enhanced – that, had they been asked, the trained subjects would have been better able to distinguish between Lucky and Widget, while still categorizing both as male. This is certainly true of human faces – we have no trouble in telling Ann from Zoe, and Andrew from Zach, while still distinguishing male from female.

The issue has been investigated experimentally, using complex artificial stimuli, by McLaren et al. (1994); see also McLaren, 1997). The stimuli used were based on the checkerboards shown in **Figure 3**.

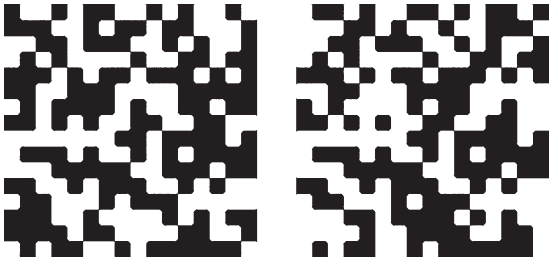

Figure 3 Checkerboard stimuli used by McLaren et al. (1994). Those shown were the prototypes. Discrimination was between exemplars, produced by changing a proportion of the elements of each prototype from black to white, or vice versa. Image courtesy of I. McLaren.

Changing a proportion of the elements from black to white, or vice versa, produced two sets of exemplars of these prototypes. Training consisted of a categorization task in which subjects learned to assign exemplars to type 1 or to type 2. In the test, two stimuli were presented side by side, and the subjects were reinforced for reliably choosing one rather than the other. They proved to be very good at this discrimination when the two stimuli were the original prototypes and also when the test stimuli were novel exemplars of these prototypes not used in the original training. But although the effect of preexposure was less profound in this case, the participants were also at an advantage when required to discriminate between two exemplars drawn from the same category. These effects are not confined to our own species. Aitken et al. (1996), using similar stimuli, generated essentially the same pattern of result in an experiment that used pigeons as the subjects.

1.07.2.4 Taste and Smell

Unusually among psychologists, students of perceptual learning have paid almost as much attention to the chemical senses as to vision and hearing. Perhaps this derives in part from the fact that some of the most dramatic examples of acquired perceptual skills are found in these modalities. Foremost among these are the well-documented achievements of expert wine tasters (e.g., Soloman, 1990), but who can forget William James's description of "the blind-deaf mute . . . Julia Brace [who] is said to have been employed in the Hartford Asylum to sort the linen of its multitudinous inmates, after it came from the wash, by her wonderfully educated sense of smell" (James, 1890, pp. 509–510).

These are obviously very special cases, but evidence that an approach to skills of this sort can be established in any of us comes from experimental studies. Thus, Peron and Allen (1988) found that novice beer drinkers, who were initially unable to tell one brand from another, became able to do so after training in which they simply sampled a range of beers and reflected on the flavor qualities that came to mind. (Training with the specialist vocabulary of master brewers conveyed no special advantage; see also Melcher and Schooler, 1996.) Rabin (1988), who asked subjects to make same/different judgments after sniffing two unusual odors, found that discrimination was enhanced when the subjects had been given prior exposure to the odors (and in this case, training in which a distinctive

label was attached to each odor during preexposure was found to help). Preexposure is not always beneficial, however. Stevenson (2001) gave training in which his subjects sniffed two odor mixtures (call them AX and BY). Subsequent discrimination between components of separate pairs (e.g., A vs. B) was good, but discrimination between components of a compound (e.g., A vs. X) was poor.

Studies of perceptual learning using animal subjects have made extensive use of tastes – adding a flavor to the drinking water of a thirsty rat ensures that the animal receives full exposure to the relevant stimulus, and the flavor-aversion conditioning technique provides an effective way of assessing discrimination. **Figure 4** shows the results of one study (by Symonds and Hall, 1995, Experiment 1) that made use of this procedure. All the rats received a test phase consisting of aversion conditioning with flavor A, followed by a test with flavor B (A and B were solutions of salt and sugar, rendered more similar by the addition of the sour taste of acid to each). In rats given no previous experience of the flavors (group W in the figure), the aversion conditioned to A generalized readily to B; that is, they failed to discriminate between A and B. The same was true of rats given prior exposure either to A or to B. But rats given prior exposure consisting of alternating presentations of A and B (group A/B in the figure) showed poor generalization (i.e., an enhanced ability to discriminate). As in the experiments by Lavis and Mitchell (2006), described earlier, this alternating

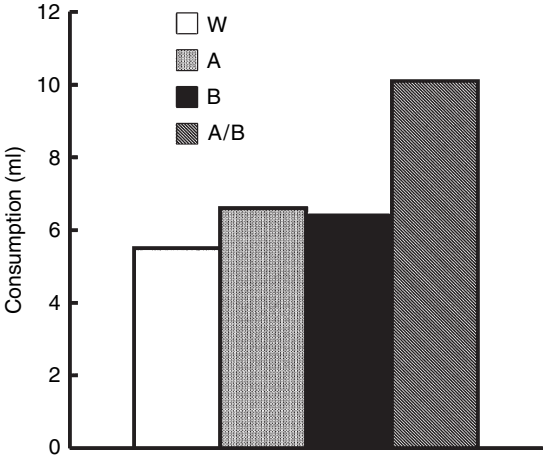

Figure 4 Group mean scores for consumption of flavor B, after aversion conditioning with flavor in the experiment by Symonds and Hall (1995). Before conditioning, different groups had received exposure to A, to B, to both (A/B), or just to plain water (W).

arrangement turned out to be critical. A subsequent study (Symonds and Hall, 1995, Experiment 3) showed that preexposure consisting of a block of A trials followed by a block of B trials (or vice versa) did not produce the same enhancement. It is interesting to note that closely parallel effects have been obtained with human subjects given initial training in which they tasted the compound flavors saline-lemon and sucrose-lemon either on alternating trials or on separate blocks of trials. Subsequent same/different judgments were found to be more accurate in those given the alternating schedule during preexposure (Dwyer et al., 2004).

1.07.2.5 Acquired Distinctiveness

The experiments discussed so far have commonly used a procedure in which the subjects received explicit discrimination training, with feedback or knowledge of results being given. But (as the authors of several of these studies have noted), it can often be difficult to be sure of the source of the improved discriminative performance that is obtained in these conditions. Is there a change in the way in which the stimuli are being perceived, or is the learning occurring at a later stage in the sequence of processes that connect input to response? Transfer studies in which the stimuli are presented to a different set of receptors (e.g., at a different retinal location) constitute one way of addressing this issue. It is usually assumed that effects that fail to transfer cannot be a consequence of some general learning process, but must be specific to the stimuli used in training. An alternative strategy is to retain the original stimuli, but to require the subjects to learn some new discrimination on the basis of them. In this case, we are looking for positive transfer. Such transfer could not be based on knowledge of general task requirements (these having been changed) but must be a consequence of some learned change in the properties of the stimuli.

This experimental design was first introduced in the classic study of animal discrimination learning reported by Lawrence (1949). In outline, rats were trained initially on a food-rewarded choice discrimination between black and white. They were then shifted to a new task involving the same cues but with a different response requirement – when given two black cues, they were required to choose the left (for example), and when given two white cues to choose the right. The responses acquired in the first stage (e.g., to approach black and avoid white) will be irrelevant in this new task; nonetheless, Lawrence

found positive transfer from stage 1 to stage 2, transfer, he said, that must depend on something that has been learned about the stimuli. The effect has been called the acquired distinctiveness of cues, the implication being that the initial training in which black and white were associated with different responses and different outcomes had rendered those stimuli more distinguishable. The idea that training in which cues are associated with differing outcomes will enhance their subsequent discriminability goes back at least as far as James (1890).

Lawrence's (1949) study was followed by a rush of similar transfer-of-training experiments, some with animal subjects (reviewed by Sutherland and Mackintosh, 1971) and many with human participants. The latter, for the most part, concentrated on a procedure in which participants learned to apply verbal labels in the first stage of training followed by a discrimination involving overt motor responses to the same stimuli in the second stage. It was usually found (see Hall, 1991, for a review) that stage-1 training facilitated learning of the second task, although whether or not this effect was a consequence of changes in the distinctiveness of the cues is open to debate. In many of the classic experiments (e.g., Battig, 1956; Gagné and Baker, 1950; Holton and Goss, 1956), comparison was made with a control condition given no stage-1 training, raising the possibility that the advantage shown by the experimental condition simply reflected some general facilitatory effect produced by the first stage of training. What is needed is to compare the effects of initial discrimination training with those of some control stage-1 procedure that will be equally effective in producing general transfer effects but that does not involve the consistent stimulus-outcome associations characteristic of the experimental condition.

One strategy, illustrated in a study by Goldstone (1994), is to use compound stimuli. In Goldstone's experiment, the stimuli were squares, differing in size and brightness. One aspect of the stimuli (e.g., their size) was irrelevant to the discrimination (based, e.g., on brightness) trained in stage 1. Thus, when it came to the test phase (involving either a further brightness discrimination or one based on shape), all subjects were familiar with the stimuli and had received discriminative pretraining. It was found that the test task was performed more readily by those subjects for whom the same dimension (brightness, in this example) was relevant in both stages. Analogous effects, which have been interpreted as reflecting an acquired enhancement of the distinctiveness of an entire dimension of stimulus variation, have been obtained in studies of animal discrimination learning (see, e.g., Mackintosh and Little, 1969).

An alternative strategy (also based on a design successfully used with animal subjects; see Bonardi et al., 1993) is presented schematically in **Table 1**. (At this point, we are concerned only with the first two stages shown in the table; the implications of Stage 3 will be taken up later). In this example, which comes from an experiment by Hall et al. (2003), people received stage-1 training with four different stimuli, four different geometrical shapes (A–D in the table). Two, A and B, were followed by one outcome (presentation of a red rectangle); two (C and D) by another outcome (a green rectangle). No overt response was required at this stage. Stage 2 consisted of a discrimination learning task in which the subjects had to learn to make one motor response rather than another to each of the shapes. Performance was good when the subjects were required to make different responses to stimuli that had been associated with different outcomes in Stage 1 (the consistent condition of the table) but was relatively poor in the inconsistent condition, when they had to make the same responses to shapes previously associated with different outcomes. Positive transfer in the consistent condition is what would be expected if cues associated with different outcomes had acquired distinctiveness.

It should be noted that with this experimental design (as with most others in this area; see Hall,

Table 1 Experimental design used by Hall et al. (2003)

Stage 1	*Stage 2*	*Stage 3*
Group Consistent		
A → red	A → left	
B → red	B → left	red → left/right?
C → green	C → right	green → left/right?
D → green	D → right	
Group Inconsistent		
A → red	A → left	
B → red	B → right	red → left/right?
C → green	C → left	green → left/right?
D → green	D → right	

Note: A, B, C, and D represent visual stimuli presented on a computer monitor; red and green refer to colored rectangles. Left and right refer to keyboard response required (left = backslash; right = forward slash). Feedback was given after responses in Stage 2. All subjects in a given group received all types of trial listed under a given stage of training. Source of data: Hall G, Mitchell C, Graham S, and Lavis Y (2003) Acquired equivalence and distinctiveness in human discrimination learning: Evidence for associative mediation. *J. Exp. Psychol. Gen*. 132: 266–276.

1991), it is possible that the effect derives, in whole or in part, from negative transfer in the inconsistent condition – that associating cues with the same outcome renders them less distinctive (an effect referred to as acquired equivalence; Miller and Dollard, 1941). But it should also be noted that acquired equivalence in itself constitutes an example of perceptual learning, one that is worth our attention. Our topic is how experience can change the way in which things are perceived. Although in almost all the examples given so far, the change has been for the better (that is, discriminability has been enhanced by experience) there is no reason why this should always be the case, and our analysis of the phenomenon would be incomplete if it failed to encompass acquired reductions in discriminability.

1.07.3 Theoretical Issues

In all the experiments described earlier, the test task requires the subject to discriminate between two similar stimuli. The situation is presented schematically in **Figure 5**. Each circle represents the set of features or elements that define or constitute a particular stimulus, A or B. Each stimulus will have a set of unique features that are not found in the other (and these are represented by the areas containing the elements labeled a and b). The fact that A and B are similar is represented by the overlap – the area marked containing the c elements designates a set of features that they hold in common. Successful discrimination is evident when the subject shows the ability to make one response to A (i.e., ac) and a different response to B (bc). It follows that the job of a theory of perceptual learning is that of explaining

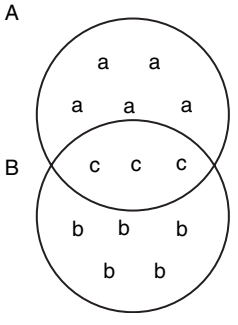

Figure 5 Each circle represents a stimulus (A or B) that is made up of a set of features (or elements). Some features are unique to a given stimulus (the a elements for A; the b elements for B); other features (c) are held in common and thus fall into the area of overlap of A and B.

how experience or training allows behavior to come to be controlled by the unique features (a and b), rather than by the common features (c). The behavior in question may be a gross overt movement, as when a rat approaches one stimulus object and avoids another; or it might be as minor as the verbal response of "higher" from a human participant presented with one of a pair of tones.

The scheme shown in **Figure 5** can be applied quite generally. It may need slight modification when the stimuli are drawn from a simple continuum, such as tonal frequency – here a given tone might be regarded as consisting of the elements a, b, c, d (say); its neighbor by the elements b, c, d, e; its neighbor by the elements c, d, e, f; and so on – but the principle remains the same; discrimination between adjacent (similar) tones requires control by the unique elements that distinguish between stimuli.

This characterization prompts an attempt at a definition of perceptual learning. It is the learning process (or processes) that increases the effectiveness of unique stimulus elements and/or reduces that of common stimulus elements, thus facilitating discrimination between similar stimuli. (Although this will serve for almost all the cases discussed in the previous section, we should note the possibility of instances in which training reduces discriminability. For these we must assume that the effectiveness of common elements is increased, that of the unique elements is reduced, or both; *See* Chapter 1.08 for configural processing).

This definition may allow us to rule out, as instances of true perceptual learning, some of the processes that result in improved performance during practice on a discrimination task. It is often found, for instance, that performance on even the simplest difference threshold task can show a dramatic improvement on the early trials, and this improvement could well be a consequence of the participant learning to deal with the requirements of the procedure. If, for example, the subject is initially a little unclear as to which button to press for the high tone and which for the low tone, practice will establish the relevant associations and remove one obstacle to accurate performance – but this improvement would not be a consequence of a change in the effectiveness of the unique or common features of the stimuli and thus would not count as perceptual learning.

This is not to say, however, that we would want to dismiss what some (e.g., Liu and Weinshull, 2000) have called 'cognitive' learning, as a possible

mechanism of perceptual learning. For example, McLaren and Mackintosh (2000) suggest that we should exclude from consideration cases in which participants, instructed to look for differences between stimuli, learn to focus on aspects that enable them to solve the task required of them. But surely a strategy as simple as learning to attend to or fixate on a particular part of a visual display deserves to be regarded as a mechanism of perceptual learning if it serves to increase the effectiveness of unique features (which happen to be located at a particular point in space). Again, Bruce and Burton (2002), discussing the discrimination of faces, question the extent to which enhanced discrimination depends on the acquisition of verbal labels for the different faces as opposed to reflecting (true) perceptual learning. But the distinction may not be a useful one. It has long been thought (e.g., James, 1890) that the acquisition of associates, such as verbal labels, might be a way of increasing the range and number of distinctive features activated by the presentation of a given stimulus; if this is so, the consequent improvement in discrimination becomes an example of perceptual learning under our definition.

The general point is that our proposed definition is silent as to the nature of the learning processes involved. The approach taken here contrasts with that sometimes taken by other students of perceptual learning. For example, Fahle and Poggio (2002) began their survey of the topic by ruling out, by means of their definition of perceptual learning, a number of possibilities that we would want to consider. Perceptual learning, they say, is independent from conscious experience and leads to implicit memory; it is not declarative, as it does not consist of consciously memorized facts or events; it is not associative, as it does not bind things together, and does not rely on the mechanisms of classical and operant conditioning; it differs from other forms of learning in that it principally involves functional and anatomical changes in primary sensory cortex. It may well be that some of the examples of enhanced discrimination that were described earlier in this chapter are a consequence of the type of learning envisaged by Fahle and Poggio (and we will try to identify them in subsequent sections of the chapter). But declining to subscribe to such a restrictive definition leaves us free to consider a range of other possible mechanisms, including several (such as various cognitive, associative, and attentional learning processes) that have been well studied in other contexts. We begin by considering the extent to which the associative analysis of learning can supply an explanation for perceptual learning phenomena.

1.07.4 The Role of Associative Processes

It may seem odd to give pride of place to a learning process that some have emphatically asserted is not responsible for perceptual learning effects; thus Gibson and Levin (1975, p. 23) wrote: "this simple and ancient notion does not work for perceptual learning, because what is learned [in perceptual learning] is not addition of something but rather extraction of something." There are, however, at least two good reasons for doing so. First, associative learning theory, in its modern form (see, e.g., Wagner, 1981; Mackintosh, 1983), provides by far the best worked out and most comprehensive account of basic learning mechanisms, and it seems a sensible first step to attempt to explain some (supposedly) new form of learning in terms of what we already know about learning more generally. Second, one of the earliest attempts to explain an instance of perceptual learning was, in fact, precisely in terms of the notion that it depended on the associative 'addition of something.'

1.07.4.1 Acquired Distinctiveness and Acquired Equivalence

Figure 6 (top part) presents a schematic version of the associative account offered by James (1890) for the acquired distinctiveness of cues. Recall that in this procedure, discrimination training, in which the cues are followed by different outcomes, enhances their subsequent discriminability. **Figure 6** shows the associations assumed to be formed when two similar cues (A and B) have been given training in which each has become linked to a different associate (X and Y); the associates are less similar (they share few common features) than are A and B. Discrimination between A and B prior to training will be difficult as they share many common elements. But the formation of the associative links means that presentation of A will produce associative activation of the representation of X, and presentation of B will associatively activate Y. As a result, discrimination between A and B will be enhanced because the proportion of common features present in the overall patterns of activation produced by these stimuli will

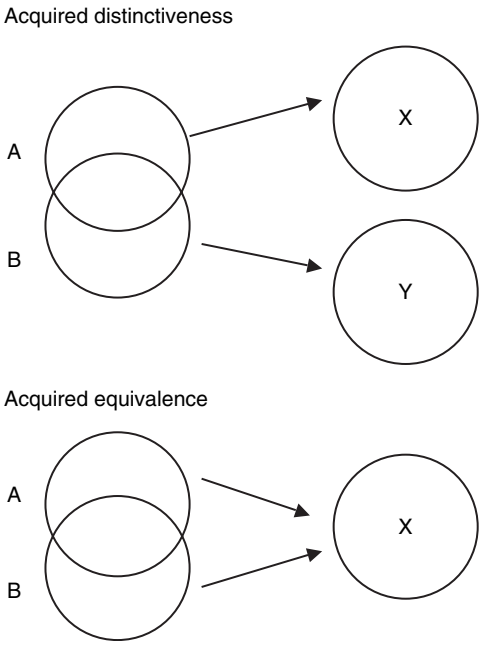

Figure 6 Associative structures in acquired distinctiveness and acquired equivalence. The overlapping circles represent two similar stimuli, A and B (see Figure 5); arrows represent associative links. In the acquired distinctiveness case, A and B have formed associations with quite different stimuli (X and Y). In the acquired equivalence case, both have become associated with the same stimulus.

be low, given the distinctiveness of their associates. The lower part of **Figure 6** shows the situation for the acquired equivalence procedure in which the two stimuli are given the same associate. Here the proportion of common elements is increased, and discriminability should go down. James focused on the case in which the associates were distinctive names, but the analysis applies (and the effect is found) when other events are used (as in experiments with animal subjects, or that presented in **Table 1**).

That stimuli can acquire new, and potentially distinctive, properties by associative means is nicely demonstrated by studies of odor perception. It has been noted that an odor such as vanilla is often described as smelling sweet, even though it is in itself tasteless. The suggestion that this quality is acquired as a result of associative learning (vanilla is often present in sweet foods) is supported by the results of a study by Stevenson et al. (1998), who gave subjects presentations of a novel odor (e.g., lychee) along with a sweet taste. Their subjects started to describe lychee as smelling sweet, a phenomenon that Stevenson et al. referred to as learned synesthesia. (Parallel effects have also been found with sour and bitter tastes.)

Whether associations of this sort are actually responsible for acquired distinctiveness effects is another matter. Gibson (1969), in her influential discussion of the topic, argued that although associations might indeed be formed during training designed to establish acquired distinctiveness, they did not provide the mechanism for the effect. Rather, the role of discrimination training was simply to ensure that the subjects concentrated on the stimuli, noting their similarities and differences, with the result that there was an increase in the perceptual effectiveness of (and attention paid to) their intrinsic distinctive features. This is what was meant by the phrase 'extraction of something,' rather than 'addition of something.' The evidence that is currently available to us does not allow a clear choice between the alternatives, but suggests, rather, that both processes may play a role.

Evidence that the associations formed during initial training can influence subsequent discrimination performance comes from the experiment by Hall et al. (2003), outlined in **Table 1**. Why, in Stage 2, did subjects in the inconsistent condition find it difficult to assign different responses to cues (such as A and B) that had shared a common associate (red) in Stage 1? According to the associative theory, this is because the representation of red was activated by both A and B during Stage 2. When subjects learned to respond left to A, this response would come under the control both of the cue actually presented (A) and its associate (red). Small wonder, then, that they found it difficult to respond right to B, given that its associate already controlled a tendency to make a different response. For subjects in the consistent condition, on the other hand, the response tendency acquired by red on a trial with A would allow the correct response to emerge immediately when B was presented. In a final test (Stage 3 of the table), subjects were asked to choose left or right when presented with the colors used as associates in Stage 1. Those in the consistent condition made appropriate choices (choosing left for red and right for green, in our example), as would be expected if the associatively activated representations of these colors had been involved in the discrimination test of Stage 2.

A reason to think that associative processes are not wholly responsible for acquired distinctiveness effects comes from the observation that mere exposure to a pair of similar stimuli (if this is appropriately arranged – as we have seen, alternating presentations

that give the opportunity for comparison to occur are particularly effective) can enhance subsequent discriminability. That is, distinctiveness can be acquired when the preexposure procedure seems to preclude the formation of links with distinctive associates. For humans, this argument may not be particularly convincing – members of this species may well provide their own associates (names or labels), no matter what the experimenter tries to arrange. But the effect is also seen with animal subjects (rats given alternating presentations of two flavors are better able subsequently to discriminate between them). If another, nonassociative, perceptual learning process is at work during mere exposure, might it not also be active when the exposure phase involves explicit discrimination training? Evidence in favor of this view comes from a study by Bonardi et al. (2005), who modified the procedure used by Hall et al. (2003) to rule out associative mediation and yet still found an acquired distinctiveness effect. Bonardi et al. concluded that the preexposure procedure had, in addition to establishing associations, modified the perceptual effectiveness of the various elements of the stimuli (specifically had enhanced the attention controlled by their distinctive features). The learning mechanisms that might be responsible for such an effect will be taken up in the final major section of this chapter, after we have considered the contribution of other associative mechanisms to perceptual learning effects.

1.07.4.2 Unitization

It has frequently been suggested (e.g., Goldstone, 2000; McLaren et al., 1989) that a process of unitization is, in part, responsible for perceptual learning effects. The idea has been expressed in a variety of different ways, but the central notion is that exposure to a complex and multifaceted stimulus will result in the formation of a unitary representation of that stimulus, in which the various features are somehow bound together. The concept has been much used in the study of complex visual perception (with its talk of object representations, face representations, and so on) but can readily be applied to other modalities (it forms the basis of the account of odor perception proposed by Stevenson and Boakes, 2003), and to seemingly simple stimuli (even a pure tone, for example, has many features – frequency, intensity, location, duration, etc).

When it comes to specifying the learning mechanism responsible for unitization, the only developed proposal has been in terms of associative processes (see Goldstone, 1998). Presentation of a complex stimulus will, it is assumed, activate a set of units that correspond to its various constituent features. Concurrent activation of these units will lead to the formation of a network of excitatory links among them, and it is this network, in the simplest interpretation of the idea, that is taken to constitute the unitized representation. In terms of the diagram of **Figure 5**, experience of stimulus A will establish connections among all the a elements. (It will also, initially, allow the formation of a–c connections too; but because the c elements may also be activated in the absence of the a elements, when a stimulus such as B is presented, these will be weakened and eventually drop out of the picture.) A slightly more elaborate version of this analysis supposes that experience of the stimulus results in the formation of a separate configural unit (see, e.g., Pearce, 1994) that is not directly activated by any feature of the stimulus itself but that comes to receive its input from the units that correspond to those features.

The formation of a simple network of excitatory associations is enough in itself to explain a range of perceptual learning phenomena. As we have already seen, the learned synesthesia of Stevenson et al. (1998) depends on associations formed between two aspects of a compound stimulus (its taste and smell). And our difficulty in discriminating among the aspects of a previously experienced compound odor (Stevenson, 2001) can be explained in similar terms. When people have sniffed the AX compound, excitatory associations will form between A and X. Discrimination between A and X will be poor because the presentation of A will produce associative activation of the representation of X, and presentation of X will associatively activate A. It is usually assumed that the state produced by associative activation of a representation will be distinguishable from that produced by direct activation (if nothing else, the intensity of activation is likely to be less; see Hall, 1996), and accordingly, discrimination between A and X should still be possible. But the existence of association between A and X will increase the similarity of the overall patterns of activation elicited by A and X and render the task more difficult.

Further support for this interpretation of unitization comes from its ability to explain the finding that preexposure to a complex stimulus or event can enhance the ease with which that complex is subsequently learned about. An example from animal

conditioning is supplied by Kiernan and Westbrook (1993), who gave rats a few minutes of exposure to a distinctive context prior to a session in which the context was paired with shock. On a subsequent test, these rats showed more evidence of learned fear in that context than did rats not given preexposure. (See also Fanselow, 1990; Bennett et al., 1996.) The only additional assumption needed to deal with this finding is that the animal's capacity to process all aspects of a complex stimulus will be limited, so that only a subset will be sampled at any one time. As the rat explores the context during preexposure, it will sample a range of contextual features, and connections will form among them. On the subsequent conditioning trial, only some of these features will be sampled, and only these will become associated with shock. A different set may be sampled on the test trial, but for animals given preexposure, the conditioned response should still be evoked, as those sampled on the test will be able to activate those that formed links with the shock during conditioning. Although presented in modern associative terminology, the central idea is essentially that popularized long ago as redintegration (by Hollingworth, 1928).

It will be apparent that the principle illustrated by this example from fear conditioning in the rat will be applicable to any case in which animals (including people) are given exposure to a stimulus containing more features than can be processed all at once. Appropriate response to a complex visual event (such as putting a name to a face) will be able to proceed more rapidly if inspection of one part of the display is able to activate representations of other features, or to activate a configural unit that is connected to the response-output mechanism. In the case of faces (and indeed other complex visual stimuli), it must be assumed that the configural unit is sensitive not just to the co-occurrence of various features but also to their spatial relationships. One of the classic findings from studies of face recognition is the inversion effect – the finding that faces are so much less well recognized when upside down; or, put another way, are especially well dealt with when they are the usual way up (Yin, 1969). This phenomenon is explained by assuming that experience of a face in the normal orientation establishes a configural unit that encodes the spatial relationships of the various features; when the face is inverted, these relationships are disrupted, and the unit will not be activated. Evidence for this interpretation comes from a study by Gauthier and Tarr (1997), who required people to learn the names of nonsense objects of the sort shown in **Figure 7**. An inversion effect was found for these stimuli too – performance was poor when the stimuli were presented upside down – but only for people who had received extensive prior experience with the cues. The effect is specific not to faces but to complex familiar visual cues.

Figure 7 Examples of the figures (referred to as Greebles) used by Gauthier and Tarr (1997) and of the names that subjects had to learn to apply to them. Used with permission.

1.07.4.3 Associative Inhibition

McLaren et al. (1989; see also McLaren and Mackintosh, 2000) have pointed out that standard associative theory predicts that certain schedules of exposure to a pair of similar stimuli (such as A and B of **Figure 5**) will allow the formation of other associations, in addition to those considered so far. They were concerned in particular with a preexposure schedule in which A and B were presented in alternation. Gibson (1969) has argued that a process of comparison plays an important part in the enhancement of discrimination that follows exposure to similar stimuli. The exact nature of the comparison process was not specified, but it can be agreed that alternating presentations are likely to enhance its operation, making this schedule of special interest.

We have already discussed how exposure to the stimuli will allow unitization to occur, with the various elements of each stimulus becoming linked together. McLaren et al. (1989) noted that the within-stimulus links formed would include excitatory connections between common and unique elements (which we may summarize as c–a, for stimulus A, and c–b, for stimulus B). As a consequence, presentation of A would, by way of the c–a association, be able to activate the representation of the unique features of B (the b elements); similarly, presentation of B by way of the c–a link would activate the a elements. On the face of things, therefore, preexposure might be expected to hinder subsequent discrimination, as these excitatory links would render the patterns of activation produced by A and B more similar to one another. Such indeed would be the outcome but for another factor that comes into play when the alternating schedule is

used – this schedule will allow the formation of inhibitory associative links. According to standard associative theory (e.g., Wagner, 1981; espoused by McLaren and Mackintosh, 2000) an inhibitory link will form between a directly activated stimulus representation and one that is activated associatively. The alternating schedule ensures that the subject experiences a sequence of trials in which a is associatively activated in the presence of b, and b is associatively activated in the presence of a. In these circumstances, inhibitory associations will form between a and b. Activation of an inhibitory link is assumed to oppose the effects of excitatory influences acting on a given representation, restricting the ability of that representation to be activated.

This analysis can supply an explanation for the results of the experiments described previously, in which discrimination was enhanced by intermixed preexposure (compared to a control condition in which the stimuli were presented in separate blocks of trials). Consider the experiment by Lavis and Mitchell (2006). When asked to make same–different judgments about stimuli like those shown in **Figure 2**, the excitatory links formed by subjects in the control condition mean that each stimulus would tend to activate, to some degree, the unique features of the other. Presentation of A would activate a and c directly and b associatively; presentation of B would activate b and c directly and a associatively. That is, all the same units would be activated in each case, and the judgment would therefore have to be made in terms of degree (or type) of activation. For subjects given intermixed exposure, on the other hand, inhibition between a and b would mean that A would activate only a and c, and B would activate only b and c, qualitatively different patterns of activation.

The difference between intermixed and blocked preexposure schedules has also been obtained in studies of animal conditioning (e.g., Symonds and Hall, 1995; see also Bennett and Mackintosh, 1999; Mondragón and Hall, 2002; Dwyer et al., 2004), and the associative inhibition analysis applies readily to this case too. In these experiments, subjects received conditioning with stimulus A followed by a generalization test with stimulus B. The poor discrimination (good generalization) produced by blocked exposure is explained in terms of the within-stimulus excitatory links formed during preexposure; specifically when tested with B, the c–a link will allow this cue to activate a stimulus representation (a) that had acquired associative strength during conditioning with A. For the intermixed condition, on the other hand, the inhibitory association between b and a established during preexposure would prevent activation of a on the test trial, eliminating its contribution to responding and thus reducing the degree of generalization.

The role of associative inhibition as a mechanism of perceptual learning deserves our serious attention because it provides one of the few fully worked out accounts of the processes by which stimulus comparison might have its effects. It remains to be determined, however, whether or not this theoretical possibility is, in fact, responsible for the effects observed. There is some supportive evidence from a study by Dwyer et al. (2001; see also Dwyer and Mackintosh, 2002). Rats were exposed to intermixed presentations of the compound flavors, AX and BX, where A and B represent unique flavors, and X an explicitly added common element. According to the associative principles outlined earlier, alternating presentations of AX and BX should establish inhibition between A and B (just as alternating presentations of ac and bc establish inhibition between a and b). Dwyer et al. found that after extensive intermixed exposure, animals were retarded in learning an excitatory association between A and B – what would be expected if the prior training had established inhibitory associations between these cues. It seems likely that associative inhibition plays a role in the perceptual learning effect under these training conditions.

It should be noted, however, that this example of perceptual learning (better discrimination after intermixed than after blocked preexposure) can be obtained in the absence of associative inhibition. Inhibitory learning can take many trials to develop (Dwyer et al., 2001, gave extensive initial training), but the perceptual learning effect can be obtained after just a few preexposure trials, well before there is any evidence of inhibition between A and B (Artigas et al., 2006). What is more, the effect can be observed when the training procedure is modified so as to preclude the formation of inhibitory links. **Table 2** (Experiment 1) shows the design of an experiment (Blair and Hall, 2003, Experiment 1a) devised to demonstrate the basic effect using a within-subject design and a conditioning procedure. The subjects (rats) received intermixed preexposure to the compound stimuli AX and BX and a separate block of trials with CX. A response subsequently conditioned to AX was found to generalize less readily to BX (the stimulus presented intermixed with AX during preexposure) than to the control stimulus CX. This result in itself is compatible with the suggestion that the

Table 2 Experimental designs used to investigate the effects of preexposure on flavor discrimination in rats

	Preexposure	*Conditioning*	*Test*
Experiment 1	AX/BX _ CX	AX +	BX and CX
Experiment 2	AX/BX _ CX	X +	BX and CX
Experiment 3	X/BX _ CX	AX +	BX and CX
Experiment 4	AX/BX _ CX	—	B+ or C+

Note: A, B, C represent flavors that could be presented in compound with flavor X. In preexposure, AX (or X, in Experiment 3) was presented in alternation with BX. The CX compound was presented in a separate block of trials. In the conditioning phase of experiments 1–3 and the test phase of Experiment 4, flavors were presented along with (+) an aversive reinforcer. The test phase measured the extent of the aversion shown to the test stimuli (a generalized aversion in the case of experiments 1–3). Experiments 1 and 2 were fully reported by Blair CAJ and Hall G (2003) Perceptual learning in flavor aversion: Evidence for learned changes in stimulus effectiveness. *J. Exp. Psychol. Anim. B*. 29: 39–48; Experiment 3 by Hall G, Blair CAJ, and Artigas AA (2006) Associative activation of stimulus representations restores lost salience: Implications for perceptual learning. *J. Exp. Psychol. Anim. B*. 32: 145–155; and Experiment 4 by Blair CAJ, Wilkinson A, and Hall G (2004) Assessments of changes in the effective salience of stimulus elements as a result of stimulus preexposure. *J. Exp. Psychol. Anim. B*. 30: 317–324.

presence of B in the test inhibits activation of the representation of the (conditioned) A element. But this argument cannot apply to the modified design shown as Experiment 2 in the table (Blair and Hall, Experiment 5a). Here the same result was found (less generalization to BX than to CX) despite the fact that conditioning was given to the X element alone. If A has not been conditioned, then any ability that B might have to inhibit activation of the A representation would be irrelevant to the outcome of the procedure. The experiment presented as Experiment 3 in the table (Hall et al., 2006) makes the same point in a different way. Here the intermixed preexposure procedure involved alternation, not of AX and BX, but of BX and X alone. Obviously, inhibition between A and B cannot be established with this procedure. Nonetheless, conditioning to AX was still found to generalize less well to BX than to CX on the test (see also Rodriguez and Alonso, 2004).

The new results just described serve to support Gibson's (1969) suggestion that a preexposure procedure that allows comparison between two similar stimuli (such as alternating presentations of AX and BX) is particularly effective in enhancing discrimination between them. They also show that associative inhibition mechanism can supply only a partial explanation for these effects. Gibson's own interpretation was that comparison served to enhance the perceptual effectiveness of the distinguishing features of the stimuli (A and B in this case). This notion can help explain the results produced by the experiments summarized in Table 2. In these experiments, performance on the generalization test will be largely determined by the response controlled by the X element, the response that was established during the conditioning phase. To the extent that the presence of another element (such as B or C) detracts from the ability of the animals to perceive stimulus X, the magnitude of the response will be reduced. It follows that if alternating preexposure enhances the perceptual effectiveness of the B element, this element will be better able to interfere with the ability of X to evoke its response on test and generalization will be restricted – the result obtained. What we need to consider now, therefore, is the mechanisms by which the perceptual effectiveness of stimuli might be modified; this issue is taken up in the next section of the chapter.

1.07.5 Attentional Learning Processes

The only learning process utilized so far has been one that results in the formation of links (excitatory or inhibitory) between the central representations of stimulus elements. Perhaps surprisingly, this notion has proved helpful in explaining some perceptual effects. But however powerful an explanatory tool this "simple and ancient notion" may be, it is not, on its own, enough to explain even simple associative learning. A number of learning theorists (e.g., Mackintosh, 1975; Pearce and Hall, 1980; McLaren and Mackintosh, 2000) have argued that the associative principle needs to be supplemented by another learning process, one that is capable of changing the properties of the stimulus representation, modulating its sensitivity to activation, for example, or modulating the readiness with which it will enter into association.

Because these theories are usually described as involving a process of attentional learning, it would be useful to clarify what is meant by attentional in his context, as the use of the term (which is really more of a chapter heading than a well-defined psychological construct) can vary widely. It is not meant to indicate a form of learning that occurs only when learners focus their attention on the task at hand (a characteristic that, in any case, would be difficult to identify in the experiments using animal subjects that will be considered shortly). Rather, it indicates a form of learning that modifies the processing that a

stimulus will receive. As for the nature of the modification, two principal suggestions have been advanced. One is that experience might change the effective salience of a stimulus (making a dim light function as if it were bright or a loud noise as if it were soft) and thus modify the ability of the stimulus to command attention. The other, not necessarily alternative, suggestion is that experience might change the associability of a stimulus, the readiness with which it will be learned about. (A significant event might deserve attention, even if its salience is low.) Both of these possibilities merit the description attentional, but it will be noted that they have somewhat different implications, and where necessary, they will be distinguished in what follows.

1.07.5.1 Latent Inhibition and Associability Modulation

Prior exposure to an event that is to be used as the conditioned stimulus (CS) in a classical conditioning procedure produces a marked retardation in the subsequent rate of learning. The source of this phenomenon (known as latent inhibition; Lubow, 1989) is still a matter for debate (some possibilities will be considered shortly). For our present purposes, however, we may simply note that mere exposure to a stimulus can produce a reduction in the readiness with which it can be learned about, and then go on to explore the implications of this fact of perceptual learning.

McLaren et al. (1989) have proposed an interpretation of perceptual learning effects in which latent inhibition plays an important part. They point out that it is important to distinguish between the ease with which a stimulus can be learned about and the ease with which it can be discriminated from other similar stimuli (which is our major concern). A process that reduced the former might enhance the latter. Consider the stimuli of **Figure 5**. A response conditioned to A will generalize to B (i.e., a failure of discrimination will occur) because the common (c) elements acquire strength during conditioning with A (ac) and are present in the test stimulus B (bc). Prior exposure to A will reduce generalization (enhance discrimination) because the c elements will suffer latent inhibition and thus acquire little strength during conditioning. This effect will be most marked if the subjects are given preexposure to both A and B, as the c elements will be present in both types of exposure trial, thus having twice the opportunity to acquire latent inhibition. Generalization should, therefore, be particularly weak after preexposure to both A and B – just the result obtained by Symonds and Hall (1995) and shown in **Figure 4**.

This simple notion generates an interesting prediction that has received experimental support. A perceptual learning effect (enhanced discrimination after preexposure) should only be found when the stimuli share a substantial number of common elements. When the stimuli are very different (as, in the limiting case, when A consists only of a elements and B only of b elements), latent inhibition of the c elements can play no part, and discrimination between A and B will be poor, as the latent inhibition suffered by the a and b elements will retard the acquisition of the (different) responses required to these stimuli. Trobalon et al. (1991; see also Prados et al., 1999) have demonstrated this result in a study of maze learning in rats. When the two maze arms that the rats had to choose from were very similar, preexposure to these arms facilitated discrimination learning; when the arms were made distinctively different, preexposure hindered learning.

Latent inhibition can provide an explanation for the result reported by McLaren et al. (1994) that people who had learned to assign checkerboard patterns (**Figure 2**) to different categories showed an enhanced ability to discriminate between new examples drawn from the same category. This result is unexpected, given that examples from the same category will have a common associate, in that both will elicit the same category label. Associative processes might be expected act to hinder discrimination between stimuli that have a common associate (the acquired equivalence effect). But this is to reckon without latent inhibition. McLaren et al. (1994) point out that during initial categorization training, the features common to all exemplars of that category occur on every trial. These features will therefore suffer extensive latent inhibition. Performance on the within-category discrimination will be facilitated, as this task requires precisely that the choice response should come under the control of features that distinguish the displays rather than features they hold in common.

The arguments just advanced hold whatever the mechanism of latent inhibition. Of the various possibilities (see Hall, 1991, for a review), perhaps the most widely accepted is that it reflects a loss of associability, this being expressed in formal terms as a reduction in the value of a stimulus-specific learning rate parameter (symbolized alpha in the influential learning model of Rescorla and Wagner,

1972; see Pearce and Hall, 1980). To adopt this interpretation raises a further interesting possibility. Latent inhibition itself involves only reduction in associability, but might it not be possible for the alpha-value of a stimulus to be increased under appropriate conditions? Mackintosh (1975) has adopted this proposal and devised a theory in which the associability of a stimulus is held to increase as a result of training in which it is a reliable predictor of its consequences (see also Kruschke, 2001). Direct tests of the validity of this proposal have generated mixed results (e.g., Hall and Pearce, 1979; Le Pelley, 2004). But if it could be confirmed, it would usefully extend the explanatory reach of the associability concept. In particular, it could supply an explanation for acquired distinctiveness effects. The acquired distinctiveness training procedure is one in which the subject experiences each of the critical stimuli in reliable association with another event (in **Table 1**, for example, A reliably predicts red, and B reliably predicts green). In addition to any associations that may be formed, Mackintosh's theory says that the associability of A and B will go up under these conditions. Subsequent discrimination between these cues would be enhanced, even in circumstances in which associative mechanisms do not seem to operate (Bonardi et al., 2005).

Whatever the fate of Mackintosh's (1975) theory of associability change, it will be evident that the basic latent inhibition process plays an important part in many perceptual learning effects. There is, however, one critical version of perceptual learning that defies explanation in terms of latent inhibition. This is the well-established, and already much-discussed, finding that discriminability is especially enhanced when the subject is able to compare the stimuli during preexposure. **Table 2** (Experiment 1) presents a simple experimental demonstration of the effect. In this experiment, the subjects can compare A and B during preexposure (they are presented on alternate trials), but will be less able to compare C with the others, as this stimulus is presented on a separate block of trials. But because the subjects experience the critical cues, A, B, and C, the same number of times, all three cues should acquire latent inhibition to the same extent. There are no grounds, therefore, for the latent inhibition account to predict the result obtained – poorer generalization from AX to BX than to CX. Further analysis of this finding is one of the topics of the final section of the chapter.

1.07.5.2 Habituation and Salience Modulation

Whatever other factors may play a part, there is no doubt that the effectiveness of a stimulus depends on its intensity. A strong stimulus will normally elicit a more vigorous response than a weak one (we show a bigger startle response to a loud noise than a soft one); associative learning occurs more rapidly when the events to be associated are intense. Formal theories of these phenomena (see, e.g., Hall, 1994) incorporate a notion of salience, a parameter associated with each stimulus and set by its intensity.

Stimulus salience will influence performance on the tests used in studies of perceptual learning. Subjects will be best able to discriminate (on a same-different test, say) between A and B when the unique features (a and b) are intense, and the common features (c) are not. And generalization between such stimuli will be poor, as the a element will dominate during conditioning with A, restricting the acquisition of control by the c element, and the b element will dominate on test with stimulus B, restricting the ability of c to influence performance. In most studies of the topic, we use stimuli with nonsalient unique features and salient common features; that is, we study the effects of experience on discriminations that are difficult. Perceptual learning effects would be obtained, then, if experience with stimuli was capable of boosting the effective salience of the unique features of stimuli (or of lowering that of common features, or both). What evidence is there that effective salience can change? We have discussed how simple exposure to repeated presentations of a stimulus can produce a loss of associability (latent inhibition), but there is reason to think that this procedure can also bring about a change in effective salience.

Repeated stimulus presentation results in habituation – the waning of the response unconditionally elicited by that stimulus. Explanation of this simple phenomenon turns out to be surprisingly complex (see Hall, 1991, for a review in the context of perceptual learning). But what we need to note for our present purposes is that the habituation procedure makes a salient stimulus behave like a less-salient one. After extensive habituation training, the startle response evoked by a loud noise will be much the same as the (weaker) response evoked by the first presentation of a softer noise. The habituation effect is most easily observed with motivationally significant events, as these evoke obvious responses; but the

learning process responsible for it presumably operates for any stimulus, including those used as cues in experiments on perceptual learning. For a few of these, the effect can in fact be observed directly. Rats show neophobia to foods (that is, they are reluctant to consume a substance with a novel taste), and habituation of neophobia is commonly observed over the preexposure phase of perceptual learning experiments using flavor stimuli (Blair et al., 2004). Observations like these make it a reasonable presumption that the (unobservable) response evoked by presentation of a checkerboard, say, will also undergo habituation, or, in other words, that these stimuli too will lose effective salience with repeated exposure.

Blair et al. (2004) have investigated the role of salience modulation in perceptual learning, focusing on the differing effects of intermixed and blocked preexposure. Recall for the experiment shown as Experiment 1 in **Table 2** that rats consume more of BX than of CX on test, and that this difference is explained by the fact that at the end of preexposure, B has greater effective salience than C (the more salient a cue, the more it will interfere with expression of the response controlled by X). Blair et al. (2004, Experiment 3) tested the salience of B and C using the design shown in as Experiment 4 in **Table 2**. After preexposure, some rats received conditioning trials with B alone as the conditioned stimulus (CS); others received C alone as the CS. Acquisition occurred more rapidly to B than to C, as would be expected if B were higher in salience than C. In a further study, Blair et al. simply monitored the unconditioned response evoked by B and C at the end of preexposure. The neophobic reaction evoked by these flavors was found to have habituated to some extent over the course of preexposure, but it was still observable, particularly for flavor B. Thus the effective salience of both B and C was reduced by preexposure, but critically, the reduction was less for the cue presented in alternation with a similar cue in preexposure.

The conclusion that emerges from these and related studies (Hall, 2003; Hall et al., 2006) is that mere exposure to a stimulus will cause a loss of effective salience, but that with some schedules of preexposure, this loss can be attenuated or reversed. The critical arrangement appears to be one in which the cue in question is presented in alternation with another similar cue. Why this schedule should have the effects it does is not yet clear. There is some evidence from the experiments by Hall et al. (2006) to suggest that an important feature of this schedule is that on each trial the subject is likely to be (slightly) surprised at the omission of one of the unique features and at the occurrence of the other. (With the blocked schedule the same stimulus occurs trial after trial.) It seems plausible that an event that evokes surprise might also maintain its salience, but the precise learning mechanisms that might underlie such an effect remain to be specified.

1.07.6 Conclusions

The material reviewed in this chapter has covered a wide range; this is true both for the empirical phenomena considered in the first part and the theoretical analyses dealt with in the second part. The latter point might seem to be a cause for concern, given our customary aspiration to achieve parsimony in explanation. But the concern would probably be misplaced. There is every reason to think that perceptual learning effects are the product (usually the joint product) of several different processes. On the basis of the evidence reviewed in this chapter, a place should be found for associatively mediated acquired equivalence and distinctiveness, effects based on within-stimulus association (unitization) and between-stimulus associations (associative inhibition), latent inhibition (and possibly other learned changes in associability), habituation, and salience modulation.

It may have been noticed that the operation of these various learning mechanisms in perceptual learning has been demonstrated for only a subset of the phenomena described in the first section of the chapter. Analytic studies have, for the most part, made use of just a few well-established and tractable experimental procedures. This may raise the fear that detailed exploration of other paradigms would uncover a whole new set of explanatory principles, in addition to those already listed. But this fear is not justified. As was suggested earlier, the job of a theory of perceptual learning is to explain how experience of similar stimuli can enhance the perceptual effectiveness of features that distinguish them and reduce the perceptual effectiveness of features that they have in common. This description is valid generally – it applies equally, for example, to rats learning to discriminate between flavors and to people learning to distinguish between speech sounds. We have every reason to hope that explanatory principles established in one of these paradigms will also apply in the other.

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1.08 Discrimination and Generalization

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1.08.1 Introduction

Studies using discrimination and generalization methods have been intimately intertwined with research and theory in learning and memory. All or nearly all the other articles in this volume will describe findings based on discrimination and generalization tasks (*See* Chapters 1.03, 1.05, 1.07, 1.11, 1.12, 1.22). From a biological perspective, learning to respond distinctively to different stimuli and transferring that learning to new situations are essential to survival in all but the most static of environments. From a cognitive perspective, understanding distinctions and perceiving similarities underpins processes of attention, perception, recognition, categorization, and reasoning, all of which usually require learning and memory for developing their content and expression.

In brief, discrimination training tests how well a learner can distinguish among stimuli, while generalization procedures test how well training with one stimulus transfers to another stimulus. These two procedures have been used in three major ways. First, they provide psychophysical techniques for

revealing the processes of stimulus encoding. Second, they have been used in delineating the roles of excitation and inhibition in learning. Third, they have contributed to the investigation of cognitive processes, particularly in nonverbal creatures. In the present article, key examples of each of these uses will be outlined after the basic methods have been described.

1.08.2 The Basics

This section describes the basic procedures and outcomes of discrimination training and generalization testing. In addition, this section treats generalization testing in the wider context of transfer of training.

1.08.2.1 Discrimination Learning

Basic discrimination training entails the presentation of two stimuli, one of which is associated with the reinforcer (S+), and one of which is not (S−). In classical conditioning, one conditioned stimulus (CS+) is consistently paired with the unconditioned stimulus (US), and the other conditioned stimulus (CS−) is not paired with US. Similarly, in operant conditioning, the target response is reinforced whenever one discriminative stimulus (SD) is present, and the target response is not reinforced whenever the other discriminative stimulus (SΔ) is present. Discrimination learning is said to occur when the level of responding is greater during S+ than S−. **Figure 1** shows discrimination learning curves obtained under different circumstances in different species.

Discrimination training using stimuli that differ along a single dimension is commonly called intradimensional training. When the S+ and S− differ along multiple dimensions, including their sensory modalities, the procedure is called either interdimensional or extradimensional training. Across investigators, there is no apparent convention for using these latter two terms.

Go/no-go discriminations. **Figures 1(a)** and **1(b)** illustrate differential classical conditioning of the eyeblink response in rabbits and humans (Gynther, 1957; Moore, 1972). For the rabbits, the stimuli were tones of different frequencies (400 Hz, 1600 Hz), and for the humans, red and white lights were used. The assignment of the two stimuli as CS+ and CS− was counterbalanced across subjects. In both cases, the likelihood of a conditioned response (CR) to both CS+ and CS− increased during the early trials of training. Thereafter, respondes to CS+ continued to rise, while respondes to CS− either stabilized or declined to a lower level.

Figure 1(c) shows results of a free-operant discrimination procedure. Rats were initially trained to press a lever bar for intermittent reinforcement (Herrick et al., 1959). Discrimination training was conducted by switching between periods in which an indicator lamp was on (S+) and periods in which the lamp was off (S−). During the S+ periods, barpresses were intermittently reinforced. During the S− periods, barpresses were never reinforced. The rate of barpressing during S+ rose steadily and approached a level around 70 responses per minute. In contrast, responses during S− gradually disappeared.

Choice discriminations. The examples of discrimination learning shown in **Figures 1(a)–1(c)** are all based on procedures in which the S+ and S− stimuli are alternated in a successive fashion. Such successive discriminations are also labeled as *go/no-go discriminations*, because the subject's task is to either display or withhold the target response, depending on the stimulus. Operant conditioning procedures also allow for *choice discriminations*, in which S+ and S− are presented simultaneously in separate locations. Most commonly, visual stimuli are used. The subject is reinforced for responding toward S+, but not if it responds toward S−. Thus, responding entails choice behavior, in which both correct responses and errors can be observed. Learning is expressed as an increase in 'percent correct responses,' that is, the proportion of trials on which the subject responds toward S+.

Choice discrimination tasks have been investigated in a wide number of species, ranging from honeybees (Couvillon and Bitterman, 1985) to elephants (Nissani et al., 2005). **Figures 1(d)–1(f)** show examples of choice discrimination learning. Specifically, **Figure 1(d)** shows learning curves obtained using a T-maze. As the name implies, the apparatus consisted of three narrow boxes arranged in a T-shaped pattern. The subjects were mice from two strains, specifically, a control strain and a transgenic strain that mimics Huntington's Disease (Lione et al., 1999). On each trial, a mouse was placed in the central arm. The subject then moved of its own accord to the junction of the maze, where it encountered S+ in one arm and S− in the other arm. In this case, the stimuli were created by making

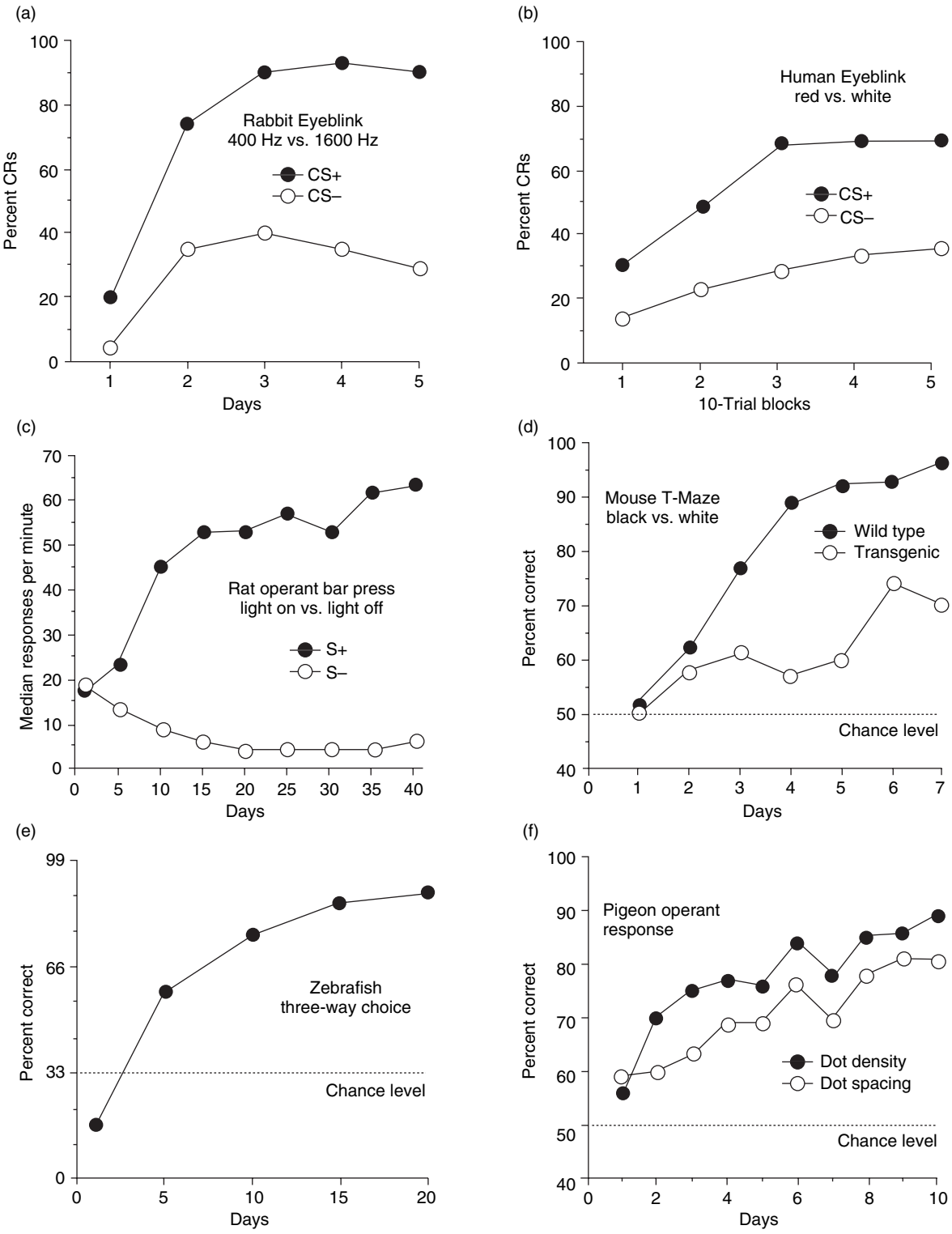

Figure 1 Examples of basic discrimination learning tasks: (a) go/no-go discrimination in rabbit eyeblink conditioning (Moore, 1972); (b) go/no-go discrimination in human eyeblink conditioning (Gynther, 1957); (c) go/no-go discrimination in operant barpress conditioning in rats (Herrick et al., 1959); (d) choice discrimination in two types of mice (Lione et al., 1999); (e) three-way choice in zebrafish (Bilotta et al., 2005); (f) visual on-screen choice in pigeons (Cook, 2001). All figures adapted with permission. CS, conditioned stimulus.

the ceiling of one arm black, and the ceiling of the other arm white. If the subject moved up the S+ arm, it would find a food pellet. If the subject moved up the other arm, there was no food pellet. As can be seen in **Figure 1(d)**, the likelihood of a correct response in the control strain gradually rose from a chance level of 50% correct to a level near 100%, while the transgenic mice only reached a level around 70%.

Figure 1(e) shows the results of a three-way choice discrimination in zebra fish (Bilotta et al., 2005). Each fish was presented with a choice between three windows, one of which was illuminated. The subject was reinforced for swimming through the illuminated window but not the other windows. The location of the illuminated window was randomly altered from trial to trial. Initially, performance was below chance level, because trials on which a subject failed to make any choice were counted. Across days of training, the likelihood of correct responding rose to 80%, at which point training was stopped.

Modern technology allows for the projection of complex visual stimuli on video screens. In some cases, the subject can indicate its choice by touching specific regions on the screen. **Figure 1(f)** shows an example of an on-screen discrimination in which pigeons were presented with a field of regularly spaced dots. The S+ was a square containing either more densely packed dots or irregularly spaced dots. The S+ was placed at a random location on the screen, and the pigeon was reinforced only if it pecked at the S+. As can be seen, the likelihood of pecking at the S+ region reached a level near 90% (Cook, 2001).

In choice discrimination tasks, subjects often display response patterns that prevent them from coming into contact with the discriminative contingencies among the stimuli, responses, and reinforcers. Subjects often adopt a position habit, such as always choosing the left-hand response. Because the S+ and S− appear equally often in left and right positions, a left-hand response will be reinforced 50% of the time. Although this rate of reinforcement is less than the maximum the subjects could obtain, it is sufficient to maintain a position habit. To ensure that the subjects do encounter all the contingencies, a correction procedure may be used. In this procedure, the choice of S− is followed by a *correction trial*, in which only S+ is presented and the subject can only make the correct response. For example, during a correction trial in a T-maze, only the arm associated with S+ will be open.

1.08.2.2 Generalization and Transfer of Training

1.08.2.2.1 Stimulus generalization: immediate, specific transfer

Basic generalization testing entails two stages. The first stage entails reinforced training in which a single stimulus (S+) is used. Once responding to S+ has reached a high level, the second stage begins. In this stage, the subject is presented with a set of test stimuli that differ systematically from S+.

Figure 2 shows three alternative plots for the results of generalization testing (Moore, 1972). In this experiment, three groups of rabbits received eyeblink conditioning in which a 1200-Hz tone was paired with the US (CS+). One group (T1) received single-stimulus training, in which only CS+ trials occurred. The second group (T1-T2) received discrimination training, in which CS+ trials were intermixed with CS− trials using another tone (2400 Hz). The third group (T-L) received training in which CS− was from a different modality, specifically, a brief illumination of the chamber. Following initial training, all three groups were tested with tones of 400 Hz, 800 Hz, 1200 Hz (CS+), 1600 Hz, and 2000 Hz.

Figure 2(a) shows the mean likelihood of a CR as a function of tone frequency. As can be seen, the highest level of responding in all three groups was elicited by the 1200-Hz CS+. As the frequencies of the test tones increasingly deviated from 1200 Hz, responding fell off in a graded fashion. However, the three groups showed some differences in both the absolute height and steepness of their gradients. To make it easier to compare the slopes of the gradients, **Figures 2(b)** and **2(c)** show two types of plots that depict *relative generalization gradients*, which adjust for differences in the level of responding to CS+.

In **Figure 2(b)**, the level of responding to each stimulus is plotted in proportion to the level of responding to CS+. Thus, responding to CS+ is always set to 1.00, and the slopes of the gradients can be readily seen. **Figure 2(c)** shows a method of plotting that emphasizes *stimulus control*, that is, how specific responding is to CS+ relative to the other test stimuli. In this method, the total amount of responding to all tested stimuli is added together, and responding to each stimulus is expressed as a proportion of that total. Thus, taller, sharper gradients indicate greater stimulus control but, conversely, less generalization. Inspection of **Figures 2(b)** and **2(c)** indicate that both discrimination groups showed less generalization, and hence greater stimulus control, than Group T1.

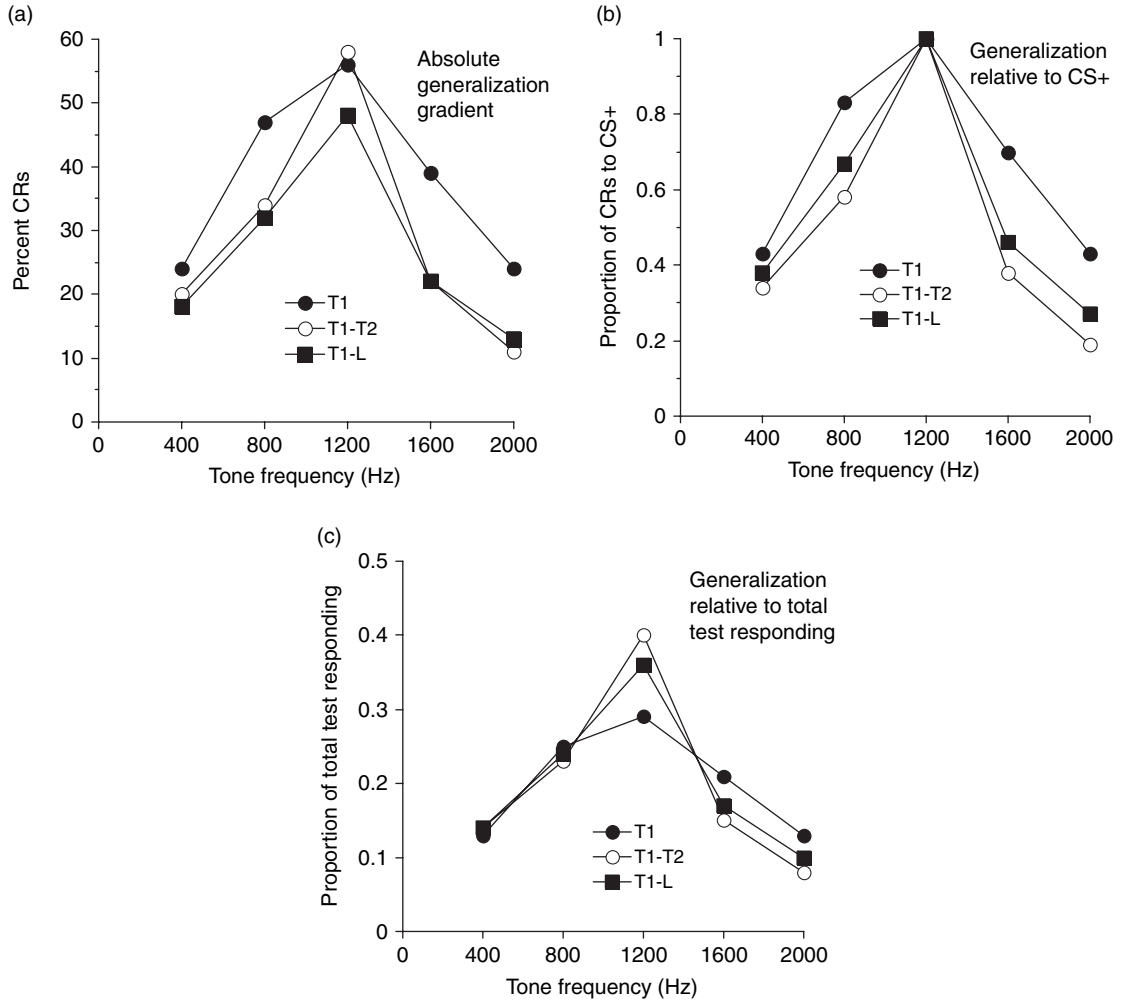

Figure 2 Alternative depictions of generalization gradients in rabbit eyeblink conditioning: (a) Absolute percentage conditioned response (CR) to each stimulus, (b) Responding to each test stimulus as a proportion of responding to CS+, (c) Proportion of total responding to each stimulus, including CS+ (Moore, 1972). All figures adapted with permission. CS, conditioned stimulus.

As shown in **Figure 2**, discrimination training tends to sharpen generalization gradients. Conversely, when efforts have been made to eliminate all potential sources of discrimination training, generalization gradients can be virtually flat. For example, **Figure 3** shows the results of generalization testing after two kinds of operant conditioning in which pigeons were reinforced for pecking a lighted keyswitch (Jenkins and Harrison, 1960). In *nondifferential training*, a 1000-Hz tone (S+) was present whenever the key was lit, which was for 33 s out of every 40 s. The 7-s period without the keylight precluded the pigeons from making the target response. In contrast, in *differential training*, the subjects received training with a go/no-go discrimination, in which S+ trials with a tone were alternated randomly with S− periods in which the keylight was on but the tone was off. The 7-s blackout periods also continued to occur in this latter condition.

In subsequent testing sessions, pecking at the keylight was tested during tones ranging from no tone (0 Hz) to 3500 Hz. As can be seen in **Figure 3**, the level of responding to the test stimuli after nondifferential training was similar to that during S+. That is, there was nearly complete generalization to the test stimuli even though the birds had only ever been trained with a 1000-Hz tone. However, the pigeons were not tone deaf. After differential training, there was a sharp generalization gradient centered on S+.

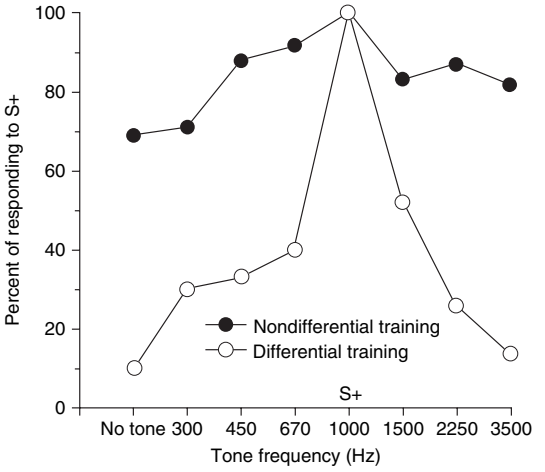

Figure 3 Relative generalization gradients in operant conditioning of pigeon's keypecking after nondifferential training with S+ only versus differential training S+ versus S− (Jenkins and Harrison, 1960). Figure adapted with permission. S, stimulus.

1.08.2.2.2 Other types of transfer

The instances of stimulus generalization described above represent only one of four basic types of transfer. Specifically, stimulus generalization testing reveals the ability of a subject to use its previous learning when it first encounters new stimuli that more or less physically resemble the stimulus used in initial training. This type of transfer can appear immediately on the first test and is specific to stimuli that differ from the training stimulus along one or more sensory dimensions (see Blough, 2001a, for an extensive discussion of the concept of *similarity*.) Provided the appropriate response is retrieved, such immediate specific transfer can be extraordinarily valuable to the survival of subjects when presented with fleeting opportunities and imminent threats. Likewise, human society highly prizes the abilities of paramedics confronted by an accident victim, pilots in an emergency, students in an examination, and even contestants in a quiz show to respond quickly and correctly to situations that are variations on their previous experience.

Despite the premium placed on immediate specific transfer, it is not the sole type of transfer. There are three other ways that transfer can appear. The second type of transfer is immediate but nonspecific. That is, previous learning is applied in situations that bear little similarity at a sensory level to the situations used in training. Instead, nonspecific transfer can be based on structural similarities between the training situation and test situation, for example, analogical reasoning in humans (Gick and Holyoak, 1980; Novick and Holyoak, 1991; Reeves and Weisberg, 1994). The third and fourth types of transfer do not appear on the first encounter with a new situation. Rather, the effect of the previous training is latent and is detected as an increase in the rate of response acquisition during reinforced training in the new situation. A specific form of latent transfer is seen whenever original learning cannot be retrieved immediately but does reappear quickly during refresher training. This type of transfer is known as *savings*. Humans refer to savings whenever they say, "I can't remember how to do it, but I know I can pick it up again quickly." A nonspecific form of latent transfer can appear in situations that share some similarity in their underlying structure, but not in the sensory features of their stimuli. This latent, nonspecific transfer is labeled variously as *learning to learn, learning set*, or *general transfer* (Ellis, 1965, p. 32).

These distinctions among types of transfer are useful for exposition but should not be taken as strict dichotomies. The immediate-versus-latent distinction is relatively clear when the new situations are relatively brief and demand a rapid response. However, when a new situation has less urgent demands, latent transfer could appear during the first encounter if learning occurs as a result of false starts and tentative solutions. As for the specific-versus-nonspecific distinction, it is more accurately construed as a continuum. It is probably difficult, if not impossible, to create situations that differ in every sensory feature from those in original training. In laboratory experiments, however, the number and magnitude of changes can be systematically varied and described.

To ground these distinctions, the remainder of this section will describe examples of the different types of transfer. However, before doing so, a few sentences about the design of transfer experiments are needed. As previously described, the detection and quantification of stimulus generalization uses the level of responding to the training stimulus (S+) as the reference point for comparison to the level of responding to the test stimuli. Such within-subject comparisons are efficient, but one must be confident that there would be little or no responding to the test stimuli in the absence of prior training with S+. Otherwise, a between-subjects design is necessary. At a minimum, there are two groups. The experimental group receives the initial training with S+, and in the basic version, the other group – known as the *rest control* – receives no

training with S+. That is, while the experimental group receives its initial training, the subjects in the rest control group sit either in their home cages or in the experimental chambers. Then, both the experimental and control groups receive the test stimuli for immediate transfer plus any subsequent training with the test stimuli to detect latent transfer. The level responding in the control group provides the reference point for the experimental group. If responding in the experimental group exceeds that of the control group, *positive transfer* or *facilitation* is said to occur. If responding in the experimental group is the lesser of the two, then *negative transfer, interference*, or *retardation* is said to have occurred.

1.08.2.2.2.(i) Immediate, nonspecific transfer A textbook case of immediate, nonspecific transfer would be cross-modal generalization, in which the subjects are trained with a stimulus in one modality (e.g., tone) and then tested with a novel stimulus in another modality (e.g., light). Reports of successful cross-modal generalization are rare. It has been seen in eyeblink conditioning in humans (Marlatt et al., 1966) but not rabbits (e.g., Kehoe, 1992; Weidemann and Kehoe, 2005). Something like immediate, nonspecific transfer, however, has emerged when the test stimuli have previously undergone even a tiny bit of training. **Figure 4** shows the results of two such experiments.

Figure 4(a) shows an example of cross-modal transfer in rabbit eyeblink conditioning (Schreurs and Kehoe, 1987). One group of rabbits (Expt'l) received 15 pairings of the ultimate test stimulus (CSX; e.g., tone) with the US. These CSX-US pairings were too few to yield discernible CR acquisition. At the same time, a rest control group received only exposure to the experimental apparatus. Subsequently, both groups received 240 pairings of a stimulus from another sensory modality (CSA; e.g., light).

During CSA-US training, the subjects were periodically tested with presentations of CSX. As can be seen **Figure 4**, both groups showed CR acquisition to CSA. As CRs were acquired to CSA, Group Expt'l also showed progressive increases in responding on CSX test trials to a level of 34% CRs. In contrast, Group Rest showed no generalized responding to CSX.

Figure 4(b) shows an example of transfer across different visual dimensions (Rodgers and Thomas, 1982). In this experiment, pigeons were given training in which the response key contained a line tilted at either a 60-degree or a 30-degree angle. One group was given go/no-go discrimination training, in which the pigeons were reinforced for pecking at the one line (S+) and not the other line (S−). The other group received *pseudo-discrimination training*, in which pecking at each line was reinforced for a random half of their trials. After this initial training, both

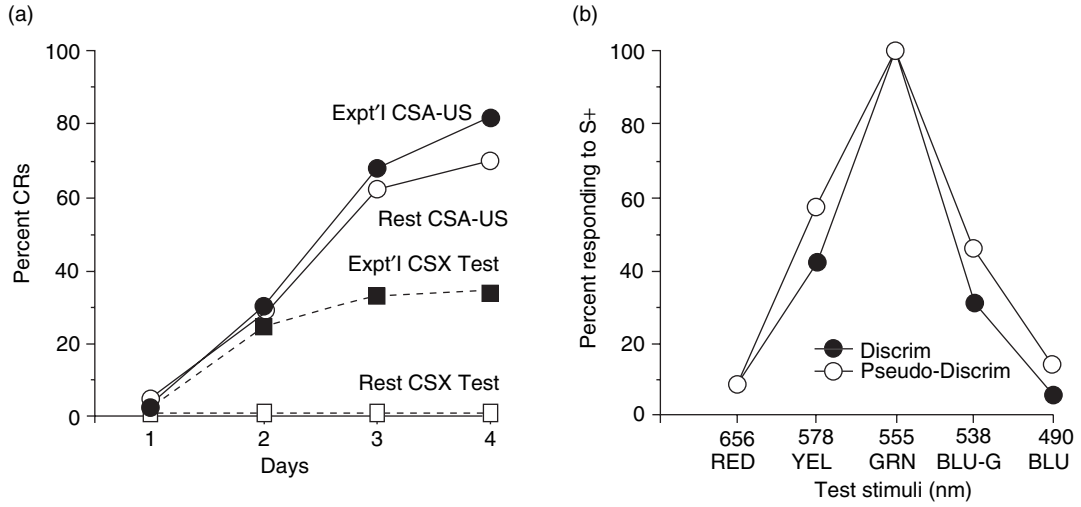

Figure 4 Examples of extradimensional transfer in rabbit eyeblink conditioning (a) and operant conditioning of the pigeon's keypeck (b). Panel (a) shows the results of testing a stimulus from one modality (CSX), while acquisition training was conducted with a stimulus from a different modality (CSA-US). The experimental group had previously received a small number of CSX-US pairings, while the control group had not (Schreurs and Kehoe, 1987). Panel (b) shows the results of generalization testing along the color dimension after pigeons had received either true discrimination training or randomized psuedo-discrimination training with two line-tilt stimuli (Rodgers and Thomas, 1982). All figures adapted with permission. CS, conditioned stimulus; US, unconditioned stimulus; YEL, yellow; GRN, green; BLU-G, blue-green; BLU, blue.

groups were given training in which they were reinforced for pecking at a green (555 nm) keylight. Finally, both groups received generalization tests along the color dimension.

Inspection of **Figure 4** reveals that the discrimination group showed a sharper gradient compared to pseudo-discrimination training. Thus, discrimination training on one dimension altered generalization along an orthogonal dimension.

1.08.2.2.2.(ii) Savings: latent, specific transfer **Figure 5**(**a**) shows two examples of savings. **Figure 5**(**a**) shows percent CRs in four successive cycles of CS-US acquisition and CS-alone extinction in rabbit eyeblink conditioning (Kehoe, 2006). **Figure 5**(**b**) shows the percent correct response in successive reversals of a choice discrimination. Specifically, rats were trained in a T-maze, in which they were reinforced for choosing one arm but not the other. Within each training session, the same choice was always reinforced, but, in the next session, the opposite choice was reinforced (Watson et al., 2006). As can be seen, in both tasks, the successive acquisitions become progressively faster.

1.08.2.2.2.(iii) Learning to learn: latent, nonspecific transfer **Figure 6**(**a**) shows the best known set of curves that demonstrate learning to learn (Harlow, 1949). Rhesus monkeys were trained with a series of 344 discrimination problems, each using a different pair of objects. In each problem, the monkeys were given six or more trials in which to choose between two objects presented on a tray; for example, two different-shaped blocks. If a subject chose the correct object (S+), there was a food reward underneath it. If the subject selected the other block (S−), there was no reward. From one trial to the next, the S+ and S− objects were randomly switched from one side to the other. After a minimum of six trials with one problem, the monkeys were then given another problem using completely different objects; for example, two small toys. Each of the learning curves shows the average percentage of correct choices for successive blocks of problems.

On Trial 1 of each problem, the choice between the two objects was necessarily random. Thus, the learning curve for each problem always starts at 50% correct for Trial 1. For the first set of problems (1–8), the rate of learning was slow. The monkeys' second choice was correct only 52% of the time, and their third choice was correct only 59% of the time. However, as the monkeys accumulated experience with the discrimination problems, the rate of learning progressively accelerated. By the final set (Problems 289–344), the monkeys made nearly 100% correct responses on Trial 2, regardless of their initial choice. In descriptive terms, the monkeys had learned a *win-stay/lose-shift strategy*. That is, if the monkeys won a reward on the Trial 1 by selecting S+, then the monkeys continued to select the same object on

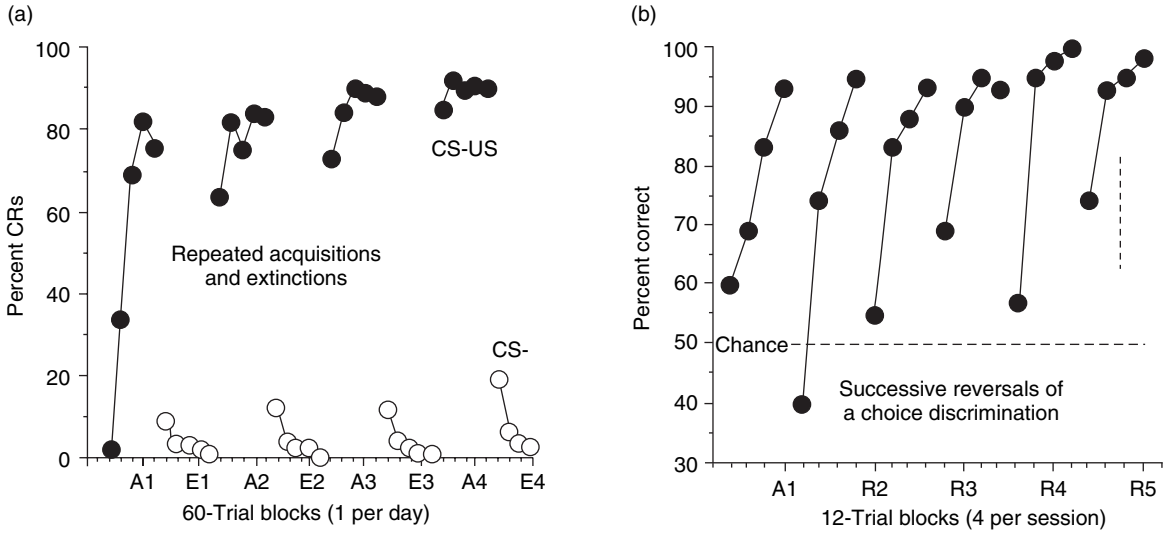

Figure 5 Examples of latent, specific transfer. (a) Repeated acquisitions (CS-US) and extinctions (CS−) in rabbit eyeblink conditioning (Kehoe, 2006). (b) Successive reversals of a choice discrimination in a T-maze by rats (Watson et al., 2006). All figures adapted with permission. CS, conditioned stimulus; US, unconditioned stimulus.

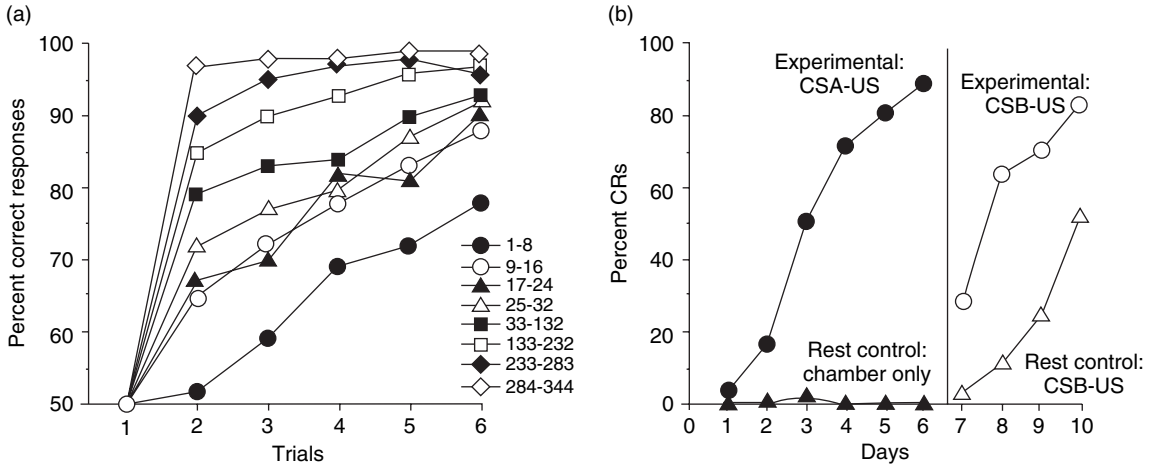

Figure 6 Examples of latent, nonspecific transfer. (a) Learning curves in successive blocks of choice discriminations in monkeys (Harlow, 1949). (b) Learning curves in two successive stages of training with one stimulus from one sensory modality (CSA-US) and a second stimulus from a different sensory modality (CSB-US)(Kehoe and Macrae, 2002). All figures adapted with permission. CS, conditioned stimulus; US, unconditioned stimulus.

subsequent trials. If, however, the monkeys lost by selecting S− on Trial 1, they shifted their choice to S+ on Trial 2 and thereafter.

Learning to learn has also been demonstrated in nonprimate species (Warren, 1960; Kamil et al., 1977). In rats, learning to learn has been obtained using visual patterns in choice discrimination (Wallace and Daniels, 1972), odors in a go/no-go discrimination (Slotnick and Hanford, 2000), and different locations of a platform in the Morris water escape task (Whishaw, 1985). Learning to learn has even been seen in classical conditioning (Kehoe and Holt, 1984; Holt and Kehoe, 1985; Kehoe and Macrae, 1997).

Figure 6(b) shows an example of learning to learn in rabbit eyeblink conditioning. In Stage 1, one group of rabbits (Experimental) received 6 days of CSA-US pairings. For half of this group, the A stimulus was a 1000-Hz tone, and for the other half, the A stimulus was a flashing light. Another group of rabbits (Rest Control) was restrained in the training apparatus without CS or US presentations. At the end of initial training, Group Experimental showed CRs on 90% of CSA-US trials, while Group Rest Control showed only a few, random blinks during blank periods corresponding to CSA.

Subsequently, both groups were tested with a new stimulus (CSB). For Group Experimental, the rabbits initially trained with the tone were tested with the light, and the rabbits initially trained with the light were tested with the tone. Correspondingly, Group Rest Control was also tested with either the light or tone. No immediate transfer was evident in these tests. However, once CSB-US pairings were started, learning to learn became evident. As can be seen in **Figure 6(b)**, Group Experimental showed rapid CR acquisition to CSB. Within the first day of Stage 2, Group Experimental showed CRs on 29% of its CSB-US trials, while Group Rest Control showed CRs on only 3% of its CSB-US trials.

Latent transfer, both in the form of savings and learning to learn, appears to be irreversible and separate from the specific contents of the original learning. **Figure 7** shows the results of two studies that illustrate this point. First, rats were initially reinforced for choosing the right arm of a T-maze (Bunch, 1939). After reaching a criterion of 10 consecutive correct responses, each rat was assigned to one of five groups. All these groups underwent reversal training in which they were reinforced for choosing the left arm of the T-maze. The groups differed in the amount of time that elapsed between initial training and reversal training. One group was immediately switched to reversal training, and the other groups waited in their home cages for 2, 7, 14, and 28 days, respectively. A sixth group was a rest control and only received training with the left-arm task.

Figure 7(a) plots the mean number of errors committed during reversal training by each group prior to achieving 10 consecutive correct responses. Relative to the rest control, Group 0, which received the immediate switch, showed negative transfer. That is, Group 0 showed more errors by persisting in

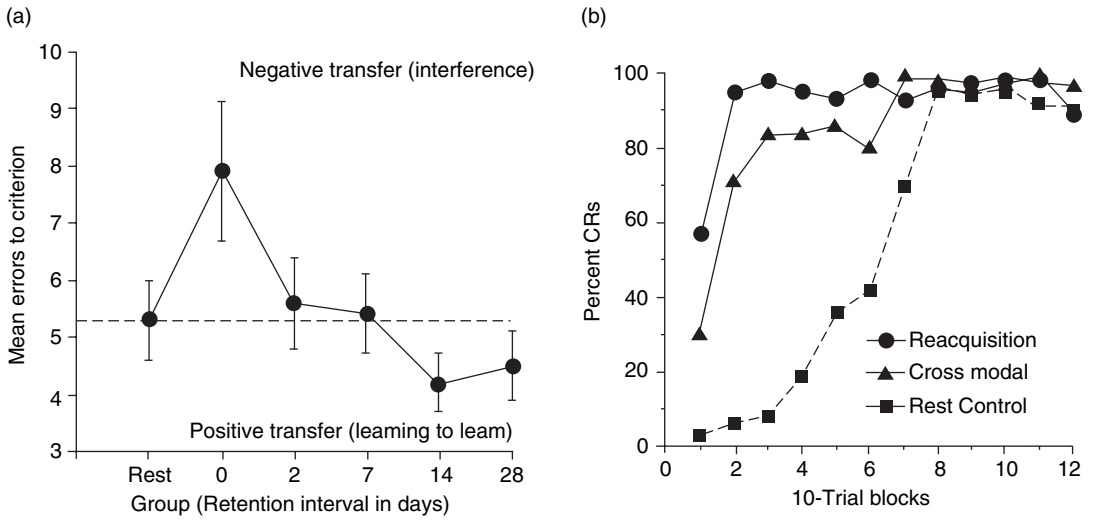

Figure 7 Examples of latent transfer after retention loss and extinction. (a) Reversal of a T-maze response in rats after a range of retention intervals (Bunch, 1939). (b) Reacquisition and cross-modal acquisition after extinction (Macrae and Kehoe, 1999). All figures adapted with permission.

performing the formerly correct response. However, as the retention interval increased, negative transfer progressively disappeared. More importantly, Groups 14 and 28 displayed positive transfer. Specifically, they showed fewer errors during the reversal than the Rest Control group. Thus, as retrieval of the original response faded, learning to learn appeared.

Figure 7(b) illustrates savings and learning to learn after an eyeblink CR was eliminated through extinction (Macrae and Kehoe, 1999). Two groups of rabbits were given initial training with either a tone or light CS. After CRs were established, both groups underwent extinction training using the same CS. After the CR had been thoroughly extinguished, one group (Reacquisition) was tested for savings by recommencing pairings of the original CS with the US. The other group (Cross Modal) was tested for learning to learn by starting pairings of the alternate CS with the US. A third group (Rest Control) was trained with either the tone or light CS. Relative to the rate of CR acquisition in the rest control group, the reacquisition group showed considerable savings. Furthermore, the cross-modal group showed strong learning to learn. In fact, acquisition to the cross-modal CS was nearly as rapid as reacquisition to the original CS.

1.08.3 Psychophysics

A central question in psychology is, "What is the stimulus for a behavior?" Psychophysical research provides the basic methods, results, and theories for answering how a stimulus input is encoded and transformed in the nervous system to eventuate in a behavioral output. In investigating the psychophysics of animals, discrimination learning and generalization testing have played a central role.

1.08.3.1 Sensory Thresholds

There are two kinds of thresholds: absolute and difference. An absolute threshold is the level of intensity of a stimulus at which a subject is able to detect the presence of the stimulus some proportion of the time, usually 50%. A difference threshold is the magnitude of the difference between two stimuli that a subject is able to detect; again, a 50% criterion is common. Figure 8 shows four examples of psychophysical curves using a combination of discrimination training and generalization testing.

1.08.3.1.1 Absolute thresholds

Figure 8(a) shows generalization tests for detecting a sinusoidal modulation in the amplitude of background noise (Kelly et al., 2006). Rats were trained on a lick suppression task, in which a mild shock to the tongue was signaled by a 3-s modulation of the background noise. Training and testing were then conducted by using combinations of modulation depths and modulation rates. The figure shows the mean percent correct as a function of modulation depths for modulation rates of 10 Hz, 100 Hz, and

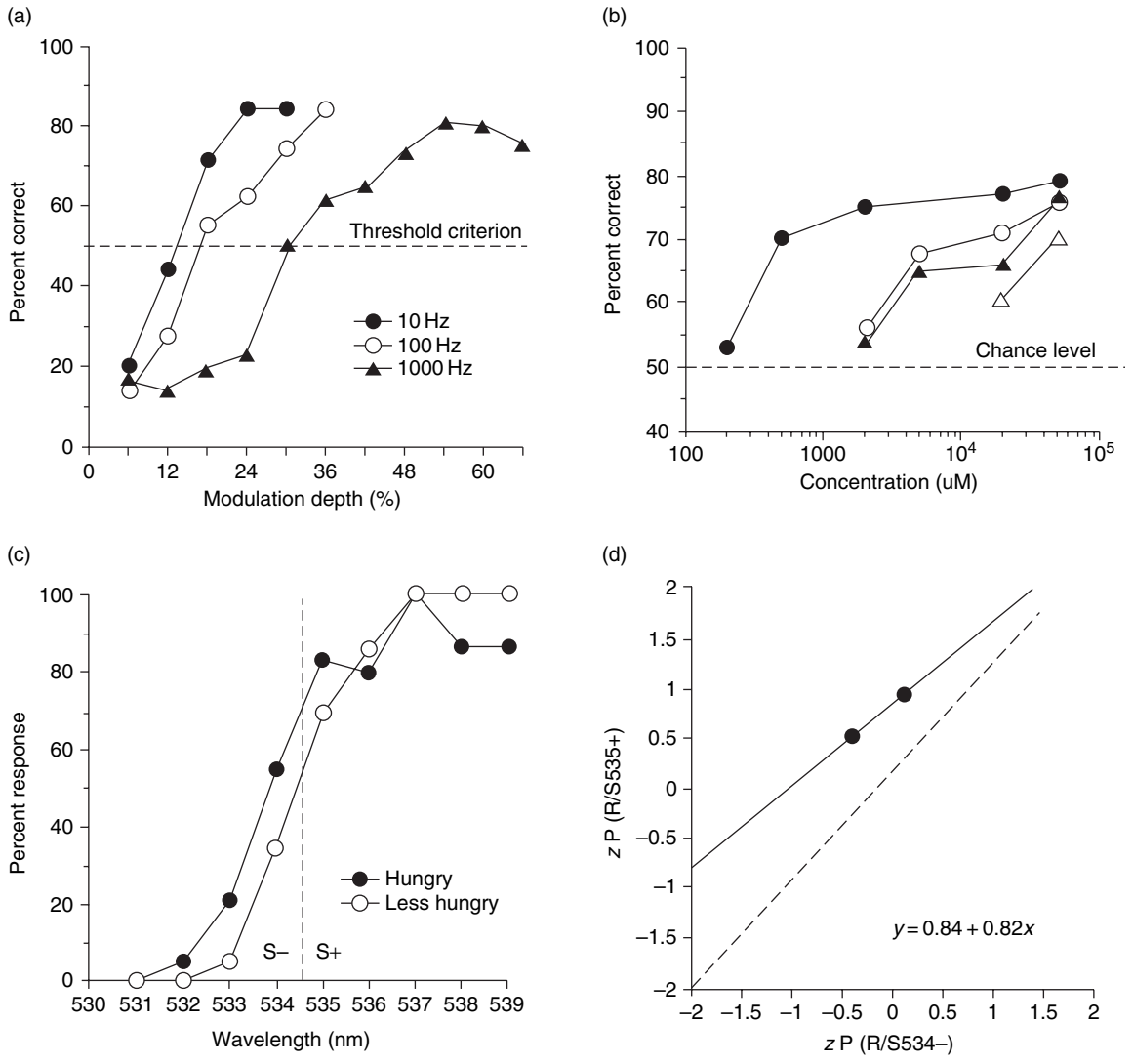

Figure 8 Examples of psychophysical curves. (a) Generalization tests for detection of a sinusoidal modulation in the amplitude of a broadband, background noise by rats (Kelly et al., 2006). (b) Curves of four squirrel monkeys tested for discrimination between increasing dilutions of an androgen-based odorant versus an odorless solvent (Laska et al., 2006). (c) Percent response in pigeons reinforced for pecking at keys of wavelengths of 535 nm and greater (Boneau and Cole, 1967). (d) The relative operating characteristic (ROC) plot for the probability of responding to the 535-nm S+ versus the 534-nm S− (Boneau and Cole, 1967). All figures adapted with permission.

1000 Hz. The absolute threshold for detecting modulation was designated as the point at which the curve for each modulation rate crossed the 50% criterion line. For example, the threshold for detecting a 100-Hz modulation was a 17% depth.

Figure 8(b) shows the individual psychophysical curves of four squirrel monkeys in a choice discrimination task. Specifically, the monkeys were tested for their ability to detect an androgen-based odorant (Laska et al., 2006). On each trial, the monkeys sniffed two strips mounted on two closed food cups. One strip was impregnated with the odorant (S+), and the other with the odorless solvent (S−). The monkeys were then allowed to open one of the cups to discover whether or not it contained the reinforcer. After the initial discrimination was established, training was continued, in which the S+ odorant was progressively diluted until a monkey failed to discriminate it from the solvent. The left-hand point in each curve represents the dilution at which the monkey's choices failed to differ significantly from the 50% chance level.

1.08.3.1.2 Difference thresholds, sensitivity, and criterion

Figures 8(c) and **8(d)** show the results of an experiment aimed at identifying the difference threshold along the color dimension in a pigeon (Boneau and Cole, 1967). The procedure was a combination of discrimination training and generalization testing. A pigeon was reinforced for pecking at wavelengths of 535 nm and above, but not for shorter wavelengths. Two levels of hunger were used on different days. **Figure 8(c)** shows the resulting psychophysical curves, which indicate that the pigeon could detect a difference as small as 1 nm. The pigeon responded more when it was hungrier, displacing the function to the left. However, the slope of the function appeared the same, suggesting that the pigeon's sensitivity to stimulus differences was unaltered. Thus, the hunger is said to bias responding but not sensitivity.

These concepts of sensitivity and bias originate in signal detection theory (SDT) (Suboski, 1967; Blough, 2001b). According to SDT, observed responding results from the joint operation of the subject's ability to detect the stimulus (sensitivity) and their bias to respond (criterion). For a given sensitivity, a change in bias will cause the psychometric function to shift, as seen in **Figure 8(c)** with the effects of hunger. However, a clearer picture of the joint effect of sensitivity and criterion appears in a different type of plot, the relative operating characteristic (ROC). The ROC plots the probability of the target response to S+ against the probability of the response to S−. **Figure 8(d)** shows the ROC for the 535-nm S+ and the 534-nm S− under the two hunger conditions. The two points are plotted on axes scaled in z scores. The two points fall on a line that lies above the diagonal. The distance of the line above the diagonal is a measure of the sensitivity, while the position of the points along the line indicates the criterion. A slope of 1.00 indicates that the sensitivity is constant across the factors that influence the criterion. Conversely, deviations in the slope indicate that a manipulation influences sensitivity. In this case, a slope less than one suggests that, in fact, sensitivity declined as hunger increased.

1.08.3.2 Psychophysics of Memory

Discrimination training and generalization testing have been extended to the study of memory, often using a type of choice discrimination. In the basic version, called delayed matching to sample (DMTS), the subject is presented with one stimulus called the sample. Then, the subject is presented with pair of target stimuli. Choosing the stimulus that matches the sample results in reinforcement (S+), while choosing the other stimulus (S−) does not yield reinforcement. By manipulating the interval between the sample stimulus and target stimuli across trials, short-term memory for the sample stimulus can be mapped.

Figure 9 plots the results of two recent DMTS studies using rats and a single monkey, respectively. The rat study entailed a two-way spatial discrimination (Harper et al., 2006). On each trial, the sample stimulus was a presentation of a retractable bar on one side or the other of a wall in the test chamber. To ensure the sample bar was attended to, the rats were trained to press the bar three times before it was withdrawn. A delay interval of 0.1, 3.0, 9.0, or 18.0 s was then imposed. To prevent the rats from simply facing the location of the sample bar during this interval, they were trained to turn around and press another bar behind them. On completion of the delay, both the left and right bars were inserted into the chamber. As soon as either bar was pressed, the bars were retracted, and if the rat had pressed the previous sample bar, the reinforcer was delivered. As can be seen in **Figure 9**, accuracy in the choice response gradually declined when the delay interval exceeded 3 s, but even after 18 s, the level of performance still exceeded the chance level (50%).

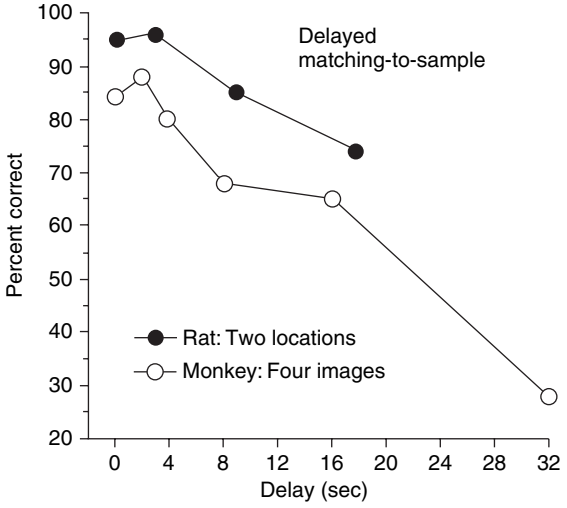

Figure 9 Percent correct choices in delayed-matching-to-sample tasks in rats (Harper et al., 2006) and a monkey (Hampton and Hampstead, 2006) as a function of delay interval between presentation of the sample and choice stimuli. All figures adapted with permission.

The other curve in Figure 9 shows the results obtained from a monkey in a four-way visual discrimination, for which the sample and test stimuli were images of 'clip art' (Hampton and Hampstead, 2006). On each trial, the sample stimulus appeared in the center of a touchscreen. After the monkey contacted this image, one of six delay intervals (0, 2, 4, 8, 16, or 32 s) was imposed. Then, four test stimuli appeared, one in each corner of the screen. Only one of the test stimuli matched the sample stimulus. A large set of images was used such that each image was seen only once in each session, which contained 48 trials. The monkey's performance showed a progressive decline but only sank to its chance level (25%) after a 32-s delay.

1.08.3.3 Psychophysics of Time

Timing is everything, and not just in comedy. Predators must pounce at just the right moment to catch their next meal, suitors must pick the right moment to make their advance, and prey must pick the right moment to dash for cover. Even a widespread, basic protective response like the eyeblink has demands on its timing. A blink that is too early or too late will not protect the eye from injury, and a blink that is too long may effectively blind an individual at a crucial moment.

Figure 10 shows a psychophysical function for stimulus duration (Church and Deluty, 1977). Rats were first trained in a choice discrimination. A press on one bar was reinforced after a 2-s light stimulus, and a press on another bar was reinforced after an 8-s light stimulus. After this training, the rats were tested with stimuli of intermediate durations. The curve in Figure 10 shows the mean percentage of tests in which the rats pressed the bar for the longer, 8-s signal. Under these conditions, the difference threshold was slightly greater than 4 s, a point approximately equal to the geometric mean of the 2-s and 8-s durations used in training.

A wide variety of studies have tracked the time course of responding based on the interval between a stimulus and a reinforcer. These studies have yielded two reliable results that are illustrated in Figure 11. Figure 11(a) shows the time course of an eyeblink CR in four groups of rabbits that were trained with four different intervals between CS onset and US onset, specifically, 125, 250, 500, and 1000 ms (Smith, 1968). The results are based on sporadic test trials in which the CS was presented without the US, and hence without any intrusion from the unconditioned

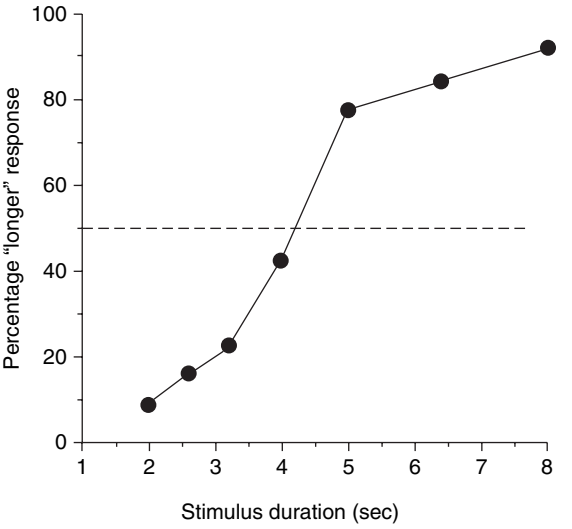

Figure 10 Psychophysical function in for stimulus duration after choice discrimination training in which rats were reinforced for pressing one lever when signaled by a 2-s stimulus and for pressing another lever when signaled by an 8-s stimulus (Church and Deluty, 1977). Figure adapted with permission.

response (UR). Similarly, Figure 11(b) shows the time course of barpress responding in a group of rats that had been trained in a temporal discrimination using a noise and a light (Roberts, 1981). For one stimulus, the first barpress after 20 s elapsed was reinforced by food. For the other stimulus, the first barpress after 40 s was reinforced. The results are based on test trials in which each stimulus was presented for 80 s without the reinforcer. Finally, Figure 11(c) shows the results of a study in which human participants were trained with stimuli of either 8, 12, or 21 s (Rakitin et al., 1998). After exposure to the target stimulus, the participants were asked to press a key when they thought that the appropriate time had passed.

Despite the large differences in the time scales, procedures, and species used in these three studies, responding showed similar temporal patterns. In each case, the peak of responding was well aligned to the interval used in training. Moreover, the variability in the placement of responding increased proportionally as the interval increased. Together, these proportional increases in the time and variability of peak placement are known as the 'scalar property' of timing. The theoretical and neural mechanisms responsible for the acquisition of response timing and its scalar property have been a matter of intensive research and theorizing in recent

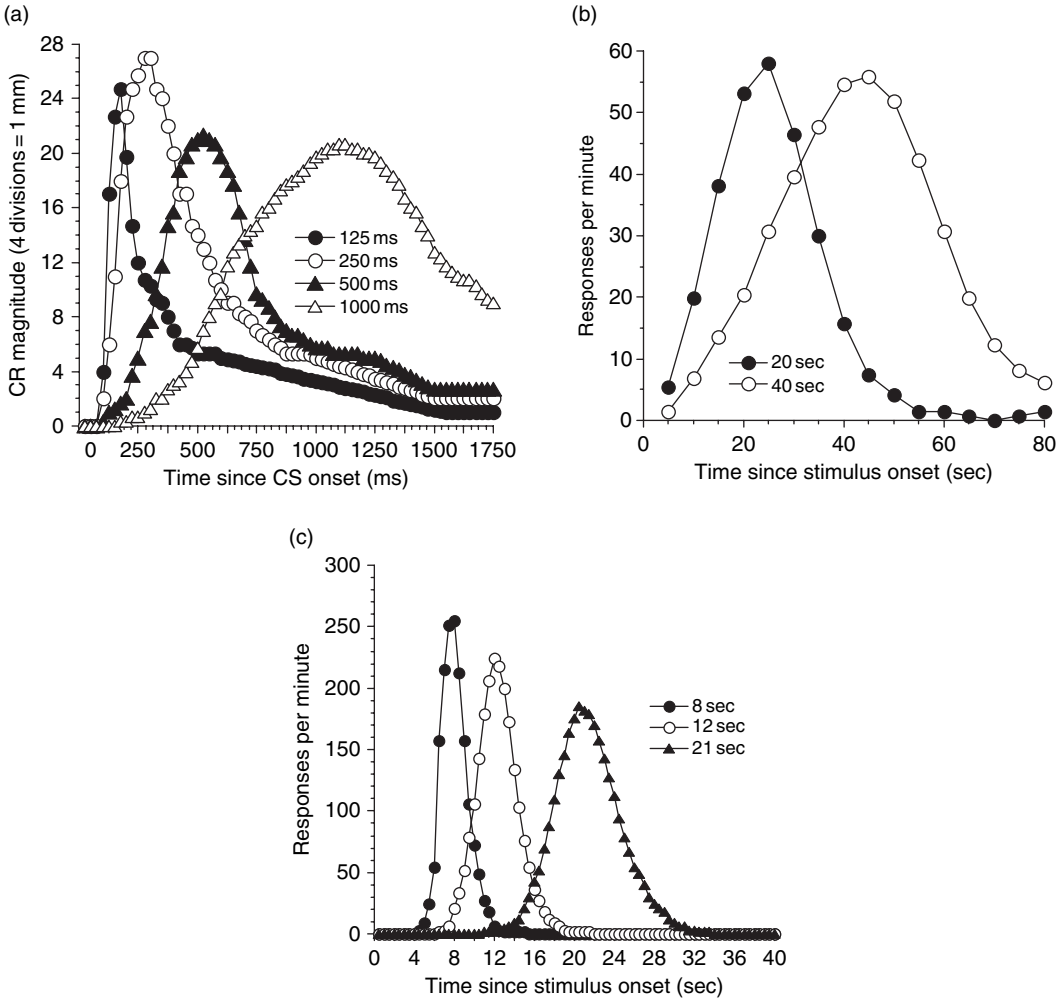

Figure 11 Examples of response timing in (a) rabbit eyeblink conditioning after training with different CS-US intervals (Smith, 1968), (b) barpressing by rats after training with two fixed intervals signaled by different discriminative stimuli (Roberts, 1981), and (c) reproduction of different stimulus intervals by humans (Rakitin et al., 1998). All figures adapted with permission. CS, conditioned stimulus; US, unconditioned stimulus.

years (e.g., Gallistel and Gibbon, 2000; McGann and Brown, 2000; Medina et al., 2000; Buhusi and Meck, 2005).

1.08.4 Excitatory and Inhibitory Learning Processes

Pavlov (1927) borrowed the terms of *excitation* and *inhibition* from neurophysiology to describe the processes underlying rises and falls in conditioned responding. Among other things, he proposed that discrimination learning could entail both excitatory learning for CS+ and inhibitory learning for CS−. Later, Spence (1936, 1937) extended these ideas to generalization testing after discrimination training. He proposed that responding to test stimuli would be influenced jointly by generalization from both S+ and S−. That is, the level of responding to each test stimulus would reflect an algebraic sum of generalized excitation from S+ and generalized inhibition from S−.

1.08.4.1 Transfer Tests of Inhibition

The measurement of inhibitory associative strength has not been a simple matter for two reasons. First, the absence of responding to a CS− may reflect a lack of excitatory learning rather than a negative association. Hence, the level of inhibitory learning

can only be inferred from the ability of the CS− to disrupt excitatory learning in a transfer test. Second, identifying transfer tests that isolate the effect of inhibitory learning from other possible disruptive factors has been difficult (Williams et al., 1992; Papini and Bitterman, 1993).

Two transfer tests have been used in tandem to determine whether a CS has acquired an inhibitory association (Brown and Jenkins, 1967; Rescorla, 1969; Cole et al., 1997). These two tests are known as the *summation* test and the *retardation* test. A candidate CS passes the summation test if it reduces CRs to another excitatory stimulus presented at the same time. Second, the candidate CS passes the retardation test if it shows a slow rate of CR acquisition when paired with the US.

Figure 12 illustrates the results of summation and retardation tests in fear conditioning in rats (Cole et al., 1997). The subjects were first given a discrimination learning task originated by Pavlov (1927), known as the *conditioned inhibition* procedure. Specifically, one stimulus was paired with an aversive US (A+), while a compound of two stimuli was presented alone (AX−). In addition, another stimulus was established as an excitatory stimulus by pairing it with the US (B+). Acquisition of excitatory fear conditioning was indexed by an appetitive response, specifically, licking water from a drinking tube. Thus, the greater the fear, the longer the time needed to complete a specified number of licks.

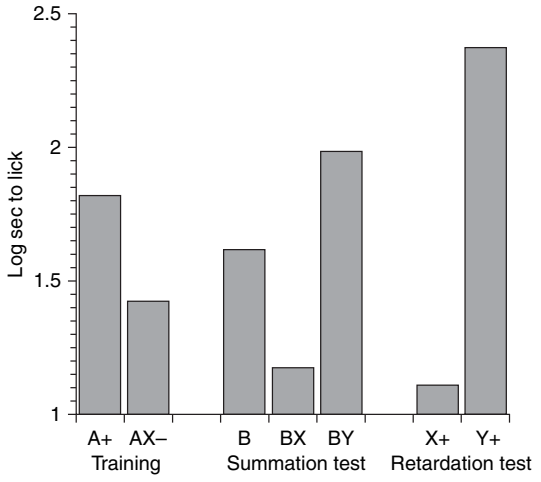

Figure 12 Results of summation and retardation tests after rats were given conditioned inhibition training, in which one stimulus was paired with an aversive US (A+), while a compound of two stimuli was presented alone (AX−). A third stimulus had also been paired with the US (B+), and a fourth stimulus was novel at the time of testing (Y) (Cole et al., 1997). Figure adapted with permission.

As can be seen in the left-hand portion of **Figure 12**, moderate discriminative responding to A+ versus AX− was achieved during initial training. Summation and retardation tests were conducted by splitting the subjects into five groups. For the summation test, one group was tested with the conditioned excitatory stimulus (B), another group was tested with a compound of the B stimulus plus the candidate inhibitor (BX), and a third group was tested with a compound of the B stimulus plus another stimulus (Y) that had not undergone any training (BY). This latter group was included to test whether compounding a neutral stimulus with B would disrupt the fear response. For the retardation test, the fourth group received pairings of the candidate inhibitor with the US (X+), and the fifth group received pairings of the untrained stimulus with the US (Y+).

Examination of **Figure 12** reveals that the candidate inhibitor (X) passed the summation test. That is, the BX compound yielded a lower level of responding than B stimulus alone. This reduction could not be attributed to unlearned effect of another stimulus, because the BY compound elicited a greater response than the B stimulus. Furthermore, the X stimulus also passed the retardation test. After X+ and Y+ pairings, the X stimulus produced a negligible response, while the Y stimulus produced a substantial response.

The two tests for associative inhibition are complementary (Rescorla, 1969). Together, they rule out different sources of response interference that do not reflect associative inhibition. The summation test reveals that the candidate CS has an antagonistic effect on responding to an excitatory stimulus. However, this antagonistic effect could have also occurred through either a division of attention between the two stimuli or a perceptual interaction between the two stimuli that would obscure the excitatory CS. The retardation test rules out these possibilities, because training with a single candidate CS is not subject to such influences from another stimulus. However, the retardation test by itself is not sufficient to demonstrate an inhibitory association, because slow CR acquisition may result from inattention to the CS (Lubow and Moore, 1959; Reiss and Wagner, 1972; Lubow, 1989; Schmajuk et al., 1996) In turn, the summation test guards against inattention, because attention to the candidate CS is needed for it to reduce responding to the excitatory CS.

The expense of conducting a thorough assay for conditioned inhibition like that of Cole et al. (1997) might seem prohibitive. However, more compact

designs are possible, albeit with some risk that multiple tests of the same subjects will alter the results. If four distinctive stimuli can be found, then a single group can be used. The subjects would first receive training identical to that used in Cole et al. (1997), specifically, A+, AX−, and B+. Subsequently, the summation test would be conducted using tests of BX versus B. If the testing, which is usually done without the US, reduces overall responding, refresher training can be conducted. Then, retardation can be assessed by pairing the candidate stimulus X with the US (X+) while a novel stimulus Y is also paired with the US (Y+). If there is mutual generalization between X+ and Y+ trials, this test will be conservative and reduce the differences in acquisition to the stimuli. If only three distinctive stimuli can be identified, then a rest control group or something like it would be needed to conduct the retardation test.

1.08.4.2 Generalization after Discrimination Training

Generalization testing has been used to infer whether or not S− has acquired inhibitory associative strength. Spence (1937) himself conducted experiments aimed at testing whether his theory could explain demonstrations of *transposition* in generalization tests after choice discrimination training. For example, chimpanzees were initially rewarded for selecting the larger of two squares, e.g., 160 cm^2 (S+) versus 100 cm^2 (S−). The subjects were then tested with other pairs of squares. Of particular interest was the pair that contained the S+ (160 cm^2) versus a larger square (256 cm^2). For this pair, the subjects selected the original S+ on only 22% of trials and, conversely, selected the larger square on 88% of the trials. Findings like this suggested that the subjects were learning a relational property of *larger than*.

Spence (1937) argued that the transposition results could be explained by the net amount of excitation and inhibition activated by each stimulus. **Figure 13** illustrates this hypothesis. In the figure, associative strength is plotted as a function of the value of stimuli along a dimension, e.g., size. There is a gradient of generalized excitation around S+ and a gradient of generalized inhibition around S−. The net amount of associative strength activated by any one stimulus is represented by the third gradient, which is the point-by-point algebraic sum of the excitatory and inhibitory strengths. As may be apparent, there is a region of stimulus values that have a larger net associative strength than the S+ (Alberts and Ehrenfreund, 1951; Ehrenfreund, 1952).

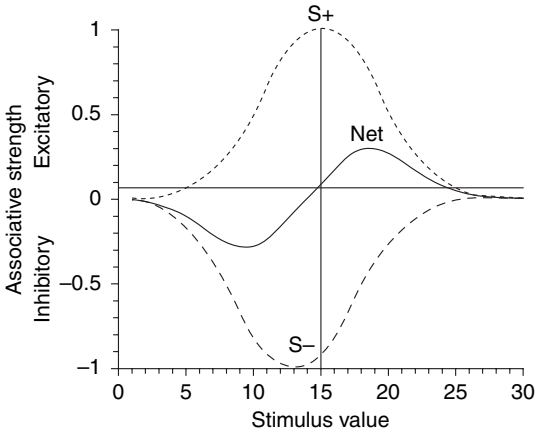

Figure 13 Theoretical net gradient based on hypothetical excitatory and inhibitory generalization gradients established during discrimination training with an S+ and S− along a stimulus dimension.

This hypothesis has also been used to explain the results of generalization testing after go/no-go discrimination training. After training with an S+ and S− along a stimulus dimension, generalization testing can produce asymmetric gradients (Ghirlanda and Enquist, 2003). In some cases, the peak of the gradient is shifted away from S+ (Hanson, 1959). In other cases, the peak of the gradient remains at S+, but the subjects respond more to test stimuli on the S+ side of the dimension than to stimuli on the S− side. This type of result is called an area shift.

Figure 14 shows an example of peak shift in a single horse (Dougherty and Lewis, 1991). Initially, the subject was reinforced with food for pressing a bar during a 60-s S+, which was a 2.5-in (63 mm) circle. After this single-stimulus training, generalization testing was conducted with a series of circles ranging in diameter from 0.5 in (13 mm) to 4.5 in (114 mm). As can be seen in **Figure 14**, the generalization gradient was more or less symmetric and centered on S+. Then, discrimination training was conducted, in which a 1.5-in (38 mm) circle was introduced as S−. After discriminative responding was established, further generalization testing occurred. The resulting gradient was shifted away from S+ and S−, and its peak was located at the 3.0-in (76 mm) circle.

The peak-shift and area-shift phenomena are consistent with the excitation-inhibition hypothesis (Spence, 1937). However, there are well-developed alternative theories that can explain these phenomena without resorting to associative inhibition (Blough,

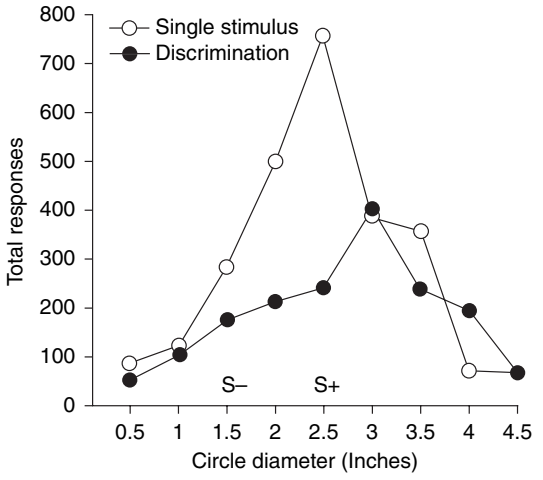

Figure 14 Peak shift after discrimination training. Generalization gradients of an operant response shown by a horse after single stimulus training and subsequent discrimination training along a size dimension (Dougherty and Lewis, 1991). Figure adapted with permission.

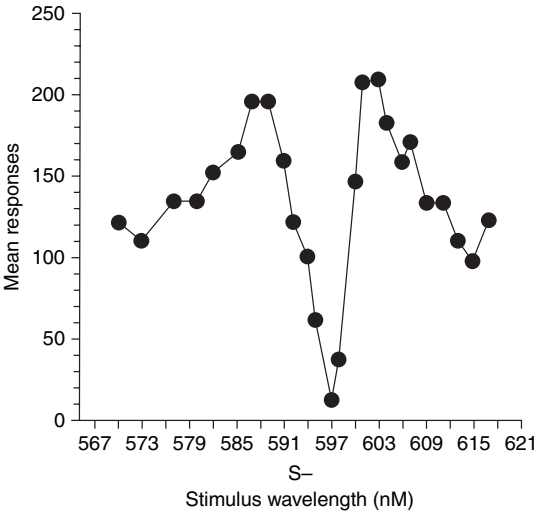

Figure 15 Generalization gradient obtained in pigeons during discrimination training in which keypecking to all stimuli along the wavelength dimension, except one (597 nm), was reinforced (Blough, 1975). Figure adapted with permission.

1975; Ghirlanda and Enquist, 2003). Furthermore, attempts to study generalization gradients during sustained operant training have yielded results contrary to the excitation-inhibition hypothesis (Blough, 1975; Hinson and Termison, 1997).

In these latter studies, discrimination training and generalization testing are conducted simultaneously, much like the psychophysical procedures described previously. Specifically, the subjects, often pigeons, are initially given reinforced training with multiple stimuli along a sensory dimension (e.g., visual wavelength). Once responding has been established to all the stimuli, discrimination training is begun. In positive discrimination training, responding to one stimulus (S+) continues to receive reinforcement, but responding to all the other stimuli along the dimension do not receive reinforcement. In the negative procedure, responding to all of the stimuli, bar one (S−), is reinforced.

Both procedures yield generalization gradients on a sustained basis. The positive discrimination yields a generalization gradient centered on S+. Conversely, the negative discrimination yields what has been described as an inhibitory gradient, in which responding is reduced at the S− value and also at nearby stimulus values (Jenkins and Harrison, 1962). An example of such an inhibitory gradient is shown in **Figure 15**.

The problem for the excitation-inhibition hypothesis arises from the shoulders that appear in the gradients at stimulus values that are at an intermediate distance from S+ or S−. In **Figure 15**, these shoulders appear for the stimulus values around 587 and 603 nm. According to the excitation-inhibition hypothesis, the negative impact of generalized inhibition from S− on responding to the excitatory stimuli should diminish in a smooth fashion. Instead, these contrast effects have been explained by models that do not use the construct of inhibition (Blough, 1975; Hinson and Termison, 1997).

1.08.4.3 Inhibition and Nonassociative Contributions to Responding

Revival of interest in learned inhibition has complicated the methodology of classical conditioning (Rescorla, 1967; Gormezano and Kehoe, 1975). In measuring excitatory conditioning, it would be ideal if each observed response were based entirely on the subject's history with the training manipulations, for example, the number of prior CS-US pairings. Not surprisingly, this ideal has never been achieved. The target response can and does occur during the CS in the absence of any CS-US pairings. The sources of this *nonassociative* responding include spontaneous occurrences of the target response, any innate tendency for the CS to evoke the target response, and pseudoconditioning, which is a sensitization-like effect arising from presentations of the US (Grant, 1943; Gormezano, 1966; Sheafor, 1975). Furthermore, in classical and also operant conditioning, performance of the learned response can be elevated or depressed

by a variety of third factors, e.g., fatigue, satiation, drugs, and individual differences in responsiveness.

In classical conditioning, a suite of control conditions has evolved for determining what proportion of responding during the CS arises from CS-US contiguity versus nonassociative sources. These control conditions traditionally include CS-alone presentations, US-alone presentations, and explicit unpairings of the CS and US. If one's goal is to obtain a pure measure of the excitatory associative strength of CS, then be prepared to be frustrated. None of these control groups provides an unbiased estimate of nonassociative responding. In particular, explicitly unpaired presentations of the CS and US, in which they are well separated in time, can foster inhibitory learning (Rescorla, 1967; Siegel and Domjan, 1971; Napier et al., 1992). Rescorla (1967) advocated a control in which the presentations of the CS and US were randomized in time. However, this truly random control itself can be biased. In particular, CS-US pairings can randomly occur. When they do, they produce excitatory conditioning and thus yield an overestimate of the nonassociative contributions (Ayres et al., 1975). Despite the possible biases, together these controls do guard against a gross overestimation of the excitatory associative effects of CS-US pairings (Kehoe and Macrae, 2002).

The implementation of the full suite of controls in separate groups of subjects dramatically increases the size of an experiment. Each subject, however, can serve as its own nonassociative control by using a discrimination procedure (Schneiderman, 1972; Schneiderman et al., 1987). Specifically, responding to CS+ reflects the total contribution of excitatory associative effects plus any nonassociative contributions. In contrast, responding to CS− would ideally reflect only nonassociative contributions. However, just as with the separate control conditions, this estimate is probably biased in two opposing ways. On the one hand, responding to CS− may be elevated by excitatory generalization from CS+. On the other hand, the explicit unpairing of CS− and the US may promote inhibitory learning that will reduce responding to CS−, and through generalization, it may also reduce responding to CS+. In summary, the difference in responding to CS+ versus CS− will be a conservative estimate of the net associative effect of excitatory and inhibitory associative learning.

1.08.4.4 Commentary

The concept of learned inhibition has been a powerful but elusive concept. Whether a form of negative association is needed to explain any or all of the phenomena remains an open question. Even the results of the conditioned inhibition procedure may be explained without assuming a negative association (Miller and Matzel, 1988). Regardless of the fate of inhibition as a theoretical construct, the empirical phenomena suggestive of inhibition provide a valuable means for investigating the ability of learning mechanisms to regulate responding downward as well upward.

1.08.5 Perception, Attention, and Cognition

Research using discrimination learning and generalization testing has progressively revealed a variety of phenomena that come under the headings of perception, attention, and cognition. There has been – and undoubtedly will continue to be – vigorous debate concerning the value of top-down versus bottom-up explanations. The transposition effect is an early example of a phenomenon that seems to require a top-down explanation, but as seen in excitation-inhibition theory (Spence, 1937), the effect may be explained by an imaginative extension of bottom-up, associative mechanisms. Other chapters in this volume describe in greater detail the theory and research surrounding the increasing number of higher-order phenomena that have been identified. The remainder of this chapter describes some key phenomena that illustrate the application of discrimination and generalization methods in these areas.

1.08.5.1 Compound Stimulus Paradigms

A compound stimulus in its simplest form consists of two elements, often a tone and a light, presented together. By varying the conditions of training for the compound and its elements in relatively small ways, a startling, diverse set of phenomena has appeared. In turn, these phenomena have driven key developments in conditioning theory since the late 1960s (Kamin, 1968; Wagner et al., 1968).

1.08.5.1.1 Compound versus element discriminations

There are four basic discrimination procedures entailing a compound and its elements, which are denoted the *feature-negative, feature-positive, negative patterning,* and *positive patterning* procedures. With the exception of the feature-positive procedure (Jenkins and Sainsbury 1969; Jenkins, 1973), they

originated with Pavlov, who used them to demonstrate both conditioned inhibition (Pavlov, 1927, pp. 68–87) and the perceptual synthesis of stimuli (Pavlov, 1927, p. 144). **Figure 16** depicts examples of the outcomes from these four procedures as seen in rabbit eyeblink conditioning, using a compound of tone and light elements (Kehoe, 1988).

1.08.5.1.1.(i) Feature-negative and feature-positive discriminations **Figures 16(a) and 16(b)** show the acquisition of feature-negative and feature-positive discriminations between a compound versus one of its elements. The feature-negative discrimination is, in fact, the same as used in the conditioned inhibition task. That is, the compound is presented alone (AX−), while one element is paired with the US (A+). Conversely, in the feature-positive procedure, the compound is paired with the US (AX+), and one element is presented alone (A−). The A stimulus is labeled as the *feature cue*, because it is the distinguishing element on AX versus X trials. The X stimulus is labeled as the *target stimulus*. In the experiments described here, the X stimulus was presented sporadically (X test) to determine the level of responding it alone would elicit.

As can be seen, discriminative responding was acquired in both tasks. The feature-negative task yielded a high level of responding on A+ trials, a low level of responding on AX− trials, and virtually no responding on X tests (Marchant et al., 1972; Solomon, 1977). In the feature-positive task, CRs were quickly acquired on AX+ trials, while responding on A− trials grew only slightly (Kehoe and Schreurs, 1986). Tests with the X stimulus revealed that it elicited nearly as much responding as did the AX+ trials.

Both these results have suggested that the level of responding to the compound reflects a summation of the separate associative strengths of the separate elements. Thus, the low level of responding on AX− trials in the feature-negative task can be attributed to the algebraic sum of A's positive strength and

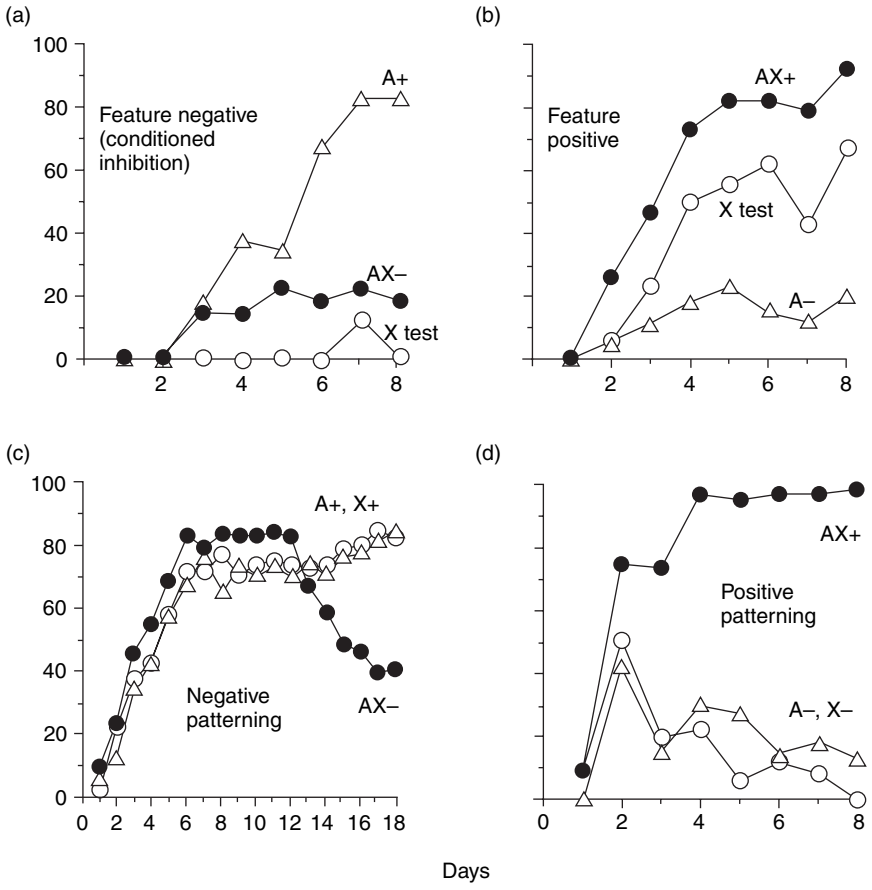

Figure 16 Compound-versus-element discrimination learning in rabbit eyeblink conditioning using tone and light elements (Kehoe, 1988). Figure adapted with permission.

X's unseen negative strength. In the feature-positive case, the high-level responding on AX+ trials can be explained as the sum of the high positive strength of the X stimulus and the low positive strength of the A stimulus. However, this elemental summation seems at most to be true only for cases like these. As will be described below, different processes appear to be engaged when these circumstances are varied (Kehoe and Gormezano, 1980).

1.08.5.1.1.(ii) Negative and positive patterning **Figures 16(c)** and **16(d)** show the results of discrimination training using a compound and both its elements. In these patterning tasks, no element is strictly a feature cue or target stimulus, but for the sake of consistency in exposition, the elements remain labeled as A and X. **Figure 16(c)** shows the results of negative patterning. The early training sessions contained a mixture of trials in which the individual elements were each repeatedly paired with the US (A+, X+). The compound without the US (AX−) was presented only occasionally. Once the CR was established on A+ and X+ trials, more AX− trials were added. By the end of the experiment, each session contained 40 AX− trials, 10 A+ trials, and 10 X+ trials. Responding on AX− trials gradually declined, while responding on A+ and X+ trials was maintained. In contrast to the slow acquisition of negative patterning, discriminative responding (**Figure 16(c)**) emerged rapidly in the positive patterning procedure, in which the compound was paired with the US (AX+), and the elements were not (A−, X−).

Patterning has attracted considerable theoretical interest. In particular, the low level of responding on AX− trials in negative patterning cannot be explained by the summation of the elements' positive associative strengths. Since the first demonstrations of patterning (Pavlov, 1927, p. 144), it has been widely supposed that there is a perceptual synthesis of the elements to form an additional configural stimulus that can acquire its own associative strength, either alongside the elemental associations (Rescorla, 1973; Kehoe, 1988) or in place of them (Bellingham et al., 1985; Pearce, 1987, 1994). As an alternative to configural theories, other theories postulate that elements and compounds have distinctive and shared features. By the appropriate assignment of excitatory and inhibitory strengths to these features, patterning can be explained (Estes and Burke, 1953; Kehoe and Gormezano, 1980; Harris, 2006). Apart from these theoretical debates, patterning procedures have been used in distinguishing the neural substrates for "simple" conditioning for single CSs from the substrates of more complex processes engaged by compound stimuli (Sutherland and Rudy, 1989; Rudy and Sutherland, 1995; O'Reilly and Rudy, 2001).

1.08.5.1.1.(iii) Serial conditional discriminations The discriminations described above all entailed compounds in which the elements were presented simultaneously. There are corresponding tasks that use stimuli presented in a serial fashion to form conditional discriminations. The delayed matching-to-sample task, previously described, is one form of a serial conditional discrimination. **Figure 17** shows the acquisition of discriminative behavior to a target stimulus (X) in three serial procedures, which are denoted, respectively, the *serial feature positive*, the *serial*

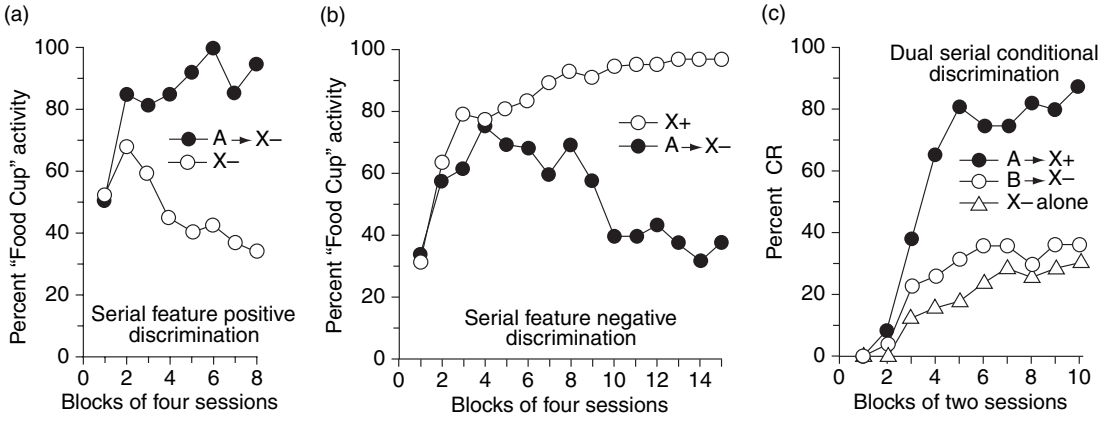

Figure 17 Acquisition of discriminative behavior to a target stimulus (X) in serial conditional discriminations, specifically a feature-positive procedure (a) and a feature-negative procedure (b) in appetitive conditioning of rats (Holland et al., 1999), plus a dual-feature procedure in rabbit eyeblink conditioning (Kehoe et al., 1987). All figures adapted with permission.

feature negative, and the *dual serial conditional* procedures. In the first two cases, the appetitive behaviors of rats for a food reinforcer were measured during the target stimulus (Holland et al., 1999). In the third case, conditioned eyeblinks in rabbits were measured during the target stimulus (Kehoe et al., 1987).

In all three cases, the feature cues (A, B) were presented before the target stimulus (X). This sequencing permits responding during the target stimulus to be measured under the influence of the prior feature cues. To denote this arrangement, the serial feature positive procedure is described as A→X+ versus X−, and the serial feature negative procedure is denoted as A→X− versus X+. For the discriminations shown here, the feature cue was a 5-s light (A) followed by a 5-s gap before the target stimulus (X), which was a 5-s tone. In the third case, both the reinforced and nonreinforced presentations of the X stimulus are preceded by a different feature cue (A→X+ vs. B→X−). In the example shown here, the feature cues were an 800-ms tone and an 800-ms noise. Each feature cue was followed by a gap of 3400 ms, and the target stimulus was a 100-ms light. On reinforced trials, the US was presented 300 ms after the light.

The discriminations shown in **Figure 17** were based on serial compounds in which there was a gap between the feature cue and target stimulus. However, similar discriminations have been established when the feature cues were extended to overlap and even extend past the target stimulus. In rabbit eyeblink conditioning, serial conditional discriminations have been reliably obtained with feature cues up to a minute in length (e.g., Brandon and Wagner, 1991; Macrae and Kehoe, 1995; Weidemann and Kehoe, 1997; Rogers and Steinmetz, 1998).

Serial conditional discriminations have been of interest in addressing several theoretical questions. The control of the feature cue over responding during the target stimulus has been thought to reveal a special type of learning. The feature cues may act indirectly on the ability of the X stimulus to elicit the CR by either (1) exercising superordinate control over memory retrieval, so-called *occasion-setting* (Holland, 1992; Schmajuk and Holland, 1998), (2) evoking an appropriate motivational state for responding (Konorski, 1967; Brandon and Wagner, 1991), or (3) perceptually fusing with the target stimulus in working memory to form a configural stimulus (Kehoe et al., 1987; Brandon and Wagner, 1998; Schmajuk et al., 1998). Finally, like delayed matching to sample, manipulations of the interval between the feature cue and target stimulus have been used to investigate working memory (Holland et al., 1997; Holland, 1998; Weidemann, 1999; Kehoe et al., 2000).

1.08.5.1.2 Compound and element testing

In investigations using compound stimuli, there are two counterparts to generalization testing after single-stimulus training. First, in *compound conditioning*, training is conducted with a compound of stimuli, and these elements are then tested separately. Second, in *stimulus compounding*, two stimuli receive separate training and are then tested by presenting them simultaneously. Since the late 1960s, investigations using compound conditioning have played a key role in the development of associative theories in which the stimuli in a compound compete for attention and/or associative strength (Kamin, 1968; Rescorla and Wagner, 1972; Mackintosh, 1975). Since then, both procedures have been used in a now-massive body of research stimulated by these contending theories, as well as newer ones (Kehoe and Gormezano, 1980; Miller et al., 1995; Wasserman and Miller, 1997; Kehoe, 1998; Blaisdell and Miller, 2001; Pearce and Bouton, 2001; Pearce, 2002).

Compound conditioning and stimulus compounding can be viewed as the endpoints of a continuum in which AX+ trials are intermixed with element trials (A+, X+) in varying proportions. For example, **Figure 18** shows conditioned eyeblink responding in

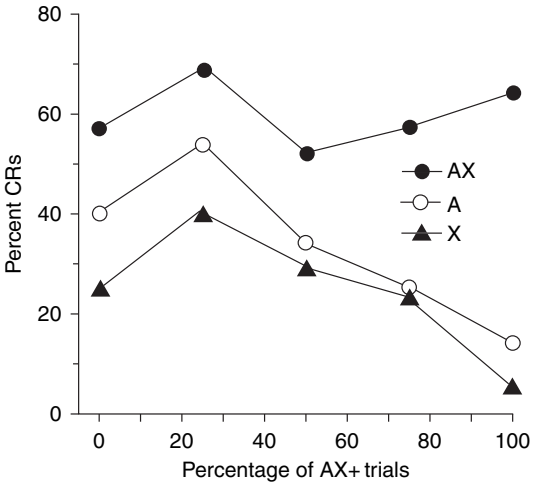

Figure 18 Conditioned eyeblink responding in rabbits to a tone+light compound (AX+) and its elements (A+, X+) as a function of the proportion of AX+ trials relative to A+ and X+ trials (Kehoe, 1986). All figures adapted with permission.

five groups of rabbits, in which the proportion of AX+ trials relative to A+ and X+ trials was varied from 0% (stimulus compounding) to 100% (compound conditioning). The A and X elements were a tone and a light. As shown in **Figure 18**, responding on AX test trials was at a high level across groups. In contrast, responding on A and X test trials was generally lower and progressively declined to negligible levels as the proportion of AX+ trials increased. These results suggest that there was summation of the associative strengths of the elements on AX trials when A+ and X+ pairs predominated. However, as the proportion of AX+ trials increased, a configural stimulus appeared to capture most of the associative strength, thus reducing the level of associative strength possessed by the A and X elements (Kehoe, 1986).

1.08.5.2 Multiple Discrimination Procedures

Multiple discrimination tasks, often presented in succession, have been used to illuminate the perceptual, memorial, navigational, and cognitive abilities of rats and pigeons in the laboratory, as well as other species in more natural environments (Roitblat et al., 1984; Bekoff et al., 2002; Spetch et al., 2003; Blaisdell et al., 2006; Hurley and Nudds, 2006). In the section on basic methods, two examples of multiple discriminations were described. One is the discrimination reversal procedure, in which two stimuli are repeatedly switched in their assignment as S+ and S−. The other is the learning set procedure, in which different pairs of stimuli are successively used as S+ and S−. This section will describe two relatively simple extensions of these procedures used to investigate higher-order processes in learning and memory in animals.

1.08.5.2.1 Overtraining reversal effect

Animals given extended discrimination training can, under many circumstances, acquire reverse discrimination more rapidly than animals given only moderate training (Reid, 1953; Sutherland and Mackintosh, 1971; Orona et al., 1982; Weiner et al., 1986; Van Golf Racht-Delatour and Massioui, 2000). This effect has seemed paradoxical, because basic learning rules predict that extra training should make the original discriminative responding more resistant, not less resistant, to reversal. This paradox has stimulated diverse theoretical accounts (e.g., Lovejoy, 1966; Siegel, 1967; Denny, 1970; Hall, 1974; Mackintosh, 1974). Among them, attentional accounts have been prominent. For example, the additional training may more firmly fix the subject's attention on the relevant stimulus dimension, which would make the reversal a relatively easy matter of switching the specific stimulus-response assignments (Sutherland and Mackintosh, 1971).

1.08.5.2.2 Oddity learning sets

Harlow (1949) argues that learning to learn "transforms the organism from a creature that adapts to a changing environment by trial and error to one that adapts by seeming hypothesis and in-sight." In this respect, learning set research foreshadows more recent investigations of the cognitive abilities of animals (Kamil, 1987). Research using the learning set procedure has, among other things, produced evidence that animals can acquire what appear to be abstract concepts (Delius, 1994; Thomas, 1996).

One example of such concept acquisition is found in oddity learning. In oddity learning, each problem involves a choice between three stimuli, two alike and one different. Selecting the different stimulus is reinforced. As is the case in the more familiar two-stimulus problems, the subjects initially show gradual improvement over trials in their ability to select the correct stimulus. The subject is said to have acquired an oddity concept when it selects the correct stimulus on the first trial of each problem, prior to any reward or nonreward for that set of stimuli. In fact, after extensive experience with oddity problems, correct choices of the odd stimulus on the first encounter with a new set of stimuli do emerge in primates (e.g., Thomas and Frost, 1983). Among nonprimate species, there has been a recent demonstration of above-chance performance on the first trial of visual oddity problems in a seal (Hille et al., 2006). However, for rats, first-trial selections of the odd stimulus have not as yet proved reliable using either visual or olfactory stimuli (Thomas and Noble, 1988; Bailey and Thomas, 1998).

1.08.5.3 Reinforcer-Related Discriminations

1.08.5.3.1 Sequential effects

Reinforcers are sensory events and thus can serve as cues in learning tasks just as well as tones and lights. In operant fixed-interval training, for example, the delivery of a reinforcer is often the only external cue for timing the next interval. Similarly, in other forms of conditioning, food has been used to signal food (Goddard, 1997), shock has been used to signal shock

(Schreurs and Alkon, 1990), water has been used to signal shock (Gormezano and Tait, 1976), and shock has been used to signal water (Gormezano and Tait, 1976).

Reinforcers have also been used extensively as feature cues in serial conditional discriminations (Capaldi, 1994). The most well-developed examples of this use may be found in investigations of the sequential features of reinforcement schedules in instrumental runway tasks using rats. These studies have revealed that the episodic memory of a reinforcer on one trial can control responding on succeeding trials. The simplest sequential effect is seen in the single alternation schedule, in which reinforced trials (R) are strictly alternated with nonreinforced trials (N). From trial to trial, the external events remain the same. If the apparatus is considered the target stimulus (X), then the single alternation schedule can be viewed as a serial conditional discrimination involving N→X+ versus R→X− trials. Through studies using variations on this basic discrimination task, the qualitative and quantitative features of reinforcers on one or more trials have been found to be effective conditional cues (Capaldi, 1994; Fountain and Benson, 2006).

1.08.5.3.2 Differential outcomes effect

The use of different reinforcers can aid discrimination learning. In the original demonstration of this effect (Trapold, 1970), two groups of rats were trained using a conditional choice discrimination. On each trial, both groups were presented with two bars to press. On half the trials, a tone (A) was presented, and presses on the right bar were reinforced. On the other half of the trials, a clicking sound (B) was presented, and presses on the left bar were reinforced. In the experimental group, different reinforcers – a food pellet and sucrose solution – were used on the A and B trials. For half the rats in this group, the two reinforced sequences were A→Right→Food and B→Left→Sucrose. The other half of the rats received sucrose on A trials, and food on B trials. In contrast, the control group received the same reinforcer on both A and B trials. Thus, half the rats in this group received the reinforced sequences of A→Right→Food and B→Left→Food. The other half of the control group received sucrose as the reinforcer.

As can be seen in **Figure 19**, the experimental group showed both faster acquisition of the correct responses and a higher asymptote of correct responding than the control group. This facilitation of discrimination learning and its terminal accuracy by using differential outcomes has been widely replicated in birds (Jones and White, 1994; Poling et al., 1996), horses (Miyashita et al., 2000), and humans (Overmier et al., 1999; Estevez et al., 2001). As well as using qualitatively different reinforcers, the effect has been obtained using different probabilities of food presentation, durations of food presentation, and locations of the food dispenser (see Jones and White, 1994, for a brief review).

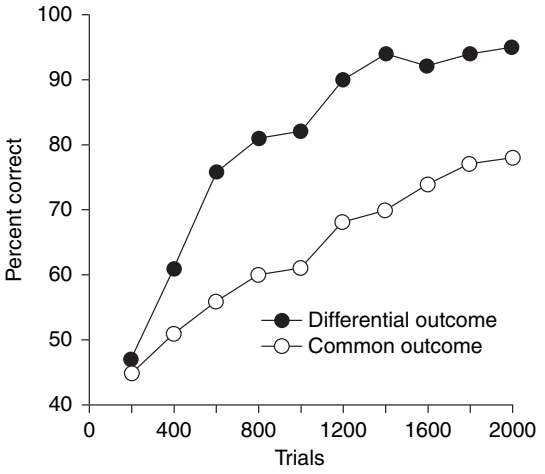

Figure 19 Acquisition of a choice discrimination in rats trained with different reinforcers (differential outcome) or the same reinforcer (common outcome) for the two responses (Trapold and Overmier, 1972). Figure adapted with permission.

The differential outcome effect and similar results have been variously attributed to 'expectancy learning,' 'prospective memory,' and 'response-outcome associations' (Rescorla, 1992; Overmier et al., 1999; Donahoe and Burgos, 2000). To objectify these notions, it has been noted that, during the reinforced sequences, the feature cue is effectively paired with the reinforcer just as in classical conditioning (Trapold and Overmier, 1972). Subsequently, like CR acquisition, the feature cue could elicit a central state describable as an expectancy or prospective memory of the reinforcer. If these central states are at all salient, they could act as additional cues that aid discriminative responding in the experimental group but interfere with discriminative responding in the control group. For example, a food-related state can be denoted X, and a sucrose-related state can be denoted Y. In subjects receiving differential outcomes, these central cues would be distinctive for each reinforced sequence, specifically, AX→Right→Food versus BY→Left→Sucrose. In contrast, for the control

group, the reinforced sequences have a common element, for example, AX→Right→Food and BX→Left→Food.

In one demonstration that expectancies acquired through classical conditioning may be responsible for the differential outcome effect, rats were first trained in a differential outcomes procedure using, once again, tone and clicker cues (A, B), plus food and sucrose as the reinforcers (Kruse et al., 1983). Second, in a different box without the operant bars, the same rats were given pairings of a white noise stimulus (C) with either food or sucrose. Third, the rats were returned to the choice discrimination task, but on test trials, the C stimulus was substituted for the A and B cues. In line with expectancy theory (Trapold and Overmier, 1972), a C stimulus that had been paired with the food caused the rats to press the bar that had been reinforced with food. Likewise, the C stimulus that had been paired with sucrose caused the rats to press the bar that had been reinforced with sucrose.

1.08.6 Conclusion

The uses of discrimination learning and generalization testing are limited only by the imagination of the investigator. This article is only a sampler of the methods that have been used and their major results. Although this article was intended to be atheoretical, it was necessary to delve into some theoretical issues in order to place particular methods in their wider context. The reader should not make any inferences about the relative merits of any particular theory and should consult wider literature cited here to understand the fully range of research and theory surrounding a specific topic. In conclusion, this article should serve as a point of departure for investigators who wish to adapt these methods to address their interests.

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1.09 Extinction: Behavioral Mechanisms and Their Implications

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Extinction is one of the best-known phenomena in all of learning theory. In Pavlovian learning, extinction occurs when the conditioned stimulus (CS) that has been associated with a biologically significant event (unconditioned stimulus, US) is now presented repeatedly without the US. In operant or instrumental learning, extinction occurs when an action or behavior that has been associated with a reinforcer is no longer reinforced. In either case, the learned performance declines. Extinction is important because it allows behavior to change and adapt as the environment also changes. Despite its fame and importance, however, it is not necessarily obvious how extinction works. One surprisingly common idea is that extinction involves the destruction of what was originally learned. Although this idea is built into several models of learning and memory (e.g., Rescorla and Wagner, 1972; McClelland and Rumelhart, 1985; see also McCloskey and Cohen, 1989), there is ample evidence that much of the original learning survives extinction (e.g., see Rescorla, 2001; Bouton, 2002, 2004; Myers and Davis, 2002; Delamater, 2004). This chapter selectively reviews results and theory from the behavioral literature in an effort to understand what is learned in extinction, what causes extinction, and how we can use our understanding of extinction to address certain clinical issues outside the laboratory.

There has been renewed interest in extinction in recent years. One reason is that as neuroscientists have made progress in understanding the brain mechanisms behind acquisition processes in learning (e.g., *See* Chapters 1.36, 3.23, 4.11), they have naturally turned their attention to extinction and inhibition too. A detailed review of the biological work is beyond the scope of this chapter, although we consider some of its implications in the final sections. Another reason for renewed interest in extinction is that it is now clearly understood to be part of cognitive behavioral therapy. That is, in clinical settings, extinction is often the basis of treatment that is used to effectively eliminate maladaptive behaviors, thoughts, or emotions (e.g., Bouton, 1988; Conklin and Tiffany, 2002). However, as this chapter highlights, extinction does not result in a permanent removal of the behavior but instead leaves

the organism vulnerable to relapse. This conclusion provides one illustration of how basic research on extinction provides information that is practically important.

The first part of this chapter introduces several extinction phenomena that any adequate theory of extinction will need to explain and accommodate. These phenomena suggest that extinction does not destroy the original learning, but instead involves new learning that is at least partly modulated by the context. They also potentially contribute to lapse and relapse effects that may occur after extinction therapies. The second part of the chapter then asks: if extinction is an example of new learning, what events reinforce or cause it? We come to the conclusion that extinction is mainly caused by generalization decrement and by new learning caused by the violation of an expectancy of reinforcement. The third part of the chapter takes knowledge from the first two sections and asks what can be done to optimize extinction learning in a way that eliminates the possibility of relapse. Our discussion in the first two sections expands and updates discussions in Bouton (2004); the last section updates a discussion in Bouton et al. (2006b).

1.09.1 Six Recovery Effects after Extinction

A number of experimental manipulations can be conducted after extinction that cause the extinguished response to return to performance. All of them are consistent with the idea that extinction involves new learning, and it therefore leaves the CS with two available meanings or associations with the US. As is true for an ambiguous word, the context is crucial in selecting between them.

1.09.1.1 Renewal

The renewal effect is perhaps the most fundamental postextinction phenomenon. In this effect (e.g., Bouton and Bolles, 1979a; Bouton and King, 1983), a change of context after extinction can cause a robust return of conditioned responding. Several versions of the renewal effect have been studied, but all cases of it support the idea that (1) extinction does not destroy the original learning and (2) the response triggered by the extinguished CS depends on the current context. In the most widely studied version, ABA renewal, conditioning is conducted in one context (Context A) and extinction is then conducted in a second one (Context B). (The contexts are typically separate and counterbalanced apparatuses housed in different rooms of the laboratory that differ in their tactile, olfactory, and visual respects.) When the CS is returned to the original conditioning context (Context A), responding to the CS returns (e.g., Bouton and Bolles, 1979a; Bouton and King, 1983; Bouton and Peck, 1989). In a second version, ABC renewal, conditioning is conducted in Context A, extinction is conducted in Context B, and then testing is conducted in a third, "neutral" context – Context C. Here again, a renewal of responding is observed (e.g., Bouton and Bolles, 1979a; Bouton and Brooks, 1993; Harris et al., 2000; Duvarci and Nader, 2004). In a final version, AAB renewal, conditioning and extinction are both conducted in the same context (Context A) and then the CS is tested in a second context (Context B). Here again, conditioned responding returns (e.g., Bouton and Ricker, 1994; Tamai and Nakajima, 2000), although there is currently less evidence of this type of renewal in operant than in Pavlovian conditioning (e.g., Nakajima et al., 2000; Crombag and Shaham, 2002).

Research on the renewal effect has helped us understand how contexts control behavior—in addition to understanding extinction itself. Several facts about the renewal effect are worth noting. First, it has been observed in virtually every conditioning preparation in which it has been investigated (see, e.g., Bouton, 2002, for a review). Second, it can occur after very extensive extinction training. In fear conditioning (conditioned suppression) in rats, Bouton and Swartzentruber (1989) observed it when 84 extinction trials followed eight conditioning trials. Other evidence suggests that it can occur after as many as 160 extinction trials (Gunther et al., 1998; Rauhut et al., 2001; Denniston et al., 2003), although at least one report suggests that it might not survive an especially massive extinction treatment (800 extinction trials after eight conditioning trials; Denniston et al., 2003). Third, the role of the context is different from the one anticipated by standard models of classical conditioning (e.g., Rescorla and Wagner, 1972; Pearce and Hall, 1980; Wagner, 1981; Wagner and Brandon, 1989, 2001). Those models accept the view that the context is merely another CS that is presented in compound with the target CS during reinforcement or nonreinforcement. It therefore enters into simple excitatory or inhibitory associations with the US. In the ABA renewal effect, for example, Context A might acquire excitatory associations with the US, and Context B might acquire

inhibitory associations. Either kind of association would summate with the CS to produce the renewal effect (inhibition in B would reduce responding to the CS, whereas excitation in A would enhance it). However, a number of experiments have shown that the renewal effect can occur in the absence of demonstrable excitation in Context A or inhibition in Context B (e.g., Bouton and King, 1983; Bouton and Swartzentruber, 1986, 1989). These findings, coupled with others showing that strong excitation in a context does not influence performance to a CS unless the CS is under the influence of extinction (described later; Bouton, 1984; Bouton and King, 1986), suggest that direct associations in a context are neither necessary nor sufficient for a context to influence responding to a CS. The implication (e.g., Bouton, 1991b; Bouton and Swartzentruber, 1986) is that the contexts modulate or set the occasion for the current CS–US or CS–no US association (e.g., Holland, 1992; Swartzentruber, 1995; Schmajuk and Holland, 1998). Put another way, they activate or retrieve the CS's current relation with the US (*See* Chapter 1.05).

Another fact about renewal is that it appears to be supported by many kinds of contexts, including physical, temporal, emotional, and physiological ones (e.g., Bouton, 2000). For example, when fear extinction is conducted in the interoceptive context provided by benzodiazepine tranquilizers chlordiazepoxide and diazepam, renewed fear was observed when the rat was tested in the original nondrug state (Bouton et al., 1990). Cunningham (1979) had reported compatible evidence with alcohol, and we have recently collected similar observations with the benzodiazepine midazolam. State-dependent learning or retention can be conceptualized as the drug playing the role of context (e.g., Overton, 1985).

A further important characteristic of the renewal effect is that it implies that extinction learning is more context specific than original conditioning. This asymmetry in context dependence between conditioning and extinction must be true if one observes ABC and AAB renewal; in either case, conditioning transfers better to the final test context than extinction does. There is typically no measurable effect of switching the context after conditioning on responding to the CS in either fear or appetitive conditioning paradigms (e.g., Bouton and King, 1983; Bouton and Peck, 1989). This is also true of taste aversion learning (e.g., Rosas and Bouton, 1998) and human causal learning, in which humans are asked to judge the causal relationship between cues and outcomes presented over a series of trials (e.g., Rosas et al., 2001). The presence of the renewal effect indicates that extinction, on the other hand, is especially context specific. Some research suggests that both conditioning and extinction become somewhat context specific after extinction has occurred (Harris et al., 2000). However, there is little question that extinction comes under relatively more contextual control than original conditioning.

The reason for the difference in the context dependence of conditioning and extinction has been the subject of recent research. Given the similarities between extinction and inhibition, Bouton and Nelson (1994) and Nelson and Bouton (1997) asked whether pure inhibition (as acquired in the feature-negative paradigm, in which a CS is paired with the US when it is presented alone, but not when it is combined with an inhibitory CS) was context specific. Inhibition acquired by the inhibitory CS transferred without disruption to a new context; thus, extinction is not context specific merely because it is a form of inhibition. A second reason why extinction might be context specific is that it is the second thing the organism learns about the CS. Nelson (2002) confirmed that excitatory conditioning (tone–food pairings) transferred undisturbed across contexts, unless the tone had first been trained as a conditioned inhibitor, as discussed earlier, in the feature-negative paradigm. Conversely, inhibition to a conditioned inhibitor also transferred across contexts unless the CS had first been trained as a conditioned excitor (through tone–food pairings). Thus, regardless of whether the association was excitatory or inhibitory, the second thing learned was more context specific than the first (cf. Rescorla, 2005). Compatible data had been shown by Swartzentruber and Bouton (1992), who found that excitatory conditioning was context specific if it had been preceded by nonreinforced preexposure to the CS.

The evidence therefore suggests that the learning and memory system treats the first association as context free, but the second association as a kind of context-specific exception to the rule. (The main exception to the second-association rule is latent inhibition, in which the first phase can be shown to exert a context-dependent influence on the second phase (e.g., Hall and Channell, 1985), despite the fact that it is arguably the first thing learned. Latent inhibition is unique, however, in that the CS is not paired with anything significant in the first phase. One possibility, therefore, is that the CS is in part encoded as a feature of the context, making it difficult to extract it from that context when it is paired with the US in Phase 2 (cf. Gluck and Myers, 1993).)

There may be functional reasons for this (Bouton, 1994). A conditioning trial provides a sample from which an animal may make inferences about the state of the world (e.g., Staddon, 1988). Statistically, if the world is composed of two types of trials (CS–US and CS–no US), then the probability of sampling a particular type of trial will reflect its true prevalence in the world. Therefore, an early run of conditioning trials would reflect its high incidence in the population; a subsequent trial of another type might reflect an exception to the rule. Learning and memory may thus be designed to treat second-learned information as conditional and context-specific. At a more mechanistic level, recent research in human predictive learning (Rosas and Callejas-Aguilera, 2006) and in taste-aversion learning with rats (Rosas and Callejas-Aguilera, 2007) suggests that ambiguity introduced by conflicting information in Phase 2 leads the participants to pay attention to the context. The key finding is that after conflicting information about one CS or predictor is introduced, other subsequently learned associations are context dependent, even if they are entirely new or learned in a separate context. Thus, the introduction of a competing, conflicting association appears to encourage the participant to pay attention to all contexts.

It is worth concluding this section by noting the direct relevance of the renewal effect to exposure therapy in humans. Several studies have now shown that an exposure treatment that diminishes fear of spiders in one context (a room or a patio outdoors) can still allow a renewal of the fear when exposure to the spider was tested in the other context (Mystkowski et al., 2002; see also, e.g., Mineka et al., 1999; Vansteenwegen et al., 2005). Similar renewal has also been reported in the study of cue exposure therapy with both alcohol (Collins and Brandon, 2002) and cigarette users (Thewissen et al., 2006). Both types of participants reported a renewed urge to use the drug when tested in a context that was different from the one in which exposure to drug-related cues (e.g., visual and/or olfactory cues associated with the drug) had taken place; the alcohol participants also demonstrated renewal of a salivation response. All such results suggest limits to the effectiveness of cue exposure therapy (see also Conklin, 2006). However, renewal effects can be attenuated if the participant is reminded of extinction just prior to the renewal test. Collins and Brandon (2002) found that presenting explicit cues (a unique pencil, eraser, and clipboard) that had been a feature of the extinction context reduced renewal of the aforementioned reactivity to alcohol cues. Mystkowski et al. (2006) reported that renewal of spider fear could be decreased if human participants mentally reinstated (imagined) stimuli from the treatment context before being tested in a different context. Both studies extended earlier findings that the renewal effect can be reduced in rats if cues that were part of the extinction context were later presented before the test (Brooks and Bouton, 1994). Renewal can be viewed as due to a failure to retrieve extinction outside the extinction context (*See* Chapter 1.05). Retrieval cues can provide a "bridge" between the extinction context and possible relapse contexts which allows for the generalization of extinction learning between the contexts (see Bouton et al., 2006b).

1.09.1.2 Spontaneous Recovery

Pavlov (1927) first observed spontaneous recovery, another well-known postextinction effect that involves recovery of the conditioned response as time passes following extinction. There are several available explanations of spontaneous recovery (for a discussion, see Brooks and Bouton, 1993; Devenport et al., 1997; Robbins, 1990; Rescorla, 2004a), and it seems likely to be multiply determined. However, we have argued (e.g., Bouton, 1988, 1993) that just as extinction is relatively specific to its physical context, it may also be specific to its temporal context. The passage of time might also bring about changes in internal and external stimulation that provide a gradually changing context. Spontaneous recovery can be seen as the renewal effect that occurs when the CS is tested outside its temporal context. Both are due to a failure to retrieve memories of extinction outside the extinction context. Consistent with this perspective, a cue that is presented intermittently during the extinction session and again just before the final test (an 'extinction cue') can attenuate both spontaneous recovery and renewal by reminding the subjects of extinction (Brooks and Bouton, 1993, 1994; Brooks, 2000). Interestingly, changing the physical and temporal contexts together can have a bigger effect than changing either context alone, as if their combination creates an even larger context change (Rosas and Bouton, 1997, 1998).

A series of experiments in appetitive conditioning with rats further suggests that temporal context can include the intertrial interval (ITI), the time between successive extinction trials (Bouton and García-Gutiérrez, 2006). Previous experiments had shown

that rats that received extinction trials spaced by either 4-min or 16-min ITIs showed equivalent spontaneous recovery when tested 72 h later (Moody et al., 2006). However, when the retention interval was 16 min rather than 72 h (Bouton and García-Guiterrez, 2006), rats that had received extinction trials separated by the 4-min interval showed spontaneous recovery, whereas rats that had received the extinction trials separated by the 16-min interval did not. These results are consistent with the possibility that the ITI was coded as part of the extinction context. Thus, analogous to a renewal effect, conditioned responding returned when the animals were tested after an interval between trials that differed from the ITI that was used in extinction. However, a mismatch between the extinction ITI and the retention interval is not always sufficient to produce renewal; rats that received extinction trials spaced by 16 min and then received a short retention interval of 4 min failed to show spontaneous recovery. This anomaly is consistent with results that emerged in discrimination experiments in which the different ITIs were used as signals about whether or not the next CS presentation would be reinforced (see Bouton and García-Gutiérrez, 2006). Specifically, rats readily learned that a 16-min ITI signaled reinforcement of a CS, whereas a 4-min ITI did not. In contrast, they had considerably more difficulty learning that a 4-min ITI signaled reinforcement and a 16-min ITI did not. The results suggest that there are interesting constraints on how the interval between trials may be coded and/or used as a context.

1.09.1.3 Rapid Reacquisition

A third effect further indicates that conditioning is not destroyed in extinction. In rapid reacquisition, when CS–US pairings are reintroduced after extinction, the reacquisition of responding can be more rapid than initial acquisition with a novel CS (e.g., Napier et al., 1992; Ricker and Bouton, 1996; Weidemann and Kehoe, 2003). Although such an effect again suggests that the original learning has been 'saved' through extinction, the early literature was often difficult to interpret because many early designs were not equipped to rule out less interesting explanations (see Bouton, 1986, for a review). To add to the complexity, studies of fear conditioning (conditioned suppression) (Bouton, 1986; Bouton and Swartzentruber, 1989) and flavor aversion learning (Danguir and Nicolaidis, 1977; Hart et al., 1995) have shown that reacquisition can be slower than acquisition with a new CS. (It is more rapid than initial acquisition with a CS that has received the same number of nonreinforced trials without conditioning (Bouton and Swartzentruber, 1989).) In fear conditioning, slow reacquisition requires extensive extinction training; more limited extinction training yields reacquisition that is neither fast nor slow (Bouton, 1986). At least part of the reason these preparations support slow reacquisition is that both typically involve very few initial conditioning trials. In contrast, procedures in which rapid reacquisition has been shown (conditioning of the rabbit nictitating membrane response (NMR) and rat appetitive conditioning) have usually involved a relatively large number of initial conditioning trials. Consistent with a role for number of trials, Ricker and Bouton (1996) demonstrated that slow reacquisition occurred in an appetitive conditioning preparation when the procedure used the number of conditioning and extinction trials that had been used in previous fear conditioning experiments. In rabbit NMR and heart rate conditioning, extensive extinction training has abolished rapid reacquisition, although slow reacquisition has yet to be observed (Weidemann and Kehoe, 2003).

Ricker and Bouton (1996) suggested that rapid reacquisition may partly be an ABA renewal effect that occurs when the animal has learned that previous USs or conditioning trials are part of the original context of conditioning. That is, when CS–US pairings are resumed after extinction (a series of CS–no US trials), they return the animal to the original conditioning context. The hypothesis is compatible with Capaldi's (1967, 1994) sequential analysis of extinction, which has made excellent use of the idea that responding on a particular trial is determined by how the animal has learned to respond in the presence of similar memories of previous trials. Presumably, conditioning preparations that employ a relatively large number of conditioning trials (e.g., rabbit NMR) allow many opportunities for the animal to learn that previous reinforced trials are part of the context of conditioning. Furthermore, Ricker and Bouton (1996) reported evidence that high responding during the reacquisition phase was more likely after a reinforced than a nonreinforced trial, which had signaled conditioning and extinction, respectively.

Bouton et al. (2004) reasoned that if rapid reacquisition is caused by recent reinforced trials generating ABA renewal, then an extinction procedure that includes occasional reinforced trials among many nonreinforced trials should slow down rapid

reacquisition by making recent reinforced trials part of the context of both conditioning and extinction. Consistent with this hypothesis, a very sparse partial reinforcement procedure in extinction slowed reacquisition compared to a group that had received simple extinction. Evidence of a similar slowed reacquisition effect has also been obtained in instrumental conditioning (Woods and Bouton, 2007) when a lever-press response was sparsely reinforced during extinction and then paired again more consistently with the reinforcer. Such a result is consistent with the idea that rapid reacquisition is at least partly an ABA renewal effect. Because the partial reinforcement treatment involved many more CS–US (or response–reinforcer) pairings than simple extinction, it is difficult to reconcile with the view that rapid reacquisition is a simple function of the strength of an association that remains after extinction (e.g., Kehoe, 1988; Kehoe and Macrae, 1997). Slowing rapid reacquisition of an instrumental response may be especially relevant from a clinical perspective, because instrumental learning is often involved in maladaptive behaviors such as substance abuse (e.g., Bouton, 2000). The evidence suggests that extinction (i.e., strict abstinence) might not be the most effective treatment to prevent relapse in all situations (cf. Alessi et al., 2004). A more successful technique might be one that permits occasional reinforcers during treatment (e.g., see Sobell and Sobell, 1973; Marlatt and Gordon, 1985) and therefore provides a bridge between the extinction and testing contexts (see also Bouton et al., 2006b).

1.09.1.4 Reinstatement

A fourth context-dependent postextinction phenomenon is reinstatement. In this effect, the extinguished response returns after extinction if the animal is merely reexposed to the US alone (e.g., Pavlov, 1927; Rescorla and Heth, 1975; Bouton and Bolles, 1979b). If testing of the CS is contemporaneous with US delivery, then the USs may cause a return of responding because they were encoded as part of the conditioning context (as earlier; see Reid, 1958; Baker et al., 1991; Bouton et al., 1993). On the other hand, in many studies of reinstatement, testing is conducted at an interval of at least 24 h after US reexposure; here one still observes reinstatement compared to controls that were not reexposed to the US (e.g., Rescorla and Heth, 1975; Bouton and Bolles, 1979b). In this case, evidence strongly suggests that the effect is due to conditioning of the context. When the US is presented after extinction, the organism associates it with the context; this contextual conditioning then creates reinstatement. For example, if the reinstating USs are presented in an irrelevant context, there is no reinstatement when the CS is tested again (e.g., Bouton and Bolles, 1979b; Bouton and King, 1983; Bouton, 1984; Baker et al., 1991; Wilson et al., 1995; Frohardt et al., 2000). Independent measures of contextual conditioning also correlate with the strength of reinstatement (Bouton and King, 1983; Bouton, 1984). Recent evidence that the effect in fear conditioning is abolished by excitotoxic lesions of the bed nucleus of the stria terminalis (Waddell et al., 2006), a brain area thought to control anxiety (e.g., Walker et al., 2003), suggests that in the fear-conditioning situation, at least, the effect may be mediated by anxiety conditioned in the context. Also, if the animal receives extensive extinction exposure to the context after the reinstatement shocks are presented, reinstatement is not observed (Bouton and Bolles, 1979b; Baker et al., 1991). These results indicate that mere reexposure to the US is not sufficient to generate reinstatement. It is necessary to test the CS in the context in which the US has been reexposed.

This effect of context conditioning is especially potent with an extinguished CS. For example, Bouton (1984) compared the effects of US exposure in the same or a different context on fear of a partially extinguished CS or another CS that had reached the same low level of fear through simple CS–US pairings (and no extinction). Although contextual conditioning enhanced fear of the extinguished CS, it had no impact on the nonextinguished CS (see also Bouton and King, 1986). This result is consistent with the effects of context switches mentioned earlier: An extinguished CS is especially sensitive to manipulations of the context. One reason is that contextual conditioning may be another feature of the conditioning context; its presence during a test may cause a return of responding after extinction because of another ABA renewal effect (Bouton et al., 1993).

1.09.1.5 Resurgence

Another recovery phenomenon has been studied exclusively in operant conditioning. In resurgence, a new behavior is reinforced at the same time the target behavior is extinguished. When reinforcement of the new behavior is discontinued, the original response can resurge. Resurgence can occur in two different forms. In one, a response (R1) is first trained and

then extinguished. After R1 is extinguished, a new response (R2) is trained and subsequently extinguished. Recovery, or resurgence, of R1 happens during the extinction of R2 (Epstein, 1983; Lieving and Lattal, 2003, Experiment 1). The other version of the procedure, which can be called the ALT-R procedure (for reinforcement of an alternative response), involves first training R1 and then reinforcing R2 during the extinction of R1. When R2 then undergoes extinction, recovery of R1 occurs (Leitenberg et al., 1970, 1975; Rawson et al., 1977; Epstein, 1985; Lieving and Lattal, 2003). Like the other recovery phenomena described earlier, both forms of resurgence support the idea that extinction does not produce unlearning.

There has been little research designed to uncover the actual mechanisms behind resurgence. One possibility is that extinction of R2 could cause an increase in behavioral variability, or frustration, that might result in an increase in any alternative behavior, not just R1 (e.g., Neuringer et al., 2001). Few studies have included a control response to show that resurgence is unique to an extinguished response. The sole exception is a study by Epstein (1983), who reported that pigeons rarely pecked an alternative, previously nonreinforced response key. An explanation with specific regard to the ALT-R procedure is that reinforcement of R2 during extinction of R1 physically prevents the animal from emitting R1 and thus prevents exposure to the R1–no reinforcer contingency (Leitenberg et al., 1975; Rawson et al., 1977). It is also possible, however, that resurgence follows from the mechanisms implicated in the other recovery effects described earlier. In particular, resurgence observed in the ALT-R procedure could be due to the fact that extinction of R1 occurs in the context of R2 responding. Then, given that extinction is context-specific, R1 would return when extinction of R2 occurs and the frequency of R2 decreases. That is, little R2 responding would return the animal to the context in which R1 had been reinforced, and thus recovery of R1 in the ALT-R procedure would be analogous to an ABA renewal effect.

A somewhat different explanation would be required to explain the form of resurgence in which R1 is extinguished before R2 training begins. In this case, testing of R1 occurs after extinction of R2, and thus responding on R2 would be minimal in both the extinction and testing conditions and thus no renewal effect should result. For this scenario, we might suggest the following explanation. The reinforcer is consistently presented during training of R2, which occurs simultaneously with extinction of R1, and thus this would continuously reinstate responding to R1 (e.g., Rescorla and Skucy, 1969; Baker et al., 1991). Reinstatement of R1 is possible due to conditioning of background context or due to the fact that the reinforcer is a discriminative stimulus signaling to make the response (e.g., Baker et al., 1991). While R2 is being reinforced, the response will interfere with the reinstated performance of R1. However, when R2 then undergoes extinction, the reinstated R1 responding (i.e., resurgence) can then be revealed. In this case, then, resurgence may be an example of the basic reinstatement effect.

1.09.1.6 Concurrent Recovery

Concurrent recovery is another effect indicating that extinction does not destroy original learning. To date, the phenomenon has been studied exclusively in rabbit NMR conditioning: In that preparation, extinguished responding to a target CS can return if a completely different CS is separately paired with the US (e.g., Weidemann and Kehoe, 2004, 2005). One interesting fact about concurrent recovery is that the effect does not necessarily depend on extinction. Rather, similar to a "learning to learn" effect in which conditioning with one CS increases the subsequent rate of conditioning with other CSs (e.g., Kehoe and Holt, 1984), responding to a weakly conditioned target CS can be increased as a result of conditioning with a different CS (Schreurs and Kehoe, 1987). Kehoe (1988) has interpreted these phenomena from a connectionist perspective. He has suggested that the effects occur because inputs from different CSs might converge on a common hidden unit. When a nontarget CS is paired with the US, it strengthens the association of the common hidden unit with the US and thereby allows more responding when the target CS is again presented. This account suggests that extinction plays no special role in enabling concurrent recovery, although the effects that reinforcing one CS has on responding to extinguished and nonextinguished target CSs have not been compared.

A different account of concurrent recovery is that it might merely be a reinstatement effect that occurs due to presentation of the US (with another CS) after extinction of the target CS. Thus, rather than depending on new CS–US pairings, the mere presence of the US alone might be enough to produce concurrent recovery and would suggest that it is similar to a basic reinstatement effect. This possibility appears unlikely in the case of NMR conditioning, because exposure to the US on its own after extinction does

not cause much reinstatement in that preparation (e.g., Napier et al., 1992). However, in the many other preparations where reinstatement does occur (e.g., fear conditioning or appetitive conditioning), a demonstration of concurrent recovery might merely be a reinstatement effect – exposure to CS–US pairings simply involves reexposure to the US (see Rescorla and Heth, 1975; Bouton and Bolles, 1979b, for evidence of reinstatement when the US is presented with or without a CS). Additional work on concurrent recovery is required to determine whether it differentially influences an extinguished CS and, importantly, whether it (like reinstatement) is context specific. Perhaps reinforcing a CS in the same context where a target CS was previously extinguished could remove the contextual inhibition that had accrued there or allow excitation to generalize to the target CS via common associations between the CSs and the context (cf. Honey and Hall, 1989).

1.09.1.7 Summary

A great deal of research thus indicates that responding to an extinguished CS is susceptible to any of a number of recovery effects, suggesting that extinction is not unlearning. Indeed, based on the results of a number of tests that allow a specific comparison of the strength of the CS–US association before and after extinction (e.g., Delamater, 1996; Rescorla, 1996a), Rescorla (2001) has suggested that extinction involves no unlearning whatsoever; the original CS–US association seems to survive essentially intact. Extinction must thus depend on other mechanisms. The renewal effect, and the fact that extinction leaves the CS so especially sensitive to manipulations of context, is consistent with the idea that extinction involves new learning that is especially context-dependent. We have therefore suggested that extinction leaves the CS under a contextually modulated form of inhibition (e.g., Bouton, 1993): The presence of the extinction context retrieves or sets the occasion for a CS–no US association.

1.09.2 What Causes Extinction?

A theoretically and clinically significant question in the field of learning and memory is what event or behavioral process actually causes the loss of responding during extinction? Several ideas have been examined and are discussed next.

1.09.2.1 Discrimination of Reinforcement Rate

One possibility is that the animal eventually learns that the rate of reinforcement in the CS is lower in extinction than it was during conditioning. Gallistel and Gibbon (2000) have argued that the animal continually decides whether or not to respond in extinction by comparing the current rate of reinforcement in the CS with its memory of the rate that prevailed in conditioning. Because rate is the reciprocal of time, the animal computes a ratio between the amount of time accumulated in the CS during extinction and the amount of time accumulated in the CS between USs during conditioning. When the ratio exceeds a threshold, the animal stops responding.

This approach has been tested in several experiments. Haselgrove and Pearce (2003) examined the impact of varying the duration of the CS during extinction; when longer CSs are used in extinction, time in the CS accumulates more quickly, and the animal should stop responding after fewer trials. In some experiments, rats were given appetitive conditioning with a 10-s CS and then given extinction exposures to a series of 10-s or 270-s presentations of the CS. When responding was examined at the start of each CS, there was an occasionally significant, but surprisingly small, effect of increasing the duration of the CS during extinction. For instance, by the 12th two-trial block, the 10-s and 270-s CS groups had similar nonzero levels of responding, even though they had accumulated a total of 4 and 108 min of exposure in the CS, respectively. On the other hand, responding did decline as a function of time within a single presentation of the 270-s CS, perhaps reflecting generalization decrement resulting from the increasing difference between the current CS and the 10-s CS employed in conditioning. Consistent with that view, when conditioning first occurred with a 60-s CS, extinction of responding occurred more rapidly with a 10-s CS than with a 60-s CS. Thus, either an increase or a decrease in the duration of the CS relative to conditioning accelerated the loss of responding. This effect of time was not anticipated by the rate-discrimination view (Gallistel and Gibbon, 2000).

Drew et al. (2004) reported compatible results in experiments on autoshaping in ring doves. Doubling or halving the duration of the CS from the 8-s value used in conditioning did not affect the number of trials required to stop responding. The fact that extinction was thus largely controlled by the number

of CS presentations is consistent with experiments that have examined the effects of the number and duration of nonreinforced trials added to conditioning schedules (Bouton and Sunsay, 2003). On the other hand, Drew et al. found that a more extreme increase in CS duration (from 8 to 32 s) increased the rate of extinction. This was attributed to the animal learning to discriminate the longer nonreinforced CS presentations from the shorter reinforced CS presentations: When 8-s CSs were presented again after extinction, birds extinguished with 4-s and 32-s CSs responded again. Animals are sensitive to time in the CS, but the number of extinction trials appears to be an important factor.

As noted by Gallistel and Gibbon (2000), the rate discrimination theory seems especially consistent with a well-known extinction phenomenon, the partial reinforcement effect (PRE; see Mackintosh, 1974, for a review). In this phenomenon, conditioning with partial reinforcement schedules (in which nonreinforced trials are intermixed with reinforced trials) creates a slower loss of responding in extinction than does conditioning with a continuous reinforcement schedule (in which every trial is reinforced). According to a rate-discrimination hypothesis (Gallistel and Gibbon, 2000), the partially reinforced subjects have learned to expect the US after more accumulated time in the CS, and it thus takes more CS time in extinction to exceed the threshold of accumulated extinction time/expected time to each US. The more traditional approach, in contrast, has been to think that partially reinforced subjects have learned to expect the US after more trials than continuously reinforced subjects have. It therefore takes more trials to stop generalizing from conditioning to extinction (e.g., Mowrer and Jones, 1945; Capaldi, 1967, 1994).

Contrary to the rate discrimination hypothesis, Haselgrove et al. (2004) and Bouton and Woods (2004) have shown that a PRE still occurs when partially and continuously reinforced subjects expect the reinforcer after the same amount of CS time. For example, both sets of investigators showed that a group that received a 10-s CS reinforced on half its presentations (accumulated CS time of 20 s) extinguished more slowly than a continuously reinforced group that received every 20-s CS presentation reinforced. Bouton and Woods (2004) further distinguished the time-discrimination account from the traditional trial-discrimination account (e.g., Mowrer and Jones, 1945; Capaldi, 1967, 1994). Rats that had every fourth 10-s CS reinforced extinguished more slowly over a series of alternating 10-s and 30-s extinction trials than rats that had received every 10-s CS reinforced. This PRE was still observed when extinction responding was plotted as a function of time units over which the US should have been expected (every 40 s for the partially reinforced group but every 10 s for the continuously reinforced group). In contrast, the PRE disappeared when extinction responding was plotted as a function of the trials over which the US should have been expected (every fourth trial for the partially reinforced group and every trial for the continuously reinforced group). Ultimately, the PRE is better captured by trial-based theories (e.g., Capaldi, 1967, 1994; see Mackintosh, 1974, for a review of the older literature).

We have already seen that responding on a particular trial occurs in the context of memories of the outcomes of previous trials – that was the explanation provided earlier of rapid reacquisition as an ABA renewal effect (Ricker and Bouton, 1996; Bouton et al., 2004). Interestingly, the recent finding that occasional reinforced trials in extinction (partial reinforcement) can slow down the rate of reacquisition (Bouton et al., 2004; Woods and Bouton, 2007) is really just the inverse of the PRE: In the PRE, nonreinforced trials in conditioning allow more generalization from conditioning to extinction, whereas Bouton et al.'s finding suggests that reinforced trials in extinction allowed for more generalization of extinction to reconditioning. Either finding suggests the importance of considering recent trials as part of the context that controls performance in extinction.

In summary, there is little support for the idea that responding extinguishes when the US is omitted because the organism detects a lower rate of reinforcement in the CS. The number of extinction trials, rather than merely the accumulating time in the CS across trials, appears to be important to the extinction process. Time in the CS can have an effect: It appears to be another dimension over which animals generalize and discriminate (Drew et al., 2004; Haselgrove and Pearce, 2003). Explanation of the PRE, however, appears to be most consistent with a view that animals utilize their memories of the outcomes of preceding trials as a dimension over which they generalize and respond (see also Mackintosh, 1974, for an extended review).

1.09.2.2 Generalization Decrement

It is possible that the animal stops responding in extinction from the point at which it stops generalizing between the stimuli that prevailed in conditioning and

those that prevail in extinction (e.g., Capaldi, 1967, 1994). This idea has had a long history in research on extinction, especially in research on the PRE. It is interesting to note that a generalization decrement theory of extinction does not imply destruction of the original learning in extinction, or indeed any new learning at all. However, there is still good reason to think that extinction also involves new learning. For instance, nonreinforcement of a food CS elicits measurable frustration, and this can be associated with stimuli present in the environment (Daly, 1974). Nonreinforcement of the CS in the related conditioned inhibition paradigm (in which a CS is nonreinforced in the presence of a second stimulus and that second stimulus acquires purely inhibitory properties) also generates measurable new learning in the form of conditioned inhibition. There is also evidence for new learning in the renewal effect. For example, either ABC renewal or AAB renewal (see earlier discussion) implies that the extinction context acquires an ability to modulate (suppress) performance to the CS. Such observations suggest that the animal has not merely stopped responding in extinction because of a failure to generalize. Instead, it appears to have learned that the CS means no US in the extinction context (see earlier).

1.09.2.3 Inhibition of the Response

Rescorla (2001) suggested that extinction might involve learning to inhibit the conditioned response. He summarized evidence from instrumental (operant) conditioning experiments indicating that the effects of extinction can be specific to the response that undergoes extinction. For example, Rescorla (1993) reinforced two operant behaviors (lever pressing and chain pulling) with food pellets and then extinguished each response in combination with a new stimulus (a light or a noise). Subsequent tests of the two responses with both light and noise indicated that each response was more depressed when it was tested in combination with the cue in which it had been extinguished (see also Rescorla, 1997a). There is thus good reason to think that the animal learns something specific about the response itself during operant extinction: It learns not to perform a particular response in a particular stimulus. One possibility is that the animal learns a simple inhibitory S–R association (Colwill, 1991). Another possibility, perhaps more consistent with the context-modulation account of extinction emphasized earlier, is that the animal learns that S sets the occasion for a response – no reinforcer relationship. Rescorla (1993: 335; 1997a: 249) has observed that the experiments do not separate the two possibilities. To our knowledge, no analogous experiments have been performed in the Pavlovian conditioning situation.

The main implication examined in Pavlovian conditioning is that extinction procedures should be especially successful at causing inhibitory S–R learning if they generate high levels of responding in extinction. This prediction may provide a reasonable rule of thumb (Rescorla, 2001). For example, when a CS is compounded with another excitatory CS and the compound is extinguished, there may be especially strong responding in extinction (due to summation between the CSs), and especially effective extinction as evidenced when the CS is tested alone (Wagner, 1969; Rescorla, 2000; Thomas and Ayres, 2004). Conversely, when the target CS is compounded with an inhibitory CS, there is relatively little responding to the compound (excitation and inhibition negatively summate), and there is also less evidence of extinction when the target is tested alone (Soltysik et al., 1983; Rescorla, 2003; Thomas and Ayres, 2004). However, although these findings are consistent with the hypothesis that the effectiveness of extinction correlates with the degree of responding, either treatment also affects the degree to which the animal's expectation of the reinforcer is violated: The stimulus compound influences the size of the error term in the Rescorla-Wagner model, and in more cognitive terms the extent to which the expectation of the US created by the compound is violated when the US does not occur. The results do not separate the response-inhibition hypothesis from an expectancy-violation hypothesis, which will be covered in the next section.

An eyeblink experiment by Krupa and Thompson (2003) manipulated the level of responding another way. During extinction, rabbits were given microinjections of the gamma-aminobutyric acid (GABA) agonist muscimol adjacent to the motor nuclei that control the conditioned response (the facial nucleus and the accessory abducens). The injection therefore eliminated the CR during extinction. However, when the subjects were then tested without muscimol, the CS evoked considerable responding, suggesting that evocation of the CR was necessary for extinction learning. Unfortunately, the muscimol microinjections also had robust stimulus effects. They caused complete inactivation of the ipsilateral facial musculature: "the external eyelids were flaccid, the left ear hung down unsupported, and no vibrissae movements

were observed on the side of the infusion" (Krupa and Thompson, 2003: 10579). In effect, the rabbits received extinction in a context that was different from the one in which conditioning and testing occurred (the ordinary state without partial facial paralysis). There are thus strong grounds for expecting a renewal effect. The hypothesis that elicitation of the CR is necessary for extinction must await further tests.

There are also data suggesting that the number of responses or level of responding in extinction does not correlate with effective extinction learning. For example, Drew et al. (2004) noted that although animals given long CSs in extinction responded many more times in extinction than animals given shorter CSs, extinction was mainly a function of the number of extinction trials. In fear conditioning experiments with mice, Cain et al. (2003) reported that extinction trials that were spaced in time produced a slower loss of freezing than extinction trials that were massed in time. Nevertheless, there was less spontaneous recovery after the massed treatment, suggesting that extinction was more effective when the treatment involved less overall responding. Experiments in our own laboratory with rat appetitive conditioning (Moody et al., 2006) suggest a similar conclusion, even though the results were different. Spaced extinction trials again yielded more responding in extinction than massed trials, but the treatments caused indistinguishable amounts of extinction learning as assessed in spontaneous recovery and reinstatement tests.

In related conditioned suppression experiments, Bouton et al. (2006a) compared the effects of extinction in multiple contexts on the strength of ABA and ABC renewal effects (discussed more in the section titled 'Other behavioral techniques to optimize extinction learning'). Rats received fear conditioning with a tone CS in Context A, and then extinction of the tone for three sessions in Context B, or a session in B, then C, and then D, before final renewal tests in the original context (Context A) or a neutral fifth context (Context E). Although the successive context switches in the BCD group caused more fear responding during extinction (due to renewal effects with each context switch), the groups showed strikingly similar renewal in either Context A or Context E. Thus, higher responding in extinction does not indicate better extinction learning; in fact, the level of responding on extinction trials was positively, rather than negatively, correlated with the level of renewal (see also Moody et al., 2006). The results seem inconsistent with a response-inhibition hypothesis. Their impact on the expectancy violation hypothesis is perhaps less clear.

Rescorla (2006, Experiment 5) provided perhaps the most direct test of whether enhanced responding or enhanced associative strength is responsible for the increased extinction (loss of responding) that typically follows compound presentation of two extinguished stimuli (see also Reberg, 1972; Hendry, 1982). He studied the effects of a diffuse excitor (e.g., a noise paired with food) and a diffuse positive occasion setter (e.g., a houselight that signaled the reinforcement of a keylight CS) on extinction of autoshaped key pecking in pigeons. When combined with a target keylight CS that was undergoing extinction, the diffuse excitor failed to increase the amount of pecking at the keylight, although it theoretically increased the animal's expectation of the US. In contrast, when combined with the target keylight CS, the diffuse occasion setter increased the amount of pecking at the CS without theoretically increasing the direct expectancy of the US. Contrary to Rescorla's (2001) rule of thumb about more responding resulting in more extinction, extinction in combination with the excitor caused a more durable extinction effect (assessed in reacquisition) than did extinction in combination with the occasion setter. The occasion setter caused no more effective extinction than extinction of the target alone. This finding suggests that the actual level of responding in extinction is not as important in determining the success of extinction as the extent to which the US is predicted and thus nonreinforcement is surprising.

In summary, although animals that receive extinction after operant conditioning may in fact learn to refrain from performing a particular response in a particular context (e.g., Rescorla, 1993, 1997a), the importance of response inhibition in Pavlovian extinction is not unequivocally supported at the present time. High responding in extinction does not guarantee more effective extinction learning, and a better explanation of the results of stimulus-compounding experiments (where the level of responding does appear to predict the success of extinction) may be the violation-of-expectation hypothesis, to which we now turn.

1.09.2.4 Violation of Reinforcer Expectation

It is commonly thought that each CS presentation arouses a sort of expectation of the US that is disconfirmed on each extinction trial. For example, in

the error-correction rule provided by Rescorla and Wagner (1972), the degree of unlearning (which we have seen can create inhibition) is provided by the difference in the overall associative strength present on a trial and the actual US that occurs on the trial. In the Pearce-Hall model (Pearce and Hall, 1980), the discrepancy was conceptualized as an event that reinforced new inhibitory learning that is overlaid on the original excitatory learning (see also Daly and Daly, 1982). Wagner's SOP ("sometimes opponent process") model (1981) accepts a similar idea. One piece of evidence that seems especially consistent with the expectation-violation view is the "overexpectation experiment," in which two CSs are separately associated with the US and then presented together in a compound that is then paired with the US. Despite the fact that the compound is paired with a US that can clearly generate excitatory learning, the two CSs undergo some extinction (e.g., Kremer, 1978; Lattal and Nakajima, 1998). The idea is that summation of the strengths of the two CSs causes a discrepancy between what the animal expects and what actually occurs, and some extinction is therefore observed. As mentioned earlier, the expectation-violation view is also consistent with the effects of compounding excitors and inhibitors with the target CS during no-US (extinction) trials (Wagner, 1969; Soltysik et al., 1983; Rescorla, 2000, 2003, 2006; Thomas and Ayres, 2004).

One theoretical challenge has been to capture the expectancy violation in real time. Gallistel and Gibbon (2000) have emphasized the fact that traditional trial-based models like the Rescorla–Wagner model have been vague about the precise point in time in a trial when the violation of expectation actually occurs. The issue is especially clear when trial-based models explain the extinction that occurs with a single extended presentation of the CS, as is the case for the context or background in conditioning protocols with very widely spaced conditioning trials. (Spaced trials are held to facilitate conditioning of the CS because long intertrial intervals allow more context extinction and thus less blocking by context.) There is good evidence that widely spaced trials do create less contextual conditioning than massed trials (e.g., Barela, 1999). To account for contextual extinction over long intertrial intervals, many trial-based models arbitrarily assume that the single long-context exposure is carved into many imaginary trials, and that more imaginary trials occur and create more extinction in longer-context exposures.

It is worth noting, however, that Wagner's SOP model (e.g., Wagner, 1981; Wagner and Brandon, 1989, 2001) is relatively specific about where in time the process that generates extinction occurs. According to that model, CS and US are represented as memory nodes that can become associated during conditioning. For the association between them to be strengthened, both nodes must be activated from inactivity to an active state, "A1," at the same time. Once the association has been formed, the presentation of the CS activates the US node to a secondarily active state, "A2." This in turn generates the CR. An inhibitory connection is formed between a CS and a US when the CS is activated to the A1 state and the US is activated to A2 rather than A1. This happens in simple extinction because the CS activates the US into A2. This process occurs in real time; thus, during any nonreinforced trial, inhibition will accrue to the CS from the point in time at which the US node is first activated to A2 until the CS leaves the A1 state, which may not occur until the CS is turned off at the end of the trial. Thus, extinction learning will proceed continuously as long as the CS is on and no US occurs on any extinction trial. A limiting factor, however, is the extent to which the CS itself is in the A2 state: The longer it remains on, the more likely the elements in the CS node will be in A2 rather than A1, making new learning about the CS more difficult. Nonetheless, extensions of the CS in extinction will have an effect, because elements in A2 eventually return to the inactive state, from where they will return to A1 because of the continued presence of the CS. SOP thus accounts for extinction in extended CSs without recourse to imaginary trials, and a recent extension of the model (Vogel et al., 2003) may also account for generalization decrement as a function of CS time (Haselgrove and Pearce, 2003; Drew et al., 2004). Although a complete analysis of SOP requires computer simulations that are beyond the scope of the present chapter, the principles contained in the model are consistent with many of the facts of extinction reviewed here. From the current point of view, its most significant problem is that it underestimates the role of context in extinction, and might not account for the negative occasion-setting function of context (e.g., Bouton and King, 1983; Bouton and Swartzentruber, 1986, 1989; Bouton and Nelson, 1998) that arguably provides the key to understanding the renewal, spontaneous recovery, rapid reacquisition, and reinstatement phenomena (for a start at addressing occasion-setting phenomena in terms of SOP, see Brandon and Wagner, 1998; Wagner and Brandon, 2001).

In fear extinction, at the physiological level, expectation violation may be mediated by activation of opioid receptors (see McNally and Westbrook, 2003). Fear conditioning is typically impaired by opioid receptor agonists (e.g., Fanselow, 1998) but facilitated by antagonists, such as naloxone. Extinction, in contrast, is facilitated by opioid receptor agonists and impaired by antagonists (McNally and Westbrook, 2003). According to McNally and Westbrook, opioid receptors may be involved in fear extinction because the omission of the expected US leads to a feeling of relief (Konorski, 1967; Dickinson and Dearing, 1978) that is mediated by opioid peptides; the relief associated with the absence of the US might countercondition fear responses. The idea is also captured in SOP theory's sometimes opponent process, A2. That is, activation of the US node in A2 reduces the effectiveness of the US and also constitutes the crucial event that leads to extinction. Activation of opioid receptors may thus play the physiological role of A2 in fear conditioning. A similar physiological mechanism has not yet been specified for appetitive conditioning, although the underlying basis of frustration is an obvious candidate.

1.09.3 Can Extinction Be Made More Permanent?

Recent research on extinction has explored several methods that might enhance extinction learning. These methods are discussed here because they provide further insight into the causes of extinction and how extinction therapies might be enhanced.

1.09.3.1 Counterconditioning

One way to optimize extinction learning might be to actually pair the CS with another US that evokes a qualitatively different (or opposite) response. In counterconditioning, a CS that has been associated with one US is associated with a second US, often incompatible with the first, in a second phase. Not surprisingly, performance corresponding to the second association replaces performance corresponding to the first. Clinical psychologists have incorporated this idea into therapies, such as in systematic desensitization, which involves the training of relaxation responses in the presence of a CS while fear to that CS extinguishes (e.g., Wolpe, 1958). Although counterconditioning may result in a quicker loss of phase-1 performance than simple extinction does (e.g., Scavio, 1974), it is another paradigm that, like extinction, involves a form of retroactive interference. Similar principles may therefore apply (Bouton, 1993). As with extinction, the original association remains intact despite training with a second outcome. This is true in both Pavlovian (Delamater, 1996; Rescorla, 1996a) and instrumental conditioning (Rescorla, 1991, 1995).

Equally important, counterconditioning procedures do not necessarily guarantee protection against relapse effects (the postextinction phenomena discussed earlier) (see also Rosas et al., 2001; García-Gutiérrez and Rosas, 2003, for compatible results in human causal learning). Renewal of fear occurs when rats receive CS–shock pairings in one context, then CS–food pairings in another, and are finally returned to the original context (Peck and Bouton, 1990). Complementary results were obtained when CS–food preceded CS–shock pairings. Spontaneous recovery occurs if time elapses between phase 2 and testing (Bouton and Peck, 1992; Rescorla, 1996b, 1997b). Finally, reinstatement has also been observed (Brooks et al., 1995): When CS–food follows CS–shock, noncontingent shocks delivered in the same context (but not in a different context) can reinstate the original fear performance. Counterconditioning thus supports at least three of the recovery effects suggesting that extinction involves context-dependent new learning.

1.09.3.2 Other Behavioral Techniques to Optimize Extinction Learning

If extinction involves new learning, then procedures that generally promote learning might also facilitate extinction. This idea has motivated recent research in several laboratories. For example, one idea is that conducting extinction in multiple contexts might connect extinction with a wider array of contextual elements and thereby increase the transfer of extinction learning to other contexts and potentially reduce the renewal effect (e.g., Bouton, 1991a). The results, however, have been mixed. Experiments in conditioned lick suppression (Gunther et al., 1998) and flavor-aversion learning with rats (Chelonis et al., 1999) have shown that extinction in multiple contexts can attenuate (but not abolish) instances of both ABC and ABA renewal relative to that observed after extinction in a single context (see also Vansteenwegen et al., 2006, for a related

example). In contrast, as discussed earlier, our own experiments using a fear conditioning method (conditioned lever-press suppression) with rats found that extinction in multiple contexts had no discernible influence on either ABA or ABC renewal (Bouton et al., 2006a). Null results (in ABA renewal) have also been reported with a fear conditioning (shock expectancy) procedure in humans (Neumann et al., 2007). All results together suggest that there are important variables that modulate the positive impact of extinction in multiple contexts on the renewal effect (see Bouton et al., 2006a, for a discussion).

Another approach to optimizing extinction learning is to space extinction trials in time. This idea has been inspired by the fact that spaced trials often yield better excitatory learning than massed trials (e.g., Spence and Norris, 1950). It is worth noting, though, that the behavioral mechanisms behind trial-spacing effects on conditioning are multiple and complex (e.g., see Bouton et al., 2006b), and that many of them focus on the facilitating effects of spacing US presentations, which obviously are not involved in extinction. Nonetheless, there are still some grounds for expecting trial spacing effects in extinction, and these have been tested in several experiments. Spaced trials often cause a slower loss of responding in extinction (e.g., Cain et al., 2003; Morris et al., 2005; Moody et al., 2006), as one might expect, for example, if long intervals between successive CS presentations allow some spontaneous recovery. However, the long-term effects of spacing extinction trials have been variable and much less clear. When responding is tested after a long retention interval, spaced extinction trials have been shown to reduce responding (e.g., Westbrook et al., 1985; Morris et al., 2005), have no effect on responding (Moody et al., 2006), and create more responding than massed extinction trials (Cain et al., 2003; Rescorla and Durlach, 1987). Another complication is the results mentioned earlier, which suggest that extinction ITI can also be part of the context that controls extinction performance (Bouton and García-Gutierrez, 2006). More work will be necessary to untangle these various effects.

Another temporal manipulation has attracted recent interest. In the fear-potentiated startle paradigm in rats, extinction conducted immediately after fear acquisition leads to seemingly more durable extinction (Myers et al., 2006). In particular, rats that received extinction 10 min or 1 h (and in some cases 24 h) after a single acquisition session later failed to exhibit reinstatement, renewal, or spontaneous recovery, whereas rats tested after a longer 72-h acquisition-to-extinction interval showed all these postextinction recovery effects. Immediate extinction thus seemed to produce a more permanent form of extinction that potentially corresponds to biological depotentiation (i.e., reversal) of potentiated synapses (e.g., see Lin et al., 2003). However, once again there are complications and boundary conditions. For example, humans that have received extinction within a few minutes of fear acquisition still show reinstatement (LaBar and Phelps, 2005) and renewal (Milad et al., 2005) when these phenomena are tested later. In rat experiments that measured freezing rather than potentiated startle, Maren and Chang (2006) found that immediate fear extinction may be less effective than delayed extinction under some conditions; immediate extinction never produced a more durable loss of freezing after delayed extinction. And in several appetitive conditioning preparations, Rescorla (2004b) independently found more spontaneous recovery (less-effective extinction) in rats when extinction occurred 1 day, rather than 8 days, after acquisition. Rescorla's methods differed substantially from those in the aforementioned studies, and it is worth noting that his extinction after a 1-day interval might already be outside the temporal window in which depotentiation is possible (e.g., see Staubli and Chun, 1996; Huang et al., 2001). But it seems clear that additional research will be required to fully understand the effects of the interval between conditioning and extinction on the long-term effects of extinction.

1.09.3.3 Chemical Adjuncts

Research on the neurobiology of conditioning and extinction suggests that certain pharmacological agents may also be used to optimize extinction learning. For example, there has been a great deal of recent interest in D-cycloserine (DCS), a compound that is a partial agonist of the *N*-methyl-D-aspartate (NMDA) glutamate receptor. The NMDA receptor is involved in long-term potentiation, a synaptic model of learning (e.g., Fanselow, 1993), and has now been shown to be involved in several examples of learning including fear conditioning (e.g., Miserendino et al., 1990; Campeau et al., 1992, see Davis and Myers, 2002; Walker and Davis, 2002). The discovery that NMDA receptor antagonists interfere with fear extinction (e.g., Falls et al., 1992; Cox and Westbrook, 1994; Baker and Azorlosa, 1996; Lee and Kim, 1998; Santini et al., 2001) supported the idea that the NMDA receptor was also involved in extinction learning. The next step was to ask whether an NMDA agonist like DCS

might correspondingly facilitate extinction. And it does; there is now evidence that administration of DCS facilitates extinction of conditioned fear in rats (Walker et al., 2002; Ledgerwood et al., 2003). And importantly, it also enhances exposure therapy in humans with acrophobia (Ressler et al., 2004) and social phobia (Hofmann et al., 2006). In each of these cases, when DCS was combined with a number of extinction trials that only partially reduced fear in a control group, it yielded more complete fear extinction as revealed during tests that were conducted without the drug.

The fact that DCS can facilitate extinction needs to be interpreted cautiously. For example, there is little in the description of how DCS works to suggest that it would do more than merely strengthen ordinary extinction learning, which, as we have shown, is relatively context specific and subject to relapse. Consistent with this possibility, although DCS facilitates the rate of fear extinction, it does not decrease the strength of the ABA renewal effect (Woods and Bouton, 2006). That is, rats for whom DCS had facilitated extinction still showed a robust return of fear when they were tested with the CS in the original conditioning context. This result indicates that DCS combined with extinction does not abolish the original learning. Woods and Bouton (2006) actually suggested that DCS might facilitate inhibitory conditioning of the context in which extinction occurs. Such a possibility is consistent with rapid extinction (enhanced contextual inhibition would decrease fear of the CS presented in it) and intact renewal (context inhibition would be gone when the CS is tested in another context). It is also consistent with other DCS effects reported in the literature. For example, DCS given during extinction can later reduce reinstatement (Ledgerwood et al., 2004); enhanced inhibition in the context would interfere with reinstatement by disrupting the development of context conditioning during US-alone presentations. DCS combined with extinction of one CS also causes less fear of a second CS tested in the same context (Ledgerwood et al., 2005); if the context were an inhibitor, it would inhibit fear of any CS tested in that context. Although DCS can have positive effects on fear extinction, it does not create unlearning.

Another compound that has been of interest is yohimbine, an alpha-2 adrenergic antagonist. This substance may cause paniclike responding when it is injected in animals or panic patients (Davis et al., 1979; Pellow et al., 1985; Johnston and File, 1989; see Stanford, 1995, for review). For that reason, it might increase the level of fear during extinction, and by thus enabling either increased response inhibition or a higher violation of reinforcer expectation (see earlier discussion), allow for better extinction learning. Consistent with this possibility, yohimbine administered before a fear extinction session can lead to better extinction learning in mice (i.e., less freezing in a subsequent test session conducted 24 hours later; Cain et al., 2003). We have replicated this effect in rats (Morris and Bouton, 2007). However, the facilitated extinction was highly context specific; rats tested in a new context or back in the original conditioning context after extinction with yohimbine still showed a strong renewal of fear. Thus, as we saw with DCS, yohimbine facilitates the rate of extinction learning without necessarily abolishing relapse. Further results suggested that presenting yohimbine on its own in a context allows that context to suppress subsequent extinguished fear performance – as if it was conditioning a form of context-specific fear inhibition. Although the exact mechanism is unclear, it seems apparent that as an adjunct to extinction, yohimbine once again may not prevent the occurrence of future relapse.

Behavioral neuroscientists have recently also become interested in memory "reconsolidation" effects (*See* Chapter 1.24) that might suggest a new way to modify previously learned memories. It has long been known that freshly learned memories may be labile and easily disrupted before they are consolidated into a stable long-term form (e.g., McGaugh, 2000; Dudai, 2004). The consolidation process requires synthesis of new proteins in the brain (e.g., Davis and Squire, 1984; Goelet et al., 1986) and can therefore be blocked by administration of a protein synthesis inhibitor, such as anisomycin (e.g., Schafe and LeDoux, 2000). In the case of fear memories, whose consolidation can be modulated by stress hormones, consolidation can also be hindered by administration of a β-adrenergic receptor blocker such as propranolol (Pitman et al., 2002; Vaiva et al., 2003; McGaugh, 2004; see also Pitman, 1989). Recent research suggests that an older memory that has recently been reactivated (for example) by a single exposure to the CS likewise temporarily returns to a labile state from which it needs to be reconsolidated (e.g., Nader et al., 2000; Sara, 2000; Walker et al., 2003; Lee et al., 2004; Suzuki et al., 2004; Alberini, 2005). Like consolidation, reconsolidation can be blocked by anisomycin (e.g., Nader et al., 2000), and in the case of a fear memory, by administration of propranolol (Przybyslawski et al., 1999; Debiec and LeDoux, 2004). In these experiments, memory is returned to a labile state by presenting the CS on a very small

number of occasions that are insufficient to produce extinction on their own (e.g., Suzuki et al., 2004). The crucial new result is that administration of anisomycin or propranolol while the memory is in this state can reduce evidence of conditioned responding when the CS is tested later. A therapeutic implication may be that one of these drugs in combination with one or two presentations of a CS may weaken an aversive fear memory. However, more basic research is needed. For example, it is not necessarily clear that a behavioral reconsolidation result involves actual modification of the original memory or mere difficulty in retrieving it (see Duvarci and Nader, 2004, for a critical analysis of these possibilities as induced by anisomycin). It seems clear that caution is necessary in interpreting the results of any effect of a drug or chemical on learning, extinction, and therapy.

1.09.3.4 Summary

A variety of manipulations have been thought to hold promise in optimizing extinction learning, but their effects have been mixed and (at this point in time) are not well understood. When investigators have specifically tested their effects on the relapse effects we reviewed in the first part of this chapter, they have often provided surprisingly little protection (see Bouton et al., 2006b, for a review). In contrast, one of the most effective and durable ways to optimize extinction learning and protect against relapse seems to be with the use of techniques that bridge the extinction and testing contexts, such as retrieval cues and presentation of occasional reinforced trials during extinction (discussed earlier). Bridging treatments accept the inherent context-specificity of extinction and work by increasing the similarity between the extinction context and test contexts where lapse and relapse may be a problem.

1.09.4 Conclusions

Extinction is a highly complex phenomenon, even when analyzed at a purely behavioral level. It is probably multiply determined. But, according to the results reviewed here, it usually does not involve destruction of the original learning. Instead, the main behavioral factors that cause the loss of responding appear to be generalization decrement and new learning that may be initiated by the violation of an expectation of the US. In SOP (e.g., Wagner, 1981; Wagner and Brandon, 2001), perhaps the most powerful and comprehensive model of associative learning that is currently available, that expectation violation takes the form of the CS activating the US node into a secondarily active (A2) state that potentially enables new inhibitory learning as long as the CS remains on and no US is presented. Importantly, this new inhibitory learning leaves the original CS–US association intact.

Bouton (1993) has argued that the fact that extinction might leave the original learning intact means that the CS emerges from extinction with two available associations with the US. It therefore has properties analogous to those of an ambiguous word, and the current performance depends on which of two associations is retrieved. Consistent with this idea, another fact that emerges from behavioral research on extinction is that it is relatively context dependent. Given this, the CS's second (inhibitory) association is especially dependent on the context for its activation or retrieval. The role of the context is usually modulatory; it activates or retrieves the CS's second (inhibitory) association, much as a negative occasion setter might (e.g., Holland, 1992). This hypothesis begins to integrate several facts about extinction and brings relapse effects like the renewal effect, spontaneous recovery, rapid reacquisition, reinstatement, and perhaps resurgence to center stage.

The major implication of behavioral research on extinction is thus that lapse and relapse are always possibilities after exposure therapy. As just reviewed, there is substantial interest among basic researchers in discovering new ways to make extinction more permanent. To date, behavioral and pharmacological methods of enhancing or optimizing extinction learning have produced lawful effects on the rate of extinction, but their effectiveness in the long term is less clear. Treatments that increase the rate at which fear is lost in therapy may not change extinction learning's inherent context specificity. At the current time, the best way to combat the various relapse phenomena reviewed here may be to consider their behavioral causes and develop techniques that might defeat them. This perspective has led to certain bridging treatments, such as the use of reminder cues or strategies or conducting extinction in the presence of the contextual cues that can lead to particular examples of relapse, which do appear to hold some promise in maintaining extinction performance in the presence of conditions that might otherwise initiate relapse.

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1.10 Cognitive Dimension of Operant Learning

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1.10.1 Introduction

1.10.1.1 Thorndike and the Law of Effect

A rat presses a lever and quickly scurries to the feeding niche to collect its meal. A crow picks up a piece of newspaper – uncovering a morsel of food dropped by a passerby. A child lifts the lid off of a cookie jar and takes a cookie. These are the types of acquired behaviors that a theory of instrumental learning seeks to explain. Instrumental learning is the acquisition of a new response through reinforcing feedback. Thorndike (1898, 1911) provided the first general theory of instrumental learning with his Law of Effect. The Law of Effect is a simple trial-and-error learning model in which the actions an individual makes in the presence of a particular stimulus or context are strengthened or weakened depending on the consequences of those actions. Actions followed by desirable consequences – such as attainment of food rewards or escape from aversive situations – become strengthened in that stimulus context, while actions followed by undesirable consequences – such as a loss of food or attainment of an aversive stimulus – become weakened in that context. His theory grew out of experiments on escape learning in animals – most famously cats. In these experiments he studied the acquisition of a new behavior in a controlled manner. A cat was placed in a cage that Thorndike called a puzzle box (**Figure 1**), with food located in view just outside. The cat could escape the box by manipulating a device.

Various puzzle boxes were rigged so that each one required a different manner of escape, such as pushing the door aside, pressing a lever, pulling a string, or a series of these behaviors. Thorndike repeatedly placed the cat inside a puzzle box and observed the latency to escape. As the cat learned which responses led to its release from the box, the latency to escape would diminish (**Figure 2**).

What struck Thorndike was that the cat initially tried all manners of escape – trying to squeeze through the bars, pawing at the door, clawing and biting and things in the box. The cat would

> strive instinctively to escape from confinement. The vigor with which it struggles is extraordinary. For eight or ten minutes it will claw and bite and squeeze incessantly. (Thorndike, 1898: 13)

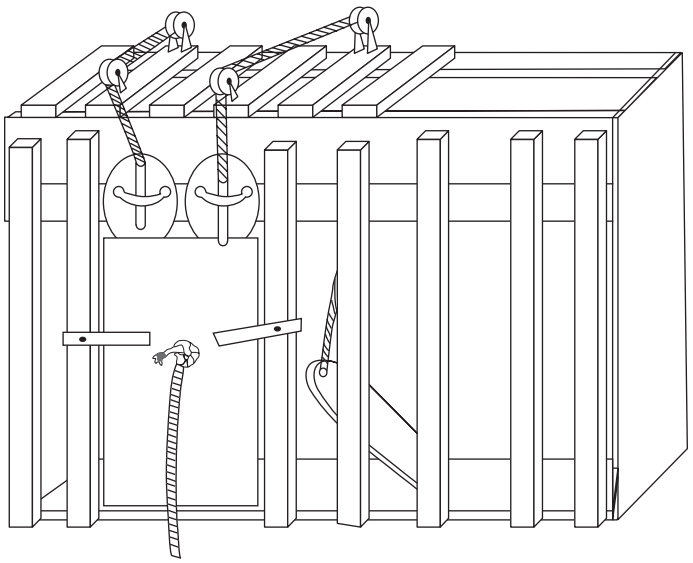

Figure 1 One of the puzzle boxes used by Thorndike to study the acquisition of new behaviors in cats. From Thorndike EL (1911) *Animal Intelligence: Experimental Studies.* New York: Macmillan.

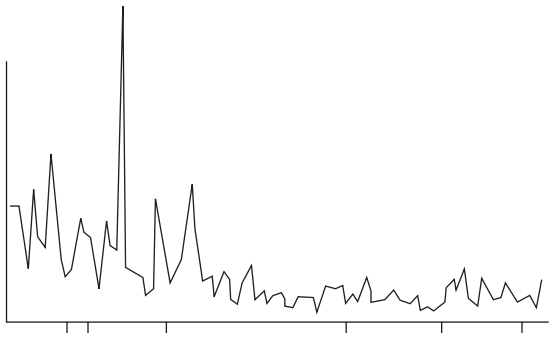

Figure 2 Escape times as a function of trials for one cat in a puzzle box experiment. From Thorndike EL (1911) *Animal Intelligence: Experimental Studies.* New York: Macmillan.

These strivings, however, diminished upon repeated exposures to the box as the cat learned which actions led to escape and which did not. The cat become more successful over repeated trials – successful escape responses that at first had been unleashed fortuitously in the random fury of its strivings became more systematically employed, while those behaviors that led to no release died away. It was this transformation from seemingly random behaviors to successfully organized ones that led Thorndike to formulate the Law of Effect.

Importantly, observations of the behavior of the cat during and after learning an escape response led Thorndike to conclude that the cat did not understand the relationship between its behavior and the consequence. Rather, the cat appeared to blindly engage in the trained action whenever it was placed in the box. Thus, in Thorndike's framework the consequences of action play an important role in the strengthening or weakening of behavior, but those consequences do not themselves enter into an association with the action or with the prevailing stimulus conditions. Rather, the subject only learns an association between the stimulus context (S) and the response (R) (**Figure 3**).

Many other behaviorists, perhaps Watson being the most famous, had their own take on S-R psychology. The behaviorist ideology eschewed references to mental terms, such as expectations, wants, and desires, and attempted to describe all acquired behavior through the objective lens of stimulus input and response output, with outcomes and consequences of behavior serving only to solidify S-R

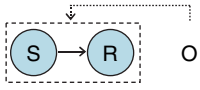

Figure 3 Theoretical associative structure underlying instrumental conditioning. S = stimulus representation, R = response representation, O = outcome. Solid arrow indicates hypothetical unidirectional excitatory association between the S and R representations. The dashed-line box envelopes the content of learning. The dotted arrow indicates that O only plays a role in reinforcing the strength of the S-R association but does not enter into an association with either S or R.

relationships (*See also* Chapters 1.03, 1.06, 1.07). Thus, to characterize the learning of a cat in a puzzle box or of a rat in a maze, the cat (or rat) is said to engage in a series of conditioned responses (muscle twitches) when they are in a certain context. The fact that these muscle twitches typically bring about a consequence is irrelevant from the cat's (or rat's) point of view. That is, the behavior of the cat or the rat is not goal directed. If one were to ask the behaviorists, "Why did the chicken cross the road?" their reply would be "Because the chicken had crossed the road a number of times in the past, and each time the crossing was followed by a satisfying outcome; whereas each failure to cross the road was followed by an unsatisfying outcome. Thus, when in the presence of the road, the experienced chicken will cross to the other side." Likewise, a child learns to lift the lid off of a cookie jar because doing so in the past has repeatedly been followed by a satisfying outcome. If the child were to be asked why they are lifting the lid off of the cookie jar, the behaviorist would expect a reply of "I dunno."

1.10.1.2 Tolman's Purposive Psychology

But doesn't it strike us as being exceedingly odd to interpret the child's act of lifting the lid off the cookie jar as NOT being due to the child's desire for a cookie – as the behaviorist would have it? Tolman agreed, and suggested that actions are performed for some purpose, that is, they are driven by expectations of an outcome (Tolman, 1932). Experiments conducted in Tolman's lab led to this conclusion. In one example, rats were given nine trials on which they learned to run down a maze and collect a desirable food reward – bran mash – in the goal box. On the tenth trial, the rats found a less desirable sunflower seed in the goal box rather than the bran mash. On the eleventh trial, the rats ran down the maze more slowly than they had on the preceding ten trials. In contrast with behaviorist theory, the rats appeared to have learned during the first nine trials to expect bran mash when they reached the goal box. The violation of this expectation on the tenth trial caused a dramatic and immediate shift in their behavior. Tolman used the behaviorist's controlled methodology and strict adherence to empirical validation to support cognitive processes as variables that intervene between stimulus and response. As a result, cognitive theories of animal learning and behavior have again become fashionable.

1.10.2 Operant Behavior: Goal Directed versus Habitual

In the past few decades, there has been a resurgence in the analysis of goal-directed behavior in psychology according to which an individual's actions are motivated by the outcome representation with which they are associated. Beyond being merely a catalyst for learning, the consequence of a response can become part of what is learned. The response–outcome (R-O) association allows the outcome to motivate the response. That is, the subject *expects* to receive O if it engages in R. A more contemporary view holds that all three elements of an instrumental learning association, S, R, and O, can be bound by associations (**Figure 4**; see also Balleine and Ostlund, 2007, for a review).

The claim that instrumental behavior can be goal-directed should not be taken to deny the existence of non-goal-directed, habitual behavior. Much of our behavior is clearly produced without the aid of any explicit representation, such as when we walk from our car to our doorstep. We may carry out such a task almost perfectly in complete darkness, or if we are otherwise distracted – such as when we are engaged in conversation with a visitor. Likewise, it is said that one way to catch a sleepwalker in the act is by rearranging the furniture in their dwelling. Presumably they will wake up when they bump into furniture that is now blocking a previously unobstructed pathway. Even when we are aware that we have rearranged the furniture in a room, it usually takes time to stop going to the corner where the easy chair used to be to have a seat. Discovering the empty corner quickly reminds us of our error. Old habits die hard, as the saying goes.

To be theoretically useful, there must be a way to empirically distinguish habitual (S-R) from goal-directed (R-O) behavior. According to several theorists (Colwill and Rescorla, 1986; Dickinson and Balleine, 1994), behavior is said to be goal directed

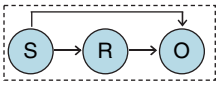

Figure 4 Theoretical associative structure underlying instrumental conditioning. S = stimulus representation, R = response representation, O = outcome representation. Arrows indicate hypothetical unidirectional excitatory associations between representations. The dashed-line box envelopes the contents of learning. Associations are hypothesized to form between S and R, between S and O, and between R and O.

if it is mediated by the instrumental contingency between the action and the outcome and by the value of the outcome (*See also* Chapters 1.03, 1.06). This operational definition is consistent with the two lines of evidence in support of R-O associations: posttraining changes in the value of the outcome and manipulations of the response–outcome contingency.

1.10.2.1 Behavioral Dissociations

1.10.2.1.1 Outcome devaluation

Perhaps the simplest way to demonstrate that the instrumental response is motivated by the expectation of the outcome is to change the value of the outcome after instrumental learning. The logic is straightforward: If desire for the outcome is motivating the instrumental response, then rendering the outcome less desirable should reduce the motivation to acquire it. Thus, reducing the value of the instrumental reinforcer after training should render it less desirable and weaken the instrumental response that had previously earned that reinforcer. Likewise, increasing the value of a reinforcer should increase the motivation to work toward obtaining that reinforcer.

Tolman and Honzik (1930) provided early empirical support for the role of the outcome expectancy in motivating the instrumental response (see also Tinklepaugh, 1928). Their study involved three groups of rats placed in a complex alley maze (**Figure 5**).

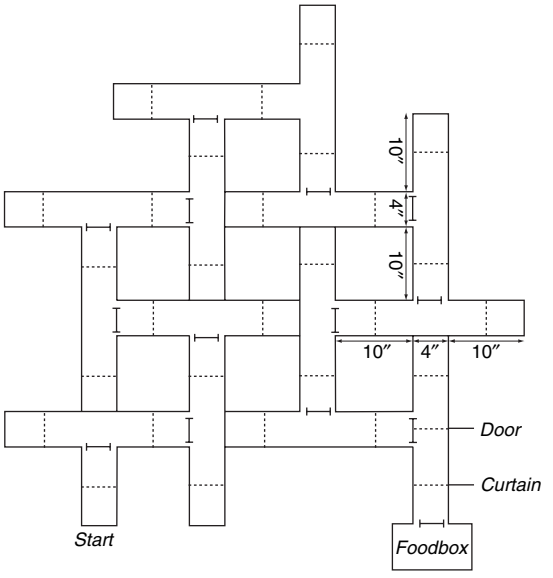

Figure 5 Maze used by Tolman and Honzik (1930) to study latent learning in rats. From Tolman EC (1948) Cognitive maps in rats and men. *Psychol. Rev.* 55: 189–208.

Each rat was placed into a start box at one end of the maze and removed from a goal box at the other end. Only one sequence of arms of the maze led from the start box to the goal box, and a number of other arms led to dead ends. Tolman and Honzik scored the number of dead-end arms rats entered ('errors') during each trip from start to goal box. One group of rats was rewarded with food when they reached the goal box, and the number of errors decreased dramatically as a function of the number of trips they made (**Figure 6**).

The second and third groups of rats received no food in the goal box for the first 10 trials in the maze. One of these groups, however, did find food in the goal box on the eleventh and subsequent trials. **Figure 6** reveals that after finding food in the goal box for the first time on the eleventh trial, the number of errors this group of rats made dramatically decreased on the twelfth and subsequent trials. This result is perhaps the most famous demonstration of latent learning in the literature, showing that rats had learned to traverse the maze efficiently (i.e., with few turns into dead ends) even in the absence of explicit food reinforcement. In fact, this learning was just as strong in the group that was not rewarded during the first 10 trials as it was for the group for which food was available from the very beginning of training. More important for our purposes, this study also demonstrates that an increase in the motivational significance of a goal (in this case the contents of the goal box) has a dramatic and immediate effect on performance. The rats in the latent group must have changed their representation of the outcome on the eleventh trial, which motivated their performance on the twelfth trial. More recent

Figure 6 Acquisition performance from Tolman and Honzik (1930). From Tolman EC (1948) Cognitive maps in rats and men. *Psychol. Rev.* 55: 189–208.

demonstrations of positive behavioral contrast effects like this provide further support for the role of outcome representations in mediating the strength of instrumental behavior (for a review see Flaherty, 1996).

Tolman and Gleitman (1949) also showed that a downshift in the cues surrounding the reinforcer can affect instrumental performance. Rats first learned to find food at either end of a T-maze. The goal box at the end of one arm was always dark, and that at the end of the other arm was always lit. Both goal boxes, however, always contained the same amount of food, and thus had equal value. Following training, the rats were taken to a separate room that was dark, in which they received foot shocks. After this experience, the rats were returned to the T-maze. Not surprisingly, the rats avoided the dark goal box in favor of the lit goal box, despite both boxes containing the same amount of food and having no shocks. This preference suggests that the rats had encoded the illumination properties of the two goal boxes and avoided the dark box in which they expected the possibility of receiving another shock.

One problem with the above studies is that they involve a change in the quality of the reinforcer, such as from no food to food in the goal box, or from food to food + shock in the dark part of the box. Perhaps the new outcome itself caused rapid changes in the instrumental response through the normal S-R mechanisms, rather than affecting the representation of the outcome mediating the response. That is, the added or altered outcome reinforces a new S-R association. This problem can be avoided by directly manipulating the value of the reinforcer itself without affecting its qualities or attributes. Adams and Dickinson (1981) demonstrated in an operant lever-pressing preparation that a downward shift in the value of the reinforcer immediately affects instrumental performance (but see Adams, 1980, 1982). Rats first learned to press a lever by reinforcing lever pressing with a food reward. After subjects had acquired the task, the reinforcer was devalued through pairings with a mild toxin that produced gastric malaise. After being paired with the toxin, rats were highly reluctant to consume the food, showing that they had acquired an aversion to it. When placed back into the conditioning chamber with the lever available that had earned the food during initial training, rats were now reluctant to press the lever as well. Importantly, the devaluation procedure was conducted away from the conditioning situation, which rules out effects of the manipulation on learning of the response and on context-illness associations. Moreover, the devaluation procedure had an immediate effect on instrumental responding when the rat was returned to the operant chamber.

More compelling evidence for the role of outcome representations in the mediation of an instrumental response comes from work by Colwill and Rescorla (1985). They demonstrated the specificity of the devaluation manipulation to the instrumental response that had earned that outcome using a choice procedure. In their study, rats were trained on two action–outcome contingencies involving two actions (lever pressing and chain pulling) and two outcomes (a sugar solution and food pellets). For each subject, one of the actions was always reinforced with one of the outcomes (e.g., lever press → sugar solution), and the other action was always reinforced with the other outcome (e.g., chain pull → food pellet). Following instrumental training of the two action → outcome sequences, one of the outcomes was devalued through pairings with a mild toxin. The effect of this devaluation procedure was to depress instrumental responding of the action that had previously earned that outcome, but not of the action that had earned the other outcome (**Figure 7**).

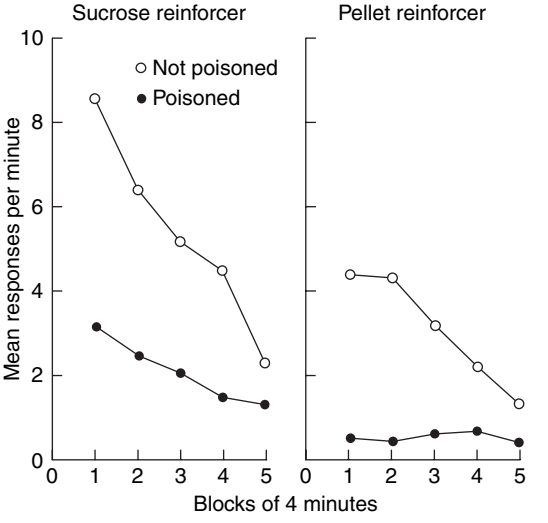

Figure 7 Experiment 1 of Colwill and Rescorla (1985). Mean response rate on the levers that had earned the poisoned (filled symbols) and nonpoisoned (open symbols) actions, for both sucrose reinforcer (left panel) and pellet reinforcer (right panel). Reprinted from Colwill RM and Rescorla RA (1985) Postconditioning devaluation of a reinforcer affects instrumental responding. *J. Exp. Psychol. Anim. Behav. Process.* 11: 120–132, with permission from the authors.

Similar results are obtained if the animal is satiated on one of the outcomes prior to the choice test session. Balleine and Dickinson (1998b) trained rats to make two instrumental responses (right and left lever presses), one for a salt-flavored and one for a lemon-flavored polycose solution. Following instrumental training, rats were given one hour to feed freely on one of the foods immediately prior to an extinction choice test in which both responses were available (but no reinforcement was delivered during the test session). Response rates were significantly lower on the lever that had earned the food reward to which they had been sated than on the lever that had earned the nonsated reward. The results of these experiments reveal the selective nature of the devaluation procedure and provide strong evidence that two separate R-O associations motivated instrumental responding. The selective nature of the devaluation treatments further show that the qualitative features of each outcome were specifically associated with the instrumental response that earned that outcome. One important caveat needs to be mentioned. In most cases, the suppressive effects of outcome devaluation on instrumental responding require the subject to reexperience the devalued food during the extinction choice test (i.e., incentive learning; Balleine, 1992; Balleine and Dickinson, 1998a,b). Initial failures to find outcome devaluation effects on instrumental responding stemmed from failures to expose the subject to the devalued outcome after devaluation treatment (Adams, 1980, 1982).

1.10.2.1.2 Manipulations of the R-O contingency

A second line of evidence for R-O associations comes from manipulations of the R-O contingency. If an instrumental response is goal directed, then by definition it should be sensitive to the relation between the action and the outcome. If the outcome is made freely available, for example, then there is no need for the individual to go through the extra effort involved in making the instrumental response to earn that outcome. Evidence showing the necessity of the R-O contingency on instrumental behavior comes from experiments using the omission procedure. In an omission procedure, a behavior is initially conditioned through pairings with reinforcement. After conditioning is established, the reinforcer is scheduled to be omitted if the subject makes a response. The reinforcer will be delivered, however, if the subject withholds responding. This procedure was developed for the purpose of dissociating instrumental from Pavlovian conditioned responding. The logic of the procedure is that Pavlovian conditioned responses (CRs) are not sensitive to the response–reinforcer contingency, and thus should not be affected by the omission of the reinforcer, whereas while instrumental responses, which by definition are sensitive to their own consequences, should be affected by the omission of the reinforcer. This procedure has been useful in discriminating acquired responses that are Pavlovian or instrumental in nature. For example, Holland (1979) showed that the acquisition of magazine approach during a tone that was paired with food developed despite the fact that food was withheld if the animal approached the food magazine during the tone. Although magazine approach appeared goal directed, it was actually shown to be insensitive to the negative response–reinforcer contingency. Rather, magazine approach appears to be a Pavlovian response (see Dickinson, 1988, for a fuller discussion of the omission procedure).

More recently, demonstrations that animals are sensitive to the response–outcome contingency have been pursued for the explicit aim of showing the goal-directedness of instrumental behavior. A simple method that demonstrates the goal-directed nature of instrumental responding is to deliver free (i.e., noncontingent) reinforcers during an instrumental session. Delivery of noncontingent food pellets during a session in which a rat is engaged in pressing a lever for food suppresses instrumental lever pressing. Moreover, if a rat has two manipulanda available, each delivering a different outcome, the suppressive effects of noncontingent delivery of one outcome is selective to the manipulandum that earns that outcome (Hammond, 1980; Dickinson and Charnock, 1985; Colwill and Rescorla, 1986; Dickinson and Mulatero, 1989). For example, Colwill and Rescorla trained rats on two instrumental responses (lever press and chain pull), each for a particular outcome (sucrose solution and food pellet). After response rates had stabilized, one of the outcomes was freely delivered in addition to being earned by the response. The rate of responding on the manipulandum that earned the noncontingent outcome decreased, while the rate of responding on the other manipulandum was unaffected. In some cases, noncontingent presentations of one outcome can depress both responses that earn that outcome and those that earn a different outcome, but the response that earns the same outcome that is made freely available shows significantly greater suppression (**Figure 8**; Balleine and Dickinson, 1998a).

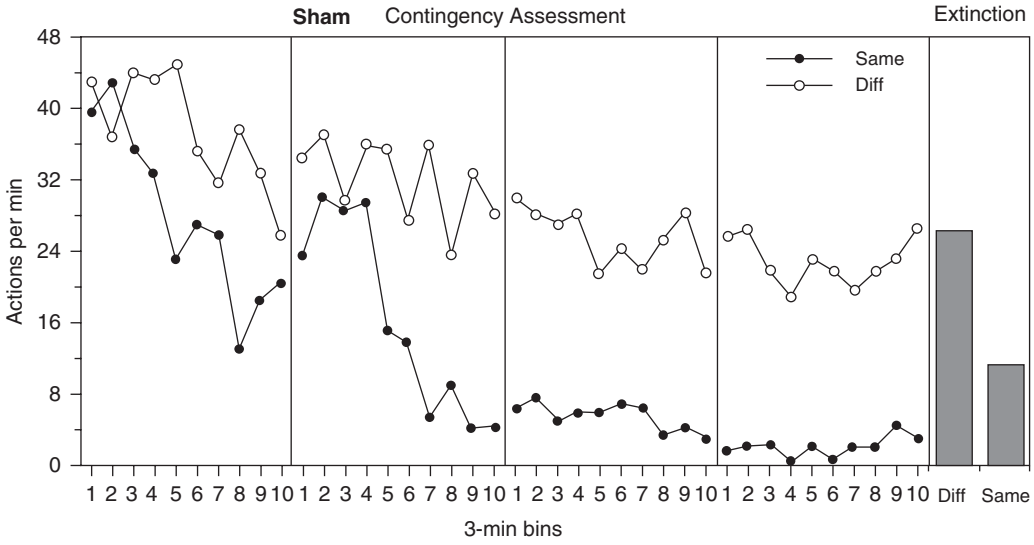

Figure 8 Figure 1 from Balleine and Dickinson (1998a). The mean number of actions (lever pressing and chain pulling) per minute during the four sessions of training under the noncontingent training and during the final extinction test (right-hand panel). The response rates are shown separately for the actions paired with the reward that was same as and different from the unpaired reward. Reprinted from Balleine BW and Dickinson A (1998) Goal-directed instrumental action: Contingency and incentive learning and their cortical substrates. *Neuropharmacology* 37: 407–419, copyright (1998), with permission from Elsevier.

1.10.2.2 Neurobiological Dissociations

The distinction between habitual and goal-directed behavior can also be made at the level of neural circuitry. Vertebrates and invertebrates both show parallel circuitry for reflexive behavior – including unconditional reflexes and species-typical fixed-action patterns on the one hand, and conditioned reflexes on the other – and voluntary behavior. For example, there are two circuits that mediate tail-flick escape behavior in the crayfish (*Procambarus clarkii*): one that mediates rapid and automatic escape responses and one that mediates slower and more flexibly controlled responses (Wine and Krasne, 1972; Edwards et al., 1999; see **Figure 9**).

The rapid escape reflex in response to abrupt stimulation, such as a sharp tap to the side of the abdomen, is a fixed-action pattern that is mediated by medial giant (MG) command neurons. Intracellular recordings from the MGs detect electrical responses in as little as 10 ms after the tap stimulus is applied. The nongiant system, which is excited by gentle prodding and pinching, mediates longer-latency responses that are under a much greater degree of control by the animal than are the immediate escape behaviors. The nongiant neural circuitry innervates and controls the same muscle systems as do the MGs, but it is much more complex (**Figure 9**) in both the interconnections and the number of layers between the sensory and motor neurons. Although much less is known about the functional control by the nongiant system, presumably it allows for a finer degree of control over the timing and direction of the movement and may even monitor actions as they are planned to allow for corrective feedback prior to execution of the action (see Section 1.10.4.1).

The distinction between the neural basis of the habit system and the voluntary or goal-directed action system can be made in vertebrates as well. There is not sufficient space here to adequately review the extensive literature on this dissociation, but it appears that, in mammals at least, S-R habit learning can be mediated at many locations within the nervous system, including the spinal cord (Chen and Wolpaw, 1995), the basal ganglia (White, 1989), and the striatum (Yin et al., 2004), whereas goal-directed R-O learning is mediated by cortical structures, such as the prelimbic area and the insular cortex (Balleine and Dickinson, 1998a).

1.10.3 Agency

1.10.3.1 Intentional Psychology: Beliefs and Desires

Now that we have established the veracity of the goal-directedness of some acquired behaviors, we can speak with some assurance about the role of

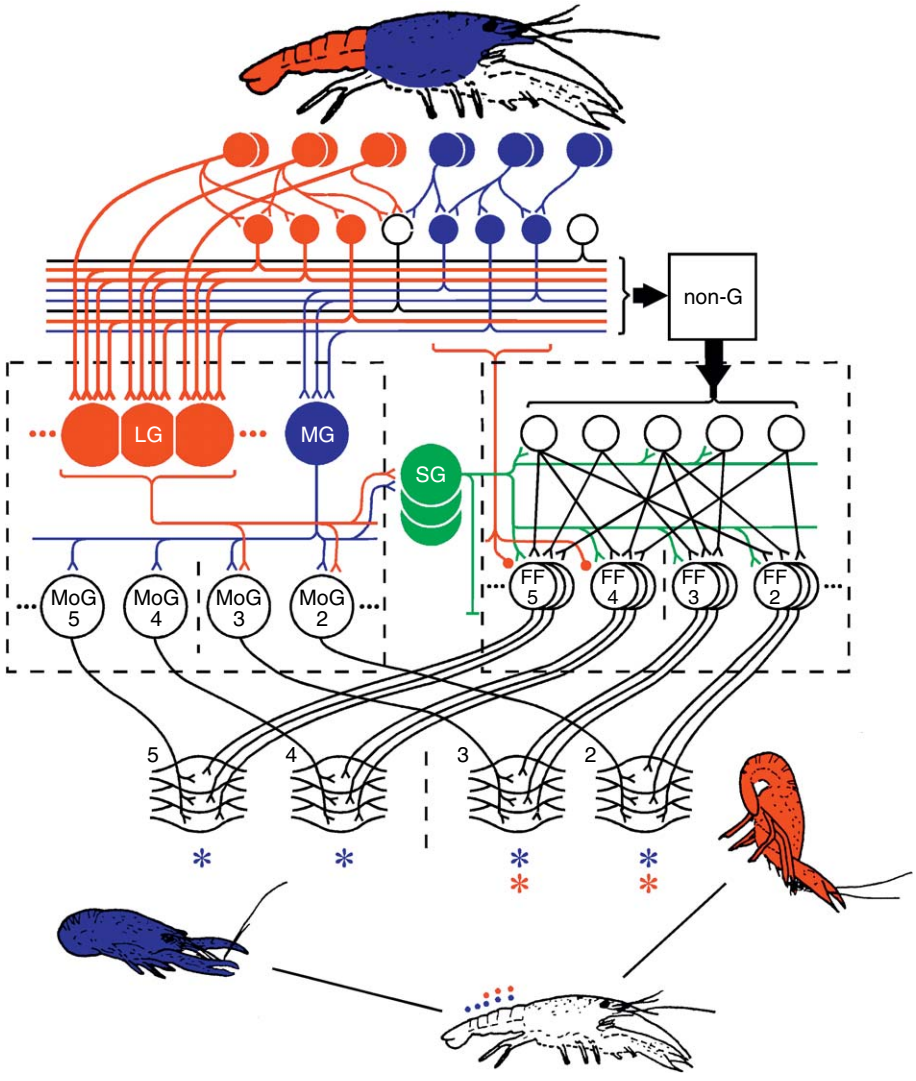

Figure 9 Neural circuitry mediating the escape reflex and volitional tail flipping in the crayfish. Giant-fiber (GF)-mediated reactions are portrayed in the drawings at the bottom of the left side of the figure: the red crayfish represents a lateral giant-axon (LG)-mediated response, and the blue crayfish represents a medial giant-axon (MG)-mediated response. The segmental joints at which bending occurs to produce these reactions are indicated by small colored circles above the white crayfish. LG-associated elements and MG-associated elements are colored in red and blue, respectively. The sensory fields (mechanosensory for LG and mechanosensory and visual for MG) for the two types of GF-mediated reactions are indicated at the top of the figure. Circuitry for GF-mediated responses is shown on the left with primary afferents, sensory interneurons, LG and MG, and giant motor neurons (MoGs) arranged from top to bottom. The multisegmental nature of the LG, which is an electrically well-coupled chain of segmental neurons, each with its own dendrites, is indicated. Colored asterisks mark phasic flexor muscles of segments 2–5 that are used in each type of GF reaction. Circuitry for responses that do not use giant neurons (non-G responses) is shown on the right. A separate population of fast flexor (FF) motor neurons generates non-G responses; uncharted circuitry (box marked non-G) and a set of partially identified premotor interneurons (open circles) mediate between sensory neurons and FF motor neurons. The segmental giant neuron (SG; green), with its blind-ending axon, allows the LG and MG to recruit non-G motor and premotor units. Lateral giant-neuron-associated sensory circuitry provides inhibitory input to caudal FFs (red) so that the SG will not cause bending at caudal joints during LG-type tail flips. Curly brackets show that multiple neurons of the population innervate the indicated target. Reprinted from Edwards DH, Heitler WJ, and Krasne FB (1999) Fifty years of a command neuron: The neurobiology of escape behavior in the crayfish. *Trends Neurosci.* 22: 153–161, copyright (1999), with permission from Elsevier.

beliefs and desires in an intentional psychology (Dickinson, 1988). Intentionality is notoriously difficult to establish in a system because so much behavior can be described using intentional language. We readily slip into intentional language to describe the behavior of our pets (Rover is barking to warn the stranger at the doorstep), computers (My laptop is searching its memory for the file), and the weather (Political leaders escaped Katrina's wrath). The language of intentional folk psychology provides a convenient shorthand to describe and explain behavior, even when we fully recognize that the behavior itself is not intentional at all (Dennett, 1987). Learning theorists themselves find it difficult not to slip into intentional language when describing the behavior of their subjects. In fact, we frequently apologize to our peers for slipping into teleological or anthropomorphic language when we catch ourselves doing so or diffuse the affront by placing the offending material in scare quotes. The rampant misuse of intentional language, however, is for the most part benign and should not devalue the scientific investigation of behavior that is truly intentional in nature. In fact, describing nonintentional behavior using intentional language may assist humankind's ability to predict and control nonintentional systems.

Given the evidence for goal-directed, intentional behavior in the animal kingdom, what is its function? That is, why would it have evolved? Couldn't a creature that lacked goal-directed behavior function just as well in its world as one that had it? Perhaps not. Explicit representation of a goal may serve to motivate behavior to bring the animal into contact with the goal. Furthermore, an animal that believes that an action produces (or prevents) a goal, and that desires (or dislikes) that goal, can be more flexible in when and how it goes about seeking and obtaining (or avoiding) the goal. For example, an omnivorous species – such as a rat, a pig, or a human – must learn which foods are good to eat and which should be avoided. A food previously discovered to be safe for consumption, however, may become spoiled or otherwise unpalatable. The animal that is capable of learning about the devalued food should be able to refrain from seeking out and consuming that food more rapidly than an animal that depended on trial-and-error learning alone. A rat is much more likely to live after one poisoning event than after many. Likewise, an animal that learns of another source where food can be obtained with much reduced effort should be able to immediately curtail exertions to acquire that food from its previous source. Such an animal can plan for the future. So far, these suppositions are no better than Kipling's (1912) *Just So Stories*. There may, however, be a more important and defensible role for intentional behavior that led to its evolution. This has to do with the concept of agency – a term that often arises in discussions of goal-directed action and intentional behavior.

1.10.3.2 Animals as Free Agents

Leslie (1995) defines an agent as an object that has three properties that distinguish it from other physical objects: mechanical, actional, and cognitive properties. The mechanical property that distinguishes agents from other types of objects is that they have an internal and renewable source of energy (or FORCE in Leslie's terminology) that allows them to *cause* things to happen in the world without themselves having to rely on external sources of energy and force (although we must acknowledge the fact that even agents must refuel). Premack (1990) has made the same distinction, invoking the term 'self-propelledness.' This property allows the agent to be a source of causation (though perhaps not the ultimate source according to adherents of philosophical determinism). To be classified as agents, objects also need to exhibit actional properties, which consist of the object's ability to act and react to events or circumstances in the world that are spatially and/or temporally distant. This property is what characterizes the agent's behavior as goal directed or intentional – at least by appearance. Note, this property of agenthood can be ascribed to an object that only appears to have intentional, purposeful, goal-directed behavior, even if the internal control mechanism itself is not goal directed. For example, a moth's suicidal plunge into a burning flame appears goal directed (the moth sought the flame) even though the control mechanism is a simple, innate (i.e., 'blind') phototaxic reflex. The third property of agency is the cognitive property. An agent's behavior is determined by its beliefs about the world (propositional knowledge). Beliefs are not only about static, coherent states of the world (semantic knowledge) but, more important, are about the causal texture of the world. To believe that A causes B asserts the belief that certain values of A (a1, a2, a3...) are determinants of certain values of B (b1, b2, b3...), and that changes in the state of A (e.g., a1 → a2) should bring about a complimentary change in the state of B (i.e., b1 → b2) (see Woodward, 2003, for a discussion of causal explanation).

1.10.4 Interventions and Causal Reasoning

1.10.4.1 Making Things Happen

What advantages does an agent gain with its ability to represent causal relationships in this manner and hold desires for particular outcomes? One obvious advantage is that such an agent could test its belief system – that is, to fact check. More important, an agent could check facts systematically (cf. Dennett's [1995] Popperian creatures) rather than through blind or random trial and error (cf. Dennett's Skinnerian creatures). The intersection of causal beliefs with desires for goals provides a creature with the ability to manipulate its environment to achieve its goals. This feature of agency provides a creature with a powerful tool – instrumental manipulation of its world. A purely Pavlovian creature can merely passively learn about the causal texture of the world through observation; such a creature is stuck in its world and can merely predict effects based on their cues and respond in anticipation. A creature with both Pavlovian and instrumental learning processes available to it could both passively learn about the causal texture of the world through observation and actively manipulate the world to directly discover its causal texture. Goal-directed instrumental learning allows the agent to actively explore its world through direct intervention. This exploration will uncover many cause–effect relationships that would have remained hidden to a purely passive observer.

Another important feature of agency is that goal-directed behaviors impart a sense of agency to the individual's intentional behaviors. That is, in contrast to reflexive habits, goal-directed behavior is accompanied by unique sensory feedback that is responsible for the sense that 'I' (the agent) control events in the outside world (Haggard, 2005). This distinction is supported by experimental (Libet, 1985) and neurobiological (Sirigu et al., 2004) evidence. These internal sensory markers of intentional behavior should be important, if not critical, to the ability to reason from causal interventions (see discussion below). That is, without the ability to distinguish effects resulting from self-generated, intentional actions from effects resulting from other causal sources (including the agent's nonvoluntary or reflexive behavior), the agent would be incapable of interventional reasoning. This suggestion is supported by imaging studies that use transcranial magnetic stimulation to temporarily inactivate a particular brain area. These studies suggest that the presupplementary motor area – which tracks the neural pathways responsible for intentional action – acts as an internal monitor of intentional action (Haggard et al., 2002; Haggard and Clark, 2003). This predicting signal allows the agent to correct errors in the execution of the action before the action itself occurs. Moreover, this predictive signal can be used to enhance the perception of non-self-generated sensations. For example, the tactile sensation that results from one finger touching the adjacent one is perceived as weaker than the same stimulus imposed from an external source. Attenuated self-generated sensations can more readily be ignored, freeing up attentional resources to focus on the external world. This attenuation of self-generated sensory experience may result from a predictive process that anticipates self-generated sensations, or a postdictive process that judges the source of the perception to be self-generated or externally generated after the sensation has been experienced.

A recent study suggests that the process is predictive (Bays et al., 2006). Participants were required to judge whether a second (comparison) tap was stronger or weaker than a first (sample) tap to their left index finger. The first tap was always the same magnitude, while the strength of the second tap was varied across trials. On most trials, the second tap followed the first by a short interval and was produced by the subject tapping with their right hand onto a button positioned above the left-hand index finger (Contact Trials, **Figure 10(a)**, top panel). This produced a sensation of tapping on one's finger through a solid object. Occasionally, subjects would receive a trial on which the second tap was artificially delayed by 500 ms after they tapped the button with their right hand. On Delay trials, the second tap was perceived as being much stronger than a tap of the same magnitude on Contact trials (**Figure 10(b)**, Group A). The increased magnitude of the perceived tap on Delay trials was not, however, a result of the absence of a right-index finger press at the time the tap was felt. Of primary importance, however, were the occasional No-Contact trials that were conducted to test whether subjects were predicting a self-generated sensation when they attempted to press the button. On No-Contact trials, the button was removed so that when the subject attempted to make a button press they failed to actually press the button (**Figure 10(a)**, bottom panel). Despite the absence of a button press, the subjects reported these No-Contact taps to be much weaker than the

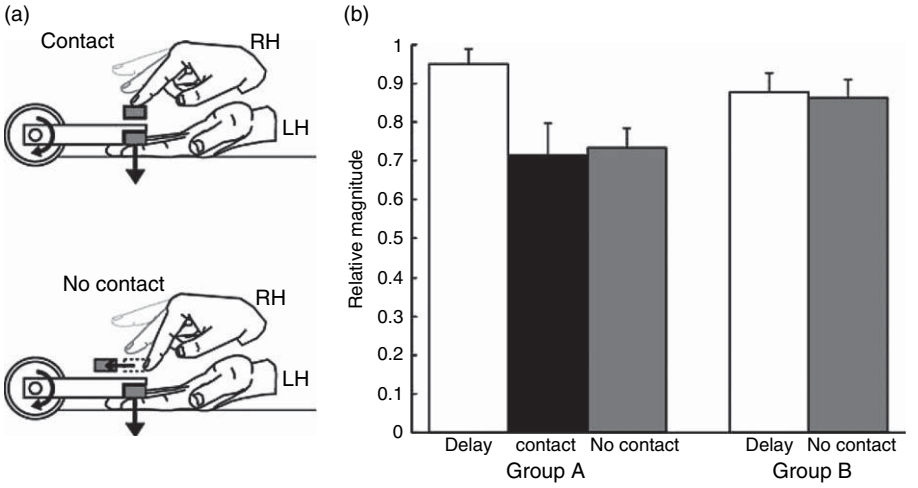

Figure 10 (a) Schematic of the apparatus and task used by Bays et al. (2006). On contact trials (top), in response to an auditory go signal, participants produced a brief force pulse with their right index finger on a force sensor fixed above their left index finger. A similar force pulse was delivered to the left index finger by a torque motor. On no-contact trials (bottom), the force sensor was moved at the start of the trial so that participants made a tapping movement with their right index finger but did not make contact. (b) Mean relative magnitude of the comparison tap to the test tap at the point of perceptual equality as a function of trial type and participant group. Error bars represent ±1 SE. Reprinted from Bays PM, Flanagan JR, and Wolpert DM (2006) Attenuation of self-generated tactile sensations is predictive, not postdictive. *PLoS Biol.* 4: e28.

taps felt on Delay trials. In fact, No-Contact taps were perceived as being the same magnitude as Contact taps (**Figure 10(b)**, Group A). These results show that the subjects anticipated the sensation of a self-generated tap, even when they were prevented from physically engaging the tap. It therefore appears that the process that monitors intentional actions is predictive and attempts to attenuate sensations arising from self-generated actions. This in turn can enhance the perception of sensations caused by an external source.

1.10.4.2 Seeing versus Doing

A burgeoning literature exploring the theoretical mechanisms of causal interventions is developing in the fields of computer science, statistical theory, philosophy, and psychology (Spirtes et al., 1993; Pearl, 2000; Steyvers et al., 2003; Woodward, 2003; Sloman, 2005; Waldmann and Hagmayer, 2005; Waldmann et al., 2006). A resulting achievement is a clear and precise language, taxonomy, and formalization of the difference between observing the cause–effect relationships among a set of variables (Seeing) and intervening on one variable to determine its causal status in relation to the other variables (Doing). These analyses describe the special status interventions have on our (human) ability to determine cause–effect relationships in the world. "If I flick this switch, the light turns on. If I don't flick the switch, the light remains dark." Such a simple cause–effect relationship can only be determined through intervention. (Note that the agent does not have to actively intervene; the agent can merely observe another agent intervening or observe a fortuitous intervention, such as a book that falls off the shelf and accidentally flicks the switch on its journey to the floor. Theoretical treatment of causal interventions does not treat these scenarios as being different in any significant way.) The ability to reason about cause–effect relationships through the intervention on a single variable is the basis for the scientific method, which gives humankind an incredible analytical power over the world. "If I put reagent X into a beaker filled with reagent Y, the mixture ignites, otherwise the mixture remains inert." "If I look at a blue-filled circle for 60 seconds, I then see a yellow, circular afterimage when I look at a white wall."

A simple example will serve to clarify the fundamental difference between Seeing and Doing and the powerful role interventions (Doing) play in causal reasoning processes. Consider the workings of a barometer (**Figure 11**).

The barometer's reading may vary upward or downward, and this variation correlates strongly with changes in the weather. If we observe an

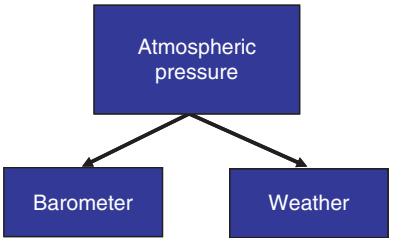

Figure 11 Causal model for how a barometer and the weather are both effects of a common cause, atmospheric pressure. Arrows indicate causal relationship directed from the cause to the effect.

increase in the barometer's reading, we can expect to see sunny skies when we look out the window. If we observe a decrease in the barometer's reading, however, we can expect to see rain clouds gathering. This tight correlation between the barometer and the weather is not the result of a direct causal relationship between the two events. Rather, changes in both the reading of the barometer and the weather are caused by a third event – changes in atmospheric pressure. If we understand the actual causal relationship among these three variables, then we would predict that tampering with the barometer (an intervention) should not affect the weather (or air pressure for that matter; Waldmann and Hagmayer, 2005). Imagine a child observing a barometer for the first time. Without prior schooling on its operation, she might at first entertain the notion – because of temporal priority – that changes in the barometer's reading cause changes in the weather. If she set the barometer to a higher reading, however, she would soon discover that she did not bring about sunny skies. Through intervention on the barometer she was able to test her hypothesis and discover a more accurate underlying causal structure. She would never have been able to discover the underlying causal relationship without access to interventional knowledge (her own or someone else's). Gopnik et al. (2004) provide another telling example:

> smoking is correlated both with having yellow fingers and with getting cancer, so having yellow fingers is correlated with getting cancer, but cleaning your hands will not keep you from getting cancer, and quitting smoking will. Knowing the right causal structure may not be essential for predicting one thing from another, but it is essential for predicting the effects of interventions that deliberately manipulate events. (Gopnik et al., 2004: 8)

Goal-directed behavior provides an important foundation for interventional reasoning. The ability to use interventions to examine causal relationships within a system is predicated on three assumptions (adapted from Gopnik and Schulz, 2004): (1) that interventions are exogenous to the system being studied and not caused by other variables within the system, (2) the intervention directly fixes one variable within the system to a specific value (e.g., a switch is moved from OFF to ON), and (3) the intervention does not affect the values of other variables within the system except through its influence on the variable that is the target of the intervention. The second and third assumptions necessarily depend on goal-directed behavior. If an individual can not represent the contingent relationship between their actions and goals, then they would not profitably use their actions – via the effects their actions have on the world – to investigate cause–effect relationships.

1.10.4.2.1 Seeing versus doing in children

Children, it turns out, do appreciate the special role of interventions in diagnosing cause–effect relationships from an early age (Gopnik et al., 2004). In one experiment, 4-year-old children were shown two or three colored rubber balls attached to sticks (hereafter referred to as 'puppets') and placed on top of a box. The children observed the puppets move up and down simultaneously. The puppets could be attached to each other – though the children could not see the attaching mechanisms – so that the experimenter could arrange for the simultaneous movement of the puppets (**Figure 12**). This enabled the children to observe the correlation of movement without observing the intervention by the experimenter. In other conditions, the experimenter could move one puppet at a time, independently of the others. In the pretraining phase of the experiment, the children watched the puppets move together simultaneously. They were then told that one of the puppets was 'special' in that it always made the other puppet(s) move.

The children were then presented with two types of tasks. In the common-effects task, the children observed two puppets (X and Y) move and then stop together simultaneously a number of times, followed by a demonstration that one of the puppets (Y) could be moved without affecting the movement of the other puppet (X). This was accomplished by the experimenter visibly moving puppet Y by moving the top of the stick to which it was attached. Finally, the children watched both puppets move

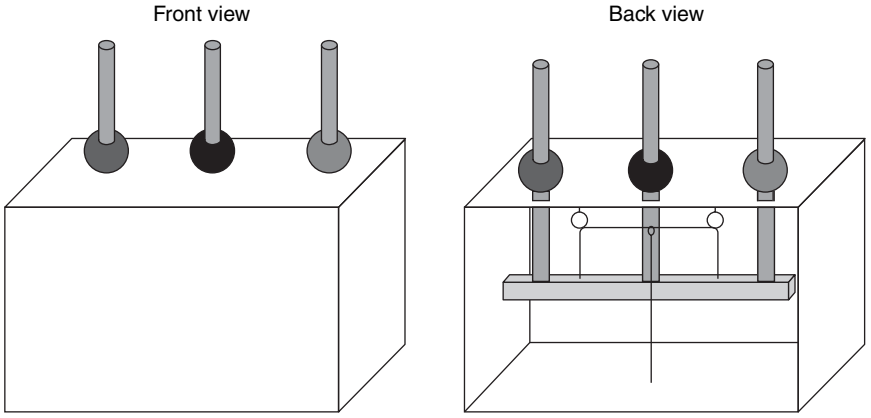

Figure 12 The puppet machine used by Gopnik et al. (2004). Reprinted from Gopnik A, Glymour C, Sobel DM, Schulz LE, Kushnir T, and Danks D (2004) A theory of causal learning in children: Causal maps and Bayes nets. *Psychol. Rev.* 111: 3–32, with permission from A. Gopnik.

together again by the experimenter invisibly moving the connecting mechanism behind the box. The children were asked "Which is the special puppet?" The children chose puppet X a majority (78%) of the time. This suggest that they drew the 'correct' causal interpretation of the relationship between the puppets; that is, that movement of Y is a common effect of both the experimenter's intervention (I) and of the movement of X. I use quotes because the movement of puppet Y was always the result of intervention by the experimenter; however, the children could not see this intervention when it was hidden by the box. Thus, given their sensory data, they observed two conditions: puppets X and Y moving without an apparent intervention by the experimenter, and puppet Y moving in the absence of the movement of puppet X when the experimenter intervened on it. The children's selection of puppet X as special indicates that they represented the causal relationships in the following way: $I \rightarrow Y \leftarrow X$, where the letters represent the events (I = intervention, Y and X = puppets) and the arrows indicate causal directionality. In causal graphs, arrows always point from the cause to the effect. Thus, in the causal graph the children inferred, Y was a common effect of both intervention by the experimenter (I) and of the movement of puppet X.

The second task involved a common-cause relationship among events. In this task, children observed three new colored puppets (X, Y, and Z) move and stop together a number of times. Then they observed the experimenter intervene to move Y independently of the other two puppets and then intervene to move Z independently of the other two puppets. The causal relationships the children should have drawn from this pattern of observations can be graphed as follows: $I \rightarrow Z \leftarrow X \rightarrow Y \leftarrow I$. This causal graph follows from the observations that puppets Z and Y could be moved independently through intervention (I) or moved simultaneously with puppet X. Thus, the children should have inferred that X was a common cause (i.e., a special puppet) of the movements of the other two puppets. This is exactly what the children reported, choosing X as the special puppet 84% of the time.

These experiments reveal how important information from interventions is for young children to reason about the causal texture of their world. If the children only had access to observations of the puppets moving together, then it would have been completely ambiguous which puppet or puppets caused the others to move, if indeed any of them were causally related to the others. It would also have been just as likely that some other, hidden force caused all of the puppets to move together (cf. changes in a barometer and the weather being driven by the unobservable air pressure). Observing that direct interventions on some of the puppets caused only those puppets to move allowed the children to select out the unlikely causal relationships among them and zero in on the most likely interpretation. That is, interventions allowed them to test their hypotheses.

1.10.4.2.2 Seeing versus doing in rats

If children can reason about cause–effect relationships from interventions, what about other animals? What is the evidence that nonhuman animals can reason about

their causal interventions? Blaisdell et al. (2006) recently investigated this question in rats using conventional conditioning procedures. In a training phase, rats observed a light followed by a tone (L → T) in one session and a light followed by food (L → F) in a second session (Blaisdell et al., 2006, Experiment 2).

One possible causal representation the rats could derive from this observational learning is that the light was a common cause of both the tone and food. No levers were present during the training phase, but during the test phase a lever was inserted into the conditioning chamber for the first time. Note that the rats had never seen this lever before and had certainly not received any training to press the lever. Nevertheless, the following contingency was put in place for half of the rats in the study: if the rat pressed the lever, the tone would come on. In contrast to this Intervention condition, the remaining rats were allocated to an Observation condition. Rats in the Observation condition also had the lever available, but pressing the lever had no effect. That is, the rats in the Observation condition had an inactive lever. The Intervention condition allowed Blaisdell et al. to assess what the rat inferred when it intervened via the lever press to turn on the tone. The Observation condition measured whether the rats expected food when they heard the tone. If rats had formed the causal model T ← L → F, then by observing the tone (Group Observe) they should diagnostically predict the light and thus also predict that food should be available (**Figure 13**, top panel, left-hand graph). By intervening on the tone, however, the rats should infer that they – and not the light – had caused the tone. Therefore, they should neither predict the light nor the food (**Figure 13**, top panel, right-hand graph). This pattern of data was exactly what Blaisdell et al. observed (**Figure 14**, left-hand bars).

If the rats were reasoning correctly about their interventions, then lever-pressing should not invariably disrupt all causal relationships between the tone and other events but only between other causes of the tone. For example, interventions on the tone should not disrupt expectations of the tone's effects. To show this, Blaisdell et al. (2006) tested another group of rats that had similar training as described above but for one key difference. Rather than receiving Light → Tone pairings in the first phase of training, they received Tone → Light pairings. This treatment, when combined with the following phase of Light → Food pairings should have taught the rats a Tone → Light → Food causal chain (**Figure 13**, bottom panels). Again the rats were divided into two test groups after receiving causal-chain training, with half the rats receiving the Intervention condition (lever pressing turned on the tone) and the remaining rats receiving the Observation condition (tones were presented independent of lever pressing). If rats had learned to treat the light as an effect of the tone, then interventions on the tone at test should still activate an expectation of the light that should then generate the expectation of food (**Figure 13**, bottom panel, right-hand graph). Thus, equivalent amounts of magazine entries should be observed in both the Intervention and Observation conditions. This is exactly what was observed (**Figure 14** central

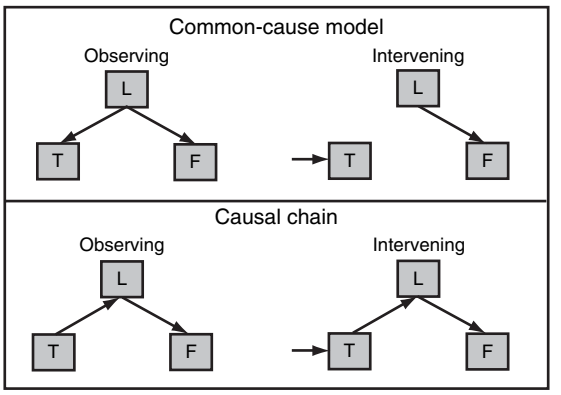

Figure 13 Causal models of the relationships among events in Experiment 2 of Blaisdell et al. (2006). Reprinted from Blaisdell AP, Sawa K, Leising KJ, and Waldmann MR (2006) Causal reasoning in rats. *Science* 311(5763): 1020–1022, with permission.

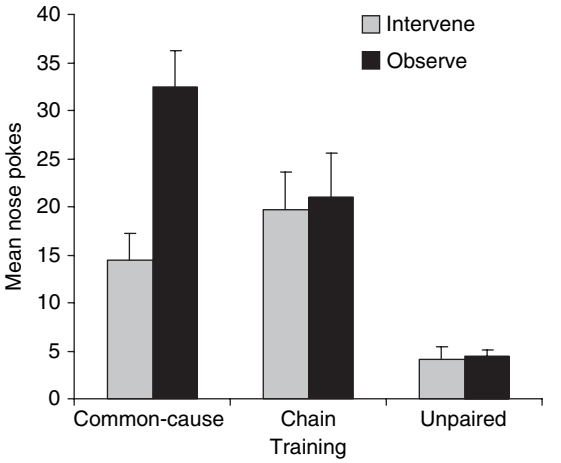

Figure 14 Data from Blaisdell et al (2006) Experiments 2a and 2b. Adapted from Blaisdell AP, Sawa K, Leising KJ, and Waldmann MR (2006) Causal reasoning in rats. *Science* 311(5763): 1020–1022, with permission. See text.

bars). It was furthermore established that magazine entries evoked by the tone depended on the tone having an indirect causal relationship to the food. This was shown through the inclusion of a third group of rats that had received unpaired presentations of the tone and light, so that the two could not enter into any kind of causal relationship, which prevented the tone from eliciting magazine entries at test (**Figure 14**, right-hand bars).

These results show that even rats can reason in a sophisticated manner about their goal-directed interventions by using them to infer the causal structure of the world. I am pitching this description of reasoning processes in rats at the computational level of analysis (Marr, 1982), which is described in terms of representations. The distinction should be made between a causal representation and the learning process though which the representation is acquired (Dickinson, 1980; Heyes and Dickinson, 1990). The learning process might take the form of an entirely algorithmic level associative process. The rats for which a lever press produced a tone did not expect food as strongly as did rats that received tones unconnected to their lever pressing behavior. A plausible explanation for this difference at the algorithmic level of description lies in the rat's knowledge about what instrumental actions do. That is, the rats in our study (and rats in general) have had lots of experience learning about the effects of their actions on the world. This learning starts in infancy (if not earlier) and continues throughout the lifetime of the rat. In fact, both 1-day-old rat pups (Johanson and Hall, 1979) and newborn human infants (DeCasper and Fifer, 1980) can learn instrumental responses. Thus, throughout their lifetimes, rats acquire a large number of action–outcome associations. This learning is typically accompanied by unique feedback cues associated with self-generated, volitional behavior (see Section 1.10.4.1). These feedback cues are presumably present while the rat is engaged in lever pressing in the study phase of the test by Blaisdell et al. (2006). If the associations between these feedback cues and prior outcomes of self-generated, goal-directed action generalize to the current test situation, then the rats that experience the tone following their lever press should discriminate the tone as being the outcome of a self-generated cause rather than the outcome of a non-self-generated cause. Therefore, the tone in the intervention condition should be treated as being caused by the rat itself, and not by the light. The light should not be expected (at a higher rate than its baseline rate of occurrence), and hence neither should the food. In other words, there are strong grounds for believing that rats can distinguish between self-generated and non-self-generated events, allowing them to learn that they can produce effects through their actions. Rats that received a tone when they pressed the lever may have generalized from their vast instrumental experience and treated the tone as the effect of their own action. This is a likely psychological mechanism candidate for reasoning about causal interventions in both nonhuman and human animals.

The analysis above suggests that, while the rats in the study by Blaisdell et al. (2006) used causal beliefs or models to reason about the source cause of the tone at test, these beliefs or models could have been acquired through conventional associative learning processes (Dickinson, 1980). Dickinson has argued that, in both animals and humans, associative learning processes can support the acquisition of causal beliefs (as distinct from associations) that then control performance through a process of practical inference (see also Heyes and Dickinson, 1990). Of course, humans also have other routes to causal knowledge available, such as verbal and instructional, but the associative (statistical) process is clearly of great importance to humans, as it is to other species.

1.10.5 Tool Use: From Crow to Cro-Magnon

The intentional, goal-directed dissection of cause–effect relationships in the world depends on two abilities: (1) the ability to discriminate self-generated intentional acts from those elicited reflexively by the environment and (2) the ability to discriminate changes in the environment resulting from one's own actions from those changes produced by other means (e.g., environmentally caused). Hence, it is only through the intentional and systematic probing and manipulation of an object that one can build a representation of its causal features. Our knowledge of everyday folk physics – that unsupported objects fall toward the ground, that pushing or pulling on an object usually moves it in the direction of the push or pull, and so on – derives from our vast lifetime experience of manipulating objects and observing the dynamic world (Gopnik et al., 2004). The ability to construct causal maps of the world through observation and planned intervention (what Gopnik et al. refer to as egocentric causal navigation) is not confined only to the human animal but is likely present in many other species as well. I already presented above

evidence that rats in a conventional laboratory setting are capable of deriving causal inferences from observational and interventional data. Below I review some of the recent literature investigating what type of causal understanding underlies tool use in nonhuman primates and in Corvids – a family of birds.

1.10.5.1 Tool Use in Primates

Tool use provides an interesting case in which to study causal knowledge and reasoning. Tools are objects with properties and affordances that convey functional value to achieving a particular goal. For example, chimpanzees (*Pan troglodytes*) in West Africa learn to use hammer-anvil stone tools to crack open palm nuts and extract the meat inside (**Figure 15**).

This behavior is transmitted culturally from one generation to the next. The full act, which takes many years to master, involves placing a round palm nut on a large, flat anvil stone and striking it hard enough with a hammer stone without causing the nut to fly off of the anvil. The learning process is motivated by many hours spent observing a proficient adult perform the act and is shaped during many hours of practice of the individual steps involved (Matsuzawa, 1994; Inoue-Nakamura and Matsuzawa, 1997; Hayashi et al., 2005). The final functional sequence eventually develops, and the skill can be usefully employed to extract the rich and nutritious meat inside the nuts.

For a tool to be functional, the user must learn about its properties and about how the user can manipulate the tool to achieve a goal. This learning might involve only a superficial understanding of the tool. For example, the animal might learn how to use the tool through procedural or habit learning without representing the underlying causal structure of the tool. The tool user may, however, acquire a deeper understanding of how the tool works. They may represent both the tool's physical properties, the rules by which those properties can be put to use (i.e., functional properties and affordances), the goal motivating the use of the tool, and the interrelationships among these domains of knowledge. This is a more complex set of relationships than what the rat was faced with in the experiment by Blaisdell et al. (2006). Perhaps this is why it has been difficult to empirically demonstrate that a tool-using animal understands the causal properties of tools and their effects.

The trap-tube task was first developed to study causal reasoning processes involved in tool use in capuchin monkeys and chimpanzees and has become a standard test for assessing causal understanding involved in tool use in nonhuman animals (Visalberghi and Limongelli, 1994; Limongelli et al., 1995; Povinelli, 2000). The trap-tube task involves the placement of a piece of food inside of a clear tube with two open ends (**Figure 16**). The subject is provided with a stick that can be used to retrieve

Figure 15 Chimpanzee using hammer and anvil stones as tools to crack open palm nuts. (Photo courtesy of Etsuko Nogami.)

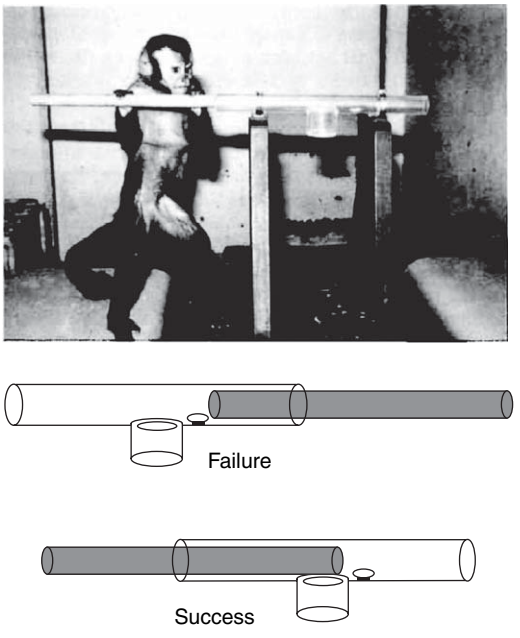

Figure 16 Trap-tube task for capuchin monkeys See text. Reprinted from Visalberghi E and Limongelli L (1994) Lack of comprehension of cause–effect relations in tool-using capuchin monkeys (*Cebus apella*). *J. Comp. Psychol.* 108: 15–22, with permission from E. Visalberghi.

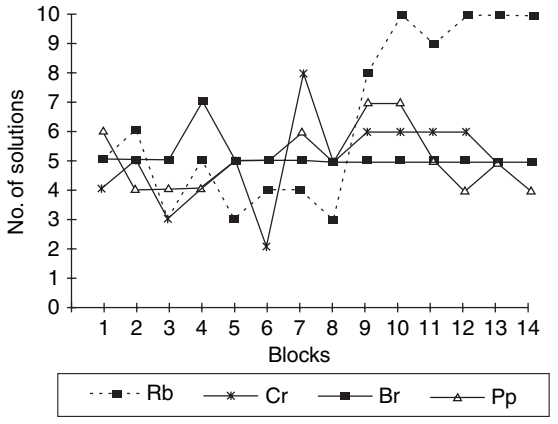

Figure 17 Number of solutions in each 10-trial block of Experiment 1 of Visalberghi and Limongelli (1994). See text. Reprinted from Visalberghi E and Limongelli L (1994) Lack of comprehension of cause–effect relations in tool-using capuchin monkeys (*Cebus apella*). *J. Comp. Psychol.* 108: 15–22, with permission from E. Visalberghi.

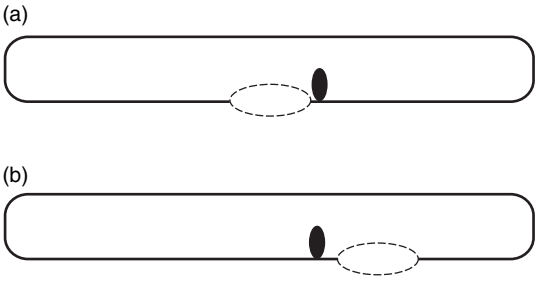

Figure 18 Trap-tube task for capuchin monkeys (Visalberghi and Limongelli, 1994) and chimpanzees (Limongelli et al., 1995). Trap Tube A is the training configuration with the trap located in the center of the tube and the food placed to one side of the trap. Trap Tube B is the transfer configuration with the trap located closer to one side of the tube and food placed in the same location within the trap that it appeared in the training tube. Whereas Trap Tube A required placing the stick in the side of the tube farthest from the reward to prevent the food from becoming trapped, Trap Tube B required changing this distance-based solution to one of inserting the stick into the side of the tube closest to the food. Reprinted from Limongelli L, Boysen ST, and Visalberghi E (1995) Comprehension of cause–effect relations in a tool-using task by chimpanzees (*Pan troglodytes*). *J. Comp. Psychol.* 109: 18–26, with permission from E. Visalberghi.

the food by pushing the food out of one end of the tube. On one side of the tube there is a hole that can trap the food if it is pushed across it. If the subject understands the nature of the trap, it should push the food out of the opposite end from the side with the trap.

In an initial study, three of four capuchins failed to learn to push the food out of the side of the tube away from the trap (see **Figure 17**), and even the successful subject only learned after about 60 trials.

Further tests revealed, however, that the successful subject did not understand how the action of the stick affected the displacement of the reward. Rather, it appeared to solve the task using a simple distance-based associative rule of placing the stick into the side of the tube farthest from the reward. A new trap tube was constructed with the trap placed closer to one end of the tube (**Figure 18(b)**). To prevent food from falling into the trap in Trap Tube B, the subject had to insert the stick into the side of the tube closest to the food rather than the side of the trap furthest from the food as was the correct solution for Trap Tube A. The successful subject, however, continued to insert the stick into the side furthest from the reward in Trap Tube B, hence causing the reward to fall into the trap. This clearly indicates that the successful monkey did not understand the relationship between the trap and the direction the food had to be moved to be successfully retrieved.

Five chimpanzees were also tested on the trap tube problem, and like the capuchins, only some of the chimpanzees learned to solve the task successfully (Limongelli et al., 1995). Unlike the capuchins, however, the two chimps that learned to solve the task did not appear to be using a simple distance-based strategy. When presented with Trap Tube B (**Figure 18(b)**), the chimps that were successful with Tube A correctly inserted the stick into the side of the trap closest to the food, thereby allowing them to push the food out of the other end of the tube (**Figure 19**).

Although the interpretation of how the chimpanzees solved the trap tube problem has been challenged with scrutiny (Tomasello and Call, 1997; Povinelli, 2000), more recent demonstrations that avoid some of the methodological problems of the earlier studies tend to support the existence of some form of appreciation of the causal nature of the task, at least by small samples of apes (Mulcahy and Call, 2006).

1.10.5.2 Tool Use in Corvids

Despite the extensive research on primates engaged in the trap tube task, some of the strongest evidence that animals can represent the causal structure underlying the tools they use comes from rooks (*Corvus frugilegus*), a member of the Corvid family, which includes crows, ravens, and jays (**Figure 20**). Although rooks are not known to use tools habitually in the wild, they will readily do so in a laboratory

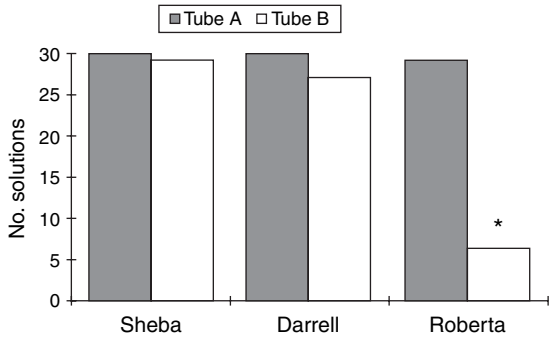

Figure 19 Performance of chimpanzees (Sheba and Darrell) and a capuchin monkey (Roberta) on Trap Tube B after having successfully learning to retrieve food from Trap Tube A. Both chimpanzees correctly inserted the stick into the side of the tube closest to the reward, allowing the successful retrieval of the food. The capuchin did not consistently insert the stick into the correct side of Tube B, and hence the food was trapped on most trials. Reprinted from Limongelli L, Boysen ST, and Visalberghi E (1995) Comprehension of cause–effect relations in a tool-using task by chimpanzees (*Pan troglodytes*). *J. Comp. Psychol.* 109: 18–26, with permission from E. Visalberghi.

Figure 20 A rook. Photo courtesy of Raven J. Brown, used with permission.

setting. In a recent study rooks were presented with a modified trap-tube task (Seed et al., 2006).

Rooks were presented with Task A, shown in **Figure 21(a)**, whereas others were presented with Task B, shown in **Figure 21(b)**. Each task involved a tube with a small piece of food located in its center. A stick was already inserted into the tube at the start of the trial, and the food was enclosed by two plastic discs. Pushing the stick forward or pulling it backward would cause the food to move in the direction of the push or pull. A 'trap' was located below the floor of both sides of the tube. One of the traps was effective in that if the food was dragged across the top it would drop inside and become trapped – preventing the subject from retrieving the food. The other trap was ineffective and could not trap the food. For the tube in **Figure 21(a)**, the trap on the right side was functional and could trap the food, and the tube on the left was ineffective. Hence, to solve the tube-trap task shown in **Figure 21(a)**, the subject should move the food over the nonfunctional trap on the left, thereby allowing the food to be retrieved. (Note, the side of the functional trap was left–right counterbalanced across trials to prevent the subject from learning a simple side-bias to solve the task.) The tube shown in **Figure 21(b)** contained a similar functional trap to that shown in **Figure 21(a)**, but the nonfunctional trap was different. Rather than pulling the food entirely across the surface of the ineffective trap, the trap was completely open so that food would fall through and out of the tube, where the subject could retrieve it.

Seven out of eight rooks learned to solve the task they were given (**Figure 22**). Task B was learned more quickly than Task A. Furthermore, transfer was almost perfect to the other task (i.e., from A to B or from B to A). This excellent performance suggests that the rooks acquired a deeper causal understanding of the task. There is, however, a simpler alternative explanation for the superb transfer between tasks. Because both tubes have the same functional trap, it is possible that the birds had simply learned to avoid moving the food in the direction of the functional trap. To test this alternative hypothesis, the birds were presented with the trap tubes shown in **Figures 21(c)** and **21(d)**. These tubes lacked the functional trap present in both Tubes A and B, and both tubes contained the nonfunctional traps from Tubes A and B. One of the originally nonfunctional traps from the initial tasks was now made functional in the new tasks. For Tube C, the nonfunctional trap from Tube A was made functional by placing bungs at the ends of the tube. Thus, the food could only be retrieved by dragging it across the trap with the opening in the bottom. For Tube D, the nonfunctional trap from Tube B was made functional by lowering the entire tube onto the floor. Thus, food dragged across the trap from Tube B would trap the food inside, while dragging the food across the trap from Tube A would allow the bird to retrieve the food.

Figure 23 shows the performance on Tubes C and D. Only one bird (open squares) consistently performed well above chance on these tubes, suggesting that this bird had acquired a deep understanding of

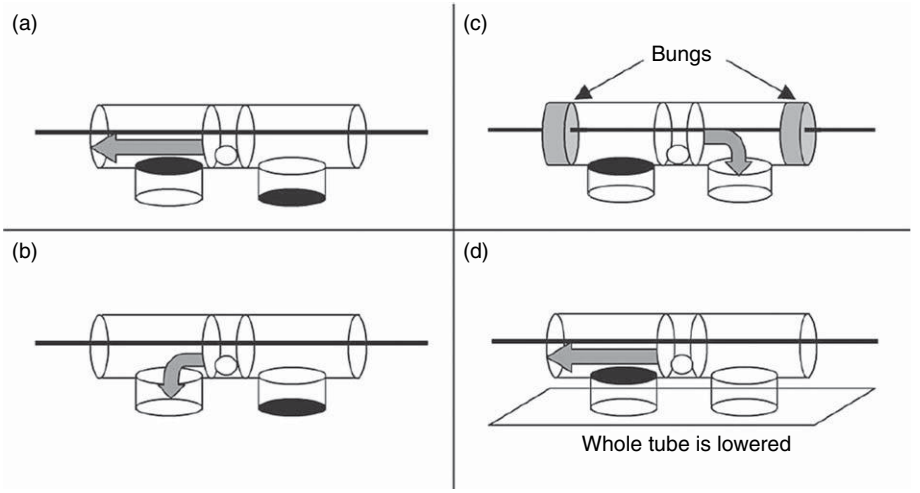

Figure 21 Trap tube problems used by Seed et al. (2006). See text. Reprinted from Seed AM, Tebbich S, Emery NJ, and Clayton NS (2006) Investigating physical cognition in rooks, *Corvus frugilegus*. *Curr. Biol.* 16: 697–701, with permission from Elsevier.

the causal structure of the trap tubes. None of the other birds were consistently above chance, and thus they likely had learned to solve Tubes A and B by avoiding the functional trap. Thus, although a majority of the birds appeared not to understand the causal properties of the tasks, one bird demonstrated knowledge of the causal properties of objects, such as that objects fall toward the earth when unsupported and that objects cannot move through physical barriers. The generalization of the knowledge about the properties of objects, and of the functional properties of the trap tube itself (i.e., how the food in the tube can be moved by moving the stick), allowed this bird to solve Tasks C and D.

The New Caledonian crow (*Corvus moneduloides*), another species of Corvid, has shown the ability to develop novel techniques to bend aluminum strips in order to use them as hook tools to retrieve food (**Figure 24**). The rapidity with which the crow achieved success not only shows that the behavior is goal directed but also conveys a deeper understanding of some of the properties of the tools and the materials and a representation of the kinds of solutions that are likely to work (Weir et al., 2002; Kenward et al., 2005). In fact, simple generalization of learned rules could not explain the excellent performance, because in most cases the solution required manipulating the tool in a way that was inconsistent or that conflicted with prior successful solutions. For example, one crow named Betty was able to correctly anticipate on four out of five trials whether bending or unbending pieces of novel materials was required to retrieve food (Weir and Kacelnik, 2007). Even this adept crow, however, often probed the recess containing the out-of-reach food with unmodified tools before modifying them or attempted to use the unmodified end of a modified tool. Though suggestive, the details of Betty's performance prevent us from determining whether she understood the physical causality underlying these tools.

1.10.5.3 Tool Use by Humankind

Although some animal species, in particular among the corvids and primates, have shown remarkable tool use abilities in the laboratory and the wild, these abilities pale in comparison to that shown by humankind. The earliest evidence for tool use among the hominids dates back by at least 2 million years, from which time modified stone tools consisting of struck flakes of volcanic rock have been found among the remains of our ancestor *Homo habilis* (**Figure 25**; Klein, 1989).

There is more extensive evidence of stone tool use by *Homo erectus* throughout Africa, Asia, and Europe dating from about 1.8 to 0.5 million years ago. By 40,000 years ago, when anatomically modern *Homo sapiens* – historically called Cro-Magnon but more appropriately referred to as *Homo sapiens sapiens* – first arrived at Europe, tool use had become quite sophisticated compared to earlier species of Homo. Furthermore, after 40,000 years ago, the evolution of tools and other technologies advanced at a very

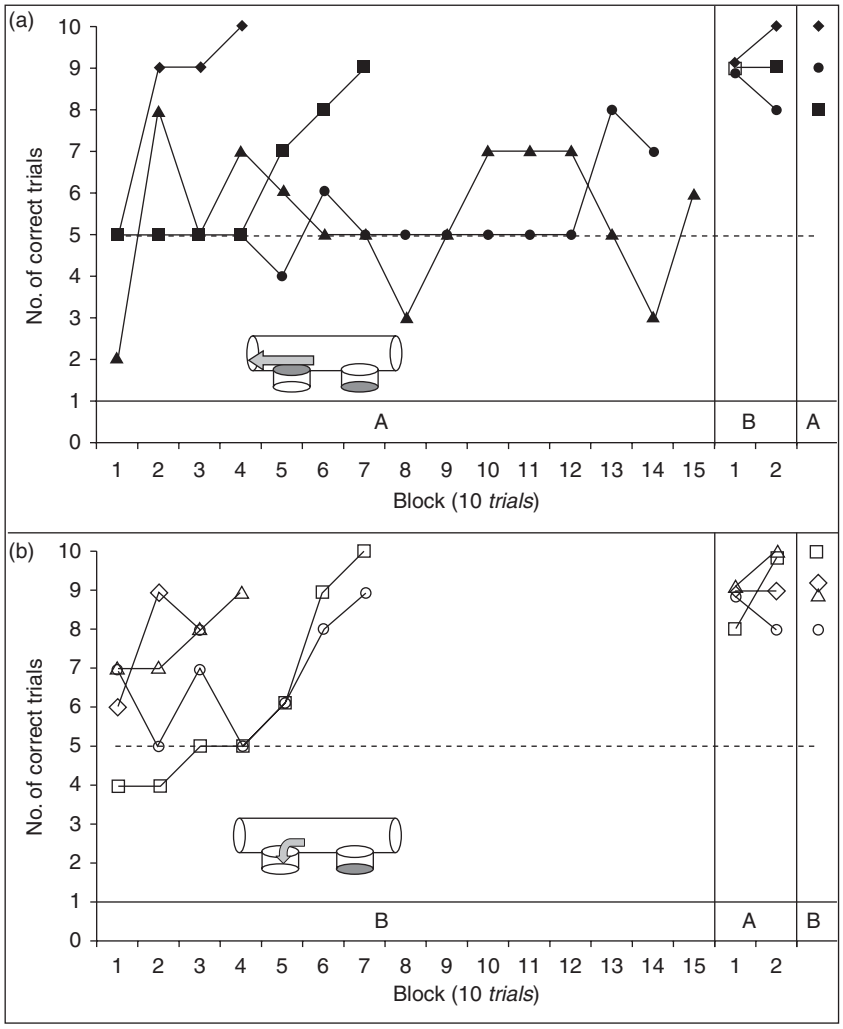

Figure 22 Results of Experiment 1 from Seed et al. (2006). See text. Reprinted from Seed AM, Tebbich S, Emery NJ, and Clayton NS (2006) Investigating physical cognition in rooks, *Corvus frugilegus*. *Curr. Biol.* 16: 697–701, with permission from Elsevier.

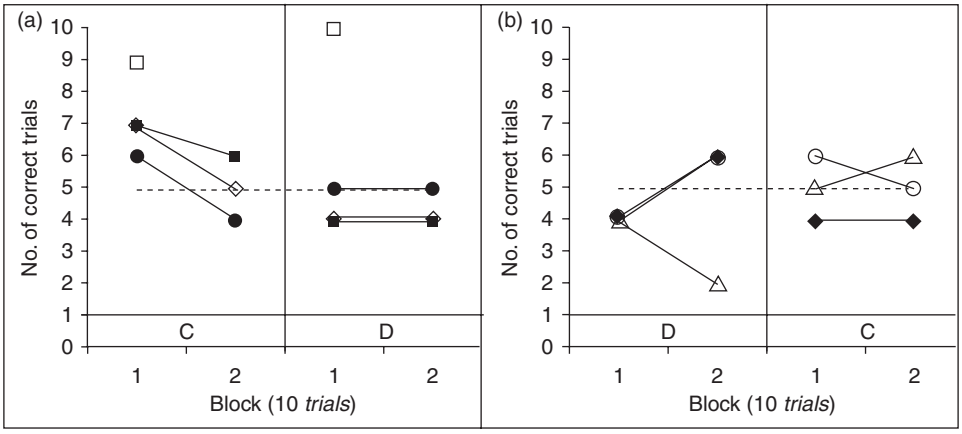

Figure 23 Results from Experiment 3 of Seed et al. (2006). See text. Reprinted from Seed AM, Tebbich S, Emery NJ, and Clayton NS (2006) Investigating physical cognition in rooks, *Corvus frugilegus*. *Curr. Biol.* 16: 697–701, with permission from Elsevier.

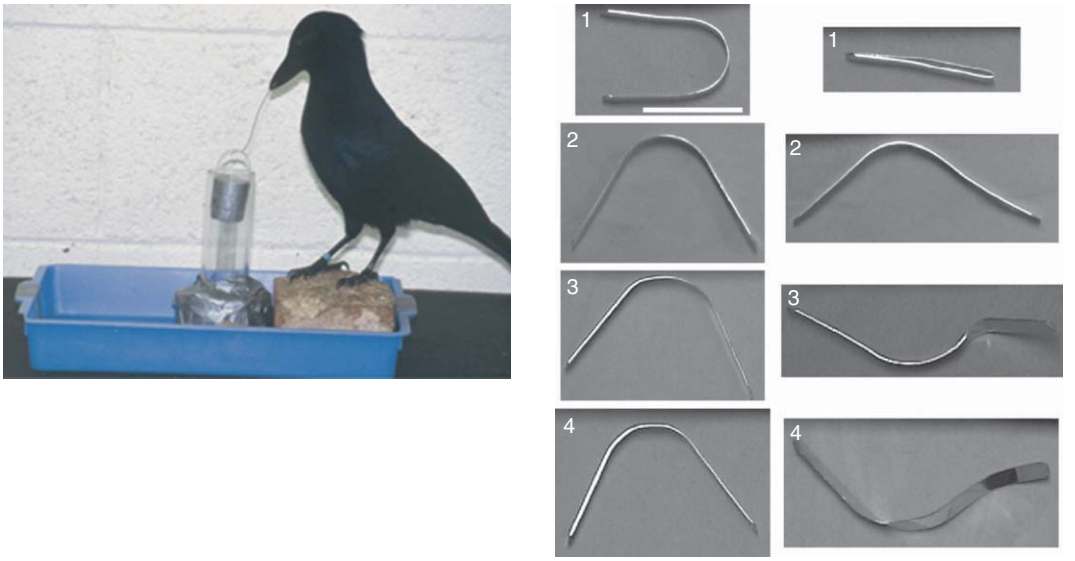

Figure 24 Left panel: Betty, a New Caledonian Crow, using a modified hook to retrieve a food reward. Right panel: Examples of hook tools modified by Betty in an experiment involving the retrieval of food. Photo courtesy of Alex Weir, used with permission.

Figure 25 Oldowan flake tools. Olduvai Gorge, Tanzania, Africa. Reproduced from University of California, Berkeley, Department of Anthropology Collection, with permission from Peter A. Bostrom.

rapid pace and showed major geographic diversification (Klein, 1989). Archeological sites throughout Europe show evidence of painting, engraving, sculpture, body ornamentation, and music. There is even indirect evidence for the weaving of wool into cloth, such as is used for clothing. Remains of dwellings, sculpture, and weapons are plentiful at many archeological sites, but perhaps Cro-Magnons are most famous for their cave paintings.

Humankind's extensive, habitual tool use, along with the evolution of language, contributes to our unique ability to adapt ourselves to life in almost every niche of the globe and to exploit a wide range of natural resources from engineered crops to nuclear energy and even allows for our occasional forays into space. This would not have been possible without our ability to represent the goals of our actions.

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1.11 Categories and Concepts in Animals

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1.11.1 Introduction and Theoretical Distinctions

> Concepts . . . give our world stability. They capture the notion that many objects or events are alike in some important respects, and hence can be thought about and responded to in ways we have already mastered. Concepts also allow us to go beyond the information given. . . . In short, concepts are critical for perceiving, remembering, talking, and thinking about objects and events in the world. (Smith and Medin, 1981: p. 1)

The ability to categorize objects and events and to extend this categorization behavior to new instances is fundamental to many human activities. We sort the objects and events around us into categories, while still being able to recognize some or all of the individual members of each category. For example, we categorize a Ferrari, a Cadillac, and a Toyota as cars, even though they do not look identical, have different engines, and are manufactured in different countries. This ability is undoubtedly beneficial. When we encounter a new model of car, we immediately recognize it as a car and engage in appropriate behavior (e.g., driving).

We also construct higher-level categories – such as clothing, tools, or furniture – whose members are perceptually disparate, but functionally similar. A violin, a piano, a flute, and a cymbal may not look alike, but we nevertheless classify them as orchestral instruments because they play music. Furthermore, we create abstract concepts that go beyond any perceptual similarities or functional interconnections; such abstract concepts entail relations between or among objects. For example, in poker, a flush entails five cards from the same suit, regardless of the values of the cards. Our everyday life contains many examples of such abstract, relational concepts: Things can be faster or slower, larger or smaller, inside or outside, same or different.

The ability to form concepts is surely adaptive, because concepts allow us to respond appropriately to novel stimuli after having experience with only a few instances from a given category. The same ability should also be beneficial for animals. One can often

observe a red-tailed hawk attacking pigeons in downtown Iowa City; undoubtedly, those downtown pigeons should soon learn to flee when they spot members of this hawk species. But, there are other, albeit rarer, hawk species which prey on pigeons, such as Cooper's Hawk or the Northern Goshawk. Clearly, the ability to recognize these less frequent predators as a threat should be advantageous for the birds, just as is our ability to recognize a Toyota as a car is advantageous for us. Charles Darwin himself in *The Descent of Man* proposed that "the senses and intuitions, the various emotions and faculties, such as ... memory, attention, ... reason, etc., of which man boasts, may be found in an incipient, or even sometimes in a well-developed condition, in the lower animals" (Darwin, 1896, p. 126). In our chapter, we review empirical studies that strongly suggest that categories and concepts are not unique to the human brain (*See* Chapters 1.07, 1.08, 1.29). Before we begin, however, we attempt to define some relevant terms.

1.11.2 Operational Definitions of Category and Concept

Since C. Lloyd Morgan (1894/1977), comparative psychologists have struggled with the following question: Do animals exhibit conceptual behavior? As is often the case with controversial questions in animal cognition, providing an operational definition of the behavior in question is the first critical step.

Cognitive psychologists often define categorization as the process of grouping objects or events into categories and responding to these categories in a similar manner (e.g., Medin and Aguilar, 2001). Concepts, on the other hand, are thought to be the elements of knowledge that assist categorization (e.g., Smith and Medin, 1981; Hampton, 2001). Many authors suggest that the term 'category' should be used to refer to classes of items, whereas the term 'concept' should be used to refer to the mental representation of those classes (Laurence and Margolis, 1999). Still others propose that the term 'concept' should be used to refer to well-defined classes characterized by a set of necessary and sufficient features and the term 'category' should be reserved for ill-defined or fuzzy classes with gradual membership (Medin, 1998). In any case, concepts and categories are treated as entities: Things to be found either in the environment or in one's mind.

Keller and Schoenfeld (1950) were first to develop an operational definition of concepts that could be made suitable for animal research. These authors began by noting that "one does not *have* a concept, just as one does not *have* extinction – rather, one demonstrates conceptual behavior, by acting in a certain way" (Keller and Schoenfeld , 1950, p. 154). What behavior, then, do we call conceptual? Keller and Schoenfeld proposed that organisms exhibit conceptual behavior when they respond similarly to members of one class of stimuli and differently to members of other classes of stimuli: "Generalization *within* classes and discrimination *between* classes – this is the essence of concepts" (Keller and Schoenfeld, 1950: p. 155). In other words, when a child says 'doggie' if she sees any dog but not if she sees a cat, or when a pigeon pecks the screen if a human being is displayed but refrains from pecking otherwise, we could say that the child and the pigeon have behaved conceptually.

The Keller-Schoenfeld definition also suggests a useful distinction between categorization and discrimination: We speak of categorization when the organism discriminates among classes of multiple stimuli rather than among individual instances of each class. So, if an organism has been trained to make one response to a single photograph of a car and to make a second response to a single photograph of a chair, then we say that the organism discriminates the car from the chair. But, if an organism has been trained to make one response to multiple exemplars of cars and to make a second response to multiple exemplars of chairs, then we say that the organism categorizes the cars and chairs. In essence, categorization entails a subset of discrimination problems in which multiple stimuli are associated with a common response.

Suppose, however, that we trained an organism to associate ten photographs of cars with one response and to associate ten photographs of chairs with a second response. Is successful learning enough to claim that conceptual behavior has been exhibited? No. Because the organism might master this task by memorizing all 20 photographs, it is obvious that we need to expand our definition: Conceptual behavior ought to be transferable to novel instances from the familiar categories. Only if the organism can produce the correct response in the presence of novel cars and novel chairs should we speak of conceptual behavior.

Indeed, even this additional requirement may not be sufficient to define conceptual behavior. What if the novel cars and novel chairs were indistinguishable from

the familiar cars and the familiar chairs seen in training? Then, the organisms' performing the correct responses in the presence of the novel testing stimuli would be a trivial failure to discriminate. A revised Keller-Schoenfeld definition of conceptual behavior should now say that an organism exhibits conceptual behavior if it can learn to respond similarly to members of one class and to respond differently to members of another class, as well as to transfer these differential report responses to novel, discriminably different members of these classes (Wasserman et al., 1988).

1.11.2.1 Types of Concepts

Humans use concepts in an extremely broad and flexible manner, classifying objects and events because they look alike, because they perform the same function, or because they entail the same relationship. For example, people may classify an object as a Ferrari (subordinate level based on subgroups within basic-level categories), a car (basic level), or a vehicle (superordinate level based on aggregation of several basic-level categories). Consequently, many researchers place concepts into three broad types (e.g., Zentall et al., 2002). Perceptual concepts (e.g., German Shepard, tree, or airplane) entail categories based on physical similarities among their members; these concepts are reviewed in the sections titled 'Perceptual concepts as basic-level categories' and 'Perceptual concepts as subordinate-level categories.' In contrast, nonsimilarity-based concepts (e.g., tools or furniture) include perceptually diverse stimuli that are grouped on the basis of common function or association; these concepts are reviewed in the section titled 'Nonsimilarity-based concepts.' Finally, abstract concepts (e.g., above or different) are based on relations between or among stimuli instead of their physical properties; these concepts are reviewed in the section titled 'Abstract concepts.'

1.11.3 Perceptual Concepts as Basic-Level Categories

Perceptual concepts have often been studied in the context of human basic-level and subordinate-level categorizations. Categorization at the basic level is generally believed to be based on high perceptual similarity among members of the same category and low perceptual similarity among members of different categories (Rosch and Mervis, 1975). In contrast, categorization at the subordinate level distinguishes subgroups within basic-level categories. So, subordinate categorization entails high within- and between-category similarity, rendering this level of categorization less preferred by humans than the basic level (Rosch and Mervis, 1975; Rosch et al., 1976; but see Tanaka and Taylor, 1991).

In this section, we review studies exploring animals' sensitivity to basic-level categories based on perceptual similarity; categorization at the subordinate level is considered in the section titled 'Perceptual concepts as subordinate-level categories.'

1.11.3.1 History of Animal Categorization Research: Herrnstein's Studies

Studies of visual categorization in animals date back to the pioneering work of Herrnstein and Loveland (1964). Using the familiar go/no-go procedure, Herrnstein and Loveland reinforced pigeons for pecking at the response plate if the photograph projected on it contained a person, but they did not reinforce the pigeons' pecks otherwise. The researchers collected 1,200 color slides, some of which contained people and some of which did not. **Figure 1** shows examples of the photographic slides used in the study.

The photographs varied in coloration and lightning. Some of them contained a group of people, whereas others depicted a single person. The depicted person or people could be partially obscured by other objects, such as trees or window frames, or could appear off center and at a distance. Nevertheless, the pigeons learned the discrimination: That is, they predominantly pecked at the photographs containing people, in spite of the great variation in content of the photographs. Moreover, the pigeons successfully categorized previously unseen photographs, thereby demonstrating an important hallmark of conceptual behavior: transfer to new exemplars from the category.

In later experiments, Herrnstein and his colleagues demonstrated that pigeons could learn tree/nontree, fish/nonfish, and even water/nonwater categories, as well as transfer their discriminative performance to novel exemplars from each category (Herrnstein et al., 1976; Herrnstein and de Villiers, 1980). Although pigeons' long-term memory can retain over 800 associations between a specific picture and a correct response (Vaughan and Greene, 1984; Cook et al., 2005), successful transfer to novel

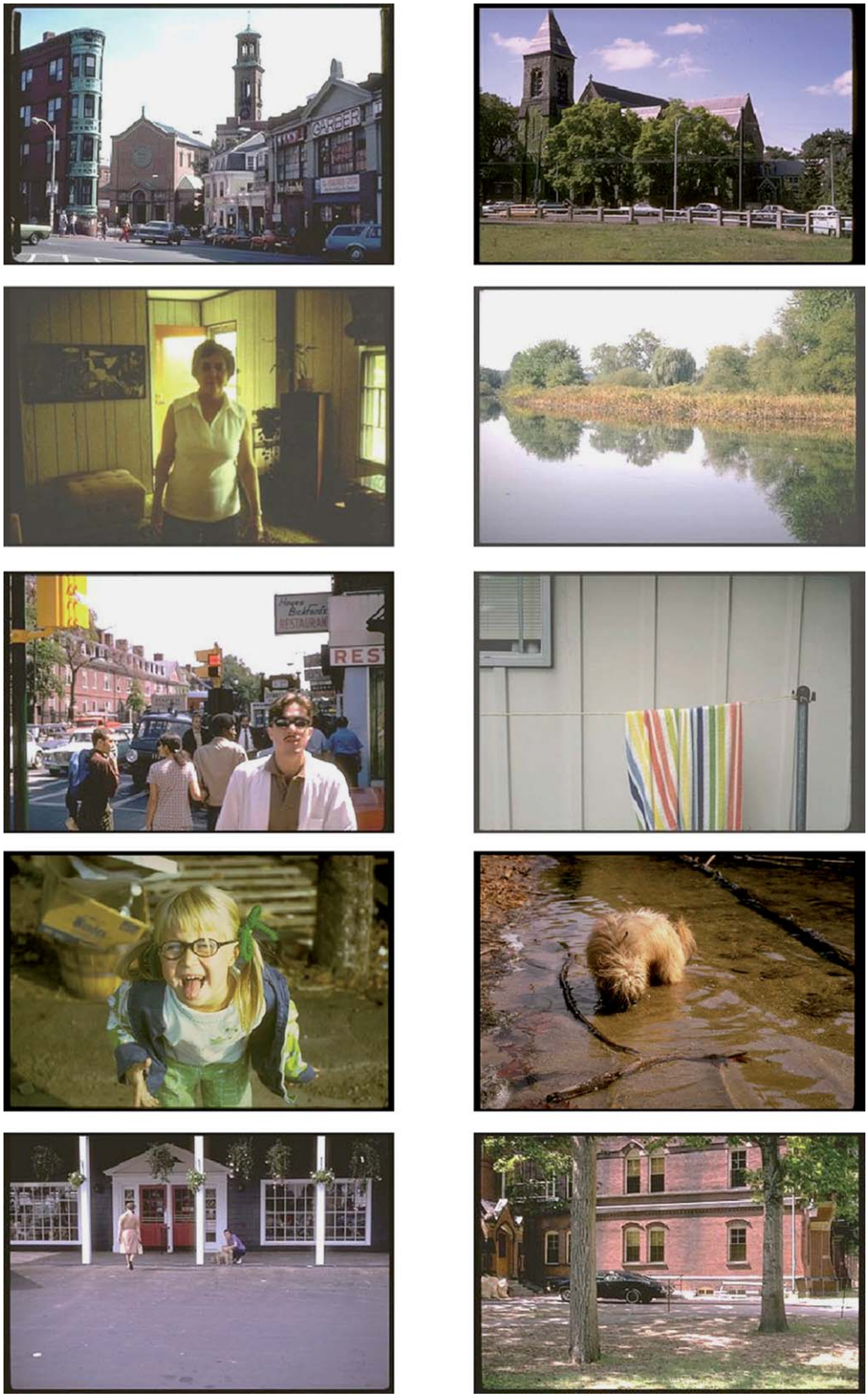

Figure 1 Representative stimuli from Herrnstein's early experiments on pigeon categorization. The left column depicts reinforced stimuli with people, and the right column depicts nonreinforced stimuli without people. Images courtesy of Will Vaughan.

members of the categories precludes simple memorization as an explanation for the pigeons' behavior.

Careful scrutiny of the slides on **Figure 1**, however, reveals a potential alternative explanation of the pigeons' performance. The backgrounds of the images in the person and nonperson categories were not matched; hence, if some features of the background (e.g., man-made objects) correlated with the presence of people, then those features might have come to control the pigeons' behavior, instead of the target object. In fact, later research has shown that, when presented with nonmatched photographs, pigeons might memorize category-relevant features, background features, or both (Greene, 1983), but when the photographs are matched, pigeons are more likely to learn category-relevant features (Edwards and Honig, 1987). Note that transfer to new exemplars can also be explained by background features being correlated with the presence or absence of the category member; such correlated features are likely to be present in the testing photographs, just as they were in the training photographs.

One could nevertheless argue that using background features together with (or instead of) the presence or absence of the target object does not mean that the pigeon cannot form the concepts of person or tree; rather, the concept formed by the pigeons may simply differ from that expected by the experimenter. Being nonverbal animals, pigeons have to discern the rules of the game from the stimulus material with which they are presented during the experiment: If the stimulus material permits an alternative strategy (e.g., trees are always located near shrubs), then the pigeons might learn to attend to such 'incorrect' cues.

Would we then conclude that pigeons' performance in these experiments fits the definition of conceptual behavior that we developed earlier? Yes. A more important and challenging question is: What features of the stimuli actually control the pigeons' behavior? The very strength of Herrnstein's approach, the use of highly varied, rich, and lifelike photographs, makes it problematic for researchers to answer this question. As we see in the next section, later studies have struggled with this problem with varying degrees of success.

1.11.3.2 Further Research on Basic-Level Categorization

Much of research that followed Herrnstein's pioneering studies continued to use the go/no-go procedure, in which one class of pictures is associated with some schedule of positive reinforcement and another class is associated with experimental extinction. Additionally, these studies generally used a single category (e.g., person) together with its complementary category (e.g., nonperson); we term this design mutually exclusive categorization. Most human language categories, however, are not of the presence/absence sort; instead, people partition the world around them into multiple clusters, such as cars, chairs, rocks, and flowers. Indeed, some authors have suggested that, although many animals can learn dichotomous presence/absence classifications, "only primates may sort the world, i.e. divide it into its indeterminately many classes" (Premack, 1976, p. 215). Although the go/no-go design can be used to study such noncomplementary categories as cats versus dogs (e.g., Ghosh et al., 2004; see also Roberts and Mazmanian, 1988), this categorization is still restricted to only two categories.

Another experimental design involves the multiple-alternative forced-choice procedure (**Figure 2**), which provides an effective means of studying categorization among multiple noncomplementary categories, such as cars, chairs, people, flowers, or cats. Using this multiple categorization scheme, pigeons have been found to be able to learn at least four noncomplementary categories of stimuli by pecking at four distinctively different choice keys and to transfer their discriminative responses to novel exemplars from those categories (Bhatt et al., 1988; Lazareva et al., 2004). Moreover, ongoing research in our laboratory suggests that pigeons' categorization is not limited to only four classes of stimuli: The birds can learn to discriminate 16 noncomplementary categories, such as ducks, bottles, trees, hats, and so on.

More than four decades of research on basic-level categorization in animals have made it clear that many animals, including primates and pigeons, can discriminate a great number of categories, including non-complementary categories (**Table 1**). Although some early reports suggested that pigeons may be unable to categorize human-made stimuli, such as cars or bottles (Herrnstein, 1985), later research has not supported this claim: Several studies have since found that pigeons can easily discriminate cars and chairs from flowers and people (Bhatt et al., 1988; Lazareva et al., 2004), and even Picasso's paintings from Monet's (Watanabe et al., 1995; Watanabe, 2001). Still, pinpointing the stimulus properties used for categorization has been elusive.

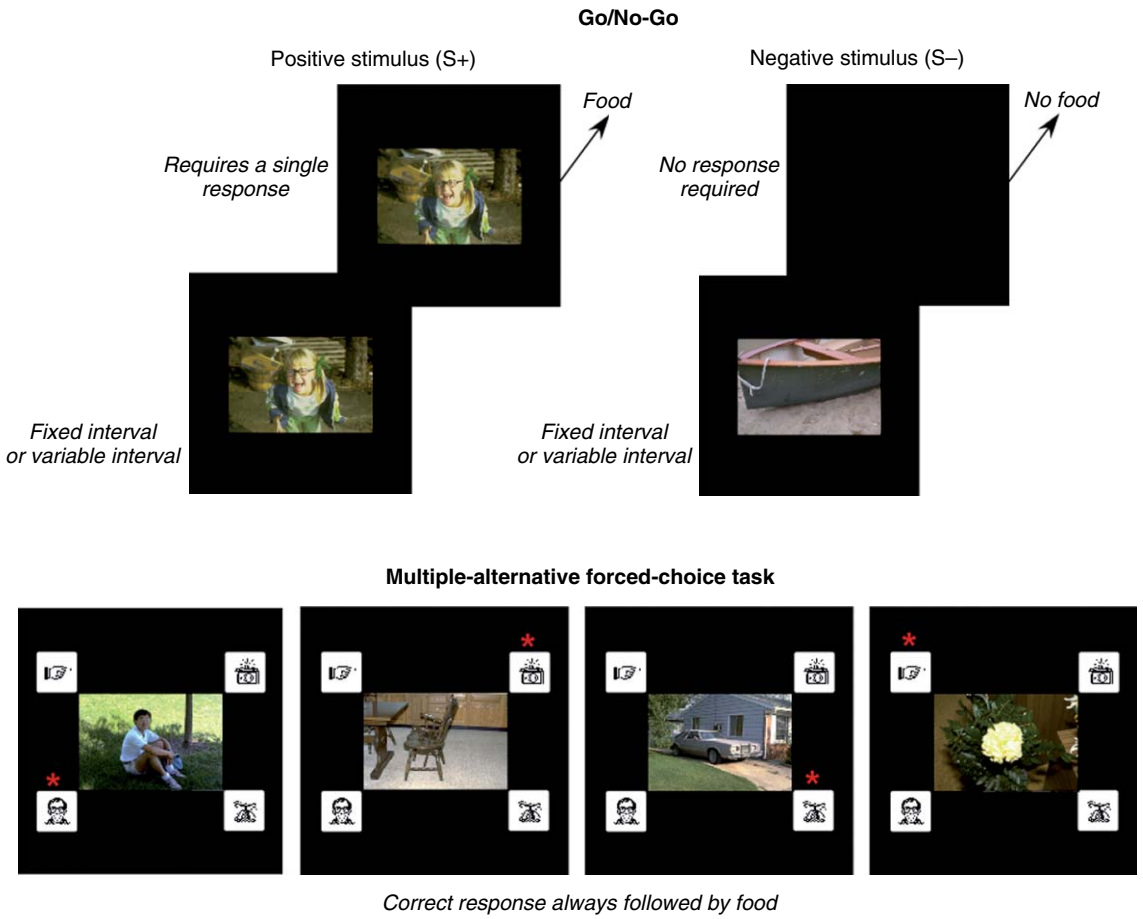

Figure 2 Comparison of go/no-go and multiple-alternative forced-choice procedures. The go/no-go procedure associates one class of stimuli (positive stimuli, or S + s) with reinforcement and the other class of stimuli (negative stimuli, or S − s) with experimental extinction. With a few exceptions, this procedure has been used with mutually exclusive categories, such as *tree/no-tree*. The multiple-alternative forced-choice procedure requires a subject to select one of the choice responses associated with a given class of stimuli (the red asterisks indicate the correct choices). This procedure has been used to study noncomplementary categories, such as people, flowers, cars, and chairs.

Table 1 Animal studies of categorization at basic level

Category	*Species*	*References*
Fish	Pigeons, rhesus monkeys	Herrnstein and de Villiers, 1980; Vogels, 1999
Trees	Pigeons, rhesus monkeys	Herrnstein et al., 1976; Vogels, 1999
Cars	Pigeons	Bhatt et al., 1988; Wasserman et al., 1988; Astley and Wasserman, 1992; Lazareva et al., 2004, 2006
Chairs	Pigeons	Bhatt et al., 1988; Wasserman et al., 1988; Astley and Wasserman, 1992; Lazareva et al., 2004;, 2006
Flowers	Pigeons	Bhatt et al., 1988; Wasserman et al., 1988; Astley and Wasserman, 1992; Lazareva et al., 2004, 2006
Humans	Pigeons, capuchin monkeys, rhesus monkeys, gorilla, baboons, chimpanzee	Fujita and Matsuzawa, 1986; Schrier and Brady, 1987; Bhatt et al., 1988; D'Amato and Van Sant, 1988; Wasserman et al., 1988; Astley and Wasserman, 1992; Watanabe, 2000; Aust and Huber, 2001, 2002; Vonk and McDonald, 2002; Aust and Huber, 2003; Lazareva et al., 2004; Matsukawa et al., 2004; Lazareva et al., 2006; Martin-Malivel et al., 2006

Studies attempting to disclose the stimulus properties controlling discriminative behavior in categorization tasks have generally used two different approaches. The first group of studies have used a *post hoc* analysis of already collected data concerning the presence or absence of some feature(s) on the target object (Schrier and Brady, 1987; D'Amato and Van Sant, 1988; Roberts and Mazmanian, 1988; Vonk and McDonald, 2004). For example, Schrier and Brady (1987) trained rhesus monkeys to discriminate photographs that contained people from photographs that did not. The photographs were rated from good (where people comprised at least 50% of the scene) to intermediate (25%–50%) to poor (less than 25%); monkeys' discrimination accuracy proved to be positively correlated with these ratings. However, other researchers using this method have often experienced difficulties in finding significant or meaningful correlations between the stimulus properties and the subjects' discriminative behavior (e.g., Roberts and Mazmanian, 1988; Vonk and McDonald, 2004).

The second and more successful group of studies has used different modifications of the training images or novel testing images varying along specific dimensions to discern the features controlling subjects' behavior (Herrnstein and Loveland, 1964; Schrier and Brady, 1987; Wasserman et al., 1988; Cook et al., 1990; Vogels, 1999; Watanabe, 2000, 2001; Aust and Huber, 2002; 2003; Ghosh et al., 2004; Lazareva et al., 2006). In a representative study, Aust and Huber (2002) trained pigeons to discriminate photographs based on the presence or absence of a person. **Figure 3** depicts some of

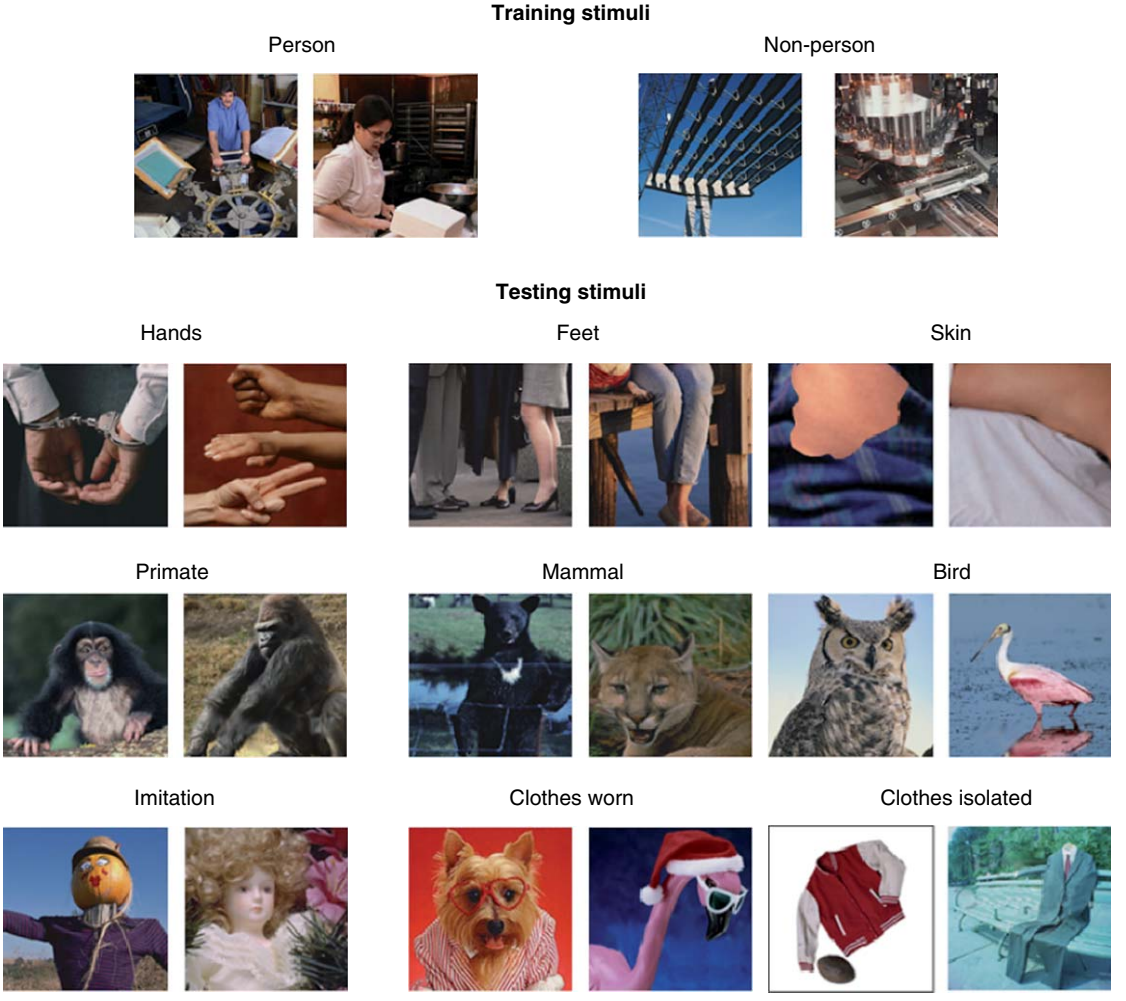

Figure 3 Examples of images used for training the *person/no-person* discrimination and for subsequent testing of features controlling pigeons' performance. Modified from Aust U and Huber L (2002) Target-defining features in a ''people-present/people-absent'' discrimination task by pigeons. *Anim. Learn. Behav.* 30: 165–176.

the images used in this study. As in earlier reports, pigeons successfully learned this discrimination. To find out which visual features controlled the pigeons' behavior, the birds were later presented with several sets of novel, nonreinforced probe stimuli that depicted some parts of the human body (hands, head, legs, and trunk) or patches of human skin of various sizes and shapes. The pigeons responded to images containing hands as if they were members of the person category, whereas images containing feet or skin patches generated the nonperson response. Aust and Huber (2002) also found that, as the similarity of the testing stimuli to the target category 'person' increased, the pigeons were more likely to classify the testing stimuli as members of the person category. Thus, artifacts imitating people (e.g., dolls or scarecrows) were more likely to produce the person response than were primates, mammals, or birds. Similarly, photographs of animals wearing clothes were more likely to be classified as members of the person category than either clothes in isolation or animals without clothes. Overall, these results suggest that pigeons' classification behavior was based on category-relevant features that were combined in an additive fashion: Exemplars with more category-relevant features were more likely to be classified as members of the person category than were exemplars with few such features.

Although some studies have been successful in identifying specific features controlling animal behavior in categorization experiments, many attempts have raised more questions than answers. For example, in our own recent study (Lazareva et al., 2006), we trained pigeons to discriminate cars, chairs, flowers, and people using a four-alternative forced-choice task. We then explored whether the pigeons' categorization behavior depended on the overall shape of the object or on its local details by using two stimulus manipulations: blurring and scrambling (**Figure 4**). Blurring

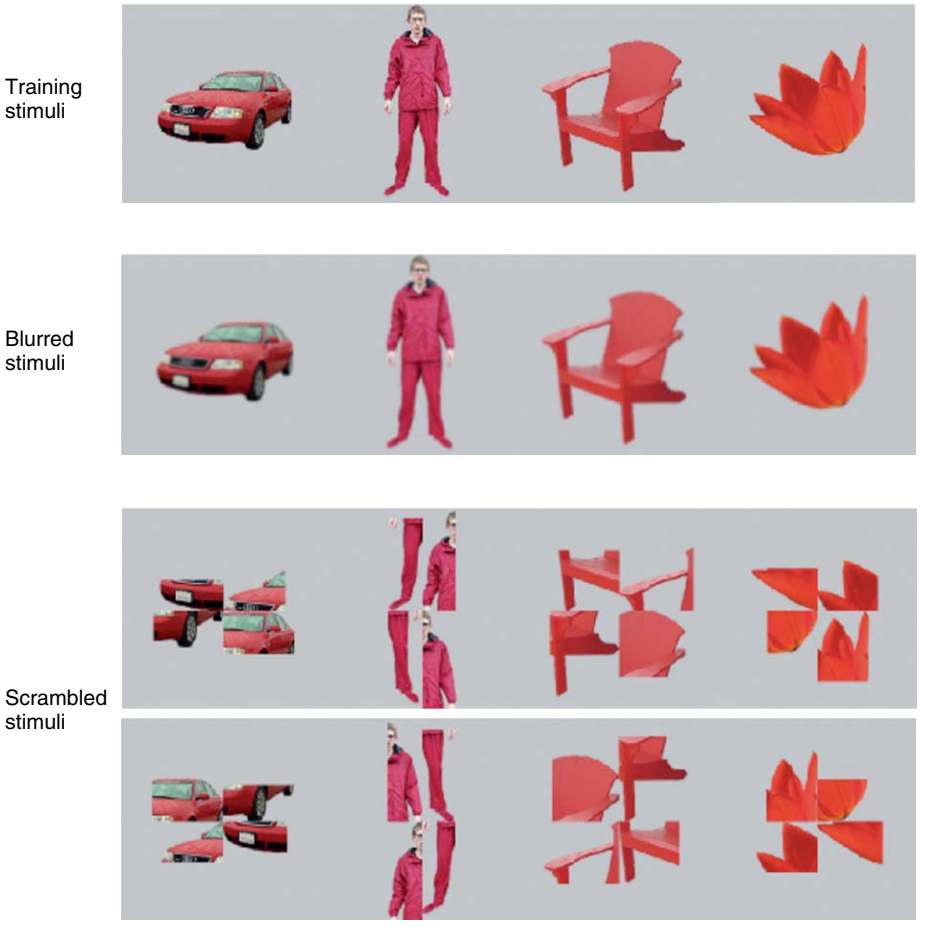

Figure 4 Examples of blurred and scrambled testing stimuli, together with the training stimuli from which they were derived. From Lazareva OF, Freiburger KL, and Wasserman EA (2006) Effects of stimulus manipulations on visual categorization in pigeons. *Behav. Process.* 72: 224–233.

distorts the fine details of the image while leaving the overall shape of the target object relatively intact, whereas four-part sectioning and scrambling leaves most of the fine details of the image unmodified but distorts the overall shape of the target object. Surprisingly, these two manipulations had decidedly different effects depending on the category of the target stimulus: Blurring impaired the discrimination of cars (and, to some extent, chairs), but it had no effect on the discrimination of flowers and people, whereas scrambling impaired the discrimination of flowers and people, but it had no effect on the discrimination of cars and chairs. These results suggest that the birds may have discriminated photographs of cars and, perhaps, chairs by using some local feature(s) and relied on the overall shape of the object when discriminating photographs of flowers and people.

But, what does it mean to say that cars and chairs were discriminated by pigeons' using some local feature? Just what might this local feature be? Similarly, what does it mean to say that pigeons relied on the overall shape of the object when discriminating flowers and people? In order to pinpoint these controlling features more precisely, additional tests, analogous to those used by Aust and Huber (Aust and Huber, 2002) are necessary. Given the large number of potential local and global features that can work alone or in combination with each other, conducting such tests for four categories would be impractical. This limitation of complex naturalistic stimuli has prompted researchers to use simpler stimuli that permit more precise analysis and manipulation to study stimulus control in categorization (see the section titled 'Artificial polymorphous categories as models of basic-level categories').

As well, innovative experimental procedures now allow researchers to efficiently isolate features of complex visual stimuli that control subjects' behavior. Martin-Malivel et al. (2006) have recently applied the reverse correlation method to disclose features controlling the categorization of human and baboon faces by humans and baboons. These researchers first trained participants to discriminate human and baboon faces at high levels of accuracy. Next, the training stimuli were warped so that the overall shape of all images was identical, a procedure that permitted the construction of a human–baboon morph. Finally, random visual noise was superimposed on the warped and morphed images to create the testing images (see top panel of **Figure 5**). The trials of interest were the morphed human–baboon images with superimposed random noise that could render the image more similar to the human face or more similar to the baboon face. The patterns of noise were then correlated with the subject's responses on a trial-by-trial basis, allowing the researchers to determine the areas of the image that were critical for categorization (see Mangini and Biederman, 2004, for procedural details).

This analysis revealed intriguing differences in the visual information utilized by people and baboons. Baboons' discrimination performance was controlled by the contrast between the eyes and the surrounding region: Lighter eye areas prompted human responses, whereas darker eye areas prompted baboon responses. People's discrimination performance, on the other hand, was controlled by this contrast information as well as by a number of other facial features, such as nose, mouth, and facial contour.

In another study deploying a different innovative technique (Gibson et al., 2005), some pigeons were trained to discriminate whether the depicted face was a man's or a woman's, whereas other pigeons were trained to discriminate whether the face had a happy or a neutral expression (see bottom panel of **Figure 5**). After pigeons' performance reached high levels of accuracy, the training stimuli were covered by a gray mask containing several openings or bubbles. The bubbles were presented at random locations on the screen, and their position determined trial difficulty. For example, the specific configuration of the mask on a 20-bubble face (**Figure 5**, bottom panel) makes it easy to determine gender, but not emotion. Thus, by correlating the locations of the bubbles with a subject's categorization responses, we can again determine the features that support reliable discrimination behavior (see Gosselin and Schyns, 2001, 2005, for details). Here, both pigeons and people were found to utilize similar pictorial information. When discriminating happy from neutral faces, both pigeons and people relied heavily on the bottom part of the face including the mouth. When discriminating male from female faces, both pigeons and people relied on regions near the eyes and the chin.

Clearly, both reverse correlation and bubbles procedures have great potential for elucidating the perceptual bases of categorization in animals (see Gosselin and Schyns, 2002, and Gosselin and Schyns, 2004, for a comparison of the techniques). Note especially that the conventional methods of modifying or deleting parts of the images to divulge which features might control performance requires some notion as to the identity of those features. In contrast, neither reverse correlation nor bubbles make such assumptions; instead, these techniques sample all of the information that is available in the images. We expect

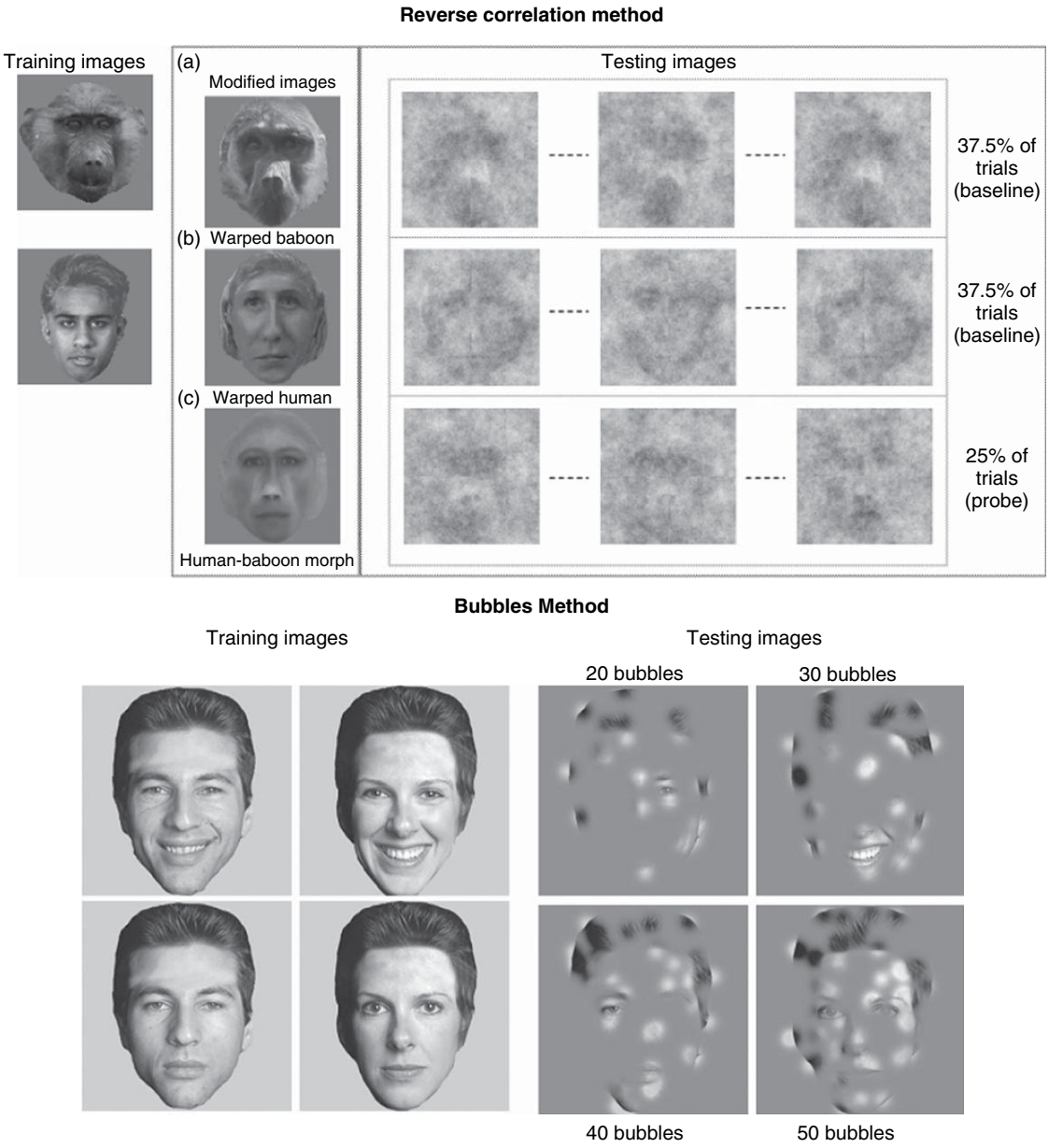

Figure 5 Illustration of training and testing stimuli for the reverse correlation method (top) and for the Bubbles method (bottom). Top: After subjects learned to discriminate human faces from baboon faces (training images), the training images were warped (modified images) or superimposed with random visual noise to obtain the testing images. Note that the human–baboon morph stimulus superimposed with random noise can look more similar to the human face on some trials and more similar to the baboon face on other trials. Modified from Martin-Malivel J, Mangini MC, Fagot J, and Biederman I (2006) Do humans and baboons use the same information when categorizing human and baboon faces? *Psychol. Sci.* 17: 599–607. Bottom: After training to report whether a face is happy or neutral or whether it belongs to a male or a female, the training stimuli were covered with a mask revealing only a portion of the stimuli through the openings or 'bubbles.' Note that the position of the bubbles randomly varied across trials so that different areas of the faces could be revealed on different trials. Modified from Gibson BM, Wasserman EA, Gosselin F, and Schyns PG (2005) Applying Bubbles to localize features that control pigeons' visual discrimination behavior. *J. Exp. Psychol. Anim. Behav. Process.* 31: 376–382.

that deploying these and other new methods will greatly advance our understanding of how animals categorize complex visual stimuli.

1.11.3.3 Do Animals Perceive the Cohesiveness of Basic-Level Categories?

Extensive experimental evidence suggests that animals can be assiduously trained to sort stimuli into different basic-level categories, including noncomplementary categories (cf. **Table 2**). But, do animals view the members of these categories as being perceptually coherent before such categorization training, as do people? In other words, do animals directly perceive the members of a basic-level category to be more similar to one another than to the members of other categories?

Several studies suggest that primates may indeed perceive basic-level categories to be perceptually cohesive clusters of stimuli. Fujita and Matsuzawa (1986) used a sensory reinforcement procedure to find out whether a chimpanzee can form the category 'person' without explicit reinforcement. The chimpanzee was shown a picture on a screen and was required to touch a response key repeatedly to keep viewing the same picture. If the response key was not touched within 10 s, a new picture was presented. Analysis of the intervals between responses and the duration of responses revealed that the chimpanzee preferred to view photographs containing people; it rarely pressed the key to repeat photographs that did not contain people. This nonpreferred, no-person category also included some ambiguous photographs in which the target object was very small as well as entirely white or entirely black photographs.

Table 2 Animal studies of artificial polymorphous categories

Species	*Technique*	*Reference*
Pigeons	Go/no-go	Lea and Harrison, 1978; von Fersen and Lea, 1990; Huber and Lenz, 1993; Jitsumori, 1993; Huber and Lenz, 1996; Jitsumori, 1996
	Two-alternative forced-choice	Lea et al., 1993
Rhesus monkeys	Go/no-go	Jitsumori, 1994
Baboons	Two-alternative forced-choice	Depy et al., 1997

In another experiment (Sands et al., 1982), rhesus monkeys were trained to move a lever in one direction if two successively shown pictures were identical and to move the lever in a different direction if the pictures were nonidentical. The set of pictures included six different exemplars of human faces, monkey faces, trees, flowers, and fruits. If the monkeys perceived members of the same category (e.g., fruit) to be more similar to each other, then they should have been more likely to erroneously respond same when a picture of an apple was followed by a picture of an orange than when it was followed by a picture from another category (e.g., an oak). Analysis of confusion errors found this to be the case: All pictures of fruit fell into the same region of multidimensional space. Interestingly, pictures of monkey faces and human faces were clustered together, as were pictures of trees and flowers, suggesting that rhesus monkeys viewed monkey faces as being similar to human faces and that they viewed trees as being similar to flowers.

What about pigeons? Several reports suggest that pigeons too perceive similarity among the members of basic-level categories (Wasserman et al., 1988; Astley and Wasserman, 1992). For example, Astley and Wasserman (1992) evaluated the perceptual coherence of basic-level categories using a go/no-go method. For some pigeons, 12 photographs of cars could serve as positive discriminative stimuli: The birds were reinforced for pecking these stimuli when they were shown. For other pigeons, 12 photographs of chairs, flowers, or people could serve as the positive discriminative stimuli. The set of negative discriminative stimuli was common to all of the pigeons and consisted of 12 photographs each of cars, chairs, flowers, and people that were different from the 12 photographs that were used as the positive discriminative stimuli. As pigeons learn this discrimination, their rate of responding to all of the negative discriminative stimuli is expected to fall. However, if pigeons see categories as collections of perceptually similar items, then the rate of responding to the negative stimuli from the same category as the positive discriminative stimuli should have fallen more slowly than to the stimuli from the other categories. Astley and Wasserman (1992) again found that the data supported such perceptual coherence: Pigeons committed most errors to the negative discriminative stimuli from the same category as the positive discriminative stimuli.

In sum, most researchers have found that, just like people, animals perceive basic-level categories to be perceptually cohesive clusters of stimuli (but see Sutton and Roberts, 2002). Recent research in our

own laboratory has also begun to explore similarities across different basic-level categories, specifically natural categories, like 'flower' or 'person,' and artificial or human-made categories, like 'chair' or 'car'. Studies of certain cerebral pathologies in humans have found an intriguing dissociation between impaired recognition and the naming of living objects and nonliving objects, suggesting that these types of categories could be represented in a qualitatively different manner (see Farah, 1999, and Martin and Caramazza, 2003, for a review). Some researchers have proposed that the categorization of artificial objects is based on their functional specifications or on the kinesthetic representation of the movements performed in using them, whereas the categorization of natural objects is based solely on their perceptual properties (Warrington and McCarthy, 1987; Damasio, 1990). Other researchers have suggested that the difference is one of degree and not of kind: The members of natural categories may be more similar to each other than are the members of artificial categories (Humphreys et al., 1988; Gaffan and Heywood, 1993; Lamberts and Shapiro, 2002; McRae and Cree, 2002).

Of course, when nonverbal animals are trained to sort photographs of cars, chairs, flowers, and people, this categorization ought to be based only on perceptual similarity. Thus, finding a natural–artificial dissociation in pigeons' categorization behavior suggests that people's categorization behavior may also be based on the perceptual coherence of the given categories. So far, our data suggest that pigeons perceive two natural categories, flowers and people, to be more similar to each other than to either cars or chairs, but they perceive cars and chairs as being just as similar to one another as they are to either flowers or people (Lazareva et al., 2004). Similarly, rhesus monkeys appear to perceive living objects as more similar to each other than to nonliving objects (Gaffan and Heywood, 1993). Although more research is needed, current data point to many potential similarities in how animals and humans perceive similarities within and between basic-level categories. The neural mechanisms of such classification behavior may be especially informed by comparative studies of the sort reviewed here.

1.11.3.4 Artificial Polymorphous Categories as Models of Basic-Level Categories

Although considerable research has demonstrated that animals can classify pictures of objects such as trees or cars, the stimulus features controlling such discriminative behavior have been much more difficult to determine (see the section titled 'Further research on basic-level categorization'). Consequently, some researchers have adopted a different approach: Instead of trying to deal with already-existing categories, they have constructed *m*-out-of-*n* artificial polymorphous categories, where a specific instance is a member of a category if it contains *m* out of *n* relevant features. Natural basic-level categories are often believed to be polymorphous; that is, there is no single feature that is necessary or sufficient for category membership. Thus, artificial polymorphous categories constructed in a similar manner might to be an effective way to model basic-level categories.

Figure 6 depicts one example of such an artificial polymorphous category studied by Jitsumori (1993). The relevant dimensions are the shape of the elements (circles or triangles), the color of the elements (white or black), and the color of the background (red or green). Here, as well as in many other similar studies, the dimensions are selected to be orthogonal, so that the contribution of each feature can be independently

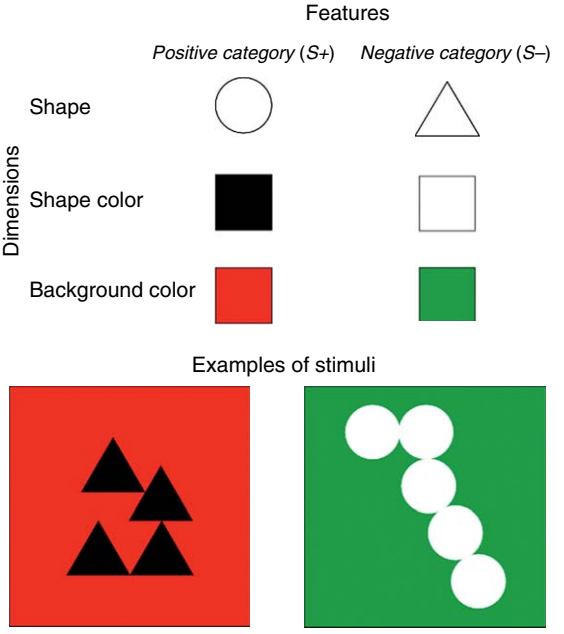

Figure 6 Construction of a two-out-of-three polymorphous category (Jitsumori, 1993). The stimuli were created by changing features from three dimensions: shape (circle or triangle), shape color (black or white), and background color (red or green). The features were randomly assigned to represent the positive and negative categories. The top panel depicts one such assignment. Stimuli with two positive and one negative feature belonged to the positive category, whereas stimuli with two negative and one positive feature belonged to the negative category.

assessed. The features are then randomly assigned to represent either a positive or a negative category. In Jitsumori's study, a stimulus with two positive and one negative features was assigned to the positive category, whereas a stimulus with two negative and one positive features was assigned to the negative category. Pigeons were then trained to respond when a member of the positive category was shown and to refrain from responding when a member of the negative category was shown, a standard go/no-go procedure. This experiment, and others listed in **Table 2**, has found that animals can learn such artificial polymorphous categories.

Despite such successful learning, other experimental data cast doubt on the similarity of artificial polymorphous categories to natural basic-level categories. Lea et al. (2006) observed that, although the discrimination of basic-level categories is easily learned, artificial polymorphous categories are often difficult to master for both animals and people. For example, pigeons took an average of 5400 trials to master the two categories depicted in **Figure 6** (Jitsumori, 1993). For comparison, pigeons trained to discriminate cars, chairs, flowers, and people in a four-alternative forced-choice task required fewer than 1000 trials to master all four categories (Bhatt et al., 1988; Wasserman et al., 1988).

Lea et al. (2006) proposed several factors that may affect the rate of learning artificial polymorphous categories. For example, in artificial polymorphous categories, each feature is equally valid: So, in the task presented in **Figure 6**, the color of the shape is as good a predictor of a category membership as is the shape itself. In contrast, the predictive value of different features identifying members of natural categories can vary. Therefore, if a subject is trained to discriminate cars from chairs, then the presence of tires on car images or the presence of thin elongated parts, such as chair legs, on chair images may be sufficient features for discrimination. Further research is needed to elucidate to what extent research on artificial polymorphous categories can be used as an effective model of basic-level categorization.

1.11.4 Perceptual Concepts as Subordinate-Level Categories

Subordinate categories are nested within basic-level categories. For example, the category monkey includes Japanese monkeys, rhesus monkeys, and capuchin monkeys. Subordinate categories have high within-category similarity, but unlike basic-level categories, they also have high between-category similarity; after all, most monkey species look quite similar to each other. Consequently, categorization at the basic level is preferred to categorization at the subordinate level (Rosch and Mervis, 1975). Nevertheless, the less-privileged status of subordinate categories can be moderated by expertise: Dog experts and bird experts identified objects at the subordinate level just as fast as at the basic level within their domain of expertise (Tanaka and Taylor, 1991; Johnson and Mervis, 1997).

Can animals learn to categorize at the subordinate level? Although categorization at the subordinate level has received far less attention than categorization at the basic or superordinate levels, several species have been successfully trained to categorize at the subordinate level (**Table 3**). Curiously, several animal studies suggest that some subordinate levels, namely, the level of species, may be a preferred level of categorization. For example, pigeons and rhesus monkeys readily learned and transferred the subordinate-level kingfisher/nonkingfisher discrimination, but they were unable to master the basic-level bird/nonbird discrimination (Roberts and Mazmanian, 1988). In another experiment (Yoshikubo, 1985), rhesus monkeys were trained to respond to photographs containing rhesus monkeys and to refrain from responding to photographs that did not contain rhesus monkeys or any other animals. In later testing, photos of Japanese monkeys were shown as probe trials. If, during initial training, rhesus monkeys formed the basic-level category 'monkey', then they should have responded to these photographs. However, they refrained from responding, suggesting that the subordinate category 'rhesus monkey' had instead been acquired. Similar data were obtained for other monkey species (Fujita, 1987).

Of course, rhesus monkeys, as well as other monkey species, have extensive experience recognizing and categorizing members of their own species. It can, thus, be argued that the shift in preferred level of categorization may be the result of high proficiency, just as in humans (Tanaka and Taylor, 1991; Johnson and Mervis, 1997). The Roberts and Mazmanian (1988) data seem to argue against this hypothesis, because both pigeons and monkeys preferred the subordinate-level category kingfisher; presumably, rhesus monkeys do not have extensive expertise with different bird species. However, other reports suggest that pigeons can, in fact, successfully master the bird/mammal discrimination (Cook et al., 1990), casting doubt on their failure to master the

Table 3 Animal studies of categorization at subordinate level

Category	*Species*	*References*
Style of painting	Pigeons	Watanabe et al., 1995; Watanabe, 2001
Oak leaves	Pigeons	Cerella, 1979
Facial expressions or gender of conspecifics	Chimpanzees, Japanese monkeys	Parr et al., 1998; Koba and Izumi, 2006
Human facial expressions or gender	Pigeons, Japanese monkeys	Jitsumori and Yoshihara, 1997; Kanazawa, 1998; Troje et al., 1999; Huber et al., 2000; Gibson et al., 2005
Conspecifics	Pigeons, chimpanzees, rhesus monkeys, Japanese monkeys, stumptailed monkeys, bonnet monkeys, pigtail monkeys, baboons	Poole and Lander, 1971; Yoshikubo, 1985; Fujita, 1987; Brown and Boysen, 2000; Watanabe, 2000; Vonk and McDonald, 2004; Martin-Malivel et al., 2006
Animal species (other than the subject's own species)	Pigeons, chimpanzees, orangutans, gorilla	Roberts and Mazmanian, 1988; Cook et al., 1990; Brown and Boysen, 2000; Vonk and McDonald, 2002; Ghosh et al., 2004;

bird/nonbird discrimination reported by Roberts and Mazmanian (1988). Future research systematically examining the preferred level of categorization across different species is needed to disclose potential differences and similarities in subordinate-level categorization in animals and humans.

1.11.5 Nonsimilarity-Based Concepts

Nonsimilarity-based concepts have often been studied in the context of human-language superordinate categories, constructed from several basic-level categories. For example, the category animal may include birds, frogs, and fish (e.g., Wasserman et al., 1992); this approach is reviewed in the section titled 'Nonsimilarity-based concepts as superordinate-level categories'. Another approach to nonsimilarity-based concepts involving equivalence class formation is reviewed in the section titled 'Nonsimilarity-based concepts as equivalence classes.'

1.11.5.1 Nonsimilarity-Based Concepts as Superordinate-Level Categories

Can animals learn to respond differentially to superordinate human-language categories? Starting with Herrnstein's studies, many animals have been found to be able to sort objects into various superordinate categories, such as bodies of water, vehicles, or food (**Table 4**). In one instance, Roberts and Mazmanian (1988) used a go/no-go task to train pigeons and squirrel monkeys to discriminate color photographs at three different levels: kingfisher versus other birds, birds versus other animals, and animals versus various outdoor scenes containing trees, furniture, houses, and other inanimate objects.

Table 4 Animal studies of categorization at the superordinate level

Category	*Species*	*References*
Bodies of water	Pigeons	Herrnstein et al., 1976
Vehicles	Chimpanzees, Japanese monkeys	Murai et al., 2004; Murai et al., 2005
Furniture	Chimpanzees, Japanese monkeys	Murai et al., 2004, 2005
Mammals, birds, or animals	Pigeons, chimpanzees, Japanese monkeys, orangutans, gorilla, rhesus monkeys	Roberts and Mazmanian, 1988; Delorme et al., 2000; Vonk and McDonald, 2002; Povinelli and Vonk, 2003; Murai et al., 2004, 2005
Man-made vs. natural, or living vs. nonliving	Pigeons, rhesus monkeys	Lubow, 1974; Roberts and Mazmanian, 1988; Gaffan and Heywood, 1993; Lazareva et al., 2004, 2006
Food	Mangabeys, gorilla, rhesus monkeys	Delorme et al., 2000; Deputte et al., 2001; Vonk and McDonald, 2002

Both pigeons and squirrel monkeys readily learned the kingfisher/nonkingfisher discrimination, and both species transferred their performance to novel exemplars. The animal/nonanimal discrimination required additional training, but it was eventually mastered. In contrast, neither monkeys nor pigeons could correctly classify novel pictures in the bird/nonbird discrimination. Similar results were obtained for a juvenile gorilla that learned the orangutan versus human, primate versus nonprimate, and animal versus food discriminations, but that animal only showed reliable transfer to novel exemplars for the orangutan versus human and the animal versus food discriminations (Vonk and McDonald, 2002). In a replication of this study, Vonk and McDonald (2004) found that, unlike the gorilla, adult orangutans readily learned all three discriminations, and they transferred their discriminative performance to novel exemplars from all three categories. It is not clear why some animals appear to have difficulty with some of the superordinate categories (e.g., birds/nonbirds) but not with others (e.g., animals/nonanimals).

Most published studies have explored animals' categorization at different levels in different experiments and usually with different visual stimuli. Yet, flexible classification of objects and events at different levels is thought to be one of the most important and perhaps the unique features of human categorization (Markman, 1989): Humans can refer to the same object as a Toyota, a car, or a vehicle, depending on context. Recently, work in our laboratory has explored whether pigeons can flexibly classify the same photographs at the basic level or at the superordinate level, depending on task demands (Lazareva et al., 2004).

As **Figure 7** illustrates, our experiment used four basic-level categories (cars, chairs, flowers, and humans) that were arranged into two superordinate-level categories (artificial: cars and chairs, natural: flowers and humans). During training, the same photograph randomly required basic-level discrimination if four choice keys were presented and superordinate-level discrimination if two different choice keys were presented. Our pigeons readily mastered both discriminations, attesting to their ability to flexibly categorize the photos; as well,

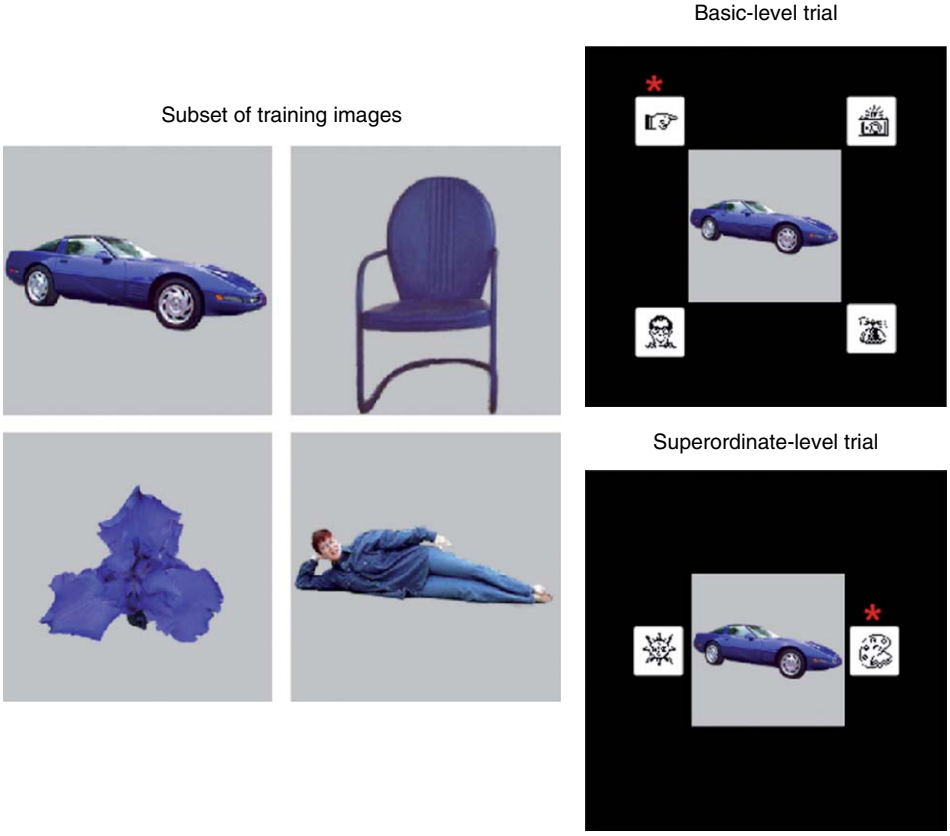

Figure 7 Examples of the photographic stimuli and the schematic layout of a basic-level trial and a superordinate-level trial used in Lazareva et al. (2004). The choice response assignments varied across birds.

our pigeons demonstrated reliable transfer to novel exemplars at both basic and superordinate levels. Flexible categorization therefore appears not to be a uniquely human ability.

1.11.5.2 Nonsimilarity-Based Concepts as Equivalence Classes

Instead of training animals to sort objects according to superordinate human-language categories, other experiments have studied how superordinate categories are created in the first place. If, by definition, superordinate categories are not based on perceptual similarity, then how and why are they formed?

One possibility (Astley et al., 2001) concerns learned or acquired equivalence: Perceptually distinct stimuli may be grouped together if all of them have been associated with the same outcome or with the same behavior. We know that the perceptually distinctive stimuli have formed a functional equivalence class if a change in response tendencies to some members of the class generalize spontaneously to other members of the class, when members of the class become interchangeable, or both (Schusterman et al., 2000; Sidman, 2000; Zentall, 2000).

Some theorists believe that functional equivalence classes need to be distinguished from stimulus equivalence classes, which represent a higher level of complexity and are empirically documented by reflexivity, symmetry, and transitivity (Hayes et al., 2001). Others argue that functional equivalence classes do not differ from stimulus equivalence classes (Sidman, 2000). Regardless of how this debate is resolved, functional equivalence classes appear to be quite close to superordinate human-language categories.

For example, we use both cars and motorcycles to travel; as cars and motorcycles each produce a common outcome (moving us from one place to another), we combine these two classes of objects into a single superordinate category, vehicle. Likewise, we call any organism that preys on other organisms (i.e., exhibits a common behavior) a predator, grouping together such diverse organisms as flatworms, ant lions, and killer whales. Is this aggregative ability uniquely human? Can animals also construct categories based on their functional, rather than perceptual, properties?

In the representative experiment depicted in **Figure 8** (Wasserman et al., 1992), pigeons were trained to peck one button when photographs of two basic-level categories (e.g., flowers and chairs) were presented and to peck a second button when photographs of other two basic-level categories (e.g., cars and people) were presented. Then, with the first two buttons unavailable, the pigeons were trained to peck a third button to photographs of flowers and to peck a fourth button to photographs of people, a procedure called reassignment training (Lea, 1984). No photographs of cars or chairs were shown during reassignment training. Finally, the researchers showed the pigeons photographs of cars and chairs with only the third and fourth buttons available. If, during original training, the pigeons grouped the two basic-level categories associated with a common response into a higher-order category, then they should have been able to select the response associated with the complementary basic-level category during reassignment training. In the testing phase, the pigeons did predominately peck the button that had been associated with the complementary basic-level category during reassignment training, thus documenting functional stimulus equivalence.

Later research in our laboratory has revealed that pigeons can form functional equivalence classes via associations with a common delay, a common probability, and a common quantity of reinforcement (Astley and Wasserman, 1999; Astley et al., 2001; see also Frank and Wasserman, 2005). For example, when cars and flowers were associated with a 15-s delay to reinforcement, or were reinforced on one out of ten trials, or were reinforced by five pellets, pigeons grouped these two basic-level categories together into a functional equivalence class.

Many other reports have documented the formation of functional equivalence classes via a many-to-one mapping procedure (e.g., Grant and Kelly, 2001; Neiman and Zentall, 2001; Urcuioli et al., 2006; see Zentall, 1998, for a review). In a typical many-to-one matching experiment, organisms are trained to make a common response after two or more entirely arbitrary stimuli. For example, pigeons could be presented with a red hue or a vertical line as an initial (or sample) stimulus; in this case, the choice of a vertical line (a comparison stimulus) is reinforced. Alternatively, a pigeon could be presented with a green hue or a horizontal line as a sample, and in this case the choice of a horizontal line is reinforced (Urcuioli et al., 1989). After that, one sample from each class is associated with a novel response: The red hue sample with the choice of a black circle and the green hue sample with the choice of a white circle. Finally, the second sample from each class, the vertical line and the horizontal line, was presented with the novel comparison stimuli, the black circle and the white circle. The pigeons selected the black circle when the vertical line was presented, and they selected the white circle when the horizontal

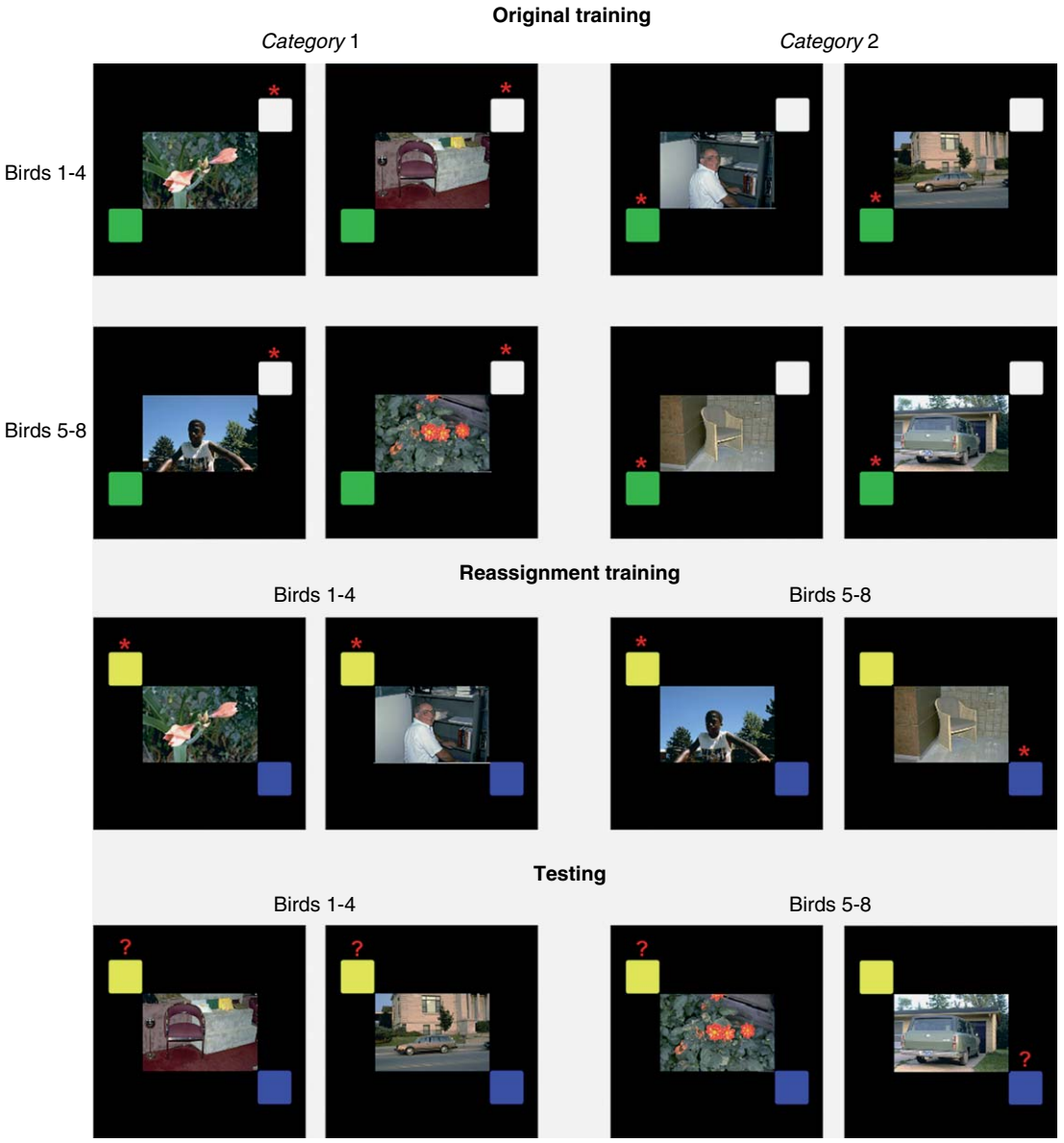

Figure 8 Schematic depiction of the training and testing procedures used in Wasserman et al. (1992). First, pigeons were trained to associate two basic-level categories with one response and two other basic-level categories with a different response (original training), creating two higher-level, superordinate categories. Later, one of the basic-level categories from each superordinate category was associated with a different response (reassignment training). Finally, the withheld basic-level categories were presented with the novel choice responses (testing). If the birds were forming superordinate-level categories during original training, then they should be able to select the correct response associated with the other class of stimuli during reassignment training. Different assignments were given to two subsets of birds in the experiment.

line was presented, divulging an emergent relation between the stimuli associated with the same event, quite similar to the experiments described earlier.

Note, however, that the functional equivalence classes in this design do not comprise several basic-level categories. Therefore, although research on many-to-one matching may have important implications for understanding superordinate categorization in animals, it does not entail many of the important features of superordinate categories, such as their hierarchical structure. To our knowledge, no research has explicitly explored to what extent superordinate-level categorization is comparable to many-to-one matching.

1.11.6 Abstract Concepts

Many theorists have drawn a distinction between natural concepts, such as tree or furniture, and abstract or relational concepts, such as above/below or same/different (Premack, 1983a; Lea, 1984; Herrnstein, 1990; Pearce, 1994; Thompson, 1995; Wasserman, 1995; Mackintosh, 2000; Wright et al., 2003; Cook and Wasserman, 2006). Members of natural concepts are grouped by perceptual, associative, or functional similarity, whereas abstract concepts are believed to go beyond the specific features of the stimuli. Instead, abstract concepts are based on relations between or among those stimuli. As C. Lloyd Morgan (1894/1977) stated in his *Introduction to Comparative Psychology*:

> Two particular objects, billiard balls for example, are perceived to be a span asunder. The balls are removed, and pieces of chalk being substituted they too are seen to be a span asunder. All sorts of small objects may be substituted for the chalk, and ... the space relationship remains unchanged. [...] We do not merely perceive those two billiard balls to be related in such a way, but we conceive the relationship in its abstract and general form. ... [T]his conception is not concrete, particular, and individual, but abstract, general, and of universal application. (Lloyd Morgan, 1894/1977, pp. 262–263)

Lloyd Morgan (1894/1977) considered whether animals can form such abstract concepts, but he later rejected this intriguing possibility, "in no dogmatic spirit, and not in support of any preconceived theory or opinion, but because the evidence now before us is not ... sufficient to justify the hypothesis" (Lloyd Morgan, 1894/1977, p. 377). Morgan's conservative assessment persists today (Premack, 1983a; Pearce, 1994; Mackintosh, 2000). Nevertheless, substantial experimental evidence now suggests that animals can, in fact, form concepts that transcend specific properties of the stimuli, such as symmetrical or same/different (see **Table 5** for more examples). We review the evidence relevant to the most extensively studied concepts in this section.

1.11.6.1 Concept of Number

Can animals count? One of the difficulties in answering this question lies in the enormous variety of behaviors that can be controlled by numerical attributes of stimuli. An organism may be trained to select the larger (or the smaller) of two arrays of items, with the experimenter controlling the nonnumerical attributes of the stimuli (e.g., area or density), so that only the number of items in the array can reliably predict reinforcement (e.g., Thompson and Chase, 1980; Zorina and Smirnova, 1996; Anderson et al., 2005). Is this evidence of counting? What about an experiment in which an organism is trained to select an array of three items, irrespective of the items' identity or configuration (e.g., Davis, 1984)? Do any of these studies represent clear evidence for the concept of number in animals?

According to Davis and Perusse (1988), who have proposed one of the most extensive theoretical analyses of numerical competence in animals, the concept of number is an abstract or modality-free numerical ability. As such, this ability ought to be revealed by means of transfer across different modalities (e.g., from visual to auditory) or across different procedures (e.g., from simultaneous to successive presentation). The concept of number is one of the attributes of true counting, "a formal enumerative process" that requires "the application of reliably ordered cardinal tags in one-to-one correspondence to the items in the array" (Davis and Perusse, 1988, p. 562). In other words, true counting requires the presence of cardinality, the one-to-one assignment of a numerical tag to an array, and the presence of ordinality, the ability to order these numerical tags. Finally, some authors have proposed transfer to new numbers as an indicator of the number concept. For example, a rat trained to press a lever two times after two light flashes and four times after four light flashes ought to be able to spontaneously press a lever three times after three light flashes with no additional training (Davis and Perusse, 1988; see also Boysen and Capaldi, 1993, and Brannon, 2005, for more recent discussion of numerical competence in animals).

To our knowledge, only one study has successfully obtained evidence for cross-modal transfer of a numerical discrimination. In this study (Church and Meck, 1984), rats were trained to press the right lever when two sounds were presented and to press the left lever when four sounds were presented. The nonnumerical features of the stimuli – such as the duration of each sound, the interval between sounds, and the total duration of the sound sequence – was controlled, so that a reliable discrimination could be based only on the number of the sounds in a sequence. After rats learned this discrimination, the sounds were replaced with light flashes; for half of the rats the response rule remained unchanged, whereas for the other half of the rats the response rule was reversed. As **Figure 9** illustrates, the rats in the nonreversal group continued to make the correct responses when sounds were

Table 5 Animal studies of abstract or relational concept learning

Concept	*Species*	*References*
Symmetry	Honeybees, pigeons, starlings	Delius and Habers, 1978; Delius and Novak, 1982; Giurfa et al., 1996; Horridge, 1996; Swaddle and Pruett-Jones, 2001
Direction or type of movement	Pigeons	Dittrich et al., 1998; Cook et al., 2002
Above/below	Baboons, capuchin monkeys	Depy et al., 1999; Spinozzi et al., 2006
Number: Cross-modal transfer	Rats	Church and Meck, 1984
Number: Cardinality	African Grey parrot, pigeons, chimpanzees	Matsuzawa, 1985; Boysen and Berntson, 1989; Rumbaugh and Washburn, 1993; Pepperberg, 1994, Biro and Matsuzawa, 2001;; Xia et al., 2000, 2001; Pepperberg, 2006
Number: Ordinality	Squirrel monkeys, rhesus monkeys	Brannon and Terrace, 1998; Brannon and Terrace, 2000; Cantlon and Brannon, 2006; Olthof et al., 1997; Washburn and Rumbaugh, 1991
Relational learning: Transposition	Pigeons, rats, chimpanzees	Gonzales et al., 1954; Lawrence and DeRivera, 1954; Riley et al., 1960; Marsh, 1967; Lazareva et al., 2005
Identity: Matching-to-sample	Honeybees, pigeons, crows, rats, harbor seal, sea lions, capuchin monkeys, orangutans, gorilla, chimpanzees	Zentall and Hogan, 1974; Fujita, 1983; Wright et al., 1988; Oden et al., 1990; Kastak and Schusterman, 1994; Smirnova et al., 2000; Giurfa et al., 2001; Barros et al., 2002; Vonk, 2003; Mauk and Dehnhardt, 2005; Pena et al., 2006
Identity: Same-different	Pigeons, African grey parrot, baboons, capuchin monkeys, rhesus monkeys	Pepperberg, 1987; Cook et al., 1995; Young et al., 1997; Wasserman et al., 2001; Young and Wasserman, 2001; Cook, 2002; Blaisdell and Cook, 2005; Gibson et al., 2006
Relation among relations	Pigeons, gorilla, orangutan, chimpanzees	Gillan et al., 1981; Premack, 1983b; Thompson et al., 1997; Fagot et al., 2001; Vonk, 2003; Cook and Wasserman, 2007

Because of the extremely large number of studies devoted to some of the concepts (e.g., the concept of number), only selected studies are listed.

replaced by flashes, although at a lower level of accuracy than in training. Moreover, the rats in the reversal group followed the previously learned rule: They continued to press the right lever in the presence of two light flashes and to press the left lever in the presence of four light flashes, even though the opposite response was now reinforced. Together, these results show that the rats' numerical discrimination was modality-free. More comparative research is needed to further explore this important competence.

Several studies provide evidence for cardinality: numerical tags that animals can use to identify arrays of items (see **Table 5**). For example, Boysen and Berntson (1989) trained the chimpanzee Sheba to select an Arabic numeral corresponding to the number of candies on a tray. Once Sheba mastered this task, she was presented with the Arabic numerals displayed on a computer monitor and was required to select the placard with the correct number of metal disks attached to it. After this training, Sheba could select the correct Arabic numeral for arrays consisting of novel, nonedible items, thereby demonstrating that she indeed learned the one-to-one correspondence between Arabic numerals and arrays of items. Similar evidence was obtained for other primates, for an African Gray parrot, and even for pigeons (see **Table 5**).

Evidence for ordinality – the ability to order numerical tags – was considerably more difficult to obtain. In order to conclude that animals are ordering numerical tags, one must eliminate the possibility that the ordering is based on other stimulus dimensions, such as density or surface area or the hedonic value of reinforcement. The latter possibility is particularly important because many studies have used

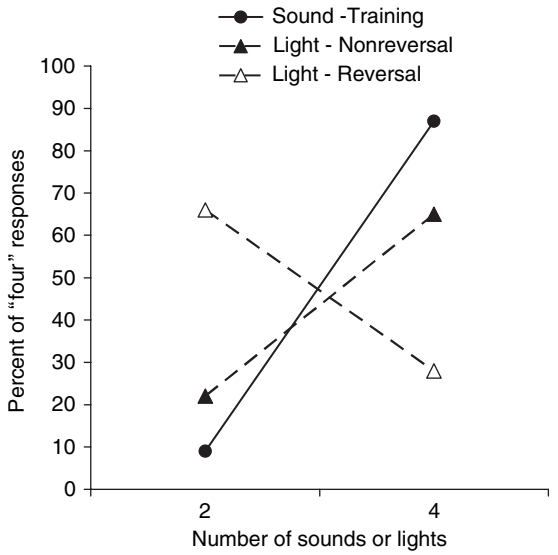

Figure 9 Transfer of the 'two' vs. 'four' discrimination from the number of sounds to the number of light flashes. For half of the rats, the response rule remained the same (Light – Nonreversal), whereas for the other half of the rats, the response rule was reversed (Light – Reversal). Data compiled from Church RM and Meck WH (1984) The numerical attribute of the stimuli. In: Roitblat HL, Bever TG, and Terrace HS (eds.) *Animal Cognition*, pp. 445–46. Hillsdale, NJ: Erlbaum.

the number of food items to establish a one-to-one correspondence between the number of items and the numerical tags (e.g., Washburn and Rumbaugh, 1991; Boyson et al., 1993; Olthof et al., 1997). In this case, the animals could have used the hedonic value of reinforcement to order the numerical tags instead of the number of items in the corresponding array.

Recently, Brannon, Terrace, and colleagues (Brannon and Terrace, 1998, 2000; Cantlon and Brannon, 2006) adopted a new approach for studying ordinality. Instead of training animals to use numerical tags, the researchers focused on the extent to which a nonverbal system for representing numbers in animals is similar to a nonverbal system for representing numbers in humans. When humans compare single-digit numbers or random-dot arrays, they exhibit a numerical distance effect (e.g., 1 vs. 7 is easier than 1 vs. 2) and a numerical magnitude effect (e.g., 1 vs. 2 is easier than 6 vs. 7), suggesting that number discriminability conforms to the Weber-Fechner Law (Dehaene et al., 1990). Does the discriminative behavior of rhesus monkeys exhibit the same regularities?

To find out, Brannon and Terrace (1998, 2000) trained monkeys to touch simultaneously presented arrays of one, two, three, and four items on a computer screen in ascending order to receive juice reinforcement. As **Figure 10** illustrates, the non-numerical attributes of the stimuli (e.g., area, density, or shape of the items) were carefully controlled, so that successful discrimination could be based only on the number of items in the array. Monkeys successfully learned the task and transferred their discriminative performance to novel arrays of five to nine items, providing strong evidence for ordinality. In a follow-up experiment, Cantlon and Brannon (2006) slightly modified the task, so that only two arrays were simultaneously presented; in addition, the number of items in the array varied from 1 to 30. The monkeys were still required to touch the arrays in ascending order. For comparison, human participants were trained to

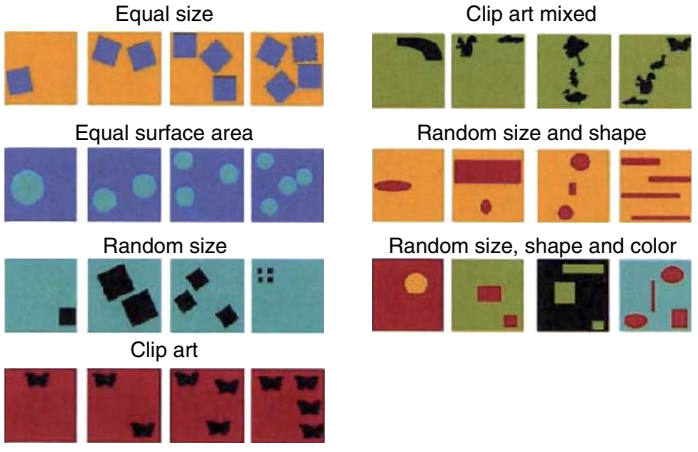

Figure 10 Examples of stimuli used during training in Bannon and Terrace (1998, 2000). Brannon EM and Terrace HS (2000) Representation of the numerosities 1–9 by rhesus macaques (*Macaca mulatta*). *J. Exp. Psychol. Anim. Behav. Process.* 26: 31–49.

perform a similar task. Cantlon and Brannon (2006) found that monkeys' performance exhibited both the numerical distance effect and the numerical magnitude effect. Moreover, monkeys' performance and humans' performance was similar not only qualitatively but also quantitatively, suggesting that monkeys and humans share the same nonverbal system for representing number. More comparative research is needed to establish whether this nonverbal system for number representation is shared by other animal species.

1.11.6.2 Relational Concepts in the Transposition Paradigm

Can animals respond to the relations between or among stimuli, rather than to the absolute properties of the stimuli? Perhaps the most famous attempt to experimentally examine this possibility comes from the case of transposition, first explored by Köhler (1918/1938). Suppose that an organism is given a simultaneous discrimination task in which the positive discriminative stimulus (S+) is a 2-cm-diameter circle and the negative discriminative stimulus (S–) is a 3-cm-diameter circle. When the organism masters the visual discrimination, just what has it learned? Has it learned that only the 2-cm circle signals food? Or has it learned the concept of smaller?

A possible answer to this question comes by considering how the organism ought to respond to new pairs of stimuli that are presented during a postdiscrimination test. If the former S+ is presented along with the even smaller 1-cm-diameter circle, then having learned to respond to the relation between the circles, the organism may actually choose the untrained stimulus over the former S+. In his pioneering studies, Köhler (1929, 1918/1938) found that chickens and chimpanzees did indeed respond in this relational manner, a result that led him to propose that stimuli are not judged in absolute terms but as relative to one another.

Does choice of the untrained stimulus over the former S+ necessarily indicate control by the relation between the stimuli rather than by specific features of these stimuli? Kenneth W. Spence (1937) famously demonstrated that relational responding in the transposition paradigm can be explained as the result of the interaction of learned tendencies to make responses to S+ and to withhold responses to S–.

If both training stimuli are located on the same dimension, then the algebraic summation of those learned tendencies leads to two peak shifts: The peak of maximal associative strength shifts away from the former S–, and the peak of minimal associative strength shifts away from the former S+. In other words, the organism may prefer the 1-cm circle because the associative strength of this stimulus is actually higher than associative strength of the formerly reinforced 2-cm circle. So, according to Spence's generalization theory, the preference for the novel stimulus over the former S+ found by Köhler was no more relational than was the tendency to approach S+ and the tendency to avoid S– after standard discrimination training. Spence's stimulus generalization theory yielded several important predictions that have been confirmed in different species, including humans, and in different experimental situations (e.g., Ehrenfreund, 1952; Honig, 1962; Cheng, 1999; Cheng and Spetch, 2002; reviewed by Reese, 1968; Riley, 1968; Purtle, 1973).

Several reports have provided data that have challenged generalization theory as the sole account of an organism's behavior in transposition tasks (**Table 5**). Consider the intermediate stimulus problem, in which an animal is presented with 1-cm, 2-cm, and 3-cm circles and is required to select the 2-cm circle to obtain reinforcement. In the test, the animal is presented with 2-cm, 3-cm, and 4-cm circles; if the animal had previously learned to respond according to the relation among the stimuli, then it ought to select the previously nonreinforced 3-cm circle. Generalization theory predicts that the animal ought to select the previously reinforced (2-cm circle) stimulus instead of the middle value. Nonetheless, chimpanzees have been found to transpose the solution of the intermediate-size problem (Gonzales et al., 1954).

Other studies have compared transposition after simultaneous and successive discrimination training. Unlike successive discrimination training, simultaneous discrimination training affords the subject the opportunity to compare the training stimuli at the same time; therefore, relational responding ought to be enhanced. Yet, stimulus generalization theory makes no distinction between simultaneous and successive modes of presentation. Several studies have indeed reported higher transposition after simultaneous training than after successive training (Riley et al., 1960; Marsh, 1967), suggesting that direct comparison does enhance relational learning (but see Hebert and Krantz, 1965; Wills and Mackintosh, 1999).

Finally, several reports, including our own, have found that presentation of multiple pairs of discriminative stimuli instead of a single pair enhances relational responding in pigeons, even when such responding is

contrary to the predictions of stimulus generalization theory (Marsh, 1967; Lazareva et al., 2005). It is well known that increasing the number of exemplars leads to better acquisition of a concept in many species of animals, including pigeons (Wright et al., 1988; Wright, 1997; Cook, 2002; Katz et al., 2002). Similarly, pigeons may need to encounter multiple instances of a rule in order to exhibit strong relational responding. Unfortunately, we do not yet know whether the same is true for other animal species. (*See* Chapter 1.29 for a corresponding experiment with honeybees).

1.11.6.3 Concept of Identity

The concept of identity, or sameness and difference, is perhaps the most extensively studied concept in animals. The most frequently used experimental procedures are matching-to-sample and same-different discrimination (**Figure 11**). In the standard matching-to-sample task, an animal first views a single stimulus, or sample, and performs several observing responses to it (e.g., touching or pecking the sample). After that, the animal is shown two comparison stimuli and is required to select the comparison that matches the sample (identity matching) or that does not match the sample (oddity matching). The simultaneous same-different discrimination task requires the choice of one response if the two presented stimuli are identical and a second response if the two stimuli are different.

Regardless of training procedure, evidence of successful transfer to novel stimuli is necessary to demonstrate the identity concept. The term full-concept learning is used when an animal discriminates the novel stimuli as accurately as the training stimuli, most strongly documenting the abstract nature of the acquired behavior. Partial concept learning refers to cases in which the novel stimuli are discriminated less accurately than the training stimuli, signifying the stimulus-dependent nature of the acquired behavior, even when discriminative performance is reliably above chance. Early research suggested that some species may only be capable of partial concept learning (e.g., Premack, 1983a). However, recent reports have shown that the number of stimuli used during training is an important parameter affecting identity concept learning, a sensible finding, as an increase in the number of training stimuli makes memorizing individual stimulus associations a more costly strategy. Different species, though, may require different numbers of training stimuli to achieve full identity concept learning, with as few as 16 stimuli for great apes and as many as 512 stimuli for pigeons (**Figure 12**; Wright et al., 2003).

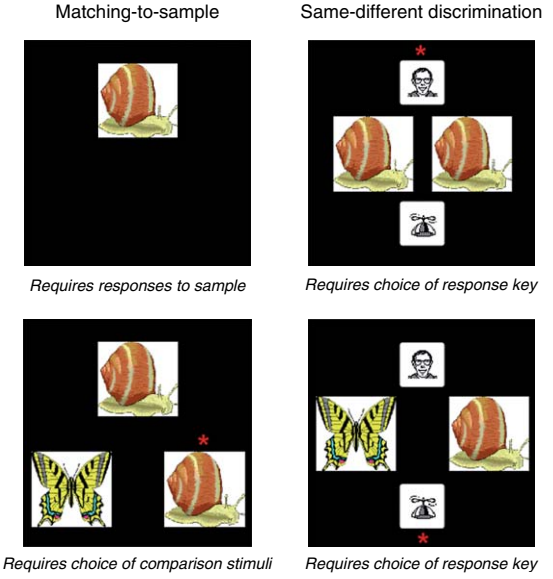

Figure 11 Comparison of matching-to-sample and same-different discrimination. In the (identity) matching-to-sample paradigm, the animal is required to select the correct (identical) comparison stimulus (the red asterisks indicate the correct choices). In the same-different discrimination, the animal is required to select one response key if the items are the same and the other response key of the items are different.

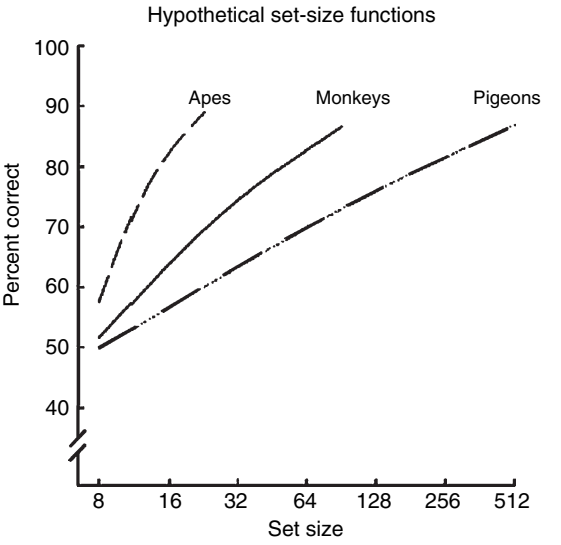

Figure 12 Hypothetical acquisition functions for apes, monkeys, and pigeons, showing low abstract-concept learning might vary as a function of training set size. From Wright AA, Rivera JU, Katz JS, and Bachevalier J (2003) Abstract-concept learning and list-memory processing by capuchin and rhesus monkeys. *J. Exp. Psychol. Anim. Behav. Process.* 29: 184–198.

Another important parameter affecting identity concept learning is the type of training procedure. Pigeons trained using simultaneous same-different discrimination or matching-to-sample procedures require hundreds of training stimuli to achieve full identity concept learning (Wright et al., 1988; Wright et al., 2003), but pigeons can also attain full identity concept learning when only two training stimuli are used (Cook et al., 2003).

What was the secret in successfully training pigeons with a small number of stimuli? In this experiment, pigeons were presented with stimuli that were alternated several times within a single trial, so that the birds had multiple opportunities to compare them. It is possible that these extended sequences were critical for the initial acquisition of the abstract concept, although the available experimental data are insufficient for substantiating this conclusion.

Interestingly, another experiment that used a similar procedure found only partial concept acquisition in pigeons (Young et al., 1997). In this experiment, pigeons were shown 16 computer icons, one at a time; either all 16 icons were nonidentical or all 16 icons were identical. Just as in the previous experiment, this procedure allowed for multiple opportunities to discern the relationship among the items. Nonetheless, pigeons' transfer to new icons, although well above chance, was below performance with the icons used in training. Clearly, additional research is needed to elucidate the factors affecting acquisition of the abstract same-different concept.

Another important consideration is the nature of the concept learned in different types of experimental procedures. Given the variety of stimuli and experimental approaches that have thus far been used, it is important to establish whether the same processes control animals' behavior in different tasks. In a representative study (Cook, 2002), pigeons were trained using a simultaneous same-different discrimination task with different types of stimuli (**Figure 13**). For some birds, all same displays and all different displays were associated with consistent report responses, regardless of the display type. For example, both different texture and different photo displays required the choice of the left key. This group was called the consistent group. In the inconsistent group, response assignment differed by display type; for example, all different texture displays required the choice of the left key, whereas all different photo displays required the choice of the right key. If pigeons learned a set of specific rules for each type of display, then they should have learned the inconsistent task just as easily as the consistent task. In fact, the consistent group learned the task faster and reached higher levels of accuracy by the end of the training than the inconsistent group. Moreover, only the consistent group demonstrated significant transfer to novel stimuli, indicative of concept formation. These results suggest that, despite dramatic perceptual differences among the types of displays, pigeons' discriminative performance was based on a single general rule that was abstract enough to be applicable to a wide variety of visual stimuli.

On the other hand, seemingly minor changes in experimental procedure may lead to dramatically different strategies employed for task solution as Gibson et al. (2006) demonstrated. In their study, pigeons were again trained to perform a simultaneous same-different discrimination. In the 16S versus 16D condition (**Figure 14**), the birds had to select one choice key if all 16 items in the display were the same and to select the other choice key if all 16 items in the display were different. In the 16S versus 15S:1D condition, different displays contained 15 identical items and 1 odd item, thus resembling previously described task (Cook, 2002; **Figure 14**). Although pigeons readily mastered the 16S versus 16D discrimination, they were unable to learn the 16S versus 15S:1D discrimination unless they were required to peck the odd item prior to making a choice response.

In a follow-up project, pigeons were trained to concurrently perform the already learned 16S versus 16D discrimination plus the newly introduced 16S versus 15S:1D discrimination. If the birds were using the same strategy in both tasks, then they should begin to make many more errors on same trials in the 16S versus 16D task, as the 16S displays are quite similar to the 15S:1D displays. On the contrary, the birds should make many fewer errors on different trials, as the 16D displays should appear even more different than the 16S display when the 15S:1D displays are introduced. Yet, none of these changes were observed. Acquisition of the 16S versus 15S:1D task did not come at the expense of 16S versus 16D task performance, suggesting that the pigeons may have used different strategies to solve these tasks. Other evidence also points to the conclusion that these two tasks are qualitatively different (reviewed by Cook and Wasserman, 2006).

1.11.6.4 Relations Among Relations

Can animals discriminate higher-order relations between multiple first-order relations? Suppose that an animal is shown a sample stimulus with two equal-size circles and comparison stimuli with two equal-size

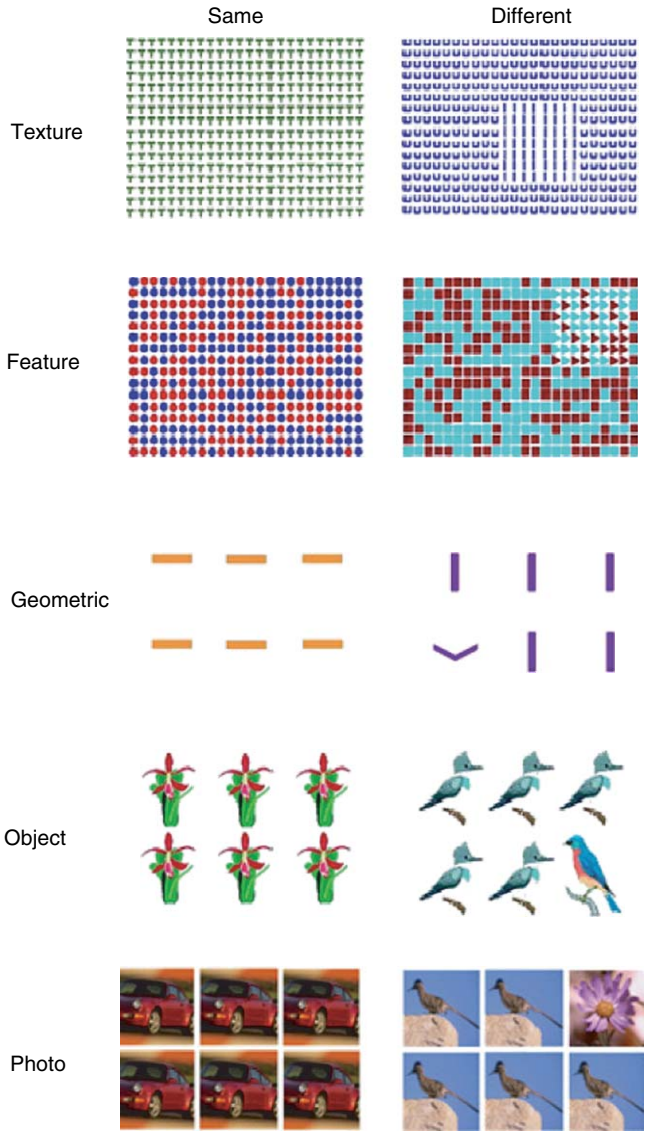

Figure 13 Examples of displays used by Cook (2002). The left column shows examples of same displays, and the right column shows examples of different displays for each display type. From Cook RG (2002) The structure of pigeon multiple-class same-different learning. *J. Exp. Anal. Behav.* 78: 345–364.

triangles and two unequal-size squares (**Figure 15**). Can the animal learn to select the comparison stimulus that entails the same relation (equal size), even though none of the shapes is identical? The ability to detect such higher-order relations is similar to human analogical reasoning, which is believed to be one of the central components of human cognition (Gentner et al., 2001).

Early research suggested that only language-trained chimpanzees can process second-order relations, whereas non-language-trained chimpanzees perform first-order same-different discriminations only on the basis of physical similarities (Premack, 1983b). Later research has found, however, that this conclusion was premature. Using a two-item display (**Figure 15**, left panel) task, several ape species have been found to respond in accord with second-order relations (Thompson et al., 1997; Vonk, 2003).

Two other studies have used a multiple-item display task (**Figure 15**, right panel) in which a sample contains an array of 16 items that are either identical or nonidentical. The comparison stimuli also contain arrays of 16 identical or nonidentical items; however, none of the items used for the sample display are

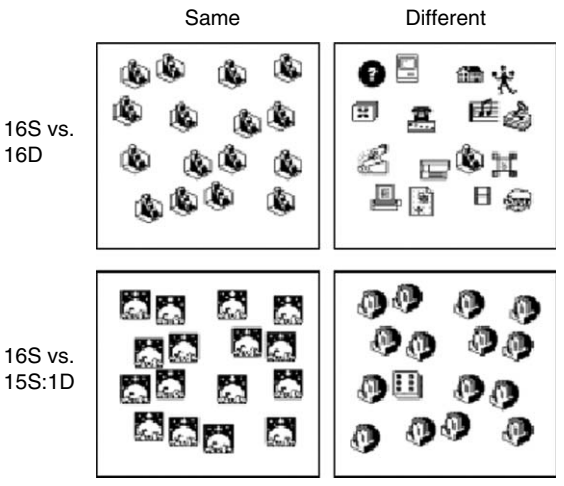

Figure 14 Examples of displays used in the 16S vs. 16D task and in the 16S vs. 15S:1D task. Modified from Gibson BM, Wasserman EA, and Cook RG (2006) Not all same-different discriminations are created equal: Evidence contrary to a unidimensional account of same-different learning. *Learn. Motiv.* 37: 189–208.

repeated in the comparison displays. Therefore, the correct response is only possible if the animal is able to detect the higher-order relations between the sample and comparison arrays. Both baboons (Fagot et al., 2001) and pigeons (Cook and Wasserman, 2006) have successfully learned this task and transferred their discriminations to arrays of novel items. It remains to be seen whether the mastery of two-item display tasks and multiple-item display tasks is based on the same cognitive mechanisms. In any case, these data suggest that the perceptual and cognitive foundations for analogical reasoning may exist not only in the mammalian brain but also in the avian brain.

1.11.7 Conclusion: What Does It All Mean?

Early in this chapter, we offered a definition of conceptual behavior that involves two key components: (1) the ability to respond similarly to members of one class of stimuli and to respond differently to members of other classes of stimuli and (2) the ability to transfer these differential responses to novel, discriminably different members of these classes of stimuli. The extensive evidence that we have reviewed here strongly supports the conclusion that concepts are not unique to human beings. Animals too form concepts based on perceptual similarity among their members, classifying objects into basic-level categories and subordinate-level categories. Moreover, animals appear to sense the perceptual structure of their environment, viewing members of basic-level categories (such as humans or trees) as being more similar to one another than to members of other categories. Animals can sort objects into non-similarity-based, superordinate categories, flexibly switching from basic-level categorization to superordinate-level categorization. Even the ability to form abstract concepts based on the relation between or among stimuli is not exclusively human; animals can respond to first-order and even to second-order relations between or among stimuli.

What does it mean to say that we share cognitive abilities with many animal species, from honeybees to chimpanzees? We are still far from answering that question. Most of the research until now has focused on whether a particular species is able to form a specific concept. Although such studies are an important first step in our understanding of categorization and conceptualization in animals, they do not tell us

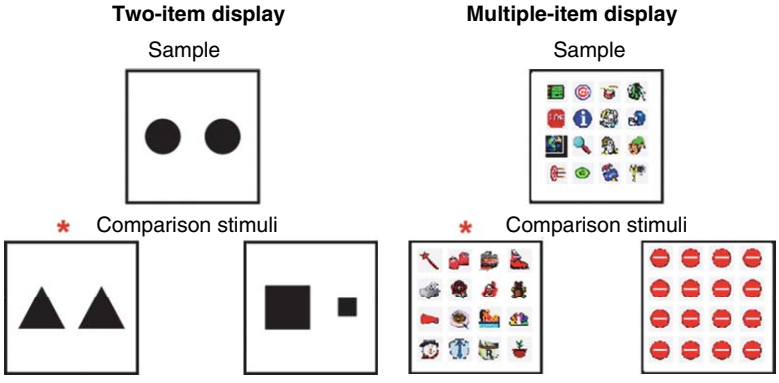

Figure 15 Examples of displays used for testing relational matching. For both types of displays, the correct choice is the comparison stimulus that involves the same relation as the sample stimulus (the red asterisks indicate the correct choices).

how the species attains concept mastery. With a few possible exceptions (e.g., Cantlon and Brannon, 2006), the mechanisms of concept formation in humans and animals remain mysterious.

Why do we need to understand the mechanisms of concept formation in animals? At least two reasons, one theoretical and one applied, can be suggested. The theoretical rationale centers on the relationship between the human mind and the minds of other animals. One exciting possibility is that the cognitive abilities of humans and animals have a common origin; that is, they may be based on some primitive trait shared by many species. For example, it is possible that conceptualization is based at least in part on fundamental laws of associative learning (Wasserman, 1995; Wasserman and Miller, 1997; Mackintosh, 2000), laws that apply to many species which are widely separated in evolutionary history. On the over hand, we may be witnessing another spectacular example of convergent evolution, similar to the bird's wing, the bat's wing, and the insect's wing. Although an adaptation for a similar function, flight, induced similar overall structures of those wings, there are enough differences in details (e.g., the absence of fused fingers in the bat's wings) that help us recognize it as an analogy (a gross similarity caused by similarity in function) rather than a homology (a detailed similarity in organization caused by common origin).

Perhaps conceptualization in humans and animals is similar only at the gross level but is dramatically different in details. Indeed, there may be dramatic differences between different animal species: Maybe the acquisition of the concept of sameness in honeybees with their compound eyes has nothing in common with the acquisition of the concept of sameness in pigeons with their lens eyes. Until we learn much more about the mechanisms of cognition in different species, we will not be able to answer this intriguing question.

From the applied perspective, sound knowledge of the mechanisms of cognition in different species may provide fresh insights for applied researchers. Indeed, research with animals may be of particular interest to applied mental health researchers, because that work usually explores the conditions under which concept formation is or is not likely to occur. For example, learning that the simultaneous presentation of stimuli and multiple instantiations of the relationship among stimuli each strengthen relational responding in pigeons may help applied mental health workers develop better techniques for teaching relational learning in humans, especially individuals who are otherwise inclined to respond to the absolute properties of stimuli instead of the relations between or among them (Carlin et al., 2003; Happe and Frith, 2006).

Fortunately, the focus of recent research has begun to shift to the mechanisms underlying the conceptual abilities of different species (Wasserman et al., 2004). Some remarkable similarities, as well as some remarkable differences, have already been revealed (e.g., Cook and Wasserman, 2006; Wright et al., 2003), and undoubtedly more await discovery. Such future research should provide essential information for elucidating the evolutionary origins of cognition. Together with Stewart Hulse (2006), we hope for "less and less research on existence proof, that is, whether or not a given species has the same cognitive capacity as we, or some other species, do" (Hulse, 2006, p. 674) and for more and more research on how these cognitive capacities operate.

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1.12 Learning and Representation

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In a representational theory of learning, the brain computes a representation of the experienced world, and behavior is informed by that representation. By contrast, in associative theories of learning, which dominate neurobiological thinking, experience causes a plastic brain to rewire itself to make behavior better adapted to the experienced world, without the brain's computing a representation of that world (Pavlov, 1928; Hull, 1952; Hawkins and Kandel, 1984; Rumelhart and McClelland, 1986; Smolensky, 1986). The computation of a representation seems to require a functional architecture that is not transparently consistent with our current understanding of neurobiological mechanisms (Gallistel, 2006), which is why representational theories of learning have not found favor among neurobiologists. Associative theories, in contrast, have been strongly influenced by neurobiological considerations for more than a century. For them, consistency with the current understanding of neurobiology is a major consideration (e.g., Gluck et al., 2005), which is why they predominate in neurobiological thinking about learning.

The results of behavioral experiments on nonhuman animals have increasingly implied that much learned behavior is informed by enduring temporal and spatial representations (e.g., Menzel et al., 2005), as even some prominent advocates of associative theories have recently acknowledged (Clayton et al., 2006a). Moreover, direct electrophysiological observation of neural activity has shown that the nervous system represents where the animal is and has been within the environment (O'Keefe and Nadel, 1978; Muller, 1996; Lee et al., 2004; Hafting et al., 2005; Foster and Wilson, 2006; *See* Chapter 1.21), how it is oriented (Ranck, 1984; Golob et al., 2001; Sargolini et al., 2006; Yoganarasimha et al., 2006), where objects are in relation to it (Muller et al., 2005; Campos et al., 2006; Goossens and Van Opstal, 2006), and the timing of repetitive or predictable events (Schultz and Dickinson, 2000; Leon and Shadlen, 2003; Meck, 2003; Ivry and Spencer, 2004; Penney, 2004; *See* Chapter 1.19). Thus, there is a conceptual tension between the behavioral and electrophysiological findings that seem to imply a computational–representational architecture, on the one hand, and on the other hand, current conceptions of neural structure and mechanism, in which seemingly essential elements of the requisite functional architecture appear to be absent.

The extent to which one believes that consistency with currently understood neurobiological mechanisms should be a constraint on models of learning depends on whether one believes that those mechanisms provide, or could provide, satisfactory accounts of the behaviorally well-documented phenomena that are the focus of this chapter – dead reckoning in insect navigation, the capacity of insects to record landmark 'snapshots,' and the capacity of food-caching jays to remember and make versatile use of a large number of episode-specific facts about each cache. They also depend on whether one believes that computation in the brain must be consistent with the principles that computer scientists believe constrain physically realizable computations. Perhaps, as many neuroscientists believe, the brain escapes the limitations and requirements that computer scientists believe are imposed by mathematics, logic, and physics.

Instructive instances can be found in the history of science where findings and analyses at a higher level

of inquiry seemed to require mechanisms at a more basic level for which there was then no explanation. Throughout the latter part of the nineteenth century, the eminent physicists William Thompson (Lord Kelvin) and P. G. Tait argued that Darwin and the geologists must be grossly in error in their estimates of the age of the earth, because no heat-generation process known to physics was consistent with a solar age of more than 100 million years. (Tait thought the upper limit imposed by physical considerations was 10 million years – see Lindley (2004) for an account.) Thompson (1862) wrote, for example, "It is impossible that hypotheses assuming an equibility of sun and storms for 1,000,000 years can be wholly true." Importantly, Kelvin did not have a satisfactory theory of where the sun's heat came from – he worked on the problem off and on throughout his career – but he was confident that a satisfactory explanation could be based on physical principles and phenomena that were then understood. Radioactivity was not discovered until 1896, and it was only in 1903 that the Curies showed that it was accompanied by the liberation of heat. The following contemporary quote gives an idea of just how revolutionary this discovery was:

> [this phenomenon] can barely be distinguished from the discovery of perpetual motion, which it is an axiom of science to call impossible, [and] has left every chemist and physicist in a state of bewilderment. (Lindley, 2004: 302)

This discovery of something that Kelvin and Tait literally could not imagine bore tellingly on their argument with Darwin and the geologists.

Whether we are in such a situation now remains, of course, to be seen. However, I argue in this chapter that the behavioral evidence for representation and computation, together with basic insights in computer science about the centrality of a read-write memory mechanism in physically realized computation, implies the existence of a neurobiological read-write memory mechanism. Given the centrality of such a mechanism to computation, as computer scientists understand it, the discovery of such a mechanism may someday have an impact on neuroscience comparable to the impact of the discovery of radioactivity on physics.

1.12.1 Representations: Definition and Explication

From the perspective of cognitive science, the brain is an organ of computation (Newell and Simon, 1975; Marr, 1982). What it computes are representations of selected aspects of the world and the animal's relations to it (the distal stimuli). It computes these representations from the signals engendered in sensory organs by the stimuli that impinge on them (the proximal stimuli) and from signals generated by the motor system, which carry information about how the animal is moving (efference copy signals; Holst and Mittelstaedt, 1950).

A representation consists of signals, symbols, and the operations on them (see **Figure 1**). The signals and symbols carry information about properties of the experienced world. The operations on those signals and symbols enable the brain to compute explicit

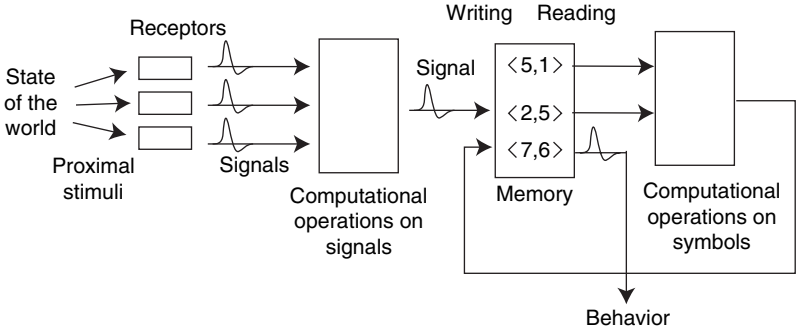

Figure 1 Schematic representation of the flow of information in a neurobiologically realized representational system. Proximal stimuli deriving from a state of the world (distal stimulus) act on sensory receptors to generate sensory signals, from which a perceptual signal specifying that state of the world is computed. The perceptual signal conveys the information to memory, where it is written into a symbol, which carries the information forward in time. Computational operations combine that symbol with other symbols to create further symbols and symbol strings in memory. The information contained in a symbol is read from memory and converted to signals in the motor system that give form to behavior.

representations from implicit ones and to anticipate behaviorally relevant states of the world.

Information is an abstract quantity carried by any signal or symbol that can reduce the brain's uncertainty about the present state of some aspect of the world (Shannon, 1948; Rieke et al., 1997). Information cannot be communicated to a device that has no representation of the world because the measure of the amount of information communicated is the reduction in the receiver's uncertainty about the state of the world. A receiver that has no representation of states of the world cannot have uncertainty about those states; more technically, it cannot have a probability distribution defined on those states. Thus, a receiver incapable of representing at least some states of the world is not something to which information can be communicated; it cannot extract from information-bearing signals the information that they carry.

Signals carry information from one place to another (from one spatial location to another). In a computer, the signals are current pulses. In the nervous system, they are action potentials, synaptic transmitters, and hormones. Symbols carry information forward in time (from one temporal location to a later one). In a computer, the symbols are bit patterns in a memory register. We do not know the physical realization of symbols in the nervous system. One school of thought doubts that they exist (Rumelhart and McClelland, 1986; Wallace and Fountain, 2003; Hay and Baayen, 2005).

The essential features of a physically realized symbol are that it encodes information about something else (to which it refers) and that it enters into symbol processing operations appropriate to the information that it encodes. Base-pair sequences in DNA are biological examples of physically realized symbols. The sequence of exon codons (base-pair triplets) between a start codon and a stop codon encodes the sequence of amino acids that compose a protein. These codon sequences carry forward in time evolutionarily accumulated information about functional amino acid sequences. They enter into combinatorial operations that recreate the sequences they encode. Base-pair sequences also encode promoters. The binding and unbinding of translation factors (themselves usually proteins) to one another and to promoters control the timing and amounts of protein synthesis. The base-pair sequences in promoters carry forward in time evolutionarily accumulated information about functional patterns of protein synthesis (when and where it is functional to synthesize which proteins). The two kinds of information carried by base-pair sequences in DNA – exon information and promoter information – are roughly analogous to the two kinds of information carried in computer memory – data and program information.

In order to refer, the signals and symbols in a representational system must be causally connected to the things or states of the world to which they refer. In a process-control computer, the causal connection is effected by means of the transducers that generate signals proportional to critical variables, such as temperature, torque, concentration, velocity, and force. Often these signals are analog signals (voltages), but these are usually converted almost immediately to symbols (bit patterns stored in memory buffers) by analog-to-digital converters. The bit patterns are then converted to digital signals (current pulses) that are transmitted over signal lines to the input registers (memory buffers) of the computer, where they write the bit patterns into memory registers. This chain of causes and effects causally connects the bit pattern in a memory register that represents the current temperature to the temperature to which it refers. The function of the memory register is to carry that bit pattern (hence, the information about temperature that it encodes) forward indefinitely in time for use in later computations. When it is to be used in a computation, the pattern is read from the register. In short, a symbolic memory register is written to by impinging information-conveying signals and read from by computational processes.

In the nervous system, inputs acting on, for example, the retina of the eye or the basilar membrane of the ear are converted first into analog signals (receptor potentials) and then into digital action potentials, which carry the information into the central nervous system. The behavioral evidence implies that the nervous system possesses a read-write memory mechanism that performs the same essential function performed by the memory registers in a computer, but we do not yet know what that mechanism is.

Because a symbolic memory mechanism has not so far been identified, it is often assumed not to exist. It is assumed that "memory [elements in the brain take the] form of modifiable interconnections within the computational substrate" so that "no separate 'fetch' [read] and 'store' [write] cycles are necessary" (Koch and Hepp, 2006). These modifiable interconnections (synaptic conductances) are thought to be the physical realization of the associations in

associative learning theory (Fanselow, 1993; *See* Chapters 1.33, 1.34, 1.35). There are, however, no proposals about how either associations in the abstract or experientially modified synaptic conductances (physically realized associations) can encode acquired information in a computationally accessible form. There are, for example, no proposals about how associations could specify the coordinates of a remembered location. Generally speaking, for associative theories of learning, this is not a problem. Because they are nonrepresentational theories, they do not require a symbolic memory mechanism.

For representational theories of learning, however, the absence of a symbolic memory mechanism is a problem, because a mechanism functionally equivalent to the tape in Turing's abstract conception of a general purpose computing machine (Turing, 1936, 1950) is essential to computation and representation (Gallistel, 2006). Representations are computed by combining information that arrives in dribs and drabs spread out in time, as is illustrated shortly with the example of the dead reckoning process. For new information to be combined with old information, a mechanism must carry the old information forward in time in a computationally accessible form.

Symbols and the processes that operate on them create functioning homomorphisms between the symbol system and the aspects of the world to which the symbols refer (Gallistel, 1990). A homomorphism between two systems is a (partial) sameness in their abstract, mathematical form. Symbolic processes and relations in the representing system formally parallel nonsymbolic processes and relations in the represented system. A functioning homomorphism is one in which the representing system exploits this parallelism to inform its interactions with the represented system.

1.12.2 Behavioral Evidence for Representations in Learning

1.12.2.1 Dead Reckoning

The position of an animal in its environment, as a function of time, is the integral of its velocity with respect to time. This is a mathematical fact about the relation between these vector variables (velocity and position). In mobile animals, a representation of their position relative to places of behavioral importance (nest or resting area, food sources, hiding places, landmarks with reference to which these other places can be located, and so on) informs their behavior in many fundamental ways. The animal brain computes a representation of the animal's position by integrating with respect to time signals that convey information about its velocity. This symbolic integration process is called path integration in mathematical work and dead reckoning in traditional texts on marine navigation. It is a foundation of animal navigation (Mittelstaedt and Mittelstaedt, 1973; Wehner and Srinivasan, 1981; Gallistel, 1990; Wehner and Srinivasan, 2003; Collett and Graham, 2004; Etienne and Jeffery, 2004; Kimchi et al., 2004; Grah et al., 2005; *See* Chapter 1.20).

Dead reckoning is a particularly simple example of representational learning – if by 'learning' we understand the process of acquiring knowledge from experience. The animal acquires knowledge of its current position (and of past positions of behavioral interest) by means of a neurobiological process that integrates experienced velocity, as conveyed by signals from sensory and motor mechanisms sensitive to correlates of velocity. The process of neurobiological integration in the brain, which is the representing system, parallels the physical integration of velocity that occurs as the animal moves within its environment. The animal's location in its environment is the represented system. In technical jargon, the symbolic processes in the brain are homomorphic to the displacement process in the system that the brain is representing, because there is a partial correspondence in the abstract (mathematical) form of the processes going on in the two systems. The resulting symbolic specification of position enables the brain to, for example, set the animal on the course for home. Insofar as the courses the brain sets actually bring the animal to its home, the symbolic representation of position informs functional behavior. This makes the neurobiological integration of velocity signals to obtain position symbols an example of a functioning homorphism, that is, a representation.

Dead reckoning provides a particularly clear and simple example of the need for a symbolic memory in computation. The essence of dead reckoning is the adding of the current displacement to the sum of the previous displacements. For this to be possible, there must be a mechanism that carries the sum of the previous displacements forward in time in a form that makes it possible to add to it. The mechanism that carries the sum forward in time must not leak; the sum must not get smaller simply from the passage of time. It must not get smaller because it is the physical realization of the symbol that specifies the

subject's displacement from the origin of the reference frame, typically the nest. If the sum gets smaller simply with the passage of time, then the brain represents the animal as getting closer to home just by sitting still.

1.12.2.2 Learning the Solar Ephemeris

The integration of the velocity vector in dead reckoning is meaningless unless the velocity vector is represented in a stable geocentric coordinate framework. The velocity vector specifies the subject's rate of displacement in two orthogonal directions (for example, latitudinal displacement and longitudinal displacement or radial displacement and tangential displacement – see Gallistel, 1990, Chapter 4, for a detailed explication of path integration computations in different coordinate frameworks). Thus, a consistent directional reference must be maintained. In navigational jargon, this is called carrying the parallel, because lines of direction at different locations (e.g., lines running north–south) are parallel. The line from the observer to any nearby landmark changes its direction as the observer moves. This is called parallax. The farther away the landmark is, the less the parallax, and hence the better it functions as a directional referent. Extraterrestrial landmarks like the sun and the stars are, for practical purposes, infinitely far away, so they have negligible parallax. These extraterrestrial landmarks are strongly preferred as directional referents by animals of many different species (Gallistel, 1990). However, they have one drawback: Because of the earth's rotation about its own axis, their terrestrial direction changes continuously.

The solar ephemeris is the direction of the sun in a terrestrial frame of reference as a function of the time of day (that is, as a function of the angular position of the earth in its daily rotational cycle; *See* Chapter 1.25). To use the sun as a directional referent in dead reckoning, the brain must learn the azimuthal component of the solar ephemeris (Schmidt-Koenig, 1960). This function (solar compass direction versus time of day, see **Figure 2**) varies greatly, depending on which hemisphere the observer is in (northern or southern), its latitude (angular distance from the equator), and the season of the year. Thus, it is a highly contingent fact about the animal's environment, the sort of thing that must be learned from experience.

There is an experimental literature on the learning of the solar ephemeris by homing pigeons and

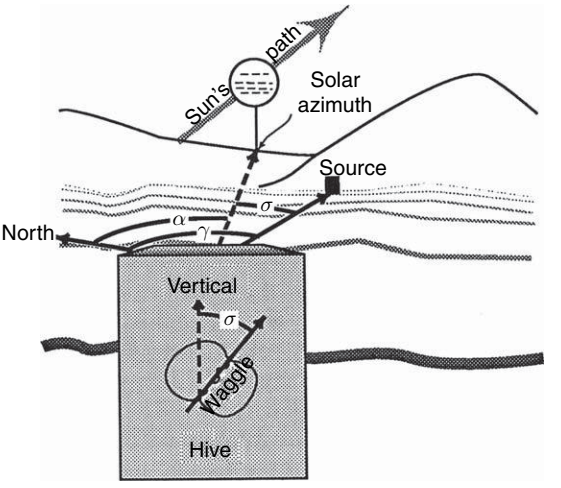

Figure 2 In the waggle dance, the returned forager circles in alternate directions from one waggle run to the next, forming a figure 8. The angle of the repeated waggle runs, relative to vertical, indicates the solar bearing of the source from which the forager has returned. The number of waggles indicates its distance. $\alpha =$ the solar azimuth, its compass direction; $\sigma =$ the solar bearing of the source, its direction relative to the direction of the sun; $\gamma =$ the compass direction of the source. Note that the compass direction of the source is the sum of the solar azimuth and the solar bearing: $\gamma = \alpha + \sigma$. (Reproduced by permission of the author and publisher from Gallistel CR (1998) Symbolic processes in the brain: The case of insect navigation. In: Scarborough D and Sternberg S (eds.) *An Invitation to Cognitive Science,* vol. 4, pp. 1–51. Cambridge, MA: MIT Press.)

foraging bees (e.g., Budzynski et al., 2000; Dyer, 2002; Towne et al., 2005). To learn the solar ephemeris is to learn, for some point of view (e.g., the hive entrance or the nest), what parts of the local horizon profile the sun is over as a function of the time of day (see **Figure 2**). To learn that, the brain must represent the horizon profile, the position of the sun relative to this horizon profile, and the time of day. The time-of-day signal is provided by the animal's circadian clock (Mouritsen and Frost, 2002; Giunchi et al., 2003; Homberg, 2004; Stalleicken et al., 2005). The universally valid parameter of the circadian clock, its period, is genetically specified. The clock parameter that must be adjusted based on local experience is the phase. The entrainment mechanism adjusts the phase of the clock in response to the rapid changes in photon flux that occur at dawn and dusk (Takahashi et al., 1984; Foster et al., 2003; Bertolucci and Foa, 2004; Foster and Bellingham, 2004).

The learning of the relation between the time-of-day signal provided by the circadian clock and the

position of the sun relative to the horizon profile at the chosen point of view appears to be a curve-fitting process (Dyer and Dickinson, 1994, 1996; Dickinson and Dyer, 1996). Built into the learning mechanism is a parameterized dynamic process – the physical realization of an equation (function) specifying the relation between two circular variables: time of day and position on the horizon. This built-in equation specifies what is universally true about the solar ephemeris, namely, that the sun is somewhere to the east all morning and somewhere to the west all afternoon. Incubator-raised bees that have seen the sun only in the late afternoon when it is declining in the west nonetheless represent it as being due east all morning long, stepping abruptly to due west around noon (Dyer and Dickinson, 1994). This default form for the ephemeris function is in fact valid only near the equator.

Notice that the information about where the sun is in the morning cannot have come from these incubator-raised bees' experience, because they never saw it in the morning. It is carried forward in time from the remote evolutionary past by the genes that code for the built-in dynamic process relating the horizon position cycle to the circadian clock's cycle, just as the information specifying the period of the circadian clock is carried forward in time by the genes that specify its molecular structure. In experienced bees, which have observed the sun at the hive at several different times of day, the parameters of the built-in function are adjusted so that the function specifies locally appropriate horizon positions for the sun throughout the day. In the summer at midlatitudes in the northern hemisphere, where the Dyer and Dickinson (1994) experiments were conducted, the sun rises north of east, moves continuously along the horizon in the clockwise direction to reach due south at noon and then on through west to north of west in the late afternoon. (By contrast, in winter at midsouthern latitudes, it rises north of east and moves continuously counterclockwise along the horizon to due north at noon and then on to north of west in the late afternoon.)

What bees learn from their experience of the sun's position over the horizon at different times of day is the locally appropriate values for the parameters of their built-in solar ephemeris function. These parameter settings are not explicitly specified by the bees' experiences of the sun's position above the horizon profile. There is no first-order sensory signal generated by any aspect of the bees' experiences that directly specifies appropriate parameter values in the way in which, for example, the signal from a photoreceptor explicitly specifies the number of photons captured by that receptor. Even the positional data – where the sun is over the profile – are only implicitly present in the spatiotemporal distribution of action potentials from the retina. The sun's profile-position coordinate must be computed from this pattern. And even then, the appropriate values for the parameters of the ephemeris equation are only implicit in data specifying several different profile positions at several different times of day. Thus, the explicit parameter values, the actual settings of the parameters, must be computed from these positional data. Moreover, the positional data are not given all at once. Experience provides different data points at different times of day. When it has extracted from the incoming retinal signals an explicit representation of the sun's profile position, the brain requires a symbolic memory to carry that information forward in time, so that it may be computationally combined with the positional data obtained later in the day to determine the appropriate values of the ephemeris parameters.

The symbolic information provided by the learned ephemeris informs not only the dead reckoning process but other behaviors as well. When a foraging bee returns from a rich source, it does a waggle dance on the vertical surface of the hive, which symbolically specifies the direction and distance of the source (**Figure 2**). The direction is specified by the angle of the waggle run relative to vertical in the solar bearing of the source, the angle at which an outbound forager must hold the sun to fly toward the source. The solar bearing communicated by the dance is not the bearing that the returning forager has just flown; rather, it is the inverse of that bearing. If the returning forager flew with the sun at its back to reach the hive, then its dance tells the other bees to fly toward the sun. This information about the relation between the direction of the source from the hive and the direction of the sun from the hive was acquired many minutes earlier. A symbolic memory is required to carry this direction vector forward in time until it is used to inform the waggle dance (cf. Menzel et al., 2006; *See* Chapter 1.25).

The information communicated by the dance is carried forward in the nervous systems of both the dancer and the observers of the dance in a computationally accessible form. If the hive is closed for several hours and then opened, outward-bound foragers do not fly the solar bearing indicated by the dance they witnessed hours earlier; rather, they fly a

time-compensated solar bearing, a bearing that takes into account the intervening change in the sun's compass direction (Frisch, 1967). This implies that either (1) a recruited bee remembers the danced solar bearing and uses its solar ephemeris to compute the expected change in solar bearing over the interval since the dance, or (2) the remembered solar ephemeris was used at the time of the dance to compute a compass bearing or the indicated location on the cognitive map (map coordinates). The remembered compass bearing (or remembered map coordinates) must then be combined with the solar ephemeris function at the time of the outward-bound flight to compute an appropriate solar bearing (how to fly relative to the sun to fly a remembered direction relative to the earth).

The learning and use of the solar ephemeris illustrates the manner in which different bits of information, gathered from experience at widely different times in the past, are computationally combined to obtain the explicit information (the current solar bearing of the food source) that informs current behavior.

1.12.2.3 The Cognitive Map

A map is a record of two or more locations in a common coordinate framework. It gives access to other (nonpositional) information about those locations (for example, information that enables the navigator to recognize surrounding landmarks and information about the food to be found there or the dangers to be wary of). A cognitive map is a map of experienced terrain computed by a brain and recorded in its (presumed) symbolic memory. Dead reckoning and the construction and use of a cognitive map are intimately interrelated (Gallistel, 1990, 1998; Collett et al., 1999; McNaughton et al., 2006; *See* Chapter 1.25).

The information that dead reckoning provides about the animal's current position becomes more valuable as the number of locations on the cognitive map increases. It also becomes more valuable as the brain records more information that will enable it to recognize those places on subsequent occasions (for example, 'snapshots' of surrounding landmarks, together with their direction and distance from the point of interest; Cartwright and Collett, 1979, 1983, 1987; Collett et al., 1986). The information about current position becomes more valuable as the map becomes richer in information, because a major function of the cognitive map is to enable the brain to compute the range (distance from the animal) and bearing (direction from the animal) of points of behavioral importance like food and the nest (Collett et al., 1999). The directional information extracted by this vector computation is critical to the setting of a course toward or away from those points. The distance information is critical to the making of decisions based on estimates of the time it will take to reach a point.

Often, dead reckoning itself provides the estimate of the animal's position on its map, but sometimes either natural circumstances (gusts of wind) or experimental intervention renders the dead reckoning position useless, in which cases the brain must rely on landmark recognition to reestablish its position on its cognitive map and compute the course back to home or to the destination it had before it was displaced (Fukushi and Wehner, 2004; Menzel et al., 2005, 2006).

Conversely, the better the positional information supplied by the dead reckoning process, the more accurate a cognitive map becomes and the easier it is to recognize relevant landmarks (Cartwright and Collett, 1979, 1983, 1987; Durier et al., 2003; Graham et al., 2004). The map becomes more accurate as dead reckoning improves because it is likely that dead reckoning is the principal determinant of the position vectors recorded on the map. When the animal finds a point of behavioral interest, such as a food source, the position vector that represents that location on the brain's cognitive map is the dead reckoning position vector (the symbol that specifies where it is in the frame of reference used for dead reckoning, for example, 50 m south and 22 m east of the nest) plus the egocentric position vector for the point of interest (the symbol that specifies the location in the egocentric frame of reference, for example, 30° left and 2 m away). Combining these two symbols is an exercise in coordinate transformation. The computation is spelled out in Gallistel (1990: 106ff; see also Gallistel, 1999). Thus, errors in the dead reckoning become errors in recorded locations. This is a major reason for the inaccuracies in early marine charts; in those charts, too, estimates of longitude were largely based on dead reckoning.

It becomes easier to recognize landmarks as dead reckoning becomes more accurate because estimates of one's location play a major role in the process. To recognize a landmark is to identify what is now perceived with something recorded on the chart. In both human and animal navigation, position confers identity (Gallistel, 1990: 140, 168ff). That is, for a

navigator to recognize a landmark, the navigator's estimate of its location must be within his uncertainty about where he is in relation to it on his cognitive map (Cartwright and Collett, 1983; Collett et al., 2002; Dale et al., 2005). A sailor on Long Island Sound seeing to her east something that looks exactly like Mount Vesuvius will not conclude that she is in Italy. She will ponder either why she never knew there was any part of Long Island that looked anything like Mount Vesuvius or, more likely, how remarkable it is that a cloud formation could look exactly like Mount Vesuvius. Thus, contrary to the common assumption, a major factor in the recognition of a landmark is that it be roughly where it is supposed to be. Being where it is supposed to be is an essential aspect of its identity. If it is not where it is supposed to be, it is not that landmark.

In short, dead reckoning provides major input to the computations that determine the recorded locations (position vectors) of points on the cognitive map. The recognition of landmarks along familiar routes (these are called waypoints in traditional navigation) is a substantial aid to dead reckoning (Kohler and Wehner, 2005). However, the animal's estimate of its position and orientation on the cognitive map is a major determinant of whether it looks for a given landmark and whether it accepts a possible landmark as the one it is looking for. Finding a looked-for landmark helps to correct error in the dead reckoning (Etienne et al., 2004) and get the animal back onto a familiar route (Kohler and Wehner, 2005).

All of this – all of our understanding of animal navigation – presupposes a symbolic memory, a memory capable of encoding information about the world and the animal's relation to it and carrying that information forward in time in a computationally accessible form. The physical realization of the geocentric position vector in the dead reckoning machinery must be such that it can be computationally combined with the physical realization of the egocentric position vector in the machinery that assigns egocentric positions to perceived landmarks (see Gallistel, 1999, for a review of the neurobiology of coordinate transformations). The resulting vector symbol (the physical realization of the geocentric coordinates of a perceived landmark) must preserve the information about the location of the landmark on the cognitive map. It must do so in a form that makes it possible to add and subtract that position vector from other position vectors, because that is what is required in computing the range and bearing of one location from another location (cf. Collett et al., 1999).

In order to recognize a landmark, the brain must previously have made a record of what that landmark looks like (a 'snapshot,' cf. Collett and Collett, 2002; Graham et al., 2004). The record must contain enough information to distinguish between that landmark and other landmarks the animal has encountered in that vicinity. The number of distinguishable snapshots that the brain of an ant or bee might make is for all practical purposes infinite – larger than the number of elementary particles in the universe. Thus, there cannot be for every possible snapshot a genetically specified neural circuit excited by that snapshot and only that snapshot. There cannot be a "gnostic neuron" (Konorski, 1967) for every possible visual scene the ant or bee may need to record. This is derisively known as the grandmother-neuron theory of perceptual encoding.

When applied to, for example, a digital camera, the grandmother-neuron theory is that the factory builds into each camera all possible pictures that might ever be taken. When you press the shutter release, the camera detects the correspondence between the image on the camera's retina and one of the images built into it at that factory, so the 'neuron' for that picture is activated. In this way, the camera (or the brain) 'recognizes' the picture that you are taking.

Clearly this will not do; the possible snapshots cannot be genetically specified and prewired into the nervous system of the ant or the bee. There must be some decompositional encoding scheme that permits finite representational resources to encode the finite number of actual snapshots taken in any one lifetime. No matter how enthusiastic a photographer you are, the pictures you actually take are a negligible fraction of all the possible pictures! A digital camera decomposes images into pixels. It uses symbolic memory to record the image values for each pixel. Thus, it encodes in memory only the pictures actually encountered, not all the pictures that might ever be encountered.

There are much more efficient encodings, as we learn when we compress image files. The use of basis functions, as in Fourier decomposition, is an example of a less obvious and potentially much more efficient encoding. Efficient encodings reduce complex inputs to vectors (in the loose sense of ordered strings of numbers) that specify parameter values for the functions that form a basis of the encoded space. Another

example of the use of finite resources to encode an infinite range of possibilities is the encoding of to-be-printed pages of drawings and text using Postscript. The number of distinguishable pages that may be printed is infinite. However, the file for any actual page is surprisingly short, which is a tribute to the efficiency of the encoding scheme.

This approach to understanding how the decidedly finite nervous system of an ant might preserve the information in a snapshot presupposes that there is a symbolic memory mechanism capable of preserving the decomposition of a snapshot (a visual scene) for later computational use. The decomposition made when the landmark was first encountered must be available when the animal encounters that landmark again, and it must be available in a form that allows it to be compared to the decomposition of the currently viewed landmark. The computational architecture should allow for the generation of a measure of similarity between the two encodings being compared (the encoding of the old image in memory and the encoding of the image now on the retina). Moreover, the making of a snapshot, as the term implies, cannot require repeated presentation of the image to be recorded; it must be a one-off process, like the process in a digital camera, which makes fundamental use of a symbolic memory mechanism (but *See* Chapter 1.33).

Recognition is, of course, what attractor networks do (*See* Chapters 1.33, 1.34, 1.35) and they do not have a symbolic memory. (The lack of a symbolic memory is what distinguishes a neural net from a conventional computing machine.) However, because it lacks a symbolic memory, a neural net cannot compute measures of similarity or relative probability. Thus, a neural net recognizer cannot provide information about two different possible matches at the same time. Activity in the net must migrate to one attractor state or another. The net cannot be in two different activity states at the same time. When it is not in one attractor state or another, its activity state does not specify anything about the input. Activity states intermediate between two attractor states do not specify, for example, the relative probabilities of two different possible matches (two different attractor states that the net might be in but is not).

The net's inability to specify a probability distribution over possible states of the world (possible matches between the current image and previous images) makes it difficult to combine the evidence from different computational procedures. For example, it makes it difficult to combine the evidence from image comparison (the probability distribution on the possible matching images, considering only the information in the images themselves) with the evidence from dead reckoning (the prior probability distribution over the possible matching images in memory, given the probability distribution on the animal's position and orientation on its cognitive map).

This last point is important because it relates to recent findings demonstrating that (1) the cognitive map gives access to other information about the world, and (2) even insects engage in what looks like Bayesian inference, combining probabilistic information acquired at different times from different sources. In these experiments (Gould and Gould, 1988; Tautz et al., 2004), foraging bees return from a food source on a rowboat in the middle of a pond or small lake. The returning foragers do the waggle dance, indicating the range and bearing of the food source, but the dance fails to recruit other foragers. This implies that (1) what the dance really communicates is not flying instructions (range and solar bearing) but rather map coordinates, and (2) the bees that observe the dance consult their cognitive maps before deciding whether to act on it (*See* Chapter 1.25). If their own past experience, as recorded on their map of where they have found food, indicates that no food is to be found anywhere near the location indicated by the dance, they decide not to go. Most interestingly, when the boat with the food source is moved close to an island, then the dance of the returning foragers does recruit new foragers. However, the recruits do not come to the boat but rather to the shore near the boat (Tautz et al., 2004). This implies that they have combined the probability distribution for the source location indicated by the dance (the approximate location of the source implied by the dance) with the prior probability distribution (based on their experience, as recorded on their map) to arrive at a posterior distribution, whose modal point is the nearby shore (the most probable location, all considered).

These findings suggest how grossly we may have underestimated the representational and computational capacities of even very small brains. Our gross underestimation of the representational capacity of brains as small as the head of a pin has allowed us to suppose that brains can get along without the symbolic memory mechanism that makes representation possible.

1.12.2.4 The Representation of Past Episodes

Our memory for episodes is another example of one-off memory for specific occurrences, where the number of possible episodes is infinite. Episodes by definition happen only once. We often recall them, however, long after they were encoded and committed to memory (albeit not as accurately as we like to think). More importantly, we combine the information from one episode with the information from another to draw conclusions not justified by any single episode: The fourth time you see a man dining with an attractive woman not his wife, you begin to think he might be a philanderer. When this conclusion becomes a fixed belief, it is an example of a declarative memory ("The man's a philanderer") inferred from a sequence of episodic memories. This does not, of course, imply that the episodic memories are forgotten. Indeed, we may call on them to justify our inference to others. Recent ingeniously constructed experiments with food-caching jays show that we are not alone in our ability to remember episodes, nor to draw behaviorally informing inferences and declarative memories from them.

In times of plenty, many birds gather food and store it in caches (*See* Chapters 1.22, 1.23). Western scrub-jays are particularly remarkable cachers. They make more than 30 000 different caches spread over square miles of the landscape (Vander Wall, 1990). Weeks and months later, during the winter when food is scarce, they retrieve food from these caches, one by one. This is another illustration of the vast demands on memory made by a cognitive map. It also emphasizes the critical importance of a computational architecture that can effect the same computation (vector subtraction) on many different symbol combinations (pairs of vectors) without having a different neural circuit for each possible symbol combination. If we suppose that the jay can set a course toward the remembered location of any one cache while at the location of any other, then, given 30 000 locations, there are on the order of 10^{10} possible course computations (vector subtractions with distinct pairs of vectors formed from the 30 000 vectors in memory). Whatever the computational architecture of the nervous system, it cannot be such as to require 10^{10} different genetically specified neural circuits to effect these 10^{10} different computations.

A long series of ingenious experiments by Clayton and Dickinson and their collaborators have shown that jays remember much more than simply the locations of their caches (Clayton and Dickinson, 1998, 1999; Emery and Clayton, 2001; Clayton et al., 2003a,b; Emery et al., 2004; Dally et al., 2005a,b; de Kort et al., 2005; Clayton et al., 2006b). They remember what kind of prey they hid in each cache, when they made each cache, which other jay, if any, was watching when they made that cache, and whether they have subsequently emptied that cache. They also remember whether they themselves have pilfered the caches of another bird. They remember the intervals that have elapsed in the past between the hiding and retrieval of a given kind of food and whether the food, when retrieved, had or had not rotted. All of this remembered information combines to determine the order in which they will visit caches they have made. The information drawn from memory that is combined to inform current behavior comes from a mixture of episodic memories ("Three days ago, I hid meal worms there, there and there, and 5 days ago, I hid peanuts there, there and there") and declarative memories ("Meal worms rot in 2 days; peanuts don't rot").

These experiments demonstrate a rich representation of the jay's past experience and the ability to compute with the symbols that carry the information gained from that experience forward in time. For example, the birds compute from the current date-time and the remembered date-time at which they made a particular cache the time elapsed since they made that cache. They compare the time elapsed since that cache was made with the remembered time that it takes the contents of that cache to rot. If the computed time elapsed is greater than the remembered rotting time, they visit first the caches where they put the peanuts, even though they prefer (unrotted) meal worms to peanuts, and even though they made the meal worm caches more recently.

When their caching is observed by another jay, they combine the memory of which particular jay it was with a memory of their own much earlier thieving (from which they appear to infer the existence of evil) and their memory for the social status of that jay. If it was a dominant jay and if their experience of their own behavior (their own pilfering) has made them believe in the evil nature of jays, then they are likely to return to the cache when no one is looking, retrieve the food, and hide it elsewhere.

The rich representation of past episodes implied by these results again implies a decompositional scheme of some kind, because the number of possible caching episodes is infinite. Indeed, it suggests the

kind of representation of our experience that appears in our linguistic descriptions of episodes (agents, actions, objects of actions, locations, markers of temporal position relative to the present and to other events, and so on). Perhaps we should not be surprised to find evidence for such representations in nonverbal animals. Our ability to use language to communicate to others the information we have extracted from our own experience of the world is itself astonishing. It would be far more astonishing if we assumed that our nonverbal ancestors had no high-level decompositional representation of the world prior to the emergence of language.

1.12.3 Implications for Neurobiology

As mentioned at the outset, the hypothesis that learning depends on the brain's computing representations of selected aspects of the experienced world is controversial because we do not know what the neurobiological realization of key components of the requisite machinery is. Indeed, we do not even have a clear notion of what this realization might be, let alone persuasive evidence that a hypothesized realization is in fact the realization. The keystone in any symbolic computation is a symbolic read-write memory mechanism. That is a mechanism to which information conveyed in signals can be written and that will carry this information forward into the more or less indefinite future, until such time as it is needed in further computation. From a neurobiological perspective (that is, from a material reduction perspective), symbols are the enduring physical changes in the nervous system that carry the information forward in time.

The behavioral evidence implies that a vast amount of acquired information is carried forward by this mechanism, even in the brains of birds and insects. This constrains physically plausible mechanisms (Gallistel, 2002). Whatever the mechanism is, it must realize a very high density of information storage; it must pack gigabytes into cubic microns. It must be thermodynamically stable, so that the preservation of the information has little or no metabolic cost. One cannot pack a great deal of information into a very small volume using a mechanism that depends on the expenditure of metabolic energy to preserve the information, because (1) there is no way to supply the requisite amount of energy within the small volume, and (2) if there were, there would be no way to dissipate the heat generated.

In short, we must remember that the laws of thermodynamics apply to whatever symbolic memory mechanism we may imagine. These thermodynamic considerations make reverberating circuits an implausible physical realization of a mechanism whose essential function is to store large amounts of information for long periods of time. This is important because reverberating activity is the mechanism for storing previously computed information in recurrent neural net models, for example, in moving-activity-bump models for dead reckoning (Samsonovich and McNaughton, 1997; Stringer et al., 2002; Conklin and Eliasmith, 2005; Song and Wang, 2005).

Another constraint on the physical realization of the symbolic memory is that it must both be capable of encoding information and be in a readable form. That is, it must be possible to envision how the enduring physical change hypothesized to be the physical realization of a symbol could in principle specify a fact about the world and how that fact could be recovered (read) from it. It is this consideration that makes Hebbian synapses implausible as the physical realization of symbolic memory. Hebbian synapses are synapses that enduringly change their conductance as the result of the pairing of pre- and postsynaptic activity. The conductance of a synapse is the amplitude and duration of the transient change in the postsynaptic membrane potential when a spike arrives at the presynaptic terminal.

The first thing to be noted in this connection is that there are few if any published suggestions that it is the synaptic conductances themselves that encode information about the world (as opposed to the gnostic neurons that are connected through those conductances). As already noted, changes in synaptic conductances are the hypothesized physical realization of changes in the strengths of associative bonds. Associative theories of learning have traditionally not been representational theories, for the simple reason that it is hard to make a symbol out of an association.

Traditionally, associative strengths have been assumed to change slowly and in a manner dependent on many different aspects of a repeated experience: how close two stimuli are in time, how strong each of them is, how often they have occurred in close temporal conjunction, and so on. Thus, the strength of an association, that is, the conductance of a modifiable synapse, is the product of many different aspects of experience. This means that it cannot encode any one of those aspects. Mathematically speaking, the mapping from experience to an

associative strength is a many-one function, and many-one functions are not invertible; you cannot recover the many from the one.

Even if the process of modifying a synaptic conductance were somehow constrained in such a manner that the conductance of a memory synapse could be made, for example, proportional to a to-be-remembered distance, the architecture of the nervous system, as currently understood, would not permit the conductance of that synapse to be read. The postsynaptic signal is a product (or joint function) of the presynaptic signal and the synaptic conductance The synaptic conductance is usually called the synaptic weight, because it is a multiplicative constant that weights or scales the presynaptic signal to determine what is seen by the postsynaptic integration process. Unless the postsynaptic mechanism has independent access to the presynaptic signal (unless it has information about that signal by a pathway other than the presynaptic pathway), it cannot estimate from the postsynaptic signal what the conductance of the synapse is. Thus, even if experience had made the strength of a synaptic conductance proportional to the distance to the food, it is hard to see how that information could be recovered by the postsynaptic integration process.

The mathematical impossibility of recovering the conductance of a synapse from the postsynaptic effect of an unknown presynaptic signal does not go away when one considers instead the problem of recovering the synaptic conductances (weights) in a neural network from the activity in its output neurons. If the postsynaptic effects are linear in the presynaptic signal strengths, then the activities of the output neurons may be regarded as the known values in a linear algebra problem, in which the input signal strengths and the intervening synaptic conductances are the unknown values. Unless there are as many knowns (output signals) as there are unknowns (input signals and synaptic conductances), it is a basic algebraic truth that the values of the unknowns cannot be recovered.

If the network is a richly connected one, with more synapses than inputs and outputs combined, then the conductances of the synapses cannot be recovered, even when both the output and the input signals are known. If postsynaptic effects are nonlinear in the inputs, the problem is still worse, because there is loss of information in nonlinear operations. Intuitively, this is because when there are thresholds, one cannot estimate from the signal on the other side of the threshold what is going on below the threshold. Thus, recognizing that the synapses whose alteration is supposed to carry information forward in time are embedded in complex networks does not on the face of it make the problem of readability less of a problem; it makes it more of a problem.

This is not to say that some particular network architecture may not solve the problem. A particular architecture dictated by the logic of the problem is just what we see in the read-write memory of a computer. One might imagine, for example, that modifiable synapses only had two states, conducting and nonconducting, and that information about facts like distance and direction was encoded in banks of such synapses, using the same binary code by which distance and direction information is encoded in the memory registers of conventional computers. However, this, too, would require revising our conception of the functional architecture of the nervous system to enable write and read operations. As indicated by the quotation from Koch (Koch and Hepp, 2006), the current understanding of the nervous system has led to the conclusion that this is precisely what its architecture does not support. Moreover, as the Koch quotation indicates, this is taken to be an advantage of the architecture, not a deficiency. Thus, within our current understanding of the functional architecture of the nervous system, there does not appear to be a way to make modifiable synaptic conductances be the mechanism of symbolic memory. This accounts in some measure for the strong strain of antirepresentational theorizing in neurobiologically inspired models of learning.

The presence of a read-write memory mechanism has far-reaching architectural implications. There is no point in writing information to memory if you cannot find it later. Thus, the existence of a read-write mechanism implies or presupposes that the architecture of the system supports memory addressing. It makes no functional sense to have a read-write memory in a machine whose architecture does not support memory addressing.

It is memory addressing that makes possible (1) the distinction between a variable and the value of that variable, (2) the ability to bind a value to a variable, and therefore, (3) the creation of symbolic structures (data structures). Memory addressing makes all this possible because the address in memory at which a given piece of information (a symbol) is to be found is itself information that may be written to memory at another address. This leads to indirect addressing in which the value to be operated on is specified not by its address but, rather, by the address where its address is stored. The bit pattern specifying

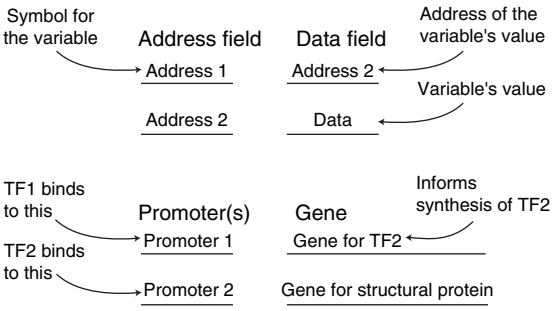

Figure 3 Indirect addressing in computers and the genome. TF = transcription factor. In both examples, the entry in the bottom right field ('Data' or 'Gene for structural protein') may be yet another address (another gene for a TF). This recursive addressing logic makes possible hierarchical data structures with a (potentially) unlimited number of levels.

the first address (Address 1 in **Figure 3**) is the symbol for the variable itself (e.g., the distance to the food source), not for the value that the variable happens to have at any one time (e.g., 10 m). The symbol for the variable gives access to the value of the variable by means of the bit pattern stored in the data field at the address specified by the symbol for the variable. The datum stored in that field is the address of the value (Address 2 in **Figure 3**). The bit pattern (symbol) that specifies the value is in the data field at the second address. To change the value of the variable, the computer changes the bit pattern stored at the second address, not the bit pattern for the first address (the symbol for the variable) nor the bit pattern stored at the first address (the address of the variable's value).

This sounds far removed from biological reality, but, as shown in **Figure 3**, the information stored in DNA is retrieved by the same functional architecture, implemented now at the molecular level, which is to say more compactly than in current computer memory. The address at which a datum (the codon sequence specifying a protein) is stored is the promoter for that gene. Transcription factors initiate the reading of that information (transcription) by binding to the promoter. When a transcription factor binds to the promoter for a gene, the amino acid sequence coded for by that gene is used by the molecular machinery that synthesizes proteins to control the synthesis of the protein defined by that sequence. The protein thereby synthesized may be either a structural protein (a building block of cellular structure, analogous to the actual value of variable) or a transcription factor (an address). Addressing the promoter of a transcription factor (synthesizing a protein that binds to its address) gives access to the addresses (promoters) of the genes to which that transcription factor binds. As that transcription factor is synthesized, it binds to those promoters, leading to the synthesis of the proteins coded by their genes, many of which proteins may themselves be yet further transcription factors. This indirect addressing makes possible the hierarchical structure of the genome. It makes it possible to have an 'eye' gene that, when activated (addressed by the transcription factor for its promoter), leads to the development of an entire eye (Halder et al., 1995). The eye gene codes only for one protein, but that protein does not itself appear anywhere in the structure of the eye. It is a transcription factor. It gives access to the addresses of other transcription factors and, eventually, through them, to the addresses of the proteins from which the special tissues of the eye are built and to the transcription factors whose concentration gradients govern how those tissues are arranged to make an organ.

What is needed for a brain to have learned representations is a similar architecture for accessing the information acquired from experience, an architecture in which it is possible to write information into biochemically realized symbols as well as read it from them.

The other critical components of a machine capable of representation that is missing from our current conception of the nervous system are the machines that operate on symbols. Needed are mechanisms that perform unary operations, such as negation, and binary operations, such as the arithmetic operations, string-building operations (e.g., concatenation), and logical operations (e.g., AND and OR). The existence of mechanisms operating on symbols would be implied by the existence of a symbolic memory mechanism, because it does not make functional sense to have a read-write memory if there are no mechanisms for operating on the symbols in it. (It's like having chromosomes without ribosomes, which are the machines that put the amino acids together to form a protein.) What makes a representation are the symbols, together with the operations on them. The operations enable the extraction of explicit representations from information that is only implicitly present in the symbols already computed. A representation, that is, a functioning homomorphism, exists only when a machine can operate on its symbols to construct new symbols, symbol strings, and data structures.

A constraint on the mechanisms that operate on symbols is that there not be as many different

mechanisms as there are distinct variables. As noted earlier in connection with the 30 000 cache locations that a jay can remember, it is not plausible to imagine an architecture in which there is a separate neural circuit dedicated *a priori* to each different vector subtraction that might ever have to be performed. The reason is once again the infinitude of the possible; there are too many possibilities.

This last constraint may seem almost too obvious to mention. However, in many neural net models of even simple computations, such as those involved in dead reckoning, the combinatorial operations are implemented by a table-look-up architecture (e.g., Samsonovich and McNaughton, 1997). There is a separate look-up table for each instance of a given kind of operation – a different table for each different case in which two variables must be added. A given table can effect the multiplication of all possible values of two variables, but it can operate only on those two variables. Moreover, each such table is composed of tens of thousands of neurons because each different combination of values for the two variables is effected by the neurons that compose the corresponding cell in the table. In short, there are as many different look-up tables as there are pairs of variables whose values may have to be combined, and within each such table, there are as many different neural subcircuits as there are pairs of values for the two variables. Such an architecture is prodigally wasteful of material resources. It is nakedly exposed to combinatorial explosions that lurk behind every tree in the computational forest. That appears to be the price that must be paid for doing without a symbolic memory.

These considerations suggest that the nervous system may in fact contain a yet-to-be discovered read-write memory mechanism. They suggest, moreover, that the mechanism is likely to be found at the level of molecular structure, rather than at the level of cellular structure. Finally, they suggest that the discovery of such a mechanism and the functional architecture that is required to make it effective would change our conception of how the brain works in fundamental ways.

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1.13 Attention and Memory in Mammals and Primates

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1.13.1 Introduction

Our brains continuously receive huge amounts of sensory information concerning the busy and complicated world in which we live. In fact, Koch and Tsuchiya (2007) recently estimated that primates receive on the order of 1 megabyte of raw information per second through their eyes alone. In order to make sense of all of this sensory input, and to act upon it appropriately, it is essential that we are able to focus our attention selectively on certain stimuli at the expense of others (*See also* Chapters 1.07, 1.08). For example, as you read this sentence, you will probably be ignoring the feeling of the clothes on your body (see Graziano et al., 2002) and any number of background sounds from the world outside. In this chapter, we consider the extent to which our brains can achieve such selectivity of information processing in the visual, auditory, and tactile modalities and the means by which they might do so.

Empirical evidence from many different experimental methodologies now supports the claim that unattended sensory information is processed less thoroughly by the brain than is attended information. The results of many behavioral studies have demonstrated that unattended sensory information is perceived and/or remembered less successfully than attended information. From a neurophysiological viewpoint, attentional modulation of neural activity has now been observed both in humans and in other animals. Here, we review the evidence from both of these lines of research, integrating the findings that have emerged from studies examining the cognitive constraints on information processing in audition, vision, and touch – the sensory modalities where attention has, to date, been most widely studied. We also draw out a number of the parallels in the mechanisms of selective attention as they affect the processing of information in the different senses.

Our focus for this chapter relates to what has perhaps been one of the most enduring questions in the study of selective attention, namely, the stage of processing at which unattended information is excluded. It is generally agreed that all information must be processed to some level, so that it can, at the very least, be designated as being either potentially relevant or irrelevant. However, estimates of the extent to which unattended information is processed in the brain have varied widely. The lively debate over this issue has continued apace for more than 50 years and is still a topic of interest for many attention researchers today. Below, we describe the origins of the debate and highlight some of the most important experimental evidence that has emerged over the years from studies of this issue in audition, vision, and touch.

1.13.2 Behavioral Evidence for Early Selection

Many of the earliest studies of attention focused on the selection taking place within the auditory modality. This research began in the early 1950s with the

development of the dichotic listening paradigm (Cherry, 1953, 1954). The participants in these early studies were typically presented with two different auditory messages, one to either ear. They had to repeat out loud (i.e., 'shadow') the message presented to a specified ear while trying to ignore the message presented simultaneously to the other (unattended) ear. The typical finding reported in many different studies was that following the shadowing task, participants were unable to remember anything of the unattended message apart from its most basic physical characteristics. For example, participants failed to notice (or at least to recall) changes to the language being spoken but were often able to recall changes in the pitch of the speaker's voice (e.g., Cherry, 1953). Indeed, Moray (1959) reported that participants often failed to recognize words that had been repeated as many as 35 times in the unattended channel. In the light of such evidence, researchers argued that attentional selection must occur at a relatively early stage of information processing, such that unattended stimuli were rejected as soon as their simple physical characteristics (such as their location, frequency, etc.) had been determined, and were not subject to any significant further processing (Broadbent, 1958). We note, however, that the definition of a "simple, physical characteristic" may not be as straightforward as it might at first seem (Allport, 1992). For example, properties that are coded early in processing in one sensory modality (such as location in vision) may only be derived much later in other sensory modalities (e.g., location is derived comparatively late in auditory processing; see Spence and Driver, 1994).

It is important to emphasize that these demonstrations of early selection assessed the extent of processing of the unattended information in terms of participants' subsequent memory for the content of the unattended stream. This approach was soon criticized on the grounds that, by the time the participants were asked about the contents of the unattended stream, they may simply have forgotten the information, rather than never having perceived it in the first place (see Wolfe, 1999, for a discussion of this critical distinction between inattentional amnesia and genuine inattentional blindness).

Nevertheless, the proposal that unattended stimuli were ruled out at a relatively early stage of information processing was also supported by subsequent studies examining the effectiveness of selective attention within the visual modality. At first, these visual studies drew inspiration from the auditory studies described above and were therefore typically designed to create a more or less direct visual analogue of the dichotic listening task. For example, in one of the earliest studies of visual selective attention, reported by Neisser and Becklen (1975), participants were presented with two superimposed video streams, one showing two people playing a hand-slapping game and the other showing three people passing a basketball between one another. The participants in this study had to attend to one of the two video streams in order to carry out a task on this attended information (such as counting the number of ball passes or hand slaps) while ignoring the other stream (in which unexpected events, such as the appearance of a new person carrying an umbrella, occasionally occurred). Just as had been found in previous auditory studies, the participants in Neisser and Becklen's study were unable to recall any of the unusual events in the unattended visual stream, despite the fact that this stream had been presented from the same location as the attended stream.

However, one might argue that the failure to remember the unattended information in Neisser and Becklen's (1975) seminal study could reflect the fact that participants would have been focusing their overt attention on the attended stream and hence may have been making eye movements to keep track of the action occurring in that stream. Any such eye movements may simply have reduced the visibility of the unattended stream (due to retinal smearing). Note that this criticism also applies to a number of the more recent demonstrations of inattentional blindness, in which participants have typically been asked to carry out a task based on the events contained within a single video (in contrast to earlier studies involving superimposed videos). The striking finding to have emerged from this type of research is that participants can remain seemingly unaware of apparently salient visual events (e.g., the appearance of a person in a gorilla outfit) occurring in the same video as the visual task (e.g., counting the number of ball passes occurring in a basketball game) (Simons and Chabris, 1999). However, because the unexpected visual event never completely coincides spatially with the attended events, these findings are also open to alternative explanations in terms of participants' eye movements.

We note that early studies of selective attention may have used auditory stimuli (at least in part) in an attempt to rule out potential alternative explanations of any attentional effects obtained in terms of overt

orienting involving eye movements. However, subsequent research has now demonstrated that eye movements can in fact influence auditory attentional allocation during dichotic listening tasks (Gopher, 1973; Hynd et al., 1986), so this assumption may not, after all, have been valid.

Nevertheless, results in favor of early selection have also been found in studies that have controlled for the potential influence of eye movements. For example, Rock and Gutman (1981) presented the participants in their study with pairs of superimposed outline drawings (either familiar or nonsense shapes) in different colors. The participants had to focus their attention on the line drawings that were presented in a particular color (with the dummy task of rating each drawing's pleasantness) while attempting to ignore the drawings presented in the other color. In a subsequent surprise memory test, the participants were often unable to recognize any of the unattended shapes, even if the test occurred no more than a second after the presentation of the shape in question (see Rock and Gutman's Experiment 4). By contrast, participants' performance on a similar memory test for the attended shapes was fairly good. The use of static presentation in this experimental design helped to reduce the likelihood that eye movements played any role in determining the pattern of results obtained (an assertion that was confirmed by the monitoring of the eye position of a subset of the participants in Rock and Gutman's Experiment 1).

However, all of the above findings of early visual selection (along with any research that attempted to assess the processing of unattended information in terms of the participants' memories for that information) are open to the criticism, already mentioned in relation to the research on auditory selective attention, that the unattended information may have been perceived but then rapidly forgotten, rather than not having been perceived at all (Wolfe, 1999). Nevertheless, the recent development of brain imaging techniques has allowed researchers to assess the neural processing of unattended information more directly, thereby removing the need to rely on participants' memory for the information. The neuroimaging approach has also provided evidence in favor of early selection. For example, Rees et al. (1999) presented the participants in their study with a series of pictures, each one superimposed with a string of letters (which formed a familiar word on certain trials but consisted of a meaningless string of letters on others). Note that the use of superimposed visual stimuli in this experimental design is similar to that of Rock and Gutman (1981) described earlier. The participants in Rees et al.'s study had to detect immediate stimulus repetitions, either in the picture stream or in the letter stream. When the participants attended to the stream of letter stimuli, the neural activity elicited by the words differed from that elicited by the nonwords (i.e., the random letter strings). However, when the participants were attending to the pictures, the neural activity elicited by the words and nonwords did not differ significantly. This result suggests that the unattended words were not even being processed to the level at which they would have been differentiated from nonwords, despite the fact that the participants were looking directly at them (because they were presented at fixation). Thus it seems that unattended visual stimuli can be eliminated from processing at a relatively early stage, at least under the particular experimental conditions used by Rees et al.

It should, however, be noted that a more recent event-related potential (ERP) study by Ruz et al. (2005), based closely on the experimental design reported by Rees et al. (1999) in their functional magnetic resonance imaging (fMRI) study, found a somewhat different pattern of results. Specifically, they observed that the patterns of ERP components elicited by words and nonwords differed significantly, even when the letter stimuli were not attended. This result would appear to suggest that unattended stimuli can be processed to a semantic (and therefore relatively 'late') level after all. Given that the experimental designs used in the two studies were so similar, this difference in the pattern of results seems likely to be due to the differences in the relative sensitivity of fMRI and ERP techniques. Note, however, that Ruz et al. found that the nature of the word processing (as indicated by the differences in patterns of ERP components elicited by words and by nonwords) was different in the unattended and attended conditions. Thus, even though Ruz et al.'s results suggest that semantic processing of unattended stimuli can occur to some extent, they nevertheless suggest that unattended stimuli are processed differently (and presumably less thoroughly) than attended stimuli. The suggestion that unattended stimuli might in fact be processed to a semantic level of information processing is supported by a large amount of behavioral evidence, as described in the following section.

1.13.3 Behavioral Evidence for Late Selection

Despite the above evidence in favor of early selection, behavioral research has also provided much evidence to suggest that supposedly 'unattended' stimuli can in fact be processed to a degree beyond that of the simple registration of their physical characteristics. As with the evidence for early selection, these observations began with research on the dichotic listening paradigm. For example, Moray (1959) reported that many of the participants in his now classic study remembered hearing their own name when it was presented unexpectedly in the unattended channel (along with some instructions that followed the name in that channel). By contrast, the participants could not remember those same unattended instructions if they were not preceded by the participant's name (indeed, Moray demonstrated that participants could not even remember words that had actually been presented 35 times in the unattended stream).

In addition, other researchers found that participants could often be influenced implicitly by the information being presented in the unattended stream, even if they could not later recall it explicitly. For example, Mackay (1973) presented ambiguous sentences to the participant's attended ear (e.g., "they threw stones toward the bank yesterday"), along with disambiguating information to the unattended ear (e.g., 'river' or 'money'). A subsequent memory test (based this time on recognition of the attended information) suggested that the participants had resolved the meaning of the ambiguous sentences in line with the information presented to the unattended ear, despite being unable to recall the content of this stream explicitly.

Corteen and Dunn (1974; see also Corteen and Wood, 1972) also reported an elegant study in which they showed that people could process unattended stimuli to the level at which semantic information became available, without any explicit awareness of having done so. In particular, they conditioned their participants to expect a small electric shock upon hearing a city name. Then, during dichotic listening, they presented city names to the participant's unattended ear, with the additional instruction that participants should press a button every time they heard a city name. Less than 1% of the city names were explicitly detected by the participants. However more than 30% of the city names elicited a galvanic skin response (GSR), consistent with the sound of the city name triggering a small psychophysiological fear response, presumably linked with the expectation of the electric shock. This finding suggests that the information in the unattended ear had been processed to a semantic level. Importantly, GSRs were even found for city names that had not been presented during the training phase of the experiment, thus demonstrating that the unattended names had been processed to a level at which semantic generalization had become possible. Based on findings such as these, late selection theorists (e.g., Deutsch and Deutsch, 1963) proposed that information was processed to a semantic level before being filtered out of the processing stream.

This proposal soon received support from studies of visual selective attention, as significant behavioral evidence emerged to suggest that, in many cases, unattended visual stimuli could be processed to a deeper level than had at first been thought. For example, many studies have now shown that participants' responses can be primed by the semantic content of apparently ignored visual stimuli. For instance, Tipper (1985) presented participants with superimposed line drawings and asked them to attend to the drawing in one color and to ignore the drawing presented in the other color. The participants had to try and name the object represented by each of the attended pictures. Tipper reported that participants' responses to a particular attended stimulus were affected by the identity of the stimulus presented on the immediately preceding trial, whether or not that previous stimulus had been attended. For example, the participants were able to name a picture of a dog more rapidly if they had named a dog (or a semantically related item, such as a cat) on the preceding trial than if they had previously named a neutral stimulus (such as a chair). This effect is often referred to as positive priming. In contrast, if participants had previously ignored a picture of a dog (or a semantically related item), they were found to be slower to name the picture of the dog on a subsequent trial (as compared with having previously ignored a neutral stimulus) – a result that is now commonly referred to as negative priming. Thus, even though participants were unable to recall the unattended stimuli explicitly (as shown on occasional catch trials in which they were asked to report the unattended stimulus but frequently failed to do so), their performance was nevertheless influenced by these stimuli at a semantic level. This result suggests that unattended visual stimuli can often be processed to a

relatively advanced stage. In fact, Tipper (1985) suggested that the relatively poor subsequent recall of the unattended items in his study reflected the fact that the representation of these stimuli had been inhibited, following the relatively extensive processing necessary for producing priming effects (see also Tipper and Cranton, 1985; Tipper and Driver, 1988). Tipper went on to suggest that this inhibitory process might have been responsible for people's failures to remember the unattended information when subsequently tested (e.g., as found in the early dichotic listening studies). This view would appear to support an account of the early selection findings in terms of inattentional amnesia (aided by the inhibition of the representation of unattended stimuli), rather than inattentional blindness.

Another very popular task developed by cognitive psychologists in the attempt to determine the extent to which irrelevant distractors are processed has become known as the flanker task (Eriksen and Eriksen, 1974). Participants in a typical study are asked to respond to a central target letter, which is flanked by one or more distractor letters (which participants have to try and ignore). The distractor letters can be associated with responses that are congruent, incongruent, or neutral with respect to the target response. If the unattended distractors received no processing whatsoever, one would expect there to be no difference in target responses (in terms of participants' speeded response latencies or accuracy) as a function of the type of distractor that has been presented. If, however, the distractors are processed to some extent, then target performance would be expected to be better on congruent trials and worse on incongruent trials, as compared with performance on neutral trials. Thus, cognitive psychologists can infer the extent to which distractor stimuli are processed from the magnitude of any congruency effects observed in the target discrimination task (where congruency effects are usually calculated in terms of the impairment of performance seen on incongruent trials relative to that seen on the neutral or congruent trials). Eriksen and Eriksen's original study (along with many subsequent studies) demonstrated significant congruency effects due to the visual distractors, implying that, at least under certain conditions, the distractors could be processed to the level at which responses are programmed despite being irrelevant to the task.

The flanker paradigm has recently been extended to the auditory modality. Chan et al. (2005) presented participants with three simultaneous voices, each saying a different word from a different spatial location. On each trial, the participants had to make a speeded discrimination response regarding the identity of the word spoken by the central speaker, while trying to ignore the distracting words being spoken by the speakers situated to either side of the central speaker (at spatial separations varying between 30 and 90 degrees). Just as shown previously in the visual modality, the participants responded significantly more slowly (and less accurately) on the incongruent trials than on the congruent distractor trials, thus showing interference by task-irrelevant distractors in the auditory modality.

Generally speaking, there has been much less research on selective attention within the tactile modality than there has been in either vision or audition. However, this area of research has begun to grow over the last few years and is now attracting considerable empirical interest (see Johansen-Berg and Lloyd, 2000; Spence, 2002; Gallace and Spence, 2007) related, at least in part, to the very real practical implications that are now emerging in the area of tactile interface design (see Gallace et al., in press, for a recent review). For example, laboratory-based research has established tactile analogues of the Eriksen flanker paradigm already described. In one such study, Evans and Craig (1992) asked their participants to discriminate the direction of a moving tactile stimulus on one finger while ignoring a moving distractor presented to another finger. The participants responded more rapidly and more accurately when the direction of the distractor stimulus was congruent (vs. incongruent) with that of the target stimulus, suggesting that the tactile distractor was processed to a level at which it was able to influence responses, despite being clearly irrelevant to the task.

More recently, Soto-Faraco et al. (2004) developed a tactile response competition paradigm using static (rather than moving) tactile stimuli. The participants in their study had to discriminate the elevation of a series of continuous target vibrations (presented to either the top or bottom of a foam cube held in one hand) whilst trying to ignore pulsed distractor vibrations (presented to the other hand at an elevation that was either congruent or incongruent with that of the target stimulus). In line with the results of Evans and Craig's (1992) earlier study, Soto-Faraco et al. observed significantly better performance when the elevation of the vibrotactile distractor was congruent (vs. incongruent) with that of the tactile target, suggesting that the distractors

were processed despite being entirely irrelevant to the participants' task (see also Driver and Grossenbacher, 1996). Note that, although these distractor congruency effects were most pronounced when the target hand was unpredictable, the same pattern of results (although somewhat reduced in magnitude) was also found when the target hand was made predictable throughout a block of trials. Thus tactile distractors can interfere with the processing of tactile targets, even when targets and distractors are made clearly distinguishable from one another spatially (thus allowing for the focusing of a participant's selective attention on the target hand). This might be taken as preliminary evidence that attentional selection within the tactile modality occurs relatively late in information processing. Indeed, subsequent research by Soto-Faraco et al. found that the magnitude of the tactile interference effect was modulated both by actual changes in the separation between the participant's hands (implying a role for proprioception in guiding selection) and by illusory changes in hand separation, induced by a mirror illusion (implying a role for visual information prior to tactile selection). This suggests that tactile selective attention was operating upon a representation of the tactile stimuli that was derived subsequent to the multisensory integration of tactile, proprioceptive, and visual information. This finding supports the idea that tactile selection occurs relatively late in information processing. However, it should be noted that little of the research on tactile selective attention has been designed specifically to address questions of early versus late selection. More research on this question might therefore be useful in the future.

Taken together, there is now a considerable body of evidence from research in audition, vision, and touch to suggest that supposedly unattended information can in fact be processed beyond the level of the simple registration of its physical features. However, as was the case with the research taken to support early selection, these findings have been subject to several alternative interpretations. For example, one particularly important criticism relating to all of the experiments that have provided evidence in favor of late selection is that although participants are asked to ignore the irrelevant information (and presumably attempt to do as they have been instructed), they may nevertheless fail to ignore this information completely, despite their best intentions. Any observations of semantic processing of the unattended information could therefore reflect failures in attentional focus, rather than necessarily indicating extensive processing of truly unattended material (e.g., Holender, 1986).

Indeed, there is some evidence to suggest that, under certain conditions, the apparent processing of unattended information can be prevented when controls are taken to ensure that the unattended information remains genuinely unattended. For example, Lachter et al. (2004) asked the participants in their study to decide on each trial whether a target stimulus constituted a word or a nonword (this is often referred to as a lexical decision task). Each target was preceded by a prime word, which was presented very briefly and then 'masked' (i.e., replaced by other visual stimuli to ensure that no trace of the original visual stimulus remained). The prime word was either identical to the target word or unrelated to it. Participants in this type of experiment are typically faster and more accurate on the lexical decision task when the target and prime are identical, as compared with when they are unrelated (an effect known as repetition priming). However, Lachter et al. (2004) demonstrated that this effect could be eliminated if the participants were prevented from paying any attention to the prime stimuli (e.g., by presenting the primes at an unattended location and for a duration that was too short to allow a shift of attention to that location). This suggests, in line with earlier results (e.g., Rees et al., 1999), that genuinely unattended words can be eliminated from information processing at a reasonably early stage. Indeed, Lachter et al. put forward a robust defense of Broadbent's (1958) original model of early selection based on their data and on the argument (mentioned above) that all evidence in favor of late selection can actually be explained in terms of a process they term slippage, in which attention is inadvertently paid to task-irrelevant items (see also Wood and Cowan (1995a,b) for evidence to suggest that hearing one's own name in the unattended stream during dichotic listening might reflect the consequences of a temporary failure of selective attention, rather than the semantic processing of unattended information). It should be noted that to date, the findings to support Lachter et al.'s claims relate only to very specific experimental paradigms and conditions. It will be interesting to see whether the finding that late selection results can be eliminated by preventing slips of attention can be generalized more widely.

1.13.4 Neural Mechanisms of Selective Attention

The behavioral evidence outlined in the previous two sections suggests that, under some circumstances, unattended information can be eliminated from information processing at a very early stage (see also Driver, 2001, for a review). This idea has been supported by neuroscientific findings suggesting that spatial attention can modulate even the very early stages of neural information processing in the auditory, visual, and tactile modalities.

One important advantage of the neuroscientific approach (as compared to the cognitive psychological approach described earlier) is that it has allowed researchers to examine whether the activation of specific brain areas at identifiable points in particular hierarchies of information processing can be modulated by attention. This approach might therefore appear to be able to provide more concrete definitions of early and late selection effects than those provided by the cognitive approach (see Allport (1992) for a discussion of some of the problems faced by cognitive researchers in this regard). However, it is important to emphasize that the notion that information passes sequentially through strict neural processing hierarchies has little support these days. For example, it is now generally agreed that visual information processing involves feedback (or 'recurrent') processing, in which later areas feed information back to earlier areas (Lamme et al., 1998). Indeed, some researchers have argued that such recurrent processing is essential for visual awareness (Lamme, 2003). In addition, there is reliable evidence to suggest that different aspects of incoming information can be processed in parallel by the brain, as demonstrated in vision (e.g., see Goodale and Milner (1992) for a review of evidence to support the existence of two parallel processing streams – known as the 'ventral' and 'dorsal' streams – in the neural processing of visual information), in hearing (see Rauschecker and Tian (2000) and Alain et al. (2001) for suggestions that a similar distinction may also apply to the processing of auditory stimuli), and in touch (see Pons et al. (1992) for early work on the possible parallel processing of different aspects of tactile information).

Nevertheless, the mounting evidence that even primary sensory areas (through which it is agreed that all incoming information from that particular sensory modality must pass) can be subject to attentional modulation is often taken to support an early selection view. In addition, researchers have sought to demonstrate that attentional modulations can occur soon after stimulus onset, providing a definition of early selection in terms of processing time rather than brain region. Below we discuss the evidence for attentional modulations of visual, auditory, and tactile information processing at stages that are defined as early either in terms of brain area or in terms of processing time, or both. We then examine the contribution that these findings have made to the question of the extent to which unattended information is processed. Note that our discussion here is focused on the neural mechanisms of spatially selective attention (rather than attention to other, nonspatial stimulus attributes) simply because this is the area in which the majority of the research has been carried out.

1.13.4.1 Audition

There is now considerable evidence to suggest that spatial attention can affect the early stages of auditory information processing (see Giard et al. (2000) for a review). One fruitful line of research has used ERP recording to assess neural processing of auditory information. In a typical design, participants are asked to attend to auditory stimuli presented at a particular location while attempting to ignore task-irrelevant stimuli at another spatial location. ERPs elicited by stimuli presented at the attended location are then compared with the ERPs elicited by the same stimuli when that location is unattended. Although there is no physical difference between the stimuli, they typically elicit different ERP patterns even at relatively short latencies after stimulus presentation, thus suggesting that auditory selective attention can have an important effect even on early perceptual processes. Specifically, the amplitudes of certain early, sensory-related ERP components elicited by attended stimuli have been shown to be greater than those elicited by unattended stimuli, with these differences starting as early as 60–80 ms after stimulus presentation (Hillyard et al., 1973; Näätänen et al., 1978; Hansen and Hillyard, 1983). There is now even some evidence to suggest that, under certain conditions, these differences can occur as early as 20–50 ms after stimulus presentation (Hoormann et al., 2000).

Neuroimaging studies of spatially selective auditory attention have also demonstrated that paying attention to the location of auditory stimuli

(as compared with ignoring those very same stimuli) can activate areas of primary auditory cortex as well as temporal lobe auditory association areas (O'Leary et al., 1997; Alho et al., 1999). In line with the ERP research described above, this research implies that selective attention can have effects on relatively early stages of auditory information processing.

This assertion is further strengthened by studies using single-cell recording techniques in nonhuman primates, in which researchers have been able to measure the responses of individual neurons while an animal performs a particular task. This type of research has also demonstrated an attentional modulation of neuronal responses in auditory cortex (Benson and Hienz, 1978). Thus, overall, the neuroscientific research appears to agree that attentional modulations can occur very soon after stimulus onset and in brain areas that are involved in the early processing of auditory information.

1.13.4.2 Vision

There is also a consensus in the neuroscience community that spatial attention can modulate the early stages of processing of visual information. For example, O'Connor et al. (2002) carried out an fMRI study in which the participants either had to attend to a checkerboard stimulus or else attend to the other side of the screen (while the checkerboard remained present but was relatively unattended). Activity levels in the visual cortex (pooled in this case across several early visual processing areas, including primary visual cortex) were found to be higher in the attended condition than in the unattended condition. This result has been replicated frequently throughout the literature (see Kastner (2004) for a review). However, O'Connor et al. extended previous findings by demonstrating that this attentional modulation effect could also be found in the lateral geniculate nucleus (LGN) of the thalamus – a brain area that is known to be involved in the very early processing of visual information.

The finding that spatial attention can modulate brain areas that are involved in the early processing of visual information has been accompanied by findings of attentional modulations of certain visual ERP components soon after stimulus onset (for reviews see Mangun, 1995; Hillyard et al., 1998). Several studies have also combined brain imaging with ERP recording in order to exploit the high temporal resolution of ERP techniques as well as the relatively high spatial resolution of PET or fMRI. For example, Heinze et al. (1994) used positron emission tomography (PET) and ERP recording to demonstrate an attentional modulation of responses in certain areas of visual cortex within 80–130 ms of stimulus onset.

Similar findings have also emerged from studies of attentional function at the neuronal level using single-cell recording techniques in nonhuman primates. In fact, there is now reliable evidence to suggest that spatial attention can modulate the responses of individual neurons throughout the macaque visual system (see Treue (2001) for a review). However, findings relating to the issue of whether or not neural responses in primary visual cortex (V1) are subject to attentional modulation have, until fairly recently, been rather mixed (see Posner and Gilbert (1999) for a review). For example, Moran and Desimone (1985) and Luck et al. (1997) both failed to find any attentional modulation of the activity of V1 neurons, despite finding clear evidence for attentional effects occurring in other early visual areas (e.g., in V2 and V4). Nevertheless, there is now an increasing body of evidence that V1 neurons can be subject to attentional modulation, at least under certain conditions (see Treue (2001) for a review). For example, Roelfsema et al. (1998) devised a task in which their monkeys had to fixate a dot, which was then joined to one of two curves, each leading to another dot. The monkey's task was to make an eye movement to the dot that was joined by the curve originating from the fixation dot. Using this task, a curve passing through a particular area of the visual field could either be made a target (by connecting it to the fixation dot) or a distractor (by not connecting it). Thus, the same cell's receptive field (RF) could fall on the target curve in one trial and on the distractor curve in another trial. Recordings were taken from 45 neurons in the primary visual cortex of two monkeys. Overall, firing rates were higher when the RF fell on a target curve than when it fell on a distractor curve, indicating that neuronal responses in primary visual cortex could be modulated by the attentional demands of the monkey's task.

Given the variability of the results concerning the attentional modulation of V1, it is perhaps not so surprising that studies looking at the possible attentional modulation of LGN neurons have also reported mixed results. Although there is some limited evidence to suggest that the activity of neurons within the LGN can be modulated by attention (Vanduffel et al., 2000), the few single-cell recording

studies in this area have usually failed to find attentional effects at this very early level of information processing (e.g., Mehta et al., 2000; Bender and Youakim, 2001). Nevertheless, taken together, the findings from visual neuroscience studies in humans and nonhuman primates appear to agree that the attentional modulation of neural processing can occur in brain areas involved in the early stages of visual information processing and within around 100 ms of stimulus onset.

1.13.4.3 Touch

Just as we have seen to be the case for both audition and vision, the neuroscientific evidence also suggests that attention can affect the early stages of the neural processing of tactile information (see Johansen-Berg and Lloyd (2000) for a review). For example, researchers have demonstrated attentional modulations of ERP components relating to tactile perception as early as 80 ms after the onset of a tactile stimulus (Eimer and Forster, 2003).

Similarly, attentional effects in primary somatosensory cortex (S1) have been demonstrated in humans using fMRI (Johansen-Berg et al., 2000) and PET (Meyer et al., 1991). Note, however, that these results might depend on the exact parameters of the experimental design used, as other studies have failed to demonstrate clear attentional effects on activity in S1 (Mima et al., 1998; Burton et al., 1999), although these studies have nevertheless often demonstrated attentional modulation of other (later) somatosensory areas (e.g., S2).

This overall pattern of results is supported by a small number of single-cell recording studies demonstrating a significant attentional modulation of neuronal responses in primary somatosensory cortex (Hyvarinen et al., 1980; Hsiao et al., 1993). Thus, although there has been less research performed on the neural mechanisms of tactile selective attention than there has been on the mechanisms of visual and auditory attention, the available research appears to agree that attention can modulate the early stages of tactile information processing.

1.13.4.4 Implications for the Early versus Late Debate

Overall, there is a significant body of evidence to suggest that attentional modulation of visual, auditory, and tactile information can occur in brain areas involved with early information processing and at times very soon after stimulus onset (see also Driver and Frackowiak (2001) for a review). However, the fact that the brain differentiates between attended and unattended inputs at an early stage of processing does not necessarily mean that unattended information is ruled out of processing altogether at that stage. Instead, these results simply demonstrate that attended and unattended stimuli are differentiated from this stage of processing onward. In fact, most of the studies described above demonstrated an attenuated response to unattended stimuli, rather than no response at all. Nevertheless, the research might suggest that unattended stimuli become less and less well represented as processing proceeds. For example, the extent to which attention can modulate neuronal activity has been seen to increase throughout the visual system, such that the attentional modulations of activity in V1 are typically smaller in magnitude than those elicited by the same experimental manipulation to activity in a higher visual area such as, for example, V4 (Kastner et al., 1998; Martinez et al., 1999; Mehta et al., 2000; O'Connor et al., 2002; Vibell et al., 2007). Recall that the research looking at possible attentional modulations of the neural processing of tactile information also found more reliable modulations in secondary somatosensory cortex than in primary somatosensory cortex (Mima et al., 1998). Such results might be taken to suggest that the neural representation of unattended stimuli is gradually reduced as processing progresses, rather than unattended stimuli necessarily being completely eliminated at one fixed point in information processing.

This suggestion would fit with the biased competition model of visual selective attention put forward by Desimone and Duncan (1995; reviewed in Duncan, 2006). According to this model, neuronal activity can be biased in favor of those neurons that respond to a particular attended stimulus. This initial prioritization of selected neurons gives them an advantage in their subsequent interactions with neurons that are not preferentially activated (because they do not respond to the attended stimulus). Thus, according to this model, unattended information would not be ruled out of processing at a particular point but would instead be subject to a continued bias against it throughout processing. This intermediate position is echoed in recent proposals for a resolution to the early versus late selection debate, as outlined here.

1.13.5 Possible Resolutions to the Debate

The studies reviewed so far all agree that, at some stage, attended information is processed with a higher priority than is unattended information. However, many of these studies differ in their estimates of the effects that this process of prioritization might have on the level to which the unattended information is processed. In contrast to the extreme positions espoused by the early selection theorists (e.g., Broadbent, 1958) and the late selection theorists (e.g., Deutsch and Deutsch, 1963), several researchers noted the variability of the findings and proposed compromise positions (see Shulman (1990) for a discussion of the dangers of failing to acknowledge the extent of the variability of the results in this research area). For example, Treisman (1960) proposed that unattended information was attenuated early in processing, rather than being filtered out completely. Her model accommodated the findings concerning the limited processing of unattended stimuli but also allowed for the possibility of further processing of this information if it were sufficiently salient (in which case, according to the model, it would be able to reach consciousness despite having been attenuated, because the thresholds for activating salient information were lower than for other information). This proposal of variable thresholds for triggering the processing of incoming information allowed for different participants to have different threshold levels for different types of information. Treisman's model was therefore capable of accounting for the observations of individual differences in the efficiency of selective attention, as described earlier (recall, for example, that Moray (1959) found that, although a subset of his participants noticed their own names when they were presented in the unattended ear, a significant number of participants did not). However, whereas these ideas relate mainly to the nature of the irrelevant information, subsequent research has indicated that the nature of the relevant information can also play a part in determining the amount of processing that unattended stimuli receive.

For example, Lavie (1995) reinvigorated the early/late selection debate by putting forward the suggestion that selection could occur either early or late in information processing, depending on the perceptual load of the relevant task (i.e., the demands placed on the perceptual system by that task; see Johnston and Heinz (1978) for an earlier hybrid model of selection). Lavie argued that the perceptual system has a limited capacity, but that all stimuli are automatically processed unless that capacity is exceeded. According to this view, selection can only occur at an early stage of information processing if the relevant task uses up all of the available processing capacity (i.e., under conditions of high perceptual load). If the perceptual load of the task is insufficient to exhaust the processing capacity, then distractors will be processed automatically, and selection will be seen to occur at a later stage of information processing.

In order to provide a direct test of this theory, Lavie (1995) assessed the performance of participants on a response competition task similar to that designed by Eriksen and Eriksen (1974; and described in the section titled 'Behavioral evidence for late selection'). Distractor interference was measured in terms of distractor congruency effects (in which responses are typically shown to be slower when distractors are associated with responses that are incongruent, vs. congruent, with the target response). Lavie also manipulated the demands of the target task, such that the perceptual load was either high (e.g., searching for an N among several other letters) or low (e.g., searching for an N among Os). Lavie reported that the participants were able to ignore the distractors more effectively (implying early selection) under conditions of high perceptual load than under conditions of low perceptual load (implying later selection in this case).

The idea that the stage of processing at which selection occurs can vary depending on the perceptual load of the relevant task has now been supported by a number of subsequent studies (Lavie, 2000; 2005). For example, Rees et al. (1997) carried out an fMRI study in which the participants viewed a peripheral display of moving dots that was irrelevant to a word-based task presented at fixation. Neural activity in motion areas MT/MST was reduced when the word-based task was highly demanding (i.e., identifying words containing two syllables) as compared to when it was less demanding (i.e., identifying words written in uppercase vs. in lowercase letters). This suggests, in line with perceptual load theory, that the neural processing of the irrelevant information was modulated by the perceptual demands of the relevant task. Similar effects have now been shown as early in visual processing as the LGN (O'Connor et al., 2002).

Given the wide range of experimental evidence now supporting perceptual load theory, it is often considered to have provided something of a resolution to the long-running early/late selection debate (although see Eltiti et al. (2005) for a possible alternative account of certain findings in terms of distractor salience). However, it is important to note that, as yet, no independent measure of perceptual load has been provided. Instead, researchers have had to rely on operational definitions, based on observations that a given manipulation of perceptual load worked in a given experimental setting. (Note that this raises particular problems when trying to interpret the results of studies where a manipulation of perceptual load is found to have no effect on distractor processing.)

Another possible limitation of the research in this area so far is that it has concentrated almost exclusively on perceptual load within the visual modality (although see Alain and Izenberg (2003) for evidence that ERP components elicited by deviant – yet task-irrelevant – auditory stimuli can be reduced under a high (vs. low) perceptual load in a relevant auditory task). Given that this line of investigation began with experiments looking at audition (with the dichotic listening paradigm) and has since expanded to look at attentional processes within the somatosensory modality, it would seem important to test the predictions of the perceptual load theory within those sensory modalities, as well as between combinations of different sensory modalities.

Despite a relative lack of unimodal studies of perceptual load effects in audition and touch, there have been a small number of recent studies that have investigated the possibility of crossmodal perceptual load effects. One possible advantage of this line of investigation is that it might provide a test of whether the limited perceptual capacity described within the perceptual load theory is modality specific (Wickens, 1984, 1992) or whether instead it consists of a common pool of processing resources that is shared between the different sensory modalities.

There is some variability in the results of the small amount of research published on this question so far, with some studies having failed to find reliable crossmodal effects of perceptual load (Rees et al., 2001; Tellinghuisen and Nowak, 2003), while others have found some suggestion of crossmodal effects (e.g., Otten et al., 2000). This variation in the results of the studies of crossmodal perceptual load might be related to the lack of an independent measure of the extent of the load imposed on the perceptual system by a particular task. It could, for example, be argued that any failures to demonstrate significant crossmodal perceptual load effects may have been due to the studies in question simply using too weak a manipulation of perceptual load. For example, the auditory discrimination of the number of syllables, used in a crossmodal study by Rees et al. (2001), is likely to have been much easier than the discrimination of the number of syllables in words presented visually, which was used in their successful intramodal study of perceptual load in vision (Rees et al., 1997). Given these limitations of the research to date, it would seem premature to draw firm conclusions at the present time, especially given the general variability in the literature on the question of whether or not attentional resources are shared between the different sensory modalities (e.g., see Treisman and Davies, 1973; Martin, 1980; Spence et al., 2001; Soto-Faraco et al., 2002; Arnell, 2006, for successful demonstrations of crossmodal competition for attention; but see Duncan et al., 1997; Soto-Faraco and Spence, 2002; and Alais et al, 2006, for a failure to find any such crossmodal competition under slightly different experimental conditions).

Earlier, we outlined how the neuroscientific evidence appears to support a compromise position, suggesting that unattended stimuli might be gradually filtered out throughout information processing, rather than being completely eliminated from processing at one fixed point in the system. This suggestion from neuroscience, along with the behavioral evidence that unattended information receives more or less processing depending on the specific perceptual demands of the attended task (e.g., Lavie, 1995), would appear to agree that there is no fixed point at which unattended information is excluded from processing, but rather that the prioritization of processing is flexible, depending on the salience of the stimuli involved, the particular demands of the task at hand, and the current goals of the observer.

1.13.6 Working Memory and the Locus of Selection

There is also evidence to suggest that the extent to which unattended information is processed can vary from participant to participant. Recall, for example, that only one in three of Moray's (1959) participants noticed their own names being presented in the unattended stream. There is now evidence to suggest

that this interparticipant variability might be related to individual differences in working memory capacity. For example, Conway et al. (2001) have shown that participants with lower working memory capacities, as assessed by their performance on the operation span task, in which participants are asked to remember short lists of words while carrying out mathematical operations (Turner and Engle, 1989), are more likely to notice their own name in the unattended stream in a dichotic listening experiment than participants with higher working memory capabilities. This finding suggests that working memory plays a key role in controlling attentional allocation, such that people with lower working memory capacities find it harder to control the deployment of their attentional resources. Note that such an interpretation might also imply, in line with the proposals of Holender (1986) and Lachter et al. (2004) mentioned earlier, that the semantic processing necessary to recognize one's own name in the unattended stream occurs as a result of the unintentional allocation of attention toward that stream (because if it simply reflected true semantic processing of all unattended information, there would have been no effect of individual working memory capacity on the likelihood of noticing the name). We note, however, that because Conway et al.'s study is correlational in nature, it cannot demonstrate a causal role for working memory in successful auditory selective attention (as it is also possible that participants' attentional abilities determined their performance on both tasks).

Nevertheless, further evidence has recently begun to emerge to support the suggestion of a causal role for working memory in control of selective attention, at least in the visual modality (De Fockert et al., 2001; Lavie et al., 2004). For example, Lavie et al. (2004) asked the participants in their study to respond to the identity of a target letter (X or Z) while ignoring a concurrently presented distractor letter, which could either be congruent with respect to the target letter (e.g., an X when the target was also an X) or incongruent (e.g., a Z when the target was an X). The participants carried out this task under conditions of either high working memory load (where they were asked to remember six randomly chosen digits) or low working memory load (where they only had to remember one digit). Incongruent distractors produced greater interference (by comparison with congruent distractors) under conditions of high working memory load than under low load conditions, suggesting that working memory availability is important for minimizing interference by irrelevant stimuli, presumably through the active maintenance of current stimulus-processing priorities (see Lavie, 2005).

The latest research to emerge from our laboratory has suggested that working memory availability might also be important for the successful control of tactile selective attention. Dalton et al. (2006) asked the participants in their study to carry out a tactile response competition task similar to that used by Soto-Faraco et al. (2004), described earlier, under conditions of either high working memory load (in which the participants were asked to remember six randomly chosen digits) or low working memory load (in which the memory set always consisted of the digits 1–6 presented in ascending numerical order). In line with the results of the visual studies carried out by Lavie et al. (2004), Dalton et al. found that distractor congruency effects within the tactile modality were significantly larger under high working memory load than under low load, suggesting that working memory is also important for the control of tactile selective attention. Similar results have also emerged from a very recent study of the role of working memory in auditory selective attention (Dalton, Santangelo, and Spence, in preparation).

1.13.7 Summary

We have considered visual, auditory, and tactile research that has addressed the question of the extent to which unattended information is processed. While certain studies reported that participants are able to recall very little about information they had ignored (e.g., Cherry, 1953; Rees et al., 1999), other studies found evidence suggesting that supposedly ignored information had in fact been processed to a relatively late stage, involving semantic processing and/or response selection (e.g., Corteen and Dunn, 1974; Ruz et al., 2005). The emergence of these conflicting findings has led to a long-lasting debate in the literature over the exact stage of processing at which unattended information is excluded.

Proponents of early selection (e.g., Broadbent, 1958; Lachter et al., 2004) have argued that unattended information is filtered out early on in information processing, on the basis of simple physical characteristics. By contrast, late selection theorists (e.g., Deutsch and Deutsch, 1963) proposed that information was processed to a much deeper level before being excluded from the processing stream.

More recently, many researchers have converged on a more flexible position, in which the locus of selection can vary according to the task demands (Lavie, 2005), the salience of the stimuli (Eltiti et al., 2004), and the individual characteristics of the participant (Conway et al., 2001). When deeper processing of unattended stimuli is observed, it seems likely that it results from some level of attentional allocation toward the unattended stimuli, either due to inadvertent slips of attentional focus (e.g., Holender, 1986; Lachter et al., 2004) or due to automatic processing of irrelevant information under conditions of low perceptual load (e.g., Lavie, 2005). Research in recent years has also identified a key role for working memory in the control of selective attention, such that selection is more effective when working memory resources are available for the attention task than when they are not (e.g., Lavie et al., 2004).

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1.14 Amnesia: Point and Counterpoint

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1.14.1 Introduction

In 1953, a man known by his initials, H.M., underwent an experimental brain operation to relieve his intractable epilepsy. This bilateral removal of medial temporal lobe (MTL) structures (see Corkin et al., 1997, for detailed report of his lesion location) was successful in lessening the frequency and severity of his epileptic seizures; however, it left him with a profound anterograde amnesia and an extensive retrograde amnesia extending to at least a decade before his surgery (Corkin, 1984; Scoville and Milner, 1957). Although H.M.'s intellectual abilities remained intact after the operation, and he was able to acquire new skills, he has not been able to consciously record any new experiences (see **Figure 1**; Corkin, 2002 for review).

H.M.'s pattern of spared and impaired abilities indicated not only that declarative (explicit, conscious) memory is dissociable from other forms of memory (e.g., implicit, unconscious) and from other perceptual and cognitive processes but also that the hippocampus and other MTL structures play a role in declarative, but not nondeclarative, memory formation (Scoville and Milner, 1957; Milner, 1962; Corkin, 1968, 1984, 2002). These realizations have spurred decades of research focusing on the role of MTL structures in learning and memory. Although initial studies focused primarily on the importance of the hippocampus in episodic memory, subsequent research has demonstrated that the surrounding cortical regions also are essential for normal declarative memory performance. Thus, patients with damage limited to the hippocampus tend not to show the magnitude of amnesia demonstrated in patients with more extensive MTL damage (e.g., Mishkin, 1978; Zola-Morgan and Squire, 1986; Meunier et al., 1993; Zola-Morgan et al., 1993; Barense et al., 2005). While these findings suggest that regions outside of the hippocampus proper contribute to declarative memory, the extent to which the different MTL structures make divergent contributions to learning and memory – and the nature of those regional specializations is hotly debated.

This chapter begins with a description of the anatomy of the MTL. We then discuss three ongoing debates concerning the role of different MTL

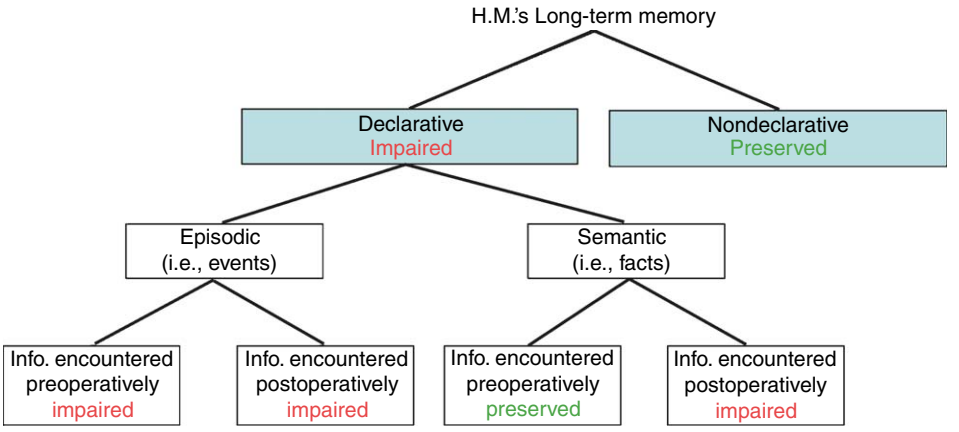

Figure 1 Pattern of spared and impaired learning following MTL resection in the amnesic patient H.M. Nondeclarative (implicit) learning is preserved in H.M., whereas declarative learning is markedly impaired. He does, however, show evidence of residual declarative learning. For example, he sometimes has demonstrated normal recognition memory performance (Freed et al., 1987; Freed and Corkin, 1988) and also has provided some evidence of new semantic learning following his operation (Corkin, 2002; O'Kane et al., 2004; Skotko et al., 2004).

structures in episodic memory. We focus on debates that have arisen from studies of patients with amnesia and from animal models of amnesia, but we also incorporate broader evidence from neuropsychology and neuroimaging.

In the first section, we describe a controversy regarding the extent to which MTL structures function together as an inseparable network that guides learning and memory versus the extent to which dissociations exist among the functioning of the different regions. At the heart of this debate is the question of whether the hippocampus performs specialized memory functions, or whether it carries out mnemonic functions redundant with those of the surrounding MTL cortices. Although some research has focused on examining whether the hippocampus plays a specialized role in spatial memory (e.g., Jarrard, 1995; O'Keefe, 1999; Aggleton and Brown, 2005; Eacott and Gaffan, 2005; O'Keefe and Burgess, 2005; *See also* Chapters 1.23, 1.33), we focus here on a controversy over the extent to which memory for interitem associations and individual items are differentially supported by the hippocampus proper and the surrounding medial temporal cortices, respectively.

In the second section, we outline a debate as to whether the perirhinal cortex plays a circumscribed role in object memory or a broader role in object perception and discrimination. According to some authors, perirhinal cortex is dedicated to memory and does not contribute to stimulus perception (e.g., Buffalo et al., 1999). In this view, stimulus perception is dependent entirely on earlier cortical areas that make up the ventral processing stream. In contrast, other researchers have provided evidence that the perirhinal cortex may not be dedicated exclusively to mnemonic processing but, rather, may support performance on some perceptual discrimination tasks as well (e.g., Eacott et al., 1994; Buckley and Gaffan, 1997; *See* Chapter 1.08).

In the third section, we discuss the pattern of retrograde amnesia following MTL damage. According to one account of memory consolidation (often referred to as the standard model; Alvarez and Squire, 1994; Squire and Alvarez, 1995; Squire and Zola, 1998; Squire et al., 2004), MTL structures play a time-limited role in declarative memory: They are essential for the initial storage and retrieval of semantic and episodic memory traces, but they become unnecessary once memory traces are established permanently in the neocortex. According to this model, following MTL lesions, semantic and episodic memory will be affected equally, and each will show a temporal gradient, with remote memories relatively preserved.

An alternative model of consolidation (termed the multiple trace model; Nadel and Moscovitch, 1997; Moscovitch and Nadel, 1998; Nadel et al., 2000; *See* Chapter 1.04) proposes that the MTL serves as a mnemonic index, or as a pointer, to the neocortical areas that store the sensory and affective qualities associated with episodic events. Each time a memory is retrieved, a new mnemonic index, or pointer, is created. According to this model, a pointer always is needed for retrieval of episodic detail; thus, MTL

structures provide lasting support during the retrieval of episodic memories. A temporal gradient could occur with partial MTL lesions, however, because remote memories tend to have more pointers than recent ones (because, on average, remote memories have been retrieved more often than recent memories). Thus, a partial MTL lesion is more likely to damage all pointers for a recent memory than for a remote memory. The multiple trace theory postulates that retrieval of semantic memories, unlike episodic memories, can occur without the MTL pointer. Because these memories are void of episodic detail, they may become consolidated and independent of the MTL.

Both models, therefore, make the same prediction for retrieval of semantic memories in amnesia: Semantic retrieval should show a temporal gradient, with better memory for remote than for recent facts. In contrast, the models make divergent predictions for the retrieval of autobiographical information. The standard model predicts that retrieval of remote memories should be preserved, whereas the multiple trace model predicts that all autobiographical retrieval should be impaired. We focus on the questions of whether amnesic patients are able to recall autobiographical memories from time periods prior to the onset of their MTL injury and, if so, whether the remote memories retrieved by amnesic patients are qualitatively the same as those retrieved by healthy individuals.

In each of these sections, we present key evidence supporting each viewpoint. We also speculate on the methodological considerations that may allow future research to help resolve these controversies. Although a consensus concerning these issues is still far in the future, the following sections highlight the contribution that studies of amnesia have made to conceptualizations of memory processes and MTL function.

1.14.2 Anatomy of the Medial Temporal Lobe

The MTL is composed of multiple structures: the amygdaloid complex, hippocampal formation (composed of the dentate gyrus, hippocampus, subicular complex, and entorhinal cortex), perirhinal cortex, and parahippocampal cortex (Suzuki and Amaral, 2003, 2004; **Figure 2**). The amygdala, an almond-shaped collection of nuclei, sits at the most anterior portion of the MTL, with direct connections to the hippocampus and surrounding cortices (Stefanacci et al., 1996). The hippocampus (so-named for its sea horse shape) is located posterior to the amygdala. Information flow through the hippocampal formation proceeds from the entorhinal cortex to the dentate gyrus through the CA fields of the hippocampus proper and then to the subiculum. The entorhinal cortex (Brodmann's areas 28 and 34), located in the anterior and medial portion of the temporal lobe (medial to the rhinal fissure), serves as the principal source of input into the hippocampus, via the perforant path (Amaral et al., 1984; Amaral and Insausti, 1990). Information from unimodal and polymodal association cortices enters the MTL mainly through the perirhinal and parahippocampal cortices. The perirhinal cortex (Brodmann's areas 35 and 36) is located in the anterior and medial portion of the ventral temporal lobe, lateral to the entorhinal cortex. Posterior to the perirhinal cortex lies the parahippocampal cortex (von Economo's areas TH and TF), lining the banks of the collateral sulcus (which separates the fusiform and parahippocampal gyri) (Van Hoesen, 1995; Amaral, 1999; Pruessner et al., 2002; Suzuki and Amaral, 2003). The perirhinal cortex receives prominent input from lateral inferotemporal cortex (unimodal visual areas) and from the polymodal parahippocampal cortex (for a detailed review of the anatomical connectivity of perirhinal cortex, see Lavenex and Amaral, 2000). The parahippocampal cortex, caudal to the perirhinal cortex, receives its strongest input from the polymodal dorsal visuospatial processing stream (Goldman-Rakic et al., 1984; Selemon and Goldman-Rakic, 1988; Cavada and Goldman-Rakic, 1989; Suzuki and Amaral, 1994). Both the perirhinal and parahippocampal cortices send strong projections to the entorhinal cortex (**Figure 2**).

1.14.3 Do Medial Temporal Lobe Structures Have Dissociable Roles?

Although H.M.'s MTL lesion is large, his resultant amnesia originally was believed to be the result of damage to the amygdala and the hippocampus (e.g., Mishkin, 1978). For this reason, early animal models of amnesia focused primarily on the role of these structures in declarative memory. Early work, using aspiration lesions, indicated that damage to the hippocampus alone was sufficient to produce severe deficits in declarative memory (e.g., Zola-Morgan et al., 1982; Zola-Morgan and Squire, 1986), lending credence to the idea that these structures were

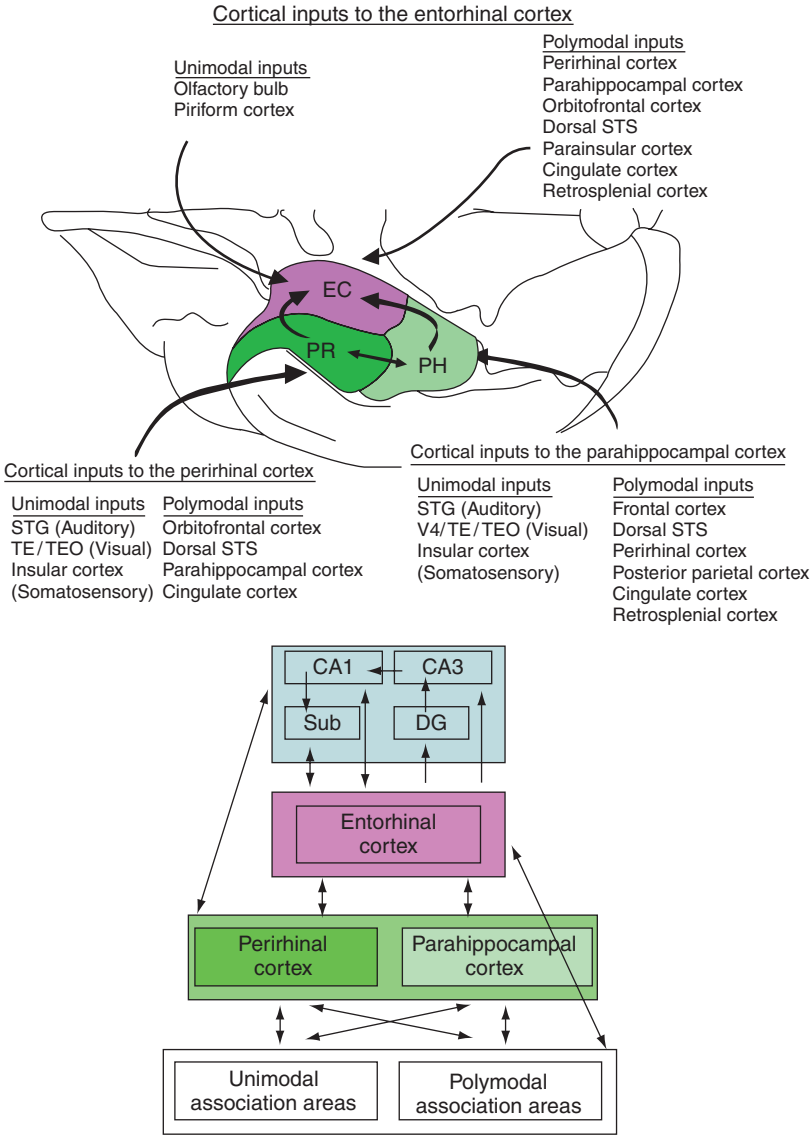

Figure 2 A schematic of MTL anatomy (top panel) and of projections within the medial temporal lobe (bottom panel). Colors reflect levels of integration within the MTL (bottom panel; green = first level of integration, pink = second level, blue = third level). Adapted from Lavenex P and Amaral DG (2000) Hippocampal-neocortical interaction: A hierarchy of associativity. *Hippocampus* 10: 420–430.

critical for declarative learning. It soon became apparent, however, that this view was too simplified: Several studies reported that recognition memory was impaired in animals with lesions that spared the amygdala or the hippocampus (e.g., Mahut et al., 1982; Murray and Mishkin, 1986; Zola-Morgan and Squire, 1986; Zola-Morgan et al., 1989). Researchers soon realized that part of the reason for the extensive declarative memory deficits following simultaneous aspiration lesions to the amygdala and hippocampus stemmed not from direct damage to those regions but, rather, from the resultant damage to the underlying rhinal cortex and to the efferents to the hippocampus. Subsequent animal research using selective lesioning techniques and electrophysiological methods has confirmed that other regions, including the perirhinal and parahippocampal cortices, also play an important role in declarative memory formation (Zola-Morgan et al., 1989; Otto and Eichenbaum, 1992; Meunier et al., 1993; Mumby and Pinel, 1994; Squire et al., 2004). Studies in monkeys and rats have shown that damage limited to perirhinal cortex, or

including perirhinal and entorhinal cortices, can lead to severe memory impairment on recognition memory tasks (Meunier et al., 1993; Eacott et al., 1994; Buffalo et al., 1999), and that such lesions also can exacerbate the memory deficits seen following lesions restricted to the hippocampus (Zola-Morgan et al., 1993; Wiig and Bilkey, 1995).

These results emphasize that MTL regions beyond the hippocampus play an essential role in declarative learning and memory. They do not, however, clarify the extent to which the different MTL structures make specialized contributions to learning and memory. It is clear that not all MTL structures support the same mnemonic functions. For example, although debate occurred for decades regarding the amygdala's role in memory, its focal role in emotion-related modulation of memory now has been well-established (Kensinger, 2004; Phelps, 2004; Phelps and LeDoux, 2005). In contrast, less consensus exists on the specialized contributions of other MTL structures to declarative memory.

In this section, we focus on a debate regarding the extent to which the hippocampus proper supports mnemonic functions that are independent of those mediated by surrounding neocortical regions. At the core of this debate is the question of whether recognition memory for individual items is dependent on the integrity of the hippocampus, or whether the hippocampus plays a specialized role in the recollection of interitem associations (relational memory), while memory for individual items (and intraitem features) can be mediated by adjacent cortical regions, such as perirhinal cortex.

1.14.3.1 Point: The Hippocampus Supports Mnemonic Functions That Are Independent of Those Mediated by Adjacent Neocortical Regions

If all MTL structures contribute equally to all forms of declarative learning, then it should follow that amnesic patients with MTL lesions should show similar magnitudes of impairment across a range of declarative memory tasks. Under certain encoding and retrieval conditions, however, amnesic patients show surprisingly good recognition memory coupled with poor recall ability (e.g., Johnson and Kim, 1985; Freed et al., 1987; Freed and Corkin, 1988; Green and Kopelman, 2002). For example, after studying a series of indoor and outdoor scenes, H.M. showed normal forced-choice recognition at delay intervals ranging from 10 min to 6 months (Freed et al., 1987; Freed and Corkin, 1988; **Figure 3**). In contrast, H.M.'s ability to recall items (or item locations) is at chance (e.g., Smith, 1988; reviewed in Corkin, 2002). Amnesic patients' rate of forgetting also differs based on the

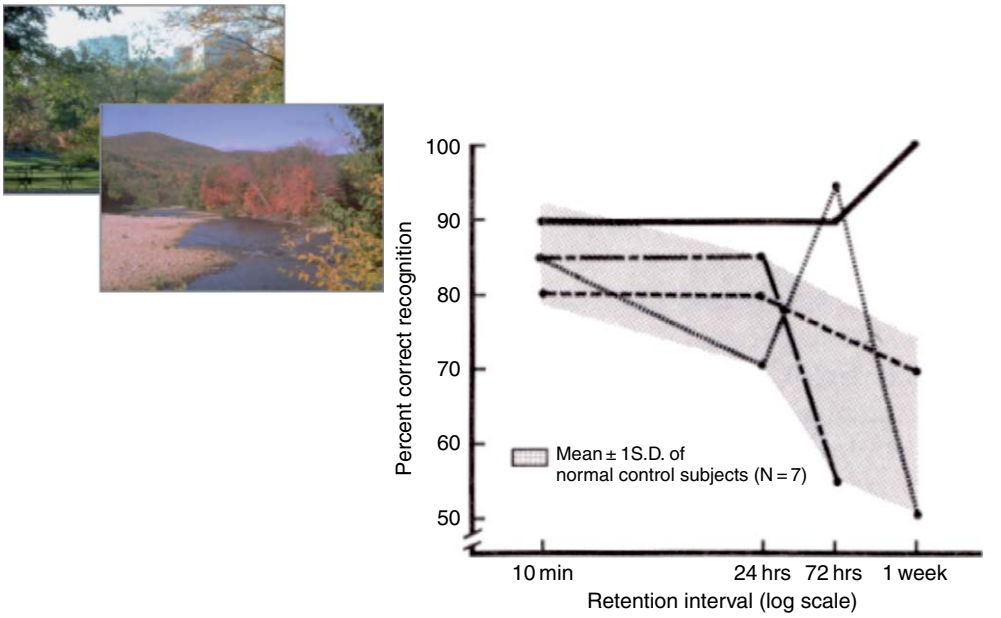

Figure 3 H.M.'s recognition memory performance. H.M. was within the normal range when asked to distinguish presented from nonpresented visual scenes. These results provide evidence of preserved learning despite his large MTL lesion. Modified from Freed DM, Corkin S, and Cohen NJ (1987) Forgetting in HM: A second look. *Neuropsychologia* 25: 461–471.

retrieval demands of the task. Recognition tasks often reveal normal rates of forgetting (Freed et al., 1987; Green and Kopelman, 2002), whereas recall tasks elicit accelerated forgetting (Green and Kopelman, 2002).

Direct comparisons between recall and recognition performance can be difficult to make, however. The two memory measures often are not matched in difficulty (recognition tasks typically are easier than recall tasks), and they always differ in their scales of measurement (with recognition tasks measuring percentage correct and recall assessing the number of items generated). To circumvent these problems, researchers often try to equate amnesic patients and control participants on recognition memory performance (e.g., by giving amnesic patients additional repetitions of studied stimuli, or by lengthening the delay after which the control participants' memory is tested), and then to assess whether, under these conditions of equated recognition, recall performance also is matched.

The initial reports using this approach revealed inconsistent results. Some investigations showed that when recognition was equated between amnesic patients and control participants, the patients' recall remained impaired (Hirst et al., 1986, 1988). Other studies, however, found that once patients' recognition memory was matched to that of control participants, recall memory was equated as well (Shimamura and Squire, 1988; Haist et al., 1992; MacAndrew et al., 1994; Kopelman and Stanhope, 1998). A study by Giovanello and Verfaellie (2001) has shed light on at least part of the reason for these inconsistencies: The relation between amnesic patients' recall and recognition performance depends, at least in part, on the method by which their recognition performance is matched to that of controls. In one experiment, Giovanello and Verfaellie (2001) tested amnesic and control participants after the same delay, but they provided amnesic patients with additional study exposures to equate their recognition memory. In a second experiment, amnesic patients' memory performance was matched with control participants' performance by testing the amnesic patients after a short delay and the control participants after a 24-h delay. These two methods for equating performance led to different patterns of results. In the first experiment, amnesic patients' recall remained impaired, even though their recognition memory was equated. In the second experiment, recall and recognition both were equated across the amnesic and control groups.

At first blush, these findings are perplexing: Why would changing the method used to match amnesic and control participants' memory performance alter the pattern of memory preservation in amnesic patients? To understand this pattern of results, consider that different processes can contribute to a correct endorsement on a recognition memory task (Mandler, 1980; Tulving, 1985; **Figure 4**). On the one hand, a participant can recollect information from the

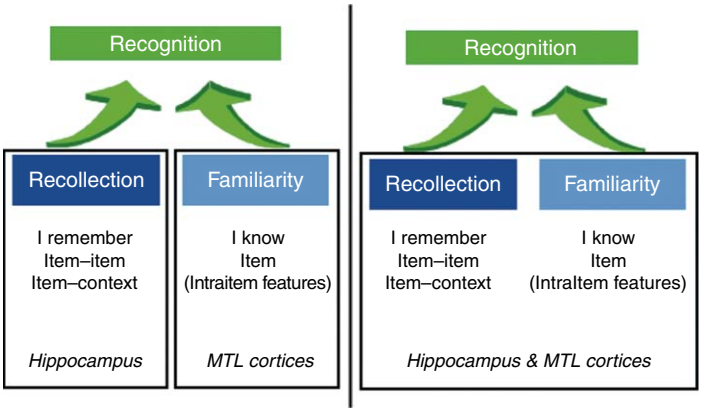

Figure 4 Processes contributing to recognition performance. When presented with an item on a recognition task, participants can use recollection to make their decision (i.e., they can determine that an item was studied because they remember something particular about the encoding episode for that item). Participants also can rely on stimulus familiarity: If a particular item seems more familiar than some other items on the list, it is likely that the enhanced familiarity stems from the fact that the item was presented on a recent study list. Debate exists as to whether these two processes are supported by different MTL circuits (with the hippocampus playing a critical role in recollection and the surrounding perirhinal and parahippocampal cortices being sufficient to support familiarity – left panel) or by the same MTL processes (with all regions of the MTL contributing to both recollection and familiarity – right panel).

study episode: Upon seeing a picture of a meadow on the recognition task, she may remember thinking about how peaceful the scene made her feel, she may recall sneezing while the picture was on the screen, or she may remember that she thought the picture of the meadow was juxtaposed with the prior picture of a crowded city street. Memory for any of this contextual information would allow the participant to confidently endorse the meadow as previously studied. On the other hand, a participant also can correctly endorse the meadow in the absence of any recollection, so long as she has a feeling of stimulus familiarity: If the meadow seems more familiar than other items that have appeared on the recognition task, it is likely that the enhanced familiarity has resulted from a recent encounter with the stimulus (i.e., that the meadow was present on the study list). In contrast, on a recall task, participants cannot rely on stimulus familiarity; they must generate the information themselves, and they therefore must be able to recollect the information and to attribute it to the study episode.

Keeping in mind that these two different processes can contribute to recognition performance, but that only recollective processes support accurate recall, let us return to the two different methods used to equate amnesic patients' performance. In the first experiment, Giovanello and Verfaellie (2001) equated recognition memory by altering the number of study repetitions (one for controls and many for amnesic patients) and by testing memory after a short delay. The fact that the two groups could be equated on recognition, but not on recall, can be explained by considering that control participants' recognition performance was likely to be supported primarily by recollective processes. The multiple item repetitions for the amnesic patients, in contrast, may have primarily boosted familiarity-based processes. Thus, while amnesic and control participants showed comparable recognition memory performance, that performance was supported by different processes in the two groups (more reliance on recollection in controls, and more reliance on familiarity in the amnesic patients). On a recall task, however, in which only recollection and not familiarity can support accurate retrieval, control participants performed better. In contrast, in the second experiment, in which Giovanello and Verfaellie (2001) equated amnesic and control participants' recognition performance by increasing the retention interval for the control participants, it is likely that the manipulation served primarily to decrease control participants' ability to rely on recollection. This reduction in recollection is probable because recollection is believed to fade quickly over a delay, while familiarity is more stable over time (Tulving, 1985; Gardiner and Java, 1991). Because control participants' reliance on recollection was reduced, the equated performance on the recognition task probably resulted from a true equating of processes (e.g., similar reliance on recollection and familiarity in the control participants and the amnesic patients). Because control participants' reliance on recollection was reduced to a level comparable to that of the amnesic patients, performance was equated not only on a recognition memory task but also on a recall task.

These results emphasize the importance of analyzing amnesic patients' performance not just in terms of the type of task used to assess memory (e.g., recall or recognition) but by considering the underlying processes as well. The evidence for a disproportionate impairment in recollection in the study by Giovanello and Verfaellie (2001) was indirect (i.e., the authors did not directly measure the contribution of recollection and familiarity to recognition task performance), but more recent investigations have tackled this question head-on.

In many of these experiments, researchers have relied on participants' self-reports as to what types of information contributed to their recognition response. With this type of assessment (referred to as the remember-know procedure; *See* Chapter 2.17), participants are asked to not only decide whether information was presented in a study list but also to indicate whether they believe the information was presented because they remember something about the context in which the item was presented (i.e., they recollect details from the study episode) or because they simply know that they studied the item (i.e., the item is familiar but they are unable to recollect any information about its presentation; Mandler, 1980; Tulving, 1985; Rajaram, 1993). Evidence using the remember–know paradigm and other dual-process procedures suggests that the familiarity-based contribution to recognition memory performance is preserved in amnesic patients, whereas the recollective-based component is drastically impaired (e.g., Bastin et al., 2004; King et al., 2004; Aggleton et al., 2005; but see Medved and Hirst, 2006, for evidence of vivid recollection in amnesic patients). Amnesic patients often give far fewer remember responses than control participants, and their distribution of remember responses across different conditions differs from that of control participants. In contrast, the distribution of know responses for amnesic patients tends to be more in line with that of control participants (e.g., Rajaram et al., 2002).

The remember–know paradigm, however, has limitations. The first is that it relies on participants' subjective reports. It is likely that amnesic patients' subjective sense of remembering may not be qualitatively similar to the sense of remembering experienced by control participants (see Maguire et al., 2001b; Rajaram et al., 2002, for further discussion). The second is that, when a participant endorses an item as remembered, it is not clear what types of information the participant has retrieved. Depending on instructions used by investigators, a remember response can signify retrieval of information ranging from a wide variety of contextual elements (e.g., remembering a feeling elicited by the stimulus, or remembering the item that preceded the stimulus) to memory for the exact features of the presented stimulus (e.g., remembering the font in which the word was written). It is likely that memory for these different types of information (e.g., item–context associations, item–item associations, and memory for intraitem features) is supported by different processes; the remember–know procedure does not provide an easy way in which to parse their distinct contributions.

To circumvent these issues, researchers have adopted paradigms that more directly compare memory for associations or relations with memory for isolated items. One commonly used paradigm asks participants to distinguish between studied associations (e.g., fork–bench, pig–limousine, often referred to as intact pairs), novel associations between previously studied items (e.g., fork–limousine, recombined pairs), and novel associations between unstudied items (e.g., canary–tool, novel pairs). The ability to distinguish intact from recombined pairs (a measure of associative memory) has been compared with the ability to distinguish either of those pairs from novel pairs (a measure of item memory). Cognitive studies in young adults have provided empirical support for a dissociation between these two types of memory measures, with associative recognition being tied to recollective processes and item memory more reliant on familiarity signals (e.g., Hockley and Consoli, 1999).

Amnesic patients often show profound impairment on associative memory measures, even under conditions in which memory for items is equated (e.g., Giovanello et al., 2003; Turriziani et al., 2004). Not only are these associative memory deficits apparent on tasks that directly ask participants whether items were studied together but they also are apparent using eye tracking measures to indirectly assess memory for item relations. Cohen and colleagues (Ryan et al., 2000; Ryan and Cohen, 2004) have found that control participants tend to look longer at components of scenes that have been moved, whereas amnesic patients do not show these effects. These data provide further evidence that amnesia may result in a fundamental deficit in processing the relations or associations among different stimuli.

The results discussed so far have suggested that amnesia does not always result in a declarative memory deficit that is equally pervasive across all memory measures. Rather, patients with amnesia are typically disproportionately impaired on recall tasks compared to recognition tasks, on tasks that require recollection as compared to those that can be supported by familiarity, and on tasks that rely on memory for associations as compared to those that can be supported by memory for items void of their context. Although these findings provide evidence for dissociations in the magnitude of mnemonic impairment based on the type of information retrieved, they do not speak to the precise regions within the MTL that may contribute differentially to different types of mnemonic functions.

Initial evidence for dissociations of function between the hippocampus proper and the surrounding MTL cortices came from animal models of amnesia. In a number of investigations, complete hippocampal removal had little to no effect on object recognition memory, even when memory was assessed after relatively long delays (Orbach et al., 1960; Stepien et al., 1960; Correll and Scoville, 1965; Meunier et al., 1993; Murray and Mishkin, 1998; Winters et al., 2004; Forwood et al., 2005). In fact, one study reported an inverse correlation between the amount of hippocampal damage and the magnitude of recognition memory impairment (Baxter and Murray, 2001). In contrast, lesions in perirhinal cortex can result in large decrements in object recognition memory (Mumby and Pinel, 1994; Brown and Aggleton, 2001; Mumby, 2001). Similar dissociations of function between the hippocampus and perirhinal cortex have been revealed in rats (Aggleton and Brown, 2005) by examining the expression of the immediate early gene *c-fos* as a marker of neural activity.

The advent of functional magnetic resonance imaging (fMRI) has made it possible to delineate the neural circuits that contribute to distinct memory processes in humans, and to examine the extent to which dissociations between the function of the hippocampus and perirhinal cortex explain dissociations in recollective and familiarity-based performance in

amnesic patients. A number of studies employing the remember–know procedure and associative learning paradigms have now provided evidence that the hippocampus proper supports the encoding and retrieval of recollective information, whereas perirhinal cortex mediates the encoding and retrieval of familiarity-based memory traces (Wan et al., 1999; Davachi and Wagner, 2002; Davachi et al., 2003; Giovanello et al., 2004; Ranganath et al., 2004; Kensinger and Schacter, 2006).

Although these studies suggest a dissociation in the contribution of the hippocampus and surrounding cortical regions in recollection- and familiarity-based processing, respectively, these neuroimaging studies cannot speak to the necessity of particular regions for these memory functions. To fill this void, several studies have investigated the degree to which lesion locus or extent affects the likelihood that amnesic patients are impaired specifically on the recollective or associative components of a memory task. If the hippocampal formation is disproportionately engaged during recollective or associative processing, compared to familiarity-based item processing, then amnesic patients with damage limited to the hippocampus should show sparing of item recognition relative to associative recognition. In fact, patients with damage limited to the hippocampus sometimes slow preserved familiarity-based responding (Aggleton et al., 2005) and relatively normal item recognition performance (Vargha-Khadem et al., 1997; Holdstock et al., 2002; Mayes et al., 2002), despite impaired free recall (Henke et al., 1999; Duzel et al., 2001; Holdstock et al., 2005). These findings are consistent with the hypothesis that familiarity-based item memory can be supported by cortical regions outside of the hippocampus proper (e.g., perirhinal and parahippocampal cortices), while the hippocampus is critical for recollective and associative-memory performance.

Even this associative/item dichotomy may be too broad, however. Evidence suggests that the hippocampus is not equally engaged in all forms of recollective or associative memory. Rather, it may play a selective role in the learning of arbitrary associations between stimuli (O'Reilly and Rudy, 2001; Ryan and Cohen, 2004). Meaningful and preestablished relations between stimuli, in contrast, may be processed more as intraitem features, and memory for those associations may be mediated by regions outside of the hippocampus proper (Aggleton and Brown, 1999). Thus, amnesic patients with extensive hippocampal damage are able to learn some stimulus pairings when those pairing are logical and build from preexisting knowledge (e.g., learning word pairs such as baby–cries or rose–red; Skotko et al., 2004). They show dramatic deficits, however, when attempting to learn stimulus associations that cannot build on such prior knowledge (e.g., apple–tent, balloon–fork; see Gilbert and Kesner, 2004, for similar results in rats with hippocampal lesions).

Evidence to support this conclusion comes from a study assessing memory for compound stimuli (e.g., pinpoint), which can be thought of as a class of nonarbitrary stimulus pairings. All amnesic patients showed a recognition advantage for these compound stimuli as compared to stimuli with no preexisting associations (Giovanello et al., 2006). Moreover, the advantage for the compound words was numerically greater for patients with lesions limited to the hippocampus than for patients with more widespread MTL damage. In other words, patients with circumscribed hippocampal lesions were able to take advantage of the preexisting associations of the compound stimuli to a greater degree than were patients whose lesions included the surrounding MTL cortices. This pattern of results is consistent with the hypothesis that regions outside of the hippocampus proper contribute to memory for nonarbitrary stimulus associations, whereas the hippocampus itself is essential for binding arbitrarily related stimuli together.

In summary, these findings provide strong evidence for dissociations of function between the hippocampus and surrounding cortical regions. In particular, they highlight instances in which the hippocampus proper plays a particular role in memory for recollective and associative information, allowing memory for an item to be bound to its context or to other items present during the encoding episode. This role of the hippocampus may be particularly critical when associations among items, or between an item and its context, do not hold preexisting significance but, rather, represent the arbitrary merging of previously unrelated stimuli.

1.14.3.2 Counterpoint: The Hippocampus and Surrounding Cortices Support Both Item and Relational Memory

Although the research described above provides compelling evidence for a dissociation of function between the hippocampus and surrounding cortical regions, other researchers argue that the hippocampus and surrounding cortices are broadly important

for all forms of declarative learning, ranging from those that can rely on judgments of stimulus familiarity to those that depend on forming associations between previously unrelated stimuli (e.g., Reed and Squire, 1999; Stark and Squire, 2001). According to this view, any single dichotomy (e.g., recollection vs. familiarity or associative vs. item memory) is too simplistic to capture the division of labor between the hippocampus and the adjacent cortical structures in the MTL, although dissociations in function between different MTL structures may exist (e.g., Stark and Squire, 2003). In this section, we describe some of the neuroimaging and neuropsychological evidence suggesting that the dichotomies described above may not always hold.

If the hippocampus performs separable functions from the surrounding MTL cortices, then it should be difficult, if not impossible, to find patients with lesions limited to the hippocampus who do not show disproportionate deficits on tasks that assess the types of memory that the hippocampus mediates. In other words, patients with circumscribed hippocampal damage always should be disproportionately impaired on tasks that assess recall compared to recognition memory, recollection compared to familiarity, and memory for associations compared to isolated items. While these dissociations often occur in patients with focal hippocampal lesions (Henke et al., 1999; Holdstock et al., 2002, 2005; Mayes et al., 2002), they are not always present: Such patients can be equally impaired on measures of recall and recognition (Reed and Squire, 1997; Manns and Squire, 1999; Cipolotti et al., 2001; Manns, et al., 2003), and they can show comparable deficits on memory assessments for single items and for item conjunctions (Stark et al., 2002; Stark and Squire, 2003). For example, Stark et al. (2002) equated item recognition between patients with discrete hippocampal damage and control participants and then examined whether associative recognition was matched as well. When item recognition was equated between groups by providing hippocampal patients with eight study exposures, no impairment in associative recognition was observed for the patient group (although ceiling effects in patients' item recognition may have masked a disproportionate impairment in associative recognition; see Kensinger and Giovanello, 2005, for further discussion).

Moreover, if the hippocampus plays little to no role in familiarity-based processing of single items, then lesions limited to the hippocampus proper should largely spare performance on tasks that can be supported by these familiarity-based processes. As discussed in the preceding section, familiarity-based processing often is spared in amnesic patients with focal hippocampal damage. Some reports, however, indicate that damage circumscribed to the hippocampus can lead to marked deficits in recognizing factual knowledge about events that occurred after the amnesia onset (Manns et al., 2003; see also Reed and Squire 1998; Kapur and Brooks 1999; Holdstock et al. 2002) and in endorsing items as previously encountered (Beason-Held et al., 1999; Zola et al., 2000; Stark and Squire, 2003). These findings violate the prediction that performance on tasks that can be supported by familiarity alone should be intact after hippocampal lesions.

The reason for the discrepant findings across studies is a topic of heated discussion. Although any number of factors may contribute (e.g., differences in the tasks used, differences in lesion location, reorganization of function, deafferentation of regions due to fiber tract damage; see Mayes et al., 2002, 2004; Holdstock et al., 2005 for further discussion), we focus here on a few possibilities that we believe are particularly viable.

One feasible contributor to the conflicting findings is that patients' lesions likely are not circumscribed to functionally monolithic regions. Rather, the lesions probably encroach on multiple subregions that subserve distinct processes. For example, considering the entire hippocampus as a single functional entity, and expecting damage to any location within the hippocampus to result in similar mnemonic deficits, is probably an oversimplification. In fact, recent neuroimaging evidence has suggested that not all regions of the hippocampus play equivalent roles in associative memory (e.g., Giovanello et al., 2004; Kohler et al., 2005). For example, Kohler and colleagues (2005) found that while a middle hippocampal region responded more to novel associations than to novel objects, the same was not true of all hippocampal regions (and see Henson, 2005, for discussion of whether there is an anterior–posterior gradient within the hippocampus).

Similarly, it is likely that collapsing all extrahippocampal medial temporal-lobe cortical regions into one functional category overlooks critical divisions of labor. For example, patient and neuroimaging studies have suggested that the parahippocampal cortex plays an important role in memory for spatial and topographical information (e.g., Bohbot et al., 1998; Epstein and Kanwisher, 1998; Kohler et al., 1998; Maguire et al., 1998; Epstein et al., 1999; Ploner et al., 1999, 2000; Barrash et al., 2000), whereas the

perirhinal cortex may play a more dominant role in the processing of object information (e.g., Aggleton and Brown, 2005; Buckley, 2005). Moreover, functional neuroimaging evidence suggests that the perirhinal cortex and parahippocampal gyrus may serve functionally distinct roles in familiarity-based and recollective-based retrieval, respectively. Thus, while perirhinal activity often corresponds with familiarity-based memory for isolated items or for intraitem features (Davachi et al. 2003; Dobbins et al. 2003; Henson et al. 2003; Kirwan and Stark 2004; Ranganath et al., 2004), activity in the posterior parahippocampal cortex often appears to relate to recollection and to memory for item context or item associations (Henke et al., 1999; Yonelinas et al., 2001; Davachi et al., 2003; Dobbins et al., 2003; Duzel et al., 2003; Kirwan and Stark, 2004; Ranganath et al., 2004).

It is also probable that differences between the role of the hippocampus proper and that of the surrounding cortices reflect gradations of specialization rather than complete dissociations. In other words, the function of adjacent MTL structures may overlap somewhat: Differences may exist in the proportion of particular neural networks within regions that subserve mnemonic functions, rather than absolute differences in the functions supported by the different structures. Electrophysiological studies back this interpretation. Although evidence indicates that neuronal activity in the hippocampus often provides a signal for conjunctive coding of items in a context (e.g., Wood et al., 2000), whereas perirhinal cortex neurons tend to respond in a stimulus-specific fashion (e.g., Suzuki et al., 1997; Young et al., 1997; Suzuki and Eichenbaum, 2000), one can find exceptions to these generalizations. For example, although most hippocampal cells respond based on stimulus-context conjunctions, stimulus-specific representations (i.e., responses to the stimulus regardless of its context) occasionally evoke hippocampal activity (e.g., Fried et al., 1997). Conversely, evidence suggests that at least in some instances, neural activity throughout the MTL cortices can correspond with memory for item associations (e.g., Buckmaster et al., 2004).

A study by Cipolotti et al. (2006) provided a strong warning that ignoring the types of stimuli for which memory is assessed leads to discrepant results regarding the role of the hippocampus in memory. These authors tested a patient with a focal hippocampal lesion on tasks assessing memory for a range of verbal, visual, and topographical information. Their results suggested that the hippocampus plays a role in both recollective- and familiarity-based processing of verbal and topographical stimuli but is not necessary for recollection and familiarity-based memory of human faces. Although future studies will be needed to confirm the generality of these findings, and to clarify whether they extend to patients with damage located anywhere in the hippocampus or whether they may have arisen due to the precise location of this patient's hippocampal lesion, the data urge caution in trying to compare results across multiple studies using different stimulus types.

In summary, the results presented here caution against adopting too simplistic a division of labor between the hippocampus and the MTL cortices. While it is likely that these regions do make independent contributions to declarative learning and memory, a consensus has yet to be reached regarding the nature of those contributions.

1.14.4 The Role of the Perirhinal Cortex in Object Memory and Object Perception

In addition to debates regarding the distribution of mnemonic function across MTL structures, disagreement exists regarding the extent to which MTL structures play an isolated role in memory. In this section, we focus on a controversy regarding the role of the perirhinal cortex in memory vs. perception. As discussed earlier in this chapter, in order for animal models of amnesia to recreate the dense anterograde memory loss demonstrated in patients such as H.M., damage has often extended outside of the hippocampus proper and into the perirhinal cortex (e.g., Orbach et al., 1960; Mishkin, 1978; Zola-Morgan and Squire, 1985; Zola-Morgan et al., 1993; Murray and Mishkin, 1998). These studies emphasize that the perirhinal cortex must play an influential role in memory.

An ongoing debate, however, regards the extent to which the perirhinal cortex is important specifically for visual memory and the extent to which the perirhinal cortex plays a broader role in visual discrimination and object perception. Perirhinal cortex has the characteristics, in terms of anatomical connections, to support memory and perception side by side (e.g., Suzuki, 1996). Although perirhinal cortex is often considered part of the hippocampal-based memory system, with strong connections to the hippocampus via the entorhinal cortex (Suzuki, 1996), perirhinal cortex is a polymodal area with strong connections to regions within the ventral visual processing stream that are

specialized for object identification (e.g., Burwell et al., 1995; Murray and Bussey, 1999; Goulet and Murray, 2001). Neurons in perirhinal cortex also have properties that could support both object memory and perception (e.g., large receptive fields that respond selectively to complex visual stimuli; Logothetis, 1998; Jagadeesh et al., 2001). In this section, we present evidence for each side of the debate: According to one view, the perirhinal cortex plays a role encompassing memory and perception (e.g., Eacott et al., 1994; Eacott and Heywood, 1995; Murray and Bussey, 1999; Buckley and Gaffan, 2000; Murray and Richmond, 2001; Bussey and Saksida, 2002); according to another view, the contribution of perirhinal cortex is limited to memory, with stimulus perception dependent entirely upon cortical areas earlier in the ventral visual processing stream (e.g., Squire and Zola-Morgan, 1991; Zola-Morgan et al., 1994; Buffalo et al., 1998; **Table 1**).

1.14.4.1 Point: The Perirhinal Cortex Supports Visual Perception

Perception refers to the process of acquiring, interpreting, and representing incoming sensory information. Memory, in contrast, refers to the retention and retrieval of these representations in the absence of the sensory information. A deficit restricted to memory, therefore, should be observed only when an individual is required to distinguish alternatives after they have been withdrawn from view. In contrast, a deficit in perception should be apparent when an individual is asked to distinguish objects in plain sight.

Some of the first evidence that perirhinal cortex may have a role in perception came from Eacott et al. (1994). They tested macaque monkeys on a matching-to-sample task. In this task, monkeys first learn that a particular object (e.g., a red square) is associated with a reward (the sample phase). They then are shown the rewarded object and a second object (e.g., a red square and a green circle), and the monkeys must choose the initially rewarded object (the match phase). Critically, Eacott et al. (1994) included simultaneous match-to-sample and delayed match-to-sample conditions. While deficits in the delayed condition could result either from perceptual or mnemonic impairments, deficits in the simultaneous condition should reflect perceptual difficulties (because the objects remain in view). They found that when there was a large perceptual load in the task (i.e., when they used many different types of stimuli across all of the trials), the monkeys with perirhinal lesions showed impairments even in the simultaneous condition. These results support the interpretation that perirhinal cortex mediates visual perception.

Further delineating the exact role of perirhinal cortex in visual perception, however, has proved challenging. For example, Eacott and colleagues' (1994) study further showed that perirhinal cortex is not needed for all forms of object perception. When perceptual load was relatively low (i.e., when only a small set of items was used across all of the trials), monkeys with perirhinal lesions showed a delay-dependent deficit: They were impaired relative to control monkeys at the long delay but performed normally at the shortest delay. This pattern of performance is consistent with a mnemonic, and not a perceptual, deficit.

Several additional studies, using discrimination of pairs of visual stimuli, showed inconsistent results. Damage to the perirhinal cortex can lead to impairments in visual discrimination under some conditions, but it does not lead to a pervasive deficit across all assessments of visual discrimination. Thus, Buckley and Gaffan (1997), like Eacott et al. (1994), reported that monkeys with perirhinal cortex lesions performed as well as control monkeys when learning a small number of concurrent visual discriminations (see also Aggleton et al., 1997; Thornton et al., 1997; Buffalo et al., 1999; Baxter and Murray, 2001) but

Table 1 Predictions made by the perception-and-memory view of perirhinal cortex, compared to the mnemonic-only view, based on the stimulus characteristics and delay interval used on tasks such as matching-to-sample or visual discrimination learning

		Perirhinal cortex necessary?	
Stimulus characteristics	*Delay*	*Perception view*	*Mnemonic-only view*
Few stimuli, distinguishable by single object features	No	No	No
	Yes	Yes	Yes
Stimuli distinguishable only by conjunctions of intra-object features	No	Yes	No
	Yes	Yes	Yes

were impaired when required to learn a large number of discriminations (see also Buckley and Gaffan, 1998).

At a broad level, these results suggest that activity in perirhinal cortex may enhance the specificity of an object's representation (i.e., the level or amount of visual detail). When the task includes relatively few stimuli, the representation for each stimulus does not have to be precise or specific for discrimination to be successful. In contrast, when the stimuli are numerous and potentially confusable, each one must be represented precisely to avoid stimulus confusion (see Murray and Bussey, 1999; Bussey and Saksida, 2002, for further discussion). By this view, perirhinal lesions selectively impair object discrimination on tasks that employ large stimulus sets.

A study by Eacott et al. (2001), however, suggested that the perirhinal cortex is not critical for representing all fine-grained discriminations between objects. In their study, Eacott and colleagues required rats to distinguish between squares and rectangles, and they modulated the difference in side length between the two shapes such that the rats had to maintain a precise representation of the shapes to distinguish the square from the rectangle. Even with perirhinal cortex lesions, the rats were able to perform the task successfully. Critically, successful performance of this task could be based on representation of a single object feature: edge length. It is likely that this single-feature representation can be supported by areas earlier in the visual processing stream, such as inferotemporal cortex (see Buckley et al., 1997), and does not require engagement of perirhinal cortex (see also Buckley et al., 1997, 2001). Rather, perirhinal cortex may become necessary only when the representation of feature conjunctions is required.

In an influential model (the perceptual-mnemonic/feature-conjunction model), Bussey, Murray, and Saksida (e.g., Murray and Bussey, 1999; Bussey and Saksida, 2002) have proposed that perirhinal cortex serves as the final processor in the ventral visual processing stream (Desimone and Ungerleider, 1986; Ungerleider and Haxby, 1994), coding for complex visual representations. Within this framework, the results described above can be understood by considering the degree of feature ambiguity present across the different stimuli. The greater the number of object pairs to be discriminated, the greater the likelihood that a particular object feature will be rewarded when it is part of one object but not when it is part of another object (i.e., the greater the feature ambiguity). Because of the increased probability that a feature will be present among multiple stimuli, the representation of conjunctions of complex features in perirhinal cortex will be critical for successful task performance. The preservation of function in the study by Eacott et al. (2001) can be explained by reliance on a precise representation of a single feature (edge length) rather than on a need to integrate multiple intraitem features. Thus, the role of perirhinal cortex may be best described as representing configural relations among features, or conjunctions of intraobject features.

To test this hypothesis directly, Eacott et al. (2001) designed a visual discrimination task in which combinations of features, rather than any single object feature, signaled the rewarded object. Performance on this task was compared to performance on a task in which visual discrimination among stimuli could occur based on single features. Rats with perirhinal cortex lesions were impaired only when successful performance required discrimination based on the configuration of features; when performance could rely on representation of single features, the perirhinal-lesioned rats performed normally. Bussey et al. (2002, 2003) have found similar results when assessing visual discrimination in nonhuman primates: Monkeys with perirhinal lesions were impaired on tasks that required disambiguating shared features, but not on tasks that could be solved by discrimination of single object features. These results are consistent with the conclusion that perirhinal cortex functions as part of the ventral visual processing stream and plays a critical role in feature integration (see Bussey et al., 2005).

Further corroborating evidence has come from a study demonstrating that the requirement for feature integration can exacerbate the mnemonic deficits demonstrated after perirhinal damage. In a spontaneous recognition task, Norman and Eacott (2004) used rats' natural tendencies to explore novel items as a means to assess their ability to remember which objects had been encountered previously. The features of the novel objects were manipulated so that some differed from familiar objects in single features, whereas others differed from familiar objects in the conjunction of features. The critical finding was that perirhinal-lesioned animals showed an exaggerated memory deficit for the feature-ambiguous stimuli compared to the feature-unique stimuli. In fact, even after relatively short delays, the perirhinal-lesioned animals were at chance in distinguishing between the novel and familiar feature-ambiguous objects (Norman and Eacott, 2004).

These findings have led to the proposal that perirhinal cortex represents the association between intraitem features (Gaffan, 1994) or the gestalt representation of a whole object (Murray and Bussey, 1999). These putative functions of perirhinal cortex are consistent with its location among high-order processing regions in the ventral visual stream. These regions tend to respond to the whole object rather than to the individual features that comprise the object (Baker et al., 2002). Two open questions are whether perirhinal cortex plays a role in configural learning of all intraitem features and whether its role is limited to intraitem feature integration or also extends to the association of features that are spatially or temporally separable (see Alvarado and Bachevalier, 2005, for evidence of dissociable roles of the perirhinal and parahippocampal cortices in object vs. temporal configural memory; see Lee et al., 2005a,b, for evidence of dissociable roles of the hippocampus and MTL cortices in spatial vs. object perception; see Shaw et al., 1990, for evidence that perirhinal cortex may be important for cross-modal object processing). These questions are closely tied to the debate, discussed in the first section of this chapter, regarding the extent to which the roles of perirhinal cortex and hippocampus can be dissociated (e.g., with perirhinal cortex representing intraitem feature integration and the hippocampus representing item–item and item–context relations).

1.14.4.2 Counterpoint: The Perirhinal Cortex Supports Memory but Not Perception

As already discussed, perirhinal cortex is not required for all object perception. Patients with MTL lesions that include perirhinal cortex are not impaired on simultaneous visual recognition memory tasks (Milner et al., 1968; Buffalo et al., 1998; Holdstock et al., 2000) or on a wide variety of visual perception tasks (Lee et al., 2005b). Where debate arises, however, is in whether perirhinal cortex serves any role in visual perception. Although the evidence described in the preceding section suggests that this area does play a fundamental role in perception, at least for some classes of stimuli, other researchers have questioned the validity of these claims.

One concern relates to the way in which researchers analyzed their data to arrive at the conclusion that perirhinal cortex is critical for visual perception. For example, in a reanalysis of the data presented by Buckley and Gaffan (1997), Hampton (2005) demonstrated that the conclusions reached differ depending on whether the measure of assessment is considered to be errors made per problem or total number of errors. Animals with perirhinal lesions appear disproportionately impaired on tasks with larger stimulus sets when performance is measured by the total number of errors. The number of errors per problem, however, remains stable across different set sizes. Although extensive discussion has probed the extent to which these differences in analyses can account for all of the discrepant findings (Buckley, 2005; Lee et al., 2005b), at the least, the debate emphasizes the difficulty inherent in comparing perceptual ability across tasks with differently sized stimulus sets.

Another complaint has been that the studies described in the prior section have not always cleanly teased apart the perceptual and mnemonic demands of the task (Levy et al., 2005). Part of this conflation has stemmed from the fact that most of the studies have assessed the role of perirhinal cortex in rats or in monkeys. Teasing apart perception from memory is difficult in these animals. For example, failure to correctly identify a rewarded target could stem not only from difficulties perceiving the target but also from difficulties remembering which item is rewarded (e.g., whether the novel object or the familiar object is rewarded). It is much easier to dissociate perceptual and mnemonic processes in humans because explicit reminders about the task instructions can be given (e.g., "which of these stimuli matches this target object?"), thereby eliminating any mnemonic load.

A number of studies, therefore, have assessed the performance of amnesic patients with damage to perirhinal cortex on perceptual discrimination tasks. Buffalo et al. (1998) and Holdstock et al. (2000) used a matching-to-sample paradigm to assess object perception and recognition memory in amnesic patients, including those with focal damage to the perirhinal cortex. In the study by Buffalo et al. (1998), participants viewed four visual designs and then, following a variable delay, indicated whether an image presented alone had been one of the four previously seen. The results revealed that at short delays (0–2 s), patients with perirhinal lesions performed well on the task, whereas they were impaired at longer delays (6 s or more).

Similar results were revealed in the study by Holdstock et al. (2000), in which performance was compared in simultaneous and delayed matching-to-sample tasks. In the simultaneous matching condition, participants viewed a probe (an abstract pattern) along with possible targets and were asked to indicate which target matched the probe. In the delay conditions, the

probe was shown and then removed from the screen. Following a delay (ranging from 0 to 30 s), participants saw the possible targets and were asked to select the one that matched the probe image. Holdstock and colleagues found that the patients with perirhinal lesions were impaired only at long delays (10 s or longer) and performed within the normal range with shorter delays (less than 5 s) and in the simultaneous matching condition. The fact that performance was impaired at longer delays but not at shorter ones suggests that perirhinal lesions primarily caused mnemonic deficits and not perceptual ones.

Further evidence in support of the conclusion that perirhinal damage does not impair perceptual ability comes from studies by Squire and colleagues. Stark and Squire (2000) tested three patients with MTL lesions that included all of the perirhinal cortex on the oddity task developed by Buckley and colleagues (Buckley and Gaffan, 1998, p. 15; Buckley et al., 2001). In this task, participants are shown five different views of the same object, and one view of a different object. Participants must indicate which object is the odd one. Obscuring the objects with a white-noise mask makes the object discrimination task quite difficult. Stark and Squire (2000) tested the amnesic participants on seven versions of the oddity task. Across each version, the patients performed as well as control participants.

Levy et al. (2005) also reported results suggesting that perirhinal lesions do not lead to perceptual deficits in humans. The authors tested two of the amnesic patients who had been assessed on the oddity tasks on a task that required discrimination of complex feature-ambiguous stimuli (using the stimulus manipulations of Bussey et al., 2003). Just as with the oddity task, these patients performed normally on similarity judgment tasks (indicating whether pairs of images were the same or different) and on simultaneous matching-to-sample tasks (indicating which of two blended images was closest to a target image). These results suggest that complex visual discrimination performance and discrimination between images that have high feature ambiguity can occur even in the absence of perirhinal cortex function.

One potential difficulty in interpreting the results of Levy et al. (2005) is that the scores obtained by the control participants were very close to ceiling. It is possible, therefore, that a mild deficit would be revealed in the patients if their perceptual abilities were assessed using tasks that resulted in poorer performance by everyone (see Lee et al., 2005, for further discussion). In the task used by Stark and Squire (2000), however, the control participants' performance was well below ceiling, providing suggestive evidence that ceiling effects may not fully explain the findings.

Taken together, these results indicate that on a range of tasks, monkeys with perirhinal lesions fail, but humans with perirhinal lesions succeed. A few viable explanations for these contrary findings come to mind. First, the monkeys may have had damage to regions outside of perirhinal cortex. Damage to lateral visual areas is known to produce visual perception deficits (e.g., Mishkin, 1982; Miyashita, 1993; Buffalo et al., 1999), and it is possible that, in at least some of the studies in nonhuman primates, the lesions encroached on these lateral visual areas (Buffalo et al., 1999). Previous studies showed that the extent of inadvertent damage to lateral temporal areas can correlate more strongly with a monkey's ability to learn concurrent object discriminations than the extent of intentional damage to perirhinal cortex (Buffalo et al., 1998). Second, the function of perirhinal cortex with regard to visual perception may be distinct in humans and in nonhuman primates, or other regions within the human ventral processing stream may serve redundant functions with the perirhinal cortex in terms of processing feature conjunctions. Third, even stronger demands for processing high-level feature conjunctions may be required to witness impairments in humans with perirhinal damage; perhaps, on the tasks used to date, engagement of other types of strategies by human participants (i.e., other than discrimination based on feature conjunctions) has increased the likelihood that regions beyond perirhinal cortex can support successful task performance. Related to this last point, human perirhinal cortex may subserve visual perception only for a subset of object stimuli. For example, Lee et al. (2005c) demonstrated that patients with MTL damage were impaired at discriminating scenes and faces, but were less impaired at discriminating single objects, and were unimpaired at discriminating art (see also Lee et al., 2005a, for importance of stimulus type). A challenge for future studies is to determine whether any of these explanations can account for the conflicting results.

1.14.5 Retrieval of Autobiographical Memories in Amnesia

So far in this chapter, we have focused on the role of MTL structures in acquiring new declarative memories. In addition, considerable debate exists regarding

their roles in retrieving remote memories. While the hallmark of bilateral MTL lesions is anterograde amnesia, this inability to form new memories typically is accompanied by difficulty retrieving memories from some time period prior to the onset of the amnesia (i.e., retrograde memory loss). As was first noted by the French psychologist Théodule Ribot in 1881 (Ribot, 1881), the retrograde memory loss often follows a temporal gradient: It is more pronounced for recently formed memories and is less pervasive for older memories (now referred to as Ribot's Law). Consistent with Ribot's law, initial studies reported that, following MTL damage, retrograde amnesia often was limited to only the few years prior to the injury, with more remote memories relatively preserved (e.g., Milner et al., 1968; Corkin, 1984).

These findings suggested that MTL structures were not required for permanent storage and retrieval of memories. Rather, the region seemed to play a time-limited role in memory storage and retrieval. Based on this evidence, the presence of consolidation processes were proposed: physiological changes that take place in the brain to stabilize memories and to make them resilient to disruption. Memory consolidation can be described on two levels. The first is a rapid, molecular-level consolidation during which the long-term conductivity of synapses is affected by experience with particular stimuli or pairings of stimuli and responses. The existence of this phase of consolidation is widely accepted and will not be considered further in this chapter (for more on molecular consolidation see McGaugh, 2000; Kandel, 2001; Lee et al., 2004). Memory consolidation can also be described at the system or network level. Broadly, this level of consolidation is believed to take place over the long term (days to decades), with MTL structures critical for mnemonic storage and retrieval only until the consolidation process is complete (e.g., Squire, 1992). Two primary models for this level of consolidation have been proposed: the standard model and the multiple trace model.

According to the standard model of consolidation, a prolonged process of system consolidation may last for a decade or more (e.g., Squire and Alvarez, 1995; Dudai, 2004). During the initial phases of this consolidation process, the MTL is required for the storage and recovery of a memory trace. Over time, however, these structures become unnecessary as neocortical activity becomes sufficient to support storage and retrieval of memory traces. This standard model, therefore, provides a mechanism to account for the temporal gradient of retrograde amnesia. Retrieval of recent events is impaired because the MTL is still required for their retrieval, whereas retrieval of remote events is preserved because the MTL is not essential for their retrieval.

According to this standard model of consolidation, the MTL is required during the consolidation period for all declarative memories (i.e., both episodic and semantic) and becomes unnecessary after consolidation processes have terminated. Thus, this model predicts that retrograde memory loss should show the same temporal gradient regardless of whether the retrieved memories are semantic or episodic in nature (Squire, 1992; Squire and Alvarez, 1995).

In contrast to the standard model of consolidation, the multiple trace model (Nadel and Moscovitch, 1997) proposes that the MTL serves as a pointer, or index, to the neocortical areas that store the representations of information present during an encoding episode (see also Teyler and DiScenna, 1986). Thus, activity in a network of MTL neurons provides a means to bind together the different pieces of information that were attended to during a particular experience. Each time a memory trace is activated (e.g., through reminiscence), another pointer is created. These new pointers bind together many of the same representations as the original pointer, but they also may include some additional representations (reflecting new associations made during the reminiscence) and may neglect others (those not activated during the reminiscence). Because the exact content retrieved about an episodic memory varies somewhat in the representations retrieved, over time each autobiographical event will become represented by multiple, distinct neuronal networks distributed throughout the MTL. Because remote autobiographical memories tend to be activated a greater number of times than more recent ones, the number of neuronal networks representing a remote autobiographical memory usually will be greater than the number of neural networks representing a recent autobiographical memory. Probabilistically, damage to a subset of MTL neurons, therefore, will be more likely to eliminate all of the networks associated with recent memories than to erase all traces of remote memories. The multiple trace model, in contrast to the standard model, explains the temporal gradient of retrograde memory loss not in terms of a change in the necessity of the MTL for retrieval of the memories but, rather, in terms of a change in the likelihood that all of the MTL traces representing a memory would be damaged as a result of brain injury. The multiple trace model does, however, postulate

that over time, semantic memories (factual knowledge void of context) can become represented solely in the neocortex. This shift can occur because, although these facts initially were acquired in specific contexts (e.g., we learned that the Eiffel Tower is in Paris by listening to our second grade teacher, we were reminded of it by reading a tour book, etc.), over time, the factual knowledge becomes extracted from the context in which it was learned. At the point where retrieval of this factual knowledge can occur without retrieval of its encoding context, MTL structures are no longer needed.

The standard model and the multiple trace model both predict that retrieval of remote semantic memories can occur in the absence of functional MTL structures. Indeed, abundant evidence shows that retrograde amnesia for semantic information tends to follow a temporal gradient (e.g., Kapur and Brooks, 1999; Fuji et al., 2000) and that retrieval of remote semantic knowledge often is preserved following extensive MTL damage (e.g., Kensinger et al., 2001; Schmolck et al., 2002). The two models, however, diverge in their predictions for whether MTL structures are always required for the retrieval of autobiographical memories (as proposed by the multiple trace model) or whether, over time, the role of those structures is eliminated such that retrieval of remote autobiographical memories can be supported by activity in other neocortical regions (as postulated by the standard model; **Figure 5**). In this section, we examine the evidence for and against the conclusion that retrieval of remote autobiographical memories requires the MTL.

1.14.5.1 Point: Retrieval of Remote Autobiographical Memories Does Not Require the Medial Temporal Lobe

If the retrieval of remote autobiographical memories can proceed in the absence of MTL function, then amnesic patients should show a temporal gradient in autobiographical memory (i.e., better memory for remote events than for recent events, in contrast to the pattern of results typically displayed by control participants) and relatively preserved memory for remote events. Both of these predictions have been upheld in a number of studies. Often, amnesic patients show a temporally graded retrograde amnesia for autobiographical memory, similar to the gradation shown for semantic knowledge (e.g., Reed and Squire, 1998; Kapur and Brooks, 1999). Particularly in individuals with damage limited to the hippocampus, memory for events sometimes is affected only for recent time points, within a decade of amnesia onset (Reed and Squire, 1998). In contrast, patients with more extensive temporal lobe damage (including the temporal neocortex) often show more extensive retrograde memory loss (Reed and Squire, 1998).

Further, amnesic patients can also show a preserved ability to retrieve remote autobiographical experiences. Several studies have reported that amnesic patients recollect detailed, autobiographical experiences from time points removed from their amnesia onset (e.g., Schnider et al., 1995; Rempel-Clower et al., 1996; Reed and Squire, 1998; Fujii et al., 1999; Kapur and Brooks, 1999; Bayley et al., 2003, 2005). Particularly in patients with damage limited to the hippocampus, the level of detail recalled about these remote life events has been reported to be indistinguishable from that recalled by healthy individuals (Reed and Squire, 1998). In contrast, patients with larger lesions extending into the temporal neocortex often show pervasive impairments in retrieving autobiographical memories from remote time periods (Bayley et al., 2005).

The fact that hippocampal damage disproportionately affects memory for recent events, while relatively sparing memory for remote events, is consistent with the standard model's proposal that the hippocampus plays a time-limited role in memory retrieval. The correspondence of more extensive damage of the temporal neocortex to disruption of retrieval of remote autobiographical memories is consistent with the proposal that retrieval of these remote events is dependent on networks distributed throughout the neocortex.

Additional support for these claims comes from comparing the performance of patients with progressive MTL damage (e.g., patients with mild Alzheimer's disease) to the performance of patients with progressive damage to neocortical regions (e.g., patients with semantic dementia). Patients with Alzheimer's disease tend to show a temporally graded retrograde amnesia, with memory for recent events more impaired than memory for remote events (Piolino et al., 2003). In fact, a deficit in retrieval of remote memories typically occurs only later in the disease process, once the neuropathological changes have begun to appear in the neocortex. In semantic dementia, the opposite pattern of results has been demonstrated: a relative preservation of memories for recent events and a disruption in memories for remote events. Because semantic dementia causes

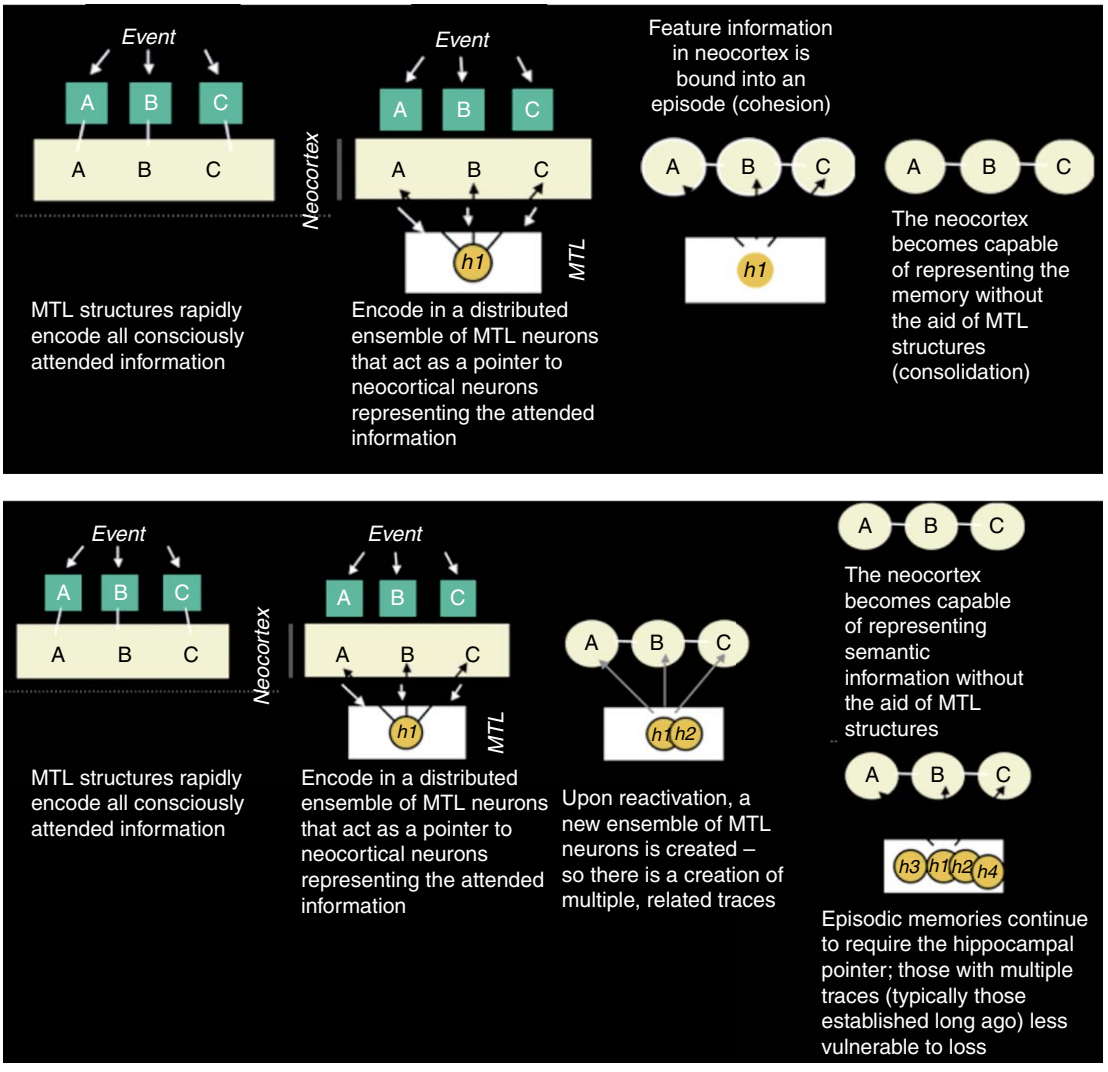

Figure 5 Phases of the consolidation process according to the standard model (top panel) and the multiple trace model (bottom panel). Adapted from Nadel L and Moscovitch M (1998) Hippocampal contributions to cortical plasticity. *Neuropharmacology* 37: 431–439.

progressive atrophy to the temporal neocortex, this pattern is consistent with the standard model's tenet that the hippocampus is needed for retrieval of recent memories but not for remote memories, while the temporal neocortex is critical for retrieval of remote memories (Murre et al., 2001; Nestor et al., 2002).

A study by Kopelman et al. (2003) reached a similar conclusion after assessing the correspondence between regional brain volume and autobiographical recall in 40 individuals with brain damage. They found that frontal lobe volume, and not MTL volume, accounted for individual differences in the retrieval of remote autobiographical memories. Corroborating evidence was revealed in a study by Eustache et al. (2004), in which resting-state glucose utilization in the hippocampus correlated with amnesic patients' ability to remember autobiographical events from recent time periods, while their ability to retrieve remote memories corresponded with glucose utilization in frontal cortex. Similar findings were reported in two functional neuroimaging studies of healthy volunteers. Piefke et al. (2003) found greater hippocampal activity during retrieval of recent autobiographical memories than during retrieval of remote memories; Takashima et al. (2006) found decreasing hippocampal activity (but increasing prefrontal activity) as a function of a memory's age (ranging from 0 to 3 months). These data are

consistent with the standard model's postulate that the hippocampus becomes less important as the age of a memory increases.

These findings all converge on the conclusion that MTL structures may not be required for retrieval of all autobiographical memories. While the function of MTL structures seems to be essential for retrieval of recently experienced events, remote memories appear to be retrieved in the absence of hippocampal function. Instead, retrieval of these events from one's past may rely on networks distributed throughout the neocortex.

1.14.5.2 Counterpoint: Medial Temporal Lobe Structures Are Required for Retrieval of Autobiographical Memories from All Time Periods

The story is not as straightforward as outlined above, however. A number of findings go against the claim that the MTL plays only a time-limited role in retrieval of autobiographical memories. For one, retrieval of remote autobiographical memories is not always spared in amnesia (e.g., Nadel and Moscovitch, 1997; Kopelman et al., 1999; Westmacott et al., 2001). For example, using family photos, Westmacott et al. (2001) found that the amnesic patient K.C. demonstrated a complete loss of memory for autobiographical episodes, even from remote time points (and see Rosenbaum et al., 2005, for similar findings). Steinvorth et al. (2005) similarly found that two amnesic patients (H.M. and W.R.) were severely impaired in retrieving remote autobiographical memories.

Second, amnesic patients often do not show a temporal gradient with regard to their autobiographical memories. This observation was made early on by Kinsbourne and Wood (1975), who suggested that amnesia affects autobiographical memories from recent and remote time points equally. This conclusion was corroborated in a series of studies by Warrington and colleagues, who demonstrated that amnesic patients often show a shallow temporal gradient (e.g., Warrington and McCarthy, 1988; Warrington, 1996). A few recent studies also have provided evidence of a flat temporal gradient for retrieval of autobiographical experiences (e.g., Cipolotti et al., 2001; Steinvorth et al., 2005).

How can one account for the discrepant findings in the amnesia literature, with some studies showing steep temporal gradients for autobiographical memory retrieval (with retrieval of remote memories relatively spared) and others showing shallower gradients (with retrieval of remote memories impaired)? One point of reconciliation relates to the specific locus of the amnesic patients' lesions. It is possible that some of the autobiographical retrieval deficits stem not from damage to the MTL but from damage to neocortical regions (see Squire et al., 2004, for more discussion). While extra-MTL damage should be considered, it does not appear to account for all discrepant findings. In at least some instances, damage specifically done to the MTL seems to correspond with difficulty retrieving remote autobiographical memories. Thus, even a patient with a lesion restricted to the hippocampal formation showed a flat temporal gradient and impaired retrieval of remote autobiographical memories (Cipolotti et al., 2001). This result suggests that flat temporal gradients cannot be ascribed merely to damage beyond the MTL. Further evidence suggesting a role of the MTL in retrieval of remote autobiographical memories came from a study examining whether the extent of MTL damage, as compared to damage to anterior and lateral temporal cortex, corresponded with the ability of Alzheimer's patients to retrieve autobiographical and semantic memories. Gilboa et al. (2005) found that while memory for semantic information was associated with damage to temporal neocortex, retrieval of autobiographical episodes was associated with the degree of MTL atrophy. Further, the strength of the association between MTL pathology and autobiographical memory retrieval was strong, even for childhood memories. These findings provide converging evidence that MTL regions remain important for autobiographical memory retrieval, regardless of the age of the memories being retrieved.

It is doubtful, therefore, that extra-MTL damage alone can account for all of the inconsistencies in the literature. Another possibility is that all regions within the MTL may not be equally important for retrieval of remote autobiographical memories. Squire and colleagues have argued that the hippocampus proper plays a critical time-limited role in retrieval of autobiographical memories (e.g., Squire, 1975, 1992; Squire et al., 1984). In their view, the surrounding MTL cortices may continue to be important for retrieval even of remote memories.

Because of the difficulties confirming that damage is circumscribed to the hippocampus and does not result in any functional damage to surrounding regions (e.g., through disruption of white matter fiber tracts), this hypothesis is difficult to address through testing of amnesic patients. Some evidence suggests that patients with damage limited to the

hippocampus show preserved retrieval of remote autobiographical memories (Levy et al., 2005), consistent with the claim that regions beyond the hippocampus are critical for retrieval of remote autobiographical experiences. Other studies, however, have reported impaired performance in patients with damage limited to the hippocampus (Cipolotti et al., 2001).

Neuroimaging studies in healthy individuals have provided one means to assess whether regions within the MTL are disproportionately active during retrieval of recent as compared to remote memories. As described in the previous section, some studies show that hippocampal activity is modulated by the age of the memory being retrieved (greater for recent than for remote memories; Piefke et al., 2003; Takashima et al., 2006). Increasing evidence, however, shows that hippocampal activity often is unrelated to the age of the retrieved memories. A number of studies have shown equivalent hippocampal activity during the retrieval of recent and remote autobiographical memories (Nadel et al., 2000; Maguire et al., 2001b; Ryan et al., 2001; Gilboa et al., 2004). One criticism of some of these studies is that the memories tested in the scanner were identified during a prescan interview; it is possible, therefore, that the true age of the memories was altered during this interview process. An fMRI study by Steinvorth et al. (2006) circumvented this problem. By assessing participants' memories for events written about in their diaries, or related by family members, these investigators measured brain activity associated with retrieval of memories that participants likely had not rehearsed recently. Although they found a large network of regions selective to autobiographical retrieval, the critical finding was that no MTL regions were more active during retrieval of recent autobiographical memories compared to remote ones. Although these studies cannot speak to the necessity of the regions, the fact that no MTL regions were less active during the retrieval of remote memories than during the retrieval of recent memories is inconsistent with the theory that the hippocampus plays a time-limited role in retrieval of autobiographical memories.

These results, therefore, suggest that differences in lesion location are unlikely to explain all of the contrary results within the amnesia literature. A more viable resolution may relate to a blurring of the contributions of semantic and episodic memory to autobiographical memory retrieval. Many of the studies that have assessed amnesic patients' ability to retrieve autobiographical memories have used procedures that do not differentiate memories that are semantic, or based on general knowledge about one's life, from those that are rich in contextual and temporal detail. Either of these types of information can allow one to recreate an autobiographical experience. For example, it is possible to remember one's sixth birthday party by reliving the tastes, sounds, and event locations. It also is possible to recall information about one's sixth birthday party by remembering the people present in a repeatedly viewed photograph, by repeating information often included in stories about the event, or by relying on general knowledge about the types of events that took place at most grade-school birthday parties.

Researchers have proposed that the ability to recall autobiographical information based on general knowledge relies on neocortical structures, just as do other forms of semantic memory (Nadel et al., 2000; Moscovitch et al., 2005). Thus, it is plausible that in studies that did not distinguish semantic from episodic contributions to autobiographical retrieval, amnesic patients appeared to have a preserved ability to retrieve autobiographical memories from remote time periods because those events had been rehearsed sufficiently to create a semantic memory, or because participants were able to rely on general knowledge about the event (Moscovitch et al., 2005).

The contribution of semantic and episodic memory to autobiographical retrieval has been assessed directly in studies that have asked participants to distinguish whether they vividly recollect a prior experience or simply know that the event has occurred. These studies have provided evidence that damage to MTL structures disrupts the ability to recall episodic details from events. A study with a patient with developmental damage to the hippocampus has clearly shown that such patients can retrieve autobiographical memories based not on episodic details but on semantic knowledge: The patient reported that there were some events from his life that he knew had happened, but that he could not recollect (Maguire et al., 2001a). Similar findings have been reported in a study of a patient who acquired MTL damage later in life (Hirano et al., 2002). In addition, Steinvorth and colleagues (2005) elicited memories for autobiographical events from two amnesic patients and from a number of age- and education-matched control participants. The investigators carefully scored the information generated by participants to distinguish recall of details based on semantic knowledge (e.g., the couch faced the television) from recall of details based on episodic knowledge (e.g., I felt the heat of the stove).

Neither patient could recall autobiographical memories with the same level of episodic detail as control participants. Their memories appeared to be constructed from semantic knowledge and were not associated with the feeling of being transported back in time (Steinvorth et al., 2005).

Further evidence to support the conclusion that many of the discrepancies in the literature relate to the amount of episodic detail remembered comes from comparing the findings of neuroimaging studies that reported greater hippocampal activity for recent than for remote memories with the findings from studies that found equivalent MTL activity regardless of a memory's age. In one study that demonstrated increased hippocampal activity for recent memories, those memories were associated with more episodic detail than were the remote memories (Piefke et al., 2003). The enhanced hippocampal activity, therefore, may relate not to the memory's age per se but, rather, to the amount of episodic detail retrieved. This hypothesis is consistent with studies that demonstrated greater hippocampal activity for memories retrieved with more episodic detail, both in patients with MTL damage (Maguire et al., 2001a) and in healthy individuals (e.g., Fink et al., 1996). In addition, a study by Addis et al. (2004) provided strong evidence that level of detail retrieved, rather than a memory's age, may be the strongest predictor of hippocampal engagement. Like Piefke et al. (2003), Addis and colleagues found that hippocampal activity was greater for recent memories than for remote ones. This finding, however, held only when the amount of episodic details recalled was not considered. Once level of detail was controlled, hippocampal activity no longer varied based on the age of the memories.

In another study that found greater hippocampal activation for recent than for remote memories, recognition memory was assessed over intervals ranging from 0 to 3 months (Takashima et al., 2006). As discussed earlier, two processes can contribute to recognition performance: episodic recollection or stimulus familiarity (Mandler, 1980; Tulving, 1985). Because recollection tends to decay over a delay faster than familiarity (Gardiner and Java, 1991), it is plausible that the decreasing hippocampal activity over time corresponded not with its reduced role in episodic recollection but with participants' increased reliance on stimulus familiarity (rather than on detailed recollection) after the delay. Support for this hypothesis is garnered from the fact that participants' performance after the delay was poor (corrected recognition performance was around 16%; Takashima et al., 2006).

These findings emphasize the need to assess not only the age of a memory but also the amount and quality of episodic detail retrieved about the memory. Future research will be needed to examine whether contradictions regarding amnesic patients' performance can be explained by considering the level of episodic (vs. semantic) detail retrieved. Recently developed interview techniques have emphasized the distinction between episodic and semantic details and have encouraged participants to generate as much information as possible about the event (e.g., to recall the sounds, colors, etc., that were present; Levine et al., 2002). These careful interview techniques make it possible to investigate the extent to which MTL damage (and damage to specific MTL regions, such as the hippocampus) disrupts the ability to recall episodic details from remote as well as recent time periods.

1.14.6 Conclusions

Since Scoville and Milner's landmark report on the amnesic patient H.M., a great deal has been learned about the types of memories that are preserved and impaired following MTL damage. As this chapter has highlighted, however, many open questions remain to be answered. Although resolution of the debates described here is a distant goal, this chapter has raised plausible hypotheses for the contradictions in the current literature and has provided a few pointers for further investigation. A common theme has been the need to examine structure–function correlations at a more fine-grained level than has been done to date. Future research will make great strides in elucidating the contribution of different MTL structures to human learning and memory if investigators examine the roles of distinct MTL structures, parse memory function into different categories based on retrieval demands and stimulus characteristics, and document the strategies that participants use for task performance.

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1.15 The Nature of Infantile Amnesia

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1.15.1 Introduction

Infantile amnesia, a term first used by Freud (1905/1953) over 100 years ago, refers to a unique memory phenomenon that occurs in humans and nonhumans alike. Essentially, infantile amnesia refers to a period very early in an organism's life when memories that are formed tend to be short-lived or inaccessible after a relatively short time frame. Historically, explanations have tended to fall into two camps. The first, or retrieval, camp holds that information that is stored early in life remains intact in storage and that infantile amnesia is the result of fluctuations in the retrievability of that information (Hoffding, 1891; Freud, 1914, 1938). The alternative, or storage, camp holds that memory storage is fragile early in life and that infantile amnesia is the direct result of this labile storage system (Kohler, 1929, 1941).

Modern theorists echo similar storage-failure and retrieval-failure conjectures about the nature of infantile amnesia. Proponents of this latter view argue that if storage is permanent and the inability to recall early experiences is simply a matter of retrieval failure, then such failures of remembering should be alleviated, given the appropriate reinstatement of retrieval cues (e.g., see Nash's (1987) discussion of the hypnotic age regression literature). This argument is fundamentally an encoding specificity one in which contextually dependent memories survive intact and can be recovered once the encoding context (internal and external) is reinstated. Of course it is difficult, if not impossible, to reinstate the context of infancy, and other data (reviewed later in this chapter) rule out a pure retrieval-based explanation in any event. Storage-based explanations have faired somewhat better. There is considerable evidence that although memory is much better in infancy than originally thought, storage in the immature organism is relatively fragile (Rovee-Collier et al., 2001). If storage is not permanent, particularly in the immature organism, then no matter what retrieval remedies are brought to bear, recall of early experiences may be impossible. Indeed, the general volatility of memory storage has been well documented over the years (e.g., Loftus and Loftus, 1980) and that it is somehow fundamentally different in infancy is equally well known (Hayne, 2004).

The issue concerning the fate of early memories is central to many theories of development, as most theories place special formative emphasis on experiences that occur early in life. This particular relevance of early experience was pivotal in Freudian theory as well as many subsequent theories of social, emotional, and personality development (e.g., see Ainsworth and Bowlby, 1991). Indeed, some early research demonstrated the profound effects of early experience (in this case, social isolation) on subsequent development (Skeels, 1966; Harlow et al., 1971). The question is whether memories of very early experiences that are alleged to result in aberrant adult outcomes are still available to consciousness or are lost to infantile amnesia, potentially exerting their effects without the person's awareness (also see Kagan, 1996).

In the nonhuman animal literature, infantile amnesia refers to the faster forgetting that is associated with immature organisms than with more mature members of the species. This more rapid forgetting in younger than older animals occurs even when levels of initial learning have been equated (see Campbell and Spear, 1972; Arnold and Spear, 1997). Much of the animal research on accelerated forgetting during infancy has been

conducted on the rat, with the early consensus being that young pups were more susceptible to interference effects in memory (Smith and Spear, 1981), a condition also thought to pertain to young humans (Kail, 2002).

Although increased susceptibility to interference effects is certainly one possible contributor to the frailty of early memory, it is by no means the sole factor. Indeed, depending upon encoding and testing conditions, young humans may be no more susceptible to interference than older children and adults (e.g., Howe, 2000; Rovee-Collier et al., 2001). As we will see, the waning of infantile amnesia is the result of changes to a number of basic memory processes, ones that emerge from correlated changes in cognitive structures. To see how, I review the latest research concerning neurobiological and behavioral underpinnings of infantile amnesia in human and nonhuman animal populations.

1.15.2 Neurobiological Factors

To begin, consider how neural maturation impacts memory. Because of the high degree of overlap between human and nonhuman animal species in the development, anatomy, and function of the hippocampus (and parahippocampal regions; e.g., Ferbinteanu et al., 2006; Manns and Eichenbaum, 2006), the results of this research will be considered together. Given space limitations, I present only a brief overview of the basics of the neural underpinnings of memory development and will touch on more specific aspects as needed (more detailed reviews can be found in Serres, 2001; Gogtay et al., 2006).

Nelson (1997, 2000) has argued that there are at least two memory systems that have different developmental trajectories. The earliest to emerge is implicit (or procedural) memory, a system that is capable of performing recognition tasks such as novelty preference, habituation, classical and operant conditioning, and visual expectancy tasks. The neurological substrates that are involved in implicit memory (e.g., the hippocampus, striatum, cerebellum, and olivary–cerebellar complex) are sufficiently developed very early in postnatal life to support this form of memory basically from birth (and perhaps before).

Explicit (or declarative) memory not only depends on hippocampal structures but is also thought to depend on inputs from elaborate networks of neocortex that converge on parahippocampal structures (e.g., entorhinal cortex; Serres, 2001). These diverse inputs are then organized into a cohesive memory trace in the hippocampus. Although some of the neural structures related to explicit or declarative memory develop early, others have a more protracted developmental course, with significant changes occurring at the end of the first postnatal year and the beginning of the second year (Serres, 2001). Of particular interest is the dentate gyrus of the hippocampus, because this serves as a critical link in the circuitry that connects the parahippocampal structures to the hippocampus (specifically to the CA3 and CA1 regions). Developments are also occurring in the frontal cortex and in the reciprocal connections between the neocortex and the hippocampus (Serres, 2001). Although neurogenesis in the dentate gyrus continues throughout life (e.g., Aimone et al., 2006), the network necessary to sustain explicit memory reaches functional maturity (in humans) late in the first year of life (Nelson, 1997, 2000; Serres, 2001). Although additional refinements occur throughout development, by the beginning of the second year of postnatal life, humans are capable of sustaining explicit or declarative recollection.

From this brief review, it would seem that the timing of these neurological changes (at least in humans) is at odds with the oft-cited empirical finding that the earliest autobiographical memories begin around the end of the second year of life (see Howe and Courage, 1993, 1997). As will become clear in later sections, neurobiological constraints may not be at the source of infantile amnesia at all. Although there are clear advances in neocortical structures that continue during early maturation, some of which might contribute, albeit indirectly, to the demise of infantile amnesia (see later discussion; Levine, 2004), I have argued previously (Howe and Courage, 1993, 1997; Howe, 2000, 2004), as has Bauer (2004), that neither age nor neurological developments control memory longevity after the basic neural 'hardware' has been laid down. Instead, the termination of infantile amnesia, like changes in other areas of memory, is controlled by alterations in the basic processes of encoding, storage, retention, and retrieval that drive memory across development and perhaps species. Although such changes may have obvious neurobiological correlates, their origins may have more to do with the development of an organism's knowledge (cognitive) structures than with additional neurobiological change by itself.

1.15.3 Behavioral and Cognitive Factors

1.15.3.1 Nonhuman Animal Populations

Research on behavioral and cognitive manifestations of infantile amnesia has focused on both faster forgetting in immature organisms and whether nonhuman animals possess autobiographical (or even episodic) memory in the first place. This latter question asks whether animals are 'stuck in time' (for a review, see Roberts, 2002). If so, then they cannot travel cognitively into the past and hence do not have what Tulving (1985, 1989, 1993) refers to as episodic memory. Tulving contrasts episodic memory and its concomitant autonoetic consciousness (personal awareness of remembering) with semantic memory and noetic consciousness in which organisms possess general information but do not experience a specific awareness of experiencing it in time. This distinction has also been linked to remember–know judgments, where remember judgments involve episodic memory because the organism remembers a particular experience having occurred in a past context, whereas know judgments involve a sense of familiarity but not a sense of personal, past experience. Although this sense of time also refers to planning for the future, the main point is whether nonhuman animals do possess the what, where, when, and perhaps who of past events. Tulving argues that, although nonhuman animals do have a well-developed knowledge of the world (e.g., general relationships between stimuli and events) derived from specific episodes, they do not code temporal information that allows them to travel back and reexperience the past episodes – that is, they have semantic memory but not episodic memory.

Clearly, it is unlikely that we will ever establish whether nonhuman animals (or preverbal humans) have autonoetic consciousness. This is because there are no agreed-upon behavioral markers of conscious experience (Griffiths et al., 1999). Hence, any model of episodic memory that requires conscious awareness must apply solely to language-using organisms. As Clayton et al. (2003a) point out, with the exception of putatively language-savvy apes and parrots, there is no litmus test that would establish that an organism was reexperiencing the past when remembering an episode.

Recognizing the futility of using phenomenological criteria in language-challenged organisms, researchers have focused on behavioral criteria in an attempt to establish that nonhuman animals (and preverbal humans) do have episodic-like memory. Specifically, there are three behavioral criteria that are consistent with Tulving's definition of episodic memory: (1) memory content, recollecting what happened where and when; (2) memory structure, having an integrated what-where-when representation; and (3) memory flexibility, deploying information in a flexible fashion (e.g., see Clayton et al., 2003a). Concerning the first criteria, the when of what-where-when seems to be particularly important in establishing that the memory is episodic. That is, a number of different episodes can all share what and where, but they cannot share when. Concerning the second criteria, what, where, and when cannot simply be linearly connected, but rather all three need to be in an integrated representation, so that retrieving any one retrieves the others. The third criteria simply states that, because episodic memories are declarative, they are by definition flexible (unlike nondeclarative memories, which are inflexible and inaccessible to consciousness). Therefore, animals should be able to flexibly deploy memories in new situations.

To date, episodic-like memories have been demonstrated in birds (e.g., western scrub jays, *Aphelocoma californica*; Clayton and Dickinson, 1998, 1999; Clayton et al., 2001, 2003c, 2005; Dally et al., 2006), rats (*Ratus norvegicus*; Eacott et al., 2005; Norman and Eacott, 2005), and gorillas (*Gorilla gorilla gorilla*; Schwartz et al., 2005). In this latter study, King, an adult male western lowland gorilla, remembered the order of past events as well as where the events occurred. Dally et al. (2006) have recently shown that western scrub jays remember not only the what-where-when of specific food caching episodes but also who (which other scrub jay) was watching them while they were hiding food, altering their recaching behavior accordingly. Episodic-like memories have also been demonstrated in pigeons (*Columba livia*; Zentall et al., 2001) and dolphins (*Tursiops truncates*; Mercado et al., 1998). Indeed, pigeons exhibited trace flexibility by accurately reporting whether they had recently been pecking when such reports were not anticipated, and dolphins too showed this flexibility by correctly reproducing actions recently performed (for a review, see Hampton and Schwartz, 2004).

Although some remain skeptical about whether these memories are episodic or even episodic-like (e.g., Roberts, 2002; Suddendorf and Busby, 2003a,b; but see Clayton et al., 2003b), questions concerning nonhuman animals' potential for autobiographical memory, at least as measured by behavioral criteria, remain an area of intense scientific debate and

research. Indeed, there are those who suggest that the anatomical details of the hippocampus and parahippocampal regions of the brain are conserved across (human and nonhuman) mammals (Manns and Eichenbaum, 2006). Moreover, these authors also claim that the functional role of these neural regions in declarative memory is also conserved across species. To the extent that the hippocampus and parahippocampal regions are critical for declarative, episodic memory (e.g., Tulving and Markowitsch, 1998; de Hoz and Wood, 2006), these across-species similarities are consistent with the claim that episodic (and perhaps autobiographical) memory may exist in a variety of mammalian species and is not special to humans. Perhaps the autonoetic component of human conscious recollection is epiphenomenal and is not a requirement of the memory itself. Indeed, Ferbinteanu et al. (2006) argue that autonoetic experience is a feature of human consciousness and not an aspect of episodic memory per se. Hence, episodic memory may exist in many species and can be measured behaviorally in human and nonhuman species alike. Certainly, as Manns and Eichenbaum (2006) point out, there are differences across species in the psychological properties of episodic memories (perhaps including autonoetic experiences), but these may be due to other differences in neocortical inputs to the hippocampus and parahippocampal regions, as well as the neocortical circuitry that mediates their outputs, determining their behavioral expression, rather than to differences between species in the hippocampal and parahippocampal regions themselves.

Although there may be some additional research needed before we can close the book on episodic memory in nonhuman animals, that these same organisms experience infantile amnesia is not contentious, although like episodic memory itself, the cause of this amnesia may not be the same across human and nonhuman animal species. Research by Richardson and colleagues (Richardson et al., 1983, 1986; Westbrook et al., 2002; Yap et al., 2005; Kim et al., 2006; Weber et al., 2006) with rat pups has shown more rapid forgetting in younger rats than older rats. More important, this research has shown that many of the behavioral markers indexing infantile amnesia in human infants are similar to those observed in nonhuman animals. For example, both human and nonhuman infants show faster forgetting than older members of the species and increased recollection following reinstatement treatments. This latter phenomenon was thought to rule out storage-failure hypotheses of infantile amnesia and instead favor retrieval failure mechanisms, because memories cannot be reactivated if they are not somehow present in storage. For example, the effects of infantile amnesia have been mitigated by injecting naloxone prior to training a fear response (Weber et al., 2006), indicating that central opioid receptors regulate retrieval of fear memories in rat pups. Similarly, in a recent article, Kim et al. (2006) have shown that, although younger rat pups retain fear conditioning for relatively short periods of time, administration of the gamma-aminobutyric $acid_A$ ($GABA_A$) receptor partial inverse agonist FG7142 alleviated infantile amnesia. It appears as though FG7142 facilitated the retrieval of a forgotten memory, showing for the first time that GABA receptors play a central role in the forgetting of fear memories from infancy.

Theoretically, these findings are critical to our thinking about infantile amnesia and memory continuity. Specifically, because GABA receptors play a role more generally in forgetting regardless of the age of the organism, perhaps forgetting early in life (infantile amnesia) and forgetting in adulthood simply represent quantitative variation rather than qualitative differences in forgetting mechanisms. Although certainly not conclusive, the fact that the same treatment that facilitates retrieval in adults also facilitates infant memory is more consistent with the memory-continuity hypothesis.

Of course, there is considerable evidence that storage factors play a role as well, because of the more rapid forgetting of information by more immature organisms. The longer an early memory has been in storage without reminders, the more likely it is to have decayed. Indeed, early memories that have been forgotten for longer periods of time are more difficult to recover than memories stored for shorter periods of time (e.g., Joh et al., 2002). Moreover, there is evidence that posttraining injections of pharmacological agents (glucose, epinephrine, norepinephrine) known to facilitate storage and consolidation in adult rats (Gold et al., 1982; Flint and Riccio, 1999) also mitigate the effects of infantile amnesia in rat pups. Thus, both storage and retrieval play roles in infantile amnesia, at least in nonhuman animals.

Finally, Richardson and his colleagues have shown that despite infantile amnesia, induction of fear responses in rat pups remains time-locked in memory. Specifically, for rats, fear is expressed in age-appropriate ways, ones that unfold with development (Yap et al., 2005). As it turns out, learned fear is expressed in terms of the age at which the animal was trained (time

of encoding), not at the age at which it was tested (Richardson et al., 2000; Richardson and Fan, 2002). Interestingly, these memories can be updated by subsequent experience. That is, memory updating can occur, and the expression of fear can be recalibrated to a more mature response, if a similar fear experience occurs at an older age (Yap et al., 2005). These results have implications for whether and how single versus multiple fear events (including acute versus chronic adverse experiences) occurring early in life may be remembered and expressed. Moreover, for language-using organisms, these results raise the question of whether experiences that have occurred before the vocabulary (receptive or productive) necessary to communicate those experiences can ever be expressed linguistically. If responses are truly time-locked at encoding, and language is not available at that time, then the answer to this question is no. I return to this point in the next section, when I consider behavioral and cognitive factors relevant to infantile amnesia human populations.

1.15.3.2 Human Populations

Like their nonhuman counterparts, studies of memory with preverbal human infants are difficult at the best of times. This is because memory tasks must be nonverbal in nature, in terms of both the task instructions and also the types of responses indexing remembering. Moreover, like nonhuman animals, preverbal human infants cannot provide us with an unambiguous indication of their conscious state during recollection. Despite these difficulties, like studies with nonhuman animals, experiments using a wide range of tasks have established beyond a reasonable doubt that human infants do possess declarative, episodic memory if not before the end of the first year of life, shortly thereafter.

Although a complete review of techniques and outcomes from infant memory research is beyond the scope of this chapter, comprehensive overviews are readily accessible (Rovee-Collier et al., 2001; Howe et al., 2003; Courage and Howe, 2004; Hayne, 2004). Instead, I will focus on several key paradigms and outcomes that illustrate the precocity of infant memory and auger well for our discussion of infantile amnesia. To begin, there are a variety of reports concerning memory for operantly conditioned events very early in life, including memory for *in utero* conditioning. For example, DeCasper and his associates have shown that newborn infants can recognize the prosodic characteristics of a story heard in the last trimester of their prenatal life and have determined the factors that affect this recognition memory (DeCasper and Fifer, 1980; Decasper and Prescott, 1984; DeCasper and Spence, 1986, 1991; Spence, 1996; Spence and Freeman, 1996).

Infants are also willing to imitate motor activities performed by an adult or peer. For example, Meltzoff and colleagues demonstrated that 6-week-olds reproduce various facial expressions and head movements modeled by an adult over a 24-h retention interval (Meltzoff and Moore, 1994). As infants mature, they can model more and more complex sequences over longer and longer delays (for a review, see Meltzoff, 1995). A strength of Meltzoff's work is that the activities performed were novel, were modeled without instruction, and were not performed by infants prior to the retention test. Thus, imitation was likely based on stored representations of previous experience and as such indexes recall (declarative memory) not recognition.

In all, experiments using conjugate reinforcement (Rovee-Collier et al., 2001), novelty preference (Courage and Howe, 1998; Courage et al., 2004), deferred imitation (Meltzoff, 1995; Hayne, 2004), elicited imitation (Bauer, 2004), and behavioral reenactment (McDonough and Mandler, 1994; Sheffield and Hudson, 1994) indicate that, like the findings for nonhuman animals: (1) when initial learning is equated, infant age and longevity of memory are positively correlated; (2) length of retention is affected by factors such as distribution of practice (spaced better than massed) and the match between proximal and distal cues at encoding and test; and (3) retention can be prolonged given appropriate reinstatement treatments. Thus, although fragile and short lasting, even very young infants can demonstrate declarative memory. As the neural structures that subserve declarative memory mature (see earlier sections in this chapter), declarative memories become longer lasting and less susceptible to decay and interference.

Although impressive, infants' encoding, storage, retention, and retrieval feats are still not as robust as those of children who have matured beyond the infantile amnesia threshold. Indeed, experiences before the age of 2 years appear to remain mysteriously 'time-locked' and inaccessible to conscious recall. Recollection, if any, is disjointed and fragmentary at best (Bruce et al., 2005) and may not be verbalizable (Simcock and Hayne, 2002). However, as has been known for a long time, at around the age of 2 years, memories become more stable and can be retrieved later in childhood and adulthood (Dudycha and

Dudycha, 1933; Kihlstrom and Harackiewicz, 1982; Usher and Neisser, 1993; Eacott and Crawley, 1998; Newcombe et al., 2000; Crawley and Eacott, 2006). Although often researchers will probe memories for specific, ostensibly significant events (e.g., birth of a sibling, death, traumatic experience), there does not appear to be any specific trend in the content of earliest memories. That is, although such recollections are not of mundane, everyday events (e.g., what one had for breakfast on the third day of December when one was 2 years old), neither do they have to be particularly traumatic (e.g., Bruce et al., 2005). Indeed, the recollections we have from around the age of two are often decontextualized segments of the past that are simple recollections of sensory experiences, behaviors and actions, or a feeling (Bruce et al., 2005).

As seen earlier in the neurobiological section, neurological changes can certainly drive many of the developments in memory that occur during the first postnatal year, but they cannot, by themselves, explain the changes in memory longevity observed in human episodic memory. Instead, we have to examine corresponding changes in cognitive development. Although many such changes exist around this transitional period (see Courage and Howe, 2002), the key development driving autobiographical memory is the onset of the cognitive self (Howe and Courage, 1993, 1997).

Briefly, consistent with trace-integrity theory (Howe, 2000), storage and retrieval lie on a single continuum, where traces consist of collections of primitive elements (e.g., features, nodes) that are integrated into a single cohesive (neural) structure in memory. The better integrated a trace is, the better it is retained over time and the less susceptible it is to interference. The less integrated a trace is, the more likely it is to recede into the background noise of other faded memories, losing its distinctiveness and hence its inherent memorability. Although these assumptions are consistent with most models of memory, it does organize a rather large literature on children's memory development more broadly and accounts for the offset of infantile amnesia and the onset of autobiographical memory, at least in humans. More specifically, as our proficiency with organizing information increases with changes in our knowledge structures, we are better able to group information in memory into cohesive structures that are more stable and relatively permanent. The key event relevant to autobiographical memory is the self – that is, you cannot have an autobiography until you have a self. As it turns out, there is considerable research that indicates that there is a dramatic shift in the development of the self that occurs around 18 to 24 months, one that results in the advent of the cognitive self (for a recent review, see Howe et al., in press). Although the specific details are not critical here, the main point is that, because a recognizable self contributes features that increase the cohesion of a memory trace, such traces are no longer event memories but memories of events that happened to me. That is, they are autobiographical.

Interestingly, it may be no coincidence that there exist corresponding neurological developments relevant to this expanding knowledge base of the self. Indeed, prefrontal areas related to autobiographical recall and the self are undergoing developmental change – for example, cortical gray matter volume increases until age four and then declines (synatogenesis followed by pruning; see Pfefferbaum et al., 1994). Functional neuroimaging studies have shown that portions of the anteromedial prefrontal cortex are involved in processing self-related information and autobiographical recall (see Levine, 2004). Although I would not argue that there is any direct link between these neurobiological changes and the offset of infantile amnesia, given that the mind is somehow a function of the brain (Levine, 2004), the cognitive advances that drive the onset of autobiographical memory may have their neural correlates in early changes in the prefrontal cortex.

Behavioral evidence has been accumulating over the past decade or so that also indicates that the self plays a key role in the onset of human autobiographical memory. For example, Harley and Reese (1999) found that the critical event related to the onset of autobiographical memory was the advent of the cognitive self (as measured by mirror self-recognition) and not performance on language measures, deferred imitation tests, or sociolinguistic measures (e.g., style of maternal reminiscing). More recently, Howe et al. (2003) reported that long-term retention of a unique event was also contingent on children having a cognitive self and not on other, related measures (e.g., language). Most recently, Prudhomme (2005) found that the cognitive self was essential for early declarative, autobiographical memory. Indeed, Prudhomme found that children with a cognitive self were not only better than those without a cognitive self on an elicited memory task, but they were also much more flexible when retrieving information.

Consistent with the theoretical approach advocated by Howe and Courage (Howe and Courage 1993, 1997; Howe et al., in press), this research makes clear that early autobiographical memory in

humans is contingent on cognitive advances, specifically the advent of the cognitive self. As children mature, the number of autobiographical events that can be retained increases due to a whole host of reasons related to memory development more generally, a discussion of which is beyond the mandate of this chapter (but see Howe, 2004; Howe et al., in press). However, an important question remains, namely what happens to memories from the infantile amnesia period. Although already acknowledged as being fragmentary at best, do they remain time-locked, or can they be later retrieved and recoded using newly acquired language skills? For humans, the period of infantile amnesia is also one in which language skills are relatively impoverished. Language acquisition was thought by many to herald the end of infantile amnesia (see Allport, 1937; Schachtel, 1947). Some current theories of infantile amnesia also emphasize the importance of language (see Harley and Reese, 1999). However, to the extent that infantile amnesia is similar in human and nonhuman animals, the role of language in the offset of infantile amnesia may be epiphenomenal. Indeed, the current evidence indicates that language does not play a causative role in the offset of infantile amnesia. However, language is one way in which humans rehearse, elaborate, preserve, and communicate memories, including autobiographical ones. So although it may not be germane to our nonhuman counterparts, it may be one of those neocortical inputs and outputs to the hippocampal and parahippocampal areas that varies across species and is important to later developments in autobiographical memory.

Curiously, although infants can recall past events behaviorally, there is little evidence that their memories are accessible to verbal report if they were laid down prior to the offset of infantile amnesia. Some studies that have shown verbal reports of early memories tested recall under conditions of high contextual support (e.g., Bauer and Wewerka, 1995, 1997). Other studies in which the degree of contextual support was much less have failed to produce verbal reports of early memories (Simcock and Hayne, 2002, 2003). Interestingly, in these latter studies, although children clearly had acquired the vocabulary to report the prior events, verbal ability was not related to memory performance. That is, children's sparse verbal reports of previous events were not due to poor memory per se, as these same children were able to accurately recognize photographs of the previous event as well as reproduce them behaviorally.

Simcock and Hayne's (2002, 2003) research provided considerably less contextual support than Bauer and Wewerka's (1995, 1997) studies. Could it be that contextual support is the key to 'helping' memories make the transition across the infantile amnesia barrier? More recent studies suggest this might be the case. For example, Bauer et al. (2004) found evidence of verbal reports of early memories only when event-related props, but not color photographs (as in the Simcock and Hayne studies), were provided as cues. Interestingly, verbal reports were not obtained from children who only experienced the events once. What this might indicate is that multiple exposures are necessary for events to cross the infantile amnesia threshold. Of course rehearsal is important in memory generally, and events that are repeated are better remembered than those that are not repeated.

More importantly, verbal evidence of memory was seen only in those children who were 20 months old at the time of the original experience and not those who were 13 or 16 months old at that time. Similar findings have been reported in other studies (e.g., Bauer et al., 1998, 2002) and are critical to the argument presented here concerning the importance of the cognitive self. Although language measures have been obtained in each of these studies (to insure adequate vocabulary to express the events), measures of the cognitive self have not been secured. However, it is clear from the vast literature on the development of the cognitive self (for a review, see Courage et al., 2004; Howe et al., in press) that the 13- and 16-month-olds who failed to exhibit later verbal recall were below this cutoff at the time the event was first experienced, whereas those who did evince verbal recall, the 20-month-olds, were above this cognitive self threshold. A similar finding has been reported by Morris and Baker-Ward (2007) using physical props (e.g., a bubble-making machine) to reinstate the encoding context in 2-year-olds; however, again, a test of the children's cognitive self was not administered.

1.15.4 Conclusions

Several conclusions emerge from this review of infantile amnesia. First, the recent literature on neural structures related to memory (particularly the hippocampus and parahippocampal regions) makes it clear that we share certain anatomical, functional, and developmental similarities with nonhuman animal species. Although there is clearly room for across-species variation in performance and

the manner in which memories are expressed, due to well-documented and substantial differences in neocortical inputs and outputs, the marked similarity in hippocampal (and parahippocampal) anatomy, function, and development is unmistakable.

Second, perhaps because of our common hippocampal (and parahippocampal) neuroanatomy, functionality, and developmental trajectory, we share with our nonhuman animal counterparts episodic or episodic-like memory, a memory like others that develops with maturation. Regardless of species, human and nonhuman animals alike can code the what, where, when, and even who of past events and use that information to guide behavior. Although the conscious, autonoetic component of recollecting autobiographical memories may be uniquely human, something that may arise given the considerable across-species differences in neocortical structures, that we share episodic memory with other species is not in doubt.

Third, and perhaps because of our common episodic memory capabilities, we also share with other species one of the limitations of early episodic memory, infantile amnesia. That is, there is faster forgetting of information than in more mature organisms, memories that are laid down are more susceptible to interference in younger than older members of the species, and for those species that recall past events, memories for early events are less accessible later in life than memories for events that occur when we are older. For humans, this infantile amnesia abates at around 2 years of age and is aided by the advent of the cognitive self. This perhaps uniquely human construct may have its roots in additional neural development in the prefrontal cortex. This is consistent with the neurobiological evidence reviewed in this chapter, as well as with the idea that across-species variation in memory (and infantile amnesia) is due to across-species variation in neocortical structures.

Because of this across-species similarity in episodic memory and infantile amnesia, it would seem to be intellectual folly to explain the end of infantile amnesia in uniquely human terms. For example, Hayne (2004) has argued that such similarities force a basic process explanation. That is, the waning of infantile amnesia must occur at the level of changes to encoding, storage, consolidation, or retrieval from episodic memory. To the extent that species-unique capacities play a role in the ebbing of infantile amnesia, they do so only inasmuch as they affect these basic processes.

This memory-continuity view has been favored by a number of early memory researchers (see Howe and Courage, 1993, 1997; Howe, 2000, 2004; Bauer, 2004, 2005; Howe et al., in press). For humans, the capacity to better organize information in memory is the key to most, if not all, memory advances in childhood. Advances in children's knowledge structures, whether they have to do with the self or other constructs (e.g., animals, vehicles), aid children's organization and storage of incoming information, making memory traces more integrated and hence more resistant to forgetting. As we have seen in this chapter, the neurological components necessary for declarative/episodic memory are in place and operating by the end of the first year of life if not before. What drives memory development are changes in knowledge (cognitive) structures that subserve encoding, storage, retention, and retrieval, making these basic processes more robust. For the offset of infantile amnesia and the onset of autobiographical memory, this knowledge structure is the cognitive self.

It is perhaps of more than passing interest to note that the cognitive self may be present in species other than humans. The quintessential test for the cognitive self is mirror self-recognition. Results of this test have been reported for a number of nonhuman species of fish, birds, and mammals (for a review, see Gallup, 1979). Although most creatures respond toward the image as if they were viewing a conspecific, nonhuman primates (given a period of exposure to mirrors) will respond to the contingent movement cues and use the reflective properties to locate objects. However, chimpanzees and orangutans demonstrate self-recognition like humans. Although speculative, it may be that the end of infantile amnesia in some other species is also heralded by the acquisition of the cognitive self. Regardless, the common ingredient that brings about the end of infantile amnesia is some change in the organisms' ability to encode, store, consolidate, retain, or retrieve event memories. Whether that is through the advent of new knowledge (cognitive) structures or some other (cognitive) mechanism is unknown. That the various species possess different neocortical structures that moderate hippocampal (and parahippocampal) functions may provide some insight as to what those mechanisms are that modulate basic episodic memory processing and end infantile amnesia. This is the grist for future research.

To close, I refer to another unresolved issue that needs further research, and that is: what is the fate of early memories? As we saw earlier, events that were experienced once were not subsequently recollected (at least not verbally) in Bauer et al.'s (2004) study, nor were events that took place earlier than 20 months

of age. Of course, these were not traumatic or life-changing experiences, but rather, innocuous laboratory-contrived events. However, there is converging evidence from adult participants that was also reviewed here that showed that recall of events, traumatic and otherwise, was limited by infantile amnesia across the first 2 years of life (e.g., Usher and Neisser, 1993; Eacott and Crawley, 1998). So the question remains, do memories for early events remain in storage despite our inability to bring them to consciousness during later childhood or adulthood?

Recall Kagan's (1996) discussion of the fate of early experience and how many theorists believe that, despite our inability to recollect early life events, they remain with us and shape our future behaviors. It is clear that early experience, by itself, does not determine future outcomes, as subsequent experience can alter that path. Even the bizarre behavior of 6-month-old isolated macaques was altered by placing them with younger female monkeys over a 4- to 5-month period (Suomi and Harlow, 1972). Indeed, there is a vast literature that shows that a strong form of early experiential determinism is not tenable (also see Howe, 2000). In light of this, there may be little reason to believe that these early experiences still exist in memory and have not been updated by more recent (and perhaps relevant) experiences.

However, recall from the discussion about neural development that in humans, at least, implicit memory precedes the development of explicit or declarative memory. Is it possible that implicit memories that have not been altered by subsequent experience (should such a thing exist) are still accessible implicitly and form the basis of our adult responses? Although there is some evidence that early implicit memories may still exist in humans (Newcombe et al., 2000), stronger evidence was presented earlier in this chapter for early fear responses in rats (e.g., Yap et al., 2005). If early postnatal fear responses are not altered by subsequent experience, then it is likely that earlier, immature conditioned responses might still predominate in the more mature organism. Because these response patterns are implicit, the organism would not be aware of the early experience, nor would it know why a particular response was occurring, something that may be akin to the apparent, sudden emergence of a phobic response. More importantly, implicit (as well as explicit) memories of early adverse experiences might be one source for later psychopathology in adulthood.

Unfortunately, here again, the evidence is wanting – however 'pleasing' such ideas may be, the literature is replete with examples that infirm rather than confirm such intuitions (for reviews, see Hardt and Rutter, 2004; Howe et al., 2006). What is not clear is how such memories are altered by subsequent experience and what the parameters are that drive such dynamic change in memory, regardless of species. Only subsequent research will clarify the fate of these early memories, particularly the ones that are laid down during the period of infantile amnesia. I suspect their fate is similar to that of early explicit memories and that they are as malleable and updatable as all memories throughout an organism's development.

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1.16 Transmission of Acquired Information in Nonhuman Primates

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1.16.1 Introduction

The development of human culture and technology largely rests on our ability to accumulate information about past experiences, store it, and transmit it to others, thus enabling a cumulative cultural evolution where innovations are based on previous innovations. Literally speaking, the ability to transmit acquired information spares us having to reinvent the wheel over and over again. What is the biological basis for this ability? Are any of these abilities shared with other species? Is there evidence for 'teaching' among animals? Do animals possess different cultures, and if so, how do they come about? Are animals informing each other about past experiences or events? These are the questions that I will address in the present article. My overview will focus on nonhuman primates because they are our closest living relatives, and thus, any comparative study will have to ask which of our abilities are shared with them; in addition, most of the evidence has indeed been collected on this taxon. To maintain a broad perspective, I will draw from studies on other animals whenever feasible.

The first part of this article will be devoted to the question of animal culture. I will begin with a discussion of the terminology used in this field and then review the evidence accumulated over the last five decades. The second part charts the communicative abilities of nonhuman primates, particularly in the vocal domain – one of the most important modes of information transmission in humans. This section will begin with the question of whether nonhuman primate signals refer to objects or events in the animals' environment, discuss the issue of vocal learning as well as the importance of auditory feedback, and

then review studies that addressed the issue of whether monkeys or apes intentionally provide information to others. The third part of this article turns to the mechanisms that give rise to the observed variations in behavior, including the question of teaching among animals. My review will conclude with a discussion of how communicative skills and the development of culture are intertwined. I will argue that, in both animal communication and animal culture, there is a fundamental difference between the sender (or actor) on the one hand, and the listener (or observer) on the other. It appears that animals are good at seeking information, processing it, and adjusting their behavior accordingly, but what they apparently do not do is to provide information with the intention to alter the knowledge state of others. To get cumulative cultural evolution off the ground, more is needed than social learning; among other things, an understanding of other individuals' knowledge states and intentions, as well as a symbol system to represent and transmit accumulated knowledge. To date, this appears to be found only in our own species.

1.16.2 Animal Culture

1.16.2.1 Definition

How one approaches the question of the evolution of culture depends very much on the framework used. An anthropocentric research program might begin with a definition of human culture and then go on to ask which aspects of this phenomenon are shared with animals. An ecological research program instead might begin with charting behavioral diversity and then go on to identify the factors that give rise to this variation. Both approaches would greatly benefit from a clear definition of the phenomenon under study; however, there is no single agreed definition to work with. One frequently cited definition (e.g., Goodall, 1973; Van Schaik et al., 2003; McGrew, 2004) is that by Edward Tylor, who described culture as "that complex whole which includes knowledge, belief, art, law, morals, custom, and any other capabilities and habits acquired by man as a member of society" (Tylor, 1871, p. 1). Early on, it became clear that this definition was of little use to people who studied animal behavioral diversity. In the 1950s, the Japanese primatologist Kinji Imanishi proposed a broader definition in which he described as cultural behaviors all flexible behavior patterns that were socially transmitted (cf. Jolly, 2001). Hans Kummer, one of the pioneers of primatological research, followed a similar line of argument. He suggested considering all behaviors as cultural that were socially transmitted and which remained in the populations ("If such social modification spreads and perpetuates a particular behavioral variant over many generations, then we have 'culture' in the broad sense in which a student of animals can use the term"; Kummer, 1971, p. 13). This view still resonates today, for instance in the definition by Laland and Janik, who describe as cultural all "group-typical behavior patterns, shared by members of animal communities, that are to some degree reliant on socially learned and transmitted information" (Laland and Janik, 2006, p. 542). The eminent cultural primatologist Bill McGrew took a somewhat different stance and defined culture as "the way we do things" (McGrew, 2004, p. 25), specifically referring to forms of behavior or artifacts that are standardized, or even stylized, and socially significant.

Not surprisingly, these broad definitions were later contested (Tomasello, 1994), and there is an ongoing and sometimes fierce debate about the terminology used when people chart and discuss behavioral diversity (Fragaszy and Perry, 2003). Andy Whiten argued that it is just an issue of definition whether or not we label the outcome of social learning as culture, while Kevin Laland and Vincent Janik find a broad definition simply more stimulating (Laland and Janik, 2006). The problem with a broad definition begins when people aim to study the evolution of human culture and implicitly operate with the same terminology for two possibly quite different phenomena. While some earlier researchers used the terms protoculture or preculture when they referred to animal behavioral diversity, this specification was at some point dropped. As a result, even proponents of a broad definition struggle with the distinction of their findings for animals versus for humans, and not surprisingly, our own species has recently been described as 'super-cultural' (Whiten, 2005, p. 54).

In addition, it is obviously logically flawed to herald observations of animal culture as signs for sharing the trait 'culture' with humans, as long as two different definitions are applied. For want of a better definition, I will in the following use the term animal culture to refer to behavioral variation among animals that appears to be socially mediated. I do not assume that animal culture and human culture are homologous, and I may note here that, in my view, human culture as a phenomenon is too complex and elusive to lend itself to a meaningful phylogenetic reconstruction. Instead, it seems more productive to

investigate the mechanisms that give rise to animal culture or human culture. In a second step, one may then identify which of these traits are shared among taxa, constituting either homologies or convergences that may have arisen to similar ecological pressures. This eventually leads to an understanding of which aspects constitute derived traits of the human lineage.

1.16.2.2 History

The interest in animal culture was sparked when Japanese researchers reported that Japanese macaques, *Macaca fuscata*, on the island of Koshima had begun to wash the sweet potatoes that had been fed to them to habituate them to the presence of human observers and lure them to the beach, where they could be more easily observed. One day, a young female named Imo waded into the water and dipped the potato into the water to get rid of the sand. This habit slowly spread through the group – 2 years later, four more monkeys were regularly washing their potatoes, and 1 additional year on, 11 out of the 30 group members had been observed to clean their food that way (Kawamura, 1959). At the end of the 1950s, the researchers observed another food processing technique that slowly spread trough the study group. Again, it was Imo who first threw wheat that had been spread on the beach into the water. The sticky sand was thus washed off, and she could scoop the clean grains out of the water. Both habits did not spread randomly within the group; subjects that interacted more frequently with one another tended to adopt this new behavior earlier than subjects with only infrequent contacts (Kawai, 1965). Kawai was the first to introduce the term 'precultural behavior' into the debate. In the Western world, the question of animal culture became a hot topic in the 1970s. At the fourth conference of the International Primatological Society in Nairobi in 1971, a workshop was devoted to the topic of "Precultural Primate Behavior." Jane Goodall, who had begun her studies of the Gombe chimpanzees, *Pan troglodytes*, in 1960, presented a paper entitled "Cultural Elements in a Chimpanzee Community" (Goodall, 1973), thus advancing this great ape species to center stage.

1.16.2.3 Recent Advances

In the last 15 years, the field of primatology has experienced an explosion of publications on the topic of cultural behavior among nonhuman primates. In his 1992 book, *Chimpanzee Material Culture*, Bill McGrew gave an overview on the differences in tool use in different chimpanzee populations (McGrew, 1992). West African chimpanzees, for instance, crack nuts using stones as hammers and other stones or roots as anvils (**Figure 1**). East African chimpanzees, in contrast, have never been observed to crack nuts, despite the fact that nuts occur in their habitat. Another behavior pattern is the so-called 'grooming hand clasp,' which was routinely observed at Mahale but absent at Gombe. There are also different variations of termite fishing. For instance, subjects at Taï use rather short sticks and insert them into termite mounds, and they pull the sticks through the mouth to harvest the termites, while subjects at Gombe pick up the termites with their hands (reviewed in McGrew, 2004). A comprehensive survey of the different variants of tool use and social behavior was initiated by Andy Whiten from the University of St. Andrews. Researchers who had observed wild chimpanzees for decades in seven different areas compiled a catalogue of behavioral patterns. They identified 39 different behavioral patterns which were either observed in only one or a few of the sites taken into account in this study and – importantly – could apparently not be related to the ecological conditions or genetic variation among subjects. Thus, the observation of behavioral variation could be well substantiated (Whiten et al., 1999). In the meantime, the interest in the so-called cultural variation has been extended to other species such as orangutans, *Pongo pygmaeus* (Van Schaik et al., 2003), and capuchin monkeys, *Cebus spp.* (Perry et al.,

Figure 1 Nut cracking in West African chimpanzees. Drawing by JF from a photograph taken by Roman Wittig, with permission.

2003; Fragaszy et al., 2004). However, the so-called 'ethnographic record' has recently been criticized by Laland and Janik (2006), who argue that it is logically impossible to rule out that some unknown ecological or genetic factor may account for the observed variation.

Cultural behavior (in the broad sense) is not restricted to the primate order. New Caledonian crows, *Corvus moneduloides*, for instance, manufacture tools from pandanus leaves (Hunt, 1996). With astonishing precision, the crows cut and tear the edges of barbed leaves to produce flat tools that are broad at one end and thin at the other, where they can be used for probing holes to harvest food. In addition, they make tools from a range of materials like tree twigs, small stems, and vines (Hunt, 1996). Hunt and Gray (2003) found that crows from different areas of New Caledonia produced leaf tools of different complexity. The authors concluded that different subpopulations apparently had developed varying degrees of expertise, indicative of a cumulative cultural transmission of the techniques used to manufacture these tools (Hunt and Gray, 2003). The crows' manufacturing skills were recently tested experimentally by Kacelnik and colleagues at Oxford University. Their studies indicate that social learning is not necessarily a prerequisite to develop the skill to manufacture hooks or choose the appropriate tool (Kenward et al., 2005; Weir et al., 2002, reviewed in Kacelnik et al., 2006), casting some doubt whether the observed variation in leaf production is indeed a sign of cumulative cultural evolution.

1.16.2.4 Dialects

Depending on the definition employed, dialects or regional variations in vocal behavior may be subsumed under the label of cultural variation. Several studies have suggested the existence of group-specific call characteristics in nonhuman primates that apparently could not be attributed to ecological or genetic variation (but see Laland and Janik, 2006). One of the earliest such reports came from Green (1975), who observed that three different populations of Japanese macaques produced acoustically different calls. Pygmy marmosets, *Cebuella pygmaea*, modified their trill vocalizations when placed with a new partner in such a way that the calls of both partners became more similar to each other than before pairing (Snowdon and Elowson, 1999). The alarm calls given by members of two populations of Barbary macaques, *M. sylvanus*, revealed significant variation between sites. Playback experiments in which calls from their own or the other population were broadcast suggested that this observed variation was perceptually salient (Fischer et al., 1998). Chimpanzees also reveal differences in the acoustic structure of their pant-hoots (e.g., Mitani et al., 1992; Crockford et al., 2004).

Since learning is not a prerequisite to develop the species-specific repertoire, it is not quite clear how group-specific calls come about. Possible mechanisms will be discussed below. In contrast, whenever learning from models is mandatory, such as in the acquisition of bird song, local variation is almost inevitable. Songbirds (of the temperate regions) learn their songs as nestlings, either from their father or other singing males in the neighborhood (*See* Chapter 1.17), and hence, specific local variation, which may initially occur as a result of a copying error, can be transmitted to subsequent generations. In addition, song elements copied from other species may be incorporated into the vocal repertoire of a species (Catchpole and Slater, 1995). Another example for a regional dialect is the song of killer whales or orcas. Orcas live in groups or pods of several matrilines, and their vocalizations reveal group-specific as well as population-specific signatures (Deecke et al., 2000; Riesch et al., 2006).

1.16.3 Primate Communication

1.16.3.1 Referential Signaling

Do monkeys or apes talk about things? Are there any indications that they refer to objects and events in the external world? Does their communication have a semantic quality? These questions experienced a boost when Tom Struhsaker reported that vervet monkeys, *Chlorocebus aethiops*, living in the Amboseli National Park in Kenya produce three acoustically distinct alarm calls and three different adaptive escape strategies in response to their three main predators: leopards, eagles, and snakes (Struhsaker, 1967). This led to the question of whether the vervets denote the predator type when they give a predator-specific alarm call. Playback experiments revealed that upon hearing an alarm call, the monkeys selected the appropriate response, even when there was no predator around. The authors concluded that the vervets denoted the predator type, or that they encoded the required response (Seyfarth et al., 1980). Either way, the alarm calls were deemed to be referential, because they apparently referred to either objects or escape strategies external to the

signaler herself. In due course, the question of semantic communication became a hot topic, and numerous studies were initiated that addressed the question of the meaning of animal signals (Zuberbühler, 2006).

While general theoretical accounts of communication (e.g., Shannon and Weaver, 1949) explicitly abstained from addressing the issue of signal meaning, such analyses as well as the work by John Smith (1977) stressed the importance of distinguishing between the role of the sender who generates the message and the recipient who interprets the signal. Accordingly, the problem with the initial conclusion (that callers may have denoted the predator type) was that the information provided in the signal was inferred from listeners' responses. The early studies on semantic or referential communication failed to differentiate between these two roles, and traces of this confusion can still be found today. Subsequent analyses, however, pointed out that, for such a seemingly referential communication system to function, signals simply have to be sufficiently specific (Macedonia and Evans, 1993). If this is the case, recipients can use these signals to predict subsequent events and choose their responses accordingly (Macedonia and Evans, 1993; Seyfarth and Cheney, 2003). Two sets of questions follow from this insight: first, how much control do primates have over their vocal production to produce specific sounds in specific situations; and second, how do primates (or other animals) attach meaning to sounds?

1.16.3.2 Learning

1.16.3.2.1 Vocal Production

Studies of the ontogeny of vocal production as well as the neurobiological foundations of vocal control in nonhuman primates suggest that the structure of primate vocalizations is largely innate. Unlike in most songbirds (*See* Chapter 1.17), exposure to species-specific calls is not a prerequisite for the proper development of the vocal repertoire (reviewed in Fischer, 2002). Nevertheless, developmental modifications occur. These can be mainly related to growth in body size. For instance, from the first week of life, rhesus macaque, *M. mulatta*, infants produced coo calls with a largely adult-like structure. With increasing age, however, the fundamental frequency dropped in pitch and the modulation of the fundamental frequency and the call amplitude decreased, while call duration slightly increased. Weight was the best predictor for almost all of the observed age-related changes (Hammerschmidt et al., 2000). Using a larger age range of subjects, Ey and colleagues analyzed the changes in the structure of contact barks in a cross-sectional study of 58 free-ranging Chacma baboons, *Papio hamadryas ursinus*. Baboon clear calls are tonal and harmonically rich (**Figure 2**). An acoustic analysis revealed that the duration of the calls and the mean fundamental frequency varied significantly with age. With increasing age, animals uttered longer calls with a lower fundamental frequency. These two variables also showed a significant interaction between age and sex, indicating that the profiles of age-related variations differed between the sexes (Ey et al., in press). The emergence of sexual differences corresponds to the onset of sexual dimorphism in body size and mass, suggesting that testosterone levels may play a role in driving changes in acoustic structure. In sum, there is ample evidence that primate vocalizations experience only minor structural changes during ontogeny. Most changes can be attributed to growth, and some also to hormonal changes during puberty, which may affect the usage and structure of certain signals.

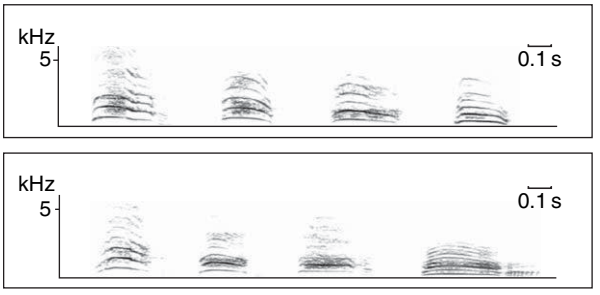

Figure 2 Spectrograms of Chacma baboon contact barks (frequency on the y-axis, time on the x-axis). The top row shows examples of calls recorded from females of different age classes (from left to right: infant, juvenile, adolescent, adult). The bottom row shows examples of male calls from the corresponding age classes.

1.16.3.2.2 Comprehension

The previous sections on modifications in the production and/or usage of calls showed that nonhuman primates apparently exhibit only little plasticity in their calls. In contrast, studies that examined the development of the comprehension of and correct responses to calls, indicated that subjects undergo pronounced changes in development. Most of the earlier work has focused on the development of vervet infants' responses to different alarm calls given to the main predators. Playback experiments showed that infants gradually develop the appropriate responses to alarm calls, and at an age of about 6 months, young vervets behaved like adults. Further evidence for a gradual development of responses comes from a study of infant baboons. Subjects developed the ability to discriminate between calls that fall along a graded acoustic continuum, as evidenced by the time spent looking toward the concealed speaker. At 2.5 months of age, infants did not respond at all to the playback of alarm or contact barks. At 4 months of age, they sometimes responded, but irrespective of the call type presented. By 6 months of age, infants reliably discriminated between typical variants of alarm and contact barks (Fischer et al., 2000). Further experiments showed that infants of 6 months and older exhibited a graded series of responses to intermediate call variants. They responded most strongly to typical alarm barks, less strongly to intermediate alarm calls, less strongly still to intermediate contact barks, and hardly at all to typical contact barks (Fischer et al., 2000). There appears to be some flexibility, because vervet infants who are exposed to specific alarm calls frequently develop the appropriate response earlier than infants who were rarely exposed to it (Hauser, 1988). Furthermore, a study of the development of maternal recognition showed that, from as early as 10 weeks of age, Barbary macaque infants responded significantly more strongly to playbacks of their mothers' calls than to playbacks of unrelated females from the same social group (Fischer, 2004). Apparently, infants are able to recognize their mothers by voice from this early age onward. Taken together, these findings corroborate the assumption that the structure of the vocalizations is largely innate, whereas call comprehension is based on learning (Seyfarth and Cheney, 1997). This asymmetry is shared with most other terrestrial mammals and taken to some extreme in the example of Rico, the domestic dog who was shown to be able to remember the names of over 200 toys. In addition, Rico was able to identify the referent of a new word by exclusion learning (Fischer et al., 2004; Kaminski et al., 2004).

How do infants learn to attach the correct meaning to sounds in their environment? To date, there is only indirect and partly contradictory evidence to what degree infant responses to calls are influenced by adult behavior. For instance, Seyfarth and Cheney (1986) reported that vervet infants were more likely to respond correctly to the different alarm calls when they had first looked at an adult. In contrast, infant baboons responded to intermediate alarm and contact barks which were typically ignored by adults, suggesting that in these situations, infants did not simply copy adult behavior (Fischer et al., 2000). In Barbary macaques, infant responses – looking toward the speaker – were not influenced by the behavior of their alloparental caretakers, that is, whether or not he or she looked toward the speaker (Fischer, 2004). Similarly, after playback of conspecific alarm calls, infant and juvenile Barbary macaques more frequently ran away or climbed into trees than did adults (Fischer et al., 1995; Fischer and Hammerschmidt, 2001). Apparently, social learning is not a prerequisite for the development of appropriate responses.

1.16.3.3 Neural Control of Vocalizations

The acoustic structure of nonhuman primate calls is determined by oscillation of the vocal folds and sometimes the vocal lip, articulatory gestures that influence the filtering characteristics of the vocal tract, and respiration (reviewed in Fitch and Hauser, 1995). Vocal behavior requires a coordination of all of these components, while vocal adjustment may take place at the level of laryngeal sound production, at the level of articulation, or both. Current evidence suggests that, in nonhuman primates, the anterior cingulate cortex serves to control the initiation of vocalizations, facilitating voluntary control over call emission and onset (Jürgens, 2002, for a review; Hammerschmidt and Fischer, in press). The periaqueductal grey (PAG) appears to serve as a relay station (Jürgens, 1998). Electrical stimulation of the PAG yields natural sounding, species-specific vocalizations. In a study with squirrel monkeys, most of the neurons in the PAG fired only before the start of vocalizations and did not show any vocalization-correlated activity (Düsterhöft et al., 2000). Recent retrograde tracing studies (Dujardin and Jürgens, 2005, 2006) revealed that vocalization-eliciting sites of the PAG receive widespread input from cortical and subcortical areas

of the forebrain, large parts of the midbrain, as well as the pons and medulla oblongata. The actual motor patterns appear to be generated in a discrete area in the reticular formation just before the olivary complex. Neurons in this area increased their activity just before and during vocalizations and showed significant correlations with the syllable structure of these vocalizations (Hage and Jürgens, 2006a,b).

1.16.3.4 Auditory Feedback

While the structure of primate vocalizations appears strongly genetically determined, some experimental studies suggest an influence of auditory feedback on vocal output. For instance, Japanese macaques' coo calls produced in response to a playback coo were more similar to the playback coo than to spontaneous coos (Sugiura, 1998). Common marmosets, *Callithrix jacchus*, increase their sound level as well as their call duration in response to increased levels of white noise in their environment (Brumm et al., 2004). Cotton-top tamarins, *Saguinus oedipus*, uttered shorter combination long calls with fewer pulses when they were exposed to white noise. In contrast, when they were exposed to modified real-time versions of their own vocal output, their calls became louder and had longer interpulse intervals (Egnor et al., 2006). It seems likely that most of these adjustments are mediated in the lower brainstem (Hage et al., 2006).

A study by Eliades and Wang (2003) on common marmosets showed that self-initiated vocalizations led to the suppression of the majority of auditory cortical neurons, while a smaller population showed an increased firing rate after the onset of the call, probably as a result of auditory feedback. Furthermore, exposure to modified self-generated vocalizations over longer periods of time may alter the response properties of neurons in the primary auditory cortex of common marmosets (Cheung et al., 2005). Whether the modulation of the auditory system is achieved via forward or inverse control, or a combination of both, needs to be investigated. In any case, this line of research eventually may help us to understand how vocal production and perception are integrated and how response priming or other related mechanisms may account for the emergence of group-specific calls.

In conclusion, there are apparently two sources that contribute to the adjustment of vocal output: one socially mediated and probably effective over longer periods, leading to group-specific calls, and the other an immediate adjustment to perturbations of auditory feedback. Whatever the details of small-scale adjustments of vocal output, learning is not a prerequisite for the development of the species-specific acoustic structure. To date, there is no reliable evidence that nonhuman primates incorporate novel sounds into their repertoire. In contrast, marine mammals such as bottlenose dolphins, *Tursiops truncatus*, were found to be able to imitate the whistles of other conspecifics (Janik, 2000); parrots, *Psittacus erithacus*, can be taught to imitate human speech sounds (Pepperberg, 2000); mocking birds mimic all sorts of sounds in their environment; and there is even an odd anecdote of Hoover, the harbor seal who was reported to be talking, albeit with a heavy Bostonian accent (cf. Fitch, 2000). That is, flexible vocal production has evolved several times independently, but we do not appear to share it with our closest living relatives (Fischer, 2003; Egnor and Hauser, 2004).

1.16.3.5 Gestures

Possibly as a consequence of the frustration with the lack of elaboration in the vocal production of nonhuman primates, researchers have become more interested in the gestural communication of apes, and recently also monkeys. The reasoning is that, while primates lack voluntary control over their vocal output, they do have excellent voluntary control over their hands. Therefore, it has been hypothesized that human speech possibly evolved via a proto-sign language (Corballis, 2002; Arbib, 2005). In addition, the brain area in monkeys (area F5) that controls manual movements is supposed to be homologous to Broca's area, which is involved in speech production, further fueling the interest in this modality of communication.

In this context, it is important to distinguish between enculturated subjects raised by humans, captive animals, and wild animals. One enculturated ape, the chimpanzee Lana, was taught to use certain gestures like 'open' and 'more,' which she used in a range of different contexts (Kellogg, 1968). Detailed studies of the signaling behavior of chimpanzees, bonobos, *Pan paniscus*, gorillas, *Gorilla gorilla*, and siamangs, *Symphalangus syndactylus*, in captivity, in contrast, did not provide any evidence that gestures referred to certain objects or events in the subjects' environment (see chapters in Call and Tomasello, in press). The majority of flexibly used gestures occurred in the play context – a context that by its definition is characterized by highly variable behaviors. The evidence for gestural communication among wild subjects is still somewhat sketchy. Recently, a study of the gesturing

behavior of wild chimpanzees indicated that subjects used one specific gesture referentially (Pika and Mitani, 2006). Specifically, during grooming, groomees frequently used a "loud and exaggerated scratching movement" (Pika and Mitani, 2006, R191) on a part of their bodies. In 64% of all observed cases, the groomer then groomed that particular spot. The authors concluded that the exaggerated scratch constitutes a communicative signal. It seems equally likely, however, that the sequence of events is an example of either local enhancement (see following) and/or 'ontogenetic ritualization' (Tomasello, 2004), where both parties of the dyad learn about the consequences of certain actions.

1.16.3.6 Intentional Signaling

One important issue for the present purpose is the question of whether the usage of nonhuman primate vocal or gestural signals can be considered as intentional. In the domain of animal communication, researchers frequently invoke the definition of Daniel Dennett (1971). Dennett described different stages of intentionality, where zero-order intentionality would apply to simple expressions of emotion or fixed action patterns given in response to sign stimuli. First-order intentionality describes communicative acts employed in order to alter the behavior of the recipient. This does not necessarily imply that the signaler is conscious of her own behavior or mental state (Bruner, 1981), in the sense that the sender knows that she does have such an intention. Second-order intentionality would apply to cases where the sender intends to alter the knowledge state of the other, and not necessarily his or her immediate behavior. For second-order intentionality to apply, the sender must know that the receiver's mental state can be different from her own mental state. So far, there is no convincing evidence for second (or higher) order intentional communication in the animal realm (Seyfarth and Cheney, 2003), so most of the studies simply ask whether primates use signals with the intention to alter their group mates' behavior. Tomasello et al. (1997) listed a set of diagnostics of intentionality: (1) 'means-end dissociation' (Bruner, 1981), i.e., different signals are used to achieve the same goal while the same signal may be used to accomplish different goals; (2) 'persistence of the goal,' i.e., the sender continues to use the same signal or enhances his signaling behavior if the desired behavior cannot be achieved; (3) sensitivity to the social context, i.e., intentional signals should not only be addressed to a recipient, but the sender should also take into account his attentional state; (4) flexibility, i.e., signals may be combined (relatively) freely. While these criteria appear all quite straightforward, it is not a trivial task to operationalize them. How, for instance, does one determine the 'goal' of the sender? The goal must be inferred from the recipients' response, opening up the same problem as mentioned for the identification of message content in the vocal domain. Hesler and Fischer (2007) used an alternative approach and aimed to identify the function of a signal by examining the contingency between signal use and responses in others. However, this approach does not give any insight into the sender's intention either. Despite its intuitive appeal, therefore, the identification of the signaler's goal remains rather elusive. This aside, it appears that apes choose appropriate signal modalities according to the state of the receiver (see chapters in Call and Tomasello, in press). Moreover, they meet the criterion of means–end dissociation in the sense that they use different signals to achieve the same 'goal' or may use the same signal to achieve different 'goals.' This ability is not restricted to apes. Despite the fact that great and small apes produce a larger diversity of manual postures than monkeys, Barbary macaques also fulfill the above-mentioned criteria (Hesler and Fischer, 2007). One difference between apes and monkeys may be that, while there is some flexibility in signal usage in monkeys, it is nevertheless quite predictable. In other words, certain patterns are frequently combined with certain other patterns (e.g., rounded open mouth threat with head bob and ground slap, but not with 'present hindquarters'). Tomasello (in press) maintains that nonhuman primate gestural communication shows much more flexibility than primate vocal communication. He supposes that this might be the case because gestures are used in "less evolutionarily urgent activities." However, except for alarm calls, the immediate survival value of most vocalizations is not very obvious either. In addition, gestural signals may be simply more variable and flexible because they have more degrees of freedom: movements can be described in terms of space and time (i.e., four dimensions), while vocalizations vary – depending on the definition – in two (amplitude and time) or three (amplitude, frequency, and time) dimensions only.

Moreover, the Barbary macaques' vocal communication also fulfills the criteria for intentional signaling listed earlier: the monkeys use the same call type in different contexts, while in one context, different call types may be uttered; if a pant is not

sufficient to threaten a group member, subjects may begin to scream; when they approach another group member, they use vocal signals if the recipient is not looking at them, while they tend to use facial expressions if they do, and they may combine different call types in one call bout (Hesler and Fischer, 2007). To summarize, at least for Barbary macaques there seems to be no substantial difference between the vocal and nonvocal domain in terms of the criteria listed. In light of the view that most communicative acts serve to either decrease or increase the distance between social partners, this may not be so surprising. In conclusion, despite a high degree of voluntary control over their hands, there is no convincing evidence that manual gestures or other nonverbal signals are used (by wild apes or monkeys) to communicate about things or provide information to others (*See* Chapter 1.15 for nonverbal communication in humans). This finding suggests that a lack of voluntary control over the vocal apparatus is only one of the constraints that prevent a more elaborate communication among our closest living relatives.

1.16.4 Social Learning

So far we have seen that a variety of animal species have developed a certain degree of behavioral traditions. Apparently, vocal or gestural communication plays no particular role when such traditions are established or maintained (except when these traditions consider the signal repertoire itself). Nevertheless, the information transmission is socially mediated. Research during the last two decades has identified different social learning mechanisms that encompass a wide range of different forms with varying degrees of cognitive complexity. The common denominator is that the behavior of one subject facilitates or influences the behavior of another subject (see Heyes and Galef, 1996; Perry and Fragaszy, 2003, for comprehensive reviews). Note that the terminology is not always used consistently (Hurley and Chater, 2005).

1.16.4.1 Social Facilitation, Stimulus, and Local Enhancement

Social facilitation is invoked when an individual's learning is affected by the activity of another animal. Animals typically pay a lot of attention to what others, particularly their group mates, are doing. This may lead to stimulus enhancement, i.e., an increase in salience of stimuli others are paying attention to, as well as local enhancement, i.e., the subject learns something about the contingencies of a specific local situation simply because it is near an individual who does something particular (Heyes, 2001). Therefore, the potato-washing behavior of Japanese macaques can be viewed as a consequence of local enhancement: subjects who were closely associated with Imo also spent more time near water and near potatoes. Hence, they may simply have individually learned how delicious potatoes tasted that had been dipped in seawater.

1.16.4.2 Emulation

Emulation has been defined as a form of social learning when the subject learns – through observation – something about the consequences of a certain action. It does not imply an understanding of the causal relationship underlying action and outcome, nor does it require an understanding of the agent's intention (Hurley and Chater, 2005). For instance, if an animal observes another subject that is manipulating a bolt at a box and then sees that the box can be opened, they associate the action (manipulating) with the outcome (opening). In other words, the animal may understand that manipulating the bolt leads to opening (the end of the action). Emulation does not require that the animal understands or follows the exact procedure (the means) of the action, nor does it entail an understanding of the other subject's intention.

1.16.4.3 Copying

Within the framework of the means–ends structure, copying would be the rendition of some other individuals' behavior with no understanding of what it is good for. In this sense, one follows the exact procedure of the model, but also follows any dysfunctional (or accidental) aspects of it. Copying has been invoked in a large array of situations, including mate choice where females may prefer males that have already mated successfully, bird song learning where songsters dutifully produce exact renditions of song models heard earlier, or the acquisition of food preferences (see Dugatkin, 1996; Zentall, 1996).

1.16.4.4 Imitation

Edward Thorndike was the first to note that most animals learn by trial and error, and not by imitation (*See* Chapters 1.06 and 1.10 for operant learning).

Indeed, thorough analyses of imitation led to the view that this capacity should be viewed as a relatively complex ability that requires an understanding of the means as well as the end of an action (Hurley and Chater, 2005). Researchers interested in imitation distinguish between perceptually opaque and perceptually transparent imitation. The former, for instance, applies to imitating someone else's facial expression, where one cannot see one's own face. Most phenomena discussed earlier, in contrast, should be viewed as perceptually transparent, because they involve hand movements and vocalizations (Heyes and Ray, 2000). For the present purposes, I will therefore not discuss models put forward to address the opacity problem.

Horner and Whiten initiated a series of experimental tests to assess the transmission of acquired knowledge in captive chimpanzees and human children. Subjects watched a human demonstrator first stab a stick into a hole at the top of an opaque box, then remove the stick and insert it into a second hole at the front panel to obtain a food reward (**Figure 3**). Both the chimpanzees (aged 2–6 years) and the children (aged about 4 years) followed this sequence of actions. However, when presented with a transparent box which revealed that sticking the tool into the top hole was inefficient, the chimpanzees switched from copying to a more goal-oriented emulation and simply used the stick to gather the reward. In contrast, the children continued to follow the demonstrated procedure of first inserting the stick into the top and then into the front hole. The authors viewed this result as evidence for the strong conformity bias of our own species (Horner and Whiten, 2005).

In a second set of experiments, Whiten and colleagues examined whether a novel technique would spread in a group of chimpanzees (Whiten et al., 2005). They first trained two female chimpanzees, each from a different social group, to use one of two different techniques (poke vs. lift) to obtain a food reward (**Figure 4**). After reintroducing the females into their groups, all but two of their group members adopted the specific strategy used by the model. Interestingly, some chimps in the lift group accidentally discovered the other technique as well. However, when retested, these chimps continued to use the originally seeded technique nevertheless, which led the authors to the conclusion that chimps show a conformity bias as well (Whiten et al., 2005).

What are the requirements to achieve the somewhat simpler case of transparent imitation? Associationist models propose that the capacity to imitate derives from the experience of simultaneously observing and executing that action in the past. Transformational theories, in contrast, suggest that an observed action is transformed into a sensory representation that is then used as an internal model for the motor program (see Heyes and Ray, 2000; Brass and Heyes, 2005, for detailed discussions). At a somewhat different level, two competing frameworks have been put forward to explain how imitation may be achieved: the sensorimotor and the ideomotor frameworks (see Prinz, 2005, for a review). In the sensorimotor model, perception and action are subserved by two distinct representational structures. Rule-based mappings connect the two. According to this model, an observer who perceives a given action needs some way of translating this into

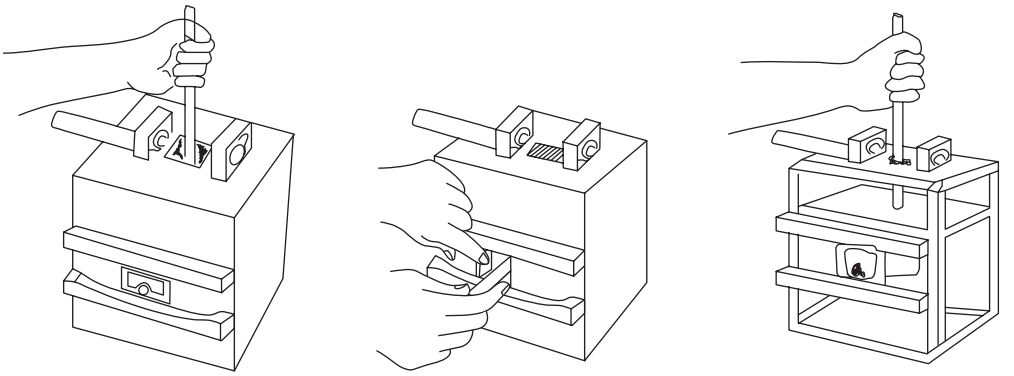

Figure 3 Opaque and transparent boxes used in the experiments with chimpanzees and children. Subjects first saw a familiar human stab a stick tool into the top and then into the front hole to recover food. In a second condition, the transparent box revealed that the first move (stabbing from the top) had no effect. In this second condition, chimpanzees switched from copying the sequence to emulation, while children continued to follow the human model. Adapted from Whiten A (2005) The second inheritance system of chimpanzees and humans. *Nature* 437: 52–55, with permission from Macmillan Publishers Ltd: NATURE.

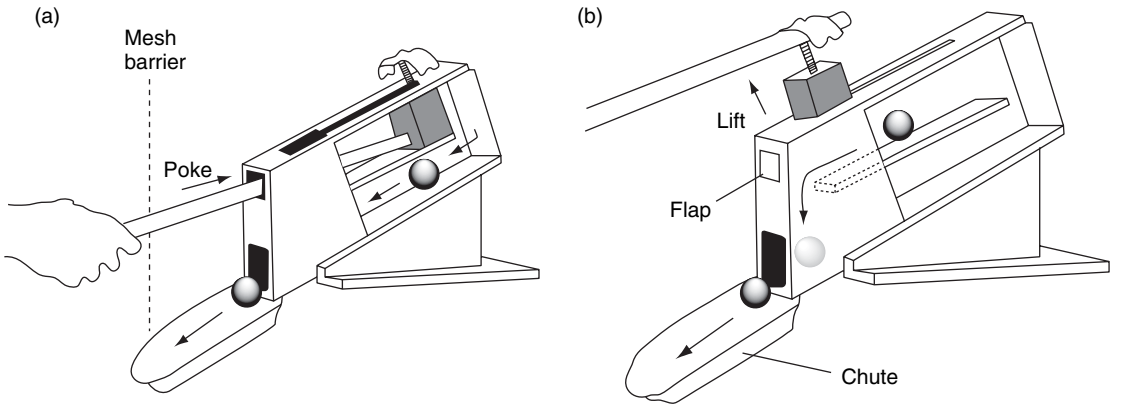

Figure 4 Two techniques for gaining food: (a) in the poke method, the stick tool is inserted into the apparatus and used to push (an invisible) blockage back along a ramp so that the food falls down and rolls forward underneath; (b) in the lift method, the stick tool is passed under hooks, allowing the blockage to be lifted and the food to roll forward. Redrawn version, adapted from Whiten A, Horner V, and De Waal FBM (2005) Conformity to cultural norms of tool use in chimpanzees. *Nature* 437: 737–740, with permission from Macmillan Publishers Ltd: NATURE.

her own motor planning. Feedback on the consequences of one's own movements can be used to correct the execution of that plan. This is generally compatible with the transformational model mentioned earlier. According to the ideomotor (common coding) framework, in contrast, perception and action are represented by the same system. This has two effects: first, to predict an ongoing action's perceivable consequences; second, to select a certain act in order to achieve certain effects (Prinz, 2005). Thus, both forward (predicting the outcome) and inverse models (using feedback) may play a role in imitation. Effectively, an understanding of imitation is part of understanding motor control and simulation and also bears on the issue of how mental state attribution is conceptualized.

While there is now some evidence that chimpanzees may be able to imitate, human children are 'imitation machines' (Tomasello, 1999, p. 159), and they have a strong tendency to imitate inefficient actions like pushing a light switch with the head instead of with the hand, or using rakes the wrong way around if the demonstrator did so. Thus, it appears that, to human children, imitation is rewarding per se. While imitation is difficult to distinguish phenomenologically from copying, true imitation requires an understanding of someone else's intention. That is, not only does the observer need to understand the goal (turning on the light) but also needs to assume that it matters to the demonstrator how the light is turned on. Thus, a certain form of perspective taking seems to be required to achieve full-fledged imitation (Tomasello, 1999).

1.16.4.5 Gaze Following

There are several studies showing that nonhuman primates follow the gaze of others regularly (reviewed in Tomasello and Call, 2006), while fewer studies systematically examined the ontogeny of gaze following in nonhuman primates. Tomasello and colleagues (2001) investigated the age at which rhesus macaque and chimpanzee infants began to follow the gaze of the human experimenter. In rhesus macaques, subjects followed the gaze of the experimenter at 5.5 months of age. Barbary macaque infants up to an age of 10 weeks did not follow the gaze of others (Fischer, 2004), but a more systematic study of the development of gaze following among nonhuman primates is still lacking.

1.16.4.6 Teaching

An assessment of teaching entails a change in perspective; all of the aforementioned social learning mechanisms tried to explain the behavior of observer or information seeker. An investigation of teaching, in contrast, focuses on the subject that provides the information. In an influential article, Caro and Hauser (1992) formulated the following criteria for the occurrence of teaching:

> An individual actor A can be said to teach if it modifies its behavior only in the presence of a naïve observer, B, at some cost or at least without obtaining an immediate benefit for itself. A's behavior thereby encourages or punishes B's behavior, or provides B with experience, or sets an example

> for B. As a result, B acquires knowledge or learns a skill earlier in life or more rapidly or efficiently than it might otherwise do, or that it would not learn at all. (Caro and Hauser, 1992, p. 153)

This rather broad definition does not assume instruction; instead the authors argue that teaching does not depend on higher-order intentionality or attribution of mental states. Nevertheless, evidence for teaching in animals remains equivocal. In a recent study, Thornton and McAuliffe (2006) investigated the transfer of skills in free-ranging meerkats, *Suricata suricatta*. After emerging from the burrow, meerkat pups spend much of their time following helpers who capture and process prey for them. Helpers killed or disabled prey more frequently when pups were young, while they fed mostly intact prey to older pups. The authors also conducted an experiment where they presented live scorpions to helpers who then removed the sting and fed the pup (all of which then also bit into the scorpion). In contrast, only half of the pups bit into the prey when no helper was around (Thornton and McAuliffe, 2006). While the helpers' behavior conforms with the criteria listed above and, thus, can be defined as a form of teaching, it is questionable whether it helps us to understand different forms of information transmission. For instance, helpers may have learned that young pups cannot handle intact prey, so they have to disable or kill it. In addition, the outcome of the experiment can easily be explained by social facilitation. To date, there is no convincing evidence for active punishment or encouragement. Obviously, however, socially living animals do provide each other with experiences that they might not have if they lived alone, so this criterion does not help much to distinguish teaching from other forms of social learning, such as social facilitation. In other words, despite some superficial similarity of the observed meerkat helper behavior and the expected outcome of teaching (in the more canonical sense of the word), it is probably a good idea to consider the form of information transmission (active or passive) when teaching is investigated.

The same needs to be said about the acquisition of the diverse cultural behavior of chimpanzees or other primates. There is to date no indication that older animals correct or help youngsters to acquire a certain skill, e.g., nut cracking. Observations of the ontogenetic development of nut cracking in chimpanzees indicated that youngsters had a strong interest in their mothers' nut cracking activities. Nevertheless, they needed to develop that ability through trial-and-error learning (Matsuzawa et al., 2001). The young chimpanzees' attention was drawn to hammer, anvil, and nut, but they could not acquire the appropriate technique through observation alone. There is no record that mothers ever corrected or punished youngsters' incorrect behavior. Therefore, it seems more plausible that the acquisition of nut cracking can be explained through a combination of social facilitation, emulation, and trial-and-error learning.

1.16.5 Conclusions

There is now convergent evidence to support the notion of a strong asymmetry between animals' intentional abilities to transmit versus to acquire information, and this is true for communication as well as social learning. Despite the fact that the structure of primate vocalizations appears fixed from birth, primate calls nevertheless provide rich information about sender attributes such as size, age, sex, hormonal levels, and also motivational state. Specific relations between motivational state and certain contextual situations allow listeners to use signals as predictors of upcoming events. There is, however, no conclusive evidence that signalers have the intention to provide this information to others. While nonvocal signals such as gestures and postures show a higher degree of variability (particularly in apes), there is no convincing evidence that gestures are used to intentionally communicate about objects or events, either. Similarly, the behavioral diversity observed among nonhuman primates can largely be attributed to social learning mechanisms where observers pay attention to and are influenced by the actors' behavior (social facilitation and goal emulation), but there is no evidence for teaching or instruction. Apparently, nonhuman primates lack the ability to attribute knowledge states to each other.

Social learning is clearly a prerequisite for the development of human culture, but it does not explain its complexity. One of the hallmarks of human culture is its rich symbolic structure (Jablonka and Lamb, 2005; *See also* Chapter 1.38). Moreover, cumulative cultural evolution (Boyd and Richerson, 2005) requires a system to represent and transmit knowledge. One such system is human speech: speech encompasses external reference as well as temporal and spatial transcendence. Once such a symbolic communication system is in place, it is possible to transmit information and knowledge

without forcing the individuals to reenact each action every time information needs to be conveyed. Speech generally and writing in particular can be used to compress information transmission, store acquired knowledge, and drive technological development. In addition, the symbolic nature of speech allows for an increasing complexity of other human symbol systems. In conclusion, while both animals and humans share a number of social learning mechanisms, humans in addition are apt imitators, attribute knowledge to others, and are in command of a complex communication system. All of these contribute to the rich symbolic structure that characterizes human cultures. Future research should be devoted to the question of how mental state attribution and information transfer are linked, and how both of these are related to the formation of a symbol system.

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1.17 Bird Song Learning

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1.17.1 Introduction

All birds have a repertoire of calls for communication about danger, food, sex, and group movements, and their calls are used for many other purposes as well. Within this repertoire of a dozen or more sound signals, a distinction can be made between calls, which are usually brief and often monosyllabic, and song, which is a more extended pattern of sound, sometimes raucous, often tonal and melodic, and not infrequently a source of pleasure for human listeners (Catchpole and Slater, 1995). Defined in this way, virtually all birds have something that we can call a song, whether it be the crow of the cock or the music of nightingales. In close to half of all 9000 species of birds, the song is learned. Most of the 4000 or so probable learner species come from just one of the 27 orders into which taxonomists classify living birds. Excluded are waders, chicken-like birds, woodpeckers, cuckoos, doves, hawks, owls, and all marine and freshwater birds. The only groups that qualify are parrots, hummingbirds, and above all the very populous suborder of the Passeriforms known as the oscines, characterized by the complexity of their vocal organs and by their distinctive forebrain circuitry expressly evolved to sustain song learning. Parrots and perhaps hummingbirds seem to have independently achieved the same end with equivalent but neuroanatomically distinct brain circuitry (e.g., Striedter, 1994).

Songs are usually, though not always, a male, androgen-dependent prerogative. In species that are learners, the associated brain circuitry is often sexually dimorphic, and there is an extensive literature on the extent and functional significance of this dimorphism and its developmental basis (Nottebohm and Arnold, 1976; DeVoogd, 1991; Arnold, 1990; Vicario, 1991; Brenowitz and Kroodsma, 1996). A larger song system in male brains clearly implies a greater commitment to song learning, and the correlation between the size of song system nuclei, the presence of song, and song repertoire size extends not only to comparisons between the sexes but also between species, subspecies, and even individuals (Nottebohm et al., 1981; Canady et al., 1984; DeVoogd et al., 1993).

Unlike calls, which are occasionally learned but are typically innate, oscine songs always develop abnormally if a young male is reared out of hearing of adults. A common consequence of dependence on learning is the emergence of local song dialects, often varying on much the same geographic scale as dialects in human speech (Baker and Cunningham, 1985). Their distinctiveness depends on such factors as patterns of dispersal from the birthplace and the timing of song learning. When dialects are well defined, it seems to be a general rule that songs like a bird's own dialect are especially potent as vocal signals, both to territorial males and to females

ready to mate. A bird's dialect may or may not correspond to that which prevails in the natal area.

Many analogies can be struck between song learning and the acquisition of human speech (Doupe and Kuhl, 1999). There is no nonhuman primate in which vocal patterns are culturally transmitted from generation to generation. Aside from cetaceans, which also engage in vocal learning, birds provide the most valuable animal model we have for studying the behavioral, hormonal, and neural basis of vocal plasticity (Konishi, 1985; Arnold, 1990; Marler, 1991; Nottebohm, 1993; Zeigler and Marler, 2004).

1.17.2 The Physiology of Song Learning

1.17.2.1 Special Brain Mechanisms

Although many important issues remain unexplored, much has been learned over the past 30 years about the specialized neural mechanisms that mediate avian vocal plasticity since the song system was discovered in the canary brain (Nottebohm et al., 1976; *See also* Chapter 3.23). There are two main functional requirements for controlling song production. Appropriate configurations of the articulators must be set up, primarily the syrinx and the postsyringeal vocal tract. Also, the required pattern of air flow must be generated to set the articulators vibrating. These controls are required whether a song is learned or innate, and as might be predicted, some of the circuitry responsible is relatively conservative, located in the midbrain and the brain stem (Wild, 1994). The added requirements for songs to be learned are met by two major circuits in the forebrain, one to sustain the actual learning process, the so-called anterior forebrain pathway, and the other, the motor pathway, for control of the production of learned songs (Nottebohm, 1993; **Figure 1**). Disruption of the motor pathway always results in the distortion and even the elimination of learned songs, whereas the anterior forebrain pathway seems to be redundant except during actual song development. As with innate songs, the motor pathway must have access both to the vocal tract and the syringeal musculature, mapped in one part of the motor pathway and also to the respiratory system, mapped in another part, both in the robust nucleus of the archistriatum, or RA (Vicario, 1991). Demands on the motor pathway circuit are complicated by the existence of two semiindependent vibrators in the songbird syrinx, requiring precise coordination, since both participate in the production of learned songs (Greenewalt, 1968; Suthers, 1990).

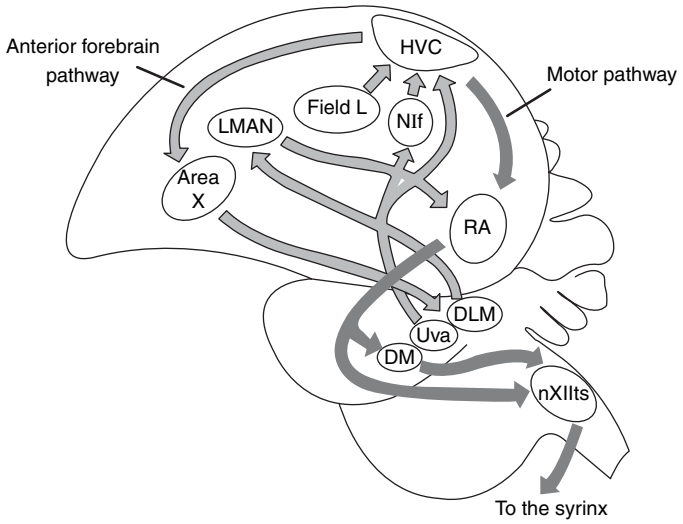

Figure 1 A diagram of the two major circuits in the song system of the oscine brain. One is for control of movements of the vocal apparatus in learned songs (motor pathway), the other for song learning (anterior forebrain pathway). There is a fork in the motor pathway from RA (robust nucleus of the arcopallium) to the hypoglossal nucleus in the brainstem, and to the vocal apparatus (nXIIts). One branch goes directly, the other passes through DM (dorsal medial nucleus) in the midbrain, thought to be involved in the control of respiration. A different circuit for the production of innate songs, involving the midbrain nucleus ICO (intercollicular complex), is not shown. Inputs to the higher vocal center (HVC, no formal name) include field L, the primary auditory forebrain area. Another auditory projection area close by that also projects to HVC. NCM (caudal medial mesopallium) is not shown (see text). Also labeled is LMAN (lateral magnocellular nucleus of the anterior nidopallium).

The hearing of birds has been extensively studied in relation to their vocal behavior (Dooling, 1982). Many parts of the song system are responsive to song stimulation. Strangely, one of the strongest stimuli, especially in the higher vocal center (HVC) of adult males, but also elsewhere, is the bird's own individually distinctive crystallized song (Margoliash, 1986). This finding has promoted the plausible speculation that adult males of some species use auditory feedback from their own song as a yardstick to calibrate the potency of songs of others when they hear them, especially those of conspecific neighbors and strangers.

Attunement of a male's song system to his own individual song, including all of its personal quirks, only emerges as songs crystallize (Doupe, 1993). Unfortunately, little attention has been paid to responsiveness of the brain of young birds to song stimuli prior to the production of plastic and crystallized song. Patterns of auditory responsiveness of the song bird brain during the earliest memorization phase of song learning remain almost unexplored (but see Whaling et al., 1995). Behavioral tests at this age demonstrate innate responsiveness to many features of song, especially those of the bird's own species (Nelson and Marler, 1993; Whaling et al., 1997). Details of the process of song memorization are consistent with involvement of the set of innate auditory predispositions that each species deploys, serving as a guide to the process of selective song learning. The neural mechanisms responsible still await neurophysiological investigation, and until they are elucidated, a full understanding of the physiological basis of song learning will continue to elude us. At present we do not know how songs are memorized by a young male, nor where they are stored in the brain in preparation for the guidance of song production.

One remarkable feature of the bird brain is the continuing development of new neurons in many parts of the brain, both in infancy and in adulthood, including the song system (Goldman and Nottebohm, 1983). Although it is not clear what relevance neurogenesis has to song learning, there are many fascinating hints, including the demonstration, in something of a technical tour de force, that some new neurons are actually inserted into the song system and become active there (Paton and Nottebohm, 1984; Alvarez-Buylla et al., 1990). It may be that neuronal turnover in the motor pathway prepares the way for the song plasticity that some birds such as the canary (Nottebohm and Nottebohm, 1978) display in adulthood.

Another place to look for song-related activity is in the forebrain auditory circuits concerned with the perceptual processing of song stimuli. One of these (caudal medial mesopallium, NCM) has been identified as the site of gene activation in response to conspecific stimulation and as a location where experience-dependent changes in electrophysiological responsiveness to song stimuli are occurring (Mello et al., 1992; Mello and Clayton, 1994). Circuits extrinsic to the song system may prove to be more involved in song learning than has been thought.

1.17.3 Sensitive Periods for Learning

In song development, as in the ontogeny of other kinds of behavior, the rapidity and precision with which learning takes place varies from one stage of the life cycle to another. All song birds seem to share a similar sequence of stages in the process of learning to sing. First is the acquisition phase often restricted to a short period, when a bird hears songs and commits some of them to memory. After storage for a period that ranges in duration from days to months, depending on the species, songs are recalled from memory, and imitations begin to emerge. Renditions are relatively faithful to the original model in some species, but others depart from it quite radically, with vocal inventiveness playing a significant role.

The separation in time between the acquisition or sensory phase and the production or sensorimotor phase, ending with the production of crystallized, adult song, is a significant one. In many birds, the sequence occurs just once, often in early adolescence. In such cases, adult song patterns remain fixed thereafter, their production waxing and waning with the passing of each breeding season. In other species, the sequence recurs repeatedly during adulthood, so that song patterns may continue to change. Even close relatives, such as canaries and sparrows, can differ in this respect.

Several species of sparrows have a sensitive period for song acquisition beginning at about 2 weeks of age, soon after they leave the nest, and ending 6–8 weeks later (Nelson et al., 1995). The precise duration varies, even in different populations of the same species (**Figure 2**). Sensitive periods for learning are not fixed but are changeable, within limits, depending on such things as the strength of song stimulation; access to a live, interactive tutor (Baptista and Petrinovich, 1986); and physiological factors, such as hormonal states, that vary with the season.

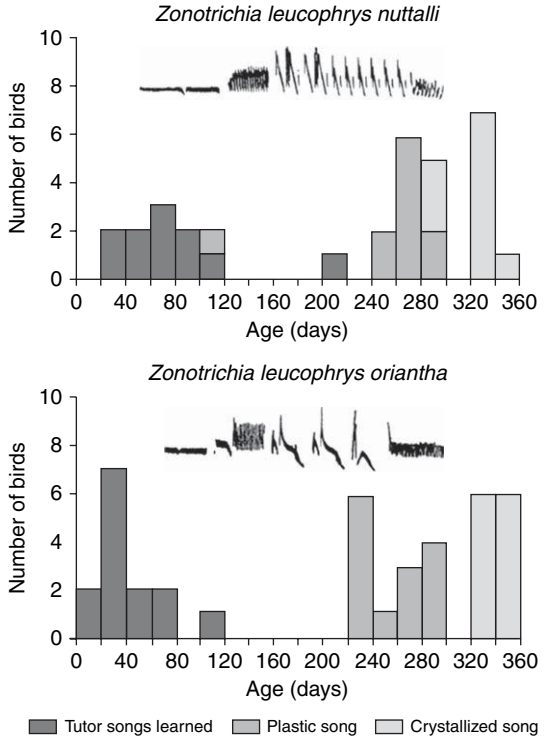

Figure 2 The timing of song production and song acquisition from constantly changing programs of tape recordings throughout the first year of life in male white-crowned sparrows raised in the laboratory. The birds represent two populations: one resident year-round on the coast (subspecies nuttalli), the other migratory in the high Sierras (oriantha). The migratory birds began acquiring song earlier (peaking at 33 days vs. 52 days), and the acquisition period was significantly shorter. A representative song of each is shown. Both are about 2 s in duration. From: Nelson DA, Marler P, and Palleroni A (1995) A comparative approach to vocal learning: Intra-specific variation in the learning process. *Anim. Behav.* 50: 83–97.

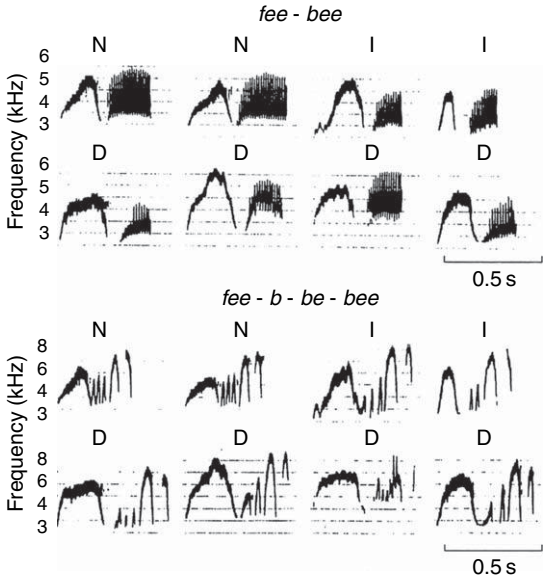

Figure 3 Within the passeriform perching birds, the oscine songbirds all have learned songs, but the suboscines, which lack the special song-system brain circuitry, do not. Here are examples of the innate song of a suboscine passerine, the eastern phoebe. Some developed normally in the wild (N), some in isolation (I), and some after deafening (D). Two song types are represented, the fee-bee and the fee-b-be-bee. Unlike oscines, whose songs are drastically modified by isolation or deafening, both isolated and deafened phoebes developed normal songs. From Kroodsma DE and Konishi M (1991) A suboscine bird (Eastern phoebe, *Sayornis phoebe*) develops normal song without auditory feedback. *Anim. Behav*. 42: 477–487.

Closure of the acquisition period can sometimes be delayed if songs of the bird's own species are withheld (Kroodsma and Pickert, 1980), as though termination of the sensory phase is delayed until adequate song stimulation has been experienced. If young are hatched late in the season and singing has already ceased for that year, the sensitive period may be extended until the following spring in some species, though this delay does not occur in all cases.

1.17.4 Vocal Development

1.17.4.1 The Ontogeny of Learned Song

In contrast with song learners, nonlearners develop normal song when raised in isolation, even after deafening (Kroodsma, 1984; Kroodsma and Konishi, 1991). This is true of a male dove, a cockerel, and even of those passerines that are nonoscines, such as the New World flycatchers (**Figure 3**). In such cases, when a young bird starts to sing, its very first efforts are clearly identifiable as immature versions of what will ultimately be normal adult song. Early attempts may be noisy and fragmented, but there is a clear linear progression in the development of innate songs.

In birds that learn their songs, the developmental progression is quite different. They begin with the unique vocal pattern called subsong, a quiet, variable warbling that can begin as early as 3 or 4 weeks of age. It is reminiscent of the early babbling stage of speech development (Locke, 1993). Typically it seems to bear no resemblance to mature song, although we know less about its structure and function than any other aspect of song development. As defined, there are no imitations in subsong. In song learners, there is a kind of vocal metamorphosis between subsong and later stages of song development. The amorphous structure and noisy spectral organization of subsong are consistent

with a role in the acquisition of the general motor skills of singing, in coping with the complexities of the oscine syrinx, and also in honing the ability to guide the voice by the ear, a prerequisite for vocal imitation (Thorpe, 1961; Nottebohm, 1972; Marler and Peters, 1982b). Duration of the subsong period varies greatly, even within a species, depending on individual histories. In deaf birds and social isolates, it may last for weeks, but a bird with adequate tutoring in youth may progress rapidly from subsong to the next phase, plastic song, in a few days.

In plastic song, signs of mature song structure first appear. Previously memorized song patterns emerge and begin to stabilize, first gradually, then rapidly, until adult, crystallized song is achieved, and the young male is ready to launch a full season of mature singing (**Figure 4**).

1.17.4.2 Overproduction, Attrition, and Action-Based Learning

Song learning has been traditionally viewed as memory based. Songs are acquired and memorized and then used later to guide motor development by auditory feedback, with no practice at the time of memorization. Another kind of vocal plasticity, involving only working memory, and with a key role for motor practice, occurs during the plastic song phase.

Several learner species overproduce during plastic song, generating more than they need for mature song, and discarding excess plastic songs as the time for song crystallization approaches (**Figure 5**). Some overproduced songs are invented, but many are imitated, and the richness of early song experience is one factor in determining the extent of the overproduced repertoire (Nelson et al., 1996). However, overproduction and attrition also occur in birds tutored and raised in individual isolation in the laboratory (Marler and Peters, 1982a). In this case, the decision about which songs to discard seems to be random, whereas in nature it is guided by social experience.

The attrition phase in which overproduced songs are discarded from the repertoire coincides with the efforts of young male sparrows to establish their first territory. As it competes with adults, those songs of the young bird that match song types of older rivals, even if only approximately, are most effective in eliciting counter singing and perhaps in territorial defense (Beecher, 1996). Young males tend to retain and crystallize those plastic songs that are the closest match to those of their rivals, and to discard the rest.

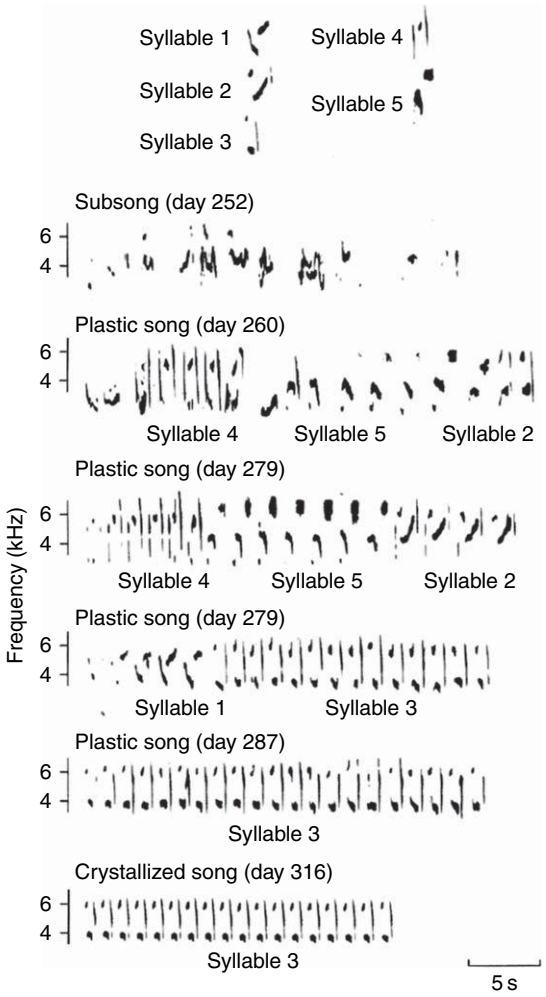

Figure 4 Development of the song of a male swamp sparrow raised in the laboratory and tutored with tape recordings of song from 20–60 days of age. Some of his training song syllables are shown at the bottom right. The first emergence of imitations can be seen in his plastic song from day 260, 200 days after the end of tutoring. His mature song crystallized around 300 days of age (see **Figure 5**).

Effects of male-to-male territorial interactions, either real or simulated, on song attrition have been described in several species of sparrows (Nelson, 1992; Nelson and Marler, 1994). In another species, the brown-headed cowbird, responses of females serve to shape male decisions about which overproduced songs to retain (West and King, 1988). The process by which a repertoire of varied motor patterns is selectively winnowed, with the selection process based on social feedback, has been termed action-based learning (Marler and Nelson, 1993). There are probably parallels with the development of other kinds of motor behavior, including play.

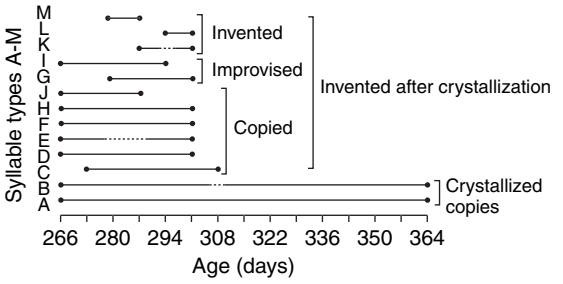

Figure 5 A diagram of song development in the same male swamp sparrow shown in **Figure 4**. In addition to the eight tape-recorded songs he imitated, he invented three more and modified two by improvisation. His crystallized adult repertoire of two song types is typical for this species.

Use-dependent processes of selective attrition may be widespread in the development of many kinds of behavioral activities. The actions that are discarded from the repertoire may themselves have functional significance. For example, songs that are practiced in youth and then rejected may provide a potential memory bank for the future, perhaps consulted in assessing the songs of others. In species that retain vocal plasticity into adulthood, discarded songs may be redeployed as the basis for producing new songs later in life.

The life-cycle timetable for action-based learning is different from that for memory-based learning. Action-based learning has the potential to recur whenever, for whatever reason; progression through a phase of variable action patterns is then followed by use-dependent winnowing of the variants. A mechanism is required for selective reinforcement of certain motor variants over others. Unlike memory-based learning, highly specialized brain mechanisms such as the oscine song system are not required. As a consequence, action-based learning is likely to have a wide phylogenetic distribution. For example, although nonhuman primates seem to be incapable of memory-based vocal learning, they do have a limited degree of vocal plasticity, possibly mediated by action-based learning.

1.17.4.3 Effects of Isolation and Deafness

Regardless of the opportunity to learn, certain aspects of the normal singing behavior of the species will always develop in oscines. When sparrows are raised in isolation, for example, the note structure and tonal quality of their songs is aberrant, but aspects of basic song syntax develop normally (**Figure 6**). Certain song features of the species are produced despite their absence from the tutoring songs a male has experienced, but with the important proviso that hearing must be intact.

The singing patterns of a hearing male oscine raised in isolation and of a male deafened early in life and raised in isolation are quite different. Typically, deaf male songs are highly degraded and variable, lacking most of the few normal features that an intact male can develop in isolation (Konishi, 1965). Songs of early-deafened males are not completely amorphous. This is true even when the critical step has been taken to be sure that there has been no song practice prior to deafening. This step

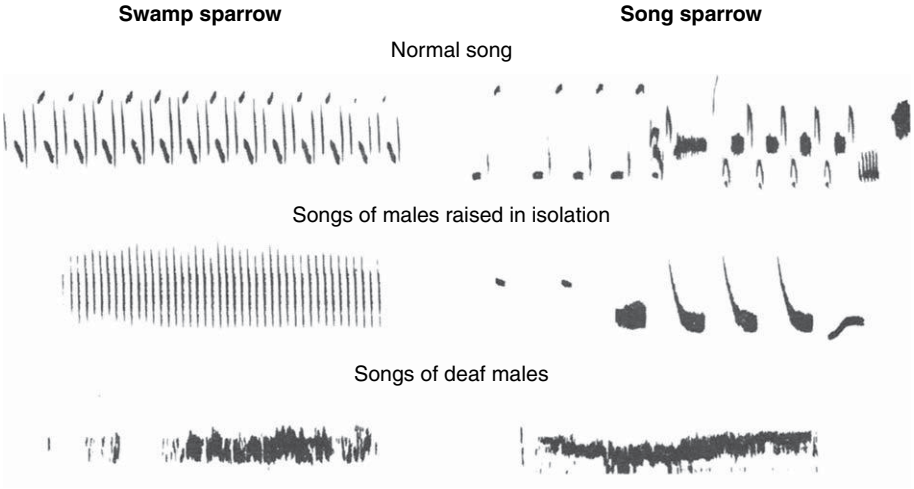

Figure 6 Normal songs of swamp and song sparrows compared with those developed by males raised in social isolation and by males deafened early in life. The simplicity of the note structure of the isolate songs is typical for sparrows. The high degree of species specificity, so evident in normal song, is largely erased from the songs of deaf males.

was lacking in some of the earliest studies. The emergence of some elements of species-specific song syntax in males deafened very early in life seems to implicate innate motor programs in song development. The highly abnormal song patterns characteristic of deaf birds develop both if a male becomes deaf before tutoring and if he is deafened after the tutoring but before singing has developed. There seems to be no internal brain circuitry that makes memorized songs directly available to guide vocal development. To transform a memorized song into a produced song, the bird must be able to hear its own voice. We can infer that some aspects of song production are guided by auditory templates for song, located in the brain, with specifications that vary from species to species (Konishi, 1965; Marler and Sherman, 1983). Neurobiologists have yet to demonstrate where these templates are located and how they operate (but see Whaling et al., 1997).

Like ornithologists, wild birds rely heavily on song for species identification. Each bird has its own distinctive way of singing, and this is recognized by other species members. Songs of early-deafened song birds, on the other hand, are often indistinguishable between species and are largely, though as noted not entirely, lacking in species specificity (**Figure 6**). Songs of males raised in isolation but with hearing intact fall somewhere between. They retain some species-typical features, and they lack others.

When tape-recorded songs are played to wild territorial males or to females in hormonally induced estrus, experienced birds of both sexes respond strongly to normal song of their species but completely disregard deaf songs as social signals (Searcy et al., 1985; Searcy and Marler, 1987). Similarly, songs of other species elicit none of the strong aggressive responses of territorial males, or the courtship of estradiol-primed females, that conspecific song evokes. But those of social isolates do retain a minimal level of effectiveness. Thus the efficacy of bird song as a social signal is augmented during development both by learning and by ontogenetic involvement of innate auditory templates that encode certain species-specific features of normal song.

1.17.4.4 Learning Preferences

Learning theorists once believed that any stimulus could be learned, given appropriate conditions; differences in stimulus potency, although present, were regarded as unimportant enough to be ignored in theorizing about the mechanisms underlying learning. We now know that innate predispositions play crucial roles in the adaptiveness of natural learning processes, and song learning in birds provides many illustrations of this principle. While birds are capable of learning a multiplicity of different sounds, including those of many other species (**Figure 7**), other-species mimicry is relatively rare in the wild (Kroodsma and Baylis, 1982). Leaving aside mimics, which only account for about 15% of songbirds, it is adaptive for most song birds to avoid other-species songs as models for learning.

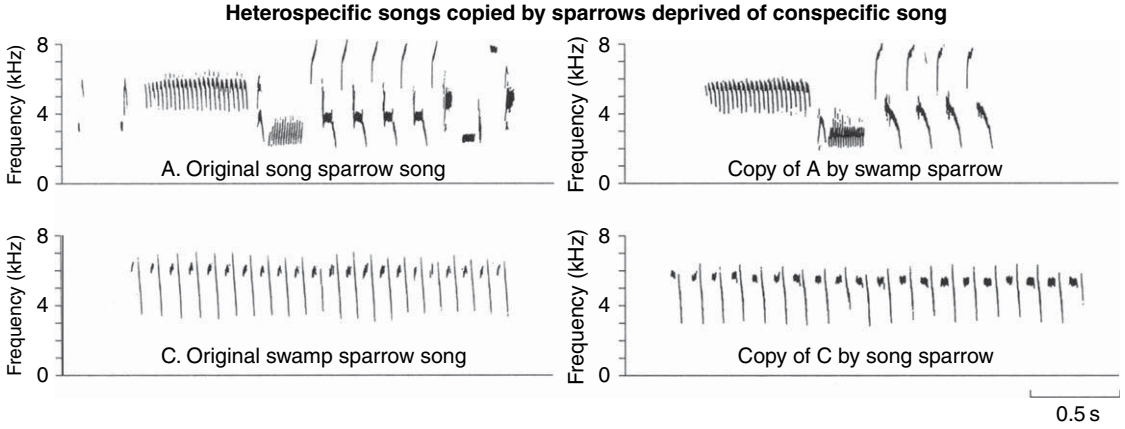

Figure 7 Although sparrows typically reject each other's songs as models for learning, they will sometimes accept them if given no other choice. Here male swamp and song sparrows learned a tape-recorded song of the other species when given no conspecific songs to listen to. Evidently the vocal apparatus is able to articulate the sounds of the other species song.

If males of two different species, such as swamp and song sparrows, are brought into the laboratory and exposed to identical tape recordings that include both species' songs, each displays a clear preference for song of its own kind, even if it has never been heard before (Marler and Peters, 1988, 1989). This preference does not arise from an inability to produce the other species song. An alien song that a male rejects, when given a choice, may become acceptable if it is the only option (**Figure 7**), especially when it is presented in a highly interactive situation by a live tutor caged nearby (Baptista and Petrinovich, 1986). But given a choice, there is typically a preference for conspecific song.

1.17.5 The Role of Innate Knowledge in Song Development

The view of the brain as a *tabula rasa*, a blank slate, all too long a basis for the thinking of learning theorists, is patently absurd. Whatever the task, every brain brings to bear a set of neurally based predispositions, each with its own evolutionary and experiential history, about how to proceed most efficiently in dealing with a given set of learning problems. Some of these predispositions are generic and pan-specific, and others are highly singular and even species specific, as with the innate processes that the human infant brings to bear on the development of speech behavior and language. Such is the case with birdsong.

The young bird embarks on the process of developing song armed with innate predispositions about how best to proceed. Some are generic, such as the widely shared tendency of songbirds to sing tonal sounds, evident even when they render imitations of sounds that were not originally strictly tonal (Nowicki et al., 1992). Others are species specific. When sparrows learn to sing, they favor conspecific song. They do so on the basis of not a single ethological sign stimulus but a range of song features. Naive young birds, recently fledged, are highly discriminating with regard to the song stimuli they find most potent, as revealed by their tendency to give more begging calls to them in response to playback (Nelson and Marler, 1993). By playing experimentally modified songs to young birds, innate responsiveness has been demonstrated to a range of conspecific song attributes. It is clear from their behavior that young birds possess extensive foreknowledge about the song of their species before they embark on the process of learning to sing. The evidence indicates that they know much more than you might guess solely on the basis of the songs that a male produces when raised in social isolation. They behave as though auditory experience that matches their innate knowledge is required before that knowledge can be fully brought to bear on song development (Marler, 1984).

One way to view song learning is as a process of validation by use of a subset of innate knowledge, drawn from a more extensive library encoded in the brain that details the rules for the singing behavior for each species (Marler and Nelson, 1992). This innate library is shared by all species members. The species-universal rules encoded there are sufficiently flexible to provide almost infinite opportunities for differences in individual behavior as a function of personal experience, and yet firm enough to restrict the development of excessive divergence between individuals.

There are analogies with the rules for orderly musical composition that each human culture possesses, encompassing the potential for an infinite number of different melodies (Jackendoff, 1994). The genetic underpinnings of the immune system provide another parallel, with the major histocompatibility complex providing enough alleles for a vast number of possible combinations. The imposition of limitations is important because it ensures that, however individualistic a bird's singing behavior may be, it still conforms closely enough to species-universal rules that obstacles to communication with others are minimized.

In addition to predispositions evident in the sensory phase of song memorization, others are manifest during motor development. The emergence of some species-specific basics in the song syntax of early-deafened males has already been noted. A bird tricked into learning heterospecific songs may only reject them in late stages of plastic song as species-specific syntax emerges (Marler and Peters, 1989). When young sparrows were tutored using experimentally modified songs with abnormally high syllable repetition rates, they imitated the songs but reorganized them in invented patterns that conformed more closely to the temporal organization of their normal species song (Podos, 1996). Again there is great flexibility in these motor predispositions, but even though songs with abnormal temporal organization can be taught, there is an underlying tendency to conform to species-specific norms. The individual and population differences that characterize learned birdsongs are all-pervasive, but they rarely confuse experienced bird watchers in identifying the species

responsible. With their genetic fitness at stake, the birds themselves are hardly likely to be any less perceptive.

1.17.6 Reinforcement and the Speed of Vocal Learning

Some of the distinctive features of song learning recur in other cases of developmental plasticity. It is not uncommon for developing behavioral and neural systems to exhibit sensitive phases, when the potential for plasticity is unusually great. Completion of one developmental stage is often a necessary precursor for embarking on the next.

Unlike some other widely studied forms of learning, no external reinforcement is required in song learning, even though social stimulation may augment the acquisition process. It suffices that the bird be exposed to an appropriate set of stimuli at the appointed stage of development for acquisition to occur. At the height of the sensitive period, learning can take place with great rapidity. Sometimes remarkably few exposures to a bird's own species song are sufficient. In sparrows, some acquisition can occur from 30 repetitions of a conspecific song. European blackbirds have learned from 15–20 song presentations on a single day.

The virtuoso performer in this regard is the nightingale, which can learn a sequence of song types accurately after 20 presentations – some males with as few as five. The virtuosity of nightingales is such that they can learn strings of up to 60 different songs and reproduce them in the original order. The sequencing is only retained in subsets of three to seven songs, however. After producing such a matched sequence, birds in one experiment then switched to another subset, beginning at a different point. These birds behaved as though they divide a string of many songs into manageable subsets of up to seven songs, using a strategy for memorizing long sequences reminiscent of that used by humans in memorizing strings of words (Hultsch and Todt, 1989, 1992, 2004). This is one of many avian accomplishments reminiscent of aspects of human speech behavior.

1.17.7 Song Development as a Creative Process

Perhaps most mysterious and intriguing of all is the ability of songbirds to invent new sounds. They achieve this in several different ways. Some birds first imitate the components of songs they have heard and then, as plastic song progresses, recombine them to create new sequences. In this way, parts can be exchanged within a song, between different songs, and even between songs that have been acquired many months apart (**Figure 8**). Rather than remaining faithful to the structure of imitated sounds, some birds find the temptation to improvise irresistible, especially during plastic song, so that songs originally imitated eventually become so changed as to be unrecognizable.

Not uncommonly, birds invent new sound patterns that they themselves have never heard. This is frequent in birds raised in isolation, with the result that, although the songs of isolates are abnormally simple in some respects, they are also extremely diverse. The act of producing novel and distinctively patterned sound seems to be itself reinforcing, attesting to the strong and deep-rooted commitment of many song birds to vocal inventiveness.

The drive to invention is stronger in some species than others. Certain birds remain quite rigidly faithful to their tutors, adding just sufficient individuality to personalize their song, and no more. Adherence to their models makes them especially tractable as subjects for laboratory investigation of song learning. Perhaps for this reason, the more imaginative songsters tend to be underrepresented in the roster of favored subjects for scientific study. The huge individual repertoires that males of some bird species possess, sometime with hundreds of song types,

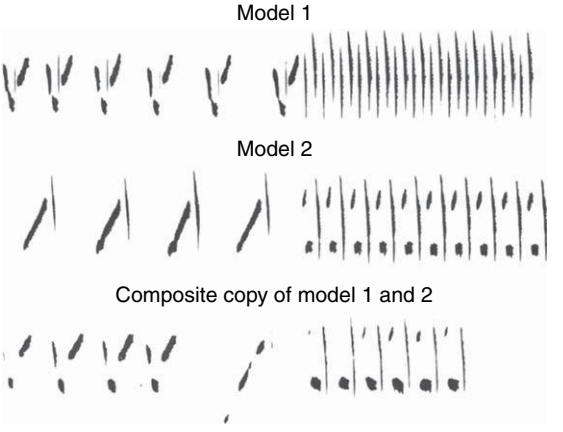

Figure 8 An example of a bird that created a new song by recombination. A captive male song sparrow was given only artificial songs to learn made up of two-parted swamp sparrow songs. He created a three-part song, approximating normal song sparrow syntax by combining parts of two different models.

built up by reiteration of these processes of imitation, segmentation, recombination, improvisation, and invention are barely manageable as subjects for scientific study. But for anyone interested in esthetic theory, the birds with large repertoires that give human listeners so much pleasure, such as mockingbirds and their relatives, the Australian lyre-bird, and certain thrushes, are unrivaled as subjects of choice. Utilitarian concerns are only one aspect of our fascination with birdsongs. If we are looking for animal models of the creative impulse, birdsong is one of the few that comes close to human standards.

1.17.8 Conclusions

As more bird species are studied, we find that each has achieved its own special solutions to the problems of developing a song. Songsters participate in a system of social communication that relies upon signals learned from others. Some species learn readily from song stimulation alone, and often remarkably few song stimuli are necessary, as though the brain is primed to be especially responsive to songs of a bird's own kind. Other species take longer to learn and require social stimulation.

Despite the possession of extensive innate foreknowledge about songs of their species, and a strong inclination to favor them if given a choice, they can be persuaded under special circumstances to learn songs of others. Certain species engage in the process of between-species mimicry naturally and create a species-specific song by imposing distinctive temporal patterning on the imitations as they are rendered. Some birds are faithful to the song they imitate, and others use imitations as a basis for improvisation, retaining certain features of the tutor and changing others.

The diversity of these patterns of behavioral development is made possible because, along with the oscine song system, the brain of each bird species brings to bear its own distinctive set of properties and predispositions to the task of learning to sing. The challenge for the neurobiologist is to understand not only the basic brain mechanisms that underlie the generic learning abilities of all song birds but also the more subtle, singular means by which a given species adds its own unique flavor to the process of developing a song, adapted to a particular set of social, ecological, and phylogenetic circumstances.

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1.18 Adaptive Specializations and Generality of the Laws of Classical and Instrumental Conditioning

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1.18.1 Introduction

How general are the laws of classical and instrumental conditioning? Empirical observations invariably involve a particular species and responses and stimuli arranged in a particular fashion. But the findings would be of little interest if they were only applicable to the specific circumstances of their discovery. Scientists strive for generality. A specific experiment is of import only because we assume that it illustrates truths about the world that go beyond the circumstances of that experiment.

Early investigators of classical and instrumental conditioning were quick to assume that the behavior changes they were discovering in the laboratory represented general laws or principles of learning applicable to a variety of species and circumstances. Thorndike, for example, formulated the Law of Effect to characterize how instrumental behavior is acquired (Thorndike, 1898, 1911). Pavlov provided principles of classical conditioning, including delayed conditioning, inhibition of delay, extinction, and spontaneous recovery (Pavlov, 1927). Skinner characterized general principles of shaping and schedules of reinforcement for operant conditioning. Hull, Guthrie, and Tolman followed suit in formulating general theories of learning which were not restricted to a particular species, response, or stimulus (Bower and Hilgard, 1981).

Formulations that ignore the species and the particular stimuli or responses that are involved in a conditioning experiment have continued to dominate learning theory in the latter part of the twentieth century. These include theories offered by Rescorla and Wagner (1972), Mackintosh (1975), and Pearce and Hall (1980), and more recently the sometimes opponent processes (SOP) theory and affective emotional SOP (AESOP) (Wagner, 1981; Wagner and Brandon, 1989), the comparator hypothesis (Miller and Matzel, 1988), the temporal coding hypothesis (Savastano and Miller, 1998), and Rate Expectancy Theory (Gallistel and Gibbon, 2000). (*See also* Chapters 1.06, 1.10, 1.12.)

The assumption of generality has shaped not only our theories but also the range of experimental preparations that we use to study classical and

instrumental conditioning. If conditioning is in fact a general process, then we should be able to discover the laws of conditioning regardless of what species or situation we examine. This rationale, explicitly stated by Skinner, encouraged investigators to select experimental preparations based primarily on convenience. At the behavioral level, investigators have settled on a small number of experimental paradigms that have gained wide usage. These include pigeons key pecking for food as the reinforcer, rats pressing a lever for food or water, and the conditioned suppression or conditioned freezing procedure for studying classical conditioning.

The assumption that the laws of learning are general and widely applicable has also provided the fundamental justification for studies of the neurobiology of learning. Although such studies may be conducted in a hippocampal slice preparation or in a marine mollusk, the experiments are assumed to reveal general principles of neural plasticity that can be extended to intact vertebrates. The present chapter will review challenges to the generality of Pavlovian and instrumental conditioning at the behavioral level and discuss theoretical efforts to incorporate these challenges into a revised general theory of conditioning (*See also* Chapters 1.17, 1.20, 1.22). The exposition will focus on studies of conditioning in vertebrate species. However, the issues raised are also relevant to studies of learning in nonvertebrate species such as the honey bee (e.g., Menzel, 1990, 1999) (*See also* Chapters 1.27, 1.29).

1.18.2 Biological Constraints on Learning

Although the general-process perspective predominated theories of conditioning through much of the twentieth century, there were serious kinks in its armor. The first prominent challenges were actually provided by Thorndike, who studied learning in 15 different puzzle boxes and found some significant differences among them. In most of the puzzle boxes, the instrumental response was manipulating a latch or pulling a string to escape and get a piece of food. However, in some of the boxes, the required response was a self care behavior (e.g., grooming or yawning). Learning did not proceed smoothly in these boxes. The instrumental response initially increased, but the gain was not sustained. The yawn or scratch became perfunctory and not as genuine as spontaneous occurrences of these behaviors. Thorndike proposed the concept of *belongingness* to characterize these failed instances of the Law of Effect. According to this concept, an instrumental response has to 'belong with' the reinforcer to show robust acquisition.

It is to Thorndike's great credit that he reported failures in instrumental conditioning and violations of his Law of Effect, along with the successes. However, the failures did not attract much attention for nearly half a century. That is not to say that learning psychologists were always successful in designing instrumental conditioning procedures. Rather, the number of common experimental paradigms quickly narrowed to three that investigators could rely on to produce orderly data (lever-pressing in rats, key pecking in pigeons, and running in a runway in rats), and the importance of 'belongingness' effects took a back seat to efficient data collection. Subsequent analyses suggested that the development of these standard conditioning paradigms required considerable tuning that helped to match the procedure to the animal's behavioral predilections (Timberlake, 1990, 2001b).

Potential problems with general principles of shaping and instrumental reinforcement only became evident when investigators stepped outside the scope of conventional procedures. Two of Skinner's graduate students ventured far from standard laboratory procedures in their efforts to create displays for amusement parks and shopping malls showing ducks, chickens, raccoons, rabbits, and pigs doing various entertaining things for food reinforcement (Breland and Breland, 1961). If the instrumental response was releasing an object or token for food (e.g., having a pig put a coin in a piggy bank), initial acquisition was followed by a serious deterioration in performance. The pig, for example, would root the coin on the ground, and the raccoon would rub the coins together or 'dunk' it into the coin slot instead of releasing it. These responses resembled the natural behavior of these animals in situations involving feeding. Therefore, Breland and Breland characterized these as instances of 'instinctive drift.'

The failures of the Law of Effect and the shortcomings of Skinnerian principles that were highlighted by the Brelands were soon followed by analogous examples in avoidance conditioning. Bolles (1970) reported that freezing and running were much easier to condition as avoidance responses than rearing, lever-pressing, or pecking a response key. Unexpected

failures of learning also appeared in the area of stimulus control. Foree and LoLordo (1973) found that pigeons were less likely to use visual cues than auditory cues as discriminative stimuli for avoidance behavior. Garcia and Koelling (1966) found that rats could not associate audiovisual cues with illness or taste cues with foot shock.

These dramatic failures or constraints on learning were obtained at the same time that investigators discovered examples of remarkably rapid learning. Tastes could be associated with illness in a single trial, even if there was a long delay between the two events (Garcia et al., 1966). Pigeons could learn to peck a key-light paired with food (Brown and Jenkins, 1968), even if the pecking behavior was not required to obtain the food (Williams and Williams, 1969). Freezing and running were readily acquired as avoidance responses (Bolles, 1970), and audiovisual cues were readily associated with foot-shock even though they could not be associated with poisoning (Garcia and Koelling, 1966).

1.18.2.1 Empirical Analyses

Although initial theoretical efforts to explain biological constraints and adaptive specializations in classical and instrumental conditioning did not provide much insight into these phenomena, empirical analyses were more informative. In general, these served to confirm the validity of the initial findings and suggested ways in which problematic phenomena might be incorporated into general process theory (Domjan, 1983).

1.18.2.1.1 Constraints on the conditioning of token-release behavior

As noted earlier, one of the major problematic findings was that animals required to deposit a token for food or water reinforcement often refused to release the token (Breland and Breland, 1961). This behavior was initially puzzling because holding onto the token was not required for reinforcement and actually negated reinforcer delivery. Although the finding was puzzling from the perspective of instrumental conditioning, it could be easily explained once investigators considered the role of Pavlovian contingencies in the procedure. Requiring contact with the token before the delivery of food resulted in pairings of the token with the food reinforcer. Such stimulus–reinforcer pairings served to condition appetitive properties to the token stimulus, and the subsequent reluctance of the animal to relinquish the token was a reflection of this Pavlovian conditioned appetitive behavior (Boakes et al., 1978). Consistent with this interpretation, Pavlovian pairings of a token stimulus with food are sufficient to generate contact and handling of the token (Timberlake et al., 1982; see also Timberlake and Washburne, 1989).

1.18.2.1.2 Constraints on the conditioning of self-care responses

Thorndike (1911) was the first of several investigators who noted that grooming responses such as scratching and yawning are difficult to increase with instrumental conditioning. Subsequent experiments with the golden hamster suggested that this constraint on learning was also due to the Pavlovian conditioning that inevitably accompanies an instrumental training procedure. Hamsters show a suppression of grooming in anticipation of daily feedings (Shettlesworth, 1975), and grooming occurs as an interim rather than a terminal response with periodic food deliveries (Anderson and Shettlesworth, 1977). Thus, Pavlovian processes tend to suppress rather than facilitate grooming. Another factor that might be responsible for constraints on the conditioning of grooming responses is lack of necessary support stimulation. Pearce et al. (1978) found that scratching in rats can be conditioned more easily as an instrumental response if the rats wear a collar that provides tactile stimulation. Scratching appears to be easier to condition in primates (Anderson et al., 1990), perhaps because primates are better able to discriminate instances of scratching (Morgan and Nicholas, 1979).

1.18.2.1.3 Constraints on the conditioning of avoidance learning

Avoidance behavior appears to be even more heavily constrained than positively reinforced behavior. The limitations operate both on the types of responses that can be learned to prevent aversive stimulation and the types of stimuli that can serve as cues for avoidance behavior. Rats readily learn to avoid aversive stimulation if the instrumental response is running in a wheel, jumping out of a shock box, or remaining still (Maatsch, 1959; Bolles, 1969; Brener and Goesling, 1970). However, they have difficultly learning to rear or press a lever to avoid shock (D'Amato and Schiff, 1964; Bolles, 1969). Pigeons have a much harder time learning to peck a response key to avoid shock (Schwartz, 1974) than they have learning to press a treadle (Foree and LoLordo, 1970).

Avoidance procedures typically provide a signal for the impending aversive stimulus, and responding during the signal cancels the scheduled shock and turns off the signal. Auditory cues tend to become conditioned more readily as warning signals than visual cues (Foree and LoLordo, 1973; Jacobs and LoLordo, 1977), whereas visual cues are more easily conditioned as signals for food. Subsequent research has shown that selective association effects can also be obtained with a single reinforcer (food or shock). In these studies, the stimulus control acquired by the auditory or visual element of a compound stimulus depended on whether the compound signaled the presence or absence of the reinforcer (Weiss et al., 1993; Panlilio and Weiss, 2005).

Initial efforts to understand biological constraints on avoidance learning emphasized the concept of species-specific defense reactions (SSDRs). Bolles (1970, 1971) proposed that, in an aversive situation, the organism's repertoire is severely limited to a set of instinctive defensive behaviors (SSDRs) that include freezing, fleeing, and aggression. Which of these responses predominates was assumed to depend on environmental factors. In the absence of an escape route, freezing was presumed to predominate. If there is a potential escape route, fleeing may be the predominant SSDR. Responses such as lever-pressing or key-pecking are never likely to be acquired as avoidance behaviors because they are not related to an SSDR.

The SSDR hypothesis was important because it emphasized for the first time that to understand avoidance behavior we first have to consider the instinctive defensive behavioral repertoire of the organism. However, the details of the SSDR hypothesis turned out to be incorrect. Studies showed that the predominant SSDR for rats was freezing whether or not a prominent escape route was available (see Fanselow and Lester, 1988). These observations led to a reformulation of the SSDR hypothesis that introduced the concept of predatory imminence (Fanselow and Lester, 1988). This revised theory assumes that which of a range of possible defensive behaviors occurs depends on the perceived imminence of danger or predation. A low level of perceived danger (or predatory imminence) may reduce the amount of time a rat spends foraging but does not generate a targeted defensive action. In contrast, a high level of perceived danger elicits freezing. When the danger (or predator) is actually encountered, a defensive circa strike response is elicited. Differences in how rapidly subjects learn various avoidance responses are explained by reference to timing of expected danger and the instinctive responses that are elicited by a particular level of predatory imminence.

1.18.2.1.4 Long-delay taste-aversion learning

Another prominent phenomenon that encouraged consideration of adaptive specializations of learning is long-delay taste-aversion learning. This effect was originally discovered during the course of research on the biological effects of exposure to X-rays. Garcia and colleagues found that rats exposed to irradiation after drinking a saccharin solution subsequently showed a strong aversion to the saccharin flavor (Garcia et al., 1966; see also Smith and Roll, 1967). This type of learning was considered to be an exception to general process learning theory because it occurred in a single trial even if the irradiation (unconditioned stimulus, US) was presented several hours after the taste (conditioned stimulus, CS) exposure. Since this type of learning occurred rapidly over a long CS-US interval, it could hardly be considered a 'constraint' or limitation on learning. Furthermore, one could hardly argue that rats evolved to learn about exposure to irradiation. However, long-delay aversion learning helped explain how animals avoid nutritionally inadequate and poisonous foods and thereby end up eating a healthy diet. Therefore, taste-aversion learning became treated as an example of an adaptive specialization of learning that plays an important role in food selection (Rozin and Kalat, 1971).

Taste-aversion learning remains unique in permitting a delay of several hours between the CS and the US. Nevertheless, efforts to understand the basis for long-delay taste-aversion learning have identified a number of important factors. One factor concerns the nature of the taste stimulus and how it is encountered. In traditional classical conditioning procedures, the subject does not have to do anything to obtain either the CS or the US. In fact, the lack of a response contingency is considered to be a defining feature of classical conditioning. In contrast, taste stimuli are usually encountered as a result of licking a drinking tube. The licking behavior controls exposure to the CS and also provides nongustatory orosensory stimulation. The licking contingency and orosensory stimulation are important for taste-aversion learning. If a flavored solution is passed over the tongue without licking (through a cannula or while the subject is paralyzed with curare), the

strength of the taste aversion is significantly attenuated (Domjan and Wilson, 1972a; Domjan, 1973).

Another factor that contributes to long-delay taste-aversion learning is that the stimuli that subjects are likely to encounter during the delay period are not readily associable with illness and therefore do not provide concurrent interference (Revusky, 1977). In the typical long-delay taste-aversion experiment, subjects are given a novel flavor to drink and are then injected with a toxin several hours later. During the interval between the taste and illness, the subjects inevitably encounter various visual, auditory, and tactile cues. However, these are not likely to interfere with conditioning of the target flavor because nongustatory stimuli are less likely to become associated with toxicosis (see following section). Consistent with this idea, introduction of other flavors during the delay interval disrupts learning an aversion to the target flavor (Kalat and Rozin, 1971; Revusky, 1977). The concurrent interference hypothesis is challenged in explaining long-delay aversion learning to the tactile or other nongustatory aspects of ingested food (e.g., Revusky and Parker, 1976; Domjan and Hanlon, 1982; Domjan et al., 1982), since these should be disrupted by other tactile stimuli encountered during the delay interval. However, these other tactile cues are not accompanied by orosensory stimuli related to eating and drinking, and therefore they may not be as available for association with illness.

1.18.2.1.5 Selective associations

Although novel tastes readily become associated with illness, novel auditory or visual cues do not. In contrast, auditory and visual cues readily become associated with foot-shock, whereas taste cues do not. These cue-consequence specificity effects were first clearly demonstrated by Garcia and Koelling (1966) and attracted a great deal of attention because they were entirely contrary to general-process learning theory as it was known at the time. Initial efforts to analyze the effect focused on various methodological issues to rule out potential artifacts. To properly compare learning about taste and audiovisual cues, the two types of stimuli have to be presented in the same manner. Garcia and Koelling (1966) equated the method of CS presentation by having rats lick a drinking tube that produced both the taste and the audiovisual stimuli. In a subsequent study, Domjan and Wilson (1972b) equated the method of stimulus presentation by having both types of CSs presented independent of behavior. Similar results were obtained with both approaches.

Another methodological concern was that shock and illness may have different nonassociative effects, and those might be responsible for cue-consequence specificity effects. That possibility was ruled out in a study in which rats were given both shock and illness unconditioned stimuli, but only one of the USs was paired with a CS (Miller and Domjan, 1981). Despite exposure to both USs, when taste and a visual cue were paired with illness only a taste aversion developed, and when the CSs were paired with shock, an aversion only to the visual cue occurred.

In all of the initial experiments, aversions were measured in terms of suppression of drinking, and some critics were concerned that this response measure was differentially sensitive to certain CS-US combinations. To evaluate that possibility, Miller (1984) recorded a variety of responses in a cue-consequence specificity experiment (freezing, rearing, grooming, chin-wiping, head shaking, gaping, and drinking). He found that a taste paired with illness elicited increased gaping, head-shaking, and chin-wiping, and an auditory cue that had been paired with shock elicited increased freezing and decreased rearing and grooming. No evidence of learning in any response measure was found if the taste had been paired with shock or the auditory cue had been paired with shock.

The aforementioned studies served to substantiate the cue-consequence specificity effect but did not prove that the phenomenon was due to adaptive specializations resulting from evolutionary selection. Because the experiments were conducted with adult rats, one could argue that their previous experiences resulted in selective associations in adulthood. During normal ontogenetic experience, tastes are correlated with interoceptive malaise, and telereceptive cues are correlated with cutaneous pain. Perhaps these historical experiences produce the cue-consequence specificity effect. This experiential hypothesis was evaluated by conducting conditioning 1 day after birth. Despite the young age of these subjects, the cue-consequence specificity effect was replicated (Gemberling and Domjan, 1982).

1.18.3 Theoretical Analyses

1.18.3.1 Biological Constraints

The examples of conditioning that were unexpected on the basis of general learning processes were initially characterized as *biological constraints* on learning (Shettlesworth, 1972; Hinde and Stevenson-Hinde,

1973). The term 'biological' was intended to capture the notion that these examples were the result of natural selection. The term 'constraint' was used because investigators found limitations on what animals could learn particularly noteworthy. However, beyond pointing out the relevance of the subject's evolutionary history, the concept of a biological constraint does little to further our understanding of these unusual learning phenomena and does little to help us discover new ones (Domjan and Galef, 1983). The various forms of learning that were identified as biologically constrained had little in common other than the fact that they were unexpected on the basis of extant general process theory. Thus, considering them in a single category did not help to identify their underlying processes. Furthermore, it is unlikely that these constraints on learning were specifically selected for. Rather, they were probably the consequence of the selection of other forms of conditioning that were especially useful for the organism and increased its reproductive fitness. Thus, constraints on learning may be epiphenomena that result from the selection of forms of conditioning that are especially effective in foraging for food or avoiding predation.

1.18.3.2 Adaptive Specializations

Characterizations of special forms of Pavlovian and instrumental conditioning as adaptive specializations also emphasized the evolutionary origins of these learning phenomena. But in this case the focus was on instances of more rapid or robust conditioning than on slow and laborious learning (Rozin and Kalat, 1971). Taste-aversion learning was frequently discussed as an adaptive specialization, since it occurs with long delays between taste and illness, can be acquired in just one trial, and is specific to taste cues. One could easily imagine how this kind of learning might have evolved, since it seems ideally suited to solve many of the challenges that omnivores face in selecting a healthy diet (Rozin and Kalat, 1971). Rapid acquisition of freezing behavior in aversive conditioning probably also evolved as a specialization of the defensive behavior system.

Although the adaptive specialization approach is more promising than the constraints approach, labeling something as an adaptive specialization is just a promissory note that requires demonstrating exactly how the conditioning process increases reproductive fitness. The promissory note has not been paid in the case of taste-aversion learning. The justification is more convincing with conditioned freezing, since animals that freeze are less likely to be detected by a predator (Arduino and Gould, 1984; Suarez and Gallup, 1981). The only direct evidence of learning increasing reproductive fitness comes from studies of sexual conditioning (Hollis et al., 1997; Adkins-Regan and MacKillop, 2003; Mahometa and Domjan, 2005; Matthews et al., in press). However, those experiments employed conventional CSs, and the authors did not argue that the increase in reproductive fitness reflected an adaptive specialization in learning. Other examples of sexual conditioning (to be described later) involved species-typical CSs and may be more accurately characterized as representing adaptive specializations.

1.18.3.3 Preparedness

Biological constraints and adaptive specializations were proposed as alternatives to a general process account of conditioning phenomena. However, they did not dissuade investigators from looking for ways to incorporate unusual forms of learning into a more general conception. The first serious proposal along those lines was the concept of preparedness proposed by Seligman (1970; see also Seligman and Hager, 1972).

According to the concept of preparedness, tasks were considered to differ in the extent to which they were compatible or incompatible with the evolutionary history of the organism. Preparedness was defined as "the degree of input necessary to produce a specified output" in a learning procedure (Seligman, 1970, p. 407). Forms of learning that occurred quickly with relatively little input were said to be highly prepared. In contrast, forms of learning that required extensive training and input were said to be unprepared or contraprepared. Thus, the concept of preparedness enabled ordering various examples of learning along a continuum of preparedness. This continuum in turn served to integrate examples of biological constraints and adaptive specializations of learning without treating either category as an exception to general principles of learning. The proposal was to supplement general process theory with the continuum of preparedness that would then help characterize all forms of learning.

The concept of preparedness provided a convenient way to talk about instances of learning that were particularly easy or difficult to learn. However, the concept did little to advance our understanding or learning processes (Domjan and Galef, 1983). As first

proposed, the concept was basically circular. Measures of the rate of learning were used to identify different levels of preparedness, at the same that preparedness was intended to explain differences in learning rate (Schwartz, 1974). Second, a common metric was not available to measure input or training across widely disparate learning tasks such as taste-aversion learning and language acquisition. Third, even if a common metric could be found, behaviors that are learned at similar rates may not share other characteristics, such as rates of extinction or sensitivity to temporal contiguity. Despite these shortcomings, the goal of defining a continuum of learning effects that differ in biological preparedness remains laudable.

1.18.3.4 Behavior Systems and Learning

One of the most systematic efforts to integrate specialized forms of learning into a general theory is the behavior systems approach. This approach provides a general framework for characterizing how evolutionary influences determine the specific behavioral outcomes of laboratory conditioning procedures. Its starting point is that behavior is organized into functional systems that evolved to deal with specific tasks.

Functional systems of behavior have been entertained by a variety of investigators. Garcia and colleagues, for example, invoked functional systems in their efforts to explain differences between taste-aversion learning and fear conditioning (Garcia et al., 1974). According to them, organisms evolved specialized behavioral and neural mechanisms to deal with interoceptive information provided by the taste of food and its postingestive consequences. The interoceptive system operates on a time scale that facilitates the association of interoceptive CSs and USs, and this is responsible for long-delayed taste-aversion learning. In contrast, different behavioral and neural mechanisms, operating on shorter time scales, are involved in organizing responses to external cues and their consequences, such distal visual and auditory input that may lead to cutaneous pain. According to Garcia et al. (1974), selective associations reflect differences in the systems that are designed to deal with the *milieu interne* as contrasted with the *milieu externe*.

The behavior systems approach is also consistent with how Bolles (1970, 1971) characterized constraints on avoidance conditioning. As was noted earlier, Bolles proposed that aversive situations activate the defensive behavior system, which constrains the response repertoire of the organism to species-specific defense reactions. This idea was subsequently fleshed out with the suggestion that the defensive behavior system is also characterized by differential sensitivity to auditory cues (Foree and LoLordo, 1973) and is organized along a temporal dimension dictated by perceptions of the imminence of danger (Fanselow and Lester, 1988).

The concept of behavior systems and how they shape the behavioral outcomes of conditioning were spelled out in greatest detail by Timberlake and his associates (e.g., Timberlake, 2001a; Timberlake and Lucas, 1989). Although Timberlake focused on the feeding system in his empirical and theoretical work, he proposed a general framework for conceptualizing behavior systems and their role in learning. According to this framework, behavior is organized into functional systems, each designed to accomplish a major biological goal (feeding, predatory defense, reproduction). Within each system, behavior is organized in terms of a hierarchy of control mechanisms. The first level consists of response modes, each of which contains a number of response modules.

Response modes have a linear temporal organization, with one mode reliably following another. For example, in the feeding system, the general search mode is followed by the focal search mode, which in turn is followed by the food/handling/consumption mode. The same linear order holds whether the sequence moves forward to ingestion of food or backward in the event that food is not found. That is, if the food/handling/consumption mode is not successful, the organism returns to the focal search mode rather than the general search mode. Most importantly for predictions of learning effects, each response mode and module is characterized by unique stimulus sensitivities and response propensities. In the focal search mode, for example, animals are more attuned to details of their environment as they look for food in a particular location. Once the food has been obtained, the response repertoire shifts to food handling behaviors, controlled by features of the food object itself.

The behavior systems approach helps us understand biological constraints on conditioning because it assumes that the ease of learning a particular task depends on the extent to which the task is consistent with the behavior system that is activated by that conditioning procedure. Food deprivation and periodic deliveries of food, for example, strongly activate the feeding system, increasing the probability of

responses and stimulus sensitivities associated with that system. If a conditioning procedure requires an instrumental response, such as grooming, that is incompatible with the feeding modes and modules, that response will be difficult to reinforce with food. Responses more relevant to the feeding behavior system will emerge with food deprivation and food reinforcement, whether these are specifically reinforced or not. The emergence of these relevant behaviors is what Breland and Breland (1961) characterized as 'instinctive drift.'

1.18.4 Natural Learning Paradigms

All approaches to studying adaptive specializations in learning assume that specialized learning effects are the products of evolution. It is important to keep in mind that evolutionary selection is an interaction between selection processes and the ecology of the organism. Adaptation is not a progression to an ideal and does not occur in a vacuum. Rather, it occurs in the particular ecology and microenvironment inhabited by the species. Natural learning paradigms focus on that microenvironment.

General theories of conditioning are invariably couched in terms of abstract stimulus and response elements (conditioned and unconditioned stimuli or instrumental responses and reinforcers). An important assumption of general process theory is that these elements are unrelated before a conditioning procedure is introduced. In fact, this initial independence has been incorporated into the definitions of the stimulus and response elements. For example, a CS is defined as being 'arbitrary' or 'neutral' before its pairings with a US (Bower and Hilgard, 1981; Staddon, 1983; Shettleworth, 1998; Papini, 2002). These terms are used to signify that the CS has no inherent relation to a US prior to the introduction of a conditioning procedure.

The lack of an initial relation between CS and US may accurately characterize laboratory procedures for Pavlovian conditioning, but such independence cannot be true for naturally occurring instances of conditioning. If a CS and US were truly unrelated to each other in nature, they would not coincide with sufficient regularity to become associated. An arbitrary or neutral CS may be paired with a US once in a while by chance, but even if such pairings occurred, they would be preceded and followed by individual encounters with the stimuli, which would undermine their subsequent association (Benedict and Ayres, 1971; Rescorla, 2000). For a CS to be reliably paired with a US outside the laboratory, the CS has to be a natural precursor of the US or a stimulus that reliably occurs in the causal chain of events that leads to the US. Thus, in natural learning paradigms, the CS has to have an inherent or preexisting relation to the US before the onset of learning. Such an inherent relation is required for the CS to be reliably paired with the US so that an association can develop.

The fact that learning outside the laboratory depends on preexisting relations between events has been recognized for some time (Dickinson, 1980; Staddon, 1988). Being able to predict events of biological significance enables organisms to cope with those events more successfully (Domjan, 2005), and the best predictors of biologically significant events are "the causes of these events, or at least detectable indices of these causes" (Dickinson, 1980) – not arbitrary or neutral stimuli.

Interestingly, the first demonstrations of classical conditioning in Pavlov's laboratory involved a procedure that could readily occur in nature (Boakes, 1984). Studying how salivation may be elicited by various substances such as dry food, Stephan Vul'fson, one of Pavlov's students, observed that the dry food elicited salivation the first time it was placed in a dog's mouth. After several tests with the dry food, the sight of the food also started to elicit salivation. In this initial demonstration, the CS was the sight of the food and the US was the food in the mouth. Notice, that the CS was not arbitrary or initially unrelated to the US. Rather, the CS was a telereceptive feature of the food object.

Much of the early literature on adaptive specializations and biological constraints on learning involved learning situations that resembled natural learning paradigms. Long-delay taste-aversion learning is a prime example. Such learning probably evolved to discourage the ingestion of poisonous foods or substances with little nutritional value (Rozin and Kalat, 1971; Rzoska, 1953). An omnivore, such as a rat, that eats a varied diet has to learn to avoid eating foods that make it sick. Given that illness is usually a delayed consequence of eating something poisonous, the learning mechanism has to operate with a long delay between the taste and the consequent malaise. Note that the CS flavor is not arbitrary, neutral, or unrelated to the consequent poisoning. Rather, the taste is a feature of the poisonous food itself. Thus, it is inevitably encountered during the chain of events that results in food-induced illness.

Learning can occur to natural precursors of the US in a variety of response systems. In interacting with a sexually receptive female, for example, a male rat first encounters the odors of the female. Sexually naive males do not have a preference for the odor of estrous females. However, a preference develops with copulatory experience (Carr et al., 1965; Ågmo, 1999), presumably because of the pairings of the odor with sexual reinforcement.

Naturalistic learning can also occur in nursing situations, where tactile cues provided to an infant by the mother typically precede nursing and can come to elicit conditioned orientation and suckling responses (Blass, 1990). Cues that precede a nursing episode are also reliable precursors of the suckling stimulation that elicits oxytocin release and the milk let-down reflex on the part of the mother. Oxytocin release and the milk let-down reflex are initially unconditioned responses to suckling. However, they can also come to be elicited as anticipatory conditioned responses to prenursing tactile and olfactory cues provided by the nursing infant (Fuchs et al., 1987; Tancin et al., 2001).

Learning no doubt also occurs to stimuli that are encountered during the course of territorial and predator–prey interactions. Although predators have evolved strategies for surprising their prey, these improvements in offense have been accompanied by the evolution of detection and defensive tactics on the part of potential prey species. Cues that precede predatory attack are not arbitrary but are related to the visual, olfactory, or auditory features of a predator that can be detected at a distance before the attack. Learning to associate these cues with the subsequent predatory encounter can improve the effectiveness of defensive behavior (Hollis, 1990). Furthermore, fear is acquired more readily to ecologically relevant cues, such as the sight of a snake, than to ecologically irrelevant cues, such as the sight of flowers (Öhman and Mineka, 2001).

Repeated drug administration by drug addicts has also been described as a natural learning paradigm in which cues related to drug administration come to be associated with the drug effects and come to elicit conditioned physiological responses in anticipation of the impending drug delivery (Siegel et al., 2000). Consistent with this model, one approach to alleviating the symptoms of drug addiction involves repeated presentations of the drug administration cues without the drug, so as to extinguish the drug-conditioned responses (Siegel and Ramos, 2002).

Siegel and his associates have extended the conditioning analysis of drug addition to learning about different components of a drug effect (Kim et al., 1999). This analysis focuses on the fact that drug effects are typically long lasting and have distinct onset properties. One cannot experience the enduring effects of a drug without first encountering its onset properties. Therefore, the early physiological effects of a drug can serve as a CS for later drug effects and can come to control conditioned anticipatory physiological adjustments, in a manner analogous to the effects of exteroceptive drug administration cues. In this paradigm the CS is clearly not arbitrary or neutral with respect to the US.

1.18.4.1 Special Properties of Naturalistic Learning

The provided examples illustrate that conditioned stimuli that are natural precursors of the US are excellent candidates for conditioning. However, these examples do not prove that learning with naturalistic CSs occurs more rapidly or is more evolutionarily prepared than learning with arbitrary CSs. Evaluation of that hypothesis requires direct comparisons of learning with naturalistic versus arbitrary cues. Such comparisons have been conducted in the context of taste-aversion learning (see reviews by Logue, 1979; Domjan, 1980) and fear conditioning (e.g., Öhman and Mineka, 2001). More recently, Domjan and associates have examined this issue in detail in studies of the sexual conditioning of male Japanese quail, *Coturnix japonica* (see review by Domjan et al., 2004).

Japanese quail are ground birds that live in the Far East and in Hawaii in areas with tall grass (Schwartz and Schwartz, 1949). Although not much is known about their natural history, when a male initially sees a female, he is likely to see only her head and some of her neck feathers. As the two get closer and closer to one another, they get to see more of each other, and that permits more proximal social interactions. Thus, the natural sequence of events that results in an intimate social interaction and copulation begins with limited visual (and perhaps auditory) contact. This sequence of events can be replicated in the laboratory in a special application of a sexual conditioning procedure.

In a sexual conditioning procedure, a signal or CS is presented shortly before a male (or female) is permitted to interact with and copulate with a sexual partner (Pfaus et al., 2001; Woodson, 2002; Krause, 2003). For basic demonstrations, the CS may be a light or an odor. However, the procedure can be also designed to model the sequence of events that potential sexual partners encounter in their natural habitat. In the case of the *Coturnix* quail, this has been done by using a three-dimensional object as the CS that included a taxidermically prepared female head with partial neck feathers. To determine how learning with such a naturalistic CS differs from learning with a more conventional arbitrary CS, an alternative CS object was constructed that was similar in size and general shape to the naturalistic CS but lacked the taxidermic head+neck component.

A basic question that has to be answered at the outset with a naturalistic CS is whether such a CS elicits behavior unconditionally, without pairings with a US. Several studies have examined the responding that develops in male *Coturnix* quail to a head+neck object that is either paired with sexual reinforcement or presented in an unpaired fashion (e.g., Köksal et al., 1994; Cusato and Domjan, 1998; Hilliard et al., 1998). These studies have shown that a head+neck CS elicits a low level of approach responding if it is unpaired with copulation, but this approach behavior is substantially enhanced if the head+neck CS is paired with the sexual reinforcer. Interestingly, this pattern of results is similar to what is typically observed in taste-aversion learning. Unpaired presentation of a novel taste with toxicosis results in a mild aversion or neophobia to the taste solution. Pairing of the taste with illness produces a much more profound aversion. In both of these naturalistic learning situations, conditioning enhances a preexisting response to the CS.

If substantial responding to a naturalistic CS requires pairings with a sexual US, how does such learning differ from learning with an arbitrary CS? The first examination of this question was conducted by Köksal et al. (1994) who compared the extent to which the blocking effect occurs if the blocked CS includes (or does not include) female head+neck cues. All of the male quail that served in the experiment were initially conditioned with an audiovisual CS as a cue for sexual reinforcement. The preconditioned audiovisual cue blocked conditioning of an arbitrary CS object but did not block conditioning if the CS object included head+neck cues. Thus, the naturalistic CS was resistant to the blocking effect.

Subsequent studies have identified a cluster of learning phenomena that serve to differentiate how sexual conditioning proceeds with naturalistic versus arbitrary CS objects. In general, conditioned responding develops more rapidly with the naturalistic CS, and a greater range of conditioned responses come to be elicited (Cusato and Domjan, 1998). In particular, whereas the arbitrary CS object only elicits conditioned approach behavior after modest levels of training (e.g., 10–15 conditioning trials), the naturalistic CS also comes to elicit vigorous copulatory attempts (grabs, mounts, and cloacal contact responses) directed at the CS. Including naturalistic features in a CS object also increases the effectiveness of that CS in producing second-order conditioning (Cusato and Domjan, 2001; also described in Domjan et al., 2004).

Another phenomenon that shows the more robust nature of learning with a naturalistic CS is that such learning is not easily disrupted by increasing the CS-US interval (Akins, 2000). Long-delay learning is one of the signature features of taste-aversion learning. Although learning in the sexual behavior system does not occur with delays on the order of several hours, the use of a naturalistic CS significantly extends the range of effective CS-US intervals in sexual conditioning. Akins found that with a 1-min CS-US interval, conditioned sexual approach behavior develops whether or not the CS includes naturalistic features. However, if the CS-US interval is increased to 20 min, conditioned approach responding develops only if the CS includes female head+neck cues.

Rate of acquisition, blocking, second-order conditioning, and manipulations of the CS-US interval are familiar ways to test the robustness of a learning phenomenon. A more recently developed manipulation involves the I/T ratio, where 'I' refers to the duration of time spent in the experimental context between trials (or the intertrial interval), and 'T' refers to the duration of the CS (or trial time). In general, CS-directed conditioned responding is less likely to develop with small I/T ratios (Gallistel and Gibbon, 2000). However, an I/T ratio that is too low to support conditioned sexual responding to an arbitrary CS will nevertheless support vigorous responding directed towards a naturalistic CS (Gean, 2006).

The original formulation of the concept of preparedness linked acquisition and extinction effects. Instances of evolutionarily prepared learning were assumed to show more rapid and robust acquisition effects as well as slower extinction effects. This

linkage of acquisition and extinction has rarely been examined empirically. In one of the few examples of such linkage, Krause et al. (2003) found that once vigorous sexual conditioned responding develops to a naturalistic CS, the responding is much more resistant to extinction than is conditioned responding to an arbitrary CS. In this experiment, responding to the no-head CS declined to near-zero levels by the 14th day of extinction, but responding to the head+-neck CS remained high through the end of the experiment on day 42. This effect may have been due to the fact that the subjects directed copulatory responses toward the naturalistic CS object. Even though this copulation occurred with an inanimate object, it might have provided some sexual reinforcement, which served to maintain responding during the extended extinction procedure (Köksal et al., 2004).

1.18.4.2 A Continuum of Learning Effects

The evidence reviewed suggests that learning in natural learning paradigms is more robust in a variety of respects as compared with learning with arbitrary cues. At least in the sexual behavior system, naturalistic learning takes place more quickly, recruits a broader range of conditioned responses, motivates stronger second-order conditioning, is resistant to blocking and extinction, and occurs with longer CS-US intervals and lower I/T ratios. This broad range of learning effects has not been examined with naturalistic and arbitrary conditioned stimuli in other response systems. Therefore, the generality of these findings remains to be evaluated. However, since evolutionary selection operates within the ecological niche of a species, it is reasonable to assume that selection for robust learning involves reactivity to naturalistic rather than arbitrary cues. Therefore, the phenomena that were observed in the sexual behavior system probably represent differences that occur more generally.

The naturalistic and arbitrary CS objects that were compared in the sexual conditioning studies represent two points along a continuum. One end of that continuum consists of stimulus objects that are entirely unrelated to a potential sexual partner. At this end of the continuum the CS has no features in common with the US. The other end consists of CS objects that have many features related to a sexual partner or the US. Such a continuum is reminiscent of the continuum of preparedness proposed by Seligman (1970). However, unlike the original preparedness concept, the continuum described here is not circular. Different points along the continuum are characterized in terms of the extent to which the CS is a natural precursor of the US, which can be determined independently of learning. Differences in learning effects can then be predicted based on positions along the continuum.

Many naturalistic learning paradigms fit the model of a CS that has some features in common with the US because unconditioned stimuli are typically complex sensory objects or events with multiple features. For example, food (whether it is inanimate or a potential prey) has visual, olfactory, and sometimes auditory features that are evident to the forager at a distance. These can act as conditioned stimuli that are predictive of other features (taste, texture, and postingestional properties) that are encountered only when the forager contacts and eats the food. The multifaceted nature of a potential food object provides opportunities to learn to predict the location, taste, and other features of the food. How this learning takes place is organized by the microecology of the foraging species. The spatial separation of the foraging animal from the food object determines the temporal order of exposure to different features of the food and determines the nature of the CS-US pairings.

Aversive conditioning during the course of predator defense can be analyzed in a similar fashion. The predator that attacks a prey species also provides a variety of telereceptive and more proximal cues. The sight, smell, or sounds of the predator can be detected at a distance and are predictive of attack and cutaneous pain if the predator manages to catch and bite its prey.

1.18.5 Conclusion

Investigators have always assumed that learning processes evolved because they provide an adaptive advantage. However, the full implications of this claim have been rarely appreciated. One important implication is that how learning occurs will depend on the details of the ecosystem in which it evolved. The generality of conditioning phenomena and processes cannot be established by proclamation or by studying arbitrary stimuli or responses. Rather, the generality of learning has to be established empirically. Furthermore, general learning effects are only apt to be evident to the extent that the demands of diverse ecosystems have common elements. In the case of Pavlovian conditioning, such generality

exists in the causal and temporal structure of the experiences that lead to encounters with biologically significant events or unconditioned stimuli. However, in these natural sequences, the antecedent or signaling events are not arbitrary or unrelated to the US. Instead they have an inherent relation to the US that is determined by the details of the organism's ecosystem. Since learning evolves in the microecology of individual organisms, stimuli and responses that are a part of that ecology will activate the learning process more effectively. Thus, learning with ecologically relevant stimuli and reposes will be more robust than learning with arbitrary cues.

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1.19 Learning to Time Intervals

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1.19.1 Introduction

Time is a fundamental dimension of life. Many animals have evolved the ability to time intervals lasting from a fraction of a second to hours. Traditionally, it is thought that a distinction must be made between circadian timing and interval timing (Gibbon et al., 1997a). Circadian timing concerns the ability to adjust to the daily cycle caused by the rotation of the Earth around its axis, which has a period of 24 h. Circadian clocks 'tick over' every 24 h or so when running 'free' (without external input about night and day), and the period is set to 24 h by external cues such as daylight. In vertebrate animals a neurological structure known as the suprachiasmatic nucleus is taken to be the 'master clock.' This chapter concerns interval timing, which is the ability to time shorter intervals, typically in the range of seconds to minutes.

We examine three experimental paradigms used to study interval timing and illustrate them with some characteristic results. Three theories of interval timing are discussed briefly. Then we turn to examining the differences between interval timing and circadian timing more closely, to show that the distinction is not so clear cut. We begin with three cases of the use of interval timing in the natural lives of animals.

1.19.2 Interval Timing in Everyday Life

The rat, normally a highly social animal, varies its eating behavior according to the size of the chunk of food it is eating. The motor patterns are so ingrained that it even does them when alone in an experimental arena, a standard operant chamber (Whishaw and Tomie, 1989; **Figure 1**). If a tiny pellet is delivered into the food magazine, the rat picks it up and eats it right at the magazine, without lowering its forepaws. With larger pellets, the rat lowers itself to the floor before eating. With still larger pellets, the rat takes the pellet and turns away from the magazine before

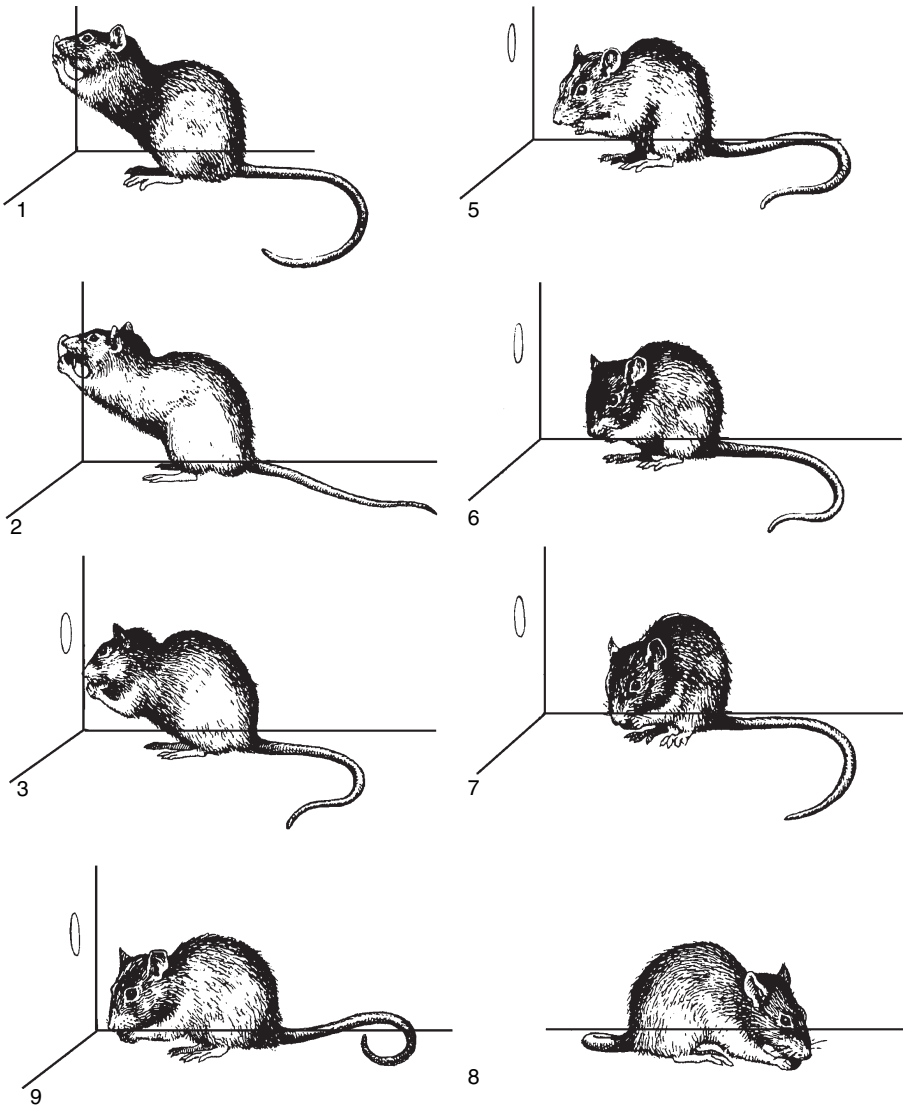

Figure 1 Illustration of what a rat does with a pellet of food of different sizes. Increasing numbers on the panels correspond to increasing food size. At the smallest sizes, the rat eats the food right at the hopper. At bigger sizes, it brings the food to ground level before eating it. At still larger food sizes, it turns away from the hopper before eating. At very large sizes (not shown), the rat hoards the food to an adjacent chamber. Reproduced with permission from Whishaw IQ and Tomie J (1989) Food-pellet size modifies the hoarding behavior of foraging rats. *Psychobiology* 17: 93–101.

eating. At the largest sizes, the rat hoards the pellet to an adjacent chamber. A further study showed that food handling time rather than food size was the determining factor in the rat's eating behavior (Whishaw, 1990). The rat varies what it does with food according to how long it takes to eat the pellet.

A foraging honeybee also needs to time short intervals regularly. Foragers rate the quality of pollen or nectar that they bring back relative to what others bring back (Seeley, 1995). This is achieved because foragers unload their booty to food storers in the hive. The length of time it takes to unload the forage thus indicates how good it is relative to what others bring back. This in turn influences other behaviors, such as whether to recruit other foragers by 'dancing' and how long to do the waggle dance (*See* Chapters 1.25, 1.29). Timing abilities in bees have also been addressed in formal experiments (Greggers and Menzel, 1993; Boisvert and Sherry, 2006).

Another example of timing in everyday life comes from a study of Rufous hummingbirds (Henderson et al., 2006). Hummingbirds adjust their visits to

flowers based on the rate at which the flowers replenish. Henderson and colleagues refilled multiple artificial flowers with sucrose solution 10 min after the bird emptied it; they refilled other flowers 20 min after the bird emptied these flowers. Throughout the day, the birds revisited the 10-min flowers sooner than they revisited the 20-min flowers. To revisit each flower at the right time throughout the day, the birds had to update their memories of when and where food was encountered for each flower and how long it has been since the last visit (Crystal, 2006a; Henderson et al., 2006).

1.19.3 Experimental Paradigms

The examples of timing just described involve learning about the temporal constraints of environmental resources. In the sections that follow, we discuss timing experiments; animals are required to learn to time target intervals in each preparation. In subsequent sections, alternative theories are discussed that make different predictions about the mechanism by which this temporal learning occurs (*See also* Chapter 1.12).

Most of the experimental work on interval timing has been done with three vertebrate species in highly controlled settings: laboratory rats, laboratory pigeons, and adult humans. Three kinds of experimental paradigms have been staples in research, and we describe them here and show some representative results.

1.19.3.1 Bisection Task

In the bisection task, also called the estimation or choice task, the subject is presented with two choices for responding, such as two levers to press for rats, two keys to peck for pigeons, or two keys to press on a keyboard for humans. During training, a single interval of time is presented on each trial. The interval is commonly signalled by the duration of a key that lights up or a tone that sounds. The interval might be one of two durations, a short duration S (e.g., 2 s) or a long duration L (e.g., 8 s). When the interval is over, the subject gets to make a choice. One of the choices is arbitrarily designated the 'short' choice and is correct for the short duration. The other choice, the 'long' choice, is correct for a long duration.

After sufficient training, subjects typically perform very well on the training intervals. Occasional tests are then given using intervals not used in training. These test intervals typically span the range of durations between the short and the long training durations. In the example, durations of 2, 3, 4, 5, 6, 7, or 8 s might be presented on test trials. Typical data from rats and humans are shown in **Figure 2**. In general, a smooth psychophysical curve is obtained, with the probability of choosing 'long' increasing in a sigmoidal fashion with the test duration presented.

Of some concern in bisection studies is the point of subjective equality (PSE) or bisection point, from which the task obtains its name. This is the stimulus duration at which a subject is equally likely to choose 'long' and 'short,' or 50% of each. The bisection point is estimated by various curve-fitting techniques from the psychophysical function that is obtained on tests. For rats and humans, the bisection point is at the geometric mean when the ratio of long to short duration is small (Church and Deluty, 1977; Allan and Gibbon, 1991). The geometric mean of two numbers, **a** and **b,** is defined as:

$$M_{geom}(\mathbf{a},\mathbf{b}) = \sqrt{(\mathbf{a} * \mathbf{b})},\ \text{the square root of } \mathbf{a} \text{ times } \mathbf{b}.$$

This is a midpoint by ratios rather than arithmetic differences, that is:

$$\mathbf{b}/M_{geom} = M_{geom}/\mathbf{a}, \quad \text{for } \mathbf{b} > \mathbf{a}.$$

In contrast, the familiar arithmetic mean (M_{arith}) of **a** and **b**, $(\mathbf{a}+\mathbf{b})/2$, is the midpoint by arithmetic differences:

$$\mathbf{b} - M_{arith} = M_{arith} - \mathbf{a}.$$

A bisection point at the geometric mean, then, suggests a comparison of ratios between the remembered reference durations and the duration presented on the test trial (more on this later).

What size ratio is small enough for a bisection point at the geometric mean differs between humans and rats. Church and Deluty (1977) found rats bisecting at the geometric mean with a L:S ratio of 4:1, as did others (Chiang et al., 2000; Santi et al., 2001; Crystal, 2002). For humans, Allan and Gibbon (1991) used L:S ratios of 2:1 or smaller and also used short durations ($\leq$4 s). With larger L:S ratios, the bisection point is no longer at the geometric mean and is often closer to the arithmetic mean. In rats, an L:S ratio larger than 4:1 led to a bisection point that differed from the geometric mean (Raslear, 1983; Siegel, 1986; Shurtleff et al., 1990; Shurtleff et al., 1992). Unfortunately, the bisection point differed between the studies. In humans bisecting short durations (<1 s), Wearden

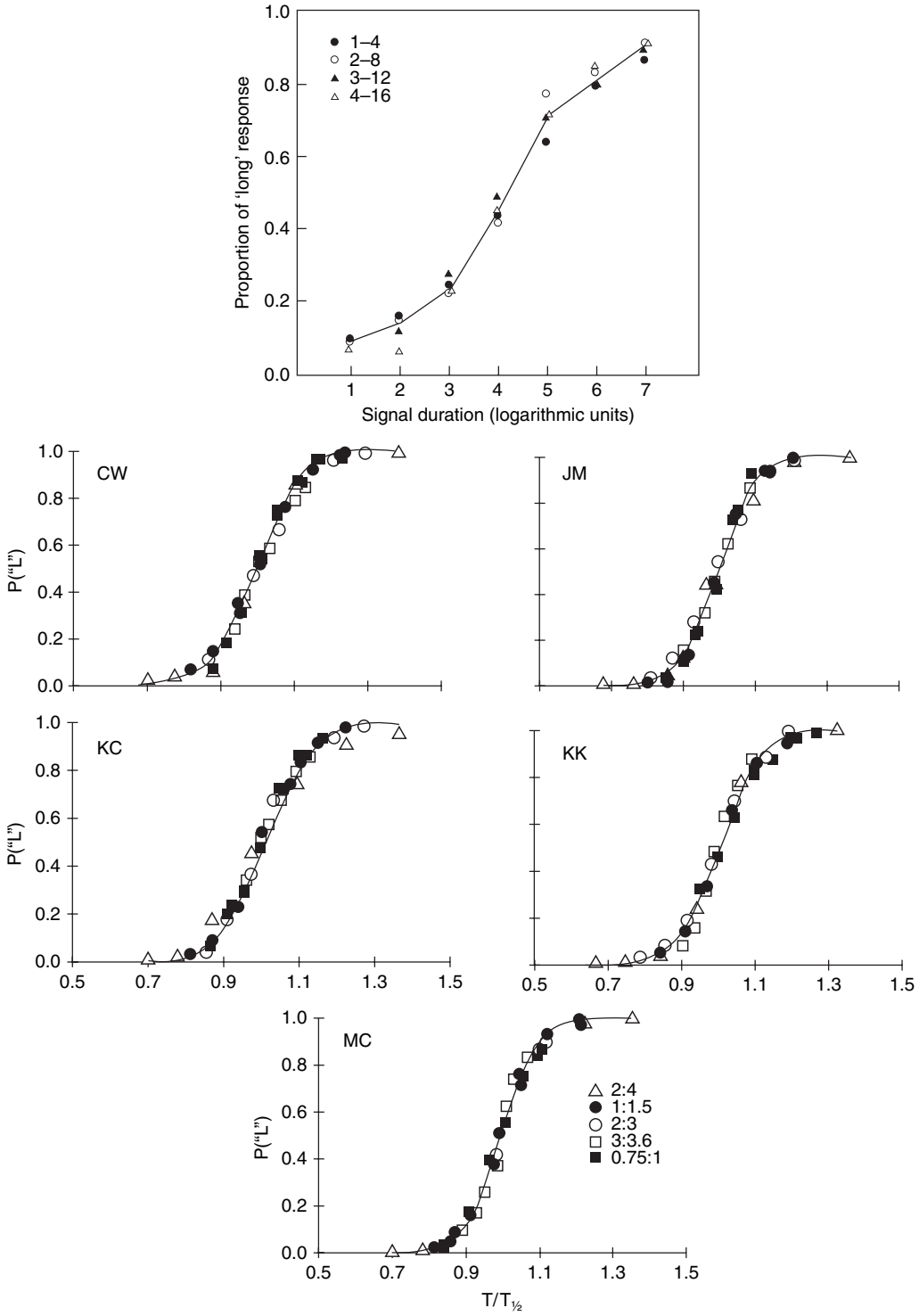

Figure 2 Representative data on the bisection task from rats (top) and individual humans (bottom). Tasks with different reference durations to be discriminated have been pooled together. Data are from unrewarded tests (for rats) and tests without feedback (for humans). Note that data from different conditions superimpose when plotted in this proportional fashion. Top, reproduced with permission from Church RM and Deluty MZ (1977) Bisection of temporal intervals. *J. Exp. Psychol. Anim. Behav. Process.* 3: 216–228; bottom, reproduced with permission from Allan LG and Gibbon J (1991) Human bisection at the geometric mean. *Learn. Motiv.* 22: 39–58.

and Ferrara (1996) found bisection at the geometric mean with an L:S ratio of 2:1, but with larger ratios, the bisection point was closer to the arithmetic mean. With longer durations in the seconds range, bisection was not at the geometric mean with any L:S ratio, including 2:1 (Wearden et al., 1997). The bisection points were closer to the arithmetic mean.

An interpretation for the variable results obtained with larger L:S ratios is that intermediate points are perceptibly different from both the L and the S durations to the subjects. A subject then in effect judges neither, and some strategic rule needs to be invoked as a basis for responding. The strategic rule is likely to vary from subject to subject, thus causing variablility in the data.

1.19.3.2 Peak Procedure

The peak procedure is an adaptation of the fixed interval schedule (**Figure 3**; *See also* Chapter 1.06). It is a production task in that the animal gets to emit responses over a stretch of time signaled by some event. Two types of trials are given to subjects. On training trials, a signal marks the start of an interval to be timed. Typically, this is a light or a tone that stays on. After a set interval has elapsed, a reward is delivered for a response (press a bar, peck a key, push a button), that is, the first response after a fixed interval from signal onset is rewarded. Responses before the fixed interval elapsed have no effect. On test trials, the signal also marks the start of a trial, but no rewards are dispensed. The signal stays on for much longer than the fixed interval on training trials. We illustrate the paradigm and typical results with some examples.

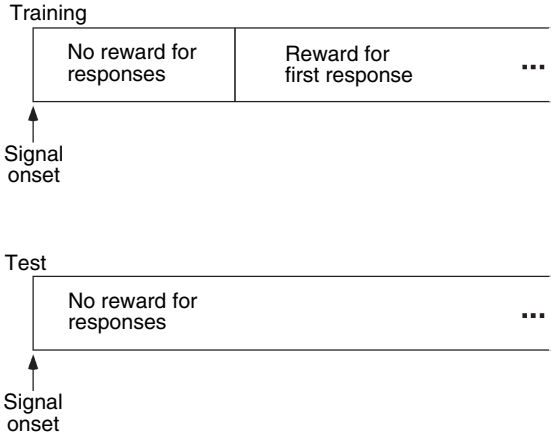

Figure 3 Schematic illustration of the peak procedure. On a training trial (top), a signal indicates the start of a trial. The first response after a fixed duration (FI) has elapsed is rewarded. On a test trial (bottom) the signal stays on for a duration much longer than the FI, and reward is withheld.

Roberts (1981) presented rats the peak procedure using fixed intervals of 20 s and 40 s. A light and a tone signaled the two fixed intervals. For example, when the tone sounded on a trial, reward was given for the first bar press after 20 s; when a light lit up on a trial, reward was given for the first bar press after 40 s. Occasionally, unrewarded tests lasting 80 s were given using the light or the tone. On these trials, the light or tone stayed on for 80 s, but no reward was dispensed. **Figure 4** shows the results on tests from the last five sessions of Roberts' (1981) experiment, averaged across rats and trials. It can be seen that the rats started responding before the fixed interval elapsed, but the probability of responding peaks at around the fixed interval assigned to each signal.

Pigeons show a similar pattern of behavior on the peak procedure (Roberts et al., 1989). In **Figure 5**, the group of pigeons was also trained with two signals, a light and a tone. When the light signaled the start of a trial, a key peck was rewarded after 15 s; when a tone signaled the start of a trial, a key peck was rewarded after 30 s. The data came from unrewarded tests with each signal that lasted 90 s. Other than peaking at around the expected time of reward on training trials, two other features are noticeable. The response distribution curves are not perfectly symmetrical. The slope is steeper on the rise to the peak on the left side than the fall from the peak on the right side. In addition, the response distribution curve for the tone shows a rise toward the end of the test trial.

In a third example, humans were tested on the peak procedure (Rakitin et al., 1998). Training fixed intervals of 8, 12, and 21 s were presented using a

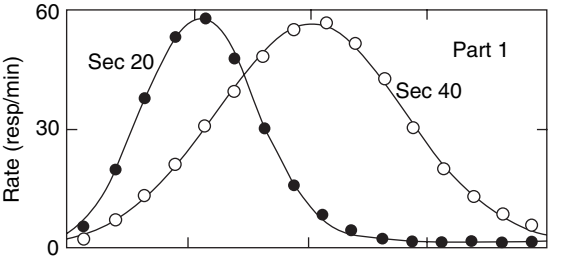

Figure 4 Representative data from rats on the peak procedure. On the *x*-axis is time elapsed because the of start of an unrewarded test trial lasting 80 s. Each tick mark represents 20 s. The peak rate of responding when averaged across trials occurs near the fixed duration. Reproduced with permission from Roberts S (1981) Isolation of an internal clock. *J. Exp. Psychol. Anim. Behav. Process.* 7: 242–268.

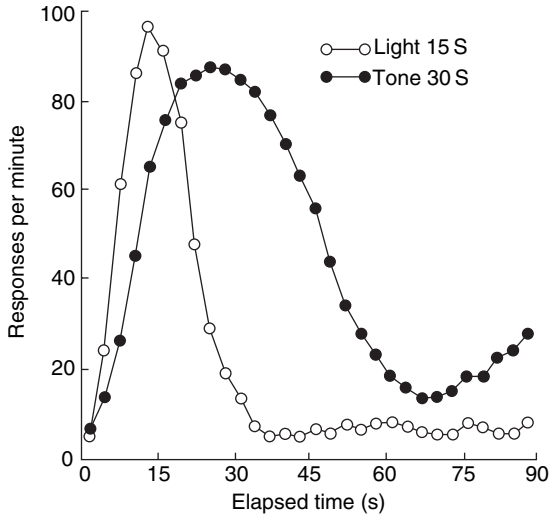

Figure 5 Representative data from pigeons on the peak procedure. These birds were trained with two different fixed intervals. A light was associated with fixed duration (FI) 15 s, whereas a tone was associated with FI 30 s. Data are from unrewarded test trials lasting 90 s. Reproduced with permission from Roberts WA, Cheng K, and Cohen JS (1989) Timing light and tone signals in pigeons. *J. Exp. Psychol. Anim. Behav. Process.* 15: 23–35.

computer. Subjects participated in multiple sessions, with the fixed interval constant in each session. Test trials were slightly different from those given to pigeons and rats. A subject could terminate a trial by pressing the enter key on the keyboard, or the tests ended automatically after the fixed interval for the session had elapsed three times. Results showed orderly near-Gaussian distributions resembling those found for rats (**Figure 6**).

1.19.3.3 Generalization

In generalization, the subject gets to answer yes or no to the 'question' of whether a presented duration is the standard or rewarded duration. For example, Church and Gibbon (1982) presented rats with a house light that stayed on for different durations. A lever was inserted after (but not before) the house light went off. A standard duration was the positive duration, for example, 4 s if the house light lasted 4 s, and a bar press would be rewarded. For other durations, bar presses went unrewarded. Results showed an orderly generalization function peaking at the positive duration and dropping off nearly symmetrically in a Gaussian fashion (**Figure 7**). The response rate did not drop to zero; at all durations, the rats responded on a proportion of the trials.

The generalization paradigm has also been used on humans (Wearden, 1992; Wearden and Towse, 1994; Wearden et al., 1997). One standard duration was presented as the positive duration. Subjects' task was to answer YES or NO according to whether the

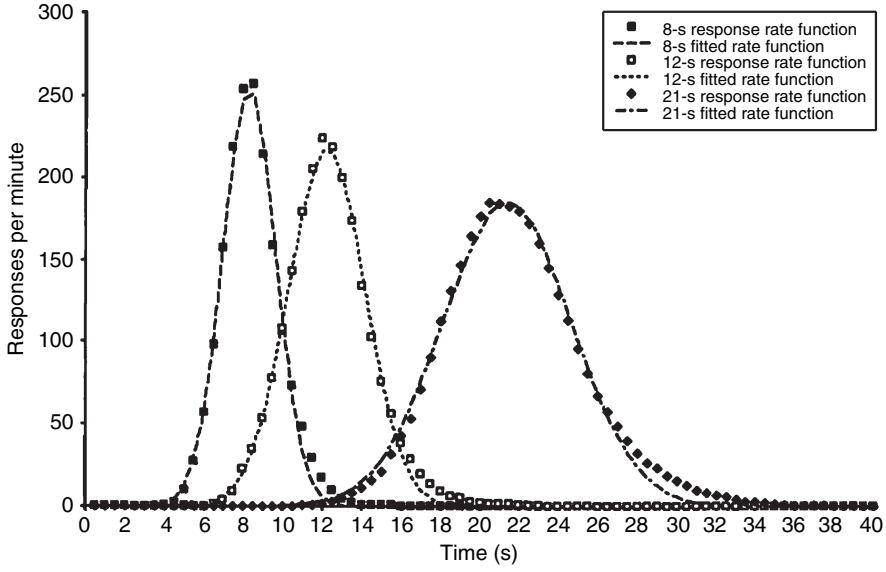

Figure 6 Representative data from humans on the peak procedure. Humans were instructed to hold down a key to 'bracket' a target fixed interval. They were to start before the interval had elapsed and stop after the interval had elapsed. Reproduced with permission from Rakitin BC, Gibbon J, Penney TB, Malapani C, Hinton SC, and Meck WH (1998) Scalar expectancy theory and peak-interval timing in humans. *J. Exp. Psychol. Anim. Behav. Process.* 24: 15–33.

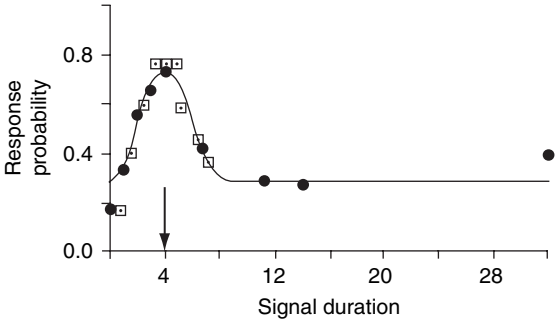

Figure 7 Representative data from rats on the generalization task. A duration of tone was presented to the rat, and then a lever was inserted. Lever presses were rewarded if the tone duration was 4 s, but not if it was any other duration. The data show a symmetric generalization gradient around the reinforced duration of 4 s. Reproduced with permission from Church RM and Gibbon J (1982) Temporal generalization. *J. Exp. Psychol. Anim. Behav. Process.* 8: 165–186.

duration presented on a trial was or was not the standard duration. Wearden (1992) and Wearden and Towse (1994) used very short durations that precluded counting. Longer durations in the seconds range were used by Wearden et al. (1997); subjects were prevented from counting by having to shadow (repeat back) an irregular list of numbers. For both short and long durations, orderly generalization gradients were obtained (**Figure 8**). A difference from those found with rats is that the gradients were asymmetric: More YES responses were given to durations longer than the standard (on the right side) than to durations shorter than the standard (on the left side).

1.19.4 Scalar Property

A property found in all the data examples that we have shown is the scalar property. Most of the data graphs are in absolute units of time. The scalar property emerges when data are plotted in units of time relative to the standard duration being measured (for the peak procedure and generalization) or the subjective middle (for the bisection task). When data are plotted in this fashion, graphs having different absolute standards superimpose, as shown in **Figure 9** for humans on the peak procedure and **Figure 2** (bottom) for humans in the bisection task. For this reason, the scalar property is also called superimposition.

Put more formally, some measure of spread, such as the standard deviation of a response distribution or the distance between the quartiles, is a constant proportion of the duration being measured in the peak procedure or generalization paradigms:

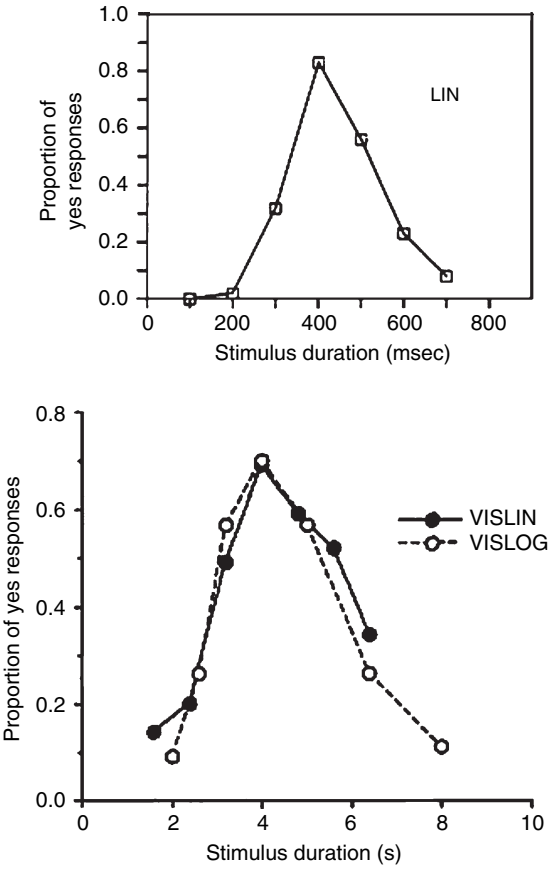

Figure 8 Representative data from humans on the generalization task with very short intervals (top) and intervals in the seconds range (bottom). Humans answered "yes" or "no" to the question of whether a presented duration matched a reference duration (400 ms in the top panel, 4 s in the bottom panel). Unlike rats, the generalization gradients are asymmetric, dropping more slowly to the right (corresponding to durations longer than the standard). Top, reproduced with permission from Wearden JH (1992) Temporal generalization in humans. *J. Exp. Psychol. Anim. Behav. Process.* 18: 134–144; bottom, reproduced with permission from Wearden JH, Denovan L, Fakhri M, and Haworth R (1997) Scalar timing in temporal generalization in humans with longer stimulus durations. *J. Exp. Psychol. Anim. Behav. Process.* 23: 502–511.

$$\text{Spread} = kX, \text{where } X \text{ is the duration being measured.}$$

This is also known as Weber's law. The constant k, which is Spread/X, is known as the coefficient of variation or Weber fraction.

1.19.5 Theories of Interval Timing

We examine briefly three different theories of interval timing; each theory makes different assumptions

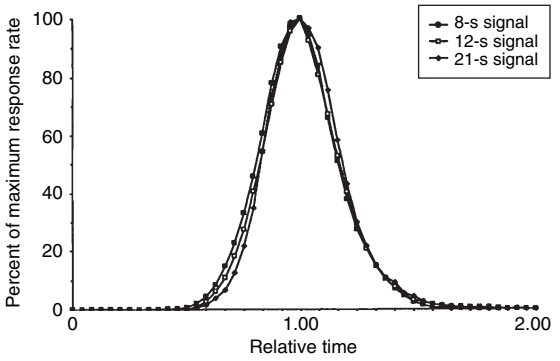

Figure 9 Superimposition in data from humans on the peak procedure. The data in Figure 6 have been replotted in relative terms, with the *x*-axis expressed as a proportion of the fixed duration. Reproduced with permission from Rakitin BC, Gibbon J, Penney TB, Malapani C, Hinton SC, and Meck WH (1998) Scalar expectancy theory and peak-interval timing in humans. *J. Exp. Psychol. Anim. Behav. Process.* 24: 15–33.

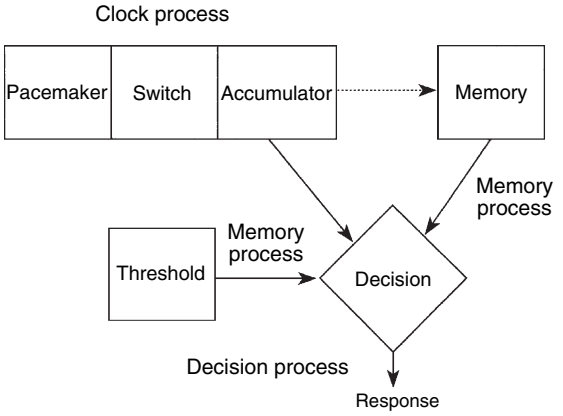

Figure 10 The Scalar Expectancy Theory (SET), consisting of three processes: a clock process, a memory process, and a decision process. The clock consists of a pacemaker that sends out regular pulses. The pulses may be gated by a switch into an accumulator for the purposes of timing a current duration. The elapsed time is compared to a standard duration retrieved from memory. If the ratio of the elapsed time to the reference time exceeds a threshold (which is also retrieved from memory for the trial), then the animal responds at a high rate (in the peak procedure) or answers "yes" in generalization. For the bisection task, two reference durations need to be retrieved, one for the long duration and one for the short duration. Ratio comparison is again made: elapsed time/S is compared with L/elapsed time. The subject chooses the reference (S or L) that generated a smaller ratio. Adapted with permission from Church RM (2003) A concise introduction to scalar timing theory. In: Meck WH (ed.) *Functional and Neural Mechanisms of Interval Timing,* pp. 3–22. Boca Raton, FL: CRC Press.

about the mechanism(s) of learning about the temporal constraints of the environment. A good number of other theories have been developed as well, but the chosen ones give some idea of the range of extant theories. At this stage, the field is far from agreeing to a single theory.

1.19.5.1 Scalar Expectancy Theory

A widely used explanation for interval timing is the Information Processing (IP) model (Gibbon et al., 1984; Church, 2003), also known as Scalar Expectancy Theory (SET; **Figure 10**). According to the IP model, timing is based on three stages of information processing: clock, memory, and decision. The clock stage of information processing consists of a pacemaker that sends pulses to an accumulator. A switch gate pulses from the pacemaker to the accumulator (or, alternatively, diverts these pulses away from the accumulator). The memory stage of information processing consists of working and reference memory storage systems. Working memory contains the current estimate of elapsed time, as indexed by the number of pulses accumulated at a particular point in time. Reference memory contains examples of previously rewarded elapsed durations that were previously transferred from working memory to reference memory. The decision stage of information processing compares a randomly selected example from reference memory to the currently elapsing duration. The decision to respond (or not) is based on the relative similarity of the estimate of current and remembered times.

The scalar property or Weber's law suggests a ratio comparison process. Consider, for example, the peak procedure. The animal is said to switch to a high rate of responding after a ratio of elapsed time (from the clock process) to the reference duration (from the memory process) has been passed. If the *average* ratio threshold is the same at different fixed durations (FIs), this would produce the scalar property. Variations from trial to trial in the thresholds and reference durations used would produce the shape of the response distribution found when many test trials are pooled.

Many timing studies have interpreted results in terms of SET. Consider, for example, the generalization results shown in **Figures 7 and 8**. Results such as those from the rat in **Figure 7** can be modeled by a decision process that compares a ratio of two durations to a threshold ratio. The numerator is the absolute difference between the duration presented

on the trial (clock process feeding working memory) and a reference duration from memory. The denominator is the reference duration. Thus, the comparison can be represented as

$$|t - T|/T < b?$$

In the inequality, T represents a reference duration from memory, whereas t represents the duration on the current trial. Such a process produces a symmetric generalization gradient. Wearden (1992) found that a modification of the comparison rule accounted for the asymmetric generalization gradients found in humans:

$$|t - T|/t < b?$$

The difference is that the denominator is the duration perceived on the current trial rather than a reference duration from memory. This shows that small modifications of SET can sometimes account for different patterns of data.

Another example of the application of SET concerns the role of attention in timing. Attention is thought to be required for the proper functioning of the clock process. We can use the analogy of filling a bucket with a hose, the bucket representing the accumulator. The hose is a pacemaker that drips constantly. The idea is that if attention is diverted, the hose will sometimes miss the bucket. One predicted result is that a longer actual duration of time must elapse under attentional demand before a subject judges that a particular reference duration has elapsed. This idea was tested on human subjects (Fortin and Massé, 2000; Fortin, 2003). Subjects had to stop a tone after 2500 ms had passed (**Figure 11**). As an added complication, the tone had a break in it at some unpredictable point, and the break was not to be timed. That is, subjects had to time 2500 ms excluding the break. The authors reasoned that anticipating the break requires attention. This led to the prediction that the later the arrival of the break, the more subjects should overestimate. It was as if the bucket were likely to be missed by the hose before the arrival of the break. The duration of the break should not make a difference, as the timing process was hypothesized to stop during the break. This was precisely the pattern of results found (**Figure 11**).

1.19.5.2 Timing Without a Clock

One might imagine that to do interval timing, one would need a clock. Models without a clock, however, have been proposed. One such model adapts a neural network to do what a clock does (Hopson, 2003). The network has an input layer, a hidden layer, and an output layer, with weights between the input layer and the hidden layer and between the hidden layer and the output layer. It is different from a standard neural network in that the hidden layer takes time to build up activation. Different units in the hidden layer build up at different rates.

The idea is to have the system build up to a maximal activation level at the predicted time of reward. Activation should come to predict probability of reward. The system learns by a back-propagation-learning algorithm that adjusts the weights. The model can account for a good number of patterns of results in the literature.

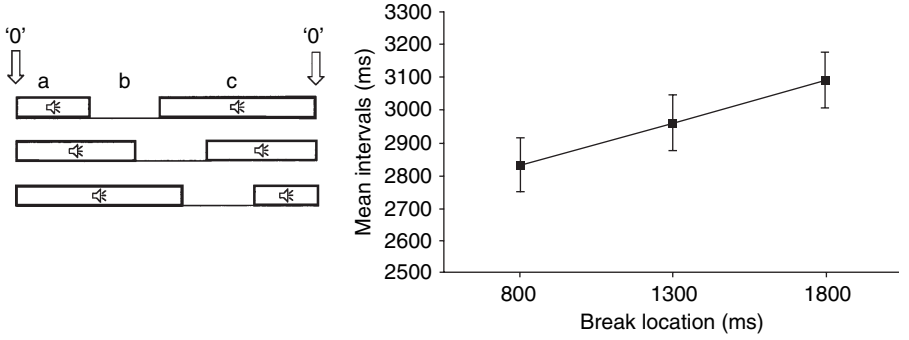

Figure 11 A dual-attention task described in Fortin C (2003) Attentional time-sharing in interval timing. In: Meck WH (ed.) *Functional and neural mechanisms of interval timing,* pp. 235–260. Boca Raton, FL: CRC Press. Reproduced with permission. Left: Humans have the task of holding down a key that produces a tone in order to produce a tone of 2500 ms duration. At some unpredictable time, the tone is interrupted by the experimenter, and the break is not to be timed. The data show that humans overproduce on this task, and the amount of overproduction varies directly with time elapsed before the break appears. The interpretation is that anticipating the break requires attention, which detracts from the task of timing. The result is that more actual time has to elapse before a perceived duration is judged to have elapsed.

1.19.5.3 Packet Theory

A recent information processing theory that makes detailed predictions is Packet Theory (Kirkpatrick, 2002; Kirkpatrick and Church, 2003; Church and Guilhardi, 2005; Guilhardi et al., 2005). It has four component processes (**Figure 12**). As an example, we consider a rat on a FI 30-s schedule of reinforcement for head entries into a food hopper. This means that 30 s after a pellet of food is delivered, the next pellet will be primed. The first head entry after the FI of 30 s results in food delivery.

The perception process is akin to the clock process in SET. It tracks the perceived time to the expected reward, about 30 s in this case. The memory in SET is a collection of reference durations from which the subject picks one for the current trial. In Packet Theory, the memory is the average expected time to reward, averaged over the reward times of the past. In the FI 30 example, this is also about 30 s. The memory process also has a threshold, in parallel with the threshold in SET. The threshold has a normal distribution about some mean proportion of time to expected reward. The decision process is based on the threshold. When the threshold has been passed, the animal switches from a low rate of response packets to a high rate of response packets.

Packets are theoretical entities that generate bouts of responding. This is signified in the response process, which turns packets into bouts of responses with particular characteristics, such as a distribution of interresponse times. The theory is thus explicit in producing an actual stream of response times in a simulated trial. Other theories are typically not quite as explicit about responses. For example, it is not clear how the activation level of the neural net in Hopson's (2003) model translates to actual responses in time.

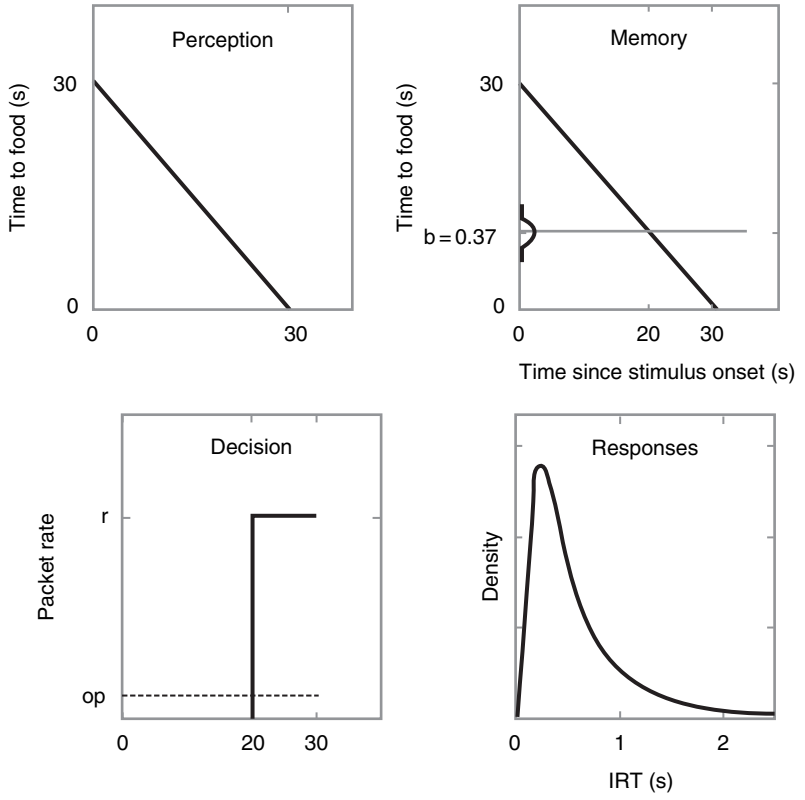

Figure 12 A schematic illustration of Packet Theory in explaining performance on a FI 30 schedule. The theory has four components. A perception unit parallels the clock process in Scalar Expectancy Theory and times the expected time to reward on the current trial. The memory process is a representation of the expected time to reward, derived from an average of past durations to reward. A variable threshold is retrieved for the current trial. The decision process switches to a high rate of production of Packets when the threshold has been crossed. Packets are theoretical underlying units that generate bouts of behavior, according to a function described in the response production unit on the right. Reproduced with permission from Church RM and Guilhardi P (2005) A Turing test of a timing theory. *Behav. Processes* 69: 45–58.

This explicit translation into responses in time means that the model can be compared against empirical data on numerous fronts, including response distributions, response rates, and interresponse times. On these multiple fronts, the model does a reasonable job of simulating the actual data obtained. Because the memory process is said to come up with an average expected time to reward, it does so even when durations to reward varied in the past, as in some random distribution about a mean. This means that the model can predict the behavior of rats in such seemingly 'nontiming' conditioning procedures. One of the strengths of the model is that it can encompass a range of conditioning procedures, and in fact, it is a new theory of conditioning (see other chapters on theories of conditioning in this volume).

A further contribution from the development of Packet Theory is an explicit recommendation for evaluating how well the model accounts for obtained data in the form of a Turing test (Church and Guilhardi, 2005). It is called a Turing test after the mathematician Alan Turing, who devised a similar test to examine whether a human can distinguish the responses of a computer-generated program from those of another human. In the case of Packet Theory, a set procedure such as FI 30 is given to rats and produces a sizeable set of trials at asymptote. The model is given the same procedure, parameters are chosen, and the model generates a set of trials. The data generated are responses in time. To test the 'fit,' one picks one trial from the rat and one trial from the model. A third trial is then picked from either the model or the rat. This trial is formally compared to the two reference trials (one from the model and one from the rat). The comparison process 'decides' whether the sample trial is more similar to the model's trial or to the rat's trial. This process can be repeated many times.

If the model generates data indistinguishable from a rat, the Turing comparison process should be at chance and be correct 50% of the time. In fact, it is correct about 60% of the time, which shows that the model, while having considerable success, may be improved by further development.

1.19.6 Connections Between Interval Timing and Circadian Timing

The examples of timing discussed earlier are referred to as interval timing (sometimes short-interval timing) to denote that the target duration is an elapsing interval. The basic idea is that an interval elapses with respect to the occurrence of some event (e.g., the onset of a light or a sound). A helpful metaphor is the familiar features of a stopwatch (Church, 1978). For example, a stopwatch may be used to time runners in a race. As the runners line up for the race, the stopwatch would be *reset* to zero. When the race begins, the stopwatch is *started*. When the runner crosses the finish line, the stopwatch is *ended*. The reading of the stopwatch provides an estimate of time to complete the event (running the race in this example). Implicit in the stopwatch metaphor is that timing an elapsed interval is quite flexible; the start and stop events are arbitrary.

One mechanism that may be used to instantiate a stopwatch mechanism is a pacemaker-accumulator. A pacemaker emits pulses as a function of time, and an accumulator integrates (i.e., counts) the number of pulses emitted. An hourglass is a familiar example of a pacemaker-accumulator; the amount of sand in the bottom chamber of an hourglass is an index of the elapsed duration since the hourglass was turned over (i.e., reset).

By contrast, circadian timing is based on the completion of a periodic process. As the name circadian implies, the periodic process is approximately a day. Therefore, time of day is indexed by the phase of a circadian periodic process. Circadian timing is widespread (Aschoff, 1981; Takahashi et al., 2001).

Unlike the pacemaker-accumulator and hourglass mechanism described for interval timing, circadian timing is based on an oscillator. The system is referred to as a circadian oscillator because the natural period of the oscillator is approximately 24 h.

1.19.6.1 Formal Properties of Interval and Circadian Timing

Gibbon et al. (1997a) described operating characteristics for interval and circadian timing systems, which is summarized in **Table 1** (the table is reproduced from Gibbon et al., 1997a). The circadian system is based on an endogenous oscillation. An oscillation is endogenous if it does not require continued periodic input to produce ongoing periodic output. For example, activity patterns occur at species-typical times of day when an animal is exposed to daily periodic light cycles (e.g., 12 h of light followed by 12 h of darkness). After the periodic light cycle is terminated (e.g., constant dim illumination), behavior 'free runs,' usually with a period that departs slightly from 24 h.

Table 1 Properties of circadian and interval timing systems

Circadian	*Interval*
Timing properties	
Endogenous oscillation: free run	Requires reset: one shot
Entrainment range: Limited	Training range: Broad
Approximately 8 h maximum	Approx. 3–4 orders of magnitude – seconds to hours
Phase shift adjustment: Slow	Phase shift immediate
Several cycles usually required	Arbitrary onset phase
Variance properties	
High level of precision:	Low level of precision:
$\sigma/\mu = 0.01-0.05$	$\sigma/\mu = 0.10-0.35$
Relationship to entrainment period (?)	Scalar property:
	Superposition in relative time $f_r(rt) = (1/r)f(t)$, $\sigma/\mu = \gamma$.

Reproduced from Gibbon J, Fairhurst S, and Goldberg B (1997a) Cooperation, conflict and compromise between circadian and interval clocks in pigeons. In: Bradshaw CM and Szabadi E (eds.) *Time and Behaviour: Psychological and Neurobehavioural Analyses*, pp. 329–384. New York: Elsevier. © 1997 Elsevier Science B.V.

Free-running behavior after the termination of periodic stimuli provides evidence that the timing system is an endogenous oscillator. In contrast, Gibbon et al. (1997a) described the interval timing system as requiring resetting. The timing system is presumed to time with respect to the occurrence of some stimulus; a single presentation of the stimulus is required to reset the interval timing system (i.e., one shot reset).

The circadian system is subject to a limited range of entrainment. For example, presentation of a periodic input is thought to entrain the endogenous oscillator only if the periodic input is within a limited range of periods near 24 h. By contrast, Gibbon et al. (1997a) described the interval timing system as having a broad training range covering 3-4 orders of magnitude from seconds to hours.

The circadian system adjusts slowly to a phase shift. A phase shift is an abrupt change in the initiation of a periodic process. A familiar example of a phase shift is the unusual wake-up times that people are exposed to after flying to a destination across several time zones. It usually requires several cycles (i.e., days) before one's activities are synchronized to the new time zone. By contrast, Gibbon et al. (1997a) stated that the interval timing system is characterized by immediate phase shift; because interval timing is not based on the phase of a periodic process, the response to a single shift in a cycle is complete adjustment or complete resetting of the timing processes (i.e., one-shot reset in Gibbon et al.'s terminology).

Temporal performance based on a circadian oscillator is highly precise as measured by cycle-to-cycle variation. For example, Gibbon et al. (1997a) summarize the coefficient of variation (CV; standard deviation of time estimates divided by the mean of time estimates) of circadian-mediated performance as approximately 1%–5%. By contrast, interval timing performance is characterized by a relatively low level of precision (coefficient of variation of 10%–35%). Therefore, it appears that a characteristic of a circadian oscillator is relatively high timing precision. In particular, a consequence of having an endogenous oscillator dedicated to timing select values within a limited range appears to be relatively high sensitivity to timing these target durations.

The variance properties of timing have played an important role in understanding interval timing (as noted in the section above about the scalar property). By contrast, the analysis of variance properties has had less impact in the study of circadian timing.

With some notable exceptions (Aschoff, 1984, 1985, 1989, 1993, 1998; Silver and Bittman, 1984; Terman et al., 1984; Aschoff and Daan, 1997; Gibbon et al., 1997a), the interaction of interval and circadian mechanisms has been largely neglected in the literature. In the sections that follow, we describe a series of empirical tests that were designed to evaluate the hypothesis that interval timing is based, at least in part, on oscillatory processes as described in **Table 1** from Gibbon et al. (1997a).

1.19.6.2 Resetting Properties of Short-Interval Timing

Figure 13 shows an example of a phase-shift manipulation applied to short-interval timing. Rats were

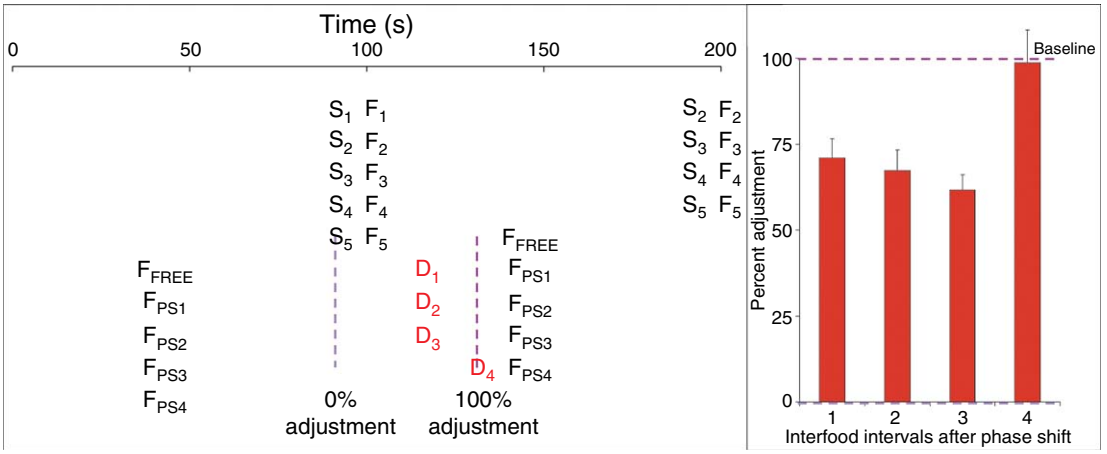

Figure 13 A phase shift produces gradual adjustment in short-interval timing. Left panel: Schematic representation of training, phase-shift manipulation, predictions, and data (double plotted to facilitate inspection of transitions across successive intervals; consecutive 100-s fixed intervals are plotted left to right and top to bottom). Rats ($n = 14$) timed 100-s intervals, and the last 5 intervals before the phase shift are shown (F = food pellet, S = start time of response burst). A 62-s phase advance (i.e., early pellet) on average was produced by the delivery of a response-independent food (F_{FREE}). All other food-to-food intervals were 100 s (F_{PS} = food post phase shift). Dashed lines indicate predictions if rats are insensitive (0% adjustment, purple) or completely sensitive (100% adjustment, pink) to the most recently delivered food pellet. A pacemaker-accumulator mechanism predicts 100% adjustment on the initial interval after the phase shift on the assumption of complete reset (Gibbon et al., 1997a). An oscillator mechanism predicts initial incomplete adjustment. Data (D) indicate incomplete adjustment on the first three trials. Right panel: Start times on the initial three trials were earlier than in preshift baseline ($t(13)$'s > 2, p's < .05). Resetting was achieved on the fourth trial ($t < 1$). Each 45-mg food pellet was contingent on a lever press after 100 s in 12-h sessions. The start of a response burst was identified on individual trials by selecting the response that maximized the goodness of fit of individual responses to a model with a low rate followed by a high rate (analysis as in Crystal et al., 1997). The same conclusions were reached by measuring the latency to the first response after food. Baseline was the average start time on the five trials before the phase shift. Left panel: Zero on the y-axis (purple dashed line) corresponds to complete failure to adjust to the phase shift; 100% (pink dashed line) corresponds to complete resetting. Error bars represent 1 SEM. Adapted from Crystal JD (2006c) Sensitivity to time: Implications for the representation of time. In: Wasserman EA and Zentall TR (eds.) *Comparative cognition: Experimental explorations of animal intelligence,* 270–284. New York: Oxford University Press. Reproduced from Crystal JD (2006d) Time, place, and content. *Comp. Cogn. Behav. Rev.* 1: 53–76. with permission.

trained to time 100 s; a food pellet was dispensed for the first response after the fixed interval of 100 s had elapsed. To implement the phase shift, an early, response-independent pellet was delivered (i.e., free food). Four food cycles were required before adjustment was complete, which is consistent with the hypothesis that short-interval timing of 100 s is based on an oscillator mechanism (Crystal, 2006c, 2006d). The prediction in the third row of **Table 1** is not supported by the data in **Figure 13**.

1.19.6.3 Endogenous Oscillations in Short-Interval Timing

The hypothesis that the timing of an interval is based on a pacemaker-accumulator or oscillator mechanism can be assessed by discontinuing periodic input (i.e., extinction) and assessing subsequent anticipatory behavior. A defining feature of an oscillator is that periodic output from the oscillator continues after the termination of periodic input. By contrast, a defining feature of a pacemaker-accumulator system is that elapsed time is measured with respect to the presentation of a stimulus, according to the classic description of this system (**Table 1**). Therefore, output of a short-interval system is periodic if presented with periodic input, but periodic output can be expected to cease if periodic input is discontinued. Groups of rats were trained to time short intervals (48, 96, and 192 s). The data from a representative individual rat in this experiment are shown in **Figure 14**, and group data are shown in **Figure 15**. As expected, periodic delivery of food produced periodic behavior during training (**Figure 15**, left column). Next, delivery of food was suspended. Behavior was periodic after termination

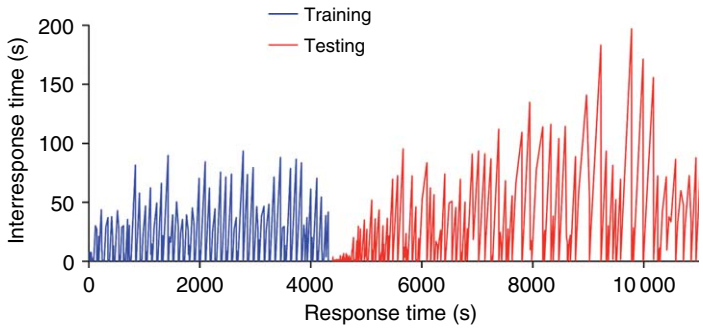

Figure 14 Many small interresponse times in short-interval timing are punctuated by much longer interresponse times, and punctuation by relatively long interresponse times continued after termination of periodic food delivery. Interresponse time (i.e., times of responses $R_{n+1} - R_n$) is plotted as a function of response time for a representative rat. During training, food was delivered on a fixed-interval 96-s schedule. During testing, food was not delivered (i.e., extinction). Extinction began at a randomly selected point in the session. The response measure was the time of occurrence of photobeam interruptions in the food trough. Reproduced from Crystal JD and Baramidze GT (2007) Endogenous oscillations in short-interval timing. *Behav. Processes*. 74: 152–158. © 2007, with permission from Elsevier.

of periodic input (**Figure 15**, right column). Importantly, the period in extinction increased as a function of the period in training, documenting that the periodic behavior in extinction was based on entrainment to the periodic feeding in training. These data suggest that short-interval timing is, at least in part, based on a self-sustaining, endogenous oscillator (Crystal and Baramidze, 2007). The prediction in the first row of **Table 1** is not supported by the data in **Figures 14** and **15**.

1.19.6.4 Timing Long Intervals

It is generally accepted that animals *cannot* anticipate intermeal intervals outside a limited range near 24 h (e.g., Stephan et al., 1979a,b; Boulos et al., 1980; Stephan, 1981; Aschoff et al., 1983; Mistlberger and Marchant, 1995; Madrid et al., 1998; White and Timberlake, 1999). However, this conclusion is based on a relatively limited data set. Indeed, caution is generally warranted when confronted with evidence for the absence of some ability (as in this case with the absence of temporal performance). An alternative explanation for the lack of evidence for timing noncircadian intervals is that timing is relatively superior near 24 h; by contrast, poorer temporal performance farther away from 24 h may make it more difficult to detect temporal performance. A major insight from interval timing in the range of seconds to minutes (Dews, 1970) is that temporal performance may be best compared across ranges if performance is expressed in relative time (elapsed time divided by the target interval, rather than absolute time in seconds; and response rates expressed as a proportion of maximal rates). The power of this data analytic approach was documented in the section titled "Scalar property."

A series of experiments investigating meal anticipation was undertaken to test the hypothesis that a circadian oscillator is characterized by a local maximum in sensitivity to time (Crystal, 2001a); according to this prediction, temporal performance is superior near 24 h, relative to intermeal intervals outside the circadian range. Food was restricted to 3-h meals, which rats earned by breaking a photobeam in the food trough. The rats inspected the food trough before meals started, thereby providing a temporal anticipation function for each intermeal interval condition. **Figure 16** shows anticipation functions for intermeal intervals near the circadian range (22 to 26 h) and outside this range (14 and 34 h). The data are plotted in relative time on the horizontal axis and proportional response rate on the vertical axis (as described by Dews, 1970). Response rates increased later into the intermeal interval for intervals near the circadian range than for intervals outside this range. Sensitivity to time was estimated by the spread of the response distributions. The spread was smaller (i.e., lower variability) for intermeal intervals near the circadian range than for interval outside this range, as shown in **Figure 17**. Note that the data in **Figure 17** document a local maximum in sensitivity to time near 24 h, consistent with the hypothesis that a property of a circadian oscillator is improved sensitivity to time (Crystal, 2001a).

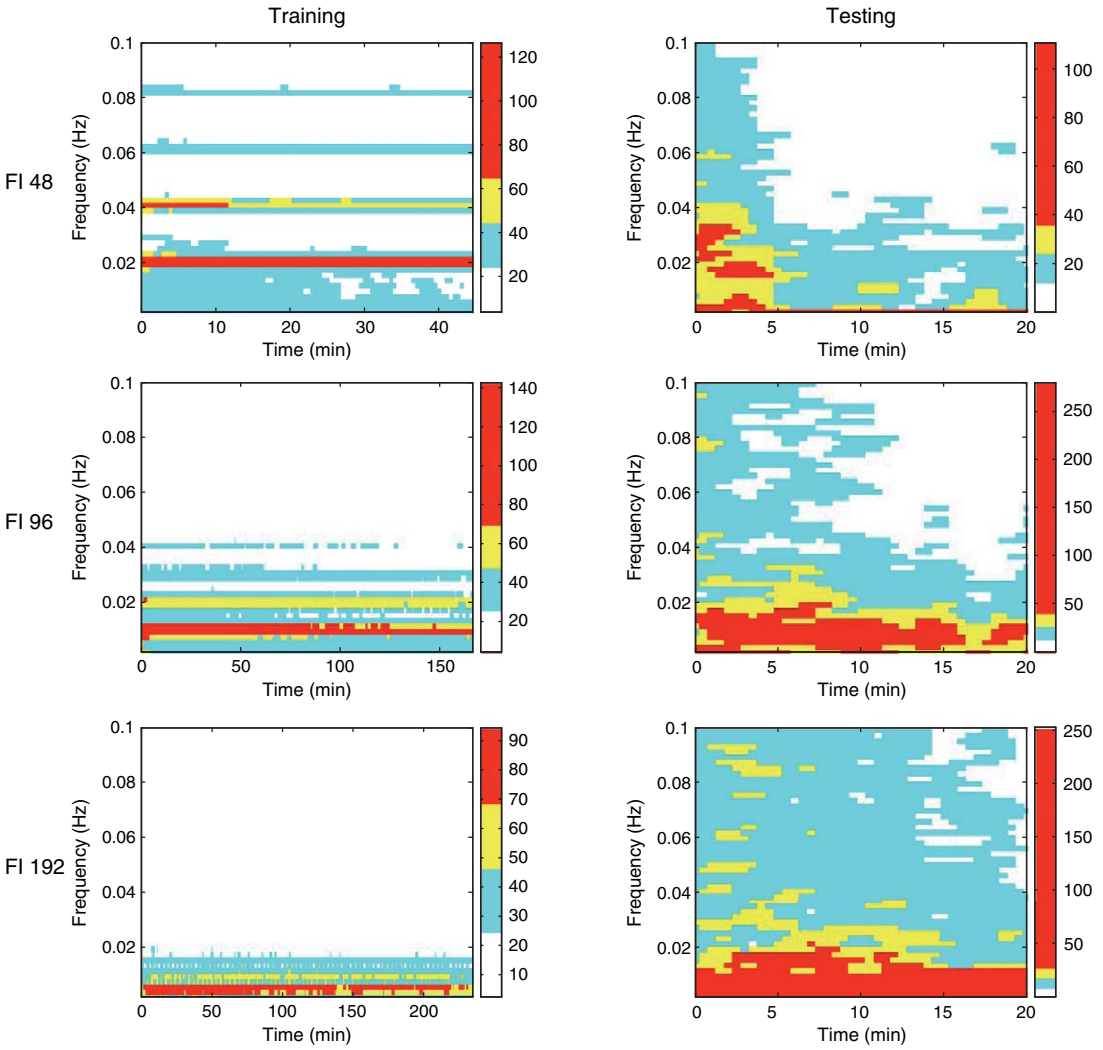

Figure 15 Endogenous oscillations in short-interval timing continue after the termination of periodic input. Short-time Fourier transforms are shown for training (left panels) and testing (right panels) conditions using fixed interval 48-, 96-, and 192-s procedures. The three-dimensional images show frequency (period = 1/frequncy) on the vertical axis as a function of time within the session along the horizontal axis; the color scheme represents the amount of power from the Fourier analysis. Concentrations of high power occur at a frequency of approximately 0.02, 0.01, and .0005, which correspond to periods of approximately 50, 100, and 200 s in top, middle, and bottom panels, respectively. Adapted from Crystal JD and Baramidze GT (2007) Endogenous oscillations in short-interval timing. *Behav. Processes*. 74: 152–158. © 2007, with permission from Elsevier.

Figure 18 shows a reanalysis of earlier published data on this problem. In particular, experiments were selected that used behaviors that are instrumental in producing food (e.g., approaching the food source or pressing a lever). Long intermeal intervals that are substantially less than 24 h are compared with a 24-h condition from each experiment. The critical part of the reanalysis was that the data were expressed in proportional time and rate. The reanalysis reveals that intervals below the circadian range (14, 18, and 19 h) are timed, but these temporal functions are less steep and have lower terminal response rates than do functions for timing 24 h. The reanalysis of published data is consistent with the hypothesis that a property of a circadian oscillator is improved sensitivity to time, as was suggested by Figures 16 and 17.

1.19.6.5 Endogenous Oscillations in Long-Interval Timing

The examples of timing noncircadian long intervals in the section titled "Timing long intervals" document

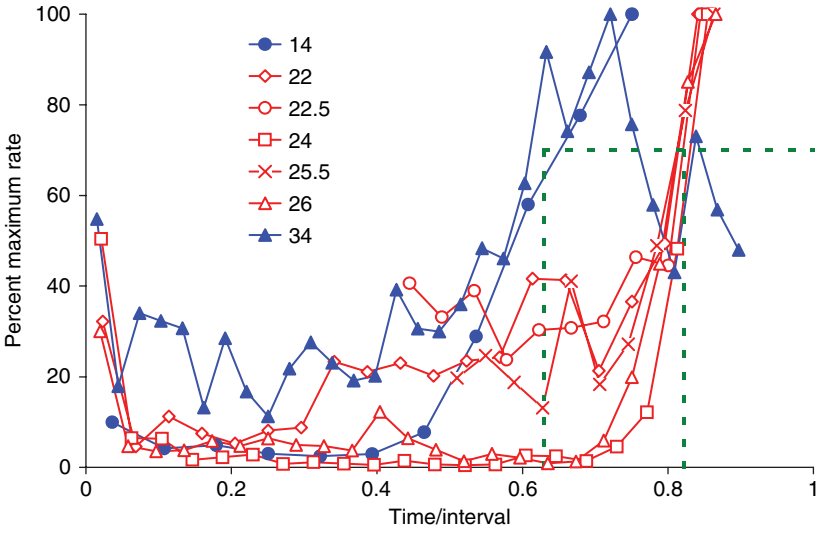

Figure 16 Response rate increased later into the interval for intermeal intervals near the circadian range (unfilled red symbols) relative to intervals outside this range (filled blue symbols); dashed lines indicate width of response rate functions. Anticipatory responses increase immediately prior to the meal for all intermeal intervals except 34 h. Each 45-mg food pellet was contingent on a photobeam break after a variable interval during 3-h meals. Intermeal intervals were tested in separate groups of rats ($n = 3–5$ per group). The end of the meal corresponds to 1 on the *x*-axis. Testing was conducted in constant darkness. Adapted from Crystal JD (2001a) Circadian time perception. *J. Exp. Psychol. Anim. Behav. Process.* 27: 68–78. Reproduced from Crystal JD (2006d) Time, place, and content. *Comp. Cogn. Behav. Rev.* 1: 53–76 with permission.

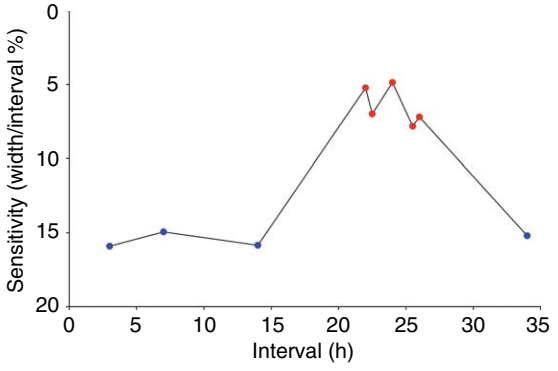

Figure 17 Intervals near the circadian range (red symbols) are characterized by higher sensitivity than intervals outside this range (blue symbols). Variability in anticipating a meal was measured as the width of the response distribution prior to the meal at 70% of the maximum rate, expressed as a percentage of the interval ($N = 29$). The interval is the time between light offset and meal onset in a 12–12 light–dark cycle (leftmost two circles) or the intermeal interval in constant darkness (all other data). The percentage width was smaller in the circadian range than outside this range ($F(1,20) = 22.65$, $p < .001$). The width/interval did not differ within the circadian ($F(4,12) = 1$) or noncircadian ($F(3,8) < 1$) ranges. The same conclusions were reached when the width was measured as 25%, 50%, and 75% of the maximum rate. The data are plotted on a reversed-order *y*-axis so that local maxima in the data correspond to high sensitivity, which facilitates comparison with other measures of sensitivity (e.g., Figure 20). Mean SEM = 2.4. Adapted from Crystal JD (2001a) Circadian time perception. *J. Exp. Psychol. Anim. Behav. Process.* 27: 68–78. Reproduced from Crystal JD (2006d) Time, place, and content. *Comp. Cogn. Behav. Rev.* 1: 53–76 with permission.

that rats can time intervals below the circadian range, but they do not identify the mechanism. In particular, the data stated earlier do not require an oscillator mechanism; an alternative is that the rats used a pacemaker-accumulator mechanism to time the long intervals. By contrast, the examples in the section titled Endogenous oscillations in short-interval timing document endogenous oscillations in timing short intervals (1–3 min) by demonstrating that timing continues after the termination of periodic input. The data described in this section document examples of endogenous oscillations in long-interval timing (16 h) by using the same experimental approach.

Rats earned food by interrupting a photobeam in a food trough during 3-h meals. The intermeal interval was 16 h. After approximately a month of experience with the intermeal interval, the meals were discontinued (Crystal, 2006b). **Figure 19** (top panel) shows that response rate increased as a function of time prior to the meals, documenting that the rats could time 16 h. To dissociate alternative timing mechanisms, it is diagnostic to consider the first two nonfood cycles. If the timing documented in the top panel of **Figure 19** is based on a pacemaker-accumulator mechanism reset by the meal, then response rate should increase as a function of time in the first nonfood cycle (as in training) because elapsed time since the last meal corresponds to the training interval. However, an

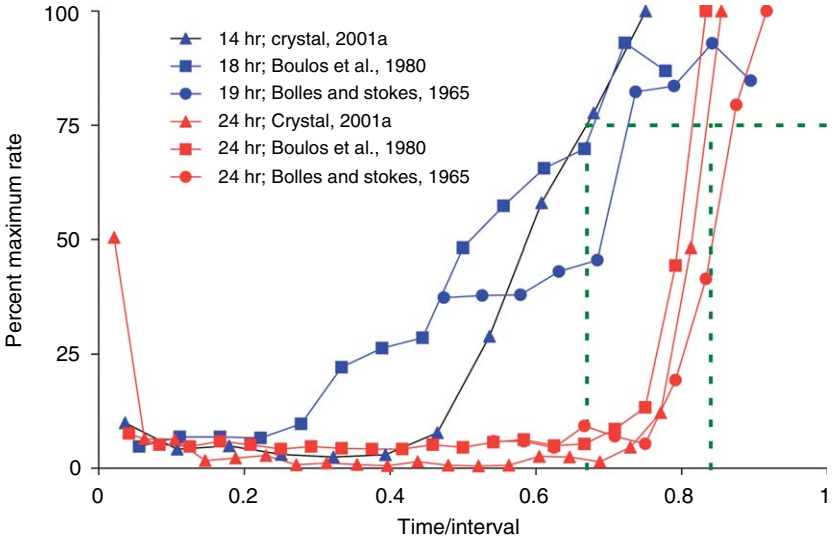

Figure 18 Rats anticipate intermeal intervals of 14, 18, and 19 h (blue symbols) with less precision (i.e., higher variability) than 24 h (red symbols); dashed lines indicate width of response rate functions. Data from Bolles and Stokes (1965) and Boulos Z, Rosenwasser AM, and Terman M (1980) Feeding schedules and the circadian organization of behavior in the rat. *Behav. Brain Res.* 1: 39–65, in which meals were earned by pressing a lever, were obtained by enlarging published figures by 200% and measuring each datum at 0.5-mm resolution. Adapted from Bolles RC and Stokes LW (1965), Rat's anticipation of diurnal and a-diurnal feeding. *J. Comp. Physiol. Psychol.* 60: 290–294 and Terman M (1980) Feeding schedules and the circadian organization of behavior in the rat. *Behav. Brain Res.* 1: 39–65, and Crystal JD (2001a) Circadian time perception. *J. Exp. Psychol. Anim. Behav. Process.* 27: 68–78. Reproduced from Crystal JD (2006b) Long-interval timing is based on a self sustaining endogenous oscillator. *Behav. Processes* 72: 149–160, © 2006, with permission from Elsevier.

estimate of the elapsed time with respect to the last meal continues to increase during the second nonfood cycle according to this proposal. Therefore, a pacemaker-accumulator does not predict an increase in response rate prior to the second skipped meal because elapsed time since the last meal is larger than the intermeal interval during the second nonfood cycle. In contrast, if the timing documented in the top panel of **Figure 19** is based on an endogenous oscillator, then response rate should increase as a function of time in both the first and second nonfood cycles because an endogenous oscillator is self-sustaining (i.e., periodic output from an oscillator is expected to continue after termination of periodic input).

The first two nonfood cycles are also shown in **Figure 19** (middle and bottom panels). Note that response rate increased as a function of time in the first and second nonfood cycles. In particular, response rate was significantly higher in the 3 h during the omitted meal relative to the 13 h prior to this point for both first and second nonfood cycles.

To characterize the period of behavior after termination of the meals, response rates were subjected to a periodogram analysis, which assesses the reliability of a periodic trend and estimates the observed period. A significant periodic trend was detected for each rat. The mean period in extinction was 20.4 ± 0.9 h (mean $\pm$ SEM), which was significantly different from 16 and 24 hr (Crystal, 2006b).

These data are consistent with the hypothesis that the natural period of the oscillator that drove behavior was 20.4 h, which is distinct from the circadian oscillator; according to this hypothesis, the two oscillatory systems are dissociated by their different characteristic periods. However, the data are also consistent with an alternative hypothesis, according to which the circadian oscillator's free-running period is modified by the periodic input to which it was previously exposed. Note that according to both of these hypotheses, long interval timing is based on a self-sustaining, endogenous oscillator; the hypotheses differ in specifying the characteristic period of the oscillator(s). Taken together, the data presented in the sections titled "Timing long intervals" and "Endogenous oscillations in long-interval timing" imply that long-interval timing is based on a self-sustaining, endogenous oscillator, which is not consistent with the prediction in the second row of **Table 1**.

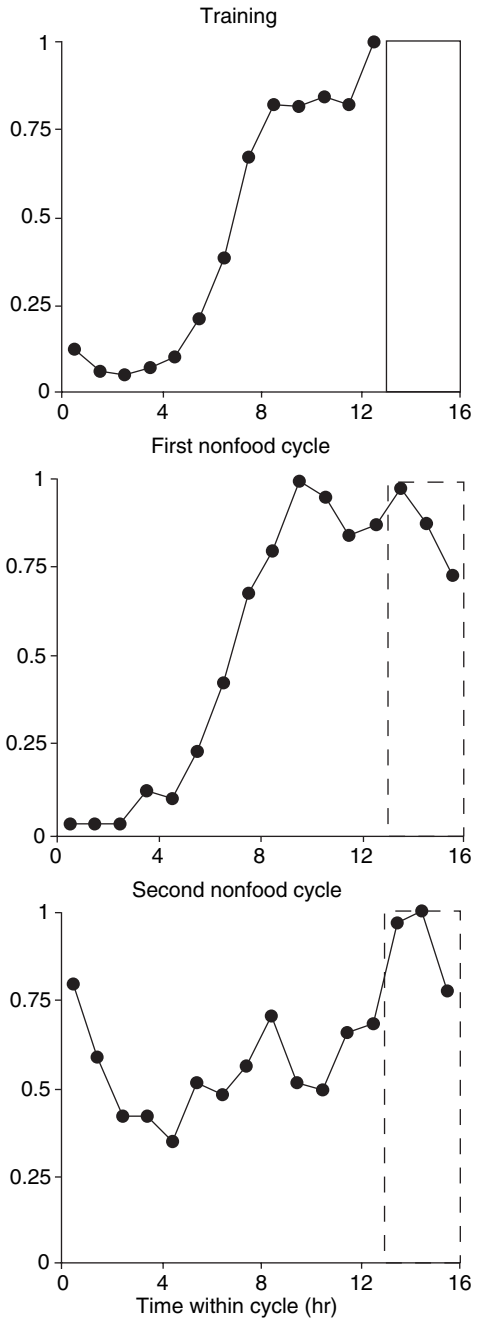

Figure 19 Endogenous oscillations in long-interval timing continue after the termination of periodic input. Response rate increased as a function of time within the 16-h intermeal interval cycle during the first and second nonfood cycle. Response rate (frequency of responses expressed as a proportion of the maximum frequency within the cycle) is plotted as a function of time within the cycle. The cycle included meals (indicated by the solid rectangle) during training (top panel). The meals were omitted (indicated by the dashed rectangles) in the first (middle panel) and second (bottom panel) nonfood cycles. Reproduced from Crystal JD (2006b) Long-interval timing is based on a self sustaining endogenous oscillator. *Behav. Processes* 72: 149–160, © 2006, with permission from Elsevier.

1.19.6.6 Variance Properties in Circadian and Short-Interval Timing

As noted in the section "Formal properties of interval and circadian timing," the study of variance properties has historically played a significant role in the development of theories of short-interval, but not circadian, timing. However, the data summarized in **Figure 17** suggest that a property of the well-established circadian oscillator is the relative improvement in sensitivity to time circadian intervals relative to noncircadian intervals. These data suggest that other putative oscillators may be identified by documenting other local maxima in sensitivity to time. Moreover, the observation that short-interval timing in the range of 1–3 min exhibits endogenous, self-sustaining patterns of behavior after the termination of periodic input reinforces the expectation that short-interval timing may be, at least in part, based on an endogenous oscillatory mechanism.

Figure 20 shows a measure of sensitivity to time plotted as a function of stimulus duration from a series of experiments that evaluated many closely spaced target intervals (Crystal, 1999, 2001b). The data suggest that sensitivity to time short intervals is characterized by multiple local maxima. The procedure involved presenting a short or long stimulus followed by the insertion of two response levers. Left or right lever presses were designated as correct after short or long stimuli. For each short duration, accuracy was maintained at approximately 75% correct by adjusting the duration of the long stimulus after blocks of discrimination trials. Sensitivity to time (as measured by signal detection theory; Macmillan and Creelman, 1991) was approximately constant for short durations from 0.1 to 34 s. However, local peaks in sensitivity to time were observed at approximately 0.3, 1.2, 12, and 24 s.

Figure 21 shows multiple local maxima in sensitivity to time across 7 orders of magnitude. The local maximum in sensitivity to time on the right side of the figure is replotted from **Figure 17**, which shows a local maximum in sensitivity to time of approximately 24 h. The local maxima in sensitivity to time on the left side of the figure are replotted from **Figure 20**. **Figure 21** documents that multiple local maxima in sensitivity to time are observed in the discrimination of time across several orders of magnitude.

To provide an independent, converging line of evidence regarding local maxima in sensitivity to time, **Figure 22** replots coefficients of variability

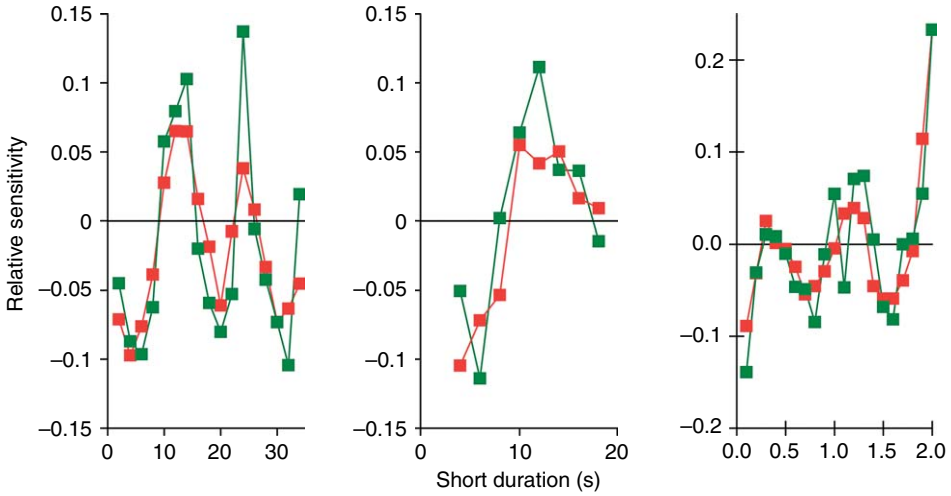

Figure 20 Sensitivity to time is characterized by local maxima at 12 and 24 s (left panel), 12 s (middle panel), and 0.3 and 1.2 s (right panel). Green symbols: average across rats. Red symbols: a running median was performed on each rat's data, and the smoothed data were averaged across rats to identify the most representative local maxima in sensitivity. Left panel: Rats discriminated short and long noise durations with the duration adjusted to maintain accuracy at approximately 75% correct. Short durations were tested in ascending order with a step size of 1 s ($n = 5$) and 2 s ($n = 5$). Sensitivity was similar across step sizes ($r(15) = .701$, $p < 01$), departed from zero based on a binomial test ($p < .001$), and was nonrandom ($r(14)_{lag1} = .710$, $p < .01$). Mean SEM = 0.03. Middle panel: Methods are the same as described in left panel, except short durations were tested in random order ($n = 7$) or with each rat receiving a single-interval condition ($n = 13$); results from these conditions did not differ. Sensitivity departed from zero based on a binomial test ($p < .001$) and was nonrandom ($r(7)_{lag1} = .860$, $p < .01$). Mean SEM = 0.02. Right panel: Methods are the same as described in left panel, except intervals were defined by gaps between 50-ms noise pulses, and short durations were tested in descending order with a step size of 0.1 s ($n = 6$). Sensitivity departed from zero based on a binomial test ($p < .001$) and was nonrandom ($r(18)_{lag1} = 0.736$, $p < .001$). Mean SEM = 0.04. Sensitivity was measured using *d'* from signal detection theory. *d'* = *z*[*p*(short response | short stimulus)] – *z*[*p*(short response | long stimulus)]. Relative sensitivity is *d'* – mean *d'*. Adapted from Crystal JD (1999) Systematic nonlinearities in the perception of temporal intervals. *J. Exp. Psychol. Anim. Behav. Process.* 25: 3–17; Crystal JD (2001b) Nonlinear time perception. *Behav. Processes* 55: 35–49; Crystal JD (2003) Nonlinearities in sensitivity to time: Implications for oscillator-based representations of interval and circadian clocks. In: Meck WH (ed.) *Functional and neural mechanisms of interval timing,* pp. 61–75. Boca Raton, FL: CRC Press. Reproduced from Crystal JD (2006d) Time, place, and content. *Comp. Cogn. Behav. Rev.* 1: 53–76 with permission.

(CV; ratio of standard deviation to the mean) as a function of the target intervals using 43 data sets from the literature selected by Gibbon et al. (1997b). The data from Gibbon and colleagues' scatter plot are replotted in the top panel of **Figure 22**, using a reverse-order vertical axis so that high points in the figure correspond to high sensitivity to time. To examine the shape of the sensitivity function, the data from Gibbon et al. were averaged in two-point blocks and subjected to a 3-point running median, which appears in the bottom panel of **Figure 22**. Sensitivity to time using Gibbon and colleagues' selection of data from the literature is characterized by multiple local maxima. The middle of the local maxima in the bottom panel of **Figure 22** occurs at approximately 0.2, 0.3, 1.2, 10, and 20 s. The top panel of **Figure 22** also shows clusters of high points near these intervals. The values of local maxima derived from Gibbon et al.'s selection of data are markedly similar to local maxima that were observed in **Figure 20**: 0.3, 1.2, 12, and 24 s (Crystal, 1999, 2001b, 2003, 2006d). Although the shapes of the sensitivity functions in **Figures 20** and **22** differ, the similarity in the locations of local maxima is significant given that the data in **Figure 22** come from 43 different data sets. Moreover, the data that appear in **Figure 22** were independently selected by Gibbon and colleagues.

1.19.6.7 Integration of Interval and Circadian Timing

The data in **Figures 20, 21,** and **22** suggest that the psychological representation of time is nonlinearly related to physical estimates of time. The existence of a local maximum near a circadian oscillator (**Figure 21**, peak on right side) and local maxima in the short-interval range (**Figure 21**, peaks on left

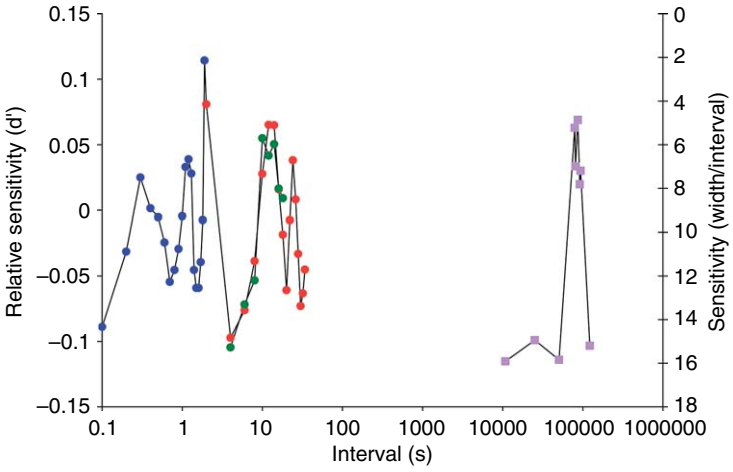

Figure 21 Multiple local maxima in sensitivity to time are observed in the discrimination of time across seven orders of magnitude. The existence of a local maximum near a circadian oscillator (peak on right side; purple squares) and other local maxima in the short-interval range (peaks on left side; blue, red, and green circles) are consistent with the hypothesis that timing is mediated by multiple oscillators. Intervals in the blank region in the center of the figure have not been tested. Left side: Rats discriminated short and long durations, with the long duration adjusted to maintain accuracy at 75% correct. Short durations were tested in sequential order (blue and red circles; $N = 26$) or independent order (green circles; $N = 20$). Circles represent relative sensitivity using d' from signal detection theory and are plotted using the y-axis on the left side of the figure. Right side: Rats received food in 3-h meals with fixed intermeal intervals by breaking a photobeam inside the food trough. The rate of photobeam interruption increased before the meal. Squares represent sensitivity, which was measured as the width of the anticipatory function at 70% of the maximum rate prior to the meal, expressed as a percentage of the interval ($N = 29$). The interval is the time between light offset and meal onset in a 12–12 light–dark cycle (leftmost two squares) or the intermeal interval in constant darkness (all other squares). Squares are plotted with respect to the reversed-order y-axis on the right side of the figure. Y-axes use different scales, and the x-axis uses a log scale. Adapted from Crystal JD (1999) Systematic nonlinearities in the perception of temporal intervals. *J. Exp. Psychol. Anim. Behav. Process.* 25: 3–17; Crystal JD (2001a) Circadian time perception. *J. Exp. Psychol. Anim. Behav. Process.* 27: 68–78; Crystal JD (2001b) Nonlinear time perception. *Behav. Processes* 55: 35–49. Reproduced from Crystal JD (2006d) Time, place, and content. *Comp. Cogn. Behav. Rev.* 1: 53–76, with permission.

side; **Figure 22**) is consistent with timing based on multiple oscillators (Church and Broadbent, 1990; Gallistel, 1990; Crystal, 1999, 2001b, 2003, 2006d). According to multiple-oscillator proposals, each oscillator is a periodic process that cycles within a fixed amount of time; an oscillator is characterized by its period (i.e., cycle duration) and phase (i.e., current point with the cycle). Each unit within a multiple oscillator system has its own period and phase. Therefore, a multiple-oscillator system includes several distinct periods. Sensitivity to time of an interval near an oscillator is expected to be higher than timing an interval farther away from the oscillator. Therefore, the multiple local maxima in sensitivity to time shown in **Figures 17, 20, 21, and 22** suggest the existence of multiple short-period oscillators.

The data reviewed in this section conflict with the predictions of **Table 1**. The first row of **Table 1** focuses on the defining feature that distinguishes oscillator and pacemaker-accumulator mechanisms. Oscillators are endogenous and self-sustaining, meaning that they continue to cycle after the termination of periodic input. By contrast, a pacemaker-accumulator requires resetting by an exogenous stimulus, and the timing system is presumed to be reset by a single event (i.e., one-shot reset). The data in **Figures 14 and 15** suggest that short-interval timing is endogenous and self-sustaining, thereby exhibiting a property of an oscillator rather than a pacemaker-accumulator.

The second row of **Table 1** focuses on the susceptibility of oscillator and pacemaker-accumulator mechanisms to time different ranges of target intervals; the former is described as subject to a limited training range, whereas the latter is subject to a broad training range. The data in **Figures 16 and 18** suggest that many long, but noncircadian, intervals can be timed, and the data in **Figure 19** suggest that long-interval timing is endogenous and self sustaining, consistent with an oscillator mechanism.

The third row of **Table 1** focuses on the responsiveness to abrupt changes in the phase of an input signal. A hallmark feature of an oscillator is gradual adjustment in phase responsiveness – meaning that

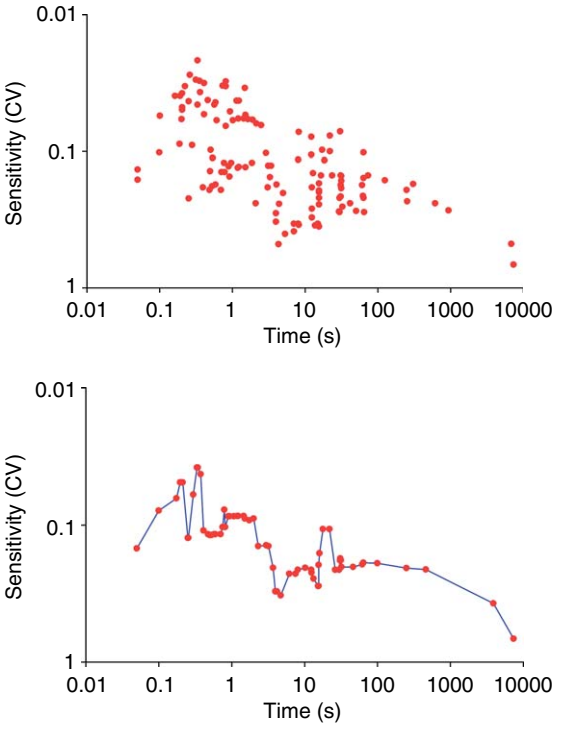

Figure 22 Top panel: Sensitivity is plotted as a function of time across six orders of magnitude. The scatter plot reveals that sensitivity to time declines as a function of increasing intervals. The data are from Figure 3 in Gibbon J, Malapani C, Dale CL, and Gallistel C (1997b) Toward a neurobiology of temporal cognition: Advances and challenges. *Curr. Opin. Neurobiol.* 7: 170–184. The published figure was enlarged by 375%, and each datum was measured at 0.5-mm resolution. The residuals from linear regression (not shown) were not random ($r(128)_{lag1} = .454$, $p < .001$). The data are plotted on a reversed-order *y*-axis to facilitate comparison with other measures of sensitivity. Bottom panel: Sensitivity is plotted as a function of time across six orders of magnitude. The data from Gibbon et al. (1997b) shown in the top panel were averaged in two-point blocks and subjected to a three-point running median. Note that sensitivity to time is characterized by local maxima at approximately 0.2-0.3, 1.2, 10, and 20 s. Note that these values are similar to the local maxima that were observed by Crystal (1999, 2001b): 0.3, 1.2, 12, and 24 s (cf. Figure 21). The residuals from linear regression (not shown) were not random ($r(63)_{lag1} = .869$, $p < .001$). The data are plotted on a reversed-order *y*-axis to facilitate comparison with other measures of sensitivity. Adapted from Gibbon J, Malapani C, Dale CL, and Gallistel C (1997b) Toward a neurobiology of temporal cognition: Advances and challenges. *Curr. Opin. Neurobiol.* 7: 170–184; and Crystal JD (2006c) Sensitivity to time: Implications for the representation of time. In: Wasserman EA and Zentall TR (eds.) *Comparative cognition: Experimental explorations of animal intelligence,* 270–284. New York: Oxford University Press. Reproduced from Crystal JD (2006d) Time, place, and content. *Comp. Cogn. Behav. Rev.* 1: 53–76. with permission.

multiple cycles under the new phase regime are required to produce complete adjustment. By contrast, a hallmark feature of a pacemaker-accumulator is immediate adjustment to a resetting stimulus — meaning that complete adjustment requires presentation of a single shifted cycle. The data in **Figure 13** suggest that short-interval timing is subject to gradual phase adjustment, consistent with an oscillator mechanism.

The fourth, and final, row in **Table 1** focuses on the variance properties of oscillator and pacemaker-accumulator timing systems. Circadian timing appears to be characterized by greater precision (i.e., lower relative variability) compared to short-interval timing. The data in **Figures 17, 20, 21, and 22** suggest that both short-interval and circadian timing are characterized by local peaks in sensitivity to time.

The data suggest substantial continuity between short-interval and circadian timing systems. Indeed, in many situations, the predictions of multiple timing mechanisms are confounded, and specific empirical tests are required to dissociate these mechanisms (e.g., Pizzo and Crystal, 2002, 2004a,b; Babb and Crystal, 2006). The data reviewed in this chapter may prompt the development of a theory of timing that encompasses the discrimination of temporal intervals across several orders of magnitude, from milliseconds to days. The proposal to integrate ideas in short-interval and circadian timing is not unprecedented given the shared genetic makeup of some behaviors over both long and short time horizons (e.g., courtship behavior and circadian period in *Drosophila melanogaster*; Roche et al., 1998).

1.19.7 Conclusions

Time is a fundamental dimension of human and animal experience, affecting behaviors that span from milliseconds to days. Indeed, timing is ubiquitous in such familiar behaviors as speech, music, and motor control in the range of milliseconds, foraging, decision making, and time perception in the seconds-to-minutes range, and sleep–wake cycles and appetite in the range of a day (Buhusi and Meck, 2005). This review has focused on (1) methods of assessing temporal performance and (2) evaluating theoretical proposals about the mechanisms responsible for temporal performance. We draw some conclusions for each of these lines of focus.

The review of experimental methods illustrates the remarkable quantitative precision that may be obtained in studying temporal performance. One of

the unifying principles is the scalar property; seemingly different patterns of performance that unfold in real time may be seen as representing cases from a single process when the data are rescaled in proportional units of time. We reviewed some of the many experiments that have demonstrated the ubiquity of this feature of temporal performance.

Nevertheless, the review of theoretical proposals suggests that the observation of superimposition does not, by itself, identify the mechanism(s) responsible for temporal performance. Although superimposition of data in relative units of time is consistent with SET (a classic pacemaker-accumulator theory of timing), other timing theories are also compatible with this feature of timing data (e.g., Hopson, 2003; Killeen and Fetterman, 1988). Moreover, we reviewed a series of experiments that sought to identify data diagnostic of pacemaker-accumulator or oscillator mechanisms. The conclusion that emerges from these lines of research is that short- and long-interval timing are characterized by oscillator-like properties.

Perhaps the greatest challenge for applying an oscillator mechanism to short-interval timing is the need to have an oscillator mechanism that is flexible enough to time intervals that span several orders of magnitude. Although this has traditionally been assumed to be compatible with a pacemaker-accumulator (but not an oscillator) mechanism, the observation of oscillator-like properties in timing intervals across several orders of magnitude encourages the view that multiple oscillators may be deployed to time a broad range of intervals (Church and Broadbent, 1990; Gallistel, 1990; Crystal, 2006d). Moreover, the data reviewed in this chapter may prompt the development of a unified theory of timing that encompasses the discrimination of time across several orders of magnitude – from milliseconds to days.

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1.20 Foraging

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1.20.1 Introduction

The oldest organisms on earth live on isolated peaks in the Great Basin. These bristlecone pines look like giant bonsai trees, twisted against the harsh conditions of the high, cold desert. The oldest of these trees are over 4500 years old. They have lived through most of recorded human history, but they do not remember. Indeed, students of learning and memory seldom give plants – whether old or young – a passing thought. It is, however, instructive to recall the differences between plants and animals and to ask whether these differences can tell us something about why one group comes equipped to learn and remember while the other does not. Nearly any introductory biology book will direct your attention to three key differences: animals move, animals have nervous systems, and animals consume living things. These three elements represent a deeply intertwined syndrome of animal features. A plant living in a flux of photons and a sea of CO_2 does not need to move. In contrast, living tissues do not flow into an animal's mouth. For animals, pastures are always greener elsewhere (often quite literally!), so animals need to move to find a fresh supply of living resources. And, of course, animals need nervous systems, and ultimately the ability to learn and remember, to control and refine these movements.

The business of acquiring and consuming living resources ranges from the spectacular – a spider

swinging a bola of sticky silk to ensnare prey – to the mundane – cows eating grass. Whatever form it takes, it is a basic feature of the animal way of life. It follows that an understanding of foraging behavior is fundamental to our understanding of animal behavior and the mechanisms that control it, including learning and memory. To begin, we consider a concrete example that illustrates the role of learning and memory in foraging.

On summer mornings from July to September masses of tiny mayflies (genus *Tricorythyodes*) emerge from cold water streams in North America. The emergence occurs in the early morning. In the hour after dawn, clouds of these tiny flies hover over every riffle in the river. These clouds are mating swarms. As the mating swarms develop, fish in the stream below move into positions downstream of the swarming flies in the workman like manner of commuters arriving at their jobs. An hour and 30 minutes after dawn the mating swarms begin to break up as the mated adults die. Their bodies cover the surface of the stream and create a feeding bonanza for the waiting fish. Although the mayflies fall indiscriminately, the currents and properties of the streambed concentrate the food. The waiting fish are clearly sensitive to these hotspots, because as the mayflies continue to cover the water, one can see patches of the stream where the surface boils with the activity of feeding fish. The ecological drama ends as suddenly as it began. A few hours after dawn the mayflies will have been swept away by the current, and the fish will have dispersed to stations throughout the river.

This predictable daily drama is typical of the situations in which learning abilities guide animal feeding behavior. Like our feeding trout, honeybees and other nectar feeders are, for example, famous for learning the temporal availability and spatial pattern of food sources (*See* Chapters 1.25, 1.29). Feeding blue jays, to take another example, learn that monarch butterflies taste bad due to cardiac glycosides that the butterflies obtain as caterpillars. Broadly speaking, foraging animals clearly learn many basic properties of their prey: how to handle them (e.g., remove spines, avoid stinging parts), where they can be found (under leaves? on tree bark?), and so on. Similarly, foraging animals use memory to guide their decisions about which patches to exploit and when to exploit them. Animals that store food for later retrieval offer an especially compelling example. Clearly, the ability to relocate hidden food items is a basic feature of animal food-caching behavior. Over the last 20 years investigators have exploited this behavior to produce new insights in how memory serves animals in naturally occurring resource gathering (*See* Chapter 1.22). Recently, for example, Clayton and Dickinson (1998) have exploited this aspect of foraging behavior to provide the first evidence that nonhumans can form 'episodic-like' memories (*See* Chapter 1.23).

As these remarks show, learning and memory play key roles in the day-to-day business of resource acquisition. In contrast, many of the classical methods for studying learning and memory seem abstract and somewhat contrived. Yet, there are deep connections between the two. One connection is procedural; food acquisition serves as a useful context with which to study learning and memory. Pavlov's dogs salivate in anticipation of food, Skinner's pigeons peck for access to food, and Olton's rats run down the arms of a radial arm maze to obtain food. It behooves students of learning and memory, therefore, to understand how the components of foraging behavior they study fit within the larger context of animal foraging. The second connection is more conceptual; a complete understanding of learning and memory will require analyses at all levels of biological organization from the molecular to the ecological, and foraging behavior stands out as one of the simplest situations in which to seek an understanding of the ecological significance of learning and memory.

1.20.1.1 Foraging Basics

Foraging refers to the business of food acquisition. Although we restrict the definition to food acquisition, ecologists commonly apply similar principles to other forms of resource acquisition (e.g., 'foraging' for mates). As a broad generalization, foraging is a relatively common and easily observed aspect of animal behavior (*See* Chapters 1.12, 1.21). As explained earlier, foraging is a fundamental component of animal existence both because it is a defining property of animals, and because it provides the fuel for the remainder of an animal's activities. Students of foraging behavior (Stephens and Krebs, 1986; Stephens et al., 2007) typically envision a three-level hierarchy of resources. At the highest level, animals forage within *habitats*. For example, a hummingbird may choose to feed in an alpine meadow or along a stream bank. Choosing a habitat determines the mix of resources that a forager will encounter at a fairly large spatial and temporal scale. Typically, we imagine that it is time consuming or costly to switch habitats. Food is virtually never spread evenly

throughout a habitat, so it is useful to imagine that food occurs in discrete *patches* within habitats. So our hummingbird would encounter clumps of flowers within the alpine meadow. In practice, of course, the 'edges' of food patches are often fuzzy, but foraging models typically ignore this. In contrast with habitats, we imagine that a forager encounters many patches during any given foraging bout, and that it may choose to exploit or ignore any given patch it encounters. However, a forager must also choose how thoroughly a given patch should be exploited. Finally, within patches we commonly imagine that the forager finds *prey* items such as seeds or insects. For our hummingbird, the flower within an inflorescence would serve as prey. While the distinction between patches and prey is usually clear-cut, it can also be subtle. Economically, the key point is that a forager can choose how to exploit a patch, but it can only choose whether to exploit a prey item. Notice finally that we call discrete food items *prey* regardless of whether they are animals or plants.

1.20.2 Basic Foraging Models

Over the last 20 years, behavioral ecologists have developed a basic set of models that make predictions about foraging behavior in a wide range of 'standard' foraging situations (Stephens and Krebs, 1986; Stephens et al., 2007). In the next few paragraphs, we present these models following the hierarchy discussed in the previous section. We begin by discussing prey choice (the lowest level), then we discuss patch exploitation, and finally we discuss habitat choice. All of these models follow the premise of rate maximization. Crudely speaking, we know both from empirical studies of food choice behavior and from first principles that both time and amount matter to foraging animals, and rate represents a simple and natural way to combine time and amount. More formally, the hypothesis of rate maximization holds that natural selection favors behaviors that lead to high rates of food intake, because animals that obtain food at a high rate will have more time and more resources to pursue other important activities (like mating, or territorial defense). One can, of course, imagine situations in which rate maximization would be inappropriate (for example, if the habitat that provides the higher rate of food intake also has the most predators). As one might expect, many papers focus on situations in which this 'standard' assumption does not hold. Nonetheless, the premise of rate maximizing represents a useful way to begin our analysis of how economic forces shape foraging behavior.

1.20.2.1 Prey Choice

Consider a bird searching for moths – prey items – along a tree trunk. We think of these items as particles dispersed through the environment that the animal 'bumps into' as it moves. Of course, there are different types of prey items. Our bird may encounter some small grey moths and some large white moths. The prey types vary in three ways. First, prey types provide different amounts of energy when consumed. To continue our example, small grey moths may provide 1.8 J when consumed, while large white moths provide 6.0 J. Second, prey take different amounts of time to pursue, handle, and consume – collectively we call this handling time. Small grey moths may require 10 s to handle, while the larger white moths take 25 s to handle. Finally, prey types have different abundances. Some types are common so the forager encounters them relatively frequently, while others are rare. This may seem like an odd way to introduce the simple idea of relative abundance, but to simplify our rate calculations we think of the average time between encounters with a particular prey type rather than using direct measures of abundance. The inverse of the time between encounters is the encounter rate, and generally speaking, abundant prey have high encounter rates. To summarize, we characterize prey items by their energy values, their handling times, and their encounter rates.

So, we have our bird hopping along a tree trunk encountering grey and white moths; now we need to think how the bird can express a choice. We will assume that the bird can decide whether to attack or ignore a given type when it is encountered. Mathematically, we represent this as a probability of attack upon encounter, and we have two such probabilities, one for the small gray moths and one for the large white moths. Now our mathematical problem is to find the attack probabilities that give the highest rate of energy intake. To do this, we need to express the intake in terms of the variables outlined here – encounter rates, handling times, energy value, and attack probabilities. This is fairly easy with one final trick. We recognize that our animal spends its time doing two things: searching and handling. By definition, if the λ_1 is the encounter rate of prey type 1, then an animal that searches for T_s units will encounter (not eat but encounter) $\lambda_1 T_s$ type 1 items, and similarly it will encounter $\lambda_2 T_s$ type 2 items.

Since our forager accepts type 1 items it encounters with probability p_1, the number it accepts in T_s units of searching is $p_1\lambda_1 T_s$; similarly the number of type 2s it accepts is $p_2\ \lambda_2 T_s$. Now we know each type 1 prey item requires h_1 time units to handle and provides e_1 calories; while each type 2 prey item takes h_2 time units to handle and provides e_2 calories. So, the rate we want to calculate is just the energy gain $(e_1p_1\lambda_1 T_s + e_2p_2\lambda_2 T_s)$ divided by the total time $(T_s + h_1p_1\lambda_1 T_s + h_2p_2\lambda_2 T_s)$. The rate of intake is

$$\frac{p_1\lambda_1 T_s e_1 + p_2\lambda_2 T_s e_2}{T_s + p_1\lambda_1 T_s h_1 + p_2\lambda_2 T_s h_2} \quad [1]$$

Since T_s is a common factor in every term in both the numerator and denominator, it cancels out to leave us with

$$\frac{p_1\lambda_1 e_1 + p_2 T_s e_2}{1 + p_1\lambda_1 h_1 + p_2\lambda_2 h_2} \quad [2]$$

More generally we have

$$\frac{\sum_i p_i\lambda_i e_i}{1 + \sum_i p_i\lambda_i h_i} \quad [3]$$

This final form is commonly called 'Holling's disc equation' (Holling, 1959) and is the conventional expression for intake rate used in foraging models. The development we followed in the preceding paragraphs shows the central assumption of Holling's disc equation; specifically, it assumes that foraging animals are either searching or handling, and that these are mutually exclusive activities. In broad overview this is a useful assumption that describes the way in which many animals feed; however, there are some important situations in which animals can handle and search simultaneously. Notably, grazing animals can often 'search for the next blade of grass' while they handle (chew) previously encountered grass blades (Spalinger and Hobbs, 1992; Illius et al., 2000).

Now that we have an expression for rate, our problem is to find the probabilities of attack that maximize this rate. This is a straightforward mathematical operation that we can solve via calculus, and which we will not pursue here. We can understand the results without working through the calculus. First, we find that the rate-maximizing value of p_i should be either zero or one; that is, our forager should always attack or always ignore the ith prey type upon encounter. Once we know this, we can ask which types should be ignored and which should be attacked. Suppose, for example, that a hypothetical forager is attacking types A and B; should it also attack type C? If we use Holling's disc equation to compare the rates of intake for a 'diet' of A and B alone to a diet of A, B, and C, then we find the effect of adding C depends on the type C's ratio of energy to handling time (e_c/h_c). We call these energy-to-handling-time ratios 'profitabilities,' and these are key variables to understanding the economics of prey choice. The basic algebraic result is that adding item C to the diet increases the overall rate of intake if C's profitability is greater than the intake rate obtained from a diet of A and B alone. This leads to the idea that foragers should rank prey types by their profitabilities. So we assign the prey type with the highest profitability rank 1, and second highest rank 2, and so on. Then, we can find the diet that maximizes intake rate by working through the prey types in rank order until we reach a situation where the rate obtained from attacking types 1 through N exceeds the profitability of the type $N+1$.

This economically derived, rate-maximizing 'diet' has several intriguing properties. First, it predicts, rather surprisingly, that animals should always take a given type upon encounter or always ignore it. This claim may surprise the casual observer, because people think of mixed-diets or 'well-balanced' diets as good diets. But this model only considers a single dimension of prey value (typically energy content), and so it cannot consider well-balanced diets. However, the idea of a 'single dimension of value' is often appropriate. In experimental studies, we often create prey types (or their logical equivalents) that value only in magnitude (e.g., big vs. small), and in nature many foragers eat a range of food items with similar nutrient contents (e.g., from the perspective of an insectivorous bird most insects have about the same balance of proteins and carbohydrates). Empirically, however, this so-called 'zero-one' rule seldom holds. Second, and most importantly, the model predicts that the better-ranked prey types control whether lower-ranked items should be attacked. When the good prey types are plentiful, foragers should ignore poorer prey types regardless of the abundance of these poorer prey types. Indeed, it is now a widely accepted principle that, in a rich environment (where foragers can easily obtain high-quality prey), animals will feed more selectively.

1.20.2.2 Patch Exploitation

At the next higher level of organization, we imagine that foragers encounter clumps of resources or

patches. Our forager finds food inside well-defined and easily recognized patches but travels between patches by crossing 'empty' space to reach the next patch. In practice, a foraging patch for a small insectivorous bird might be pine cones in which insects hide, or for an aquatic snail it might be the sunlit top of a rock on which algae grow. When we think of prey items, we think of entities that provide a given amount of food in a given amount of time, but animals exploit patches more flexibly. Our insectivorous bird might 'skim the cream' from a patch by quickly extracting the obvious and easily captured prey and then moving to another fresher patch. Alternatively, it might exploit the patch thoroughly by meticulously peering behind every scale of the pine cone before traveling to the next patch. To represent this flexibility, we plot a gain function that represents the relationship between time spent exploiting a patch and amount of food extracted from a patch. **Figure 1** shows a typical gain function.

Patches deplete, of course, so as the figure shows, we expect that gains within a patch will increase at a declining rate, so the gain functions usually bend down as the example in the figure shows. A 'cream skimmer' will leave the patch early, when the gain function is still increasing relatively steeply; while a 'thorough exploiter' will stay much longer, until the curve flattens out. Both strategies have risks, of course. The 'cream skimmer' risks spending too much time unproductively traveling between patches, while the 'thorough exploiter' risks wasting time in the current patch when there are richer fresh patches to be had.

Foraging theory's models of patch exploitation address the balance between cream skimming and thorough exploitation directly. Again, we begin by

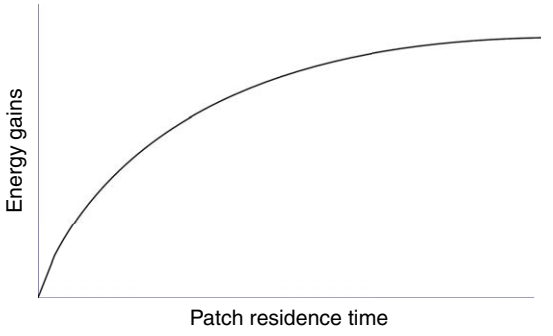

Figure 1 A typical patch gain curve. The x-axis shows the time spent exploiting a patch, and the y-axis shows the energy extracted from the patch. The forager extracts more energy as it spends more time in the patch, but this relationship tends to bend down because the patch depletes.

expressing the rate of food acquisition (again using eqn [3], Holling's disc equation). Let τ be the mean travel time, that is, the time it takes our forager to move from one patch to the next. Let t be the patch residence time. The variable t is the variable of interest here, because a small t corresponds with a cream-skimming strategy, while a large t implies thorough exploitation. Finally, let $g(t)$ be the gain function. Specifically, $g(t)$ gives the energy gains obtained when our forager exploits a patch for t units of time. So

$$\frac{g(t)}{\tau + t} \qquad [4]$$

gives the rate of energy intake for single patch type. Although one can solve this problem via calculus (find the t value that maximizes intake rate), there is an elegant and informative graphical solution.

Figure 2 shows the graphical solution.

We draw the gain function in the right-hand panel, so that patch residence time (t) is on the x-axis and energy gain is on the y-axis. Next we do something a bit unconventional. We let the negative part of the x-axis represent travel time (τ), with travel time increasing as we move away from the origin. Now for a given patch residence time, the intake rate (eqn [4]) is the slope of line drawn from travel time on the (usually) negative part of the x-axis to the point $(t, g(t))$ on the gain curve. If you imagine this point on the gain curve sliding up and down along the curve as we simultaneously maintain the straight line connecting our point to the travel time on the left side of the x-axis, then it is easy to see that the highest slope (and hence the highest rate) occurs when the line is just tangent to the gain curve.

Now that we know how to find the patch residence time that gives the highest rate, we can ask how the properties of the model determine the best patch residence time. There are two things within this simplified version of the model that we can consider: the gain function ($g(t)$) and the travel time (τ). The effects of travel time on patch exploitation have attracted the most attention. Broadly speaking, if the habitat is poor, then travel times will be long, but if the habitat is rich, then travel times will be short. If travel times are short, our tangent-construction solution predicts shorter exploitation times. Intuitively, in rich habitats foragers should adopt a cream-skimming strategy. In contrast, when travel times are long, the tangent-construction method shows that we should expect longer exploitation times. In poor habitats, then, we expect foragers to

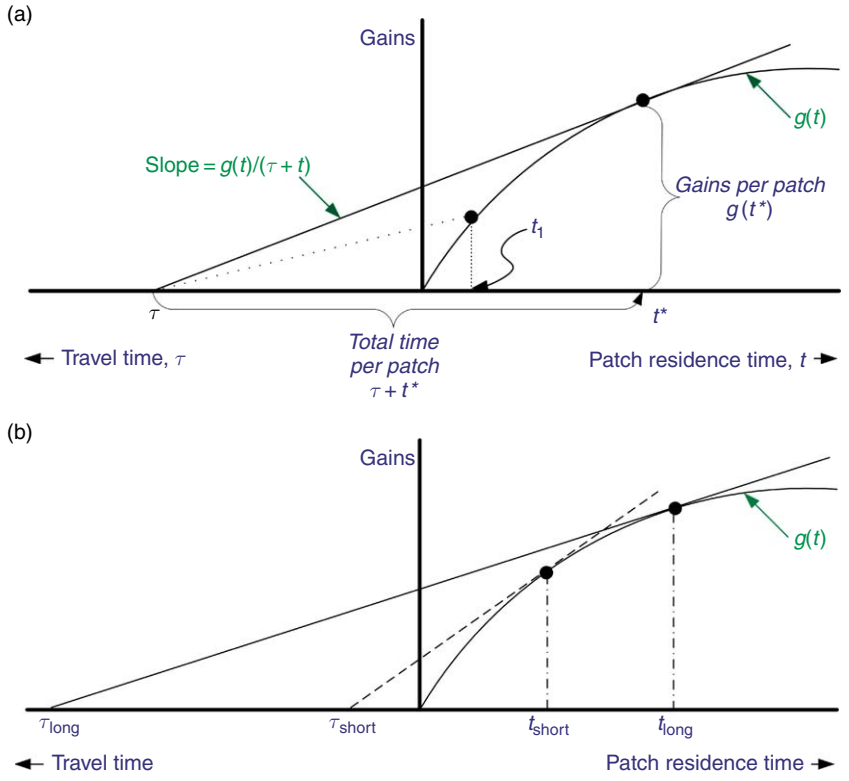

Figure 2 The graphical solution of the rate-maximizing patch exploitation time, sometimes called a Cowie plot or a rooted-tangent plot. Panel (a) shows the graphical method of find the patch residence time (t) that maximizes the forager's intake rate. On the x-axis we plot patch residence time (t) increasing to the right of the origin and travel time (τ) increasing to the left of the origin. The y-axis shows the energy gains as in **Figure 1**. The slope of a line drawn from a given travel time on the left side of the x-axis to a point on the gain curve on the right gives the intake rate associated with the corresponding patch residence time (t). Algebraically the slope of this line is $g(t)/(\tau + t)$, the quantity that our model seeks to maximize. One can readily see that the highest slope corresponds to a line that is just tangent to the gain function. The solid diagonal line illustrates this. Note that a line drawn to any other point on the gain curve will give a lower slope, as the dotted diagonal line shows. Panel (b) shows the main prediction of the model. When the travel time is long (τ_{long}), our tangent construction solution gives a long patch residence time (t_{long}), but when the travel time is short (τ_{short}), we predict a shorter patch residence time (t_{short}).

follow a 'thorough exploiter' strategy. The data provide strong support for the qualitative claim of cream-skimming in rich habitats and thorough exploiting in poor habitats. Indeed, this simple observation may be the single best-supported theoretical prediction from behavioral ecology. An appealing feature of this result is that we can understand it by comparing the value of continuing to exploit the present patch to the value of leaving to find a new patch. Short travel times increase the value of 'leaving,' and so we predict leaving earlier. The simplicity of this value of staying versus value of leaving comparison is important because, as we will see, it is the key to generalizing the model to more complex situations.

Gain functions can vary in a virtually unlimited number of ways, but our simple tangent construction method quickly shows the effects of different shapes. For example, if foragers can search systematically, their gains will follow a simple straight line up to some maximum. The gain function for systematic search will be a straight line from zero to the maximum, and then it will kink and become a flat line at the maximum food obtainable from the patch. Our tangent construction method shows the highest obtainable corresponds to a line intersecting the 'kink' (predicting that foragers should stay until they have extracted the maximum amount, but no longer) in this special case. Obviously, we would predict no travel time effect in this situation. Again, this makes sense when we consider the value of staying versus the value of leaving. There is no point in leaving until you have extracted the maximum amount of food, because the very best thing that could happen if you leave is to encounter a new patch with the same linear rate of gain.

The behaviors associated with patch exploitation have not been widely used in studies of learning and memory, and this is unfortunate, because patch exploitation is an important and well-documented aspect of natural foraging behavior. In addition, the successful family of models described here provides useful insights into the economic forces that have shaped patch exploitation.

1.20.2.3 Ideal-Free Distribution

Now consider a simple and somewhat artificial situation in which each of two sites provide food, say site 1 and site 2. Some distance separates the two sources, so that a forager cannot feed at both sites simultaneously, but must choose whether to feed at site 1 or site 2. Now, suppose that site 1 provides food at a rate of 10 J per hour (call this r_1), while site 2 delivers food at a rate of 5 J per hour (call this r_2). Economically, this is a trivial problem. Any sensible economic agent would stick with the site that delivers resources at the highest rate. This situation is not terribly interesting as it stands, but it becomes much more interesting when we imagine several foragers exploiting the same pair of feeding sites.

If several foragers all move to the single best site, they will have to share the food available there, and this raises the possibility that some of the members of the group could do better by moving to the other, less desirable site. The simplest model that addresses this question is called the ideal-free distribution. Like the two models discussed above, a review of this model provides significant insight into the economic logic of group foraging. To begin, imagine a group of six fish, with two at site 1 and four at site 2. Now if we assume that the fish at site 1 roughly share the food arriving there, then each expects to gain 10 J per hour divided by 2, or by 5 J per hour; using the same reasoning the four fish at site 2 expect to gain 1.25 J per hour per fish (i.e., 5/4). In this situation, we would expect that at least one of the fish at site 2 would be tempted to move to site 1 where it can do better, so we would have 3 fish at site 1 and 3 fish at site 2. But now the per-fish rate at site 1 would be 3.3 J per hour (10/3), while the per-fish rate at site 2 is 1.67 J per hour (5/3). So fish at site 2 are still doing worse, and at least one of them should be tempted to move to site 1, making the distribution four fish at site 1 and two fish at site 2. Now, the per-fish rate at site 1 is $10/4 = 2.5\ \mathrm{J\,h^{-1}}$, and the per-fish rate at site 2 is $5/2 = 2.5\ \mathrm{J\,h^{-1}}$. In this situation, no individuals are tempted to move to the opposite site. So we conclude that this four and two arrangement is stable, and we would expect this pattern to persist. Notice that, in our imaginary scenario, the fish have arranged themselves in a way that matches the inputs to the two sites. This 'input matching' result is the basic prediction of the ideal-free distribution model. This input-matching prediction holds reasonably well in experimental studies of animal feeding groups, even though the idealized assumptions of the model seldom hold. For example, the assumption that individuals share equally at feeding sites almost never holds, because some animals compete more effectively than others. Typically, this does not affect the input-matching result too much, because good competitors tend to occur at all feeding sites.

Logically, the ideal-free distribution's prediction of the input matching is quite similar to Herrnstein's matching law (Herrnstein, 1974), which predicts that individuals should distribute responses across two options in a way that matches the rates of food delivery associated with those responses. The matching law, however, focuses on the behavior of individuals, while the ideal-free model considers how the actions of individuals determine the properties of groups. While one derives the ideal-free model from very simple economic considerations, input matching for individuals (as the matching law predicts) only makes economic sense when rewards wait to be collected, as they commonly do in variable interval schedules.

1.20.2.4 General Principles and Conclusions

The central idea in the described models is the economics of lost opportunity. In prey selection a forager may reject a mediocre item, because to accept it means that the forager may miss an opportunity to obtain a better item. A forager exploiting patches in a rich environment leaves early, because it will lose opportunities to exploit new patches if it stays too long. A forager, feeding in a group, may choose to feed at a less productive site because competitors at the more productive site reduce the opportunities for success there. One should view these three basic models as starting points which, taken together, identify many fundamental variables in foraging behavior and draw our attention to the fundamental role of opportunity costs in foraging behavior.

Behavioral ecologists have extended these models in several significant ways. For example, these models tacitly assume that we can use averages to characterize the properties of resources (patches, prey, foraging sites). Models of risk-sensitive foraging consider the economic consequences of variability, predicting, for example, that foragers may prefer to take risks when

their energy supply is very low, even though they should avoid risks when they are well-off (Bateson and Kacelnik, 1998). Another important theme has been the effects of predation. While the described models only consider energy and time, it is often the case that the best places to forage (sites or patches) are also the most dangerous. Predators, after all, readily learn where prey are concentrated. The threat of predation can have dramatic effects on the behavior of foragers (see the section titled 'Foraging and predator avoidance').

1.20.2.5 Connections to Learning and Memory

Virtually all experimental tests of foraging models rely on learning. For example, in a now classic test of the diet model, Krebs et al. (1977) allowed foraging great tits (a small European bird) to watch prey items passing by on a small conveyor belt. They used the conveyor belt to manipulate encounter rate, and they found that the encounter rate with the highest quality items determined selectivity as the prey model predicted. Obviously great tits did not evolve collecting meal worms from conveyor belts, so their experience must have determined their choice behavior in Krebs et al.'s experiment. In light of experiments like Krebs et al.'s, behavioral ecologists – a group stolidly committed to the idea that muddy boots are required equipment for behavioral studies – came to the realization that they could design better experiments and build more testable models by attending to the large extant literature of animal learning and memory.

Some students of foraging have taken the connection with learning a step further by directly considering the fitness value of learning in foraging situations. Imagine, for example, that the quality of a foraging site changes unpredictably according to some mathematically well-defined process. One can then ask whether a forager should use its experience with this variable resource to determine whether to exploit or ignore this resource (see, for example, Krebs et al., 1978; Lima, 1985; Stephens, 1987, 1989, 1991; Tamm, 1987; Shettleworth et al., 1988). The next section discusses this approach in more detail.

1.20.3 Behavioral Ecology of Learning and Memory

Foragers live in a changing world. In the morning, the best feeding sites may be below riffles, while deep pools provide the best foraging in the evening. Competitors and predators may appear unpredictably. Foragers may encounter new prey types as the seasons progress or as the forager moves through its environment. Foragers need mechanisms that help them adjust to these changes, and surely learning and memory are a central part of this toolkit. In stark contrast to the world of change and the subsequent need for adjustment that real foragers face, the basic models of foraging (discussed in the previous section) assume that foragers can act with complete information about their options. Behavioral ecologists have used the machinery of statistical decision theory (Dall et al, 2005) to relax this complete information assumption. The family of models derived from these efforts considers the economics of adjusting to change. These 'incomplete information' models ask when and if it pays to learn about changing resources. The results, we argue, provide the foundation for an evolutionary approach to learning and memory and illustrate how an understanding of foraging can contribute to an enlarged view of the biology of learning and memory.

We will outline three basic approaches to the problems faced by an incompletely informed forager. To begin, we develop a simple model that calculates the value of information. This introduces the idea of environmental uncertainty and focuses our attention on the connection between information and action. Second, we move toward a model of learning by considering the economics of tracking a varying resource. In tracking models we explicitly consider how change generates uncertainty, and how animals may be sensitive to the properties of environmental change. Finally, we briefly discuss how environmental changes and the reliability of experience should influence memory.

1.20.3.1 The Value of Information

The working assumption of behavioral ecologists interested in learning and memory is that animals learn and remember things because acquiring and retaining particular kinds of information produces fitness benefits. Behavioral ecologists generally expect, therefore, that animals should learn and remember more valuable information more readily. But what makes information valuable and why would some types of information be more valuable than others?

To answer this question, imagine a simple situation in which some relevant state of the world (a prey type, a feeding site, . . .) can be any of several states. To be concrete, consider a squirrel foraging from pinecones.

Some pinecones are good feeding sites, others are mediocre, and still others are poor feeding sites. Our hypothetical squirrel cannot tell which is which. So when is information about pinecone type valuable? To find the value of information, we imagine two situations. First, suppose that the squirrel can recognize pinecone types perfectly. If this is true, then it should able to adopt an exploitation strategy that is appropriate for each pinecone type: aggressively exploiting good pinecones, skimming the cream from mediocre pine cones, and completely ignoring poor pinecones. Given the mix of pinecone types in the environment and our knowledge of how a 'knowledgeable' squirrel would treat them, we can – in theory – calculate the expected benefits of being completely informed about pinecone quality. Now, imagine the other extreme. Suppose that our squirrel cannot recognize pinecone quality, so it must treat all pinecones in the same way. Specifically, the best an ignorant squirrel can do is to adopt some average exploitation strategy that represents a compromise between the three types. Again, we can – in theory – calculate the expected benefit that an ignorant squirrel can achieve. The value of information about pinecone type is the difference between the expected benefits that a completely informed forager can obtain and the benefits expected by an 'ignorant' forager who must choose a single compromise tactic to suit all situations.

The general lesson here derives from the focus on action. The difference between informed and uninformed foragers is that the informed forager can treat different states differently, while the uninformed forager must adopt a single one-size-fits-all strategy. It follows that the value of information flows, in a very fundamental way, from information's potential to guide actions. Consider, for example, a situation in which the best way to exploit our hypothetical pinecones is the same regardless of type. What is the value of information then? Clearly, it must be zero. Because if the best action for all three types is the same, then an ignorant forager can adopt this single best action and do just as well as an informed forager. So, if a source of information cannot reveal something that will change the forager's action, then according to this definition it is not valuable. Valuable sources of information have the potential to change behavior.

1.20.3.2 Tracking

The value of information approach imagines a static but uncertain world – pinecones may be good or bad – and we ask whether a forager should make an effort to find out. In many important natural situations the properties of resources change: pinecones are bad now, but they might improve in the future. This is the problem of environmental tracking. It represents an important next step in models of uncertainty, because it considers the effects of environmental change. By considering change, tracking models make a more direct connection to studies of learning, because we think of learning as a mechanism that helps animals adjust to changing environments.

Tracking models usually assume that some aspect of the environment, say the quality of a feeding site, changes according to some stochastic process. A typical 'tracking' analysis might model the process of environmental change by assuming that the state stays the same from one time period to the next with a constant probability. If this persistence probability is high, the forager experiences relatively long runs of the same state (e.g., the varying site stays in the good state for a long time), but if the persistence probability is low, the state changes frequently. Finally, we imagine that the forager has some alternative but mediocre resource to exploit. So the varying site is the best choice when it is in the good state, but the mediocre alternative is best when the varying site is in the bad state. These assumptions create a basic tracking problem. The forager must somehow use its experience to decide whether to exploit the varying resource or the constant but mediocre resource. Specifically, we can ask how frequently the forager should check the state of the varying resource, given that its last observation showed that the varying resource was in the bad state. The optimal sampling frequency depends on how frequently the environment changes and on the underlying payoffs (i.e., the value of the varying resource when it is good, the value of the varying resource when it is bad, and the value of the constant-mediocre resource). A striking feature of the optimal solution is that it does not make economic sense to sample in many situations. The region which it makes economic sense to sample increases with environmental persistence. When there is no environmental persistence, the varying resource changes randomly from one time to the next, so the forager has no option but to use the resource that yields the highest gains on average (a strategy called averaging). However, when persistence increases, the varying resource switches between long runs of good and bad, and now it makes sense to keep track of the varying resource's state. Recognizing that 'averaging' is the alternative to sampling, one can readily see that

tracking will be most useful when the forager's alternatives are about the same on average. If they are quite different on average, then a nonsampling strategy that sticks with the better of the two options will often be the most sensible choice.

A handful of experimental studies have tested the predictions of these optimal tracking models (Tamm, 1987; Shettleworth et al., 1988; Inman, 1990). A recent review by Stephens (2007) found that animals tend to sample less frequently when environmental persistence is high, as these models predict. In addition, they sample less when we experimentally increase the value of the mediocre-stable resource. Again, this is as we would predict, because when the constant resource is good, there is less value in detecting the onset of a good state. On the other hand, our models predict that increasing the value of the varying resource's good state should increase the sampling rate, but the three experimental studies we have on the topic show no consistent effect of this variable. While our economic model agrees qualitatively with observations, Shettleworth et al. (1988) reported that a mechanistic model based on scalar expectancy gave a better quantitative fit to their data.

1.20.3.3 Optimal Memory

The tracking models discussed in the previous section greatly oversimplify how animals respond to experience. For example, they often assume that a single 'sample' is sufficient to instantly change a forager's behavior. An obvious next step asks how animals integrate information from many experiences, and specifically how foragers should combine past and current experience. This leads us to questions about the economics of memory in changing environments. While there is comparatively little work on the behavioral ecology of memory, those studies which have modeled memory have taken one of two approaches. One family of models (e.g., Cowie, 1977) represents memory as a sliding window, e.g., all experience from the last 4 days is weighted equally. These models typically try to understand how the optimal length of the memory window depends on the properties of environment (e.g., rates of change, reliability of experience, and so on). The second approach uses a past versus present weighting system to represent memory (McNamara and Houston, 1987). This approach is reminiscent of Bush and Mosteller's old stochastic learning rules (Bush and Mosteller, 1955). At each trial the animal's judgment (say x) of the current state of the environment is updated via a linear weighting scheme that puts some weight on the previous judgment and some weight on the animal's most recent experience. In these models, one tries to find the optimal past/present weighting. Of course, both approaches are simplistic mathematical expedients that caricature memory. Both schemes can be extended and elaborated in various ways. For example, one can create a hybrid model by adding a weighting scheme (which weights recent experience more heavily than past experience) to the memory window formulation (see, for example, Hirvonen et al., 1991).

Two economic variables should, in theory, affect how foragers should strike a balance between past and present information. The first key variable is the environmental rate of change. Animals that experience high rates of change should weight recent experience heavily and devalue past experience (i.e., they should have a short memory window). The second key variable is the reliability of experience. Consider a situation with two prey types: good and bad. However, the world is a noisy place, so our hypothetical forager experiences the good state as a distribution of qualities from very good to relatively poor. When the resource quality varies like this, a single experience will not be enough to discriminate between good and bad states. The logic here parallels the statistical concept of standard error: the variance of the measured quantity and the sample size combine to determine the error in our estimate of the mean. When experience is an unreliable guide to the underlying state, then foragers need past information (i.e., a larger sample size) to reasonably characterize the current state. So when experience is unreliable, we expect foragers to weight past experience more heavily (i.e., to have longer memory windows).

Two empirical studies support the idea that change 'shortens' an animal's time horizon. Devenport and Devenport (1994) studied ground squirrels foraging from two baited feeding stations. In one treatment the quality of the stations changed frequently (Left-best, then Right-best, and so on), but in the second treatment the good station was always the same. Next, Devenport and Devenport observed which station the squirrels selected after an experimentally imposed retention interval. Squirrels in the stable environment group always returned to the site that had been best regardless of the retention interval. However, squirrels in the varying environment treatment returned to the old 'best site' after short retention intervals but choose randomly after long intervals. In a similar set of studies Cuthill and colleagues tested the effects of varying travel time on the patch exploitation behavior of

starlings. In one study Cuthill et al. (1994) slowly changed the experimentally imposed travel time (from long to short or from short to long). In this slowly changing environment regime, they found that starlings needed experience with several 'long' travel times to appropriately change their patch exploitation behavior. In another study, however, Cuthill et al. (1990) created a rapidly changing environment by alternating long and short travel times. In this situation, they found that starlings adjusted their patch exploitation behavior after a single experience with a 'long' travel time. We readily acknowledge that these studies do not necessarily show differences in memory. It could be for example, that the Devenports' variable-environment squirrels remember the previous state perfectly, but somehow choose not to act on it (a variant of the well-known learning-performance distinction). However, the observation that environmental change influences how the animals integrate information across various time scales seems important and basic regardless of whether this happens via memory or some other mechanism. Although we do not discuss specific mechanisms here, one potential mechanism may not even be through the weighting of specific memories, but in the processes by which these memories are formed, with regards to time course, stages, and the use of multiple sites and substrates (see Menzel, 1999).

1.20.3.4 What Not to Learn and Remember

As students of learning and memory we tend to assume that these fundamental abilities serve animals effectively in many situations, including, but not limited to, foraging behavior. Yet the models reviewed here reveal many situations where it does not pay to attend to experience: there is no benefit in attending to information that cannot change behavior; when the environment changes frequently, simple fixed strategies can often outperform learning; similarly in an environment with rapid change, the contents of long-term memory will often be hopelessly out of date and misleading. Indeed, if we considered the costs of implementing learning and memory (Dukas, 1999), the conditions that favor learning would be even more restrictive. We do not want to overstate the case, however. Animals clearly derive benefits from learning and memory in many situations, but animals do not learn and remember everything. The ideas presented here provide a useful starting point for analyses that consider the ecological relevance of learning and memory. Do animals adaptively forget in changing environments? Do they filter out experiences that cannot change behavior? A research program that considers the role of learning and memory in foraging and other significant natural contexts may help answer questions like these.

1.20.4 How Learning Constrains Foraging

The previous section discussed how models and data from foraging can inform our thinking about learning and memory. The interactions between foraging and learning, however, run in two directions. This section briefly discusses how results from the study of learning and memory can refine the study of foraging. Consider a migrating bird arriving in a new location. Likely, it will be familiar with the types of prey available at the new site: beetles look very much like beetles everywhere. While many of the new beetle prey will be nutritious and edible, some will probably taste bad or spray noxious chemicals that our hypothetical immigrant has never experienced before. Clearly, the basic models of foraging theory offer us no help in trying to describe or analyze this situation. The prey model, for example, will tell us whether our bird should attack a novel noxious prey from an economic perspective, but clearly this is irrelevant until the forager has an opportunity to adjust to the properties of the prey in its new location. In most situations, these 'adjustments' will occur via learning. It follows that a complete understanding of animal foraging behavior will need to incorporate learning to accommodate situations like our migrating bird's dilemma and probably a great many others.

Unfortunately, the path connecting animal psychology and behavioral ecology has not always been smooth. Early behavioral ecologists advocated a mechanistically agnostic perspective that continues to influence many behavioral ecologists. This perspective recognized that natural selection directly favors behavioral outcomes and acts only indirectly on behavioral mechanisms. According to this view, the prey model (to take a specific example) can be correct and useful regardless of how animals adjust to the properties of prey. Biology is, of course, hierarchical, and one can offer biological explanations at many different levels (e.g., cellular, physiological, population, and so on). Behavioral ecology's mechanistic agnosticism wanders into error, in our opinion, when it seems to diminish the importance of integrating approaches from different levels of

biological explanation. The need for studies that combine foraging and psychology illustrates this point eloquently. This section asks how the properties of learning and memory constrain animal foraging behavior. Of course, learning and memory influence virtually every aspect of foraging. To reduce the problem to manageable size we will focus on the problem of prey choice.

1.20.4.1 Acquisition: The Most Basic Constraint

Our migrating bird cannot adjust to new prey types instantaneously. Learning takes time. During acquisition a learning animal's behavior becomes increasingly appropriate for the new situation. It follows that behavior during acquisition is something less than completely appropriate. Thorndike's fundamental observation that learning proceeds incrementally therefore provides the most basic mechanism through which learning constrains foraging. The next few paragraphs review several ways in which acquisition constrains foraging.

1.20.4.1.1 Search image

The insects that form the basic diet for nesting birds vary in abundance throughout the nesting season. In the 1960s Luuk Tinbergen (Tinbergen, 1960) noticed that there was a considerable lag between the first appearance of prey type and its inclusion in the diet. Tinbergen compared prey choice to prey abundance, and he observed that foragers took fewer prey than expected when few prey were present, but they took more than expected when the prey type became abundant. In a now famous paper, Tinbergen argued that this transition reflected a sort of perceptual learning that we now call search image. This is an intuitive idea for many people: when searching for your lost keys, you barely notice the pencils and paper clips on your desk. The search image hypothesis asserts that foraging animals, like the human key-loser, use a perceptual template of the items they are searching for, and as a consequence their perception of objects that do not fit the search image is reduced. Establishing that this appealing idea applies to foraging animals proved to be an arduous process. One reason for this is that many nonperceptual phenomena could account for Tinbergen's observations. For example, if emerging prey items occur in a different habitat (say under rocks), it may be quite reasonable for birds to continue to glean insects from leaves until prey 'under rocks' are abundant enough to justify a change in foraging habitat.

However, careful experimental studies (e.g., Pietrewicz and Kamil, 1979; Bond and Kamil, 1999) have demonstrated that the search image phenomenon is real. Kamil and colleagues have shown that recognition of cryptic prey improves with experience (*See* Chapter 1.22). However, these effects only appear for cryptic prey; animals do not show the same improvements with experience when tested with noncryptic stimuli of the type commonly used in psychological studies (e.g., red circles vs. green triangles). From the perspective of a cryptic prey species, this 'learning to see' phenomenon means that abundance reduces the protective effect of crypticity. In the course of evolution this can produce polymorphisms (i.e., a prey species with two or more cryptic forms), because an abundant form loses its advantage, while less abundant forms gain an advantage. In an extremely creative set of studies, Bond and Kamil (2002) have demonstrated this effect by training captive blue jays to peck at virtual 'moths' on a computer screen. Bond and Kamil arranged a situation in which the population of virtual moths evolved (in the computer). Using this technique, Bond and Kamil showed that the jay's perceptual learning did, indeed, select for prey polymorphisms.

1.20.4.1.2 Learning to handle prey

Monk's hood (genus *Aconitum*) flowers have an unusual shape. The face of the flower is normal enough, with a patch of stamens in the center; above this is the 'hood' that gives the flowers their common name. The flowers hold their nectar in a pair of receptacles that are 'under the hood' and above the face of the flower. A bee extracting nectar must land on the center of the flower (where its body touches the stamens), crawl up into the hood, and finally extend its tongue upward into the nectar receptacles. Given the complexity of this extractive task, it is not surprising that bumblebees must learn how to exploit monk's hood flowers. Indeed, studies by Laverty (1994) show classical acquisition curves for bumblebees exploiting monk's hood. Experience increases handling accuracy and reduces handling time. While Laverty's work showed that learning plays a critical role in extracting nectar from a complex flower like monk's hood, he also found that experience reduced handling time for simple cup-shaped flowers.

Situations like this must be common. Experience must often improve foragers' abilities to handle prey, but this requires a little rethinking of our standard prey selection model. Handling time is a basic parameter in the standard model, but what happens

if handling changes with experience? Which handling time matters: the handling of a naive forager or the handling that an experienced forager can achieve? Students of foraging think that this learning tends to favor diet specialization, because this process tends to make familiar items profitable (low handling times) and unfamiliar items unprofitable (high handling times). Although foragers could improve their handling times by exploiting unfamiliar prey, the comparative advantage of exploiting familiar types provides a sort of dietary inertia that encourages specialization.

This dietary inertia appears to be important in the flower exploitation choices of honeybees and bumblebees. In a phenomenon called flower-constancy or majoring, individual bees often seem to stick with a single species of flower, even when better flowers become available (Heinrich, 1976) (*See* Chapter 1.29). Given the start-up costs associated with learning to exploit a new flower and relatively short life spans of foraging workers, it is probably better (from the hive's perspective) to have older workers continue their foraging specializations while younger bees adapt to changes in the local flora by forming their own specializations. Another level of specialization is at the species level. How does acquisition apply to a species which specializes on only one type of flower? Laverty and Plowright (1988) compared the learning of a specialist species of bumblebee with that of a generalist species. They found that the specialist bumblebees acquired handling skills specific to its specialized flower more quickly than generalist bumblebees did. Many of the generalist bumblebees give up before locating the nectar, as one might expect for a species having to optimize the ability to learn about a large variety of flower morphologies.

1.20.4.1.3 The generality of the 'acquisition constraint'

These two topics – search image learning and learning to handle prey – represent only two ways in which learning can constrain animal foraging. In both cases the time lags involved in learning mean that foragers must be slightly out of sync with the current economic situation. This scenario must play out in many other contexts. To give two examples, studies of aversion learning suggest that animals are well equipped to learn that certain feeding resources should be avoided, and studies of spatial behavior show that animals learn the locations of food resources. In all these 'learning' situations the basic acquisition constraint applies. The behavior of learning foragers will often be on its way to somewhere, rather than in 'optimal' alignment with the current situation.

1.20.5 Emerging Topics

Using the basic models (discussed in the section titled 'Foraging basics') as a starting point, students of foraging have developed several new topics that make connections with learning and memory. This section reviews two of these topics – predator avoidance and social foraging. Although existing approaches to predator avoidance do not make significant connections to learning and memory, we feel there is enormous potential for studies that combine studies of appetitive learning (e.g., learning about food) with fear conditioning and avoidance learning. These two phenomena must frequently be in conflict in natural situations. Social foraging makes important connections with learning and memory because many animals forage in groups (notably many common 'model' organisms such as rats, pigeons, and humans). There is, of course, a well-developed and fascinating literature of social learning. We argue, in this section, that a deeper understanding of learning in social contexts will result when studies of social learning are informed by an understanding of the economic costs and conflicts inherent in group foraging.

1.20.5.1 Foraging and Predator Avoidance

The basic models outlined in the section titled 'Foraging basics' consider the economics of foraging decisions isolated and abstracted from the world in which foragers live. For muddy-boots biologists the most glaring oversight of this approach is that most animals live with the constant threat of predation. To make matters worse, the options that are the best economically are often the most dangerous. The site that provides the most food typically attracts the most foragers, and since these foragers are food for predators, it also attracts the most predators. In other cases the best feeding strategies expose foragers to the risk of predation. For grazing aquatic invertebrates like snails or caddis fly larvae the safest place is typically under rocks or deep in the substrate, but the algae that these organisms feed on grows in the sunlight on the exposed tops of rocks – ideal hunting conditions for a visual predator.

Animals do, of course, respond to the presence of predators. The crude effects are obvious and easily demonstrated. Guppies forage nearly 24 h per day in

streams without predators, but they restrict their foraging to the daylight hours when predators are present (Fraser et al., 2004). Juvenile sunfish forage in the weeds along lake shores even though they could obtain food at a faster rate in the open water (Werner et al., 1983; Werner and Gilliam, 1984). Tadpoles restrict their movements when predators are in the vicinity (Anholt et al., 2000). Many birds, from ostriches to chickadees, increase their vigilance (looking up) in risky situations, and clearly time 'looking up' is time that must be subtracted from feeding. Many small mammals organize their activities around a central refuge, such as a burrow or nest. Obviously, these animals quickly deplete the resources near their refuge, and so they face a trade-off between poor feeding close to their safe haven and richer pickings at riskier distances. Finally, many animals can dramatically reduce the risk of predation by foraging in groups. Groups allow foragers to spread the risk of predation and share the costs of vigilance, even though they can increase competition for food and other resources.

A striking finding is that the indirect effects of predation (e.g., the changes in behavior caused by predation) can often be more important ecologically than the direct effects of predation (i.e., the direct reduction in population that occurs when predators kill prey). For example, in 1995 the US National Park Service reintroduced wolves into Yellowstone National Park, where they could prey on the abundant herds of elk. Of course, the wolves have killed many elk, but this does not seem to be nearly as important as how wolves have changed the elk's behavior. Since the reintroduction, park biologists have observed a dramatic change in the riparian (streamside) habitats of the park: willow and aspen have proliferated, and streams meander more and beavers are more abundant. Without wolves, elk grazed everywhere, preventing regeneration of willows and aspens that beavers depend on. Now, elk spend more time in groups, more time being vigilant, and they forage less efficiently. They avoid dangerous thickets where predators might lurk (Laundre et al., 2001). In the words of Brown and Kotler (2007): "Fear can be a powerful ecological force."

1.20.5.1.1 Modeling predator avoidance

We can express the qualitative logic of foraging/predation trade-offs in succinct caveman grammar: food good, death bad. But how do we combine the value of food and threat of death by predation into logically coherent common currency? The breakthrough comes when we realize that food and death both connect to fitness via the fundamental life history parameters of survival and reproduction. Imagine, for example, that we can divide an animal's life into a sequence of equal time intervals. For an animal outside of the breeding season, we can write the benefits derived from the current interval as the product SV, where S represents the probability of survival to the next interval, and V represents the expected future reproduction of an individual who survives to the next interval. Foraging and predation combine to influence both the probability of survival and the expected value of future reproduction. To survive to the next period, an animal must obtain a minimum amount of food and avoid being killed by a predator. In addition, the animal's nutritional status and overall well-being determine the expected value of future reproduction V. Models of predation–foraging trade-offs typically proceed by specifying the relationship between foraging behavior (feeding site choice, patch use, etc.) and these two basic life history variables (survival and future reproduction). An especially illuminating case occurs with animals, like fish, that have indeterminate growth. These animals grow continuously, so food intake today leads to a larger body tomorrow, which ultimately leads to more reproduction. For these animals, reasonable assumptions about the effects of foraging on survival and future reproduction lead to the so-called μ over g rule (Gilliam, 1982; Stephens and Krebs, 1986). This formulation predicts that animals foraging under predation risk should forage in a way that minimizes the ratio of mortality rate (μ) divided by growth rate (g). To be explicit, imagine a juvenile fish that can choose to feed in any of three habitats. We can, in theory, express how risky each habitat is in terms of the mortality rate experienced there, and we can express the benefits of foraging there in terms of growth rate. The μ-over-g rule tells us that our hypothetical fish should choose the habitat with the smallest mortality rate to growth rate quotient. Although modelers derived this simple result from fairly restrictive assumptions, it seems to be fairly robust, and it provides a useful and elegant way to think about the trade-off between predation and foraging.

1.20.5.1.2 Learning about predators

Obviously enough, animals cannot learn very effectively from the experience of being eaten by a predator. Yet experience clearly plays an important role in anti-predator behavior. Vervet monkeys, for example, learn that some individuals give unreliable alarm calls (Cheney and Seyfarth, 1988). European

blackbirds learn to fear 'harmless' objects, if they observe others reacting fearfully to them (Curio et al., 1978). In widely cited studies, Cook and Mineka (1990) showed that monkeys could easily learn to fear snakes by observing the fearful reactions of conspecifics, but did not learn to fear plastic flowers from similar experiences. Although most published examples deal with social learning (see the next section), animals must learn about the risk of predation from many other unconditioned stimuli (e.g., moving shadows, the odor of a freshly killed conspecific, and so on). The study of escape responses, startle responses, and fear has a long history in the literature of learning (*See* Chapter 4.11; Bolles, 1970; Cook and Mineka, 1991; Davis, 2006). The lesson that emerges from studies of foraging is that resource acquisition and predator avoidance represent a fundamental and evolutionarily ancient trade-off. We argue, therefore, that a research program that considers the balance between learning about food and learning to avoid danger may reveal important new connections.

1.20.5.2 Social Foraging

In the autumn and winter nearly everyone in the northern hemisphere can observe large aggregations of starlings wheeling in the sky and foraging across lawns and agricultural fields like a hungry army. This is not unusual, of course; many animals feed in groups, including troops of capuchin monkeys, fishing pelicans, and swarms of army ants. Yet behavioral ecology's basic models of foraging focus on isolated individuals foraging as 'economically independent entities' (Waite and Field, 2007). In recognition of this, the topic of social foraging has been a growth area over the last 15–20 years. How does foraging in groups change the dynamics of foraging behavior? Unfortunately, we can only give a complicated answer to this simple question. As we will explain, the presence of others can influence foraging in many ways. Conspecifics can reduce feeding opportunities via competition, but they may also confer benefits such as enhanced predator avoidance and increased rates of food discovery. Perhaps the most interesting complication, from the perspective of learning and memory, is the problem of information transfer within foraging groups. A key advantage of grouping is that animals can benefit from the predator detection abilities of their group mates, and many studies show that group foragers reduce their personal vigilance (compared to individuals foraging alone). On the other hand, sharing information with group mates is not always in an animal's best interest. Group mates who are too well informed might interfere with your food discoveries or block the best escape routes when a predator appears.

1.20.5.2.1 Group size

The key to understanding the economic complexities of social foraging is a game theoretical approach. Game theoretical analyses of adaptation differ from the simpler optimization approaches because they recognize that the advantages of a forager's actions will depend on how others act. We already saw this principle when we considered the ideal-free model of habitat choice: the value of a feeding site depends on the number of competitors present. Among the most basic questions one can ask about social foraging is what determines group size? We typically address this question by plotting the relationship between the intake rate each group member obtains and the group size. In the ideal-free distribution, the simple inverse r/n describes this relationship, and the feeding rate always decreases as the group size increases. In situations like this more group mates are always bad, and we call this a 'dispersion economy.' Given that many animals are solitary foragers, this situation must be quite common in nature. Indeed, students of social foraging think that increasing group size must eventually reduce foraging benefits, even for animals adapted to large aggregations. In many situations, we believe that foraging benefits increase with group size (at least initially), and we call this an 'aggregation' economy. **Figure 3** shows the typical situation for an aggregation economy.

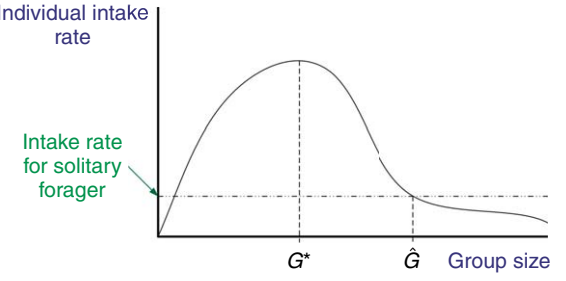

Figure 3 Graphical analysis of foraging group size. The plot shows the relationship between group size and individual intake rate for a so-called aggregation economy (see text). Initially, the per capita intake rate increases with increasing group size to a peak when the group size equals G^*. After that it declines with increasing group size. The dashed horizontal line shows the intake that a solitary forager can achieve. If the group grows beyond $\hat{G}$, then group members can benefit by leaving the group. We generally expect that stable groups should be between G^* and $\hat{G}$ in size.

Initially, individual foraging rates increase with group size, possibly due to the reduced cost of vigilance or the benefits of shared food discovery. During this increasing phase, adding a new member to the group is in the interests of current group members and prospective joiners. Indeed, existing members may even try to recruit new members during this 'increasing benefit' stage. Eventually, however, the foraging benefits will peak, and the mundane negative effects of feeding more mouths will decrease foraging benefits. One might expect groups to converge on the group size that maximizes feeding benefits (G^* in **Figure 3**), but this logic only holds up if existing members can control group entry. If, instead, individuals can join the group freely, then the group size can continue to increase because solitary foragers can still benefit by joining the group. In the language of game theory, one says that the benefit-maximizing group size is unstable. In general we expect to see groups larger than the benefit-maximizing group size (G^*) but smaller than the group size where individuals could do better by foraging on their own ($\hat{G}$ in **Figure 3**). In theory, the degree of control that group members can exert over group entry will determine stable group size: more control will push the predicted value toward the benefit-maximizing group size (G^*), and less will shift things toward larger group sizes near the theoretical maximum $\hat{G}$.

1.20.5.2.2 Producers and scroungers

While animals often benefit from foraging in groups, this does not preclude conflict between group members. The phenomenon of scrounging or kleptoparasitism provides a simple and important example. When one member of a group discovers food, others often parasitize this discovery. One can easily observe this among pigeons feeding in a city park. The mad rush of pigeons toward a successful forager often seems quite comical. From the food discoverer's (or producer's) point of view, scroungers represent a cost of group foraging, but from the scroungers' perspective this 'information sharing' is a distinct advantage. Behavioral ecologists, led primarily by Giraldeau and his colleagues (see Giraldeau and Livoreil, 1998, for review), have investigated this phenomenon in some detail both theoretically and experimentally. To begin, we imagine that group-feeding animals can choose between the producer and scrounger strategies. A producer always finds its own food, and a scrounger feeds by joining producers. So while a producer forages by looking for food, we imagine that a scrounger forages by looking for successful producers. (The reader may object that it is unreasonable to think of producer and scrounger as hard and fast categories, because individuals can easily switch between producer and scrounger tactics, and of course, that is correct, but it turns out that this premise still leads to a useful and empirically successful model.) Now, obviously, a group with only scroungers is a nonstarter, because no one would find anything to eat. The presence of producers makes scrounging a viable strategy, but what mix of producers and scroungers would we expect? Again, this requires a game theoretical approach. The stable mix depends, it turns out, on how much food a producer can obtain – a quantity that we typically call the producer's advantage. If a producer must share everything it finds equally with its group mates, then we predict a minimal number of producers. At the other extreme, if producers can completely monopolize their discoveries, then we expect all group members to act as producers and no scroungers.

In what is perhaps the most elegant study of this problem, Mottley and Giraldeau (2000) created an experimental situation that allowed only certain group members to act as producers and forced others to act as scroungers. By doing this, they established that the benefits derived from scrounging decreased as the number of scroungers increased. After establishing that the basic features of the model applied, they modified the situation to allow animals to switch between producer and scrounger. This study found, as predicted, a higher frequency of producers when producers got a larger share of discovered food and a lower frequency of producers when the experiment forced producers to share most of the discovered food with scroungers.

1.20.5.2.3 Social information use

Foraging animals, like human shoppers, seldom have complete information about their options. So they must often use experience to respond appropriately to changes in prey quality or the properties of a food patch. Often animals will use direct ('personal') experience with food resources to adjust their behavior, but group-foraging animals have another option. The behavior of group mates often provides information about resources. The producer-scrounger situation, we discussed above, provides a simple example; scroungers exploit information about food patches obtained by producers. There are, however, many other examples. Rats may change their foraging

preferences when they smell a new food type on a group mate's breath (Galef and Stein, 1985; Galef and Giraldeau, 2001). Honeybees indicate the distance and direction of food resources by performing their famous waggle dance (*See* Chapter 1.25; von Frisch, 1950). Honeybees may often return to the hive with only partially filled crops, and an interesting explanation for this is that bees foraging in suboptimal patches may be returning early to hive to gain information about better patches from other bees (Varjú and Núñez, 1991, 1993). In parallel with the distinction between personal and social sources of information, behavioral ecologists distinguish between private and public information. Every member of a social group can act on public information, but only the possessor of private information can act on private information, and of course, a key question for the private information possessor is whether to act in way that makes its 'knowledge' public (see, for example, Valone, 1989; Templeton and Giraldeau, 1995).

Social influences on foraging behavior range from the simple (e.g., local enhancement) to the comparatively elaborate (e.g., true imitation, see Galef, 1988). While we leave a complete review of social learning and related phenomena to others (Galef and Giraldeau, 2001) (*See* Chapter 1.16 for a discussion of social learning in primates), we note here that many examples of social learning involve foraging behavior: British titmice opening milk bottles (Sherry and Galef, 1990), quail pecking or stepping on a treadle to obtain food (Zentall, 2004). A full development of the connections between the behavioral ecology of foraging and social learning remains, in our view, an unexploited opportunity. The following paragraphs give two examples, both from work with foraging starlings, that suggest how one might combine social learning and foraging studies.

Templeton and Giraldeau (1996) studied the effects of a partner on patch exploitation. They created experimental patches with discrete holes in which they placed food. Patches varied in that some patches were completely empty (no holes had food), while in others some holes (1 in 10) contained food. This arrangement sets up a classic patch sampling problem that behavioral ecologists have often used to study the behavior of solitary foragers (e.g., Lima, 1983). In this situation the forager must somehow decide how many empty holes to tolerate before leaving to find a new patch. Templeton and Giraldeau's findings suggested that foragers used the explorations of the others to make patch departure decisions. They found that animals foraging in pairs typically probed fewer holes before leaving than animals foraging alone, and that they probed more holes when their companion probed few. Perhaps the most intriguing finding of this study, however, was that the complexity of the sampling task seems to influence the degree to which starlings use social information. Templeton and Giraldeau found that partners influenced patch sampling as described here when they presented patches that were rectangular arrays of 'food holes.' However, partners had no effect on patch departure when patches were simple linear arrays of holes. This suggests that the importance of social learning may depend on the costs and reliability of individual experience.

Krebs and Inman (1992) studied how the presence of a partner changed the basic problem of tracking a varying resource. As explained in more detail above, subjects in tracking studies must choose between two feeding sites. One site is always the same but mediocre, while the other changes from good (better than the mediocre site) to bad (worse than the mediocre site) unpredictably. Here the question is how frequently should one check (or sample) the varying resource? When two animals do this together, however, only one needs to sample. And Krebs and Inman found that, in pairs of starlings, one typically sampled and the other simply followed the behavior of the sampler. This is an extremely intriguing result in several ways. First, if only some members of a group act as 'information gatherers,' this would seem to diminish the information sharing advantage of group foraging. Second, if some individuals in a group simply play follow the leader, then we would expect that followers obtain lower quality information than leaders. Finally, one has to wonder how the asymmetry between followers and leader develops. How, for example, would a prospective follower choose between two tutors performing conflicting actions?

1.20.5.2.4 Intelligence, foraging, and sociality

We end our discussion of 'emerging topics' with a brief mention of possible connections between foraging and general cognitive abilities (i.e., intelligence). We use the word 'intelligence' here fairly uncritically, acknowledging that a rigorous definition eludes us but that most readers will understand our general meaning. Many students of comparative cognition ascribe to the social intelligence hypothesis, which holds that interactions within social groups have favored increased intelligence. The focus of this hypothesis has typically been on the role of

intelligence in disputes about rank and the formation of alliances (Harcourt, 1988; Dunbar, 1998; Reader and Laland, 2002). An alternative hypothesis focuses on the role of foraging and resource extraction (King, 1986). The growing sophistication of social foraging theory suggests that these may be false alternatives. Social behavior does not exist in a vacuum; social interactions are typically about resources. It does not seem far-fetched to speculate that interactions among social foragers have played a role in the evolution of advanced cognitive abilities.

1.20.6 Summary and Conclusions

Behavioral ecologists have developed a large family of foraging models that focus on costs and benefits. These models consider diet selection, patch exploitation, and habitat use. There are many important connections between foraging and learning. Experimental studies of foraging decisions typically depend on animal learning abilities, and many studies of learning focus on situations in which subjects learn about food resources. Students of foraging behavior have considered the adaptive value of learning and memory by constructing models in which hypothetical animals can use or ignore their experience. A surprising result is that it often pays to act without experience. For example, if the environment changes frequently a fixed choice strategy will often serve a forager best. Animals, of course, face many problems other than foraging. They must, for example, avoid being eaten. Unfortunately, the best places to feed are frequently also the most dangerous, so foragers must somehow balance the need to obtain food with the risk of predation. Learning clearly plays a role in predator avoidance. While behavioral ecologists have studied the trade-off between foraging and predator avoidance extensively, we know relatively little about possible interactions between avoidance learning and learning about food. Many animals forage in groups, and this introduces several complications. Group foragers can use the behavior of their group mates to find food or avoid predators, but the availability of the public information can create conflicts of interest within the group.

A complete understanding of learning and memory needs to go beyond an understanding of mechanism and develop an understanding of how these mechanisms serve animals in nature. Foraging is a defining property of animals, and as such it represents an important natural context for learning and memory. At the same time, students of foraging can benefit from an understanding of the basic principles of learning and how these apply in natural situations.

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1.21 Navigation and Episodic-Like Memory in Mammals

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1.21.1 Navigation

The word navigation is derived from the Latin 'navis' (ship) and 'agere' (to drive), and in the classic sense, refers to the science of directing a craft by determining its position, course, and distance traveled (*Encyclopedia Britannica*). In ethological terms, navigation refers to self-controlled movement in space toward an unseen goal (Alyan and Jander, 1994; Alyan and McNaughton, 1999). Although habitats vary substantially between species, the navigational demands are fundamentally similar across mammals (Mackintosh, 2002). All mammals must learn and remember locations of importance and compute trajectories that minimize risk given their size, mode of transportation, the characteristics of their environment, and their specific needs. Although such navigational behavior unfolds seamlessly, it reflects the use of multiple strategies based on different types of representation of the environment (O'Keefe and Nadel, 1978; Mackintosh, 2002; *See* Chapters 1.12, 1.20, 1.22, 1.23, 1.25, 1.26). Under some circumstances, a navigational strategy based on a detailed and flexible representation may be ideal (e.g., to find a different way to get to work when one's habitual route is blocked), whereas simpler strategies and representations may be preferable in others (e.g., to tell a tourist how to get to the zoo).

1.21.1.1 Types of Mental Representations That Can Support Navigation

1.21.1.1.1 Egocentric versus allocentric

Egocentric representations encode relations between landmarks in the environment and the organism (e.g., shelter is 30 m to my left, food source is 40 m straight ahead). In contrast, allocentric representations depict exclusively the relations among landmarks in the environment, without consideration of their relation to the organism (e.g., food source is 50 m northeast of shelter). Allocentric representations are more flexible, since a single representation can be used to calculate trajectories between any landmarks, regardless of changes in the position or orientation of the animal.

1.21.1.1.2 Route versus map

A typical example of a route representation is a set of verbal turn-by-turn directions. More formally, according to O'Keefe and Nadel (1978), a route representation consists of information for a trajectory between a start and goal location that is based on the identification of specific landmarks in the environment (e.g., a particular store on one corner) and the performance of the appropriate behavioral response in relation to each landmark (e.g., turn right; O'Keefe and Nadel, 1978). Because routes simply direct attention to particular objects and specify turns within egocentric space, they can be learned rapidly and require very little skill. However, the information provided in a route representation is very inflexible; the information must be used in the proper sequence and allows no freedom of choice. Their accuracy also depends on the stability of the landmarks (e.g., particular store not going out of business) and on the ability to adequately perceive the landmarks (e.g., darkness can complicate their use). Finally, calculating a return trajectory using a route representation is often challenging and prone to errors, an all-too-familiar experience for some.

In contrast, a mental map has the same properties as a real-world road map; namely, it is an allocentric representation of places in terms of distances and directions among items in the environment (O'Keefe and Nadel, 1978). Mental maps are built out of the integration of multiple trajectories within a specific environment, and consequently, require considerable experience with the environment in order to form. Nonetheless, their flexibility is a distinctive advantage, in that they support the calculation of detours, shortcuts, and novel trajectories. Finally, contrary to routes, maps are not disrupted by alteration or disappearance of individual cues, and provide unambiguous directions on the return trajectory (O'Keefe and Nadel, 1978).

1.21.1.2 Strategies for Navigation

Perhaps the most impressive spatial behavior is the seasonal long-distance migration observed in a number of species of birds (e.g., Berthold, 1996; *See* Chapter 1.22) and some mammals (e.g., Berger, 2004). Migrating animals use cues, such as the position of the sun and stars at specific times of the day or gradients in the Earth's magnetic field, to orient themselves and maintain that orientation over hundreds or thousands of kilometers to their destination (Sherry, 1998). Although many nonmigrating mammals, including rodents and primates, have been shown to be sensitive to those same celestial and geomagnetic cues (Baker, 1980; Mather and Baker, 1980; Levine and Bluni, 1994), mammals are not believed to rely on those strategies for their usual navigational needs, and the use of such global-reference cues will not be further discussed here (for a review see Sherry, 1998). Instead, mammals have been shown to rely on three main strategies for navigation: piloting, path integration, and the use of guidances and orientations.

1.21.1.2.1 Piloting

Piloting refers to the use of allothetic (external or distal) cues for navigation (Gallistel, 1990; Whishaw and Wallace, 2003; *See* Chapter 1.12). More specifically, it involves computing relations among cues, usually visual landmarks, to determine one's current position in the environment and plan a trajectory. Although under some circumstances mammals could use a single landmark to calculate their position, experimental manipulations have shown that they instinctively use all available landmarks as a unit (O'Keefe and Nadel, 1978; Suzuki et al., 1980). For instance, spatial performance is not impaired by removing a few of all the available distal landmarks, but it is compromised when one interferes with the relations among landmarks by randomizing their arrangement (Suzuki et al., 1980). Piloting is generally associated with, but is not limited to, navigation based on a mental map of the environment.

1.21.1.2.2 Path integration

Path integration, also known as 'dead reckoning' (from deduced reckoning, or reasoning), is a navigation strategy used by sailors hundreds of years ago to

estimate their ship's position using information about speed of movement, travel time, and directional change whenever visible landmarks were unavailable (Whishaw and Wallace, 2003). From an ethological perspective, Darwin (1873) was the first to propose such a mechanism as a navigational strategy in animals. Path integration is currently defined as the capacity to use idiothetic cues, or cues generated by the animal's movements, to calculate the updated position of the animal by monitoring its trajectory in relation to a start location (Gallistel, 1990; Whishaw and Wallace, 2003). Idiothetic cues include information about self-movement from proprioceptive and vestibular systems (Wallace et al., 2002), sensory flow (e.g., optic flow, odor, or sound gradients; Wylie et al., 1999), and perhaps efferent copies of movement commands (Whishaw and Wallace, 2003). Importantly, the use of such egocentric cues does not imply a lack of flexibility in navigation, as animals using path integration can adjust their trajectory according to unexpected obstacles or changed task demands (Whishaw and Wallace, 2003; McNaughton et al., 2006). Finally, path integration is both automatic and constant, and since it does not require the use of visible landmarks, it can support navigation in case piloting fails due to the unavailability of distal cues (Etienne and Jeffery, 2004).

1.21.1.2.3 Guidances and orientations

As first described by O'Keefe and Nadel (1978), guidances refer to landmarks to be approached or followed, while orientations consist of particular movements in egocentric space to be made in the presence of particular guidances. The defining characteristic of this strategy is that, even though it can be used to solve spatial problems, it is not a spatial strategy per se (O'Keefe and Nadel, 1978; Mackintosh, 2002). A specific example of this strategy would be navigation based on approach or avoidance of specific visible landmarks or other cues such as an odor gradient (e.g., a predator following the odor trace of a prey), or navigation based on route representations consisting of a list of stimulus–response (or guidance-orientation) associations inflexibly leading from one point to another.

It is important to note that these strategies are not necessarily used in a mutually exclusive manner; on the contrary, mammals presumably use all of them simultaneously (Etienne et al., 1998; Eichenbaum and Cohen, 2001). For instance, mammals have been shown to predominantly rely on piloting to distal cues to navigate, but if such cues unexpectedly become unreliable or unavailable, animals will readily switch to path integration and demonstrate precise navigation despite the impoverished stimulus conditions (Whishaw and Wallace, 2003). Furthermore, there are distinct advantages to the concurrent use of multiple strategies. The main limitation of piloting is that distal cues are not always available, whereas path integration has been shown to be progressively degraded by the accumulation of error (Etienne and Jeffery, 2004); optimal navigation may therefore be achieved by relying on 'episodic fixes' (Gallistel, 1990), by which the position calculated via path integration is periodically updated by the position calculated from the distal landmarks through piloting.

1.21.1.3 Neural Basis of Navigation

In recent years, navigation research has focused primarily on elucidating its neural substrate, such that there are now few purely behavioral studies. Consequently, further development in our understanding of the navigational capabilities of mammals is intimately associated with the progress of our knowledge of the contribution of different neural systems. Although crucial to all mammalian species, navigational capacities have been most extensively investigated in rodents, presumably because their small size facilitates the manipulation of their spatial environment in a laboratory setting. Naturally, this focus on rodents raises the concern that our extensive understanding of navigational behavior may not extend to other mammals. However, comparative studies have shown that, despite significant differences in niche or specific adaptations, different mammalian species are remarkably similar in terms of neuroanatomy (Sherry and Healy, 1998) and in the way they solve spatial problems (Save et al., 1998), thereby lending support to the validity of cross-species comparisons (*See* Chapters 1.22, 1.25, 1.26).

After millions of years of evolution, it seems unlikely that the brain regions responsible for navigation are a 'tabula rasa'; instead, spatial behaviors and neural systems themselves are more likely to have been preorganized by evolutionary history (Whishaw and Wallace, 2003). The result of such evolution is a set of distinct navigational strategies, which, because each entails unique types of computation, requires the use of distinct neural systems (Sherry and Schacter, 1987). This section will discuss the role of a number of neural systems in supporting navigational behavior.

1.21.1.3.1 The hippocampus as a cognitive map

The hippocampus of food-storing birds and mammals has been shown to be up to twice as large as the hippocampus of species that do not rely on retrieving food they previously cached (Krebs et al., 1989; Sherry et al., 1989; *See* Chapters 1.22, 1.23). Similarly, anatomical scans have shown that humans with advanced navigational capabilities, such as London taxi drivers, may have bigger hippocampi as well (Maguire et al., 2000). Given the biological costs associated with maintaining a larger hippocampus, these findings indicate that this neural structure must play crucial role in navigation ability.

Elucidating the specific role of the hippocampus in spatial memory has been the main focus of navigation research for almost 30 years. The main inspiration for this line of research is a highly influential book by O'Keefe and Nadel (1978) entitled *The Hippocampus as a Cognitive Map*. In this book, O'Keefe and Nadel provided an extensive review of the anatomical, electrophysiological, and neuropsychological literature pertaining to the hippocampus. Based on the evidence they reviewed, they proposed the theory that the hippocampus specifically mediates the construction and use of a cognitive map, an allocentric topographical map of the physical environment that animals use to navigate among salient locations and other important cues in a flexible manner. In the framework of the present chapter, the cognitive map of the hippocampus is best characterized as supporting the navigational strategy of piloting to landmarks (*See* Chapter 1.33).

1.21.1.3.1.(i) Anatomy of the hippocampal memory system The hippocampus is a structure located deep in the brain, with its name derived from its curved shape in a coronal section, which resembles a seahorse. It is generally defined as consisting of the CA fields (cornu ammonis; CA1-CA3) and the dentate gyrus. The hippocampus receives highly processed sensory information, and as such should be described in the context of a larger system of cortical areas (Amaral and Witter, 1989; **Figure 1(a)**). This connectivity pattern between the hippocampus and cortical areas (**Figure 1(a)**), as well as the local hippocampal architecture circuitry (**Figure 1(b)**), has been shown to be remarkably conserved across mammalian species (Manns and Eichenbaum, 2006).

Individual perceptual and mnemonic representations from virtually all higher-order cortical processing areas are funneled into the parahippocampal region, which consists of the entorhinal, perirhinal, and parahippocampal (postrhinal in rodents) cortices, and in turn, information is then funneled into the hippocampus. This strong convergence of diverse inputs to the hippocampus, combined with the rapid and stable synaptic plasticity characterizing hippocampal circuits (e.g., Bliss and Collingridge, 1993), allows the hippocampus to rapidly form stable associations among previously unrelated elements from disparate cortical areas. A critical feature of the system is that the connections are reciprocated in a topographic manner, such that a representation at every level could reactivate elements of the higher level representation that originally activated it (Amaral and Witter, 1989;

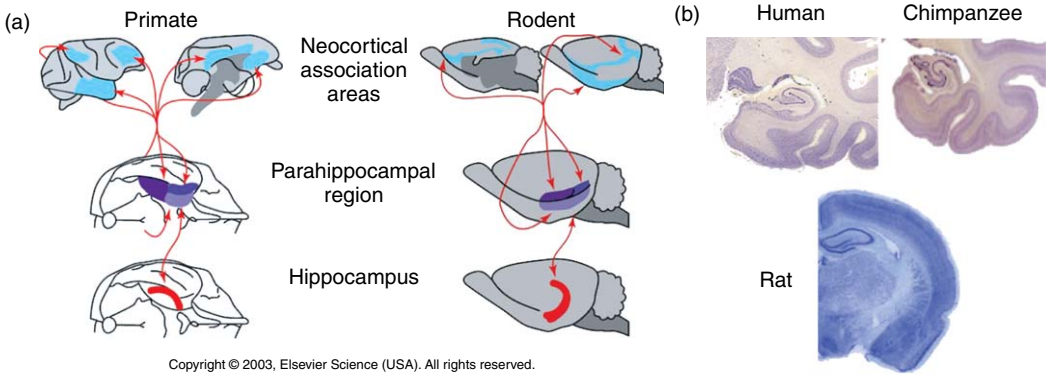

Figure 1 Similarity in the anatomy of the hippocampal system in primates and rodents. (a) Reciprocal connections between the neocortex, the parahippocampal region, and the hippocampus. From Squire LR, Bloom FE, McConnell SK, Roberts JL, Spitzer NC, and Zigmond MJ (2003) *Fundamental Neuroscience,* 2nd edn., p. 1305. New York: Academic Press, copyright Elsevier (2003). (b) Coronal sections of the hippocampus revealing the similarities in the structure of the hippocampus itself, such as the two interlocking c-shaped sectors consisting of the dense cell layers of the CA fields and dentate gyrus. The chimpanzee and human brain slices were cropped to focus on the region of interest; used with permission from www.brainmuseum.org, supported by the National Science Foundation.

Lavenex and Amaral, 2000). This organization may be important for consolidating associations formed in the hippocampus back into cortical areas (Buzsaki, 1996). These distinctive features of the anatomy of the hippocampal system suggest that it would be well suited to act as a cognitive map (O'Keefe and Nadel, 1978).

1.21.1.3.1.(ii) Experimental analysis of navigation A number of field studies have shown natural behavior indicative of navigation based on a cognitive map (see O'Keefe and Nadel, 1978). For instance, analysis of the spatial behavior of wolf packs in the wild indicates a flexible maplike representation of the environment. Wolves have been reported to return to their pups from any direction, to take necessary shortcuts or detours, and to be able to divide and regroup as a pack over large distances (presumably beyond the range of howling; Peters, 1973). Menzel (1973) has reported similar abilities in chimpanzees. When chimps were carried around an environment and allowed to observe an experimenter hiding food rewards, they subsequently ran directly between food locations and rarely returned to locations where food had already been obtained. Such efficient navigation, not shown in chimps that did not observe the experimenter hiding the food, demonstrates a clear mental representation of the food locations.

Though valuable insight has been obtained from field studies, the vast majority of studies characterizing the navigational capacities of mammals were performed in controlled experimental settings. The laboratory setting confers an advantage because it allows more control over the cues animals may use to navigate and facilitates measurements of navigation capacity. Importantly, the artificial nature of the laboratory experiment does not seem to be a significant concern, as evidenced by studies showing that spatial behavior does not differ between field and laboratory contexts (e.g., Jacobs and Shiflett, 1999). This section will review the behavioral paradigms that provided evidence suggesting that animals use a cognitive map for navigation. Consideration of all paradigms is beyond the scope of this chapter; consequently, only the most widely used will be discussed. Readers are referred to O'Keefe and Nadel (1978) and Eichenbaum and Cohen (2001) for extensive reviews.

1.21.1.3.1.(ii).(a) Evidence from rodent studies

- *Complex maze learning.* Inspired by the famous maze at Hampton Court Palace in England, Small (1901) introduced the use of complex mazes to study animal intelligence (see **Figure 2**). The method was quite simple. After being placed at the start of the maze, the rat had to learn through trial and error the trajectory that led to the goal, learning being indicated by a gradual decrease in the number of entries in blind (incorrect) alleys across trials.

  Though extensively used to study animal learning, the nature of the task emphasized the learning of series of egocentric left and right turns and as such is not optimally designed to measure navigational capacities. However, careful analysis of the behavior of animals suggested that rats 'navigated' through the maze by relying to some extent on visual landmarks in the room to locate the general direction of the reward. In fact, manipulations such as rotating the maze seemed to disrupt performance (Watson, 1907), and rats were more likely to enter blind alleys

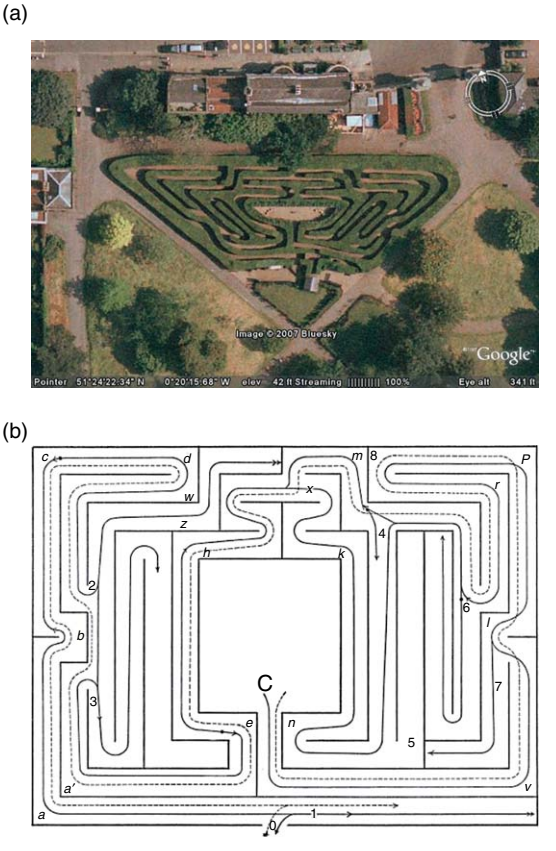

Figure 2 Complex maze learning. (a) Hampton Court Palace maze outside London, which served as inspiration; used with permission from Google Earth mapping service. (b) Diagram of one of the mazes used by Small WS (1901) Experimental study of the mental processes of the rat. II. *Am. J. Psychol.* 12: 206–239.

that pointed toward the goal than those pointing away from the goal (Tolman, 1932). Furthermore, damage to the hippocampus leads to deficits in complex maze learning in a number of studies (see table A20 in O'Keefe and Nadel, 1978), suggesting that the ability of rats to use distal cues to establish their position in relation to the goal location is an important source of information that complements the learning of left- and right-turn associations.

- *Detours and shortcuts: Cognitive maps in rats and humans.* Instead of accepting the commonly held notion from behaviorism that animal learning is based on an assortment of stimulus–response associations which are subsequently triggered by environment stimuli, Tolman demonstrated that rats can learn the spatial layout of a maze as stimulus–stimulus associations and express their knowledge in a flexible manner. In fact, Tolman and Honzik (1930) showed that rats familiar with an environment spontaneously take the shortest detour when their habitual route is blocked (**Figure 3(a)**), and subsequently, Tolman and colleagues (Tolman et al., 1946) used the 'sun-burst' maze to show that rats can select the optimal shortcut when the longer habitual route is suddenly unavailable (**Figure 3(b)**).

  Based on these findings, Tolman (1948) was the first to propose that rats (and humans) use cognitive maps of their environment to get from one place to another. The impact of Tolman's work diminished in the 1950s and 1960s but increased in the late 1970s, as his description of cognitive maps inspired the influential work of O'Keefe and Nadel (1978).
- *Radial-arm maze.* Inspired by the natural behavior of rats, Olton and colleagues (Olton and Samuelson, 1976; Olton et al., 1977) designed the radial-arm maze, a formal testing paradigm to study the ability of rats to collect food efficiently from several locations. The maze is composed of a central platform, out of which runway arms extend radially (typically eight arms). In the original version, a food reward is initially placed at the end of each arm, and optimal foraging performance would consist of running down the end of each arm only once. Since arms are not rebaited, revisiting an arm is considered an error (**Figure 4(a)**).

  Experimental manipulations have shown that distal (extra-maze) cues control behavior (Olton

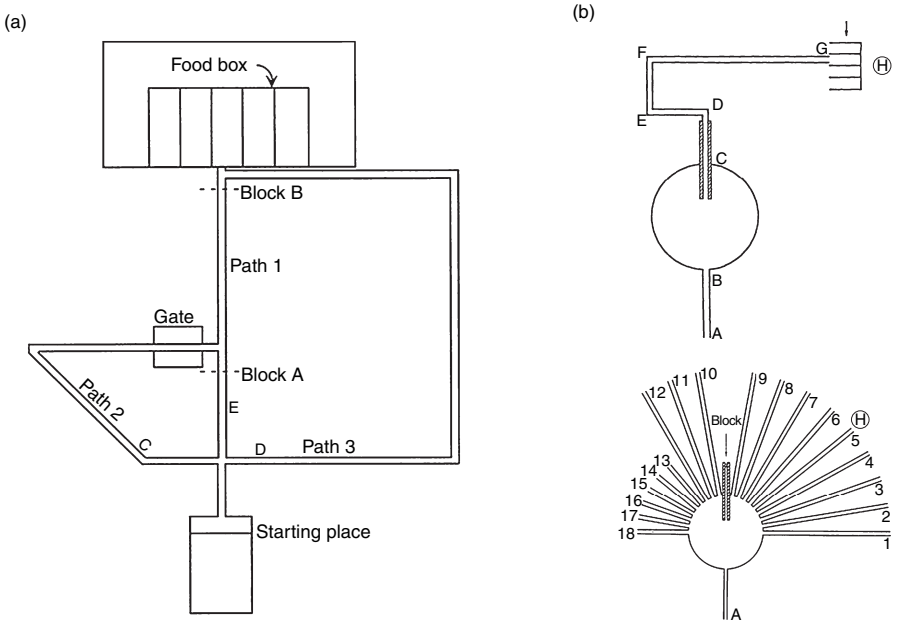

Figure 3 Detours and shortcuts as evidence for a cognitive map. (a) Diagram of the maze used to test the ability of rats to infer a required detour. From Tolman EC (1951/1966) *Collected Papers in Psychology*, p. 74. Berkeley, CA: University of California Press. (b) Diagrams of the maze used to test the ability of rats to infer a shortcut. Top: animals learned this particular trajectory to the goal (H). Bottom: The learned trajectory was blocked and rats could choose from arms 1 to 18. The largest number of rats chose arm 6, which led most directly to the trained goal site. Adapted from Tolman EC, Ritchie BF, and Kalish D (1946) Studies in spatial learning. I. Orientation and the short-cut. *J. Exp. Psychol.* 36: 13–24, with permission from the American Psychological Association. The use of APA information does not imply endorsement by the APA.

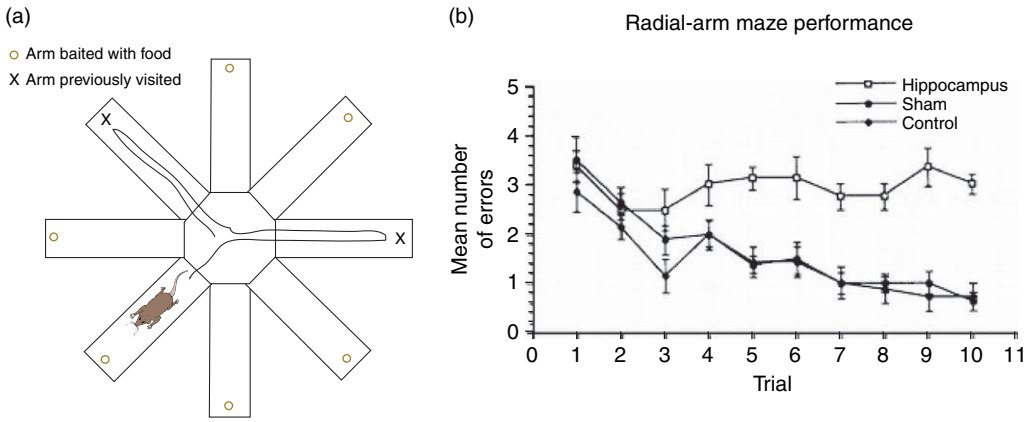

Figure 4 Radial-arm maze task. (a) The maze consists of eight arms radially extending from a central platform. Before each session, all arms were baited with a food reward, and optimal foraging performance would consist of running down the end of each arm only once. (Olton and Samuelson, 1976). (b) Animals with hippocampal damage were severely impaired in learning the task compared to control groups. Adapted from McDonald RJ and White NM (1993) A triple dissociation of memory systems: Hippocampus, amygdala, and dorsal striatum. *Behav. Neurosci.* 107(1): 3–22, with permission from the American Psychological Association. The use of APA information does not imply endorsement by the APA.

and Samuelson, 1976; Suzuki et al., 1980). Indeed, eliminating local odor cues by washing the maze does not affect performance, and animals are not affected by rotations of the maze on its central axis (i.e., they continue to use the distal cues in the room to orient themselves). Disruptions of the hippocampus severely impair performance on this task, suggesting that the hippocampus plays an important role in the processing of information about spatial location supporting navigation (Olton et al., 1978; Olton and Werz, 1978; **Figure 4(b)**). Although it was shown that the deficits of hippocampal animals in the task may not be indicative of a spatial memory deficit per se (Olton and Feustle, 1981, discussed later), this paradigm has been used extensively and represents an important step in our understanding of the neural basis of navigation.

- *Morris water maze.* The water maze task, developed by Morris (1981), is the quintessential navigational paradigm in behavioral research. It measures the ability of rats to remember the location of an escape platform in a large swimming pool (usually >2 m in diameter). Rats are capable swimmers but, as one would expect, prefer not to swim and instead search for solid ground. To ensure that animals rely on extra-maze cues to find the platform location, the water is made opaque by adding powdered milk, and the escape platform is placed arbitrarily in the pool just below the surface. Unlike spatial paradigms that rely on a limited number of choices (left or right turns, or arms in the radial-arm maze), the water maze tests spatial acuity and requires constant monitoring on the part of the animal (Morris, 1981). Furthermore, rats must use a flexible spatial representation to solve the task, since they are placed in different starting locations at the periphery of the pool on each trial, to avoid the establishment of a rigid swim route, and are also very efficient from novel starting points even if they were trained from a consistent starting point (Morris, 1981; **Figure 5(a)**).

Morris and colleagues (1982) have shown that damage to the hippocampus results in severe impairments in the water maze task (**Figure 5(b)**), providing strong support for the theory that the hippocampus implements a cognitive map of the environment. On the initial trial, control animals could take up to 1 or 2 min to find the platform, but on subsequent trials this latency was reduced considerably to approximately 10–15 s from all starting positions (**Figure 5(c)**). Rats with hippocampal damage also showed some improvement in finding the platform, but their performance reached an asymptote (~35 s), and unlike control animals, they never swam directly to the platform. The trajectories of the rats were also monitored during 'transfer tests,' during which the escape platform was removed (**Figure 5(d)**). Whereas normal rats swam around where the platform should have been (indicating strong memory), hippocampal rats showed no preference for the quadrant where the platform should have been located. Finally, in a control condition in

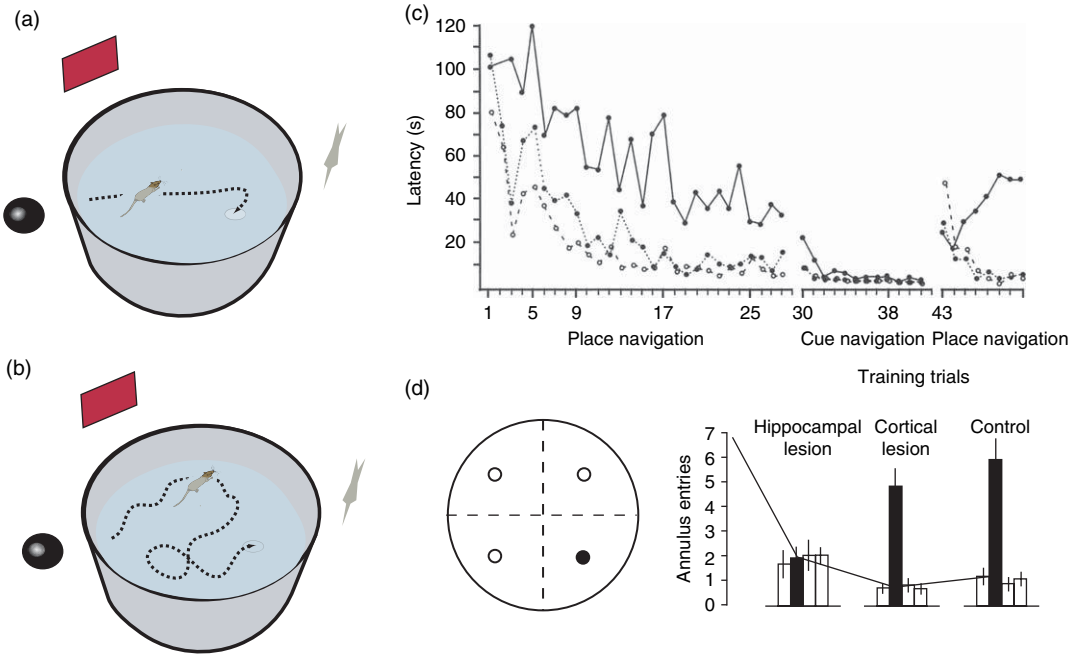

Figure 5 Morris water maze task (Morris, 1981). (a) Example swim path of a control rat. (b) Example swim path of a rat with damage to the hippocampus. (c) Performance of rats with hippocampal lesions (filled circles and lines), cortical lesions (filled circle and dashed lines), and normal controls (open circle) in acquiring the water maze task. Place navigation refers to the condition in which the platform is hidden under the surface; cue navigation refers to a control condition in which the platform is visible to the animals. (d) Performance on transfer test in which the platform was removed. Hippocampal animals were no more likely to pass over the trained location of the platform (filled circle and bar) than the corresponding location in other quadrants (open circles and bars) (c, d) Adapted from Morris RG, Garrud P, Rawlins JN, and O'Keefe J (1982) Place navigation impaired in rats with hippocampal lesions. *Nature* 297(5868): 681–683, with permission from Macmillan Publishers Ltd.

which the escape platform is visible to the rats (cue navigation; **Figure 5(c)**), both groups rapidly learned to swim directly to it, suggesting that hippocampal rats do not suffer from visual or motivational deficits.

The main assets of the water maze task are the speed of training and the robustness of the impairments shown in animals with damage to the hippocampus. For these reasons, the water maze task has become a benchmark test of hippocampal function in rodents (Eichenbaum and Cohen, 2001).

1.21.1.3.1.(ii).(b) Evidence from primate studies

A number of experimental studies in humans and monkeys have also implicated the hippocampus in learning spatial information essential for navigation. Neuropsychological studies have shown that the integrity of the hippocampus is essential for spatial memory, as demonstrated by the deficits of amnesic patients (Smith and Milner, 1981, 1989; Maguire et al., 1996) or monkeys with dysfunctional hippocampi (Murray et al., 1989; Lavenex et al., 2006) in different spatial tests. Functional neuroimaging studies have also identified the hippocampus as part of network of structures important for navigation in large-scale environments. For instance, Maguire and colleagues (1997) scanned London taxi drivers with years of experience while they recalled complex routes around the city. Compared with baseline and other nontopographical memory tasks, such route recall resulted in activation of a network of brain regions, including the right hippocampus. Furthermore, in a different study, Maguire and colleagues (1998) developed a test in which normal human subjects explored an imaginary town using virtual reality technology, and reported that activation of the right hippocampus was strongly associated with remembering where specific places were located and navigating accurately between them.

1.21.1.3.1.(iii) Electrophysiological evidence supporting the cognitive map theory

The concept of a cognitive map has been refined over the years (McNaughton et al., 1996; Muller et al., 1996; O'Keefe and Burgess, 1996) but remains fundamentally similar

to O'Keefe and Nadel's (1978) original description. Conceptually, the cognitive map is a two-dimensional Cartesian reconstruction of the environment, in that it provides metric representations of distances and angles between the relevant stimuli (*See* Chapters 1.33, 1.35).

The discovery of place cells in the hippocampus was a crucial piece of evidence supporting the cognitive map theory of the hippocampus. *Place cells* are hippocampal neurons, typically from regions CA1 and CA3, that fire at a high rate whenever the animal is in a specific location in the environment, called the place field (**Figure 6(a)**). Their existence was first described by O'Keefe and Dostrovsky (1971) in rats, but was later confirmed in numerous more systematic studies (e.g., O'Keefe, 1976; Olton et al., 1978; O'Keefe and Conway, 1980; Hill and Best, 1981; Best and Ranck, 1982) and in other mammalian species (humans: Ekstrom et al., 2003; monkeys: Ludvig et al., 2004; mice: Rotenberg et al., 1996). A number of properties of place cells are suggestive of a cognitive map representation of a specific environment. First, their firing pattern is determined by the global spatial relations among landmarks, not simply associated with a particular cue in the environment. For instance, place cell firing is maintained even if individual distal cues are removed, or if all distal cues are rotated as a unit (the place field rotates with the cues; O'Keefe and Conway, 1978; Miller and Best, 1980; Hill and Best, 1981). Second, many place cells reflect the overall topography of the environment, as they were shown to scale their size to reflect changes in the size of the environment (Muller and Kubie, 1987; O'Keefe and Burgess, 1996). Third, once established, the spatial representation of a specific environment coded by place cell is stable over long periods of time (at least 5 months; Thompson and Best, 1990).

At the conceptual level, a place cell is thought to construct the notion of a place in the environment by encoding the multisensory input pattern that can be perceived when the animal is in a specific part of the environment (O'Keefe, 1979). Each place cell is hypothesized to represent the position of the rat at a particular coordinate position in the map of the environment, and as a population, place cells could underlie a mechanism by which information about the spatial layout of the environment could be used to compute the flexible trajectories required by navigation.

1.21.1.3.2 Processing of spatial information in other brain regions

Despite an intense focus on the role of the hippocampus, it is clear that navigation is a capacity that requires other brain systems as well. In addition to the role of sensory systems to process and represent environmental stimuli, and the involvement of the prefrontal cortex in providing executive control of response selection and planning through interactions with cortico-striatal loops (e.g., De Bruin et al., 1994; Alexander et al., 1986; Dunnett et al., 2005), a number

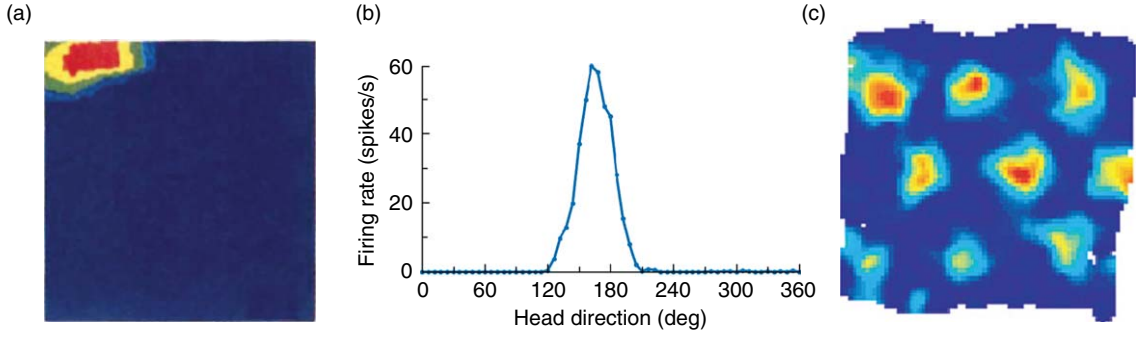

Figure 6 Neurons with spatial firing properties. (a) Place cell: Hippocampal neuron showing an increase in firing rate whenever the animal enters the North-West corner of an open-field environment. Adapted from O'Keefe J and Burgess N (1996) Geometric determinants of the place fields of hippocampal neurons. *Nature* 381(6581): 425–428, with permission from Macmillan Publishers Ltd. (b) Head-direction cell: Postsubicular neuron increasing its firing rate whenever the animal's head is facing a particular direction (60 degrees). Taken from Taube JS (2007) The head direction signal: Origins and sensory-motor integration. *Annu. Rev. Neurosci*. 30: 181–207, with permission from the Annual Review of Neuroscience. (c) Grid cell: Dorsocaudal medial entorhinal cortex neuron exhibiting multiple spatial firing fields arranged in a hexagonal grid in an open field. Taken from Hafting T, Fyhn M, Molden S, Moser MB, and Moser EI (2005) Microstructure of a spatial map in the entorhinal cortex. *Nature* 436(7052): 801–806, with permission from Macmillan Publishers Ltd.

of structures are thought to be critical for navigation, either by having a role associated with that of the hippocampus or by supporting navigational strategies independent of the hippocampus.

1.21.1.3.2.(i) Brain systems associated with the role of the hippocampus

1.21.1.3.2.(i).(a) Parahippocampal region

Although the perirhinal, parahippocampal, and entorhinal regions make significant contributions on their own to information processing (Suzuki et al., 1993; Brown and Aggleton, 2001; Burwell et al., 2004), a major role of these regions is to act as a necessary intermediary link between the complex representations in cortical association areas and the hippocampus itself. According to McClelland and Goddard (1996), the parahippocampal region is important for 'compressing' representations of the complex information contained in numerous cortical associational areas, which allows the hippocampus to access and create associations between those complex representations. This network organization would provide a mechanism by which complex visuospatial representations of the environment in the parietal and retrosplenial cortices shown to be important for spatial information processing (Kolb et al., 1994; Ennaceur et al., 1997; Maguire, 2001; Mesulam et al., 2001; Whishaw et al., 2001; Parron and Save, 2004; Vann and Aggleton, 2004; Goodrich-Hunsaker et al., 2005) could reach the hippocampus. This proposal is supported by evidence demonstrating that the entorhinal (Parron et al., 2004; Parron and Save, 2004) and postrhinal/parahippocampal regions (Maguire et al., 1997; Liu and Bilkey, 2002) are important for performance of spatial tasks.

1.21.1.3.2.(i).(b) Areas with spatial coding complementing that of the hippocampus

Though the representations are typically not as precise and sparse as those of the hippocampus, consistent place-related firing has been observed in the lateral septum (Zhou et al., 1999; Leutgeb and Mizumori, 2002), ventromedial entorhinal cortex (Mizumori et al., 1992; Quirk et al., 1992), subiculum (Sharp and Green, 1994; Martin and Ono, 2000), postsubiculum (Sharp, 1996), and parasubiculum (Taube, 1995a; Hargreaves et al., 2005). Such spatial representations in many distinct regions associated with the hippocampus may imply some degree of redundancy in the nervous system, but more likely it reflects the complementary contribution of each of those areas in representing or operating on aspects of the organism's representation of space.

Directional heading is another type of spatial coding important for navigation. It is coded by *head-direction cells,* which are neurons that discharge when the animal points its head in a particular direction and, similar to place cells, are responsive to allothetic and idiothetic cues (**Figure 6(b)**). Head direction cells were initially discovered in the postsubiculum of rats (Taube et al., 1990), but were since then reported in the parasubiculum (Taube, 1995a), anterior thalamus (Taube, 1995b), laterodorsal thalamus (Zugaro et al., 2004), retrosplenial cortex (Chen et al., 1994), and medial entorhinal cortex (Sargolini et al., 2006), as well as in the primate presubiculum (Robertson et al., 1999; see Knierim, 2006, for a review). Information about directional heading is thought to provide an 'internal compass' or an orientation framework for our representation of space, and thus to be important for navigation through piloting (Goodridge et al., 1998) and path integration (Golob and Taube, 1999; Sargolini et al., 2006).

Finally, the most recently discovered type of spatial coding is an environment-independent coordinate system. The key units of this system are *grid cells,* cells located in the dorsocaudal medial entorhinal cortex that exhibit multiple spatial firing fields arranged in a hexagonal grid (Fyhn et al., 2004; Hafting et al., 2005; **Figure 6(c)**). The grid cell representation may offer a robust metric for calculating position, and since the dorsocaudal medial entorhinal cortex projects to the hippocampus, this information must be important for the development of sparse representations of spatial information in the hippocampus.

1.21.1.3.2.(ii) Brain systems implementing alternative navigational strategies

Although navigation research has predominantly focused on the hippocampus, there is considerable evidence that efficient navigational behavior can be supported by strategies other than piloting or the use of a cognitive map.

1.21.1.3.2.(ii).(a) Guidances and orientations

Consider the example of a spatial discrimination task in which an animal is required to learn to turn left on a T-maze in order to obtain a reward. One obvious strategy would be for the animal to use cues surrounding the maze to orient himself and learn that he must choose the arm on the left side of the room in

order to be reinforced, that is, to use a piloting strategy thought to be dependent on the hippocampus. However, the animal could also use at least two other approaches to solve the task, which would fall under the guidances and orientations strategy. First, the animal could develop an attraction, or bias, toward the goal arm, approach it, and obtain a reward.

This navigational strategy of approaching or avoiding particular stimuli depends on the amygdala, a region known to be important for processing the emotional valence of stimuli (McDonald and White, 1993) and other aspects of emotional memory (McGaugh, 2005; Schafe et al., 2005). Alternatively, the animal could also solve the task by learning to perform an egocentric response (i.e., a left turn) at the choice point, a strategy that reflects the use of stimulus–response associations and depends on the integrity of the striatum (McDonald and White, 1993; Packard and McGaugh, 1996). The striatum is best known for its role in planning and modulation of movements, but has been shown to be important for associating specific stimuli or representations to complex behavioral responses, such as forming habits and skills (Graybiel, 1995; White, 1997). The parietal cortex has also been shown to be implicated in the processing of egocentric representations and computation of body turns necessary for navigation (Burgess et al., 1997; Maguire et al., 1998) and displays firing properties that are thought to underlie route representation (Nitz, 2006).

1.21.1.3.2.(ii).(b) Path integration The neural implementation of path integration is not yet well understood. In addition to the neural systems responsible for processing the idiothetic cues (proprioceptive, vestibular, and sensory flow information), behavioral evidence suggests that the parietal (Parron and Save, 2004), entorhinal (Parron and Save, 2004), and retrosplenial cortices (Cooper and Mizumori, 1999) are important for successful path integration (see Etienne and Jeffery, 2004).

The recent discovery of grid cells may help further our understanding of path integration by providing insight into its potential neuronal mechanisms. Grid cells are part of an environment-independent spatial coordinate system that integrates information about location, direction, distance, and speed. In addition to the grid cells previously reported in superficial layers (Fyhn et al., 2004; Hafting et al., 2005), the deep layers of the medial entorhinal cortex contains grid cells that are colocalized with head-direction cells, as well as conjunctive grid X head-direction cells, with all cell types modulated by running speed (Sargolini et al., 2006). Such colocalized representations of location, distance, speed, and direction information have been proposed to be necessary for the neural computations required by path integration (Fuhs and Touretzky, 2006; McNaughton et al., 2006).

The parallel nature of the contribution of distinct strategies to navigation has been cleverly demonstrated experimentally, by creating conditions that expose either their synergistic or their conflicting interactions (McDonald and White, 1993; Packard and McGaugh, 1996). For instance, McDonald and White (1993) designed a simple stimulus–response task in which rats were required to enter arms identified with a light cue (stimulus–response, or habit, learning). As expected, the performance of control animals incrementally improved with training, and animals with damage to the dorsal striatum were severely impaired in learning the task. Interestingly, animals with hippocampal damage outperformed even control animals, implying that the use of a 'hippocampal' strategy, such as trying to remember the location of specific arms entered, is counterproductive in this particular task. This facilitation effect reflects the conflicting contribution of different strategies to solve the same navigation problem.

These findings help clarify the need for distinct neural systems encoding distinct behavioral strategies. For instance, a piloting strategy supported in part by the hippocampus can be employed quickly, but it requires intensive use of the animal's cognitive demands. In contrast, a stimulus–response strategy develops more slowly, but because of its 'habit' nature can support navigation with little mental effort. The latter strategy is particularly advantageous on highly familiar terrain in that navigation can be accomplished on 'automatic pilot,' and can also explain the common human behavior of following a habitual route in error if our concentration wanders.

1.21.1.3.3 Shortcomings of the cognitive map theory: Evidence for an alternative framework

1.21.1.3.3.(i) Navigation by piloting to landmarks does not always require a cognitive map, and navigation reflecting the use of a cognitive map does not always require the hippocampus First, tasks requiring animals to pilot to landmarks do not necessarily require following a unified cognitive map representation of the environment, as different types of associative learning

may often support performance (Mackintosh, 2002). For instance, in the radial-arm maze task, animals could use information from (nonunified) local visual scenes specific to one or a few arms to remember the individual arms already visited on a given trial. Second, elaborate spatial processing indicative of a cognitive map representation can be accomplished in subjects with damage to the hippocampus. For instance, amnesics are impaired at learning new environments but can flexibly recall the spatial layout of a region learned prior to brain damage onset (Teng and Squire, 1999; Rosenbaum et al., 2000), and hippocampectomized animals are able to use the relations between distal landmarks to successfully navigate to an unseen food location (Alyan et al., 2000).

1.21.1.3.3.(ii) Neural representations in the hippocampus are not limited to a faithful Cartesian representation of the layout of the environment The only behavioral protocol in which the firing properties of place cells reflect an allocentric representation, independent of viewpoint and ongoing behavior ('true' place cells; O'Keefe, 1979), is when rats randomly forage for food in an open field environment (e.g., Muller and Kubie, 1987). This experimental protocol is unique in that every task component (e.g., location of reward, direction of trajectory, behavior) is randomized, and consequently most neurons show spatial specificity and no other behavioral correlate. In more elaborate behavioral paradigms, firing patterns of hippocampal neurons can be characterized as encoding task 'regularities' in a variety of paradigms (Eichenbaum et al., 1999). The activity of hippocampal neurons can reflect specific spatial regularities systematically embedded in the task protocol, such as particular speed and direction of movement or angle of turn (McNaughton et al., 1983; Wiener et al., 1989; Markus et al., 1995), but also extends to nonspatial regularities as well. Perhaps the clearest example was provided by Wood and colleagues (1999) in a study in which rats were required to perform the same recognition memory judgment (i.e., is the current odor a match/nonmatch to the previous odor?) in nine distinct locations in a rich spatial environment. They showed that some hippocampal cells fired only in a specific location, others only for one of the nine odors, others to the match/nonmatch status of the presented odor, and others to conjunctions of those task elements. These results indicate that hippocampal neurons encode a broad range of spatial and nonspatial stimuli, behavioral events, and contingencies that characterize the task at hand.

1.21.1.3.3.(iii) The hippocampus is critical for nonspatial memory Accumulating evidence confirms that the role of the hippocampus is not limited to the processing of spatial information. In fact, though the radial-arm maze was designed to take advantage of rats' spatial abilities and was initially discussed as supporting evidence for the role of the hippocampus in processing spatial information (Olton et al., 1978; Olton and Werz, 1978; see earlier section titled 'Radial-arm maze'), Olton's subsequent work suggests that the hippocampus is not crucial for spatial processing per se, but rather plays a specific role in remembering trial-unique information for both the spatial and nonspatial domains (Olton and Papas, 1979; Olton and Feustle, 1981). In addition, the hippocampus has been shown to be important for creating representations of nonspatial information that can be expressed flexibly, in that they support novel generalizations and inferences from the acquired knowledge (e.g., Bunsey and Eichenbaum, 1996; Dusek and Eichenbaum, 1997; Dusek and Eichenbaum, 1998; Alvarez et al., 2002). These studies will be further discussed in later sections.

1.21.1.4 Conclusions

Navigation is a capacity crucial to mammals' survival that depends on the use of multiple strategies and types of representation, and thus emerges from the contribution of distinct neural systems. Striking parallels in neuroanatomy, neurophysiology, and behavior indicate that these navigational principles are fundamentally the same across mammalian species.

The prominent theory of the neural basis of navigation proposed that the hippocampus represents a cognitive map of the environment (O'Keefe and Nadel, 1978). The hippocampus was later shown to have a more limited role in spatial processing than originally envisaged, and to process nonspatial information as well, suggesting that the hippocampus is important for, but not dedicated to, spatial computations (Mackintosh, 2002). Conversely, recent studies have demonstrated that the full extent of the contribution of extra-hippocampal areas in the service of navigation has been underappreciated. Although elements of the cognitive map theory were incorrect, it should be considered a significant accomplishment in

that it acted as a catalyst and generated considerable research evidence on the neural basis of navigation.

Finally, experimental research on navigation has provided a significant contribution to our understanding of general learning and memory principles in mammals. First, it demonstrated that animal learning is not limited to specific stimulus–response associations, but that animals can also internally reorganize information to form cognitive, or stimulus–stimulus, representations (Poucet, 1993). Second, it helped dissociate multiple memory systems by identifying some of their neural substrates and revealing conditions in which they may compete. Finally, because navigation tests such as the watermaze are easy to implement and quick to perform, they are currently being used extensively as a screening tool for learning and memory deficits for a variety of experimental groups (e.g., effects of drug compounds, effects of aging).

1.21.2 Episodic Memory

Unlike the study of navigation, which emphasized research in rodents, episodic memory research has its roots in the study of human cognitive psychology. Unfortunately, the concept of episodic memory is perhaps not as intuitive as the capacity for navigation. Navigation is a behavior that can be accomplished using distinct capacities or strategies, whereas episodic memory should be viewed as a specific memory capacity among many that are essential for adaptive behavior. The precise nature of episodic memory may be better understood by comparisons with other memory systems in a multiple memory system framework, which is the approach taken in this section.

1.21.2.1 Multiple Forms of Memory

1.21.2.1.1 Historical perspective

The existence of multiple forms of memory gained widespread acceptance rather recently, but some prescient thinkers proposed similar ideas a long time ago. Writings from Aristotle (circa 350 BC; translated in Aristotle, 1984), Maine de Biran (1804/1929), Gall (1835), William James (1890), and certainly many others have offered meritorious descriptions of the organization of memory (for reviews, see Schacter and Tulving, 1994; Eichenbaum and Cohen, 2001). Most of these proposals contrast forms of memory that support 'conscious' memory, with other forms which characteristically do not reach consciousness, that are more of a 'habit' nature. In a manner akin to current accounts, some even distinguished capacities similar to our current concepts of 'knowing' and 'recollecting' (Eichenbaum et al., 2005). Although these early accounts relied on introspection and observation rather than experiments, they successfully capture the essence of the idea of multiple forms of memory as it is known today. At the same time, they also offer a sense of the considerable progress made in recent years in refining these ideas, especially in terms of delineating their respective neural substrates.

1.21.2.1.2 Modern taxonomy of memory systems

In recent years, a new memory taxonomy has emerged from the experimental work of several laboratories (e.g., Cohen and Squire, 1980; Squire and Zola-Morgan, 1991; Schacter and Tulving, 1994). Despite evolving primarily from human studies, this classification has been shown to extend to other mammals as well (Eichenbaum and Cohen, 2001), and considerable research evidence indicates that these phenomenologically distinct forms of memory are supported by distinct memory systems (**Figure 7**; Squire, 2004). The two major types of memory, declarative and nondeclarative, are distinguished according to criteria such as the speed of information acquisition, the nature of their represented information, and the means of expression (Squire, 1992).

Declarative memory, also called explicit memory, refers to the memory for facts and events that can be brought into consciousness and expressed explicitly (Cohen and Squire, 1980; Cohen, 1984; Squire and Cohen, 1984; Cohen and Eichenbaum, 1993; Tulving and Markowitsch, 1998). The stored information is of propositional nature (i.e., can be 'declared'), such that one could describe it symbolically and infer relationships among memories. It is a large, complex, and highly structured system with a presumed unlimited capacity, which allows information from all modalities to be stored, often after a single exposure to an event or a fact. A critical feature of the declarative memory representation is its flexibly of access and expression, which allows memories to be retrieved from various logical associations and expressed through a variety of behaviors. Declarative memory is further divided into episodic and semantic memory (Tulving, 1983; Tulving, 1991; Squire et al., 1993), and their distinction has received considerable interest in recent years. In broad terms, episodic memory

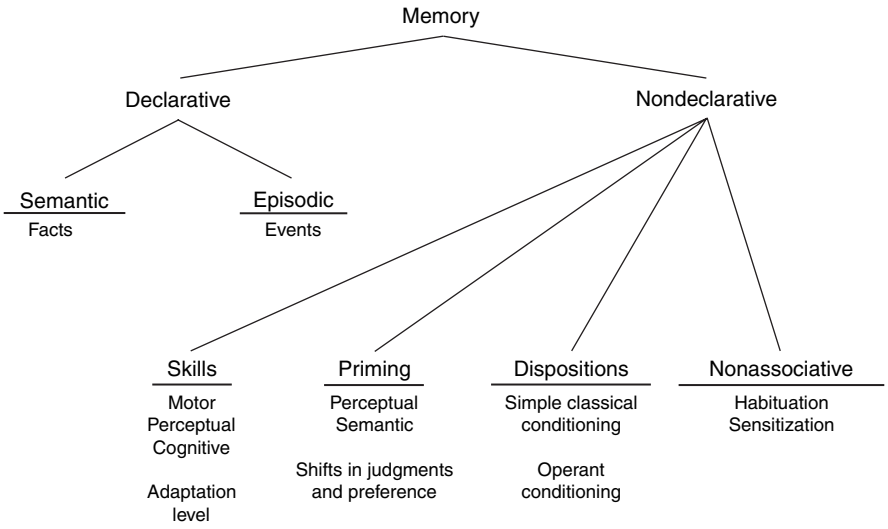

Figure 7 Taxonomy of memory systems. Adapted from Squire LR (1992) Memory and the hippocampus: A synthesis from findings with rats, monkeys, and humans. *Psychol. Rev.* 99: 195–231, with permission from the American Psychological Association. The use of APA information does not imply endorsement by the APA.

refers to the memory for personal experiences (or autobiographical memory for events), while semantic memory is the memory for facts or general knowledge about the world (Squire et al., 1993).

Nondeclarative (implicit) memory, in contrast, consists of a broad set of learning capabilities that involve acquisition of skills and preferences that can be expressed unconsciously by changes in the speed or biasing of performance; they include motor, perceptual, and cognitive skills; habituation and sensitization; sensory adaptations; and priming of perceptual and lexical stimuli (Squire, 1992). Unlike declarative memories, for which the products of retrieval can be consciously 'thought about' and may not lead to overt behavior, retrieval of nondeclarative memories must be expressed through a change in performance (Tulving and Markowitsch, 1998).

1.21.2.1.3 Episodic and semantic memory

Although these two forms of declarative memory have many characteristics in common, they are distinguishable on a number of features (Tulving, 1972, 1993a; Tulving and Markowitsch, 1998). In his earlier work, Tulving (1972) suggested that they differed in their relationship to the context in which information was acquired, in that episodic memories were intrinsically tied to the context, whereas semantic memories were essentially context-free. Recent developments in the definition of episodic memory have focused on differences in the 'experiential' nature of episodic and semantic memory as revealed by careful introspective analysis. According to Tulving and Markowitsch (1998), episodic memory involves 'remembering' specific personal past experiences, whereas semantic memory involves 'knowing' impersonal facts. More precisely, the fundamental difference between episodic and semantic memory lies in the basis of the type of awareness that accompanies recall: episodic memory requires an awareness of personal experience (autonoetic awareness), whereas semantic memory is characterized by the conscious feeling of knowing (noetic awareness; Tulving, 2002). In addition, Tulving (2002) also emphasized that episodic memory is unique in that it is the only form of memory oriented toward the past at the time of retrieval, in the sense that our 'self' must 'mentally time travel' through one's past, in order to 'reexperience' a specific event. In contrast, semantic memory, or knowing, occurs in the present, as one needs not think back to earlier experiences to confirm knowledge of a fact (e.g., knowing that Paris is the capital of France does not require memory for a specific experience).

Annual scientific conferences provide a concrete example of the distinction between the two types of declarative memory. Seeing a particular face may trigger retrieval of an episodic memory, as you think back and distinctly remember having spoken to this person in front of their research poster toward the end of the session on the first day of the meeting. In contrast, seeing another face may simply bring forth a feeling of familiarity as you know you have met this person before but cannot retrieve the

specific experience, a situation with the potential to be quite embarrassing. This ability of normal individuals to discriminate between 'remembering' and 'knowing' has been used to operationalize whether particular recalled memories originated from the episodic or semantic domain, providing evidence that the types of memory can be experimentally dissociated at the phenomenological level ('remember/know' distinction; Tulving, 1993b; Knowlton and Squire, 1995).

1.21.2.2 Role of the Hippocampus in Episodic Memory

As in the study of navigation, the main focus of episodic memory research has been to clarify its neural substrate. Therefore, progress in our understanding of the nature of episodic memory will be discussed along with developments in our knowledge of its neural implementation.

As mentioned earlier, the strong convergence of diverse inputs to the hippocampus, combined with the rapid and stable synaptic plasticity characterizing hippocampal circuits (e.g., Bliss and Collingridge, 1993), allow the hippocampus to rapidly form stable associations among previously unrelated elements from disparate cortical areas. Although such associations could be created in the service of creating a cognitive map, as discussed in the previous section, they could also support episodic memory coding by associating environmental stimuli that constitute elements of individual episodic memories.

1.21.2.2.1 Evidence from human studies

The first insight into the neural implementation of episodic memory came from case studies of neurological patients reported to have a memory deficit for daily life events (Tulving et al., 1988; Vargha-Khadem et al., 1997). In one study, patients who suffered neurological damage selective to the hippocampus demonstrated a pronounced amnesia for the episodes of everyday life, but attained relatively normal levels of speech and language competence, literacy, and factual knowledge (Vargha-Khadem et al., 1997). These findings suggested that episodic and semantic memory differ in their neural implementation, with only the episodic component being fully dependent on the hippocampus.

The evidence provided by these case studies is supported by more formal experiments. First, evidence suggests that the hippocampus is important for tests of recall, which are thought to depend exclusively on episodic memory retrieval (Mandler, 1980), but not for recognition tests, which may be solved in nonepisodic ways (for a review, see Aggleton and Shaw, 1996). Second, using the 'remember/know' method to distinguish episodic and semantic memory, Eldridge and colleagues (2000) demonstrated that the hippocampus was selectively activated when sample items were consciously recollected ('remember'), but not when they were simply recognized ('know'). Unfortunately, the use of these paradigms is unlikely to be the key to determine the role of the hippocampus in episodic memory, as similar studies have failed to show a selective role for the hippocampus in episodic processes (e.g., Squire and Zola, 1998; Manns and Squire, 1999; Manns et al., 2003). These inconsistent findings are in part due to the fact that these paradigms provide inadequate measures of episodic memory capacity. Comparisons between tests of recall and recognition are not sensitive enough to clearly distinguish the contribution of episodic and semantic processes, such that slight changes in methodological details can affect the performance of control and amnesic subjects (Squire and Zola, 1998), and although the 'remember/know' approach may capture the phenomenological distinction between episodic and semantic retrieval, its use of introspective judgments cannot be considered an infallible operational definition, as human subjects are notorious for creating false memories (see Eichenbaum and Fortin, 2003).

Recent approaches focusing on objective and measurable observations to assess episodic memory performance have shown promising results. In line with Tulving's (1972) early proposal that the context in which events are experienced is an integral part of episodic memories, functional imaging studies have shown that the hippocampus is involved in remembering the specific relationships among elements of an episodic memory (Davachi and Wagner, 2002) and the episodic context in which specific items were presented (Davachi et al., 2003). Another approach consists of using *receiver operating characteristics* (ROC) analyses to measure the relative contribution of episodic and semantic processes to recognition memory, which are based on the pattern of correct and incorrect responses across confidence levels or biases (Yonelinas, 2001, 2002; procedures described in further detail in the following). Such studies have also shown that the hippocampus is selectively important for episodic memory (Yonelinas et al., 2002; Daselaar et al., 2006).

Unfortunately, even if considerable progress were made in establishing clear experimental measures of episodic memory capacity, the exclusive use of human studies may not be sufficient to determine the precise contribution of the hippocampus to episodic and semantic processes. First, studies of neurological patients present a number of limitations. The brain damage patients suffered is rarely circumscribed to one specific area, which hinders the establishment of clear structure–function relationships. Conversely, brain damage to one specific area is rarely complete such that remaining fragments with residual function may or may not contribute to memory (Squire and Zola, 1998; Maguire et al., 2001). Second, advances in functional neuroimaging may be able to address some of those issues, but like any experimental technique, it has limitations on its own. The primary concern is that resolving the role of specific structures in different types of memory may be beyond the anatomical and temporal resolution of functional imaging techniques.

In light of these difficulties in providing unambiguous evidence from studies of amnesia or functional brain imaging in humans, it is clear that the development of an animal model of episodic memory to use in convergence with human studies is essential. As demonstrated in the navigation section earlier, animal studies directly compensate for the limitations of human studies by allowing much greater control over the anatomical specificity of the brain lesions and the amount of information learned before and after the damage. Also, the activity of individual neurons in multiple brain areas can be directly observed in animals, and the findings may reveal qualitative as well as quantitative distinctions in the nature of memory representations in these areas.

1.21.2.2.2 Evidence from nonhuman mammals

Do animals have episodic memory? One point of view, proposed by Tulving and others (Tulving, 1972, 2002; Tulving and Markowitsch, 1998), is that episodic memory is unique to humans. Tulving and Markowitsch (1998) claimed that the distinction between remembering and knowing simply does not exist in animals, and that declarative memory in animals corresponds to semantic memory in humans. The opposite position emphasizes parsimony in evolutionary design, which suggests that the antecedents of human forms of episodic memory should be found in animals (Morris, 2001; Whishaw and Wallace, 2003). Evolutionary parsimony implies that a similar algorithm is being used by the hippocampus and its related structures across mammalian species, a proposal supported by the high degree of conservation of the hippocampal system across mammalian species in terms of cell types, connectional architecture, and basic structure (Amaral and Witter, 1989; Lavenex and Amaral, 2000; Manns and Eichenbaum, 2006). Even if the information to be operated upon is different in humans because of language, consciousness, or autonoetic awareness, there are no obvious cellular, connectional, or biochemical differences that would suggest that the computations are of a different nature (Morris, 2001).

According to Tulving (2002), concepts unique to humans such as self, autonoetic awareness, subjectively sensed time, mental time travel, language, and consciousness are all critical to episodic memory. However, though it is clear that these elements of episodic memory cannot be addressed in animals, their absence does not necessarily invalidate attempts to model episodic memory in animals. For instance, language is clearly important for episodic memory in humans, but species that cannot communicate through language are not logically prevented from having private recollective experiences, as experiencing events and recalling them later is certainly independent of their overt communication to conspecifics (see Morris, 2001). In order to develop an animal model of episodic memory, we must operationally define species-independent features of episodic memory that can be tested experimentally by objective behavioral measures reflecting the subject's knowledge of its experiences. Recent theoretical work has identified a number of such features of episodic memory that can be tested in animals (Griffiths et al., 1999; Aggleton and Pierce, 2001; Morris, 2001; Roberts, 2002; Eichenbaum and Fortin, 2003; Whishaw and Wallace, 2003), and four experimental approaches testing the following features will be discussed here: (1) episodic memories are acquired in a single experience, (2) episodic memories contain information about the context in which experiences occurred, (3) episodic memories are structured sequences of events, and (4) episodic memories are characterized by a recollective process with distinct retrieval dynamics.

1.21.2.2.2.(i) Episodic memories are acquired in a single experience The first approach focuses on the fact that episodic memories are formed after a single experience (e.g., Tulving, 1972, 2002; Tulving and Markowitsch, 1998). The main experimental

paradigm used in this approach is the delayed nonmatch-to-sample task, which tests the capacity of animals to remember a single exposure to a novel stimulus (Gaffan, 1974; Mishkin and Delacour, 1975). This recognition task starts with a sample phase in which the animal is presented with a single stimulus, followed by a delay period in which the stimulus is not present, and ends with a test phase in which the animal is required to select the novel stimulus over the sample stimulus (that is, to 'nonmatch' to the sample) in order to be reinforced. A substantial literature in rats and monkeys shows that damage to the medial temporal lobe region (hippocampus and parahippocampal region), or damage selective to the parahippocampal region, produces a severe delay-dependent impairment. In fact, animals perform normally when the delay period lasts a few seconds, but their accuracy declines rapidly with longer delays (Suzuki et al., 1993; Mumby and Pinel, 1994; Zola-Morgan et al., 1994; Murray, 1996). However, selective damage to the hippocampus results in modest (Mumby et al., 1992; Zola et al., 2000; Clark et al., 2001) or no deficit on delayed performance even when the memory load is very high (Murray and Mishkin, 1998; Dudchenko et al., 2000), suggesting that parahippocampal cortical areas can support this form of simple recognition without critical hippocampal involvement.

In contrast, different results have emerged using the visual paired-comparison task, a recognition test similar in memory demands, but with slightly different behavioral procedures. In this test, animals are simply exposed to a novel visual stimulus and then, following a delay, are tested for time spent visually investigating the same stimulus versus a novel stimulus. In this test, selective damage to the hippocampus produces a severe delay-dependent impairment (Clark et al., 2000; Zola et al., 2000). These mixed findings among recognition tests in animals parallel the inconsistent results reported in recall and recognition tests in human neurological patients mentioned above.

At first, such inconsistent findings in recognition tests seemed difficult to reconcile with emerging theories of the role of the hippocampus in episodic memory. However, it has become clear that recognition paradigms can be solved by ways other than episodic memory (e.g., Griffiths et al., 1999). In fact, analogous to the 'remember/know' distinction reported earlier, performance on simple recognition tasks can be supported by at least two processes, one involving a hippocampal-dependent recollective (episodic) process and another involving hippocampal-independent familiarity-based (semantic) processing (Eichenbaum et al., 1994; Griffiths et al., 1999; Fortin et al., 2004; Brown and Aggleton, 2001). Methodological differences between recognition studies may change the degree to which subjects rely on episodic recall or on alternative strategies, a likely explanation for the conflicting results in subjects with damage to the hippocampus.

In conclusion, the formation of a memory after a single experience is not a capacity exclusive to episodic memory, as it can be supported by a number of other memory capacities (e.g., familiarity, imprinting, fear conditioning, taste aversion). Tests of memory for single experiences are not sensitive enough to distinguish the individual contribution of these processes and thus are not optimal for investigating episodic memory.

1.21.2.2.2.(ii) Episodic memories contain information about the context in which experiences occurred In order to be exclusively episodic in nature, experimental paradigms must have behavioral requirements unlikely to be fully subserved by other memory systems. A new approach pioneered by Clayton and Dickinson (1998) is to take advantage of the contextual differences between episodic and semantic memory to contrast them. According to Tulving (1972), episodic memories are tied to the context in which experiences occur, as if labeled for when and where they were acquired, whereas semantic memories are timeless and not bound to the place or other aspects of the context where knowledge was gained. Therefore, tests requiring animals to remember information about the context in which episodes occurred may be successful in measuring episodic memory capacity.

1.21.2.2.2.(ii).(a) What-where-when Clayton and Dickinson (1998) were the first to show that episodic memory may not be a uniquely human phenomenon, by providing behavioral evidence that animals can recall a unique past experience. They investigated memory for when and where events occurred in a clever experiment that utilized the natural caching behavior of scrub jays. Initially, jays cached both worms and peanuts in an array of locations. Jays prefer worms to peanuts, so if recovery is allowed within a few hours after caching, the jays will recover worms first. However, if a multiday interval is imposed between caching and recovery, the jays know that the worms are degraded and proceed to recover peanuts first. Scrub jays were capable of

selecting either type of food depending on the time since caching, leading the authors to conclude the jays remembered *what* had been cached, as well as *where* and *when* each item was cached (what-where-when). Importantly, because the stimuli are equally familiar at the time of test, subjects cannot choose on the basis of differential familiarity, unlike in radial-arm maze and delayed nonmatching tests.

This capacity to retrieve information about the time and place of occurrence of a unique experience has also been shown in rodents (Ergorul and Eichenbaum, 2004), suggesting that it extends to nonhuman mammals as well. However, there are a number of limitations to this model. First, as mentioned by Roberts (2002), the '*when*' component of the study is more a reflection of the jays' sense of how much time has passed since the caching events rather than a measure of a temporally organized memory structure representing when specific experiences occurred in relation to each other, as described by Tulving (1983). Furthermore, the capacity to judge how much time has passed since caching could be guided by signals about the trace strengths of the caching memories instead of episodic recall. Thus, jays may learn to prefer worms 4 hours post-caching when their memory for the caching memory is very strong, but not after a few days when the memory is weaker (Eichenbaum and Fortin, 2003). Second, the requirement for 'what,' 'where,' *and* 'when' is arbitrary and, with regard to the role of the hippocampus, appears inconsistent with the findings of patients and animals with hippocampal damage. In fact, subjects with damage to the hippocampus are impaired when required to remember *where or when* specific events occurred (see below), suggesting that episodic recall is needed to retrieve either type of information associated with the original experience (not necessarily a combination of the two). It therefore appears that the proposal that episodic memories consist of a conjunction of *what*, *where*, and *when* may be too stringent. Overall, however, the demonstration by Clayton and Dickinson (1998) of a possible operational measure of episodic memory has been very influential and generated considerable interest in developing a more complete animal model of episodic memory (*See* Chapter 1.23).

1.21.2.2.2.(ii).(b) Context in which experiences occur There are a number of experimental situations in which animals are required to remember the context in which specific events occurred, and evidence suggests that this capacity depends on the hippocampus. It is important to note that, in this chapter, context is broadly defined as the set of background features, external or internal to the subject, that are present when an event occurs (Ferbinteanu et al., 2006). The context of interest can be a particular visual scene (Gaffan and Parker, 1996) or the environment itself, as in the case of contextual fear conditioning paradigms in which damage to the hippocampus impairs the ability of animals to remember the testing box in which they received a foot shock (Phillips and LeDoux, 1992, 1995). Alternatively, the context may also be more abstract, in the sense that the hippocampus may be critical to remember the relevant component or feature of a particular task. For instance, the hippocampus has been shown to be important for the ability to choose the response that is correct given a specific behavioral context, such as which of two sequences is being completed (Agster et al., 2002), or to remember the task context in which a particular item was presented (e.g., source identification; Davachi et al., 2003). The context can also refer to internal sensations, as demonstrated by a study in which the hippocampus was critical to remember which behavioral response is appropriate, depending on rats' current internal motivational state (i.e., hunger or thirst; Kennedy and Shapiro, 2004).

Another line of evidence came into focus after recent models of episodic memory emphasized the importance of the spatial context of episodic memories (Clayton and Dickinson, 1998). In fact, when reinterpreted within an episodic memory framework, a number of spatial memory deficits in hippocampal animals alluded to previously in the navigation section can be better described as deficits in remembering the spatial location (spatial context) where specific events occurred. For instance, the hippocampus does not seem to be critical for spatial processing per se, but is important for remembering the spatial locations of individual arm entries in the radial-arm maze task (Olton and Papas, 1979), the trial-unique location of the escape platform in the watermaze (Steele and Morris, 1999), and the location where a trial-unique paired-associate was presented (Day et al., 2003). The relationship between navigation and episodic and semantic memory will be further discussed in the final section of this chapter.

In summary, these findings suggest that the experimental approach of testing the memory for the context in which specific events occurred, a function dependent on the hippocampus, is a feature of episodic memory that can be successfully tested in animals.

1.21.2.2.2.(iii) Episodic memories are structured as sequences of events The first two approaches were based on distinctions of episodic memory that have been emphasized explicitly by Tulving and, for the most part, described experimental paradigms already prominent in memory research. This novel approach captures a defining feature of episodic memory, but the formal details of the theory emerged from computational models of hippocampal function (Levy, 1996; Wallenstein et al., 1998; Lisman, 1999). As mentioned earlier, the neural architecture of the hippocampal system is rather unique. Of particular interest is region CA3 of the hippocampus, which has the highest density of recurrent connections in the brain and as such has been proposed to act as a heteroassociative network allowing successive events to be bound together into sequences (Levy, 1996; Wallenstein et al., 1998; Lisman, 1999). In line with Tulving's views (1983) that episodic memories are organized in the temporal dimension and that the central organizing feature of episodic memory is "one event precedes, co-occurs, or follows another," the fundamental principle underlying this approach is that each individual episode is organized as a sequence of events unfolding over time. Thus, this approach tests whether episodic memories contain not only a particular item or items that one is attempting to recall, but also the full sequence of events that precede and follow.

1.21.2.2.2.(iii).(a) Learning new sequences of events To investigate the specific role of the hippocampus in remembering the order of events in unique experiences, recent studies have employed a behavioral protocol that assesses memory for episodes composed of a unique sequence of olfactory stimuli (Fortin et al., 2002; Kesner et al., 2002). In one of these studies, memory for the sequential order of odor events was directly compared with recognition of the odors in the list independent of memory for their order (Fortin et al., 2002; **Figure 8(a)**). On each trial, animals were presented with a series of five odors, selected randomly from a large pool of common household scents. Memory for sequential order was probed using a choice test in which the animal was presented with two odors from the series and was reinforced for selecting the odor that appeared earlier. Similarly, recognition memory for the items in the series was probed using a choice between one of the odors from the series and another odor from the pool that was not in the series, and reinforcement was given for selecting the odor not presented in the series. Normal rats performed well on sequential order judgments across all lags (temporal distance between probed odors; **Figure 8(b)**), and performance on probes was dependent on the lag, indicating that order judgments were easier for more widely separated items. By contrast, rats with hippocampal lesions performed the sequential order task at near-chance levels and were impaired at all lags. However, both control rats and rats with selective hippocampal damage acquired the recognition task rapidly, and no overall difference in performance was observed between the two groups (**Figure 8(c)**).

As mentioned earlier, a potential confound in any study that employs time as a critical dimension in episodic memory is that, due to the inherent decremental nature of memory traces, memories obtained at different times are likely to differ in terms of their trace strength. However, in the study of Fortin and colleagues (2002), both the control and the hippocampal groups demonstrated a temporal gradient in recognition performance (**Figure 8(c)**), suggesting that memories were in fact stronger for the more recently presented items in each sequence (e.g., performance on E vs. X is better than on A vs. X). Therefore, their finding that only hippocampal animals were impaired in sequential order judgments (**Figure 8(b)**) suggests that this capacity cannot be fully supported by the use of relative strengths of memories traces, but rather depends on episodic recall of the odor sequence.

Contrary to the argument that animals lack episodic memory because they are 'stuck in time' (Roberts, 2002; Tulving, 2002), these observations suggest that animals have the capacity to recollect the flow of events in unique experiences. Finally, reports of a similar pattern of findings in subjects with damage to the hippocampus (Spiers et al., 2001; Downes et al., 2002; but see also Hopkins et al., 1995) support the validity of this approach as an animal model of episodic memory.

1.21.2.2.2.(iii).(b) Disambiguating learned sequences of events A major challenge for a robust model of episodic memory is the requirement for a capacity to develop representations that can distinguish two experiences that share common elements (Shapiro and Olton, 1994). Levy (1989, 1996) proposed that memory for the ordering of events mediated by the hippocampus may be especially important when the event sequences have overlapping elements through which memory of earlier elements must be remembered to complete each

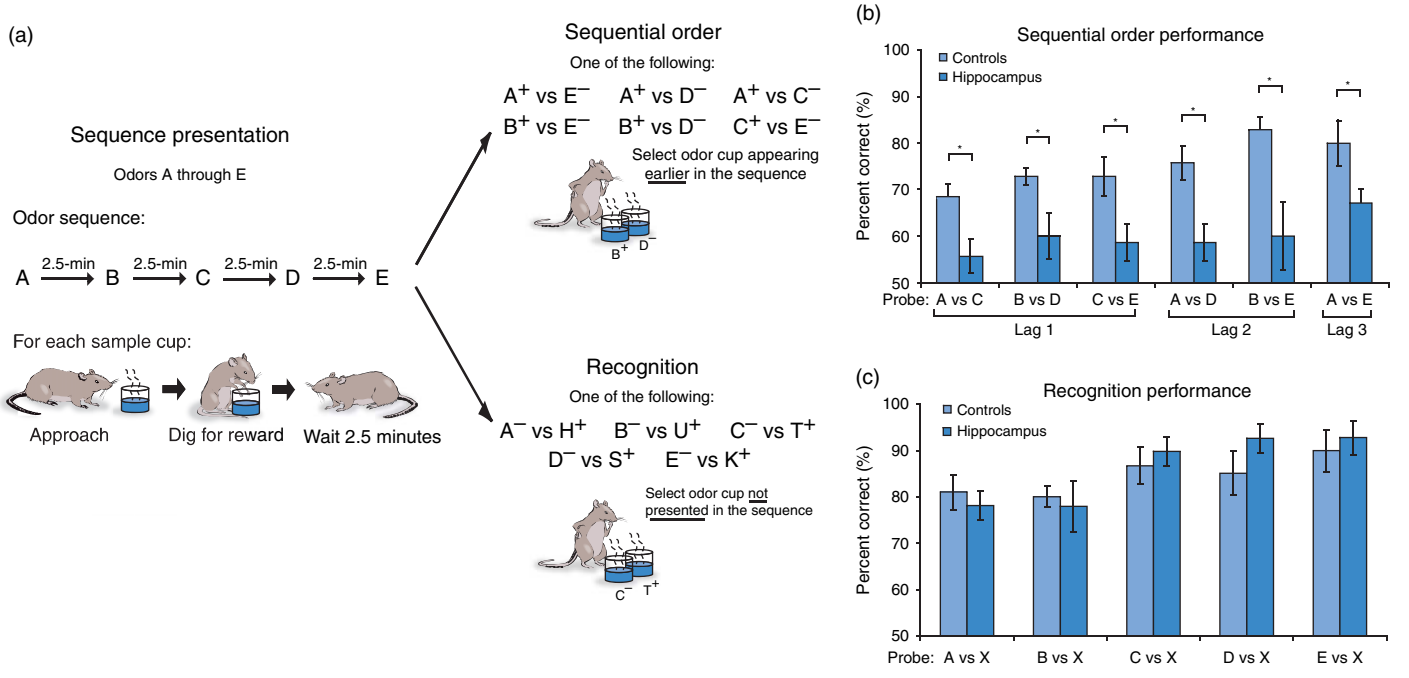

Figure 8 Sequential order and recognition tasks. (a) On each trial the animal was presented with a series of five odors (e.g., odors A through E). The animal was then either probed for its memory of the order of the items in the series (*top*) or its memory of the items presented (*bottom*). +, rewarded odor, −, nonrewarded odor. (b) Hippocampal animals were impaired on all sequential order probes. Performances on different probes are grouped according to the lag (number of intervening elements). (c) Hippocampal animals performed as well as controls on the recognition probes. 'X' designates a randomly selected odor that was not presented in the series and used as the alternative choice. *, $p < .05$. (a–c) Adapted from Fortin NJ, Agster KL, and Eichenbaum H (2002) Critical role of the hippocampus in memory for sequences of events. *Nat. Neurosci.* 5: 458–462, with permission from Macmillan Publishers Ltd.

distinct sequence. In order to test whether such sequence disambiguation is a fundamental feature of memory processing dependent on the hippocampus, Agster et al. (2002) trained rats on a sequence disambiguation task designed after Levy's (1996) formal model that involved two partially overlapping sequences of events (**Figure 9(a)**). The sequences were presented as a series of six pair-wise odor choices where, for each sequence, selection of the appropriate odor at each choice was rewarded (e.g., first choice being A vs. L). Each trial began with two forced choices that initiated production of one of the two sequences (e.g., A then B for Sequence 1). Then the animal was presented with two forced choices that were the same for both sequences (i.e., X then Y). Subsequently, the subject was allowed a free choice and was rewarded for selecting the odor assigned to the ongoing sequence (e.g., if given A→B→X→Y, then select E to complete Sequence 1). Finally the animal completed that sequence with one more forced choice (e.g., F for Sequence 1). The critical feature of this task was the free choice. On that test, animals were required to remember their choices from the first two pairings of the current sequence during the ambiguous components of the trial and then to use the earlier information to guide the correct odor selection.

Rats with damage to the hippocampus were impaired when sequences were presented in rapid succession (**Figure 9(b)**). However, hippocampal rats performed as well as controls in a version of the task in which proactive interference was reduced by increasing the inter-sequence delay (**Figure 9(c)**), but they were subsequently impaired when a 30-min delay was introduced before the free choice (**Figure 9(c)**). These findings suggest that, when memory demands are minimal, as in conditions of low proactive interference or no demand to hold information through ambiguous material, other brain systems, such as corticostriatal pathways, can succeed in coding sequences in which each segment rapidly or unambiguously leads to the next (Nissen and Bullemer, 1987; Reber and Squire, 1998). Conversely, under memory demands characteristic of episodic memory function, such as when proactive interference is high or when a substantial delay is

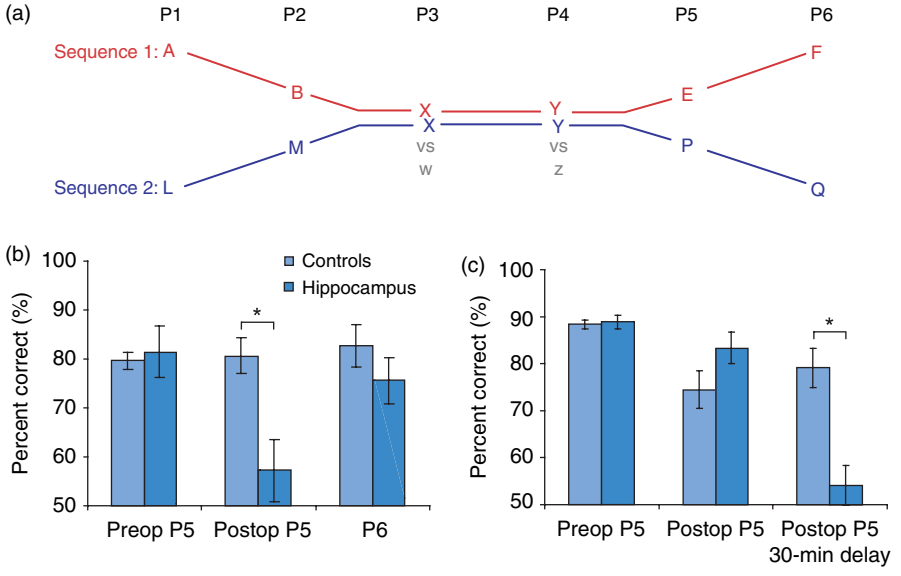

Figure 9 Sequence disambiguation task. (a) The two odor sequences are indicated by letters (Seq1: ABXYEF; Seq2: LMXYPQ). In performing each sequence, the rat selected between vertically aligned odors (e.g., for sequence 1 presentation, the rat should have selected A over L, B over M, X over W, Y over Z, E over P, and F over Q). Note that in both sequences, X was to be selected over W, and Y over Z. (b) When sequences were presented in rapid succession, hippocampal rats were impaired on P5, but not on P6, suggesting that their deficit is limited to a failure to remember the current sequence through the overlapping segment. (c) However, hippocampal rats performed as well as controls in a version of the task in which proactive interference was reduced by increasing the intersequence delay, but were subsequently impaired when a 30-min delay was introduced before the free choice. Modified from Agster KL, Fortin NJ, and Eichenbaum H (2002) The hippocampus and disambiguation of overlapping sequences. *J. Neurosci.* 22: 5760–5768, with permission from the Society for Neuroscience.

imposed between sequential experiences, a representation mediated by the hippocampus is required to disambiguate sequences of events.

In conclusion, the proposal that individual episodic memories are encoded as sequence of events, a hypothesis derived from computational models of hippocampal function, is in agreement with Tulving's view (1983) that the preservation of the temporal structure of events in experience is a central organizing feature of episodic memory. This approach has been successful in showing that the hippocampus of mammals is important for learning new sequences of events (in rodents: Fortin et al., 2002; Kesner et al., 2002; in primates: Spiers et al., 2001; Downes et al., 2002) and for disambiguating overlapping sequences of events (in rodents: Agster et al., 2002; in primates: Kumaran and Maguire, 2006).

1.21.2.2.2.(iv) Episodic memories are characterized by a recollective process with distinct retrieval dynamics The most recently developed approach to investigate episodic memory in animals focuses on mathematically deducing from objective behavioral measures the relative contribution of the two processes underlying recognition memory mentioned earlier: *recollection,* which corresponds to the recall of the original experience characteristic of episodic memory, and *familiarity,* which refers to nonepisodic recognition based on a general sense that an item has been previously experienced. In humans, signal detection techniques have been used to distinguish recollection and familiarity based on the shape of their receiver operating characteristic (ROC) curves, standard curves that represent item recognition across different levels of confidence or bias (Yonelinas, 2001; **Figures 10(a)–10(c)**). In such ROC curves, asymmetry (Y-intercept > 0) is viewed as an index of recollection, whereas the degree of curvature reflects the contribution of familiarity to recognition performance.

In order to determine whether animals also employ multiple processes in recognition memory and to explore the anatomical basis of this distinction, Fortin and colleagues (2004) adapted these techniques to examine odor recognition memory in rats (see **Figure 10(g)** for details). The ROC curve obtained in rats had asymmetrical (Y-intercept > 0) and curvilinear components, indicating the existence of both recollection and familiarity in rats (**Figure 10(d)**). Furthermore, following selective damage to the hippocampus, the ROC curve became entirely symmetrical (Y-intercept = 0) and remained curvilinear, indicating selective loss of recollection capacity but preserved familiarity (**Figure 10(e)**). To further compare the performance of the two groups, the control group was subsequently tested with a longer memory delay (75 min instead of 30 min) to have its overall level of performance (averaged across biases) match that of the hippocampal group. In contrast to the hippocampal group (**Figure 10(e)**), the ROC curve of normal rats at 75 min delay remained asymmetric but showed little curvilinearity (**Figure 10(f)**), indicative of recognition performance mostly based on recollection.

The pattern of findings in this study strongly suggest (1) that the ROC approach, developed in humans, can be used to investigate episodic memory in rodents as well; (2) that rodents also use both episodic recollection and familiarity in recognition memory, providing an explanation for the mixed findings in the recognition tests mentioned; and (3) that, as in humans (Yonelinas et al., 2002), the hippocampus plays a predominant role in episodic recollection compared to familiarity (but see also Wais et al., 2006).

1.21.2.2.3 Hippocampal neuronal mechanisms underlying episodic memory

In addition to the behavioral and neuropsychological findings described, characterizations of the firing patterns of hippocampal neurons in animals performing memory tasks have helped clarify the role of the hippocampus in episodic memory by shedding light on its information processing mechanisms. Observations from rats, monkeys, and humans, and across many different behavioral protocols, show that important attributes of episodic memory are encoded in hippocampal neuronal activity: (1) hippocampal neurons code for the context in which specific events occur, (2) hippocampal neurons encode episodes as sequences of events, and (3) hippocampal neurons disambiguate and link distinct episodic memories (see **Figure 11**).

1.21.2.2.3.(i) Coding of specific events or experiences in their context In this chapter, context is defined as the set of background features, external or internal to the subject, that are present when an event occurs (Ferbinteanu et al., 2006). It includes the spatial location where an event took place, but extends to other aspects of context as well, such as the behavioral significance of particular stimuli in the task, or the emotional or motivational states experienced during specific events. As

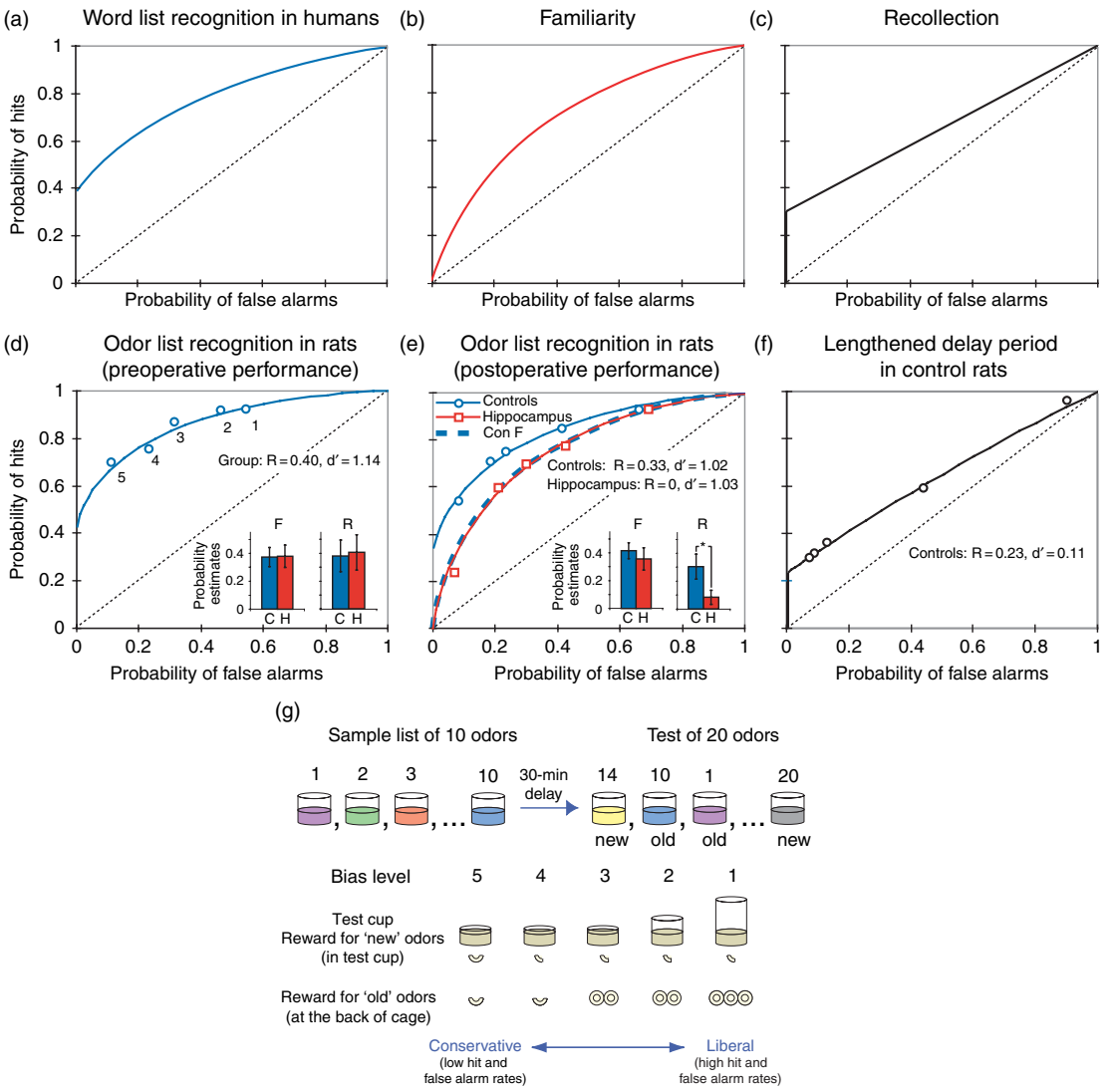

Figure 10 ROCs for recognition performance in humans and rats. (a–c) Performance of humans in verbal recognition. In a typical experiment, human subjects initially study a sample list of words and then are presented with a larger list containing the sample words intermixed with new ones and asked to identify each word as old or new. The resulting ROC analysis plots hits (correct identifications of old items) against false alarms (incorrect identifications of new items as old) across a range of confidence levels or response bias. The data points are then curve fitted by a model with two parameters (Y intercept and d′) using a least-squares method (see Yonelinas et al., 1998, for details). (d–f) Performance of rats in odor recognition (Fortin et al., 2004). (d) Normal rats tested with a 30-min delay. Insets: recollection (R) and familiarity (F) estimates. (e) Postoperative performance with a 30-min delay, including an estimated curve for controls based on familiarity alone (con F). (f) Control rats tested with a 75-min memory delay. Diagonal dotted lines represent chance performance across criterion levels. C, control group; H, hippocampal group. Error bars indicate SEM; *, $p < .05$. (g) Odor recognition task. The behavioral procedures remained fundamentally similar to those used in human studies but were adapted to take advantage of the natural behavior of rats. On each daily test session, rats initially sampled ten common household scents mixed with playground sand in a plastic cup containing a cereal reward. Following a 30-min memory delay, the same odors plus ten additional odors were presented in random order, and animals were required to identify each odor as old or new. To plot ROC curves, hit and false alarm rates were obtained under a range of response criteria, from conservative to liberal. To achieve this, different response criteria were encouraged for each daily session using a combination of variations in the height of the test cup (making it more or less difficult to respond to that cup), and manipulations of the reward magnitudes associated with correct responses to the test and the unscented cup. Top: Sequence of odor presentation. Bottom: Test cup heights and reward payoffs for each bias level. (a–g) Adapted from Fortin NJ, Wright SP, and Eichenbaum H (2004) Recollection-like memory retrieval in rats is dependent on the hippocampus. *Nature* 431(7005): 188–191, with permission from Macmillan Publishers Ltd.

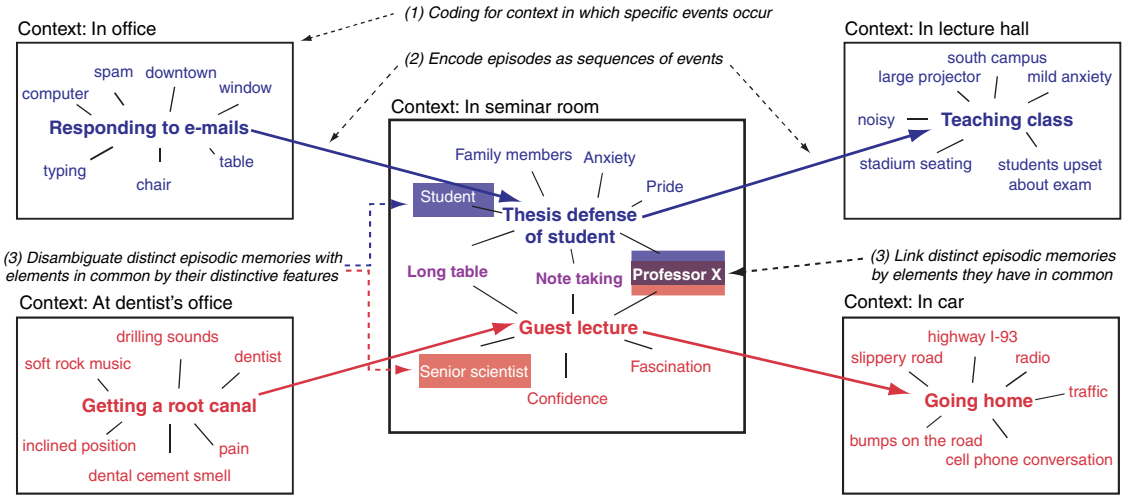

Figure 11 Hippocampal neuronal mechanisms underlying episodic memory. Characteristic features of episodic memories are encoded in hippocampal neuronal activity: (1) hippocampal neurons code for the context in which specific events occur, (2) hippocampal neurons encode episodes as sequences of events, and (3) hippocampal neurons disambiguate and link episodic memories with elements in common.

described in the previous section, a plethora of studies have described the spatial coding properties of hippocampal neurons; however, the focus of the vast majority of these studies was the coding of space itself (e.g., cognitive mapping), not the spatial context of unique experiences, and as such most paradigms had very little memory requirement.

The role of the hippocampus in representing different elements of the context in which specific events take place was first demonstrated by the study of Wood and colleagues (1999) mentioned earlier, in which rats were required to perform the same behavioral judgments (match or nonmatch to the previous odor) at many locations in the same environment. In addition to the cells coding for task regularities, such as specific locations, odors, or match/nonmatch status discussed earlier, they reported other hippocampal cells that fired in association with combinations of events and the context in which they occurred. For instance, some cells fired only if the animal began the approach from a particular location, or fired only for a particular conjunction of the odor, the place where it was sampled, and the match/nonmatch status of the odor. This conjunctive coding representing spatial and nonspatial features of specific events suggests that the hippocampus encodes the context of individual experiences.

A recent study reported very similar results in humans (Ekstrom et al., 2003). The activity of hippocampal neurons was recorded as subjects played a taxi driver game, searching for passengers picked up and dropped off at various locations in a virtual-reality town. Some cells fired when subjects viewed particular scenes, occupied particular locations, or had particular goals in finding passengers or locations for drop off. Many of these cells fired in association with specific conjunctions of a place and the view of a particular scene or a particular goal. Similar coding reflecting context-specific activity in the hippocampus has been reported in a number of other studies as well (e.g., Moita et al., 2003; Wirth et al., 2003). Thus, in rats, monkeys, and humans, a prevalent property of hippocampal firing patterns involves the representation of unique conjunctions of elements reflecting the context in which specific events occur (**Figure 11**).

1.21.2.2.3.(ii) Coding of episodes as sequences of events Computational models have hypothesized that the unique neural architecture of the hippocampus would be well suited for coding individual episodes as sequences of events (Levy, 1996; Lisman, 1999), a prediction supported by aforementioned behavioral studies (Agster et al., 2002; Fortin et al., 2002). Although unequivocal evidence for coding of sequences of individual events is not available

at this time, this hypothesis has received experimental support. In fact, hippocampal neurons show activity patterns reflecting sequential coding of locations (Mehta et al., 1997, 2000) and have been shown to preserve the sequential order in which locations were visited (Lee and Wilson, 2002; 2004; Foster and Wilson, 2006). Moreover, every behavioral event of significance is encoded by individual hippocampal neurons (e.g., presentation of a specific stimulus, response to a cue), such that different subsets of hippocampal neurons are sequentially activated as the animal performs the task at hand (Wiener et al., 1989; Eichenbaum et al., 1999), providing the hippocampal network as a whole with a representation of the full sequences of events composing individual episodes (**Figure 11**).

1.21.2.2.3.(iii) Disambiguating and linking distinct episodic memories Since a large number of episodic memories have elements in common, the neural systems responsible for the encoding and retrieval of episodic memories must use neuronal mechanisms that minimize interference between such similar episodic memories but also preserve the integrity of the relations among dissimilar episodes, which support the flexibility of access and expression as well as comparisons within the episodic memory network (Eichenbaum, 2004). According to the current framework, this can be accomplished by *disambiguating* the representation of similar episodes by emphasizing their differences, and by *linking* common elements between distinct episodic memories to emphasize their similarity (**Figure 11**).

A recent study by Wood and colleagues (2000; see also Frank et al., 2000) lent support to this characterization by recording from hippocampal neurons as rats performed a spatial alternation task in the T-maze. Performance on this task requires that animals distinguish left-turn and right-turn episodes and remember the immediately preceding episode so as to select the other option on the current trial, task demands reflecting the use of episodic memory (Olton, 1984, 1986). The key comparisons focused on the central 'stem' of the maze, which is common to both left- and right-turn trajectories (**Figure 12(a)**). They reported firing patterns that emphasize the distinctiveness between similar episodes, indicative of *disambiguation* coding. In fact, many of the cells that fired when the rat was running down the stem fired differentially on left-turn versus right-turn trials (**Figure 12(b)**), and this differential activity was shown not to be due to differences in head direction, running speed, or location on the two trial types. Thus, even though the behavior and external stimuli were held constant, these cells provided a representation that disambiguated two otherwise identical trajectories on the stem according

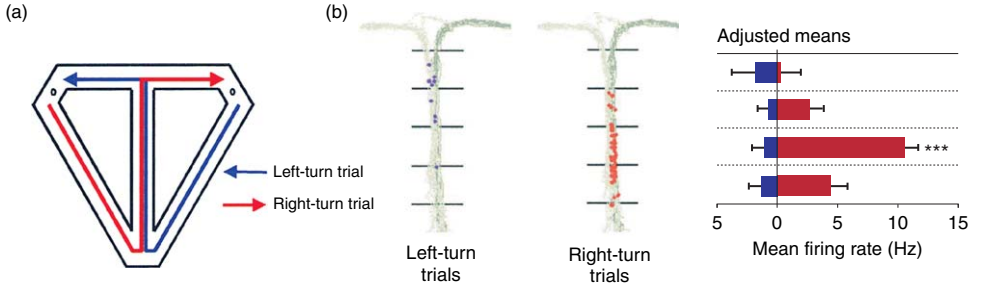

Figure 12 Disambiguation coding in hippocampal neurons. (a) Rats performed a continuous alternation task in which they traversed the central stem of the apparatus on each trial and then alternated between left and right turns at the T junction. Rewards for correct alternations were provided at water ports (small circles) on the end of each choice arm. The rat returned to the base of the stem via connecting arms and then traversed the central stem again on the next trial. For analysis of neural firing patterns, left-turn (blue arrow) and right-turn (red arrow) trials were distinguished. (b) Example of a hippocampal neuron disambiguating between left- and right-turn episodes. This cell fired almost exclusively during right-turn trials. Plots on the left show the location of the rat when individual spikes occurred for left-turn trials (blue dots) and right-turn trials (red dots). In the right panel, the mean firing rate of the cell for each sector, adjusted for variations in firing associated with covariates, is shown separately for left-turn trials (blue) and right-turn trials (red). Adapted from Wood E, Dudchenko P, Robitsek JR, and Eichenbaum H (2000) Hippocampal neurons encode information about different types of memory episodes occurring in the same location. *Neuron* 27: 623–633, with permission from Elsevier.

to the cognitive demands of the task, that is, to remember which turn was performed last. In addition, Wood and colleagues also reported coding patterns reflecting *links* between similar elements of distinct episodes. In fact, they recorded from cells that fired in the same location on the stem for both trial types, potentially providing a link between left-turn and right-turn representations by the common places traversed on both trial types.

In summary, the firing properties of hippocampal ensembles underlie episodic memory by encoding individual episodes as sequences of events as well as the common stimuli, places, and events that are shared across episodes, providing a framework for linking related memories and disambiguating overlapping ones (Eichenbaum et al., 1999; **Figure 11**).

1.21.2.3 Contribution of Other Neural Systems to Episodic Memory

In addition to the hippocampus, a number of other neural systems are important for episodic memory. Evidence from both neuropsychological studies and functional brain imaging indicates that the prefrontal cortex plays a role in the strategic or organizational aspects of episodic retrieval (Milner et al., 1985; Gershberg and Shimamura, 1993; Wheeler et al., 1995; see Buckner and Wheeler, 2001, for a review). The parahippocampal region also plays a crucial role in episodic memory by combining multimodal representations of stimulus details in neocortical areas to form representations of individual items and contextual elements (see Eichenbaum et al., 1994, for a review). These elemental representations in parahippocampal areas would then be combined in the hippocampus to form episodic memories by encoding individual experiences with the context in which they were experienced. Such representations in the parahippocampal region are also thought to underlie nonepisodic recognition memory based on a sense of familiarity, by allowing comparisons between a recently presented item and the current item (Suzuki et al., 1993; Stern et al., 1996; Henson et al., 2003).

1.21.2.4 Conclusions

The idea of multiple memory systems has now gained wide acceptance, and considerable evidence suggests that these memory systems are fundamentally similar across mammalian species. However, the episodic memory system was initially thought to be unique to humans because of its dependence on human-centric faculties such as self, mental time travel, and consciousness. This section reviewed the fundamental features of episodic memory that can be operationally defined and tested in nonhuman animals as well and presented accumulating evidence from behavioral and electrophysiological studies demonstrating that animals are capable of episodic memory, and that, as in humans, this capacity depends on the integrity of the hippocampus. These findings suggest that we are making considerable progress in developing an animal model of episodic memory function, which will help uncover its underlying neuronal mechanisms.

1.21.3 Reconciling the Role of the Hippocampus in Navigation and Episodic Memory

In the service of clarity, this chapter has described the capacities for navigation and episodic memory separately, which parallels the relative independence of the two respective domains until recently. However, accumulating evidence indicates that the two capacities are related. As mentioned in the previous section, the role of the hippocampus in episodic memory can be responsible for deficits observed in a number of spatial paradigms, such as those requiring the animal to remember the trial-unique locations where specific events occurred (Olton and Papas, 1979; Steele and Morris, 1999; Day et al., 2003), though such an account fails to fully capture the extent of all navigational deficits reported in subjects with damage to the hippocampus. In fact, other navigational deficits appear to result from a failure to create a trial-independent mental representation of the environment that the subject can use to navigate flexibly (e.g., a stable cognitive map of a particular environment), suggesting that the hippocampus is critical for some aspects of semantic memory as well.

A careful review of the literature confirmed that subjects with hippocampal damage are indeed impaired at creating a stable memory structure that supports *flexibility* of access and expression, but, more importantly, also revealed that this deficit extends to memories of both spatial and nonspatial nature

(Eichenbaum and Cohen, 2001). For instance, animals with hippocampal damage can use simple strategies to solve spatial tasks, such as learning to approach a specific set of stimuli, but are severely impaired compared to controls when the start location or the destination is changed and a novel trajectory must be conceived (Eichenbaum et al., 1990; Whishaw et al., 1995; Whishaw and Tomie, 1997). Similarly, hippocampal rats perform as well as controls in nonspatial paradigms that require learning of consistent and nonambiguous reward or response associations to individual cues, such as discrimination learning (e.g., learning to select the always-rewarded item 'A' over the never-rewarded item 'B'; e.g., Dusek and Eichenbaum, 1998; Kesner et al., 2002) and recognition memory tasks (e.g., learning that item 'C' was presented earlier in the session, but that item 'X' was not; Dudchenko et al., 2000; Fortin et al., 2002), but are impaired when required to process relations among individual stimuli and use such information in novel situations (e.g., Bunsey and Eichenbaum, 1996; Dusek and Eichenbaum, 1997). For instance, in the transitive inference task, hippocampal rats could learn a series of discriminations involving overlapping elements as well as control animals (i.e., A > B, B > C, C > D, D > E; in which '>' corresponds to 'must be selected over'). However, when confronted with novel pairings (e.g., B vs. D), only control animals could successfully process the relations among all individual items (A > B > C > D > E) and correctly identify the indirect relationship between the items (i.e., B > D; Dusek and Eichenbaum, 1997).

1.21.3.1 Cognitive Maps as Semantic Knowledge Structures Extracted from Individual Episodic Memories

Although aspects of semantic memory can be learned without the hippocampus (e.g., Vargha-Khadem et al., 1997), the hippocampus appears to be important for the process of developing relations among individual episodic and semantic memories, crucial to support the flexibility of declarative memory representations (Eichenbaum et al., 1999; Eichenbaum, 2004). According to the relational theory of hippocampal function, this process occurs by linking common elements between individual memories (e.g., a location, or a person), which ultimately leads to the creation of the large multidimensional relational network of declarative memories (Cohen, 1984; Eichenbaum et al., 1999; Eichenbaum, 2004). According to this model, spatial representations of the environment, or cognitive maps, are simply one type of semantic knowledge represented in the network.

How are mammals creating cognitive maps of their environment to optimize navigational behavior? In humans, there are two ways to create a mental representation of a specific environment (Shelton and McNamara, 2001). The first is to use a 'survey' strategy, which is characterized by an external perspective, such as a bird's eye view, and allows direct access to the global spatial layout. This strategy is predominantly used when subjects are studying an actual map to learn the layout of the environment. The alternative is a 'route-based' strategy, which is characterized by knowledge of spatial layout from the perspective of a ground-level observer navigating the environment. In this case, initial knowledge is represented as individual trajectories or routes (e.g., airport → hotel, hotel → convention center, convention center → restaurant, restaurant → hotel), but with enough experience with routes within a specific environment, subjects can successfully construct a maplike representation out of those individual routes (or episodic memories).

Since most mammals do not have access to aerial views of their environment, nor have the capacity to read a map, it is clear that the survey strategy is not common to all mammals. The route-based strategy, in contrast, takes advantage of the instinctive behavior of mammals to actively explore their environment (O'Keefe and Nadel, 1978) and uses the same circuitry in humans as it does in animals. In fact, in a recent functional neuroimaging study, the survey representation was shown to activate inferior temporal and posterior parietal regions, suggesting that such information is acquired as complex visual scenes, whereas the route-based strategy recruited regions not activated in survey encoding, including the hippocampus and parahippocampal region (Shelton and Gabrieli, 2002). The strong consistency in data on route encoding from studies on hippocampal neuronal firing patterns in rats and hippocampal activation in human functional imaging support the notion that hippocampal representations of space, like those for nonspatial memory, are created from individual sequences of events and places where they occur (see **Figure 13**).

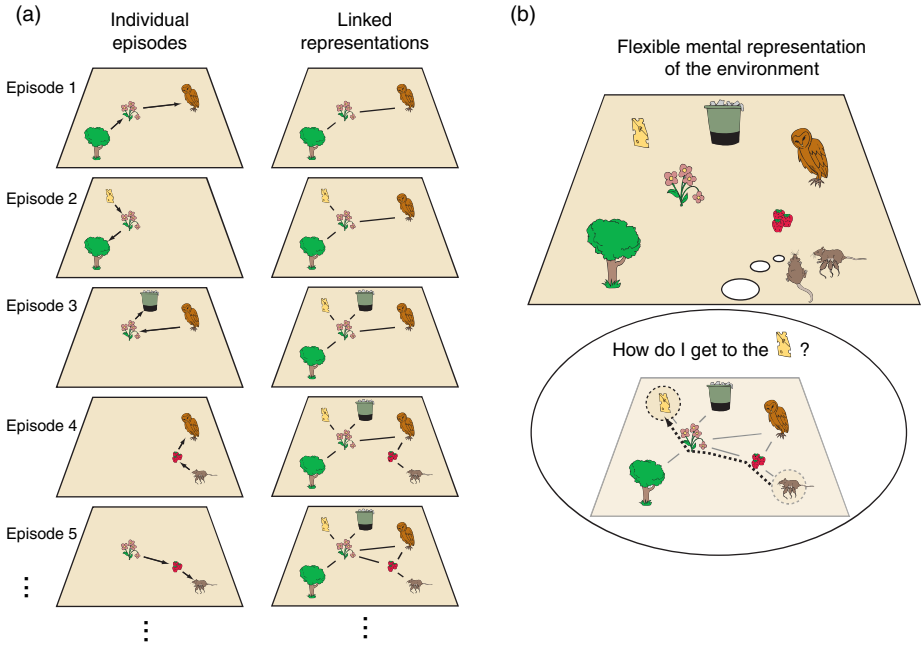

Figure 13 Cognitive map of the environment built out of individual episodes. (a) The column on the left represents individual episodes, each consisting of a particular trajectory in the animal's environment. The column on the right shows the representation that gradually forms as the common features between individual episodic memories (in this case, locations) are linked together. (b) After extensive experience in the environment, the linked representations can support flexible navigation by allowing the animal to determine a novel trajectory between two known points.

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1.22 Memory in Food Caching Animals

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1.22.1 Introduction

One of the more interesting developments in the study of learning and cognition over the past 25 years has been the realization that learning and memory play an important role in the natural world of many animals (e.g., Balda et al., 1998). As this realization led to research into animal cognition in natural settings, it became clear that such research can make important contributions to our understanding of animal and human cognition. In this chapter, we review one of the areas of research that originally stimulated interest in the role of memory in the field, the ability of many food-storing animals to remember where they have cached their food (*See also* Chapter 1.23).

We will begin our review of research in this area with a brief review of the natural history and ecological significance of food-caching. We will then review the evidence demonstrating the use of memory for accurate cache recovery, followed by a discussion of the characteristics of cache site memory and the comparative evidence for differences in memory among

caching and noncaching species. We will conclude with reviews of how caching animals encode spatial information and the neural substrates for spatial memory in caching animals.

1.22.2 The Natural History of Food Storage

Animals face many problems obtaining food. Food may vary in abundance on a daily or seasonal basis, or even unpredictably, in boom–bust cycles. Even when food is abundant, there may be fierce competition for access to the food. And animals have evolved a number of strategies to cope with these problems, such as migration, hibernation, and torpor to deal with variability and food-caching and/or territoriality to deal with competition. Food hoarding is a strategy that can help an animal cope effectively with both variability in food availability and competition. By gathering food and hiding it, an animal can simultaneously store food against lean times and gain control over food against competitors.

Food storing takes many forms, from the nest of eusocial bumble bees to the grain silos of the human farmer (see Vander Wall, 1990, for a comprehensive review). The food storage patterns seen in nature vary considerably in the degree of dispersion among caches. At one extreme is larder-hoarding, in which food is gathered during times of plenty and placed into one or a few large larders. The hive and honeycombs of the honey bee (*Apis mellifera*), the granaries of acorn woodpeckers (*Melanerpes formicivorus*), or the middens of red squirrels (*Tiamiasciuris hudsonicus*) are excellent examples of this type of food storage. Once created, of course, these large caches require defense against competitors. Indeed, it would appear that one of the potential disadvantages of larder-hoarding is that loss of a larder incurs high cost to the original hoarder, since each larder site contains a large proportion of the animal's stored food.

The other extreme, in which food is stored in a large number of widely dispersed locations, is referred to as scatter-hoarding. Examples include fox squirrels (*Sciuris niger*), which store many of their walnuts singly; chickadees and tits, which store single seeds in moss and crevices; and nutcrackers, which store one to 14 pine seeds in each cache site. Larder- and scatter-hoarding define a continuum, not a dichotomy, and some mammals such as fox squirrels and yellow pine chipmunks (*Tamias amoenus*) store both singly and in larders (Vander Wall, 1990). These two storage strategies require quite different defense strategies. The scatter-hoarder creates more caches, each containing less food, over a much larger area than does the larder-hoarder. The caches of a scatter-hoarder cannot be physically defended since they are highly dispersed, but the loss of any single cache site is much less significant for the scatter- than for the larder-hoarder. But scatter-hoarding does raise an interesting question, with intriguing cognitive possibilities. How does the scatter-hoarder relocate the large number of cache sites it has created?

Logically, there are three general classes of answer to this question. We present them in decreasing order of the cognitive demands needed for hoarders to successfully recover their own caches.

1. Memory for cache sites: If the hoarder could remember individual cache sites, it could then use this memory to recover the caches. This would, however, require considerable memory capacity.
2. Site preferences or movement rules: Suppose an animal had certain locations that it preferred to visit or specific paths which it regularly followed, then stored food in those locations or along those paths. If it searched those places, it would be able to find the stored food at a much greater rate than could be achieved by random search. This strategy would require that the animal remember the preferred sites or paths, but it would not be necessary to remember individual cache sites. If, however, the site or path preferences could be learned by an observing competitor, then the potential for loss would be great.
3. Direct cues: The hoarder could relocate its food through detection of cues (such as odor) emanating directly from the cached food itself. While this recovery mechanism would require little cognitive ability except perhaps specialized sensory capacities, it would have a large potential downside. Any animal capable of detecting the cues could recover the cache, with potentially disastrous effects for the animal that originally created the cache.

Interestingly, there appears to be a negative correlation between the cognitive demands of the strategy and the resistance of the strategy to competitors for the caches. This implies that under appropriate conditions – a high risk of loss of scatter-hoarded food – the use of increased cognitive capacities might be favored, even though

cognitive abilities involve heavy metabolic costs (Attwell and Laughlin, 2001).

The results of a field study of cache recovery and pilfering in small mammals (Vander Wall, 2000) is quite interesting from this point of view. Vander Wall allowed some yellow pine chipmunks (*Tamias amoenus*) or deer mice (*Peromyscus maniculatus*) to cache pine seeds in a large outdoor cage and then search for their own caches (knowledgeable foragers), while additional animals searched for caches created by others (naive foragers). The experiment was conducted in the Carson mountain range of western Nevada, where the climate is usually quite dry. Under dry conditions, knowledgeable animals were much more successful finding their own caches than naive animals searching for the caches of others. When conditions were wet, such as following rain, however, the chipmunks and mice found all caches, their own, those of conspecifics, or those of the other species, with equal facility (**Figure 1**). The superiority of the knowledgeable over the naive cachers under dry conditions demonstrates the advantage that detailed spatial memory can give. On the other hand, the ability of the naive foragers to locate the caches created by others demonstrates the potential liabilities of relying on direct cues to relocate cache sites.

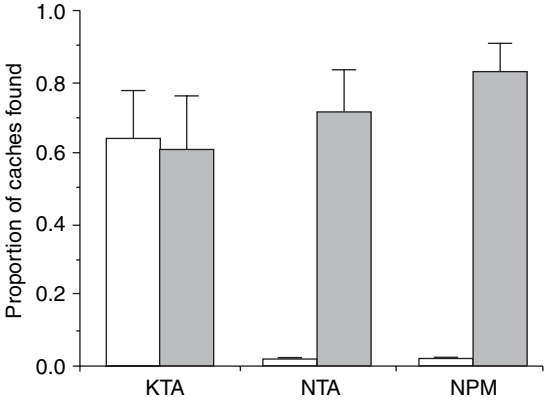

Figure 1 The proportion of caches found by yellow pine chipmunks (TA) and deer mice (PM) who either knew the locations of the caches (K) or were naive about the cache locations (N) under either dry conditions (open bars) or wet conditions (filled-in bars). From Vander Wall SB (2000) The influence of environmental conditions on cache recovery and cache pilferage by yellow pine chipmunks (*Tamias amoenus*) and deer mice (*Peromyscus maniculatus*). *Behav. Ecol.* 11: 544–549; used with permission from Oxford University Press.

1.22.3 Establishing the Role of Memory

Until recently, it was thought unlikely that scatter-hoarders used spatial memory for the recovery of cached food (e.g., Gibb, 1960). Gradually, however, evidence mounted that spatial memory for specific cache sites could be important to accurate cache recovery in a number of species of birds and mammals. Field studies provided suggestive evidence. For example, Tomback (1980) developed a technique for roughly estimating the accuracy of cache recovery by Clark's nutcrackers (*Nucifraga columbiana*) in the field. Tomback's estimated probabilities were quite high, much higher than could be expected by chance. In another field study, Cowie et al. (1981) placed mildly radioactive seeds in a feeder, and they were taken and cached by marsh tits and a scintillation counter used to locate cached seeds in the area around the feeder. A control seed was then placed 100 cm from each cached seed and survivorship of the seeds monitored. The cached seeds disappeared much more rapidly than control seeds, strongly suggesting removal by cachers and consistent with memory for specific cache sites.

MacDonald (1976) conducted similar experiments with a vixen, which almost always found dead mice she had cached but almost never found dead mice cached 1–2 m away by the experimenter.

1.22.3.1 Experimental Evidence for Spatial Memory

Although these types of field studies yielded results consistent with the use of spatial memory for specific cache sites, field studies lack the capacity for experimental control necessary to fully rule out alternative hypotheses. The breakthrough came with a series of laboratory studies that showed that many parids and corvids would cache and recover seeds under laboratory/aviary conditions. In this section, we briefly review the major findings of some of these studies that established the role of spatial memory in the relocation of cached foods.

Balda (1980) tested a single Eurasian nutcracker (*Nucifraga caryocatactes*) in a room with a dirt floor. The bird readily cached and accurately recovered seeds in this room. The search was accurate even when seeds had been removed from caches before recovery, demonstrating that cues emanating directly from caches were not necessary for recovery. Vander

Wall (1982) extended these findings by letting two Clark's nutcrackers individually cache and recover cached seeds in the same aviary. In virtually every case, each bird recovered only its own caches. This also provides strong evidence against direct cues, as well as against site preferences or paths unless these are idiosyncratic.

At the same time as studies were proceeding with nutcrackers, researchers in Canada and England were developing laboratory-based procedures to study cache recovery in chickadees and tits. Sherry et al. (1981) gave marsh tits (*Parus palustris*) sunflower seeds to store in moss-filled trays in an aviary. The birds revisited the areas of the trays used to cache seeds significantly more often than would be expected by chance 3 and 24 h after original storage of the seeds, even though the seeds had been removed. Sherry et al. (1981) also found that the probability of visiting those quadrants in which cache sites were located was higher following caching than it had been during a precaching exploratory session, suggesting that site preferences were not responsible for the performance. Sherry (1984) extended these results in an aviary study with black-capped chickadees (*Parus atricapillus*) by giving the birds specific potential cache sites (as opposed to areas in a tray). The chickadees cached readily in these sites and recovered the caches more accurately than would be expected by chance or by precaching exploratory patterns.

Kamil and Balda (1985) took a more direct approach to the control of site preferences during cache recovery by Clark's nutcrackers. They used a room with 180 holes in the floor, each of which could either be filled with sand for caching/recovery or be capped with a wooden plug. This made it possible to limit the number of sites available for caching sessions, forcing the birds to cache in sites essentially chosen by the experimenters. Even under these conditions, nutcrackers were able to recover caches accurately, demonstrating that site preferences are not necessary to accurate cache recovery by nutcrackers.

Three studies in the early 1990s demonstrated the use of spatial memory to relocate caches in scatter-hoarding rodents. Jacobs and Liman (1991) had gray squirrels cache hazelnuts in an outdoor arena. The squirrels found significantly more of their own caches than the caches of other squirrels that had stored food in the same arena. Vander Wall (1991) allowed yellow pine chipmunks to cache seeds in an arena filled with dry sand. The chipmunks were significantly more likely to find their own caches than caches of other chipmunks. Jacobs (1992) allowed Merriam's kangaroo rats to cache and retrieve sunflower seeds in an arena with plastic cups filled with sand for caching sites (much like Kamil and Balda, 1985). Before retrieval, she removed half of the caches made by each rat. The rats searched significantly more in locations where they buried seeds, whether the seeds had been removed or not. Jacobs also found that a naive rat searching in the arena found significantly fewer caches than the rat that had made the caches.

It is clear that some form of spatial memory for cache sites is used to find cached seeds by members of several taxa (see Vander Wall, 1990, for a broad survey of food-hoarding in animals). Although other mechanisms such as olfaction or site preferences may play a role, many food-storing corvids and parids and rodents can find their food when these mechanisms are controlled for or eliminated. We now turn our attention to what is known about spatial memory in food-storing species.

1.22.3.2 The Characteristics of Cache Memory and Retrieval

It seems likely that the characteristics of cache site memory in any particular species of scatter-hoarders will be a function of a complex interaction between functional and mechanistic variables. For example, as caches are created, they are necessarily created in some sequence. A large psychological literature indicates that in the case of such serial lists, the order in which the items to be remembered are presented can have important effects on how well they are remembered, the serial position effect well known to students of memory (e.g., Wright et al., 1984; Wright, 2006). On the other hand, different orders of recovery may be most adaptive under different circumstances (Andersson and Krebs, 1978). Duration of memory may be another example. In this section, we briefly survey what is known about the duration, contents, and dynamics of cache memory.

1.22.3.2.1 Memory duration

Balda and Kamil (1992) tested four groups of nutcrackers, each at a different amount of time after caching, from 11 to 285 days. All four groups performed well above chance levels. Bednekoff et al. (1997a) used a repeated-measures design in a comparative study with nutcrackers, pinyon jays (*Gymnorhinus cyanocephalus*), Mexican jays (*Aphelocoma ultramarina*), and Western scrub jays (*A. californica*; note that the classification of *Aphelocoma* species was

modified several years ago; we use the current nomenclature throughout this chapter) and found that the birds were still performing above chance after 250 days. In contrast, most studies with parids have suggested much shorter memory durations. For example, Hitchcock and Sherry (1990) found that black-capped chickadees did not find their caches at better than chance levels after postcaching intervals over 28 days, and Brodin and Kunz (1997) obtained similar results in willow tits (*Parus montanus*).

These differences in memory duration correspond with differences in natural history. Many corvids cache in the fall and then depend on their cached food throughout winter into the spring (Vander Wall, 1990). In contrast, within the parids many species cache for shorter periods of time, caching and recovering throughout the winter (e.g., marsh tits, *P. palustris*; Cowie et al., 1981), although there are some parids that cache in fall and use those caches for some months (e.g., crested tits, *P. cristatus*, Haftorn, 1954). Brodin (2005) has suggested that corvids possess a site-specific, accurate long-term memory, whereas parids may use a more general memory along with area-restricted search.

1.22.3.2.2 Memory for cache contents

Several studies indicate that cachers can remember the contents of their caches. Sherry (1984) allowed black-capped chickadees to cache two types of seed and found that they recovered the type they preferred before the nonpreferred type. Clayton and Dickinson (1999) extended this methodology by allowing Western scrub jays to cache two types of food, then prefeeding one of the foods before recovery testing. During recovery, the birds preferentially searched sites in which they had cached (but not retrieved) the food that had not been preferred (*See* Chapter 1.23, research by Clayton and her colleagues on episodic-like memory). This suggests a memory process more dynamic than a simple association of foods and the locations of their caches.

Moller et al. (2001) gave Clark's nutcrackers small and large pine seeds to cache and videotaped recovery sessions. They measured the size of the gape, the distance between the upper and lower bills when beginning to dig out the cache. Gape size was reliably larger for caches containing the larger seeds than for caches containing the smaller seeds.

1.22.3.2.3 Order of recovery

As a number of authors have pointed out (e.g., Vander Wall, 1990), since caches are created sequentially, the psychological literature on memory suggests that there ought to be some relationship between order of cache creation and order of cache recovery, either primacy (first recovering the caches that were created first) or recency (first recovering the most recently made caches). Psychological studies of memory for serial lists regularly find both of these effects in a wide variety of contexts in humans and animals (see recent review by Wright, 2006).

It has also been argued on functional grounds that there should be recency effects in cache recovery (e.g., Shettleworth and Krebs, 1982). The more time that has passed since a cache was created, the less likely that cache is to still be available to the cacher. As time passes, the probability of the cache having been pilfered increases and the chances of the cache location having been forgotten also may increase. Thus, recovery of the most recent caches first could maximize the total number retrieved. There has, however, been no consistent evidence of such effects. For example, when black-capped chickadees stored and recovered sunflower seeds in Sherry (1984, experiment 2), 24 correlation coefficients between cache and recovery orders were calculated. Two were significant in the positive direction, one was significant in the negative direction, and the remainder were not significant.

In a comparative study with Clark's nutcrackers, pinyon jays, and western scrub jays, Balda and Kamil (1989) calculated 42 correlation coefficients between cache order and recovery order. Twenty-five were above zero, one equaled zero, and the remaining 16 were below zero. Six of the positive correlations were significant, as was one of the negative correlations, indicative of a tendency toward recency effects, which was not statistically significant overall.

1.22.3.2.4 Proactive and retroactive interference

Serial position effects are often interpreted as due to the effects of interference, at least in part (see Shettleworth, 1998, for discussion; *See also* Chapters 1.06, 1.10). Two types of interference are generally recognized. If the target information was experienced before the interfering information, the effects of the interfering information are called retroactive interference. If the target was experienced after the interfering information, it is called proactive interference. As might be expected from the failure to find strong serial-position effects during cache recovery, attempts to document retroactive and proactive interference during caching have also yielded only

weak evidence for such effects. Bednekoff et al. (1997b) explicitly tested for interference in nutcrackers' cache memory by allowing caches to be made at different times. They found no evidence of interference between the two sets of caches.

Experiments using techniques other than cache recovery have found clear evidence for interference effects in parids and corvids. For example, when black-capped chickadees were presented with three-item lists in an operant associative task, they showed clear primacy and recency effects (Crystal and Shettleworth, 1994). And when Lewis and Kamil (2006) gave Clark's nutcrackers separate lists of locations to remember, they showed clear retroactive and proactive interference effects between the lists. These results raise the question of why such effects are weak in cache recovery but prevalent with other measures of memory performance.

1.22.3.2.5 Dynamism of memory

As caches are recovered, an additional problem arises: not only are there caches to remember but there are emptied caches to avoid. Do cachers avoid revisiting sites which have been emptied? Sherry (1982) examined this question by allowing marsh tits to cache in moss-filled trays then recover about half these caches 3 h later. Twenty-four hours after the initial caching, he allowed the tits to recover more caches. The birds clearly made more visits and spent more time at sites which had been cached in but not recovered from than sites that had been cached in and recovered from. Sherry (1984, experiment 2) obtained similar results in black-capped chickadees.

Frequent revisits to emptied cache sites have been observed in Clark's nutcrackers (e.g., Balda, 1980; Kamil and Balda, 1985). These studies, however, were not primarily intended to measure revisit probabilities against appropriate controls, so the implications of the frequent revisits were not clear. Balda et al. (1986) found that revisits to emptied caches by nutcrackers were much more frequent than expected by chance, and that revisit probability was not affected either by leaving signs of previous recoveries on the surface of the sand around cache sites or by reducing the number of seeds in a cache.

From a functional perspective, these observations are a puzzle since revisits increase foraging effort and may also increase predation risk. These considerations led Kamil et al. (1993) to take another look at revisits by Clark's nutcrackers, using a technique which allowed independent estimates of search accuracy and of preference. When they tested sites with cached seeds (good sites) vs. sites with cached seeds that had been removed by the birds (old sites) vs. holes that never had seeds in them (bad sites), the results clearly demonstrated that nutcrackers treated old sites differently than good sites. When they visited a cluster that contained an old site, they probed more of the alternative sites than when they visited good sites that contained seeds. In addition, they visited clusters containing good sites earlier than those containing old sites. They also found that old sites were visited earlier than clusters containing bad sites. Once the good sites have been exploited, the birds are more likely to visit old sites where they cached and then removed seeds than to visit bad sites that never contained seeds.

1.22.3.2.6 Are all caches created equal?

In the Balda and Kamil (1992) study of long-term memory for cache sites, the group of birds tested at the longest cache-retrieval interval showed an interesting pattern of errors. Their error rate per recovery was approximately equal to that of the groups with the shorter retention interval until about 75% of their caches had been recovered. After that, they began to make more errors. This pattern suggested that after 280 days, the nutcrackers had begun to forget some of their cache locations and had recovered those that they remembered most accurately first.

Kamil and Balda (1990) controlled the order of cache recovery by covering the floor in one-quarter of the caching room with canvas. Over four recovery sessions, each quarter of the room was covered once. The accuracy of recovery of this group was compared to that of a control group for whom all cache sites were always available. As predicted, the experimental group showed an initial recovery accuracy that was lower than that of the controls. But this accuracy level was constant over the four sessions, whereas the accuracy of the controls declined, so that during the fourth session the experimental group was more accurate than the controls. This clearly supports the hypothesis that some sites are remembered better than others, and then recovered first.

1.22.3.3 Coding of Cache Site Locations

When a scatter-hoarding animal remembers sites at which it has stored food, just what is it about the cache location that is remembered? Just how is the location encoded in memory? This raises basic questions about orientation and navigation. There is

an enormous literature on the cues that animals in a wide variety of taxa use to find locations during, for example, foraging, homing, or migration (*See* Chapters 1.12, 1.20, 1.25). In this section we will focus on studies that relate to how caching animals use landmarks to find locations and divide our review into studies that study cache recovery and studies that use other techniques.

1.22.3.3.1 Landmark use during the recovery of stored food

Many studies have demonstrated that landmarks play a crucial role in accurate cache recovery. For example, animals have been tested for their ability to find their caches when most or all of the landmarks present during caching have been removed from the caching area during recovery testing. If landmarks are important, this should produce substantial decrements in the ability to relocate caches, and it does (e.g., nutcrackers, Balda and Turek, 1984; parids, Herz et al., 1994). Barkley and Jacobs (1998) took a slightly different approach, allowing Merriam's kangaroo rats to cache and recover with either no or with many landmarks present. While the number of landmarks had no effect after a 1-day retention interval, there were large effects after 10 days. The kangaroo rats that had cached and recovered with no landmarks performed at much lower levels than those who had cached and recovered with 16 landmarks present. While such studies establish the role of landmarks in cache recovery, they tell us little about what mechanisms might be used. Few studies, however, have attempted to determine the mechanisms that are used during cache recovery.

The first attempt to do so of which we are aware was by Bossema and Pot (1974). They compared the routes used by individual Eurasian jays (*Garrulus glandarius*) when making and recovering caches. They found that the jays tended to use the same route during recovery as during caching more often than would be expected by chance. Bossema and Pot suggested that the birds used a snapshot of the scene from the cache site when they cached, then matched what they saw to the snapshot during recovery.

Kamil et al. (1999) found the Bossema and Pot interpretation unconvincing. There are many other reasons that could result in use of the same path during caching as during recovery. They conducted an intensive videotape study of movement patterns by nutcrackers during caching and recovery, using a technique that allowed estimates of the accuracy of recovery of each individual cache. Like Bossema and Pot (1974), Kamil et al. (1999) found that the birds tended to frequently use the same path during caching and recovery, but differing paths and body orientations were also often used. Because their procedure allowed cache-by-cache estimates of accuracy, Kamil et al. (1999) were able to determine the effect of consistency of direction on recovery accuracy. There were none: Birds were equally accurate regardless of the path used. This result argues directly against the snapshot hypothesis.

Bossema (1979) looked at the locations of caches and the accuracy of recovery relative to the positions of landmarks. He found that Eurasian jays cached more near vertical objects and were more accurate at retrieving their caches when vertical objects were available as beacons, as opposed to horizontal objects. Similar results have been found in studies in which caching animals have been trained to find food in specific locations (see following). In a second test, he taught the birds to find hidden food in a specific spatial location relative to two vertical landmarks. He performed tests in which one landmark was removed or the landmarks were moved further apart or closer together. From these tests, he concluded that the jays were using the distance between the spatial location and the line between the landmarks to orient.

Vander Wall (1982) took a different approach. After Clark's nutcrackers had cached food in an arena with an array of landmarks, he displaced the landmarks in one half of the room 20 cm from their original location during cache recovery (**Figure 2**). During the subsequent recovery session, the birds shifted their searching approximately 20 cm in the direction of the displacement in that half of the room. When the birds searched for caches made in the center of the room, where some of the nearby landmarks had been displaced and others had not, their search was displaced approximately 10 cm in the direction of the displacement. This suggests that the birds were integrating information from multiple landmarks (shifted and nonshifted) and searching at some kind of averaged location.

One of the ways to use landmarks to find a location is to use the directional relationship between the cache site and one or more landmarks (e.g., Kamil and Cheng, 2001). The use of directional information requires a compass. In a series of studies, Wiltschko and Balda (1989) and Wiltschko et al. (1999) have used clock-shift procedures in outdoor aviaries to demonstrate the use of a sun compass by scrub jays and nutcrackers. For example, Wiltschko and Balda (1989) had scrub jays cache in a 90° sector of a

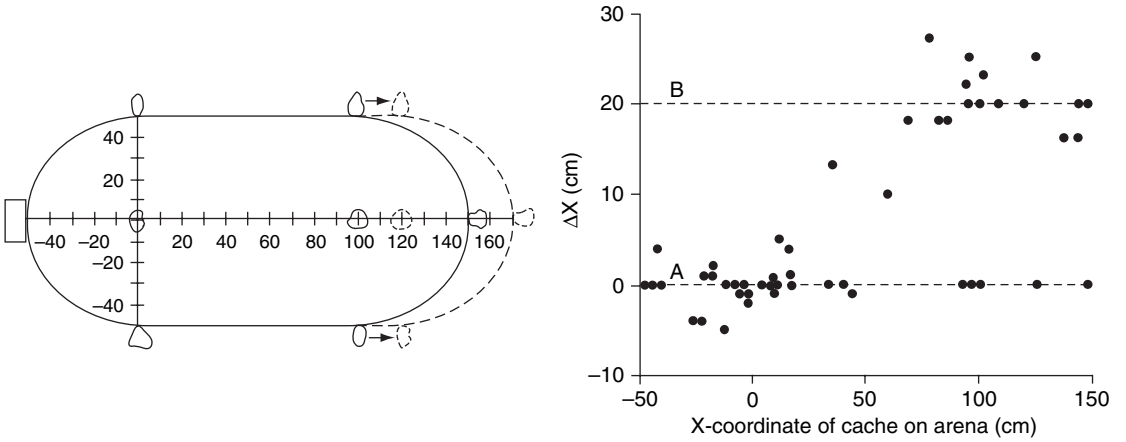

Figure 2 The left panel shows the caching arena during control (solid lines) and during landmark-shift (dashed lines) conditions. The right panel shows the distance between a probe and the nearest cache on the y-axis as a function of the original position of the cache in the x-axis. If the birds followed the shift, a Δx score of 20 cm would be expected. Reprinted from Vander Wall SB (1982) An experimental analysis of cache recovery in Clark's nutcracker. *Anim. Behav.* 30: 84–94, copyright 1982, with permission from Elsevier.

circular outdoor arena and then recover after being clock-shifted 6 h. When clock-shifted, the search was concentrated in an adjacent 90° sector, as would be expected if the sun compass was being used. Although these experiments clearly establish the use of sun compass under some conditions, caches can be recovered in the absence of information from the sun (e.g., indoors). Furthermore, when multiple landmarks are used to encode locations, clock shifts should produce conflicting effects (Kamil and Cheng, 2001).

Another issue that has been investigated both during cache recovery and during other tests of spatial memory in seed-caching animals is the relative importance of local versus global (or distal) cues. Local cues are those located relatively close to the goal location, while global or distal cues are generally larger, but further away. Several studies have found that birds seem to have some preference for caching near objects in the environment, suggesting that local cues may be quite important (e.g., Bossema, 1979). On the other hand, Balda et al. (1986) found that Clark's nutcrackers ignored local cues on the surface when making revisits to cache sites. The results of Herz et al. (1994) suggest the importance of global cues. Black-capped chickadees stored food on artificial trees placed within a symmetrical enclosure that had large global cues on each wall. There were unique color place cues located by each potential cache site. The removal of the place cues did not affect retrieval accuracy, but when the global cues were removed, search accuracy decreased. In a second study, when birds were only given global cues during caching, displacement of those cues during recovery produced a displacement of the search behavior. Watanabe (2005) has also shown the importance of global cues in remembering cache locations in Western scrub jays.

Results from mammals also suggest distal cues are important. Lavenex et al. (1998) found that fox squirrels use distal environmental cues rather than proximal cues to find food in a field experiment. Even when proximal spatial information was available, the squirrels chose to use the environment surrounding the apparatus to gain spatial information, presumably directional information or bearings. Jacobs and Shiflett (1999) devised an outdoor vertical maze to mimic the vertical structure of the squirrel's environment. They found that fox squirrels used distal cues to orient within this maze as well.

1.22.3.3.2 Landmarks and the coding of spatial locations

Cache recovery procedures are very limited for studies on how cachers encode spatial locations. As several of the studies reviewed suggest, the geometrical relationships among landmarks and between landmarks and the location of a cache are important. But it is very difficult to control location-landmark geometry when the caching animal is free to cache throughout the test arena. Therefore, many investigators have used procedures in which animals are trained to find buried food in a location defined by a set of local/global landmarks or other cues.

Bennett (1993a,b) trained Eurasian jays to find hidden food within an array of landmarks on the floor of an arena. The array consisted of landmarks that were either short or tall and either near or far from the hidden food location. He found that the birds relied more heavily on near, tall landmarks to find the food, a finding similar to that of Bossema (1979), described in the section titled 'Landmark use during the recovery of stored food.' This suggests that local cues are most important, especially if tall.

Other experiments, however, suggest that low horizontal features that define an edge can also be important. Cheng and Sherry (1992) trained black-capped chickadees to find food buried in a location defined relative to the locations of a cylindrical landmark and an edge (**Figure 3**). Then, when the landmark was shifted in a direction either parallel or perpendicular to the edge during probe trials, the birds followed parallel shifts more strongly. This was particularly clear during probe trials in which the landmark was shifted in a diagonal direction, which produced more parallel than perpendicular shifts in search. As Cheng and Sherry pointed out, these results suggest that perpendicular distance from an edge can serve as an important means of encoding spatial locations and are consistent with the results of Bossema (1979).

Similar studies with Clark's nutcrackers (Gould-Beierle and Kamil, 1996, 1998), pinyon jays, and scrub jays (Gould-Beierle and Kamil, 1998) have also found that the distance from an edge is important. In all three of these studies, birds found hidden food whose location was defined relative to the locations of an edge and a cylindrical landmark. As in the parids studied by Cheng and Sherry (1992), these corvids followed parallel shifts and shifted more readily in the parallel direction when given diagonal shifts. However, when the landmark was shifted in a direction perpendicular to the edge, nutcrackers, pinyon jays, and scrub jays did not shift their searching in that direction, in contrast to the results of Cheng and Sherry (1992).

Gould-Beierle and Kamil (1998) extended the conditions originally tested by Cheng and Sherry (1992) by testing the effects of varying the position and orientation of an edge and landmark across training trials with nutcrackers, pinyon jays, and scrub jays. Following this training, the birds were more sensitive to shifts in the position of the cylindrical landmark, shifting their search with each landmark shift to a much greater extent than birds trained with a nonshifting edge and landmark. The shifting of the relationship between the local cues (edge and cylinder) and the global cues (features of the room) appeared to result in a devaluation of both the global cues and distance from the edge. The extent to which distance from a line or edge is used thus depends upon the salience and location of other, more distal or global landmarks.

The relative importance of local versus more distal or global cues depends on context and on the distance between the local cues and the target location. Gould-Beierle and Kamil (1999) trained three groups of Clark's nutcrackers to find a hidden food site within an open room filled with wood chips. Two local cues were available near the food site, a cylindrical landmark and a horizontal piece of wood (much like the edge of a tray in previous studies). The groups varied in the distance the cylinder and edge were from the target location. They found that the

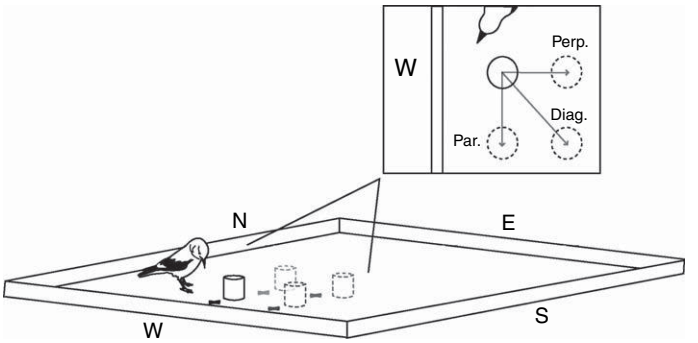

Figure 3 Typical setup (not to scale) for experiments on landmark displacement with an edge and a single landmark present. The birds are initially trained with the cylinder in the location indicated by the solid circle (top left in the inset). They are then tested with occasional nonrewarded trials at each of the three test positions, representing displacements perpendicular (Perp.), parallel (Par.), and diagonal (Diag.) to the long axis of the nearest edge. Drawing by Karina I. Helm.

group with these cues closest to the target used them more heavily to find the location, while the other two groups relied more on information from global cues within the room.

Goodyear and Kamil (2004) extended these results with a study in which different groups of Clark's nutcrackers were trained to find a buried seed at a location defined by an array of four landmarks, each of which was at a different distance from the goal, followed by probe tests with each of the individual landmarks. The groups differed in the mean distance from the landmarks to the goal location. For the group for whom the nearest landmark was quite close to the goal, the presence of the closest landmark had the greatest effect on search accuracy, an effect reminiscent of the overshadowing effect in Pavlovian conditioning (Gallistel, 1990). However, at longer goal–landmark distances, this overshadowing effect disappeared, and each landmark controlled search roughly equally.

Since geometry clearly affects search, there has been some interest in the ability of caching animals to directly learn geometric relationships. Kamil and Jones (1997) tested the ability of Clark's nutcrackers to learn a general geometric rule for spatial locations. They trained nutcrackers to find a seed that was always located halfway between two landmarks whose position in the room and interlandmark distance varied from trial to trial. The birds learned the task readily and searched extremely accurately when tested with new interlandmark distances. Follow-up studies demonstrated that nutcrackers could learn other geometrical rules (Kamil and Jones, 2000), including the use of relative bearings (Jones and Kamil, 2001). Comparative studies found that nutcrackers performed these tasks much more accurately than pigeons (Jones et al., 2002; Spetch et al., 2003).

One of the things that makes the location of cached food so interesting from a coding/navigation and orientation perspective is that successful cache recovery requires a very accurate search. Given the size of a nutcracker's beak and the size of pine seeds, for example, the bird must dig within 1–2 cm of the center of a cache in order to find it. This led Kamil and Cheng (2001) to hypothesize that nutcrackers encode the directional relationship between the goal and multiple landmarks. This was based on a combination of known features of search accuracy and a logical consideration. When nutcrackers are looking for a cache site that is not close to a landmark, the use of directional information results in a more accurate search than the use of distance information. But all compasses have error, and compensation for such error can be achieved by taking bearings to multiple landmarks. Although there are some data that support the model (Kamil et al., 2001), there are, as yet, insufficient data to fully evaluate the hypothesis.

1.22.4 The Evolution of Spatial Memory in Seed-Caching Animals

The capacity, duration, and dynamics of the memory that seed-caching animals use to relocate stored food seem quite impressive compared to the results of many studies of animal memory using standard psychological procedures such as the radial maze or matching-to-sample (*See* Chapters 1.20, 1.21, 1.23, 1.25, 1.26). This led to the development of the hypothesis that dependence on memory for the location of cached food would be associated with heightened memory abilities. This hypothesis, sometimes referred to as the ecological hypothesis, has led to many studies of memory comparing species that differ in their degree of dependence on cached food. We will divide our review of this literature by methodology, first discussing studies that used cache recovery as their measure of memory, then reviewing studies that used measures of spatial memory that do not depend on the caching and recovery of food.

1.22.4.1 Cache-Site Memory

There are relatively few comparative studies involving cache site memory. This is probably because such studies require the availability of a set of closely related species (or populations) which cache, but vary in some dimension of cache-related natural history, and there are few such instances. We are only aware of four such studies, two with corvids and two with parids.

Balda and Kamil (1989) compared the cache recovery accuracy of three corvid species that differ in their degree of dependence on stored food, Clark's nutcrackers, pinyon jays, and Western scrub jays, after a relatively short retention interval of 7 days. They found that the more cache-dependent species, nutcrackers and pinyon jays, recovered their caches more accurately and more rapidly than Western scrub jays. Bednekoff et al. (1997a) tested the same three species as well as Mexican jays after retention intervals of 10–250 days. They found that cache recovery performance of the two *Aphelocoma* species

(Mexican and Western scrub jays) was lower that that of the nutcrackers or pinyon jays, but that all four species performed with only modest accuracy levels (although still significantly better than chance) after the two longest retention intervals (of 150 and 250 days).

Healy and Suhonen (1996) compared marsh tits and willow tits. Willow tits live in harsher environments than marsh tits and are thought to retrieve their caches after longer retention intervals than marsh tits. In this study, however, no differences in the accuracy of cache recovery were found after either a short (1- to 2-h) or a long (17-day) retention interval. Pravosudov and Clayton (2002) compared two populations of black-capped chickadees, one from Alaska and the other from Colorado. They found that the birds from the harsher environment of Alaska cached more food and recovered it more efficiently than the birds from Colorado, demonstrating that different ecological pressures within this single species are correlated with differences in spatial memory ability. Thus three of these four studies found differences in cache recovery accuracy that were correlated with differences in dependence on stored food.

1.22.4.2 Noncache-Site Memory

Most comparative work on spatial memory involving scatter-hoarding species has been based on procedures that do not depend on caching. Such procedures are necessary to compare spatial memory between caching and noncaching species. In addition, data from such tests could address the question of just how specialized the spatial memory abilities of food-caching animals may be. A variety of techniques were applied, and we have organized our review of these comparative studies by the procedures used to test memory.

1.22.4.2.1 Window shopping

The window shopping task is probably the noncaching task most similar to cache memory. Instead of storing food in a location to be remembered, the bird encounters the seed, either behind a transparent window or a seed wedged into a small hole so tightly that it cannot be removed. Shettleworth et al. (1990) showed that memory for such encountered seeds appeared to be similar to that for stored seeds in black-capped chickadees and coal tits. Krebs et al. (1990) used the technique to test coal tits against nonstoring great tits. They found somewhat better performance in the storing coal tits. Coal tits were more likely than great tits to return to sites at which they had seen seeds. They were also better at discriminating between sites seen to contain seeds and those seen to be empty.

1.22.4.2.2 One-trial associative tasks

In one-trial associative tasks, two or more stimuli are all associated with a correct location. Following a single experience at that location, the subject is given test trials in which it chooses between the spatial location or the nonspatial stimuli (which are now presented in a new location; see **Figure 4**). A number of one-trial associative studies have used a variation of window shopping in which the bird finds food at a specific location which is also indicated by cues from an object. The bird is allowed to begin to eat, but is interrupted (removed from the experimental situation) before completely consuming the food. The test is to see where the birds will return to look for the seed, to the correct spatial location or the correct location based on object cues. In comparisons of food-storing and nonstoring species in both parid and corvid families (Clayton and Krebs, 1994), the food-storing birds (marsh tits and jays) went first to the correct spatial location, whereas the nonstorers (blue tits, *Parus caeruleus*, and jackdaws, *Corvus monedula*) went equally as often on their first choice to the correct spatial or object-specific location. When comparing food-storing chickadees to nonstoring dark-eyed juncos (*Junco hyemalis*), Brodbeck (1994) found similar results, with the chickadees responding preferentially to spatial cues and the juncos responding equally to spatial and object cues. These studies provide further evidence that food-storing birds focus heavily on spatial memory when returning to food sites.

In another variation on this theme, Brodbeck and Shettleworth (1995) placed spatial and object-related cues in conflict in the choice phase of test trials in a matching-to-sample experiment. With this technique, they found that while space was the first choice of food-storing chickadees, nonstoring juncos chose space and color equally. They also demonstrated that when shown a compound stimulus of both spatial location and color and tested on each element of the compound alone, chickadees performed better on the spatial element, while juncos performed equally well on both elements. This, along with the other one-trial associative memory experiments, demonstrates the importance of spatial information to food-storing birds.

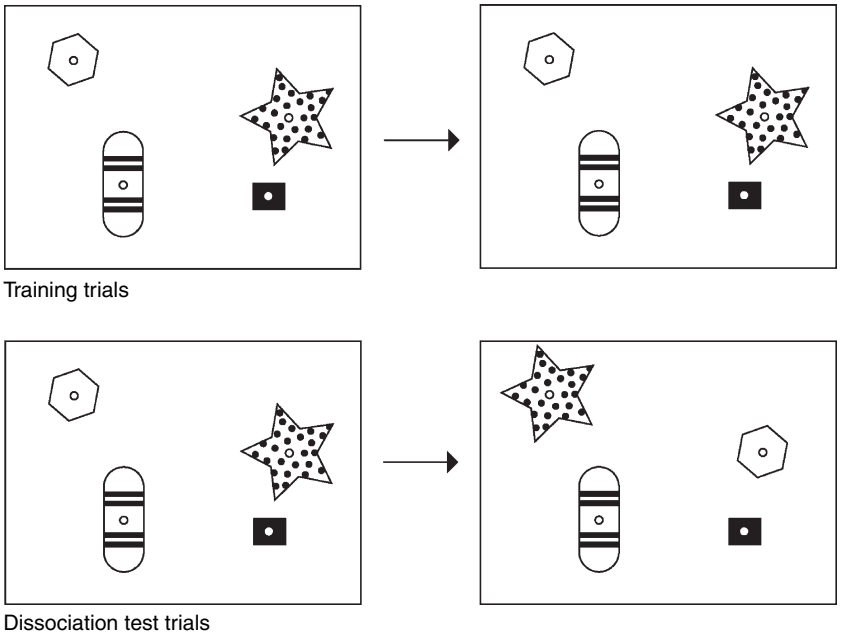

Figure 4 Diagrammatic representation of the logic of one-trial associative tasks. The top pair of figures shows training trials. One of the stimuli is randomly designated correct on each trial (with new, trial-unique stimuli used for each trial). The bird is then rewarded when it pecks the correct stimulus. The display then disappears for a retention interval, and the same display is presented for choice, and the bird is rewarded for pecking at the same stimulus. Once this training is complete, the bird receives occasional dissociation test trials, as shown in the bottom pair of figures. These trials differ from training trials in that the spatial locations of two of the stimuli are switched. If the bird pecks at the same visual stimulus (the dot-filled star, in this case), this indicates control by the stimulus. But if the bird pecks at the old location (the hexagon), it suggests spatial control. Spatial location and visual stimulus have been dissociated. Drawings by Karina I. Helm.

Lavenex et al. (1998) used an approach similar to that of one-trial associative tests in a field experiment. Although their training task involved multiple trials, they gave fox squirrels different spatial and nonspatial relational proximal cues that could be used to predict the locations of nuts buried by experimenters. The squirrels used spatial over nonspatial information to solve the task, even when both were available, a result similar to the results of laboratory one-trial associative experiments just reviewed.

1.22.4.2.3 Open-room radial maze

In an open-room radial maze procedure, Hilton and Krebs (1990) tested two storing parid species, two nonstoring parid species, and a nonstoring greenfinch in an open-room analog of the radial maze. They found decreasing performance as the retention interval increased from 30 s to 24 h. The food-storing tits (marsh and coal tits) performed above chance after 24 h, although the extent to which their performance exceeded chance was modest. In contrast, neither the nonstoring tits (blue and great tits) nor the finches performed above chance after 24 h.

Kamil et al. (1994) tested four corvids who vary in dependence on stored food in their version of an open-room analog of the radial maze. They found that the two species most dependent on stored food, Clark's nutcrackers and pinyon jays, acquired the task to higher levels than the less dependent species, Mexican and Western scrub jays. When retention intervals of 30–300 min were tested (in ascending order), the species differences tended to disappear as the retention interval got longer. Only the most dependent species, the nutcrackers, performed above chance after a 24-h retention interval, although, as in the marsh and coal tits, their performance was only modestly better than chance.

Gould-Beierle (2000) also tested four corvid species – nutcrackers, pinyon jays, Western scrub jays, and jackdaws – on a version of the open-room radial maze task. She included both a reference memory and a working memory component by having 12 holes in the floor, four of which were never correct while the other eight were used in the usual way as working memory locations. She found that pinyon jays and scrub jays performed better than nutcrackers

and jackdaws in both the working and reference memory aspects of the procedure. When looking at the first four searches in the maze, however, the nutcrackers performed as well as the two jay species in working memory and there were no species differences in reference memory. The performance of the scrub jays was not expected and suggests further exploration into the spatial memory abilities of this species. Perhaps combining both a working and reference memory task simultaneously affects spatial memory differentially in these species.

Barkley and Jacobs (2007) used an open-room task similar to a radial maze analog. They trained two species of kangaroo rat in a task in which the animal was shown four locations (randomly chosen out of 128) and then tested for their ability to remember the four 24 h later. One species was the scatter-hoarding Merriam's kangaroo rat (*Dipodomys merriami*), a species that hoards intensively. The other was the leaf-eating specialist Great Basin kangaroo rat (*D. microps*), which relies less on scatter-hoarding than Merriam's. Merriam's kangaroo rat performed considerably better than the Great Basin kangaroo rat on this task.

1.22.4.2.4 Operant tasks

A number of investigators have used several different operant tasks to measure differences in memory ability among storing species and between storing and nonstoring species. The most commonly used procedure has been spatial delayed nonmatching- or matching-to-sample. In this task, each trial consists of two parts: the presentation of the sample followed by the presentation of a choice test. Thus, for example, Olson (1989, 1991) had two keys on the front wall of an operant chamber. Each trial began with the illumination of one of those keys, chosen at random on each trial. After the bird had pecked at that key and moved to the back of the box to peck at another, single key located there (to break up any patterns of settling in front of the to-be-correct key), the bird was presented with two keys and rewarded only for pecking at the key that had not been pecked at earlier in the trial (nonmatching). Olson (1989) tested Clark's nutcrackers, scrub jays, and pigeons (*Columba livia*). Although all three learned the task with equal facility, the nutcrackers outperformed the other two species when the task was made more difficult by either titrating the delay between sample and choice test or by introducing multiple samples to be remembered.

These results were extended in a subsequent study (Olson et al., 1995) with nutcrackers, pinyon jays, scrub jays, and Mexican jays. In this study, a computer monitor and touch screen were used. When the delay interval was titrated, the nutcrackers performed at much higher levels than the other three species. After completing this spatial nonmatching test, the birds were then tested on an almost exactly equivalent nonspatial task. In this experiment, the samples could be either red or green and the bird had to remember the color rather than the location. Under these conditions, the ordering of the species changed completely, and none of the species differences were statistically significant (**Figure 5**).

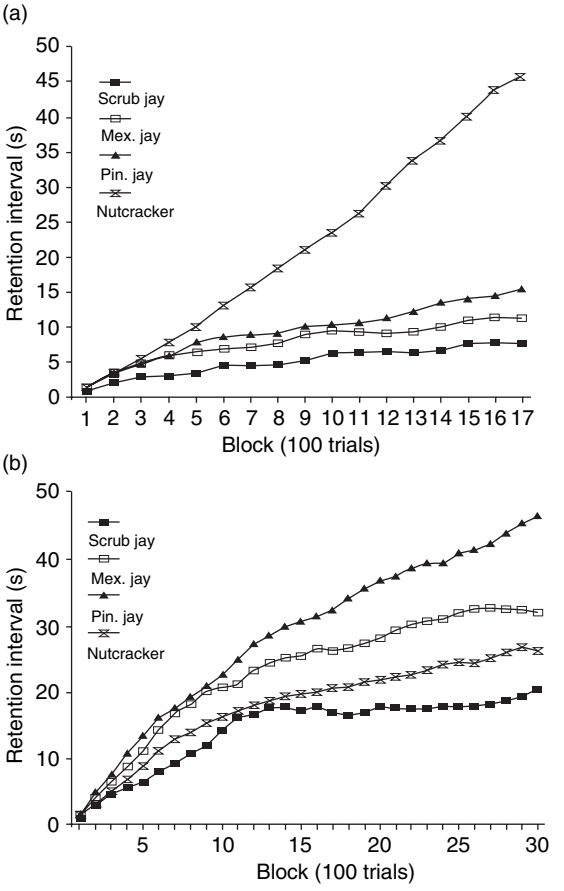

Figure 5 (a) Performance of scrub jays, Mexican jays, pinyon jays, and nutcrackers during spatial nonmatching-to-sample titration. (b) Performance of each species during color nonmatching-to-sample titration. Data are presented as averages of blocks of 100 trials. From Olson DJ, Kamil AC, Balda RP, and Nims PJ (1995) Performance of four seed-caching corvid species in operant tests of nonspatial and spatial memory. *J. Comp. Psychol.* 109: 173–181; used with permission from the American Psychological Association.

Healy and Krebs (1992) studied matching-to-sample in marsh tits and great tits, using a choice apparatus attached to the birds' home cage. The birds took a seed from the correct location, which was signaled both by location and by a visual object, then returned to their home cage to consume the object. They were then given a choice test, and the two species performed very similarly. The only significant different between the species was superior performance by the storing marsh tits early in acquisition of the task. Healy (1995) used a more traditional nonmatching-to-sample (NMTS) test on a computer monitor with four parid species, two storing species (coal and marsh tits) and two nonstoring species (blue and great tits). The birds performed well at retention intervals as long as 100 s, but there were no differences between the storers and nonstorers. This may have been due to the presence of spatial and nonspatial cues. It is also possible that storing and nonstoring tits perform similarly during matching-to-sample type procedures.

1.22.5 Neural Substrates

The central role of spatial memory in the recovery of scatter-hoarded food raises a number of questions about neural substrates. Which areas of the brain are used during cache recovery? What types of species differences in neural structure are associated with the evident differences between species in performance on cache recovery and other tests of spatial memory? In this section we review the literature relevant to these questions.

1.22.5.1 Role of the Hippocampus in Spatial Memory

O'Keefe and Nadel (1978) first proposed a central role for mammalian hippocampus in spatial memory. This hypothesis has been confirmed by experiments in many different laboratory tasks (*See* Chapters 1.33, 2.11). Most of these experiments, however, have been carried out in mammals, while most research on spatial memory in scatter-hoarders has been carried out in birds. What is known about the avian hippocampus (**Figure 6**)?

In this context, it is interesting to note that there is a radically new view of brain evolution and the structure of the avian cerebrum, a view that emphasizes the large number of avian–mammalian homologies (Jarvis et al., 2005). This view has led to a proposal for a radical revision of the nomenclature for avian cerebrum, a nomenclature that "better reflects these functions and the homologies between avian and mammalian brains" (Jarvis et al., 2005: 2). Research with scatter-hoarding birds and mammals is consistent with this revision. The dorsomedial region of the avian telencephalon has been shown to be homologous to the mammalian hippocampal formation in many regards. This includes connectivity (Krayniak and Siegel, 1978; Casini et al., 1986; Szkeley and Krebs, 1996), distribution of neuropeptides and neurotransmitters (Erichsen et al., 1991; Krebs et al., 1991; Gould et al., 2001), generation of long-term potentiation (Shapiro and Wieraszko, 1996) and a theta rhythm (Siegel et al., 2000), electrophysiology (Siegel et al., 2002), and *N*-methyl-D-aspartate (NMDA) receptor activation (Shiflett et al., 2004) and immediate early gene expression (Smulders and DeVoogd, 2000b; Shimizu et al., 2004) during spatial tasks. Behaviorally, lesions to pigeon hippocampus disrupt performance on a variety of spatial memory tasks such as learning spatial representations in homing (reviewed in Bingman et al., 2005), spatial reversal learning (Good, 1987), spatial alternation (Reilly and Good, 1987), and spatial delayed matching-to-sample (Good and Macphail, 1994).

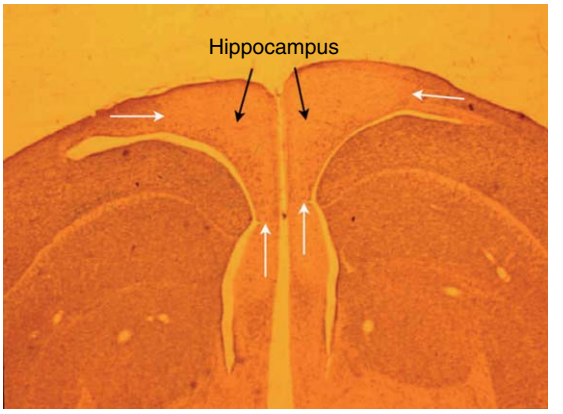

Figure 6 A photomicrograph of a coronal section through the avian hippocampus, with boundaries indicated by the white arrows. (Photograph by Kristy Gould.)

1.22.5.2 The Hippocampus in Food-Storing Birds

In the case of food-storing birds, hippocampal lesions disrupt cache retrieval. Krushinskaya (1966) lesioned the dorsomedial and dorsolateral sections of the hippocampus of Eurasian nutcrackers after they had stored

food in a dirt-floored laboratory room. When given the opportunity to recover, lesioned birds retrieved 13% of their caches while nonlesioned controls recovered around 90% of theirs. Although Krushinskaya's lesion methods were crude, and she may have inadvertently damaged areas outside of the hippocampus, later research has confirmed the role of hippocampus in cache recovery and spatial memory using lesion techniques on black-capped chickadees during both food storing (Sherry and Vaccarino, 1989) and delayed spatial matching-to-sample tasks (Hampton and Shettleworth, 1996). Temporary inactivation of the hippocampus in black-capped chickadees also produces memory impairment in a spatial associative task (Shiflett et al., 2003), indicating hippocampal involvement in storing and retrieving spatial information in the short term.

These results strongly suggest that the species differences in spatial memory and cache recovery should be reflected in differences in hippocampal structure. Comparative studies of avian hippocampus have found that species that store food have a larger relative hippocampal size than those that do not (Krebs et al, 1989; Sherry et al., 1989; Garamszegi and Eens, 2004; Lucas et al., 2004; but see Brodin and Lundberg, 2003). Correlations are also found between food storing behavior, spatial memory performance, and relative hippocampal volume for food-storing birds within corvids (Healy and Krebs, 1992; Basil et al., 1996) and parids (Hampton et al., 1995; Healy and Krebs, 1996), as well as in food-storing rodents within the kangaroo rat family (Jacobs and Spencer, 1994).

There are also population differences in hippocampal volume within species. Black-capped chickadees that live further north, in harsher climates (e.g., Alaska), store more food, perform better on spatial tasks, and have a larger hippocampus than birds living further south (e.g., Colorado) (Pravosudov and Clayton, 2002; but see Brodin et al., 1996). Similar population differences may exist in other species. For example, Pravosudov and de Kort (2006) analyzed the brains of a large number of scrub jays, which have been classified as storing fewer seeds (Balda and Kamil, 1989) and performing less accurately during many spatial memory tasks (e.g., Balda and Kamil, 1989; Olson, 1991, see previous); they have been found to have a smaller hippocampus than other food-storing corvids (Basil et al., 1996). Their data indicated a significantly larger relative hippocampal volume than the scrub jays in Basil et al. (1996). This difference in results may be due to methodological differences (paraffin-embedded vs. frozen tissue). On the other hand, the scrub jays used in the two studies came from different regions (northern Arizona and northern California), and there may be population differences within scrub jays in hippocampal size correlated with natural history.

1.22.5.3 Experience, Seasonality, and Neurogenesis in Birds

There are also important interactions between early environment, seasonality, and hippocampal growth. In at least some food-storing birds, early experience with food storing contributes to the development and ultimate size of adult hippocampus. When juvenile food-storing parids are given the opportunity to store food, they perform better on tests of spatial memory (Clayton, 1995, 2001) and develop larger hippocampi with more neurons and an increased cell proliferation rate compared to food-storing parids that are not allowed to store food (Clayton, 1996; Patel et al., 1997). Juveniles given the opportunity to perform noncaching spatial memory tasks also perform better and have a larger hippocampus than those that were not (Clayton, 1995). The developing hippocampus seems to be sensitive to experience with tasks that require the recall of spatial locations, at least in food-storing parids. However, food-storing experience during adulthood does not change the volume or number of hippocampal neurons (Cristol, 1996). This all suggests that early experience with food storing leads to the development of a larger adult hippocampus with more neurons (Healy and Krebs, 1993, Healy et al., 1994) and a high cell proliferation rate (Patel et al., 1997).

Seasonal changes in the neural tissue associated with birdsong in species that sing seasonally are well known (Nottebohm, 1981). Similar phenomena have been demonstrated in birds that cache/recover seasonally. Barnea and Nottebohm (1994) studied hippocampal neurogenesis in adult black-capped chickadees and found a seasonal difference in neuronal recruitment, with more new neurons in October than any other time of year. This corresponds to a time of seasonal diet change, from insects to seeds, with many of the seeds being stored (see Pravosudov, 2006, for discussion of a bimodal peak in food storing among parids). Barnea and Nottebohm did not, however, find a seasonal difference in total number of hippocampal neurons. They hypothesized that seasonal recruitment is part of a neuronal replacement process important for the acquisition of new spatial

memories. As seeds begin to be stored in October, new memories are established, requiring new neurons. Without a change in total neuron number, however, there must be apoptosis occurring as the new neurons are recruited.

Smulders et al. (1995) reported a seasonal change in the relative volume of the hippocampus in black-capped chickadees, with the peak in October. This seemed to complement the results of Barnea and Nottebohm (1994). Smulders et al. (2000), however, concluded that this change in volume was related to an increase in the total number of neurons in the hippocampus. Barnea and Nottebohm did not find seasonal changes in total neuron number, only in the number of new neurons. Smulders and DeVoogd (2000a) hypothesized that the overall increase in neurons they found was the mechanism allowing greater processing of spatial information in the fall. The more neurons, the more spatial information can be processed. This differs from Barnea and Nottebohm's hypothesis of neuron replacement with no net gain in number of neurons.

Hoshooley and Sherry (2004) attempted to distinguish between the hypotheses of Barnea and Nottebohm (1994) and Smulders et al. (2000) by determining if the seasonal changes in chickadee hippocampus were a result of more 'new' neurons or an increase in the actual 'production' of neurons. They found no seasonal change in hippocampal volume, total neuron number, or neuron production, which suggests enhanced survival of new neurons in the fall, not an increase in neuron production. Smulders (2006), however, has pointed out that the birds used by Hoshooley and Sherry (2004) were held in captivity for up to 2 weeks before they were sacrificed and that captivity can cause decreases in neurogenesis (Barnea and Nottebohm, 1994) and hippocampal volume (Smulders and DeVoogd, 2000a) in birds.

There appear to be two mechanisms affecting hippocampal size in food-storing parids. First, food storing experience early in life increases adult hippocampal volume by influencing the total number of neurons and the extent of cell proliferation. Second, in adults, when demand for spatial memory increases because of food-storing, either the number of hippocampal neurons increases or the number of new neurons that survive increases, resulting in a larger population of new hippocampal neurons to process new memories being formed. Further work will be necessary to fully understand the reasons for the increase in neuronal recruitment found by Barnea and Nottebohm (1994).

A possible complication is that cell proliferation is correlated with spatial memory and social status in mountain chickadees (*Parus gambeli*; Pravosudov and Omanska, 2005). Subordinate mountain chickadees performed worse on spatial memory tasks (Pravosudov et al., 2003) and also had lower cell proliferation rates (Pravosudov and Omanska, 2005) than their dominant counterparts. Individual birds that performed better on spatial memory tasks also had higher cell proliferation rates, suggesting a strong correlation between proliferation and spatial memory. However, no differences were found in hippocampal volume or total neuron number.

Other hippocampal differences have been found between food-storing and nonstoring birds. This includes larger calbindin-immunoreactive neurons in the hippocampus of food-storing than nonstoring corvids and parids (Montagnese et al., 1993) and significantly lower levels of NMDA-binding receptor sites in the hippocampus of food-storing parids (Stewart et al., 1999). How these differences might be related to food-storing is unclear. However, blocking NMDA receptors when black-capped chickadees are learning a one-trial spatial association task prevents the retrieval of the food after either 3 or 24 h. It also blocks learning about a new spatial location within the context of an already learned array of locations (Shiflett et al., 2004). This suggests that the avian hippocampus plays a role in linking new spatial locations into preexisting spatial memories (Smulders, 2006) and that NMDA receptor activation is important only in processing spatial information over the long term. Food-storing birds have fewer hippocampal NMDA receptor sites, which seems contradictory to these results. But this highlights the complexity of the relationship between the NMDA system and food-storing and the need for future work in this area.

1.22.5.4 Role of the Hippocampus in Mammals

In food-storing mammals, three studies have addressed species and seasonal differences in hippocampal neuroanatomy. Lavenex et al. (2000a,b) found no seasonal variations in hippocampal volume, total neuron number, or cell proliferation rates in the adult scatter-hoarding eastern gray squirrel (*Sciurus carolinensis*), an interesting contrast with the results from birds (Smulders et al. 1995, 2000) in terms of seasonal changes in volume and neuron number. Barker et al. (2005) compared the yellow pine chipmunk, both a

larder- and scatter-hoarder, to the scatter-hoarding eastern gray squirrel during the fall when both species were actively collecting and storing food for winter. Gray squirrels had three times the number of proliferating cells in the dentate gyrus of the hippocampus, but no significant difference in the number of new neurons compared to the yellow pine chipmunk. There was a nonsignificant trend suggesting gray squirrels had more new neurons, and Barker et al. (2005) hypothesized that the greater number of proliferating cells provided a larger population from which to recruit new neurons into the hippocampus.

The Barker et al. (2005) results were quite different from those of Lavenex et al. (2000a) in terms of cell proliferation rates, but there were two major methodological differences. While Barker et al. used free-living animals sacrificed within 2 h of capture, Lavenex et al. used animals that had been in captivity for days before sacrificing. In addition, Barker et al. analyzed endogenous proteins that are indicators of neurogenesis, proteins that would be conserved at time of capture regardless of stress due to capture. Lavenex et al. assessed neurogenesis with a mitotic marker, which can be affected by stress of captivity (Barker et al., 2005). The results of Barker et al. (2005) were also different from those of Hoshooley and Sherry (2004), in that cell proliferation was related to spatial memory, but not the number of new neurons. These differences may also be the result of differences in stress due to captivity. It is also possible that there are different mechanisms producing hippocampal seasonal changes in mammals and birds.

1.22.5.5 Other Brain Areas

Brain areas other than the hippocampus play important roles in processing spatial information. However, the contribution of these areas to the recovery of scatter-hoarded food has not been investigated very thoroughly. These areas include the parahippocampal region (or Wulst in birds), the prefrontal cortex (or caudolateral nidopallium in birds), the septum, and visual areas. All of these brain regions have connections with the hippocampus in both mammals and birds and their contribution to spatial memory in mammals has been extensively studied. But we have little understanding of their contribution to spatial memory in birds or to scatter-hoarding in general for either rodents or birds. A handful of studies show general differences in the volume of two of these structures in food-storing birds. Gould et al. (2001) show that the medial substance P receptor field within the parahippocampal area of the food-storing black-capped chickadee is larger than that found in the nonstoring blue tit and great tit. Shiflett et al. (2002) showed that the septum is larger in chickadees than in blue and great tits. What these results mean in relation to food-storing is not clear, but research investigating brain regions connected with the hippocampus and their contributing role to spatial memory should be continued.

1.22.5.6 Cognitive Pleiotropy

Like genes, cognitive abilities can affect more than one trait. Spatial memory, for example, can facilitate territoriality or migration as well as cache recovery. This complicates the analysis of the relationship between natural history and cognition/neuroanatomy. If evolution has favored larger hippocampal volumes in some species, there must be strong advantages to such investment for those species, given the high cost of maintaining neural tissue (Attwell and Laughlin, 2001). But the hippocampus undoubtedly plays a significant role in behaviors other than relocating stored food. For example, Volman et al. (1997) looked at hippocampal volume in two species in each of two genera of woodpeckers. In *Melanerpes*, they found a larger hippocampus in a scatter-hoarding species than a larder-hoarder. But in *Picoides*, they found generally large hippocampal volumes even though neither *Picoides* species scatter-hoards. They suggest that factors other than scatter-hoarding may influence hippocampal size.

One such factor is migration. Healy et al. (1996) demonstrated that experience with migration has an impact on the size of the hippocampus in Garden warblers (*Sylvia borin*), who normally migrate from Europe to tropical Africa. They found that warblers at least 1 year old that have experience migrating have a larger hippocampus after at least one migration trip than 3-month-old, naive birds. In contrast, no age effect was found in nonmigratory Sardinian warblers (*S. melanocephala momus*), who had relatively small hippocampi, suggesting that it is the migratory experience and not some other maturational factor that affects hippocampus size.

Mettke-Hofmann and Gwinner (2003) extended these results with behavioral measures. They found better long-term spatial memory in migratory garden

warblers than nonmigratory Sardinian warblers. Taken together, these studies suggest a picture for these migratory and nonmigratory congeners similar to that found for storing and nonstoring parids by Clayton and her colleagues (reviewed in the section titled 'Memory for cache contents'). The connection between migration, spatial memory, and hippocampal structure is further indicated by the research of Cristol et al. (2003).

1.22.6 Conclusions

The study of spatial memory in scatter-hoarding animals has enriched the scientific understanding of animal cognition. The duration, capacity, and dynamism of this memory have driven impressive, stimulating research into both ultimate-evolutionary and proximate-neurophysiological explanations. The most important impact of this research has probably been its contribution toward integrating biological and psychological approaches to animal cognition, combining concepts and designs from psychology with those from biology in a broadly evolutionary framework, leading to a better understanding of the complex relationships between natural history, cognition, and brain structure and function.

At another level, the cache recovery context has proved an extremely valuable setting for experiments on animal cognition. As our review has hopefully demonstrated, experiments on caching and recovery have extended ideas about the memorial capacities of animals. Many interesting questions about cache memory remain, questions such as the role of interference in forgetting, how information about the emptying of cache sites affects cache site memory, and exactly how cache site locations are encoded. In addition, caching and recovery are also providing an extremely useful context in which to study other important aspects of animal cognition such as episodic-like memory and social cognition (*See* Chapter 1.23).

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1.23 What Do Animals Remember about Their Past?

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1.23.1 Introduction

Episodic memory refers to the ability to remember specific personal happenings from the past. Ever since Tulving first made the distinction between episodic memory and other forms of declarative memory in 1972, most cognitive psychologists and neuroscientists have assumed that episodic recall is unique to humans, in part because our reminiscences are accompanied by the subjective awareness of remembering (e.g., Tulving, 1983; Suddendorf and Corballis, 1997; Wheeler, 2000; *See also* Chapters 1.02, 1.04, and 1.21). Tulving argues that, like many animals, his pet cat can acquire and retrieve all kinds of information about events that have happened in the past, but only in a way that is devoid of any awareness of remembering such events. So while his cat may know that she caught a mouse, what she does not recall is the personal experience of having caught that mouse. To be fair, humans also have many instances of knowledge acquisition in which we do not remember the episode in which we acquired that information. For example, although most of us know when and where we were born, we do not remember the birth itself or the episode in which we were told when our birthday is.

According to Tulving, the retrieval of semantic factual knowledge and episodic memories can be differentiated in terms of the remember-know distinction. To *know* that the Psychological Laboratory was opened in Cambridge in 1912 is semantic knowledge, whereas *remembering* having attended a comparative cognition lecture at Cambridge University is an episodic memory. Remembering and knowing are thought to be two separate subjective states of awareness, the former being an awareness of reliving past events in the mind's eye (what he later called mental time travel; Tulving, 2000), whereas the latter only involves an awareness of knowledge without any requirement to travel mentally back in time to reexperience the past (Gardiner and Richardson-Klavehn, 2000).

In humans, these episodic memories are often recalled vividly and contain a rich representation of the past event. Furthermore, the memories may appear quite suddenly, and out of the blue. In his autobiographical novel *Remembrance of Things Past,* Marcel Proust (1922) described such a moment of episodic recall:

> And suddenly the memory returns. . . . The taste was that of the little crumb of madeleine which . . . my aunt Léonie used to give me, dipping it first in her own cup of real or of lime-flower tea. And once I had recognized the taste of the crumb of madeleine soaked in her decoction of lime-flowers which my aunt used to give me . . . immediately the old grey house upon the street, where her room was, rose up like the scenery of a theatre to attach itself to the little pavilion, opening on to the garden, which had been built out behind it for my parents (the isolated segment which until that moment had been all that I could see); and with the house the town, from morning to night and in all weathers, the Square where I

> used to be sent before lunch, the streets along which I used to run errands, the country roads we took when it was fine.

One of the cardinal features of episodic memory is that it operates in what Tulving (2002) calls "subjective time," namely that remembering the event is always accompanied by an awareness of traveling back to the past time in which the experiences were recorded (see also Hampton and Schwartz, 2004). Episodic memory differs from all other kinds of memory in being oriented to the past, and specifically in the past of the owner of that memory. So while some factual memories do involve a datable occurrence, they are fundamentally different from episodic memories. Indeed, as William James so aptly wrote:

> Memory requires more than the mere dating of a fact in the past. It must be dated in *my* past. (James, 1890: 650)

Tulving (2002) argues that episodic remembering requires a specific form of self-consciousness, *chronesthesia,* that enables an individual to address her own, personally experienced past, which "does not reside in memory traces as such; it emerges as the phenomenally apprehended product of episodic memory system" (Tulving, 2000: 17), much like the piece of madeleine dipped in linden tea enabled Proust to consciously relive the past as a simultaneous part of his present. Many cognitive neuropsychologists have argued that this ability to travel back in time in the mind's eye to reexperience the past is unique to humans; animals, by contrast, are stuck in the seemingly eternal present (e.g., Tulving, 1983; Suddendorf and Corballis, 1997; Roberts, 2002). So according to Tulving (1983), his pet cat does not recall the personal experience of having caught the mouse, nor is she aware that the event is explicitly located in her past.

Language-based reports of episodic recall suggest not only that the retrieved experiences are explicitly located in the past but that they are also accompanied by the conscious experience of one's recollections (e.g., Wheeler, 2000), of feeling that one is the author of the memory, what Tulving (1985) called *autonoetic consciousness*. It is this feature of episodic recollection that William James referred to when describing what he called the "warmth and intimacy" of one's episodic memories (James, 1890).

This phenomenological definition makes it impossible to assess the claim that episodic memory is unique to humans, because there are no agreed nonlinguistic behavioral markers of these kinds of conscious experiences in nonhuman animals (Griffiths et al., 1999), and therefore we have no way of assessing whether Tulving's cat, or any other animal, does or does not experience an awareness of the passing of time and of reexperiencing one's own memories while retrieving information about a specific past event. This dilemma can be resolved to some degree, however, by using Tulving's original definition of episodic memory (Tulving, 1972), according to which he identified episodic recall as the retrieval of information about where a unique event occurred, what happened during the episode, and when it took place. The advantage of using this definition is that the simultaneous retrieval and integration of information about these three features of a single, unique experience may be demonstrated behaviorally in animals. We refer to this ability as *episodic-like memory* (Clayton and Dickinson, 1998) rather than episodic memory, because we have no way of knowing whether or not this form of remembering is accompanied by the various phenomenological aspects that accompany conscious recollection in humans.

1.23.2 Animal Studies

Some of the earliest evidence that animals may be capable of episodic-like recall came from studies of rats foraging in a radial arm maze (*See* Chapter 1.22). In a paper entitled "Remembrance of places passed: Spatial memory in rats," Olton and Samuelson (1976) argued their laboratory rats could remember which arms had already been chosen and/or which had not in order to forage efficiently by avoiding those arms they had visited previously. Another potential example of episodic-like memory came from studies of visual short-term memory in which rhesus monkeys (*Macaca mulatto*) had to choose, for example, either the previously presented stimulus or the novel stimulus in a delayed matching or nonmatching to sample task (Mishkin and Delacour, 1975). In both of these studies, the animals may have solved the task by remembering the specific past event – about which arms had been visited in the case of the rats, and which stimuli had been seen in the case of the monkeys. There is a simpler alternative, however, namely that the animals could have based their decision of where to search in the maze or which stimulus to choose in the matching tasks on familiarity rather than recall; for example, they could simply have learned to avoid stimuli that look familiar, arms in the case of a rat in the radial maze and objects in the

case of the monkey performing the matching task (see Griffiths et al., 1999).

There is growing evidence that familiarity and episodic recall are separate cognitive processes, both psychologically (Mandler 1980; Jacoby and Dallas, 1981; Kelley and Jacoby, 2000) and neurobiologically (Aggleton and Brown, 1999, 2006; Wheeler, 2000), and that they have different retrieval dynamics (e.g., Yonelinas, 2001; Yonelinas et al., 2002, 2005, for humans; Fortin et al., 2004, for rats). For example, Yonelinas (2001) has argued that the receiver operating characteristic (ROC) of human recognition memory consists of two components. The first is a *familiarity* component, which is mediated by a standard signal-detection process, whereas the second is an *episodic recollection* component that reflects a high-threshold process in which recollection only occurs once the strength of the episodic memory trace exceeds a threshold. Given this analysis, there is no need to appeal to anything more than the discriminative control exerted by the familiarity of an arm in the radial maze task or of the sample stimulus in a delayed matching task.

Fortin and colleagues used Yonelinas' analysis to determine whether rats were capable of episodic recollection using an odor recognition paradigm (Fortin et al., 2004). Importantly, the rats produced an asymmetrical ROC curve that is characteristic of the conjoint control of recognition by familiarity and episodic recollection. Moreover, their assessment of the two processes through the ROC analysis allowed the authors to dissociate the two processes, by both brain lesions and retention interval (*See* Chapter 1.21).

Clayton and Dickinson (1998) adopted a different approach to the question of whether or not animals other than humans are capable of episodic recall. Rather than applying a theoretically derived analysis, they considered cases in nature in which an animal might need to rely on episodic recall as opposed to other forms of memory. They suggested that the food-caching behavior of Western scrub-jays (*Aphelocoma californica*) might be one such example, because this species of bird hides both perishable food items (e.g., insect larvae) and nonperishable food items (e.g., nuts) for later consumption (for other potential candidates, see discussion by Clayton et al., 2001a). A suite of studies have shown that Western scrub-jays, like many other food-caching animals, have highly accurate and long-lasting spatial memories for the locations of their caches (Bednekoff et al. (1997) for Western scrub-jays; see review by Shettleworth (1995) for food-caching animals in general; *See also* Chapter 1.22). As these jays rely on their caches for survival in the wild, the selection pressure for remembering which caches were hidden where and how long ago might have been particularly strong (Griffiths et al., 1999), particularly since they cache year round (Curry et al., 2002). Furthermore, the birds also cache reliably in the laboratory, providing both ethological validity and experimental control (Clayton, 1999).

Rather than identifying episodic-like memory by its retrieval characteristic, Clayton and colleagues focused on the behavioral criteria for episodic-like memory, namely that the animal must be capable of remembering what happened where and when on the basis of a single past experience and in a way that cannot be explained in terms of relative familiarity. Clayton et al. (2003a) argued, however, that this criterion was insufficient; rather, an animal's *episodic-like* memory must fulfill three criteria: content, structure, and flexibility. In terms of the content of an episodic-like memory, we have argued that it is the 'when' component that is critical, since episodic memory is the only kind of memory to be explicitly located in the past. Furthermore, the what-where-and-when components form an integrated structure, and this feature of episodic memory is important because it permits discrimination between similar episodes that occurred at different times and possibly different places. Finally, the third criterion is one of flexibility, namely that the information can be represented in memory in a form that allows it to be used in a number of different ways, depending on the context. This flexibility arises from the fact that episodic memories are embedded within a larger declarative system that also encodes factual knowledge (e.g., Tulving and Markowitsch, 1998), and consequently the information can be updated and generalized across situations (*See* Chapters 1.04 and 1.21).

1.23.3 The Critical Components of Episodic-Like Memory

In the following section we discuss each of these three key features of episodic-like memory in turn in order to assess the evidence that some nonhuman animals do have episodic-like memory and, if so, which ones. We shall start our analysis by reviewing what is known about the memories of food-caching jays and then discuss subsequent studies that have been conducted on other animals, primarily rodents and primates.

1.23.3.1 The Content Criterion: The Importance of Pastness

Episodic memory is the only memory to be oriented in time (Tulving, 2000), and more precisely, in subjective time. Semantic and other memories do contain information that was acquired in the past, but without any notion of when they had been acquired, and thus these memories are timeless. As we argued in the introduction, having an awareness of the passage of time ('pastness') is a critical feature of episodic memory, one that distinguishes it from all other forms of memory, so it is therefore essential that any demonstration of episodic-like memory must show that the animal remembers when a particular event occurred in the past as well as what happened where (Clayton, 2004).

1.23.3.1.1 The what-where-and-when memories of food-caching Western scrub-jays

When investigating food-caching by Western scrub-jays as a natural candidate for episodic-like memory in animals, Clayton and Dickinson reasoned that, as these birds do not eat rotten insects, the recovery of perishable items is only valuable as long as they are still fresh. Consequently, a jay should remember not only the location of a cached food item but also the content (perishable or nonperishable food item), in addition to keeping track of the time since caching. Clayton and Dickinson (1998) gave the jays a series of trials in which they cached both their preferred food wax worms (wax moth larvae), and the less-preferred peanuts in two sand-filled ice cube trays, both of which were made visuospatially distinct and trial-unique by structures of children's building blocks (Lego Duplo) attached to the sides of the trays. Consequently, the jays cached in different pairs of trays on different trials so that each caching episode was unique (**Figure 1**). Although the birds had no cue predicting whether or not the wax worms were perished other than the passage of time, the birds quickly learnt that wax worms were available and fresh when recovered 4 h after caching, but rotten after 124 h, while peanuts were always fresh (Clayton and Dickinson, 1998).

Having received four pairs of training trials, the birds were given a pair of test trials, in which the caches were removed prior to recovery and the trays were filled with fresh sand to ensure that the birds could not use any cues emanating directly from the hidden food. The birds' search patterns at recovery demonstrated that they did remember which caches they had hidden in which particular trays and when, because they looked primarily in the places in which they had hidden the wax worms when the food had been cached 4 h ago, but redirected their search to peanut sites when the food had been cached 124 h ago. Note that the recoveries after both short and long retention intervals (RIs) always occur at the same time of day (4 h after caching on the same day as caching or 5 days after caching), and therefore neither circadian rhythms nor the state of hunger at the time of recovery could provide cues to guide the jays' searching behavior (see de Kort et al. (2005) for further discussion).

Figure 1 Sweetie Pie, one of the Western scrub-jays in Clayton's colony, is about to cache one of the wax worms in one of the visuospatially unique caching trays. Photo courtesy of Ian Cannell and Dean Alexis, University of Cambridge, UK, with permission.

At issue, however, is whether the birds really remembered the specific past caching episodes or whether they simply knew what had been cached where and when. In order to search in the correct cache sites that were unique to that specific caching event, the jays had to retain information about which caches they had placed where. However, it is possible that rather than remembering how long ago they had cached, the birds relied on familiarity judgments with the caching trays in order to decide whether to search for worms or peanuts. When caching had occurred just 4 h previously, the trays are presumably much more familiar than when caching occurred 124 h ago, and so the jays might have used a conditional rule, "if the trays look familiar search for worms, but if the trays look relatively unfamiliar search for peanuts."

To investigate this issue, Clayton and Dickinson (1999a) then gave the jays a second test trial (i.e., with no food actually present at recovery to test for memory), using an interleaved trial procedure shown in **Figure 2**. The jays were allowed to cache one food type on one side of a tray, while the other side of the tray was made inaccessible for caching by attaching a transparent strip of Plexiglas to cover all the caches sites on that side of the tray. The birds then got their trays back in the morning of the fifth day, prepared so that the birds

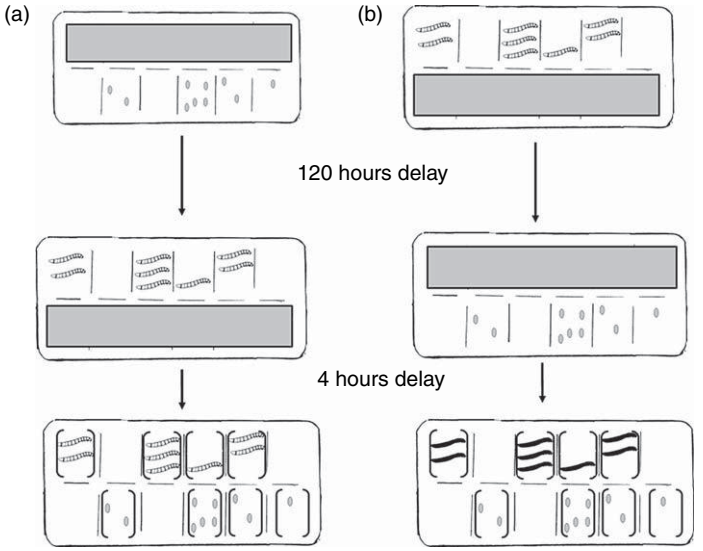

Figure 2 The food caching what-where-when memory paradigm used to test the content criterion of episodic-like memory by Western scrub-jays. Having received a series of training trials in which the birds could cache and recover peanuts and wax worms, the birds received an interleaved trials procedure in which they cached peanuts in one side of a caching tray and then wax worms in the other side of the tray 120 h later (a) or vice versa (b). On test, 4 h later, they then were given the opportunity to search in both sides of the tray. The brackets indicate the fact that the food caches were not present at recovery in order to test for memory. The open symbols represent fresh wax worms, the black symbols illustrate rotten wax worms, and the grey filled circles denote peanuts. The grey bar indicates the side of the tray that was blocked by a Plexiglas strip (the unavailable cache site) during each of the two caching events.

could cache the second food type in the previously inaccessible side of the very same tray, and they were prevented from recovering the previous caches by our attaching the Plexiglas strip to the other side of the caching tray. In the afternoon of this day 5, the jays were allowed to search in both sides of the tray. What is important here is that the interleaved procedure removes the differential relative familiarity of the tray because the temporal pattern of exposures to the tray was the same irrespective of whether or not the worms had been cached first, 124 h ago, or second, just 4 h ago. And since the caches were not present at recovery, the birds would have to rely on their memory of the previous caching episodes in order to search appropriately.

This they did: the jays spontaneously searched for the worms they had cached 4 h earlier, whereas on the trial in which they had cached the worms 5 days ago they ignored searching in the worm sites and instead searched for peanuts. This pattern of recovery searches suggests that the birds were not using tray familiarity as a cue but, rather, that they were remembering specific past caching episodes in terms of where they had hidden the peanuts and worms, and how long ago. Subsequent work established that the jays could also keep track of two perishable foods that decayed at different rates (Clayton et al., 2001b).

Several authors have argued that, as the jays had received a number of training trials in which they could cache and recover the various food items, they may have learned to solve the task by learning semantic factual rules about when to recover the particular foods rather than episodically recalling which foods had been cached where and how long ago (e.g., Zentall et al., 2001; Dere et al., 2005; Hampton et al., 2005). However, such claims misunderstand the theoretical interpretations of the role of semantic and episodic-like memory in the control of caching. Clayton and colleagues have argued that, to search in a particular tray for the perishable caches only when fresh and not when degraded, the birds must integrate a semantic-like rule about how long each food type remains fresh with a specific episodic-like memory of which caches they hid where in a given tray on a specific day (Clayton et al., 2003b).

1.23.3.1.2 Evidence of what-where-and-when memories in other animals

There have been a number of attempts to assess the content criterion of episodic-like memory in other animals (*See* Chapter 1.22). For example, Hampton and colleagues (Hampton et al., 2005) adopted our scrub-jay paradigm to test what rhesus monkeys

remember about specific foraging events. The test room contained three foraging sites, two baited and one unbaited, and what the monkeys had to learn was that all food was fresh after 1 h, but their preferred food was rotten after 25 h, while the less-preferred food remained fresh. The monkeys rapidly learned to search first for their preferred food, and to avoid the empty foraging location. However, although rhesus monkeys rejected the rotten food much like the jays did, they did not reverse their search patterns after the long delay, but instead they revisited those locations that contained their preferred food irrespective of the length of the delay. Like the jays, the monkeys remembered the what-and-where of trial-unique events; however, unlike the jays, there was no evidence that they encoded temporal information.

Perhaps the foraging paradigm might be less suitable for testing episodic-like memory in rhesus monkeys, given that they do not naturally cache perishable and nonperishable foods, although they do have specialized cheek-pouches that allow them to hoard food and eat it later in safe surroundings. But certainly their survival does not depend on them caching food for later, and their feeding ecology does not require them to keep track of decay rates, as they are primarily herbivorous (Hampton et al., 2005). Given the complexity of their social lives (e.g., Humphrey, 1976; Tomasello and Call, 1997; Whiten and Byrne, 1997), however, tests of episodic-like memory in primates that involve a social component, such as who was present in a particular social setting, may be more salient.

Roberts and colleagues found no evidence that rats could remember when they had cached various food types (Bird et al., 2003; McKenzie et al., 2005). However, most species of rat do not store much food in the wild (Vander Wall, 1990), and like primates, but unlike the jays, they do not rely on their caches for survival (Vander Wall, 1990). When viewed in this light, perhaps it is not so surprising that the rats behaved like the monkeys rather than like the jays, preferentially searching at recovery in those locations in which they had hidden food more often than other locations that had not been associated with food. Yet there was no evidence that they encoded the 'when,' because they did so even when items repeatedly degraded or were pilfered before recovery (Bird et al., 2003; McKenzie et al., 2005).

By contrast, recent work by Babb and Crystal (2005) did provide some evidence that rats could remember the what-where-and-when of specific past events. Instead of hoarding the food items themselves, the rats were trained to remember where they had previously encountered food that they could subsequently recover after either 1 h (the short RI) or after 25 h (the long RI). As shown in **Figure 3**, using a

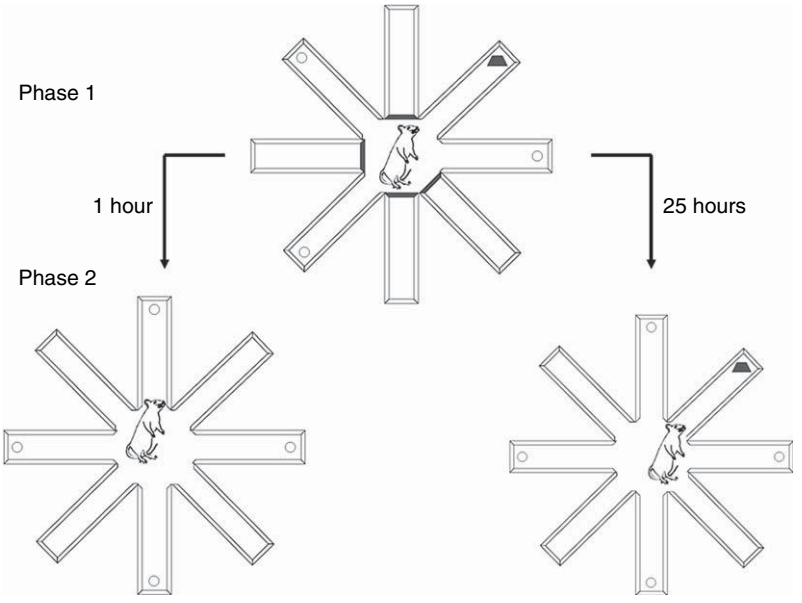

Figure 3 The what-where-when memory paradigm used to test rodents in a radial arm maze. In phase 1 the rats were given the opportunity to explore four of eight arms, three of which contain rat pellets, and one of which contains chocolate. During phase 2, the rats were allowed to explore all eight arms. The delay between phase 1 and phase 2 was either 1 h or 25 h, and rats received training with both the short and the long delay. The black bars show entrances that were inaccessible during phase 1; open circles represent rat pellets, and solid symbols denote chocolate pieces.

standard eight-arm radial maze, the rats were allowed to search during phase 1 for food located at the ends of four arms of the maze (the other four arms were blocked): three of them with regular pellets and one with highly preferred chocolate pellets. During phase 2, the rats were returned to the maze with all eight arms accessible, and the four previously inaccessible arms were baited with regular pellets. In addition the chocolate pellets were replenished if the rats were returned to the maze after a long delay, but not after a short RI. If the rats remembered which arms they had visited and eaten food from in phase 1, then they should selectively search in the previously inaccessible arms, because these are the ones that still contain food. If they also remembered which foods were available where and how long ago they had visited the maze in phase 1 then they should prefer to visit the arm containing the chocolate pellets after the long RI, but avoid that arm after the short RI.

Babb and Crystal found that the rats did learn to avoid the previously baited arms and to revisit the chocolate arm after a long RI only, demonstrating that rats could use the length of the retention interval as a cue to guide their choice of where to search, a finding that has also been replicated by Roberts' group (Naqshbandi et al., 2007). In both studies, however, the issue is whether the rats also remembered the specific contents of the encountered food. Although the rats may have remembered that a particular arm they had visited during phase 1 specifically contained chocolate, it is also possible that they simply encoded that the arm contained something more preferred than rat chow as opposed to encoding the precise type of food. Actually Clayton and Dickinson (1999b) already made a similar argument in the case of the jays' memories for what they had cached where. To establish that the jays did remember the content of their caches, the jays were given the opportunity to cache two equally preferred foods, and then just prior to recovery they were fed one of the two foods until sated. The jays searched preferentially for the non-prefed food at recovery, even on test trials in which no food items were present during recovery, thereby demonstrating that the jays did remember which particular foods they had cached where.

Babb and Crystal (2006b) addressed this question in an elegant satiation study. In phase 1, rats found three arms baited with regular food, and two of the other arms were each baited with a particular flavored food (grape, raspberry), both of which were equally preferred over regular pellets and both replenished after the long, but not short, RI. The rats were more likely to revisit the flavored arms and avoid the other previously baited arms after the long delay, just as they had done in the previous study. However, when the rats were sated on one flavor shortly before phase 2, the rats avoided that arm but revisited the arm containing the other flavored food, suggesting that they remembered not only which arms contained preferred food but that they also recalled the specific food contents of these arms. A transfer experiment using banana chips and chocolate, the latter devaluated by pairing it with a lithium chloride injection after phase 1, produced the same results.

Crystal (2006) argued that these experiments provide evidence of episodic-like memory in rats. Only by remembering what happened where and when could the rats return reliably to the replenishing chocolate arm after the long interval, but avoid this arm when the chocolate was devalued. However, this experiment does not control for relative familiarity. It is not clear whether the rats really remembered when to visit the chocolate arm or whether the rats could have solved the task by learning a rule that it is only when the arm appears to be relatively unfamiliar that the rats should search for the chocolate, or grape, or raspberry. As the lithium chloride treatment may have caused a general aversion to chocolate, the rats had to return only to the previously blocked arm, and since that arm had not been visited during phase 1, it may have been less familiar to the rat than the arms that had been visited in phase 1.

The results of the satiation study, although more convincing, can still be explained in terms of familiarity. Although rats do not use time of day or state of hunger as cues guiding their behavior (Babb and Crystal, 2006a), the relative familiarity of the maze itself could function as a cue to avoid or to revisit arms with preferred food in just the same way as we argued for the jays and their trays in the first experiment, namely that when the maze cues are highly familiar, then avoid all familiar arms, and when the maze cues are much less familiar, then revisit the arm containing the preferred food. Clearly what is needed to discriminate between these two possibilities is an interleaved trial procedure of the kind described for the jays in **Figure 2**.

Eichenbaum and colleagues used a different approach when developing their rodent model of episodic-like memory, with a focus on resolving the recollection versus familiarity dispute (*See* Chapter 1.21 for detailed discussion). Their sequence learning paradigm is based on the assumption that humans

may infer the sequence of events by their relative times of occurrence (Roberts, 2002), because "one event precedes, co-occurs, or succeeds another in time" (Tulving, 1983: 38). For example, Ergorul and Eichenbaum (2004) trained their rats to learn unique sequences of four odors in terms of which odors the rats encountered where and in what order ('when'). On test, two of the four odors were presented, and the rats were reinforced for choosing the location of the cup containing the odor that had occurred earlier in the sequence. This they did. However, when the rats were given a probe test, in which the cups that had previously contained the odors were placed in the correct locations but the odors had been removed so that they did not provide scent cues, then the rats failed to make the correct choice. Consequently, the rats may have solved this task by remembering which odors they had encountered earliest in the sequence without any recourse to remembering where.

A similar rational was adopted by Schwartz and Evans (2001), who tested the 31-year-old gorilla, King, for his ability to remember a temporal sequence of specific past feeding events. Specifically, they argued that "the animal's response should provide information about its past rather than about the current state of knowledge" (Schwartz et al., 2005: 231). King received three types of food in succession, each 5 min after finishing eating the former one. On test, a few minutes later, the experimenter asked King what he had eaten and in what order. Although King was able to answer correctly, he itemized the food in reversed order only, from the most familiar to the least familiar item. Consequently, his performance could be explained in terms of relative familiarity rather than episodic-like recall.

To summarize this section, attempts to establish models of episodic-like memory in nonhuman animals other than Western scrub-jays suggest that the 'when' component is by far the most challenging feature. In some studies, the animals failed to show any sensitivity to the temporal relationships between events (Bird et al., 2003; Hampton et al., 2005). Of course, the absence of evidence is not evidence of absence, and the fact that rats do appear to remember what happened where and how long ago when tested for the memory of food they have seen previously (Babb and Crystal, 2005, 2006b; Naqushbandi et al., 2007), but have not cached previously (Bird et al., 2003), suggests that the ecological salience of the task may be critical. One advantage of the caching paradigm when employed with Western scrub-jays is that it taps into this particular species' natural propensity – if not obsession – to cache and efficiently recover perishable as well as nonperishable food. That said, an outstanding issue in the rodent memory models is whether the animals may have solved these tasks using relative familiarity or rule learning after intensive training instead of episodic recall (Schwartz and Evans, 2001; Babb and Crystal, 2005, 2006a), and the extent to which an animal's ability to remember and discriminate between sequences (e.g., Schwartz and Evans, 2001; Agster et al., 2002; Ergorul and Eichenbaum, 2004; *see also* Chapter 1.21) depends on episodic-like recall.

1.23.3.1.3 Challenging the "when" component

Other researchers have questioned whether the encoding of the 'when' component is central to the concept of episodic-like memory, or whether in fact it is the context in which the event occurred that is critical. Although human memories are usually rich and detailed in some aspects, the quality of the temporal information may be rather poor (e.g., Friedman, 1993, 2001; Simpson et al., 1998; Eacott and Norman, 2004). This does not remove our own sense of the pastness of a memory, however, just as Proust was fully aware of the concurrence of his present and the mentally relived past. Furthermore, most humans have seemingly little difficulty in discriminating between memories that have the same 'what' and 'where' contents but different 'when' components. For example, most of us find it easy to differentiate between memories of two different meals with the same friend. In this case the 'when' component may simply be a temporal form of the occasion setting 'which,' and we distinguish between our memories of the two events by binding each episode to the different contexts provided by the two restaurants. Eacott and Norman (2004) therefore suggested that the behavioral criteria for episodic-like memory should not be restricted to what-where-and-when but should also include what-where-and-which, with the 'which' component functioning as the occasion-specific context (see also Kart-Teke et al., 2006).

The what-where-and-which concept of episodic memory has been exploited by capitalizing on the rodent's propensity to seek out novelty (e.g., Eacott and Norman, 2004; Dere et al., 2005; Norman and Eacott, 2005; Kart-Teke et al., 2006). The most convincing example is that conducted by Eacott and her colleagues (2005), because they were able to control for relative familiarity. To do so, they built a maze in the shape of an E so that they could place two different novel objects at the two outside ends of

the E-maze, out of sight of the rats at their starting point, as shown in **Figure 4**. The rats were given the opportunity to explore the two different objects in one specific context before being allowed to investigate them again in a different configuration in a second context that was texturally different from the first (plain grey and smooth to the touch versus wire mesh). Following this episode, the rats were exposed to one of the objects outside the two contexts until they had become habituated to it, an experience that enhanced their propensity to explore the other object when returned to one of the two E-maze contexts. A rat could only do so, however, if it had remembered where the objects were located in the E-maze in a particular context during the initial episode. Their rats' success at this task led Eacott and colleagues to argue that the rats recollected the object (what) and its location (where) in a particular context (which) on the basis of unique what-where-and-which memories. Note that, because the objects were out of sight at the starting point, the rat's choice to turn right or left could not have been based on relative familiarity of the object.

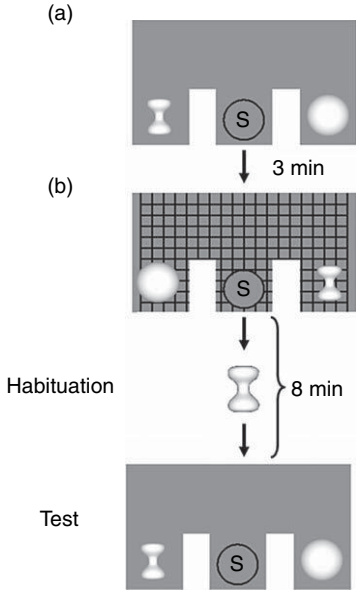

Figure 4 The what-where-which paradigm used to test rodents in the E-maze. The rats explored two different objects placed in a certain spatial configuration in a particular context (a), before investigating them again in a different configuration in a second context (b). Following this episode, the rats were exposed to one of the objects in a different place until they were habituated. On test they were placed back in either context (a) or (b), but the objects were not visible from the starting point (S) in order to test for memory.

1.23.3.1.4 Differential forgetting or remembrance of times past?

Although we have argued strongly for the importance of the 'when' component of an episodic-like memory, several researchers have suggested that demonstrations of an animal's ability to remember the what-where-and-when of a specific past event may not be sufficient to claim that the animal is capable of episodic recall. In the case of our jays, it is argued that, instead of traveling back in time, the birds might have used the strengths or ages of memory traces in order to know when to search for the food items at particular locations.

There are two ways in which a jay could use the strength or vividness of the memory trace to date its memory of a particular caching event: spontaneous forgetting and directed forgetting (for detailed discussion see de Kort et al., 2005). Consider the case of the jays that learned that if there was a short delay between caching and recovery then their worms would still be fresh at the time of recovery, whereas if a long interval had elapsed between caching and recovery then the worms would have degraded. In terms of spontaneous forgetting, the bird might simply forget the location of degraded worms because the event happened a relatively long time ago. Alternatively, rather than having an all-or-none response (remember–forget), the bird might use forgetting, just like familiarity, as a conditional cue to control its pattern of searches at recovery. The birds learn to search for the worms when they recall a vivid memory of the caching episode, whereas they learn to avoid attempting to recover the worms when they recall a less vivid or partially forgotten memory. Staddon and Higa (1999) have recently proposed an account of interval timing based upon conditional control by the strength of the memory for a time marker.

If the jays use vividness to date the caching episode, we should expect the birds to show some evidence of forgetting for other aspects of the content of the memory, specifically the location and types of caches, at longer delays. Even if the worms did not degrade with time since caching, the time-dependent forgetting of the memory representation should be accompanied by a loss in accuracy for locating the different caches of food. To assess this possibility, Clayton and colleagues (Clayton and Dickinson, 1998, 1999a; Clayton et al., 2001b) tested the cache recovery behavior of a second group of jays, whose potentially perishable caches did not in fact degrade. These birds showed no loss in accuracy of locating the food caches at the retention

intervals for which the putative forgetting should have been controlling the recovery choice of the birds whose caches did degrade. Furthermore, the jays were just as accurate at locating caches after 5 days as they were after 4 h (Clayton and Dickinson, 1999b), even though we should have to assume that the strength of the cache memory after 5 days was significantly weaker than after 4 h if this strength difference is to control a complete reversal in recovery patterns. Consequently we think that it is unlikely that the jays' temporal control of caching was mediated by memory decay.

Although spontaneous forgetting is unlikely to account for the behavior of the jays, perhaps directed forgetting (Roper and Zentall, 1993) may be more plausible. Consider, once again, the case in which jays cache and recover worms and peanuts after a short and a long delay. As the peanuts never perish, the jays always recover fresh peanuts. However, this is not the case for their worm caches, which are found to be degraded at recovery on half of the trials (i.e., those in which recovery occurs after a long delay). According to the directed forgetting account, experience with unpalatable worms on half of the recovery trials causes the jays to forget the location of these caches more rapidly. For example, perhaps having experienced degraded, unpalatable worms at recovery, the jays subsequently devote less processing of the location of these caches on subsequent caching episodes, which in turn leads to more rapid forgetting of those caches.

A test of this hypothesis would be to establish whether the jays could learn the opposite profile for when worms are edible, namely one in which worms ripen rather than degrade over time (de Kort et al., 2005). To do so the jays received a series of trials in which they could cache peanuts and worms as before, but this time the worms were degraded after the short delay but were fresh after the long delay. de Kort and colleagues found that the jays rapidly learned to avoid searching for worms after the short retention interval, while preferentially searching for them after the long one. The fact that jays' performance could not be explained by either spontaneous or directed forgetting strongly suggests that jays encode time specifically within their episodic-like memory of events (de Kort et al., 2005).

As explained in the first section, we can never ascertain whether or not an animal is aware of the past while retrieving a memory. All we know is that animals can discriminate among events which occurred at different times in the past. One way to tackle this issue would be to devise an experiment in which the animal is given the opportunity to report that it knows whether or not it has remembered using an uncertainty monitoring paradigm (Smith, 2005), just as Hampton (2001) did when testing whether his rhesus monkeys knew what they had and had not remembered.

1.23.3.2 The Structural Criterion of Episodic-Like Memories: An Integrated Representation

In humans, the content of an episodic memory is usually a rich representation of what happened, where, and when. Imagine the moment when handing over a carefully chosen present to a special person. We not only remember where and when we bought this present, and of course what we have chosen, but we may spontaneously remember a number of other details about the event, such as whether it was a rainy or a sunny day, and whether the shop was overcrowded or completely empty, and some of us might remember other seemingly trivial details such as what shoes we were wearing.

One of the cardinal features of human episodic memory is that, when we come to recall the event to mind, we retrieve all these components together as a gestalt image. This reflects the structure of episodic memory, namely that the 'what,' 'where,' and 'when' components are not encoded separately but are bound together in an integrated representation, and consequently the retrieval of one component elicits the retrieval of the others. Contrast this integrated representation with a linear one in which the components are not directly linked. If we were to remember the episode as a series of separate components, then we would not be able to distinguish between episodes that have similar contents and locations but different temporal contexts, such as family Christmas dinners that occurred in different years. It is this feature that led Clayton et al. (2003a) to argue that the criterion of remembering what-where-and-when is not, by itself, sufficient to characterize a memory as episodic-like if, by this term, we mean that the memory has the behavioral properties of human episodic memory.

To illustrate this point, let us consider another food-caching scrub-jay scenario in which the birds cache the same foods in two trays at different times. The design is illustrated in **Figure 5**. Briefly, the trained jays were allowed to cache peanuts and worms in one tray on one day, and then at a later time they cache the same food types in a second tray, after which the jays are allowed to recover from both

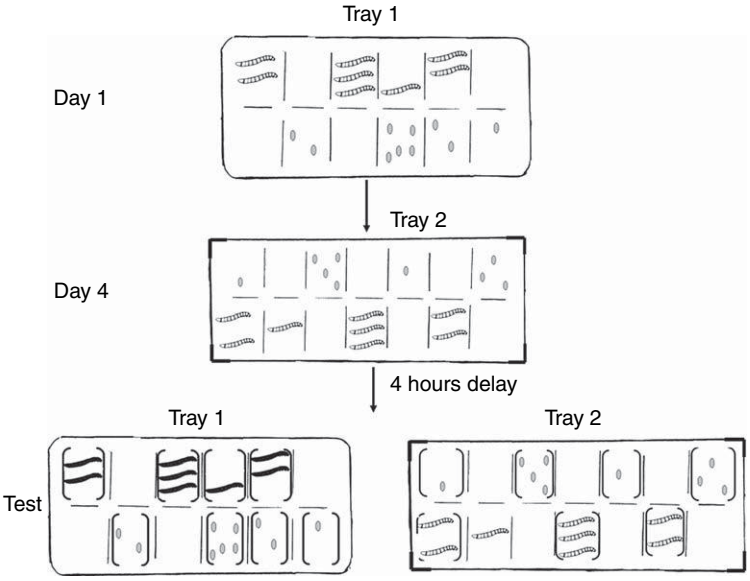

Figure 5 The food caching what-where-when memory paradigm used to test the structure criterion of episodic-like memory by Western scrub-jays. The jays cached peanuts and wax worms in one caching tray on day 1, and then again in a second tray on day 4. On test, 4 h later, the jays were given the opportunity to search in both trays. The brackets indicate the fact that the food caches were not present at recovery in order to test for memory. The open worms represent fresh wax worm caches, the black worm symbols denote rotten wax worm caches, and the grey filled circles illustrate peanut caches.

trays. The retention intervals are such that the worms will be decayed in the first tray while still being fresh in the second tray, and the critical question is whether the jays show the appropriate search pattern for each of the two trays.

If the birds retrieved the 'when' component separately, they could not have distinguished between the caching episodes because, by that account, the jays would simply associate caching the worms with a temporal tag, and the memory of caching worms at recovery would retrieve temporal tags for both the long and short RIs. In short, a linear mnemonic structure does not support the appropriate recovery pattern, namely searching for peanuts in the first tray and worms in the second tray. However, the jays do in fact search appropriately (Clayton et al., 2001b), a result that suggests that they do form integrated memories, because they can distinguish in memory between the two caching episodes in terms of their time and location, even though they involved the same food items.

Clayton and colleagues interpreted this finding as evidence that the jays' behavior met what they called the *structural* criterion for episodic-like memory (Clayton et al., 2003a,b). Not only must a memory have the what-where-when representational *content* to be episodic-like, but that content must be bound in a form that yields a unique memory for each caching episode. It is this representational binding that allows the jays, like humans, to discriminate between similar episodes that occurred at different times and possibly different places.

What of other animals? So far, the only other study to test the structural criterion of episodic-like memory is that by Shettleworth and colleagues (Skov-Rackett et al., 2006). In a series of delayed matching to sample tasks on a touch screen, they tested their pigeons' memory of all three features independently: on some trials they had to select the correct 'what' in terms of an object's identity, on other trials they had to select the correct 'where' in terms of an item's location on the screen, and on yet other trials they were tested for their memory of 'when' in terms of the time intervals passed since presentation of items. The pigeons performed well above chance on all three types of trials, which suggests that the pigeons could encode all three features during a single presentation. However, further tests established that the pigeons' memories were stored independently in a linear rather than in an integrated structure. It remains to be seen whether the rodent what-where-when and even what-where-which memories possess an integrated structure like those of jays and humans, or whether they are linear, like those of pigeons.

Further evidence for the integrated structure of scrub-jay episodic-like memories comes from studies of the social context of caching (reviewed by Clayton et al., 2007). These birds readily steal one another's caches (e.g., Clayton and Emery, 2004) and go to great lengths to protect their own caches from being stolen by another bird, hiding them behind barriers (e.g., Dally et al., 2005) and moving those caches another individual has seen them make once that other individual has left the scene (Emery and Clayton, 2001). Of particular relevance to the issue of integrated memories is the finding that these jays recognize particular individuals and remember which particular individual was watching them cache during specific past caching episodes and take protective action accordingly (Dally et al., 2006). It is this integrated structure of their episodic-like memories that allows them to discriminate between caching episodes that differed only in terms of who was watching when.

The only other published study that tested an animal's episodic-like memory with a 'who' component was conducted on the gorilla King (Schwartz et al., 2002), who was trained to associate five food types and their respective English words with five wooden cards carrying a picture of each food in question. In addition, he was trained to associate each of two human trainers who were present during the trials with a card carrying the name of that person. To test King's episodic-like social memory, the "to-be-remembered trainer" handed the gorilla one piece of food through the bars of the cage, while the second trainer was present but did not do anything. On test, either about 10 min later or on the next day, King received five cards for the various food types plus the two cards for the two trainers. In response to the questions "What did you eat?" and "Who gave you the food?" King was expected to hand over the card that corresponded to the food he had eaten and the card with the name of the trainer who had given him the food, which he did for the most part accurately (see also Schwartz et al., 2004). However, the issue remains as to whether King episodically recalled the specific past event or whether King's performance could have been based on relative familiarity, given that he simply needed to select the cards that matched the stimuli he had encountered most recently (for detailed discussion see Schwartz, 2005; Schwartz et al., 2005).

To summarize this section, we have argued in order to demonstrate episodic-like memory in an animal requires not only evidence that the animal remember the what-where-and-when of a specific past event, and in a way that cannot be explained by relative familiarity, but that the structure of this memory requires an integrated representation. To our knowledge, so far only the jay studies provide evidence for all features. Even less well studied than the structural criterion is the third key feature of episodic-like memory – its flexibility.

1.23.3.3 The Flexibility Criterion

As we argued at the end of the section titled 'Animal studies,' there is a third defining feature of episodic-like memory, namely that it should be capable of flexible deployment (Clayton et al., 2003a). This term refers to the fact that the use of the information encoded in a memory can vary depending on the context. Because episodic memories are embedded within a larger declarative memory system that also encodes factual knowledge (e.g., Tulving and Markowitsch, 1998), the information can not only be generalized across situations but also updated when new information is acquired after the encoding of the original memory.

Evidence for the updating of episodic-like memories comes from a study by Clayton and Dickinson (1999b) in which the jays cached two types of food, peanuts and dog kibble, in both of two trays. They then allowed the jays to recover the peanuts from tray A and the dog kibble from tray B. If the birds could update their original cache memories in light of these recoveries, they should have represented tray A as containing only kibble and tray B only peanuts following the recovery episodes. To test whether this was so, one of the foods was then devalued by allowing the jays to consume it to satiety before they once again searched for their caches. Evidence for mnemonic updating came from the observation that the jays searched the kibble sites when prefed peanuts and peanut sites when prefed kibble, thereby demonstrating that they integrated the memories of the caching and recovery episodes in a way that enabled them to know the identity of the food items remaining in the trays.

This study also illustrated a second form of flexibility. The fact that the jays searched preferentially for the nondevalued food types shows that the deployment of cache memories is sensitive to changes in the incentive values of the caches. There is now good evidence that rats are also capable of this form of mnemonic flexibility. Eacott and colleagues have argued that their rats do show flexibility

because the demonstration of the what-where-and-which depended upon devaluing one of the objects as a target of exploration by habituation. A similar claim was made by Babb and Crystal (2006a) on the basis of the fact that, when their rats acquired taste aversion to chocolate after the memorization phase, they subsequently avoided the chocolate arm.

A final form of flexibility illustrates the fact that episodic memory is embedded with a general declarative system and that episodic information interacts with semantic knowledge in the control of behavior. Again this point can be illustrated with the scrub-jay food-caching paradigm. Across a series of training trials, the jays learned that different types of perishable food decayed at different rates – mealworms were rotten just 1 day later, whereas crickets, like wax worms, took longer to degrade (Clayton et al., 2001b). Clayton and colleagues argued that, in order to adopt the appropriate recovery strategy of recovering perishable caches while they are still fresh but avoiding them once they have perished, the jays would need to acquire a semantic-like knowledge about the rates at which various cached foods decay and to combine this with an episodic-like memory for each particular caching event (Clayton et al., 2001b, 2003b). The basic idea is that degrade rates of the different foods are extracted across a number of caching-and-recovery bouts and stored in a semantic-like representation, but that this information needs to be coupled with a particular episodic-like memory of the caching event if the jay is to know what to search for and where. The flexibility of this declarative memory system arises from the fact that the same episodic-like memory can support different recovery strategies depending upon the jay's semantic-like knowledge of when the caches degrade.

A strong test of this declarative flexibility asks whether the deployment of a cache memory is sensitive to new information about perishability, even though this information is not available until after the caching has occurred. Clayton et al. (2003b) assessed this form of flexibility. To do so, the jays first received a series of training trials in which they learned that crickets remain fresh for 1 day but have degraded after 4 days. On the basis of a temporal generalization test, it was then established that the birds behaved as though they expected the crickets to remain fresh for up to 3 days after caching, even though they had not been trained with these retention intervals and had no direct information upon which to base these expectations, merely an interpolation from the differential training at the 1- and 4-day intervals. At issue was whether the birds would change their strategy of where to search at recovery if they obtained subsequent information after they had already cached the food that their expectation about the durability of the cricket caches was, in fact, false.

In order to provide this information, the jays were given the opportunity to cache crickets in three different trays on three successive days. The critical design of this test is illustrated in **Figure 6**. On the fourth and fifth days the jays recovered the crickets from each of the first two trays, one per day, so that the jays had experienced a 3-day retention interval between each caching and recovery episode. During these two recovery episodes, the jays discovered to their surprise that the crickets were degraded. Note that this information was acquired a long time after the birds had formed the episodic-like memory of caching crickets in a particular tray on a given day.

At issue was whether the birds in this group would integrate this new, semantic-like information with the episodic-like memory of caching in the third tray on day 3. On the sixth day the birds received the final tray back, but no caches were present in order to test for memory. All the birds avoided searching in the cricket sites on test, a result which demonstrates that such integration occurred and attests to the declarative nature of the jay's memory for caching in particular trays. By contrast, a control group whose caches perished at the expected rate showed the same recovery pattern as before, preferentially searching for crickets.

1.23.3.4 Incidental and Automatic Encoding

So far we have argued that there are three key features of the behavioral components of episodic memory. The first – in terms of content – is that the subject recalls a specific event that happened in the past and in a way that cannot be explained in terms of discrimination by relative familiarity. Second, the representation of that past event should contain multiple features (e.g., where, what, who) in addition to 'when,' which are bound in an integrated structure. The third pertains to the flexible deployment of information acquired after encoding of the original memory, which allows memories to be embedded within a broader declarative memory structure and allows the subject to keep track and update information accordingly. However, several

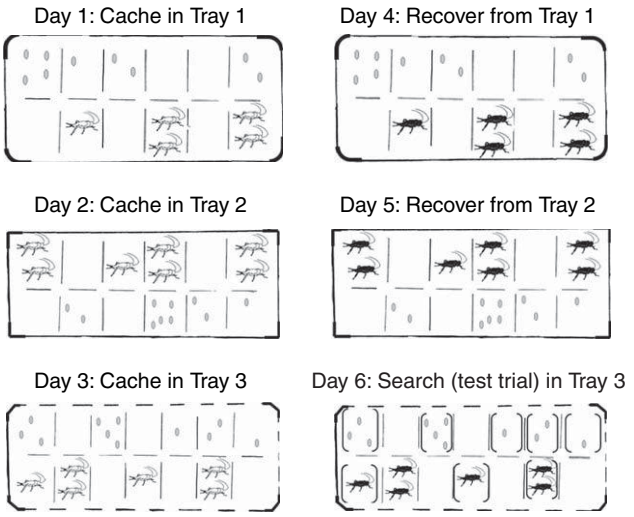

Figure 6 The food caching what-where-when memory paradigm used to test the flexibility criterion of episodic-like memory by Western scrub-jays. The jays cached peanuts and crickets in one caching tray on day 1, and then again in a second tray on day 2, and in a third tray on day 3. The birds were given the opportunity to recover the caches they had hidden in tray 1 on day 4, and in tray 2 on day 5. On test, on day 6, the jays were allowed to search in tray 3. The brackets indicate the fact that the food caches were not present at recovery on day 6 in order to test for memory. The open symbols represent fresh cricket caches, the black symbols represent rotten cricket caches, and the grey filled circles represent peanut caches.

other facts of human episodic memory remain to be explored.

When addressing the issue of an integrated representation, for example, we noted the richness of many episodic representations, of how we typically encode many seemingly incidental features of an event, without any deliberate intent to do so. So what is the origin that causes the flood of memories during episodic recall? In their "automatic recording of attended experience" hypothesis, which they invoked to explain the function of synaptic plasticity in the hippocampus, Morris and Frey (1997) emphasized this feature of episodic memory.

Zentall and colleagues (Zentall et al., 2001) have made a similar point by noting that, when animals receive a number of training trials, they may come to expect a test of their memory even though the event may be novel. For example, in the case of the jays, the birds may come to expect that their caches will be available for recovery, and that the expectation of this recovery test may lead to a semantic memory rather episodically encoding the unique features of that particular caching event. He argues that it is only when asked an unexpected question that one has to travel mentally back in time to reexperience the event in question and find the correct answer. We remain to be convinced, however, that incidental encoding is a defining feature of episodic memory. While many features of an event may indeed be encoded automatically, it does not follow that a prior request to remember specific aspects of an event preclude it from being encoded as an episodic memory. Consider a special event, a wedding, for example. Knowing that one may be asked to describe the event to others later does not prevent one from reexperiencing that episode each time one is asked to do so.

Perhaps some of the most convincing work on spontaneous episodic recall comes from C. Menzel's (1999, 2005) studies of Panzee, an 11-year-old female chimpanzee, who was trained to use a lexigram as well as gestures in her daily encounters with her human caretakers (**Figure 7**). Panzee could regularly watch from indoors how a human caretaker in the outdoor area kept a particular object in his hand and then hid it under a natural cover before leaving the outdoor area. Later, Panzee would spontaneously initiate contact with the caretaker, showing that person the lexigram of the hidden food type, and subsequently guiding the trainer outdoors, giving the gesture for 'hide,' and eventually pointing in the direction where she had observed the object being hidden. Of course Panzee's own initiative in catching the caretaker's attention and communicating is not without nonepisodic explanations (for discussion, see Menzel, 2005), but it may provide a promising avenue for future studies on this issue.

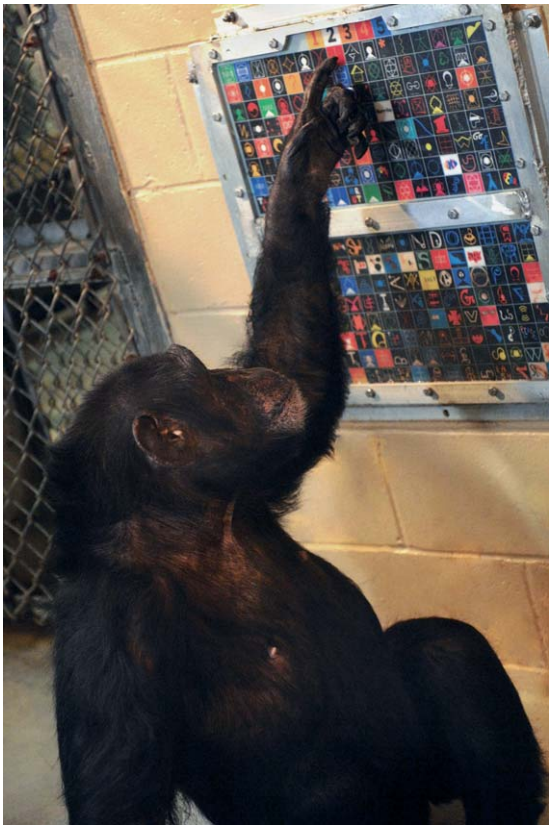

Figure 7 Panzee, the chimpanzee, using the lexigram to communicate with her human caretakers. Photo courtesy of Charles Menzel and Carolyn Richardson, Georgia State University, USA.

1.23.4 The Distribution and Evolution of Episodic Memory

Many people share Nietzsche's view that animals are stuck in the present and thus cannot episodically recall specific events that happened in their past because they have no sense of possessing a personalized past:

> they do not know what is meant by yesterday or today, they leap about, eat, rest, digest, leap about again, and so from morn till night and from day to day, fettered to the moment and its pleasure or displeasure, and thus neither melancholy nor bored. (Nietzsche, 1983: 60)

Such an ability to acquire and retrieve information about the world in a seemingly depersonalized timeless zone may have its advantages, for as Nietzsche pointed out:

> This is a hard sight for man to see; for, though he thinks himself better than the animals because he is human, he cannot help envying them in their happiness – what they have, a life neither bored nor painful, is precisely what he wants. (Nietzsche, 1983: 60)

The problem with such an account is that it is essentially untestable. As we pointed out at the start of the introduction, we can probably never know whether any nonhuman animal is capable of episodic recall, at least in the form that we humans experience, with the associated conscious experiences of chronesthesia and autonoesis.

By turning our focus to the behavioral criteria for episodic memory, we can at least assess which animals possess these elements of episodic memory that, in the absence of any assessment of phenomenological criteria, we call episodic-like memory. This perspective may in turn provide clues as to how and why episodic memory evolved. After all, it seems unlikely that episodic memory evolved in humans *de novo,* without any precursors in the rest of the animal kingdom.

What does an understanding of the distribution of episodic-like memory among animals tell us about the evolution of episodic memory? If we accept that episodic-like memory is present in at least one species of corvid (i.e., the Western scrub-jay) and at least one species of ape (i.e., humans), then it follows that this ability might have arisen through convergent evolution (similarities that arise as a result of adaptation to similar selection pressures in distantly related species) rather than through a shared common ancestor (homology). However, if we were to show that all birds and mammals possess episodic-like memory, then the parsimonious explanation would be that they share a common ancestor who also possessed the same traits. The answer to this question will only be known when studies have been conducted on a wider sample of species.

So far the attempts to establish models of episodic-like memory in nonhuman animals are still in their relative infancy, and perhaps this is not too surprising given that the initial paper on episodic-like memory in jays was published about 10 years ago (Clayton and Dickinson, 1998). Our review of the available evidence to date suggests that a strong case can be made for the scrub-jays, and a promising case for the chimpanzees, yet there is no evidence that rhesus monkeys remember when as well as what and where, and for rats the results are mixed. Rats do appear to remember what happened where and how long ago when tested for the memory of food they have seen previously (Babb and Crystal, 2005, 2006b;

Naqshbandi et al., 2006), but not that they have cached previously (Bird et al., 2003), suggesting that ecological salience may be critical in designing and developing the appropriate tasks.

As we noted earlier in this chapter, an outstanding issue in the rodent memory models is whether the animals may have solved these tasks using relative familiarity or rule learning after intensive training instead of episodic recall (Schwartz and Evans, 2001; Babb and Crystal, 2005, 2006a), and the extent to which an animals' ability to remember and discriminate between sequences (e.g., Schwartz and Evans, 2001; Agster et al., 2002; Ergorul and Eichenbaum, 2004; *See also* Chapter 1.21) depends on episodic-like recall. Furthermore, with the exception of the scrub-jay studies, most of the experiments have focused on the content criterion for episodic-like memory, rather than on its structure or flexibility of deployment. So clearly there is much more work to be done. A key question for future research will be to make sense of these apparently conflicting results, perhaps by using similar paradigms with different species. But this pattern of mixed results provides a cautionary note that even species that can remember the what, where, and when of a specific past experience may not necessarily express this ability under all conditions (Clayton, 2007).

The other point worthy of mention, and one we alluded to earlier, is that of ecological salience. Perhaps it is no coincidence that the evidence for episodic-like memory in the jays comes from studies of their natural propensity to cache and form rich representations of previous caching events in order to recover their food efficiently in the future. There are a number of features of the food-caching behavior of scrub-jays that might be rather special. The first is that there is no need to train the animals to perform a caching and recovery task, as these are behaviors that the animals do for a living. Moreover, Western scrub-jays are highly motivated to do so and will go to great lengths to protect their caches from being stolen by conspecifics (see review by Clayton et al., 2007), and so presumably there was intense selection pressure on mnemonic processing by jays. McKenzie et al. came to a similar conclusion when arguing that the observed differences in chronesthesia between rats and scrub-jays

> favor greater fitness for birds through sooner and greater acquisition of food. . . . Food hoarding and retrieval behaviors inherited by rats from rodent ancestors may not have been fine-tuned by the same demands placed on food-storing birds. (McKenzie et al., 2005: 24)

Perhaps it is not surprising that the scrub-jays show such rapid learning about the fate of their caches over just two or three trials.

Indeed, the jays cache perishable foods in an environment where the rate at which foods decay changes across the year, and from day to day, depending on the weather conditions between caching and recovery, so fast that flexible learning may be essential to their survival. For jays that live in the Central Valley (California, USA), the ambient temperatures rarely fall below 10 °C but may rise to over 40 °C between July and September. At such temperatures, caches that consist of various invertebrates, for example, will degrade rapidly in the heat and more slowly in cold. So the problem for a scrub-jay is not only to learn how quickly a particular food type degrades but also to be capable of updating information in a flexible manner, based on the ecological conditions that occur in the interim between caching the item and recovering it (de Kort et al., 2005).

Not all animals cache food, but there are a number of others that forage for food that degrades, ripens, or replenishes, providing another potential candidate for studying episodic memory that could be investigated experimentally (for suggestions of other candidates, see Clayton et al., 2001a; Griffiths and Clayton, 2001; Clayton and Griffiths, 2002). Nectar-feeding hummingbirds and bats, for example, could increase their food intake by taking into account a given flower's secretion rate to guide an individual's revisiting schedule. Henderson et al. (2006) tested whether free-living, territorial Rufous hummingbirds (*Selasphorus rufus*) could keep track of when the flowers refilled with nectar. The animals quickly learned the different refilling rates of the two types of flowers and roughly adjusted their revisiting schedules accordingly. Furthermore, the birds appeared to remember which particular flower they had emptied recently (Jones and Healy, 2006). Similarly, Gonzalez-Gomez and Vasquez (2006) found that green-backed firecrown hummingbirds (*Sephanoides sephaniodes*) remembered not only the location of a particular flower but also the locations of the most rewarding nectar sources among less-rewarding flowers. The flower bats (Phyllostomidae, Glossophaginae), for example, *Glossophaga soricina,* may also be a promising candidate for testing episodic-like memory, especially given that these bats can hold more than 40 feeder visits in working memory without indication of memory decay (Winter and Stich, 2005), and would allow a second test of convergence of episodic-like memory in birds and mammals.

If convergent evolution is the most likely process, then what are the common selective pressures shared between the ape and corvid species (see Emery and Clayton, 2004), or between the hummingbirds and bats? One direction for future research will be to characterize what advantages the possession of episodic memory might have, and this is where comparisons of different behavioral systems may be particularly informative. It is important to note at this point that similarity arising as a result of convergence need not lead to identical solutions (e.g., Salwiczek and Wickler, 2004), and therefore episodic-like memory in different groups may have similar functional properties without necessarily having similar neurobiological structures (de Kort and Clayton, 2006). A second direction for future research will be to ask questions about the brain systems necessary to support the various kinds of memory and the extent to which convergently evolved brain systems are similar in their details. For example, although there is good evidence that the hippocampus plays an important role in spatial memory processing in both birds and mammals, the two types of hippocampi differ in structure, with the avian one being nuclear and the mammalian one being laminar (see Emery and Clayton, 2005).

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1.24 Reconsolidation: Historical Perspective and Theoretical Aspects

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> *Everything flows and nothing abides; everything gives way and nothing stays fixed.* (Heraclitus 535–475 BC)

1.24.1 Historical Background: Thinking About Memory

Memory is dynamic, in that it is constantly being updated as it is retrieved. Heraclitus was the first writer to insist on this dynamic nature of memory with his metaphor cited above or with the more familiar,

> You can never step twice into the same river as other waters are ever flowing on you. (Heraclitus 535–475 BC).

William James (1892) updated this view, arguing that memory was a dynamic property of the nervous system, in constant flux as a result of being retrieved within current cognitive environments. These speculations were largely supported by the seminal experiments of Bartlett (1932), showing that information was gradually biased toward the subjects' cultural expectations as it was repeatedly recalled. Extensive evidence for this comes from laboratory studies of human memory processes over the past three decades, where old memories have been shown to be profoundly influenced by information in the retrieval environment, particularly if this information is in contradiction of the old memory (see Loftus, 2005a,b for reviews; *See also* Chapters 1.02, 1.04).

1.24.1.1 Reconsolidation: A Hypothetical Construct

Postretrieval retrograde amnesia was demonstrated experimentally in rats several decades ago, but the term 'reconsolidation' was introduced more recently, as a hypothetical construct to account for amnesia after cued recall (Pryzbyslawsky and Sara, 1997). Reconsolidation has attracted wide interest among contemporary neurobiologists for a number of reasons, including broad therapeutic applications (Pryzbyslawsky and Sara, 1997; Debiec and Ledoux, 2006). There has been a proliferation of papers appearing in the literature in the past decade, mostly addressed at understanding the molecular and cellular mechanisms underlying this still-hypothetical process. As with any area of scientific inquiry, as the number of investigators addressing the question increases, so do discrepancies in results, alternative explanations of the data, and definitions of constraints. Several reviews of this recent literature are available (Nader, 2003; Dudai, 2004, 2006; Alberini, 2005; Alberini et al., 2006; *See* Chapters 1.27, 4.14). The purpose of this chapter is to provide a deeper historical perspective from which to understand and evaluate current issues.

1.24.2 The Consolidation Hypothesis

1.24.2.1 Origins and Fate of the Consolidation Hypothesis

To understand the significance of the growing literature and issues being raised around the reconsolidation construct requires a brief reminder concerning the origins and fate of the consolidation hypothesis (for recent, more extensive, reviews see Dudai, 2004; Sara and Hars, 2006). Scientific investigation of memory processes was initiated at the end of the nineteenth century by German psychologists, first Ebbinghaus (1885) and then Mueller and Pilzecker (1900). Their studies of verbal learning and retention in human subjects led them to suggest that a memory trace was formed gradually over time after acquisition, and they introduced the term 'consolidation.' Contemporary with this were the very influential clinical observations and theoretical elaborations of the French psychiatrist, Ribot (1882). From his studies of amnesic patients, he formulated 'La loi de regression' that simply notes that as memories age, they become more resistant to trauma-induced amnesia. The origins of the neurobiological studies of memory processes can be found in early animal models of experimental amnesia (Duncan, 1945, 1948, 1949). Based on a clear temporal gradient of efficacy of the amnestic treatment, these early investigators concluded that retrograde amnesia experiments provided direct evidence for Mueller and Pilzecker's hypothesis stating that postlearning neural perseveration was necessary for consolidating memory. Electroconvulsive shock treatments (ECS) disrupted this activity, thereby preventing postacquisition memory consolidation. In the same year, and quite independently of Duncan's results, Hebb (1949) formalized the idea that propagating or recurrent impulses of a specific spatiotemporal pattern underlie initial memory. This provided the rationale for the use of ECS as an amnesic agent to study the temporal dynamics of consolidation, since such a specific spatiotemporal pattern of neural activity could hardly survive the electrical storm induced by ECS.

Thus the study of memory became, for the most part, a study of function through dysfunction. Investigators overwhelmingly relied on amnesia – either clinical studies of amnesic patients or animal models of experimental amnesia. The protocol of retrograde amnesia, indeed, opened a door on a neurobiological approach to the study of memory, evaluating the efficacy and temporal dynamics of diverse physiological treatments to disrupt memory without interfering with acquisition. The common feature of these experiments is that amnesic agents lose their ability to respectively impair memory as the interval between memory acquisition and treatment is increased, defining a temporal gradient. This large body of data supported the consolidation hypothesis, which stipulates that (1) memories are fixated or consolidated over time; (2) once consolidated, memories are then stable; and (3) acquisition of a new memory and its consolidation together form a unique event. Consolidation happens only once (McGaugh, 1966, 2000).

1.24.2.2 Challenges to the Consolidation Hypothesis

Embedded in the extensive literature on memory consolidation generated during the 1970s, however, were a myriad of studies challenging the interpretation of these retrograde amnesia experiments (*See also* Chapters 1.04, 1.05, 1.14). These include scores of demonstrations of recovery from retrograde amnesia

over time or after a reminder. Spontaneous recovery at various times after ECS-induced amnesia was reported by Cooper and Koppenaal (1964), Kohlenberg and Trabasso (1968), Young and Galluscio, (1971), and D'andrea and Kesner (1973). There were similar reports of spontaneous recovery after protein-synthesis-induced amnesia as well (Quartermain et al., 1970; Serota, 1971; Squire and Barondes, 1972). Moreover, reminders before the retention test in the form of exposure to the conditioned stimulus (CS) or the unconditioned stimulus (US) (Koppenaal et al., 1967; Galluscio, 1971; Miller and Springer, 1972; Quartermain et al., 1972) or to the training context (Quartermain et al., 1970; Sara, 1973; Sara and David-Remacle, 1974) could effectively promote expression of memory in rats that had been submitted to an amnestic treatment after learning. Later, pharmacological studies added particularly strong arguments for the contention that the amnesic agent did not prevent formation of a memory trace. Drug treatment given before the retention test could attenuate or reverse amnesia (Gordon and Spear, 1973; Sara and Remacle, 1977; Rigter and VanRiezen, 1979; Quartermain et al., 1988). If the animal could express memory after a drug treatment, with no further exposures to the elements of the learning situation, then the recovery could not be attributed to new learning.

This large and growing body of literature benefited from a thorough and thoughtful review by Donald Lewis as early as 1976, in a paper titled "A cognitive approach to experimental amnesia." His conclusion at that time was that memory 'fixation' was very rapid – a matter of seconds, and that the extended retrograde amnesia gradient was due to the effect of the treatments on retrieval (Lewis, 1976). Indeed, if memory disruption after ECS, hypothermia, or protein synthesis inhibition is alleviated by reminders or drugs given before the test, then the sparing of the original memory trace would be a logical imperative, requiring an alternative explanation for the behavioral deficit. Reconsolidation has awakened new interest in this literature, generated more than 30 years ago, and several reviews by those very investigators who contributed the initial studies 30 years ago have been published recently (Gold, 2006; Riccio, 2006; Sara and Hars, 2006).

Many clinical investigators dealing with human amnesic patients also argued convincingly that amnestic syndromes were, for the most part, due to retrieval dysfunction (*See* Chapters 1.14, 1.15). This was based on experiments showing that profoundly amnesic patients were able to benefit as much as healthy volunteers from partial cuing in a memory test of previously acquired word lists. This retrieval facilitation by cuing occurred even though the patients did not remember ever having learned the list. Such a phenomenon led to the conclusion that the memory deficit was due to a retrieval dysfunction rather than a failure to consolidate the new memory (Warrington and Weiskrantz, 1970). This naturally led to a call for consideration of retrieval, itself, as an intricate part of the memory process. Warrington and Weiskrantz went on to warn against any interpretation of behavioral deficits after amnestic treatments in animals as failure to consolidate, because it was impossible to demonstrate experimentally the absence of a memory trace. On the other hand, their studies clearly demonstrated that memory traces could be revealed by appropriate retrieval cues.

1.24.2.3 Amnesia and Forgetting As Retrieval Failure

Norman Spear took the position, with Weiskrantz, that all memory deficits, including forgetting, should be considered as retrieval failure, since it was impossible to prove the absence of a memory trace (Spear, 1971). He argued in several monographs published in the 1970s that memory studies should focus on retrieval. Remote memory is always apprehended through its retrieval and, especially in animal studies, through its expression as adaptive behavior. Thus the context in which the retention test is administered can play a determinant role in the behavioral expression of memory (or amnesia). The retrieval context includes the learning-associated environmental cues, and also the internal state of the animal, including motivational and attentional factors (Spear, 1971, 1973, 1976, 1981; Spear and Mueller, 1984). Indeed, many studies were later to confirm this hypothesis: Spontaneous forgetting of a complex maze task could be reversed by exposure to contextual cues just before the retention test (Deweer et al., 1980; Deweer and Sara, 1984; Gisquet-Verrier and Alexinsky, 1986). Furthermore, electrical stimulation of the mesencephalic reticular formation (MRF) (Sara et al., 1980; Dekeyne et al., 1987) or the noradrenergic nucleus locus coeruleus (Sara and Devauges, 1988; Devauges and Sara, 1991) also facilitated the retrieval of the a 'forgotten' maze task when administered before the retention test. All of these experiments used the same

appetitively reinforced maze task adapted from that described by Donald Lewis in his earlier 'cue-dependent amnesia' studies (see below).

While emphasizing the 'lability' of the retrieval process and its dependence on the information in the retrieval environment, Spear's main thesis was that "consolidation occurs when memory is retrieved, as well as when it was stored originally" (Spear and Mueller, 1984, p. 116; see also Spear, 1981). Nevertheless, it is nowhere specified in Spear's writings that retrieval processes trigger time-dependent neurobiological processes identical to that occurring after learning, nor does he suggest experimental protocols that would test this thesis. The experiments cited above, demonstrating a facilitation of retrieval by pretest manipulations, do not directly address the issue of consolidation occurring at retrieval, because the retention test occurs within a time frame when one would expect residual effects of the memory-modulating treatment on behavior. The adequate protocol to test treatment effects on a putative post-retrieval consolidation would be to reactivate the memory by means of a retrieval cue, administer the treatment, and then test for retention at some later time, when the effects of the treatment would have dissipated. A change in memory expression, compared with a nontreated control group, could then be attributed to reinforcement or disruption of a postretrieval consolidation process.

1.24.3 Cue-Dependent Amnesia

1.24.3.1 Seminal Studies by Donald Lewis

Such experiments, carried out independently by Donald Lewis and his colleagues, demonstrated 'cue-dependent amnesia' in the rat. These studies showed that a temporally graded retrograde amnesia could be obtained when the memory trace was activated by a reminder of the original learning event, just before the amnestic treatment. While the 'recovery' studies, discussed above, challenged the consolidation hypothesis' claim that experimental amnesia procedures block the time-dependent formation of the memory trace, the 'cue-dependent amnesia' studies challenged the corollary that consolidation occurs only once, that is, that consolidated trace is fixed and impervious to further disruption (McGaugh, 1966, 2000; Dudai, 2004).

These studies of Lewis are really at the origin of today's 'reconsolidation' hypothesis, so it is appropriate to examine these experiments in detail to determine to what extent they already addressed some of the current issues being raised. In the first series of experiments from the Lewis laboratory, rats were trained in conditioned lick-suppression protocol. Thirsty rats learned to lick a drinking spout; when this behavior was well established, a tone (conditioned stimulus, CS), followed by a footshock (unconditioned stimulus, US), was presented during the ongoing licking behavior. Subsequent presentations of the tone alone elicited suppression of licking. A day after training, when memory expression was robust and reliable in control rats, the CS was presented alone, followed by electroconvulsive shock, a treatment that produces amnesia when administered after learning. Those rats that were 'reminded' by the CS, before ECS, showed a significant behavioral deficit when tested the following day. ECS in absence of the cue had no effect on subsequent behavior. These investigators referred to the phenomenon as 'cue-dependent amnesia.' Their interpretation was that the cue reinstated the memory, putting it in an active state and making it labile, as it was immediately after acquisition (Lewis and Maher, 1965, 1966; Misanin et al., 1968; Lewis, 1969, for Review). Cue-dependent amnesia was replicated by Mactutus et al. (1979), see Lewis 1969 for review using hypothermia as the amnesic agent.

Cue-dependent amnesia could likewise be induced by protein synthesis inhibition in much the same way that newly acquired memories are. Judge and Quartermain (1982) trained mice on the conditioned lick suppression task used by Lewis. The protein synthesis inhibitor anisomycin was injected systemically at different time intervals after a single memory reactivation, consisting of a brief exposure to the training context. There was a clear renewed efficacy of the treatment after reactivation, although the temporal gradient was steeper than for that generated after initial learning. (It can be noted that the conditioned lick suppression is a Pavlovian conditioning protocol based on the tone-shock association and, as such, is perfectly analogous to the 'conditioned fear' protocol that is now almost universally used in reconsolidation studies. In both cases the response to presentation of the CS alone is behavioral inhibition; i.e., lick suppression or freezing.)

Later experiments from the Lewis laboratory showed that the phenomenon of cue-dependent amnesia was not limited to aversive Pavlovian conditioning protocols. In a series of experiments, rats were trained in a complex maze consisting of four consecutive left–right choices, using food reward as the incentive (see **Figure 1**). The training procedure

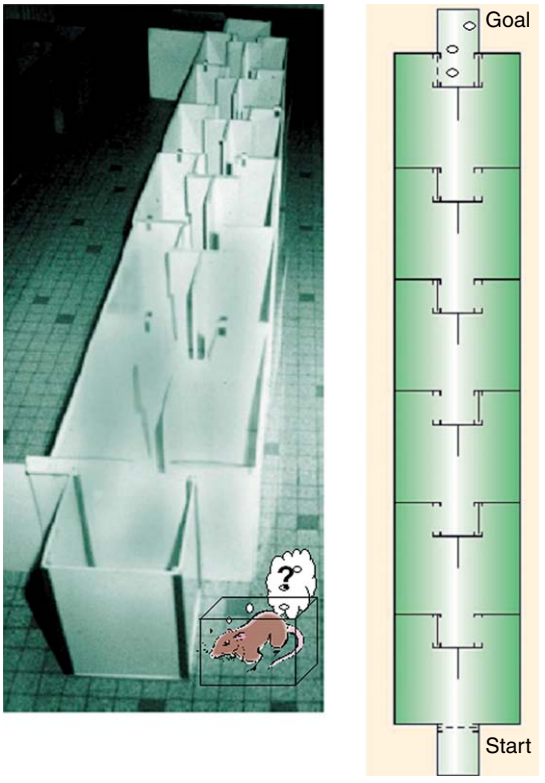

Figure 1 Krechevsky maze consisting of a series of left–right choices to reach a goal box containing palatable food. In the six-unit version, shown here, exposure to contextual cues in the experimental room, combined with stimulation of the reticular formation or the locus coeruleus just before the retention test, alleviated forgetting. A four-unit version of the task was used by Lewis to show cue-dependent amnesia. Adapted from Sara SJ (2000b) Strengthening the shaky trace through retrieval. *Nat. Rev. Neurosci.* 1: 212–213.

involved 10 days of elaborate pretraining, and then rats were trained for ten trials/day over several days until they reached the stringent behavioral criterion of 11/12 correct choices on three successive trials. During a 7-day rest period, rats were handled several times daily in order to extinguish handling-associated arousal. The following day, rats were exposed to the start box of the maze and the click of the door opening to provide reinstatement cues for the memory of the maze. This procedure was followed by electroconvulsive shock treatment. Retention was assessed 24 h later by counting the number of errors made before attaining the initial training criterion. Rats subjected to the reactivation procedure followed by ECS expressed profound amnesia compared with those subjected to ECS alone in the absence of the reactivation. A series of careful control experiments eliminated motivational and arousal confounds and explored the nature of the relevant cues in the start box. It was determined in this case that the click of the door opening in the start box was the salient feature in reinstating the memory (Lewis et al., 1972; Lewis and Bregman, 1973).

1.24.3.2 Behavioral Studies

Purely behavioral studies, in animals and humans, further confirmed that retrieval induces memory lability. Gordon and Spear (1973), in a series of experiments in rats, showed that reactivation of memory by various reminders makes it vulnerable to interference by another task, or to distortion by nonrelevant cues present at the moment of reactivation. This approach has been used recently with human subjects, showing that the memory for a list of junk objects can be distorted by intrusions from a second list presented right after the memory of the first list was reactivated by a reminder cue. An important observation in the latter series was that the disruption of memory was only expressed after a delay of 1 day; subjects tested right after learning the second list showed good retention (Hupbach et al., 2007). This experiment not only illustrates that memory can be distorted by intrusions but, on a more positive note, also shows how new information is integrated into an existing functional memory system when it is in an active state. This is the kind of analysis and approach advocated by Lewis several decades ago in his monograph entitled "A cognitive approach to experimental amnesia" (Lewis, 1976).

These studies are compatible with a long line of human studies suggesting that memory is substantially modified by the incorporation of new information during retrieval (Loftus, 1979, 1981). In the view of all these authors, the modulation of long-term memory is not an ongoing continuous process but occurs at transient windows of opportunity when the trace is in an active state. Reactivation can be spontaneous or triggered by external or internal events and, as discussed below, may even occur during sleep (see Sara and Hars, 2006, for review).

1.24.4 Cue-Dependent Amnesia: Neurobiological Hypotheses

Thus it was clearly established, by broad experimental evidence, as early as the late 1960–1970s, that well-consolidated memories are vulnerable to interference in a time-dependent manner when they were

in an active state. Unfortunately, little attempt was made at the time to integrate this phenomenon of 'cue-dependent amnesia' into the rapidly developing neurobiological hypotheses of memory formation.

1.24.4.1 NMDA Receptors in Cue-Dependent Amnesia

In 1997, our interest in cue-dependent amnesia was rekindled by some unexpected results that would provide the opportunity for such integration. The initial purpose of our experiments had been to study the effect of *N*-methyl-D-aspartate (NMDA) receptor blockade on various stages of acquisition of a spatial reference memory task to fix a critical time point during the multisession acquisition when these receptors might play a specific role. The surprising result of the initial experiment was that not only did the NMDA receptor antagonist, MK-801, disrupt performance of a well-trained spatial task but the rats continued to show a decrement on the subsequent trial, 24 h later. Follow-up experiments showed that posttrial injections of the antagonist up to 2 h after the training trial likewise induced the performance decrement the following day. Given our long-standing interest in the dynamics of memory retrieval and recovery from amnesia, we interpreted these unexpected results within Lewis' conceptual framework of 'cue-dependent amnesia.' Since there were no previous reports of long-lasting effects of MK-801, we performed a series of experiments to confirm that a well-consolidated spatial memory, acquired over many days, reactivated by a single errorless trial, was somehow dependent upon intact NMDA receptors to maintain stability. The memory deficit was robust, in that there was no spontaneous recovery 48 h later (Przybyskawski and Sara, 1997, Exp4). Amnesia was, however, only partial, in that drug-treated rats could relearn the task and attain asymptotic levels of performance in only a few massed trials (Przybyskawski and Sara, 1997, Exp3). Extrapolating from these data, we suggested that

> memory is reconsolidated, so to speak, each time it is retrieved and these reconsolidation processes are dependent on the NMDA receptor for at least 2 h after the reactivation. (Przybyskawski and Sara, 1997, p. 245)

To our knowledge, this is the first use of the term 'reconsolidation' in the literature in relation to the well-established phenomenon of cue-dependent amnesia.

1.24.4.2 Role of the Noradrenergic System

We went on to investigate the role of beta-adrenergic receptors in this putative reconsolidation process and showed that postreactivation, a systemically injected beta antagonist, propranolol, induced amnesia in the spatial memory task protocol. Memory had to be reactivated by a single reminder trial for the drug to induce amnesia, expressed 24 h later.

As the spatial discrimination task is acquired over many trials, it does not lend itself to comparison of the temporal dynamics of postretrieval reconsolidation with that of postacquisition consolidation. This is an essential step in establishing that reconsolidation involves cellular processes similar to those occurring during the initial consolidation. Injection of propranolol after reactivation of a single-trial passive avoidance task also induced amnesia. In this case the effect of the beta receptor antagonist was even more robust after reactivation than after original training (Przybyskawski et al., 1999, Figs. 4 and 5).

Both the spatial memory task and passive avoidance memory formation depend upon hippocampal activity. To investigate the temporal dynamics of NMDA and beta receptors in reconsolidation in a task that does not involve the hippocampus, we used a simple, rapidly learned odor-reward association task (Tronel and Sara, 2002). Rats were extensively handled, mildly food deprived, and familiarized with the palatable reinforcement. Three sponges, each impregnated with a different odor, are arranged symmetrically within a wooden box; the spatial configuration of the sponges within the box are changed from trial to trial. Chocokrispies are hidden in the hole of the sponge with the target odor. The response is measured as a nose poke into the correct hole. Rats are very proficient at this task, learning the three-way discrimination in only three trials (for further details see Tronel and Sara, 2002). Intracerebroventricular (ICV) injections of an NMDA receptor antagonist induce amnesia not only when the injections are made immediately after learning (Tronel and Sara, 2003) but also when drug treatment is administered after a reminder cue consisting of the target odor presented in the experimental context (Torras-Garcia et al., 2005). In this task, ICV injection of beta receptor antagonists induces amnesia when the injection is made within a narrow time window around 2 h after learning, and the rat is tested 48 h later (**Figure 2**, top). Quite strikingly, the same temporal dynamic of involvement of beta receptors is seen after memory reactivation (**Figure 2**, bottom);

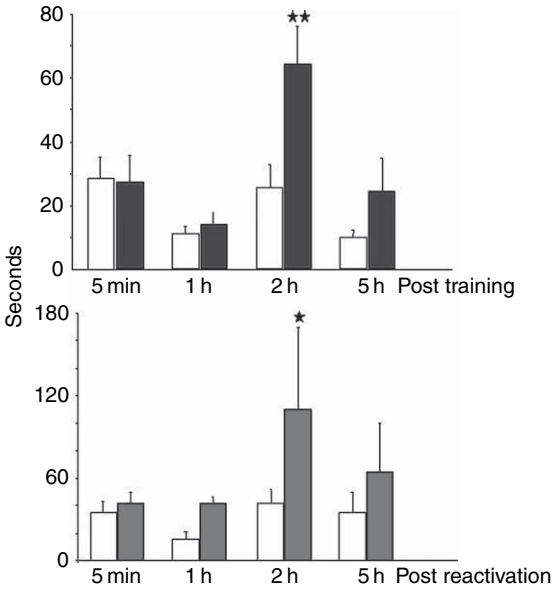

Figure 2 Retention performance in terms of latency to find the reward in an odor-reward association task 24 h after learning (top) or 24 h after memory reactivation by a brief exposure to the CS in the experimental context (bottom). Rats were injected with saline (white bars) or a beta receptor antagonist (red bars) at times indicated after training. There was a narrow time window at 2 h after training when the initial memory required beta receptors and a strikingly similar time window after reactivation when the drug treatment was effective in producing amnesia. Adapted from Tronel S, Feenstra MG, and Sara SJ (2004) Noradrenergic action in prefrontal cortex in the late stage of memory consolidation. *Learn. Mem.* 11: 453–458.

thus, the noradrenergic system appears to be involved in a late phase of memory consolidation, and again in reconsolidation (Tronel et al., 2004).

Based on the data from these studies, we proposed that treatment with propranolol in conjunction with psychotherapeutic memory reactivation could serve to attenuate the compulsive traumatic memories associated with posttraumatic stress disorder (Przybyskawski et al., 1999).

1.24.5 Rebirth of Reconsolidation

These pharmacological data, showing cue-dependent amnesia effects of NMDA and beta antagonists, merely confirmed and extended results obtained by many others nearly three decades earlier. Donald Lewis had proposed, in light of the large amount of data already available at this early date, to replace the consolidation paradigm by a conceptual framework of active and inactive memory in labile and stable states and to open the way for a more cognitive interpretation of amnestic syndromes (Lewis, 1979). It is truly ironic that the Lewis cue-dependent amnesia studies are at the origin of the current 'reconsolidation' hypothesis, as it is clear that the phenomenon of postreactivation lability cannot be understood by a simple extension of the consolidation concept. The problem is that cue-dependent amnesia is not predicted by the consolidation hypothesis and is, in fact, in direct contradiction of it. Neither Lewis nor his contemporaries used the term reconsolidation, and they were generally not interested in such questions as "does reconsolidation recapitulate consolidation?" Their aim had been merely to show that the amnesia gradient did not reflect the duration of a consolidation process and that consolidation was not a unique event. Memory was labile when in an active state, and lability was not time bound to acquisition. Indeed, the initial series of experiments was explicitly designed by Lewis and colleagues to challenge the interpretation of retrograde amnesia as consolidation failure and to inspire a more cognitive interpretation of memory function and dysfunction.

Nevertheless, there has been a surge of interest in 'reconsolidation' initiated by elegant experiments from the Ledoux laboratory. These investigators found that amnesia could be obtained after reactivation of conditioned fear by injecting, directly into the amygdala, the protein synthesis inhibitor anisomycin (Nader et al., 2000). The results obtained were similar to those of Judge and Quartermain (1982), except that now the protein synthesis inhibition was limited to a structure that is part of the neural circuit underlying the fear conditioning.

1.24.6 Neurobiological Substrates and Boundaries of Reconsolidation

The decade since this report has seen a proliferation of studies of cue-dependent amnesia that fall into two categories. One approach has been to study the cellular and molecular processes implicated in putative reconsolidation and to investigate to what extent they recapitulate consolidation processes occurring after initial learning. The second, and by far the more controversial, approach lies in an attempt to firmly establish (or repudiate) reconsolidation as a real phenomenon in memory processing by delineating the 'boundaries' within which cue-dependent amnesia can be obtained and sustained.

1.24.6.1 Neurobiological Substrates

The search for cellular and molecular substrates of reconsolidation has produced a myriad of results delineating neuromodulatory systems, neurotransmitters, intracellular signaling pathways, transcription factors, and brain regions that are necessary for both posttraining and postretrieval memory stabilization. Others seem to be specific to one or the other stage of memory processing. For example, NMDA receptors are necessary for both consolidation and reconsolidation across tasks and species (Pryzbyslawski and Sara, 1997; Perdreia et al., 2002; Torras-Garcia, 2005; Akirav and Maroun, 2006; Lee et al., 2006). The role of beta noradrenergic receptors, on the other hand, seems to be restricted to postretrieval memory processing (Debiec and LeDoux, 2006; Pryzbyslawski et al., 1999; Roullet and Sara, 1998; Diergaarde et al., 2006). Early implication of the transcription factor cAMP response element binding protein (CREB) in the stabilization of both new and reactivated fear memory was established by experiments using transgenic mice with inducible and reversible CREB repressor (Kida et al., 2002). This burgeoning literature concerning neurobiological substrates of postretrieval memory processes, focusing, for the most part, on intracellular cascades and immediate early gene expression, has been subject to several recent comprehensive reviews (Alberini, 2005; Alberini et al., 2006; Dudai and Eisenberg, 2004) and has been updated in the present volume (*See* Chapter 4.14).

1.24.6.2 Boundaries of Reconsolidation

It is becoming increasingly evident, as the literature grows, that the same old questions raised during the consolidation era, concerning the nature of the memory deficit after an amnestic treatment, persist, although the discussion lacks the strong polemics of the past generation. Does the amnestic agent block consolidation, or now reconsolidation, or does it impair retrieval (*See* Chapters 1.04, 1.05, 1.14)? The single-trial inhibitory avoidance protocol that was used almost exclusively in earlier consolidation studies has been replaced by a simplified version of conditioned fear, in which a CS is associated with footshock to produce a conditioned emotional response measured as freezing behavior. The protein synthesis inhibitor, anisomycin, has largely replaced ECS as the generic amnestic agent in these reconsolidation boundary studies.

1.24.6.2.1 A note on the action of anisomycin

The increasing use of anisomycin as a generic amnestic agent to study boundaries and temporal dynamics of postretrieval memory processing is based on the widely held assumption that *de novo* protein synthesis is the final step of the intracellular cascade triggered by a learning or retrieval event and necessary for the consolidation or reconsolidation of long-term memory. This recent literature largely ignores the fact that caution was urged by the early users of protein synthesis inhibitors as amnestic agents in behavioral experiments, because of toxicity and ability to induce behavioral aversion. Thus, special care must be taken, especially in avoidance experiments, to dissociate aversive and amnestic effects of the drugs on behavior (Squire et al., 1975; Davis et al., 1980). Furthermore, several early investigators provided evidence that memory impairment attributed to protein synthesis inhibitors could be accounted for, at least in part, by specific effects on brain catecholamine systems (Flexner and Goodman, 1975; Quartermain et al., 1977; Flood et al., 1980; Altman and Quartermain, 1983; Davis and Squire, 1984, for review). These data take on particular importance in the light of several recent studies reporting the amnestic effects of the beta adrenergic antagonist propranolol after memory retrieval (see preceding paragraph).

More recent literature has underlined the fact that anisomycin activates the MAPkinase intracellular signaling pathway and causes apoptosis at doses lower that those that inhibit protein synthesis (Routtenberg and Rekart, 2005; Rudy et al., 2006). So, while the behavioral deficits associated with administration of anisomycin after memory reactivation are quite reliable, they may be caused by effects other than the inhibition of protein synthesis. This underlines the caveats inherent in elaborating a theory of memory function relying exclusively on a single paradigm that includes fear conditioning and anisomycin-induced amnesia.

1.24.6.2.2 Permanence of cue-dependent amnesia?

Is the memory deficit permanent, or is there spontaneous recovery or the possibility of recovering the memory by further treatments or reminders? Quartermain showed early on that postretrieval anisomycin-induced memory deficits in mice were less persistent than the deficits obtained by the same dose administered after training (Judge and Quartermain, 1982). They found

spontaneous recovery of memory in mice 4 days after the reactivation-drug treatment, while behavioral expression of amnesia was still present in those mice receiving the anisomycin after training. In more recent studies, in mice submitted to context fear conditioning and systemic injection of anisomycin, amnesia is durable after acquisition, but after reactivation it is necessary to use repeated injections, and the amnesia is transitory (seen at 1 day but not at 21 days; Lattal and Abel, 2004; Prado-Alcala et al., 2006). Spontaneous recovery from cue-dependent amnesia has been confirmed by others using either systemic or locally injected anisomycin in rats, mice, chicks, or fish (Anokhin et al., 2002; Eisenberg and Dudai, 2004; Power et al., 2006). On the other hand, at least one group reports persistent amnesia even several weeks after retrieval and treatment by anisomycin (Duvarci and Nader, 2004). These discrepancies are similar to those reported decades ago concerning the nature and permanence of the memory dysfunction in experimental amnesia studies, as discussed above. Moreover, the same logical objection voiced by Warrington and Weiskrantz (1970) years ago can be raised here. Experimental amnesia studies are fatally flawed from the outset, since it is not possible to prove the null hypothesis (i.e., the absence of a memory trace).

1.24.6.2.3 Age and strength of the memory

The ease with which cue-dependent amnesia can be obtained may depend upon the age and the strength of the memory. Newer memories appear to be more susceptible to disruption after their retrieval than are older memories (Milekic and Alberini, 2002; Eisenberg and Dudai, 2004; Suzuki et al., 2004). Moreover, the strength of the memory, as revealed by the probability of its behavioral expression, also determines its vulnerability at retrieval. This has been shown by manipulating the number of unreinforced reminder trials to yield either reactivation or extinction (Eisenberg et al., 2003; Stollhoff et al., 2005; *See* Chapter 1.27). With a weak memory, resulting from a single training trial, in a conditioned taste aversion task, unreinforced presentation of the CS results in extinction. If this is followed by an amnestic treatment, extinction is blocked, and retention for initial learning is expressed at retention test. If the initial memory is strong, presentation of the CS reactivates the memory, rendering it labile, and amnesia is expressed at retention test (Eisenberg et al., 2003).

Another way to shift from retrieval to extinction in behavioral control is to modify the duration or the repetition of the cuing episode: a brief retrieval will reactivate the memory, making it labile. A long or repeated retrieval will lead to extinction (i.e., new learning, with its requirement for consolidation). Using a fear conditioning to context protocol, Suzuki et al. (2004) show that there is no amnesia with brief exposure to the CS (1 min), amnesia with a moderate exposure (3 min), or retention with a long CS exposure (30 min). This retention is interpreted as an amnesia for the extinction induced by the 30-min unreinforced CS exposure. Interestingly, they observe that the effective duration of cuing to induce lability increases with either the strength or the age of the memory.

1.24.6.2.4 Task- and species-related boundaries

Although most of the studies of cue-dependent amnesia use fear conditioning, the phenomenon can readily be obtained after different forms of appetitive and aversive learning in many species: rodents (Lewis et al., 1972; Lewis and Bregman, 1973; Pryzbyslawski and Sara, 1997; Pryzbyslawski et al., 1999; Torras-Garcia et al., 2005; Wang et al., 2005; *See* Chapter 1.09), and even in the honeybee (Stollhof et al., 2005), crab (Frenkel et al., 2005), slug (Sangha et al., 2003), and snail (Gainutdinova et al., 2005; Kemenes et al., 2006) (*See* Chapter 1.27). Behavioral tasks used in these studies have included conditioned taste aversion (Eisenberg et al., 2003; Gruest et al., 2004a,b), object recognition (Kelly et al., 2003), inhibitory avoidance (Milekic and Alberini, 2002), instrumental incentive learning (Wang et al., 2005), odor reward association (Torras-Garcia et al., 2005), and eyelid conditioning (Inda et al., 2005). Moreover, at least in the case of rodents, this aspect of memorization is already present at the beginning of life, showing that it is a fundamental aspect of memory (Gruest et al., 2004a,b).

Despite generality of the cue-dependent amnesia phenomenon across species and tasks, some important constraints have recently been reported. Biedenkapp and Rudy (2004) attempted to induce amnesia by injecting anisomycin after reactivating a memory for a context. Rats received six massed exposures to a specific context in which they would be later receive tone–shock fear conditioning. In this particular protocol, the context preexposure is necessary for the rat to subsequently learn the tone–shock

association. The authors clearly showed that intrahippocampal anisomycin induces amnesia for the context when injections are made immediately after the preexposure. However, they failed to obtain cue-dependent amnesia if the injections were made after a 5-s or a 1-min reactivation of the context memory. One explanation that they offer to account for their negative findings is the 'significant event hypothesis.' A significant event is one that has been associated with a reinforcement, giving it predictive value. This hypothesis is quite appealing in light of the strong evidence for a major role for the locus-coeruleus noradrenergic system in memory retrieval and putative reconsolidation processes, as discussed above. It would take a 'significant event' to elicit the attention or arousal response associated with the activation of neuromodulatory systems (Sara, 1985, 1991; Bouret and Sara, 2004, 2006).

A related determinant of the lability of a reactivated memory is the extent to which a new encoding mode is solicited at the time of retrieval (Morris et al., 2006). These authors show that a reactivated spatial reference memory, learned in the water maze over several days, is not susceptible to amnesia induced by injection of a protein synthesis inhibitor into the hippocampus. It is only when new information must be integrated into the existing memory that amnesia follows such injections. These data fit nicely with the 'significant event' hypothesis. New information requiring behavioral adaptation should elicit attention and activate neuromodulatory systems necessary for stabilization of the reorganized memory.

1.24.6.2.5 A note on the problem with negative results

Cue-dependent amnesia studies lead to the conclusion that memory in an active state is labile and can be disrupted by a wide range of treatments, many of which are effective in producing amnesia when applied after new learning, as well. If an animal expresses amnesia after training and amnestic treatment, one concludes that memory consolidation was blocked by the treatment. If the memory is subsequently expressed after a reminder or a pharmacological treatment, one must conclude that the trace was there, but for some reason the animal could not express it behaviorally. What about possible outcomes of experiments evaluating the putative reconsolidation processes? There the amnestic agent is applied after a reminder that is supposed to reactivate the memory. If the rat expresses amnesia on a retention test, can it be taken as proof that the treatment erased or weakened the reactivated, labile trace by preventing reconsolidation? Suppose the memory is expressed at some later test? Or after a reminder? So, as Weiskrantz warned, we are faced with the impossible challenge of proving that the memory trace does not exist. When no cue-dependent amnesia is expressed on the retention test, there are several possible conclusions: (1) the amnestic agent was not effective in blocking reconsolidation because of, for example, inappropriate dose), (2) the reactivation treatment was not sufficient to elicit the memory to put it into an active labile state, or (3) postreactivation reconsolidation processes are not necessary.

1.24.7 Beyond Cue-Dependent Amnesia: Retrieval Strengthens Memory

This large body of literature concerned with cue-dependent amnesia confirms the *lability* of a memory trace after its reactivation, but it should not lead to the counterintuitive conclusion that retrieval weakens memory. Memory lability has always been easier to document behaviorally through amnesic rather than through promnesic treatments, which is why most investigators have chosen this research strategy. We know, however, that retrieval of memory does not usually result in wiping it out or even weakening it. On the contrary, a high level of attention or arousal during retrieval, a more likely real-life scenario, should *reinforce* the labile active memory. Remembering, especially when it involves effortful retrieval, usually occurs in an attentive, motivated behavioral state. During such states, neuromodulatory systems are activated (Bouret and Sara, 2004), releasing noradrenaline, dopamine, and other neuromodulators in the forebrain structures involved in the ongoing sensory processing and retrieval. These neuromodulators act to promote synaptic plasticity and trigger intracellular processes leading to new protein synthesis, upon which stable long-term memory is dependent (Sara, 2000a,b) (*See* Chapter 1.27 for a discussion of the internal reinforcement theory).

1.24.7.1 Cue-Dependent Enhancement

1.24.7.1.1 Enhancement by MRF stimulation

Although the strengthening of a memory trace by repeated remembering seems intuitively valid, experimental documentation of retrieval-associated memory improvement is rather sparse. There are a few reports

of marked improvement of memory in the rat when retrieval is accompanied by arousal. Experiments by DeVietti et al. (1977) demonstrated that electrical stimulation of the mesencephalic reticular formation (MRF), which improves memory consolidation when administered within a short time after acquisition, improved memory for a well-consolidated conditioned fear response when it was applied after memory reactivation. The reactivation treatment consisted of a 15-s exposure to the tone in the training chamber; the rat was tested 24 h later. The shorter the interval between the reactivation and the MRF stimulation, the better the memory enhancement, the temporal gradient of efficacy being strikingly similar to the postacquisition gradient (**Figure 3**).

1.24.7.1.2 Enhancement by activation of the noradrenergic system

It has been shown repeatedly that stimulation of the noradrenergic system will enhance memory retrieval when given before the retention test (reviewed above), but it is only recently that the effects of pharmacologically increasing noradrenergic tonus on putative reconsolidation processes have been investigated. The alpha 2 receptor antagonist, idazoxan, increases firing of noradrenergic neurons of the locus coeruleus twofold at a dose that has no detectable effect on overt behavior such as locomotor activity (Sara, 1991). Rats were trained in the odor-reward association task used in the timolol studies described above. Forty-eight hours later, they were exposed to the odor in a neutral cage, located in the experimental room. This was followed by an injection of idazoxan; control rats were handled in the colony room before injection. Idazoxan enhanced performance when the rat was tested 48 h later, but only when the injection was given after memory reactivation (**Figure 4**). These data complement studies showing cue-dependent amnesia induced by beta adrenergic antagonists, discussed earlier. Together they lend support to the notion that the locus-coeruleus-noradrenergic system is activated by cues associated with target memories and contributes to the postreactivation stabilization or reinforcement of the memory (Sara, 2000b; see also Sara, 1985).

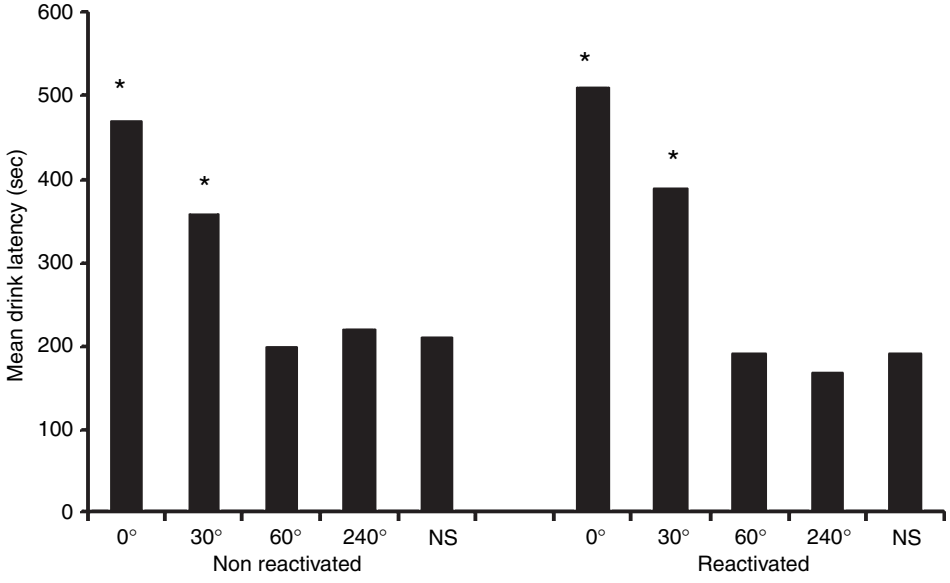

Figure 3 Retention performance in a lick suppression task after training and electrical stimulation of the mesencephalic reticular formation (MRF) (left) or after memory reactivation by the tone conditioned stimulus and (MRF) stimulation (right). In each condition one group of rats received no stimulation, to provide a baseline performance (NS). Histograms from left to right represent data from rats stimulated immediately after training or exposure to the tone or progressively longer delays. The treatment was effective in improving retention when applied up to 30 min after training; behavioral performance of rats stimulated after that was indistinguishable from that of NS rats. Note the striking similarity between the temporal gradient of efficacy after training and after reactivation. Figure adapted from Devietti TL, Conger GL, and Kirkpatrick BR (1977). Comparison of the enhancement gradients of retention obtained with stimulation of the mesencephalic reticular formation after training or memory reactivation. *Physiol. Behav.* 19: 549–554, with permission from Elsevier.

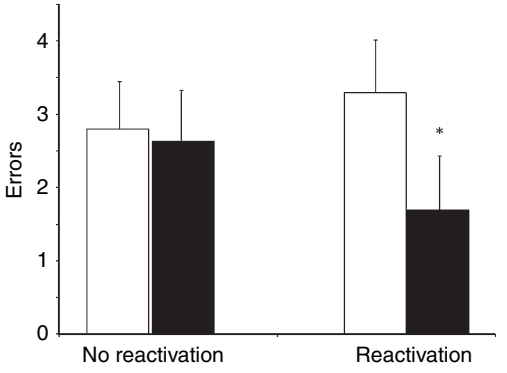

Figure 4 Retention performance in terms of errors before finding the reward in a retention test, 3 weeks after learning the odor-reward association test. Left: data from rats receiving no reactivation on the day before the test. Right: data from rats exposed for a few minutes to the target odor in the experimental room, 24 h before the retention test. White bars: saline injections after reactivation or no reactivation. Black bars: rats injected with idazoxan (an alpha 2 receptor antagonist that increases release of NE), 2 mg kg^{-1} ip, after no reactivation or reactivation.

1.24.7.1.3 Enhancement by activation of PKA

The beta adrenergic receptor is one of a family of receptors positively linked to G proteins that serve to activate the cyclic AMP intracellular signaling cascade, leading to gene induction by the transcription factor CREB. The resulting *de novo* protein synthesis is thought to be essential for the stabilization of long-term memory. Activation of protein kinase A (PKA) is an important step in this cyclic AMP intracellular signaling cascade. A recent study by Tronson et al. (2006) has shown that pharmacologically activating PKA within the rat amygdala can facilitate fear memory if and only if the memory has been reactivated by a reminder. These results, taken together with the studies showing enhancement of reactivated memory by beta adrenergic agonists, lend support to the notion that the noradrenergic system, activated at retrieval, serves to reinforce memories rendered labile by reactivation.

1.24.7.2 Clinical Significance of Cue-Dependent Enhancement

Clinical syndromes such as posttraumatic stress disorder (PTSD), phobias, obsessive compulsion, and craving in addiction share the common feature of underlying compelling, persistent memories. The possibility that memory may be rendered labile under controlled conditions has broad therapeutic applications for these disorders, accounting for the increasing number of investigators interested in this aspect of memory.

In particular, the susceptibility of reactivated memories to noradrenergic manipulation sheds some light on the underlying mechanisms of pathological persistence of memory. In the case of PTSD, it has already been established that there is greater noradrenergic (NE) activity under baseline conditions in patients with chronic PTSD than in healthy subjects, with a direct relationship of NE activity to the severity of the clinical syndrome (Geracioti et al., 2001). Further activation of this system during stress could recreate the internal state induced by the original trauma and thereby reinstate the memory (Grillon et al., 1996). The demonstrated role of the intracellular pathway activated by this system in reinforcing reactivated memories (Tronson et al., 2006) suggests that the memory recalled in the presence of a high level of NE will be reinforced each time (see also **Figure 3**).

The potential usefulness of noradrenergic receptor–blocking agents in PTSD has already been pointed out by Cahill (1997), who suggested that treatment with beta blockers as soon as possible after the traumatic event might prevent the development of PTSD. The recent 'rediscovery' of the phenomenon of lability of memory in its active state adds a new dimension to this potential use. Treatment with beta receptor antagonists, at the time of spontaneous or clinically elicited reinstatement of the traumatic memory, should serve to attenuate the active memory by blocking reconsolidation processes (Przybyskawski et al., 1999).

1.24.8 New Look at Retrieval and 'Reconsolidation'

Memory only lends itself to study through its retrieval; as William James underlined more than a century ago, "the only proof of there being retention is that recall actually takes place" (James, 1892). Although some memory retrieval is likely to occur spontaneously as a result of random fluctuations of network activity in the brain, retrieval is usually brought about with effort, as a result of integration of incoming environmental information with the 'memory network' driven by that information (Tulving and Thomson, 1973). It follows from this that the formation of new memories will always be made on the background of retrieved information. Recent functional imaging studies in humans are, indeed, confirming these earlier theoretical

speculations (e.g., Cabeza et al., 2002; Nyberg et al., 1996a,b). They are providing clear evidence that it is memory of the past that organizes and provides meaning to the present perceptual experience. Borrowing Tulving's terminology, new episodic memory, to be remembered in a meaningful way, must be consolidated within a preexisting semantic memory (Tulving, 2002).

This analysis does not allow a clear demarcation between consolidation and retrieval processes, and in this view, it can be assumed that every retrieval operation should trigger a reconsolidation process. This is a view similar to that of Spear, although he never used the term 'reconsolidation' (Spear and Mueller, 1984). It follows from this that retrieval will change the information content of the 'trace' such that memory can be viewed as an emergent, dynamic, adaptive property of the nervous system. It is in that sense that

> Everything flows and nothing abides; everything gives way and nothing stays fixed.

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1.25 Learning and Memory in Communication and Navigation in Insects

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1.25.1 Introduction

The distinction between instinctive and learned behavior is a fundamental issue in behavioral research. A major difficulty in addressing it relies on the fact that labeling behaviors as either instinctive or learned is in most cases a merely analytical approach to the problem. Behavior develops on the basis of the interplay between an animal's phylogenetic boundaries and the sources of external signals that belong to its specific sensory world (Tinbergen, 1963; Lorenz, 1981; Shettleworth, 1998; Macphail and Bolhuis, 2001). It follows that when an animal computes the differences between stimuli activating the same or different sensory modalities, its subsequent behavior will be the outcome of an unbroken succession of possible responses, whose particular boundaries have been modified by selection in the course of evolution. Learning is embedded into this continuousness, and its effects on the animal's instantaneous performance will be superimposed onto those of its specific phylogenetic boundaries. This is why it is so fundamental to focus on salient responses invariably linked to the animal's previous experience when distinguishing between instinctive and learned behaviors. From a behavioral point of view, this leads to the search for the mechanisms underlying the animal's decision, a notion that denotes the process of parsing complexes of stimuli into equivalent options and the control of the subsequent responses that arise from the corresponding choices.

A central argument advanced in this chapter is that some invertebrate taxa constitute powerful model systems for the study of the teamwork between these two modes of behaviors, instinctive and learned, and for the analysis of basic principles of learning and memory, particularly within the context of communication and spatial cognition, where the possibility of revealing decisions might be within reach. Our focus is on social insects, animals that form societies and appear to have been exposed to higher cognitive demands during the course of evolution, perhaps due to their long lifetime, the diversity and complexity of the signals involved in their social interactions, and the development of counterresponses. In fact, "if Earth's social organisms are scored by complexity of communication, division of labor and intensity of group integration, three

pinnacles of evolution stand out: humanity, the jellyfish-like siphonophores, and a select assemblage of social insect species" (Wilson, 2006). Within this insect group, emphasis is on the honeybee, *Apis mellifera*, simply because its communication and navigation skills are impressive (e.g., von Frisch, 1967; Seeley, 1995; Menzel et al., 2005). Furthermore, because their brains are small, bees appear to be suitable subjects for studying system-level neural correlates of learning and memory through robust behavioral approaches, both at the level of single neurons and neural networks.

1.25.2 Communication

Since its early days, ethology has nurtured the study of learning and memory phenomena, and a great deal of its classical ideas "emerged or crystallized from the study of animal communication" (Konishi, 1999). It follows, therefore, that communication in nonlinguistic animals has been at the center of many of the current behavioral approaches to learning and memory. The study of animal communication is concerned with the production of and the responses to signals, including adaptive advantages and mechanisms of central processing, motor coordination, and peripheral detection and filtering. In invertebrates, it is in the context of communication and navigation that learning transcends elementary forms of association in particularly clear ways (Menzel et al., 2006). The evaluating signal for storing experience must come from internal nervous system conditions at the time of learning, depends considerably on the motivational level, requires attention to a subset of stimuli, and is adjusted to the animal's own behavior in an intricate way. The signals learned are usually composed of multimodal inputs, which cannot be isolated from each other, and the motor performances involve sophisticated sequences of programs. Insects make use of all sensory channels for communication and evolved sophisticated sender–receiver systems serving mate recognition and sexual selection, predator–prey relationships, and complex social interactions. Although the sensory, ecological, and evolutionary aspects of these communication systems have long been studied in detail (see the following discussion), little is known about the cognitive dimensions of these communication systems (e.g., how innate mechanisms interact with experience-dependent developmental processes, how these mechanisms depend on internal and external conditions, and how learning actually shapes a communication process). In the present context the neural mechanisms of insect communication will henceforth be eschewed altogether. We shall describe a few examples from several taxa illustrating the dominance of innate behaviors with regard to communication with conspecifics; these examples will be listed according to the sensory modalities involved in the processing of communicating signals. We will then focus on a few examples illustrating simple forms of learning in a selected group of insects, and finally, we will focus on the study case of the honeybee dance communication system, with special emphasis on the structure and content of the spatial memory underlying such complex phenomenon.

1.25.2.1 The Dominance of Innately Programmed Responses in Communication

1.25.2.1.1 Chemical

In highly eusocial species, the interplay between innate and learned behaviors becomes evident through group recognition, which greatly depends on smell and genetically programmed responses to information gathered in specific, innately recognized behavioral contexts (Lindauer, 1961; Wilson, 1971; Michener, 1974; Barrows et al., 1975; Oster and Wilson, 1978; Fletcher and Ross, 1985; Hölldobler and Wilson, 1990). Within an insect society, conspecific individuals respond differently to age, sex, and physiological groups, and the task of recognizing queens, males, workers (both egg-layers and infertile individuals), as well as intruders, strongly relies on volatile pheromones, variations in hydrocarbon cuticular profiles, and environmental odors (e.g., Eberhard, 1969; Bell, 1974; Franks and Scovell, 1983; Wagner et al., 1998; Liebig et al., 2000).

Individually distinctive or colony odors are frequent across highly eusocial insects and constitute the basis of colony integration and social organization (Hölldobler and Michener, 1980). Foragers from many ant species, for example, resume their field excursions by following trails chemically marked with colony-specific components (Hölldobler and Wilson, 1990; Billen and Morgan, 1998). Research on the harvesting ant *Pogonomyrmex*, for example, shows how context-dependent innate responses to olfactory stimuli lead to adaptive behavioral flexibility during foraging (Greene and Gordon, 2003). A colony of these ants consists of a single queen and

several thousand workers, including foragers and patrollers. Patrollers scout the foraging area before foragers leave the nest; if they do not return from their early excursions, the foragers will not begin to work. Recently, Greene and Gordon (2003) first blocked a *Pogonomyrmex* colony's foraging activity by removing its patrollers and then presented the foragers with glass beads at the nest's entrance. These beads had previously been coated with cuticular lipids from patrollers, hydrocarbon profiles from either patrollers or within-the-nest ants, and plain solvent; they also used live patrollers as a positive control. The authors thus found that task-specific cuticular hydrocarbons from patrollers were sufficient to rescue the colony's foraging activity, and that the foragers' responses depended not only on the patrollers' hydrocarbon profiles, but also on whether or not they were presented at the nest's entrance and at the right time of day (Greene and Gordon, 2003). The question remains of to what degree simple forms of learning, such as habituation (Barrows et al., 1975), underlie these responses to colony and individually distinctive odors.

1.25.2.1.2 Visual

Visual stimuli also control innate responses in communicating insects. Fireflies use luminescent signals for attracting mates (Lloyd, 1983). Butterflies use bright colors, iridescence, and polarized light in the context of long-range mate recognition and sexual selection (e.g., Vane-Wright and Boppre, 1993; Sweeney et al., 2003). Males of the hoverfly *Syritta pipiens* closely track the movements of conspecifics (Collett and Land, 1975), a skill that seemingly serves copulatory functions. Male flies of the genus *Lispe* perform a dancelike motion pattern during courtship that is seemingly perceived through vision (Frantsevich and Gorb, 2006). Visual cues enhance recruitment orientation to food sources in stingless bees (Nieh, 2004), that is, several species of these highly social insects exhibit local enhancement and orient toward the visual presence of foraging conspecifics (Slaa et al., 2003), a phenomenon also found in honeybees (Tautz and Sandeman, 2002) and wasps (D'Adamo and Lozada, 2005). Furthermore, stingless bees also appear to visually track the piloting flights of experienced conspecifics, and these movements can guide them for at least part of the distance to a food source (Esch et al., 1965; Esch, 1967; Kerr, 1969), although the role of learning in this intriguing form of recruitment has yet to be analyzed.

1.25.2.1.3 Mechanosensory

The use of air pressure waves, substrate-born vibrations, and touching is widespread in sexual selection, alarm and defensive behavior, and complex social interactions in insects (e.g., Webster et al., 1992; Fullard and Yack, 1993; Michelsen, 1999; Hölldobler and Roces, 2001; Virant-Doberlet and Cokl, 2004). Complex behaviors involving these types of signals vary considerably across species. Female crickets, for example, orient toward males by recognizing and localizing the sound signals they produce. Their auditory orientation emerges from mechanisms detecting species-specific temporal structures of the males' sound signals, as well as reactive motor responses to individual sound pulses (Webster et al., 1992; Stumpner and von Helversen, 2001; Hedwig and Poulet, 2004). Leaf-cutting ants are highly sensitive to substrate-borne vibrations (Markl, 1965) and possess stridulatory organs that produce such vibrations when the animals are engaged in leaf-cutting (Tautz et al., 1995; Hölldobler and Roces, 2001). It has been shown that these substrate-born stridulatory vibrations operate as short-range recruitment signals that enhance the effect of recruitment pheromones (Hölldobler and Roces, 2001).

Moreover, the exchange of liquid food by mouth is widespread among highly eusocial species of insects (Wilson, 1971), and these social interactions depend strongly on intense antennal contacts that occur between donor and food-receivers (Free, 1956; Montagner and Galliot, 1982). In the honeybee, antennal interactions are also important in the transmission of waggle dance information (Rohrseitz and Tautz, 1999). Ants recruit nest-mates to newly discovered food sources as well as possible nest sites by means of tandem running (Hingston, 1928), a behavioral mechanism that strongly relies on mechanosensory cues (e.g., Wilson, 1959; Hölldobler et al., 1974; Möglich et al., 1974; Traniello and Hölldobler, 1984). Camponotus ants returning from successful field excursions stimulate nest-mates through fast directed movements of their front legs or even their entire bodies, as well as food samples; they then present the nest-mates with their gasters, and tandem running begins between pairs of leaders and followers (Hingston, 1928; Hölldobler et al., 1974; Hölldobler and Wilson, 1990). Hölldobler (1974) and colleagues demonstrated that tactile signals from the follower's antennae are sufficient to trigger an ant's leadership behavior, and that the subsequent following behavior relies on mechanical stimulation based on contact with a leader's gaster. Tandem running is especially

interesting in the context of genetically programmed mechanosensory communication because it clearly exposes the bidirectionality of the communication process. It has recently been shown through the behaviors of both the leader and the follower in a pair of tandemly running *Temnothorax* ants how they depend on each other (Franks and Richardson, 2006), that is, there is an evident feedback between both ants that relies on mechanical stimuli and helps in maximizing the speed at which the two of them can travel their path. It remains open whether and how the follower gathers path-related information during tandem running that might subsequently be used in solitary excursions.

1.25.2.2 Learning in Communication

1.25.2.2.1 Chemical

Some animals imprint on salient aspects of their sensory world pre- and postnatally. Slave-making ants provide an interesting example of imprinting. These ants invade colonies of other ant species and transport the pupae back to their own nest. Adults emerging from these pupae behave and work for the slave-making species as if it were its own species (Isingrini et al., 1985; Carlin and Schwartz, 1989). Evidence indicates that this phenomenon depends on a process of imprinting, by which the slave ants learn to recognize the slave-makers as members of their own species. This process involves learning about the slave-makers' hydrocarbon cuticular profiles, a distinctive olfactory mark of the species. Apparently, imprinting is successfully accomplished when the hydrocarbon cuticular profiles of both ant species, the slave-makers and the slaves, do not differ markedly (Lenoir et al., 2001; D'Ettorre et al., 2002).

Communicating insects also benefit from anticipatory behavior based on simple associative principles. In the honeybee, for example, and probably also in many other social species, the exchange of liquid food by mouth, called trophallaxis, allows individuals to assign nectar odors with predictive values. Animals associate the odor (as the conditioned stimulus or CS) and the sucrose (as the unconditioned stimulus or US) present in the nectar they receive through these social interactions. This form of learning leads to long-term olfactory memories after a single learning trial – even when trophallaxis is brief – and the strength of association depends on CS and US intensity, as well as on the animals' past foraging experience (Gil and De Marco, 2005). Olfactory memories established in this manner may have important implications in the organization of foraging (Gil and De Marco, 2006): First, foragers and food-receivers may benefit from learned odors in searching for a transfer partner, eliciting trophallaxis, or even avoiding it; second, currently unemployed foragers as well as nonexperienced foragers may benefit from a highly prevalent CS available within the colony to resume their subsequent foraging flights (e.g., the higher the rate of encounter with a rewarding olfactory CS the higher the probability of flying out to search for the prospective nectar source).

1.25.2.2.2 Visual

Polistes wasps provide an example of selective learning, which develops around innate responsiveness to simple sign stimuli: the yellow-black patterns of the wasps' faces and abdomens (Tibbetts, 2002). These patterns vary across individuals and correlate well with a wasp's ranking in a colony's hierarchy based on body size and dominance. Manipulating them induces aggressive responses in staged contests between pairs of unfamiliar individuals, and subordinate wasps with experimentally altered facial color patterns are targets of considerably more aggression from the dominant individuals than sham controls (Tibbetts and Dale, 2004). The question of whether these observations reveal individual recognition in insects remains open, but the wasps' behavior in the staged contests indicates that these animals learn about visual signals of quality that convey information on conspecifics on the basis of a colony's inherent hierarchy. Another interesting example of visual learning involved in communication may arise from the dance behavior of the Asian honeybee, *Apis florea*. In contrast to *Apis mellifera* bees, these animals do not dance on a vertical plane, but on the flattened tops of their open combs, which are directly exposed to a view of the sky (Lindauer, 1956; Koeniger et al., 1982; Dyer, 1985). These bees orient their dances on the horizontal plane according to both celestial cues and landmarks (Dyer, 1985), and the bees that closely follow these communicating dances might use their vision to collect information from them.

1.25.2.2.3 Mechanosensory and combined modalities

The seemingly ritualized movements or dances that honeybees use to recruit nest-mates from the colony – or the swarm – to the location of a desirable resource involve multiple signals, including mechanosensory stimuli (von Frisch, 1967). The role of learning in the context of dance communication was initially

dismissed (Lindauer, 1952), but we shall see that this system's functioning may depend strongly on the structure and content of the honeybees' spatial memory.

Karl von Frisch (1946) revealed that a highly stereotyped, still variable motion pattern that honeybees perform on the comb surface conveys to the human observer the position of a well-defined target at the endpoint of an average vector in a two-dimensional egocentric system of coordinates. Since its early days, von Frisch's (1946) discovery was recognized as one of the most impressive achievements of twentieth-century behavioral biology. This motion pattern involves finely controlled repetitive movements and can therefore be described on the basis of its inherent, well-defined features: orientation in space and tempo. The term waggle dance denotes a form of this pattern that conveys information about targets located fairly far from the hive, whereas the term round dance refers to a slightly different form that the animals perform after returning from nearby locations (von Frisch, 1967). Honeybees also use other stereotyped motion patterns when engaged in cooperative work that have also been called dances (von Frisch, 1967; Seeley, 1998). For example, a honeybee may shake its body back and forth, also rotating its body axis every second or so, and walk slowly in all directions across the comb (Seeley 1992). This type of motion pattern has been called tremble dance (von Frisch, 1923), and it helps the colony members to coordinate their activities while handling the collected nectar, both outside and inside the nest (Seeley, 1992). When a forager returns from a highly desirable nectar source and has problems searching for a food receiver (a younger bee that receives its load and eventually stores it in the honeycombs) (Doolittle, 1907; Lindauer, 1952), it usually performs a tremble dance, which may last several tens of minutes. These dances are followed by a rise in the number of available food-receivers and a drop in recruitment of additional foragers to nectar sources, thereby helping the colony to maintain its rate of nectar processing matched with its rate of nectar gathering (Seeley, 1992). In another intriguing example of these dances, a honeybee remains stationary and briefly vibrates its body laterally at a frequency of 4–9 Hz, sometimes alternating brief periods of self-grooming. This pattern has been called the grooming invitation dance (Haydak, 1945) and increases the workers' chances of being rapidly groomed by a nest-mate (Bozic and Valentincic, 1995; Land and Seeley, 2004). These two later forms of dances, however, do not convey spatial information. Our focus is therefore on the waggle dance, which does convey spatial information and is perhaps the most intriguing form of these complex, iterative movements.

The homeostasis of a honeybee colony greatly depends on cooperative work and efficient communication (e.g., Lindauer, 1961). Compelling evidence indicates that the waggle dance is embedded in a series of communication systems that enables the colony to coordinate the activity of its members during foraging and nest-site selection (e.g., Seeley, 1995). Hence dancing honeybees have their own spectators. The colony members that keep close contact with a dancing bee, usually called dance followers or recruits, appear to detect a variety of signals emitted by the dancer and process them in such a way that their ensuing behaviors may greatly vary due to the content of these signals (von Frisch, 1967). Nevertheless, the way in which the followers detect the dance signals is not yet well understood, but the diversity of these signals indicates that multiple sensory modalities are involved in dance communication (Michelsen, 1999). Mechanical stimuli derived from the body contacts between dancers and followers are certainly involved, as well as environmental chemical cues brought into the colony by the dancers, and most likely also semiochemicals coupled to the dancer's wagging movements. Three-dimensional fields of air currents surrounding the body of the dancing bees and substrate-borne vibrations caused by the wagging movements of the abdomen also seem to play a role in dance communication (Esch, 1961; Wenner, 1962; von Frisch, 1967; Michelsen et al., 1987, 1992; Bozic and Valentincic, 1991; Kirchner and Towne, 1994; Tautz, 1996; Rohrseitz and Tautz, 1999). In addition to these external stimuli, propioceptive signals enable both dancers and followers to process mechanosensory information derived from the position of their body relative to the direction of gravity (von Frisch, 1967). Because the dance in *Apis mellifera* takes place on the vertical surface of the comb, the dancers have to transfer visual information gathered during their foraging flights to a reference system primarily defined by mechanosensory stimulation, a process called transposition also found in other insects (von Frisch, 1967). We shall focus on a few selected features of the waggle dance because they illustrate how learning may be involved in this form of social communication and also pose the question of how space is represented in the honeybee brain. Obviously, both

sides of the communication process, those from dancers and followers, must be taken into account if one is to understand what a successful follower actually learns from a dancing bee and how it combines the information available via the dance signals with that of its own spatial memory.

In the waggle dance (von Frisch, 1946, 1948, 1967), the dancer moves forward on the comb surface while moving its abdomen from side to side at about 15 times per second. This straight portion of the dance is called waggle-run. Without interruption, it then moves in a semicircular trajectory and returns to the starting point of its recent waggle-run; this portion is called return-phase. Once at this position, it repeats the forward, wagging portion of the dance. The dancer also tends to alternate clockwise and counterclockwise throughout successive return-phases. The followers tend to approach the dancer's body during the return-phase, which indirectly restricts the area on the comb in which the dance takes place, and if they begin following the dance maneuvers, their movements during a given return-phase will determine their subsequent position with respect to the dancer's body during the following waggle-run. Moreover, during the return-phase, dancers and followers interact repeatedly with their antennae and mouthparts, allowing mutual stimulation through chemical and mechanical signals. Finally, consecutive waggle-runs are performed with some directional scatter, which decreases when the distance to the indicated goal increases.

A major feature of the dance is that it can be triggered by different constellations of external stimuli, thereby conveying information about different types of goals. Honeybees dance for desirable sources of nectar and pollen (von Frisch, 1967), thus improving the colony's food collection (Sherman and Visscher, 2002), and also for water, essential to down-regulate the nest's temperature when the hive gets overheated (Lindauer, 1954). This undoubtedly speaks about how versatile the dance communication system is. But perhaps the most striking example of this versatility relies on its role during swarming (Lindauer, 1951, 1953, 1955). Upon leaving their old nest during a colony's seasonal division, honeybees rely on a complex group decision-making process for selecting a new nest site. Their ultimate success depends on an accurate, fast, and unified collective decision (Seeley and Visscher, 2004). During this process, numerous colony members locate and dance on the surface of the swarm for potential nest sites. The decision process thus relies on several groups of dancers indicating different sites and recruiting uncommitted bees to follow their own dancing; most of the swarm's members remain in place until all dancers achieve unanimity by indicating the same goal, then the swarm lifts off (Seeley and Visscher, 2004).

The number of dancing events varies across dances, thereby revealing the regulatory responses and amplification phenomena that operate on the signal production side of the communication process. The strength of the dance depends on the flow rate (Núñez, 1970) and sugar content (von Frisch, 1967) of the nectar that the dancers bring into the colony; the flown distance (Seeley, 1986) and the nature of the indicated goal, that is, either a nest site or a food source (Seeley and Buhrman, 2001); the colony's nectar influx (Lindauer, 1948; Núñez, 1970; Seeley, 1995; De Marco, 2006); the dancer's past foraging experience (Raveret-Richter and Waddington, 1993; De Marco and Farina, 2001; De Marco et al., 2005); and even weather conditions (Lindauer, 1948; Boch, 1956). Honeybees also adjust the rate of waggle-run production by modifying the duration of the return-phase based on specific properties of the indicated goal (Seeley et al., 2000; Seeley and Buhrman, 2001) and by means of signals derived from their interactions with their fellow mates (Lindauer, 1948, 1954; Núñez, 1970; Seeley, 1986; De Marco, 2006) and time-based cues coupled to the current foraging status of the colony as a whole (Lindauer, 1948, 1954; Seeley, 1995). These relations enable the dance communication system to be tuned according to both colony demands and availability of resource opportunities.

1.25.2.2.4 What is the information content of the honeybee waggle dance?

So far, we have briefly described the waggle dance as an intriguing example of multisensory convergence, central processing, and motor coordination. We shall now focus on how it relates to navigation. Flying bees are able to use the sun as a reference to maintain a course, a mechanism referred to as the sun-compass (von Frisch, 1967), and also recognize the sun's azimuth by the pattern of polarized light in the blue sky (von Frisch, 1949, 1967; Rossel and Wehner, 1984). They also compensate for the sun's time-dependent movement, even when neither the sun nor the pattern of polarized light is visible (Lindauer 1957, 1959). For this task to be accomplished, they must learn the sun's azimuth as a function of the time of

the day during their initial orientation flights (Dyer and Dickinson, 1996).

A waggle dance encodes the direction and distance of a goal. First, the average orientation of the successive waggle-runs relative to the direction of gravity approximates the angle between the direction toward the goal and toward the sun (von Frisch, 1949, 1967; Lindauer, 1963). Second, the average length of the waggle-runs increases together with the distance from the hive to the goal (von Frisch and Jander, 1957). Early studies suggested that a honeybee's estimate of the flight length depends on gauging the amount of energy expended while flying (Heran, 1956; Scholze et al., 1964). Cumulating evidence now suggests that honeybees gauge and control the distance that they travel by integrating self-induced optic flow during flight (i.e., the net amount of image motion over the retina accumulated during movement) (Esch et al., 1994; Esch and Burns, 1996; Srinivasan et al., 1996, 2000; Tautz et al., 2004; De Marco and Menzel, 2005). The functioning of this mechanism is not yet fully understood, but it seems to depend on flight height and initial calibration based on landscape features (Esch and Burns, 1996; Esch et al., 2001). These two correlations convey to a human observer the circular coordinates of specific locations in a two-dimensional space and also provide a direct access into the dancer's perceptual world.

Evidence indicates that some of the followers that keep close contact with a dancing bee subsequently fly the approximate direction and distance that the dance conveys to the researcher (Lindauer, 1967; Esch and Bastian, 1970; Gould, 1975; Judd, 1995; Riley et al., 2005). They also use additional cues (i.e., semiochemicals and visual cues provided by other colony members, as well as environmental odors) to pinpoint the location of the targeted goal (e.g., von Frisch, 1967; Tautz and Sandeman, 2002). Six decades after von Frisch's (1946) original discovery, however, the process of decoding information in the dance still remains obscure (Michelsen, 1999). Some reasons are probably to be found in the striking variability of the multiple dance signals (e.g., von Frisch and Lindauer, 1961; Esch, 1978), the rather suboptimal methods that have been used so far to record the movements of both dancers and followers, and the lack of suitable tools to track the behavior of the followers after they depart from the hive. Improvements arise along with new methods (see the following discussion).

However, it is also worthwhile to consider a general aspect of the dance communication system that has received little attention, namely, the interaction between two different sources of spatial information that the followers might be able to access simultaneously: (1) the actual dance signals and (2) their own spatial memory store, as derived from their previous flights and reward experience; we shall refer to this putative store as the animal's spatial knowledge. The interaction between these two sources of information refers to a fundamental question in any process of communication. Communication depends on reproducing at one point an abstract entity selected at and sent from another point, but the entity that is finally reproduced on the receiver's side also depends on stored variants of this entity, which the receiver computes together with the signals it receives from the sender. In other words, one needs to ask whether a follower recollects stored information while decoding information in the dance.

The extent to which individual honeybees are exposed to the waggle dance throughout their foraging life has been addressed only recently (Biesmeijer and Seeley, 2005). In their study, Biesmeijer and Seeley (2005) reported that no more than a quarter of an average bee's lifetime field excursions was preceded by dance following, and in most of the instances in which the bees did follow dances before resuming their field excursions, they did so by following those that appeared to be indicating the goals that they were already familiar with. These findings are in close agreement with previous results by von Frisch (1968), who reported that the followers' response to the dance depends on their background of experience with the indicated goal, and that dances for familiar goals lead to more effective recruitment. Biesmeijer and Seeley (2005) also reported that the honeybees with field experience followed an average of only two to four dance circuits before resuming their new flights to the target. This small number of dance circuits provides spatial information only roughly to a human observer (**Figure 1**) and poses the question how informative this sample can be to the followers (Haldane and Spurway, 1954). Taken together, the results of this study suggest that the most advantageous functioning of the dance communication system will depend not only on the dancer's ability to keep record and derive spatial features of its recent field excursion, but also on the follower's ability to acquire, store, and recall specific navigational memories in the dance context (Menzel et al., 2006).

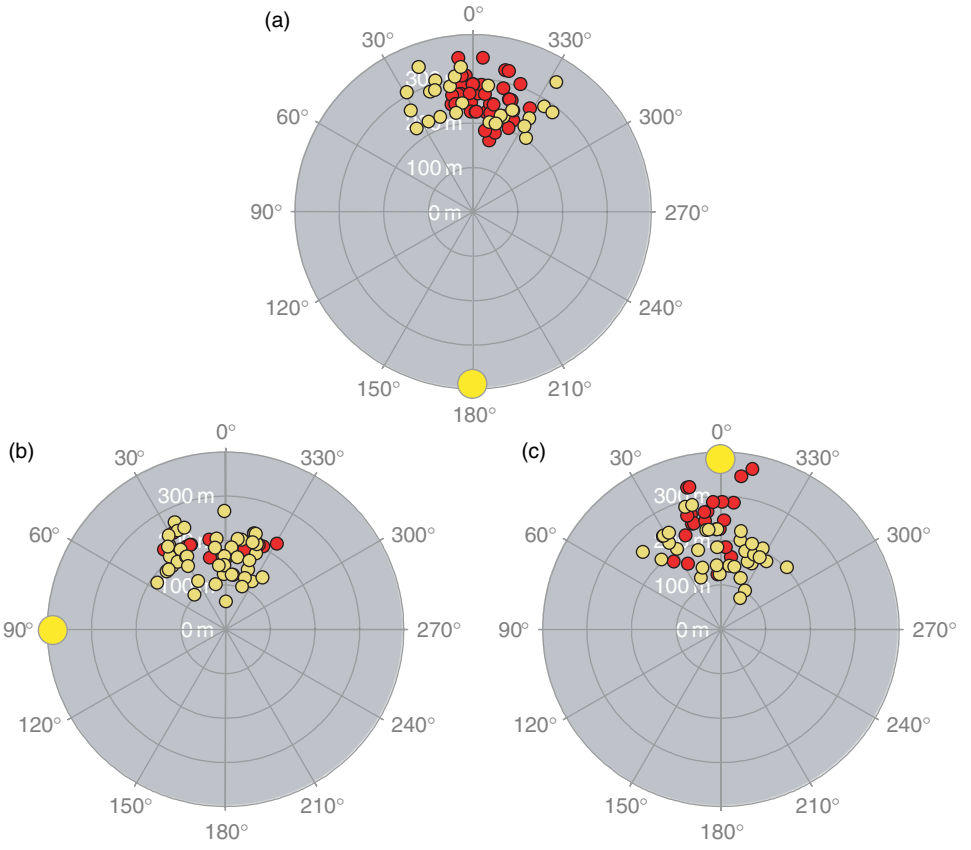


Figure 1 A waggle dance encodes the direction and distance of a goal: First, the average orientation of the successive waggle-runs relative to the direction of gravity approximates the angle between the directions toward the goal and toward the sun. Second, the average length of the waggle-runs increases together with the distance from the hive to the goal. These two correlations convey to a human observer the circular coordinates of specific locations in a two-dimensional space. The figure depicts radial maps indicating vectors' endpoints (in yellow and red) from waggle-runs performed by two dancing bees during single dances. These bees foraged regularly on a feeder placed 225 m west of an observation hive (52° 27′ 25″ N, 13° 17′ 46″ E) whose entrance pointed toward the north. In the radial maps, the direction of the feeder corresponds to 0°. Dances were video-recorded (at 88 frames s^{-1}) on the same day, in the morning (a), and the early (b) and late (c) afternoon, meaning that the sun (indicated by a yellow circle) was behind (a), to the side (b), or in front of the bees (c) during their flights toward the feeder, respectively. The coordinates indicated by the single waggle-runs are widely scattered around the actual position of the goal.

But is there any indication of some form of persisting spatial memory available to transitorily uncommitted honeybees (either dancers or followers)? Sometimes the waggle dance occurs in the absence of foraging. Under these conditions, it is performed in accordance with the current position of the sun and without any view of the sky, even during the night. These dances encode spatial information about goals that the dancers would have visited if they were guided by their sense of time (Lindauer, 1957, 1960; von Frisch, 1967). Furthermore, dancers seem to recall information related to goals visited several weeks earlier and estimate, at night, the closest goal in time after being trained to two different feeding places at two different times during the day (von Frisch, 1967). It follows, therefore, that honeybees use persisting memories to control their dances, which can be retrieved by specific stimuli (e.g., odors associated with the prospective goal) and whose content is appropriately combined with the time of day and complexes of signals that determine the animal's overall motivational state. The retrieval of long-term spatial memories has been observed in navigating bees (e.g., Menzel et al., 1998, 2000), but its appropriate incorporation into the dance context

poses additional questions. One of these questions is whether the waggle dance conveys to a follower only the approximate direction of and distance to the goal, or whether it also encodes a constellation of signals embedded in the follower's spatial knowledge, built throughout its previous flights and organized by reference to topographical features of the hive's surroundings. The structure of the spatial memory in honeybees will be addressed in the section titled 'Memory Structure'; we shall see that there is convincing evidence indicating that navigating bees may benefit from a topological representation of the environment, or a maplike spatial memory. It is thus conceivable that if honeybees are able to store spatial memories linked to specific locations in the field, and perhaps memories on specific features of their targets (e.g., food availability at a certain time of the day), the dance followers might also be able to combine information available through the dance with information from their own spatial memory, either already associated with the goals being indicated or in spatial relation to landmarks embedded in the seemingly topological structure of their spatial memory. What kind of spatial memory may be necessary for the waggle dance to encode information on past goals? How do these memories develop throughout the dancer's foraging life? These questions refer to the cognitive complexity underlying dance communication in honeybees. Future research on dance communication will certainly profit from the analysis of the interplay between the process of encoding and decoding spatial information in the dance and the structure and content of the honeybees' spatial memory.

1.25.3 Navigation

The term navigation denotes an animal's ability to efficiently travel between at least two specific distributions of concurrent signals (locations), even without having sensory access to the signals that define its targeted location. This notion removes any reference to the sensory modalities involved in gauging compass directions and distances and the control of the motor programs underlying the subject's locomotion. For the location to be reached, therefore, a navigating subject must be able to detect whether or not the immediate distribution of signals available within its current sensory horizon corresponds to the location it has been traveling to, a process that, in principle, only depends on innately stored information and programmed responses. In most animal species, however, survival involves moving regularly from and to several locations. It follows that to cope with such a complex navigational task, the single distributions of signals defining these locations (available from either idiothetic or allothetic sources or from both) must be stored in specific forms of persistent memories. Differences in the content and the organization of these complexes of memories may arise as long as task complexity varies across taxa.

1.25.3.1 Typology

Different classification schemes are used in the analysis of spatial behavior. Kühn's (1919) attempt to conceptualize orientation mechanisms on the basis of the relationship between sensory stimuli and an animal's response to them is an early example of these schemes. In recent decades, research on spatial behavior has also nurtured the development of biologically inspired artificial navigation systems. This gave researchers an opportunity to classify several theoretical accounts of spatial behavior within a single unifying framework centered on the structure and content of the information used by the navigating agents. The ensuing classification schemes are based on task complexity and experimentally tested features of an agent's spatial behavior (Trullier et al., 1997; Franz and Mallot, 2000); they tend to be purposely broad, ignore endogenously coordinated performances, and dissect complex behaviors found in nature into motor programs that can be reliably implemented in artificial systems. Although somewhat crude, these typologies provide a suitable basis for analyzing basic strategies of spatial behavior, feature detectors, and navigation learning. Their most salient characteristic is that they account for complex navigational tasks by means of hierarchically organized, interacting strategies. For example, Trullier et al. (1997) conceive taxes (Kühn, 1919; Fraenkel and Gunn, 1961) as the basic machinery of all navigating agents and then distinguish between local navigation and way-finding. Local navigation accounts for orientation in the immediate environment, where the agent acts based on information available within its perceptual range, whereas way-finding involves moving in a large-scale environment, where relevant cues lie beyond the perception range, and the goal is not in the immediate environment. Technically speaking, each of these categories can still be divided into several levels: search,

direction-following, aiming and guidance for local navigation, and recognition-triggered responses, topological, and survey navigation for way-finding (Franz and Mallot, 2000). Due to their hierarchical organization, way-finding relies on local navigation, but it is not yet clear how these strategies may interact in the brain, or how animals recognize specific locations, let alone how they may assign specific identities to these locations, a prerequisite of some high-level navigational tasks. Evidence indicates, however, that a hierarchical array of seemingly different, interacting strategies underlies the spatial behavior of some species of navigating insects (Wehner et al., 1996; Menzel et al., 2005). In the present context, we will use this basic typology to survey the structure and content of the spatial memory used by desert ants and honeybees, because data from these two navigating insects are frequently discussed using different terminologies and approached from different conceptual frameworks.

1.25.3.2 Navigation in Desert Ants

Desert ants of the genus *Cataglyphis* live in subterranean nests surrounded by relatively flat and featureless areas. They forage individually and travel over distances of hundreds of meters along circuitous paths during their foraging excursions. After grasping a food item, they quickly return in a straight line to the proximity of the starting point of their journey, where they finally break off their homeward runs and start a systematic search aimed at pinpointing the entrance of the nest; avoiding overheating is crucial in their environment. These ants primarily benefit from path integration (Mittelstaedt and Mittelstaedt, 1980), also referred as to dead-reckoning (*See* Chapter 1.12), to accomplish their remarkable homing performances (Wehner, 1992; Collett and Collett, 2000). This means that a navigating ant iteratively computes all its rotational and translational motion components, integrating them into a sort of global vector (Wehner, 2003) that connects, at any time, its current location and that of the starting point of the excursion. This navigation strategy can easily be revealed by displacing the animal over some distance; after being released, it chooses a compass direction and walks an approximate distance that brings it to a predictable, virtual reference point (Pièron, 1904; Santschi, 1911).

Desert ants appear to inexorably compute this type of vectorial information and are incapable of using more than one vector simultaneously. They benefit from inverse forms of these home vectors and efficiently move from the nest to previously visited field locations. Global vectors also appear to be stored and recalled in accordance with specific contexts, meaning, for instance, that when a returned ant is moved back to a recently visited location, it does not apply its recent home vector to once again navigate its way to the nest. Instead, it uses a systematic, time-consuming search strategy to find the nest's entrance (unless it navigates in familiar terrain offering conspicuous landmarks, as we will see). Under these circumstances, however, the information about its recent home vector does not disappear, and the ant is able to subsequently use an inverse form of it to quickly find its way to the previously visited location. This indicates, in turn, that path integration information is transferred from some form of working memory into a different, more persisting memory stage, from which it can be later recalled on the basis of context-dependent signals (Wehner, 2003).

In order to use path integration, a desert ant must be able to align its trajectory with a locally available compass direction and to reliably acquire distance information. *Cataglyphis* ants do not acquire directional information by means of idiothetic sources, such as an inertial compass or proprioreceptive signals; they do it using a celestial compass based on a specialized set of polarization-sensitive ultraviolet receptors located within a particular portion of the retina (Wehner, 1994, 1997), which detect the pattern of polarized light in the blue sky (Wehner and Müller, 2006). The functioning of this celestial compass involves an internal ephemeris function (Wehner and Müller, 1993) and demands recalibrations due to the inexorable changes in the pattern of polarized skylight that take place during the day. Desert ants do use idiothetic sources to compute translational motion: Distance information appears to be gauged by means of a step integrator (Wittlinger et al., 2006), and the control of distance by self-induced optic flow seems to be only slightly modified under specific test procedures (Ronacher and Wehner, 1995). These two path-related components – distances and directions – are combined via some sort of accumulator, the state of which encodes the ant's current coordinates relative to the reference point. The task of surveying the possible computational boundaries of this hardwired accumulator lies beyond the scope of this chapter (for comprehensive accounts of this issue, see Wehner 1997, 1999, 2003). In the present context, let us simply say that its functioning directly depends on locomotion (Seidl

et al., 2006), that it must process distance and compass information simultaneously (Sommer and Wehner, 2004), and that it allows an ant to gauge the ground distance while traveling undulating paths (Wohlgemuth et al., 2001; Grah et al., 2005). Its output can also be combined with external, sensory cues, thereby reducing search costs and improving the ants' general foraging strategy (Wolf and Wehner, 2000, 2005). This accumulator or path integrator also appears to continuously process information, that is, when homing ants are captured at the nest's entrance and displaced several times in a row to the initial position of their homeward runs, they move away from their reference location (the proximity of a virtual nest) when transferred to a featureless test channel (Andel and Wehner, 2004).

The findings described earlier illustrate how path integration, a basic local navigation strategy, enables *Cataglyphis* ants to reliably find the proximity of a virtual nest in unfamiliar terrain. Things are different in familiar terrain, however. The use of landmark-based information improves the efficiency of an ant's path integrator because the number of inaccurate alignments increases together with the length of the animal's excursion (Wehner and Wehner, 1986). Due to these unavoidable computational errors, a cross talk between path integration and guidance decreases the chance of missing the goal (Collett and Collett, 2000). Provided with an irregular environment, homing ants use landmarks while on their way to the nest's immediate surroundings (Bregy and Wehner, 2003; Knaden and Wehner, 2005), thus following well-defined paths or routes (Collett et al., 1992; Wehner et al., 1996; Kohler and Wehner, 2005). Moreover, it has recently been shown that they can also use memories of minute ground features to pinpoint the entrance of the nest (Seidl and Wehner, 2006). Guidance, therefore, leads navigating ants to locations where they have acquired a certain egocentric relationship with respect to a specific configuration of external signals (Wehner et al., 1996; Collett and Collett, 2000). They thus take advantage of a store of reliable landmark-based memories, which can be associated with specific motor routines and recalled in the appropriate context, and exhibit goal-directed movements at different locations (Collett et al., 1998; Collett and Collett, 2000, 2002; Åkesson and Wehner, 2002). Research on other ant species (e.g., Jander, 1957; Graham and Collett, 2002; Wehner et al., 2006) also provides evidence of local navigation and even more complex navigation strategies based on (1) the recognition of a catchment area from which a configuration of landmarks is perceived to be identical, (2) successful orientation within this area, and (3) the subsequent selection of a goal-directed movement. These strategies, therefore, rely on the combination of several recognizable areas associated with specific goals and directed actions (Barto and Sutton, 1981; Trullier et al., 1997; Collett and Collett, 2000).

The study of navigation in desert ants has led to a remarkable understanding of the basic mechanisms that these animals use for setting a directional bearing in the field (Wehner, 2003). Experience-dependent behavioral flexibility is conceived as a calibration process of path integration computations. Next, a context-dependent recollection of path integration coordinates may eventually lead to the ants' seemingly idiosyncratic routes. Landmarks provide information about turns to make and distances to travel next (Collett, 1996, 1998; Collett and Collett, 2000; Kohler and Wehner, 2005), such that seemingly complex performances might be based on simple rules that depend on learning sensory-motor routines. This is frequently referred to as procedural learning. Traditional thinking on ant navigation therefore conceives a toolbox of sensorimotor routines, whose stepwise application enables the animals to solve seemingly complex navigational tasks. Moreover, the recollection of single vectors does not require an overall representation of multiple locations, and the selection of goal-directed actions may exclusively depend on innately stored, calibrating information. This corresponds to the fact that ants do not appear to make decisions involving equivalent options. The question remains how ants use external signals to map several recognizable places. None of the current approaches to navigation in several ant species has yet reached the level of analysis achieved in the study of homing by desert ants, let alone the principles of navigation learning in walking insects.

Honeybees also exhibit procedural learning. They learn to negotiate complex mazes of adjacent boxes by associating colored disks with right or left turns, for example (Zhang et al., 2000; Srinivasan and Zhang, 2004), and also refer to compass directions in their dances on overcast days (von Frisch, 1967; Dyer and Gould, 1981). The latter result reveals that landmarks serve as a backup system, which conveys direction information to navigating bees and poses the question of how external signals are actually incorporated into the bees' representation of space (Gallistel, 1990). Interpretations from the study of ant navigation have often been transferred to bees. In

contrast to ants, however, bees fly over distances of a few kilometers, cruising well aboveground; they also use depth information extracted from motion parallax (Lehrer, 1996) and learn about the absolute size of landmarks (Horridge et al., 1992). Furthermore, honeybee foraging behavior involves a remarkable diversity of responses, including those underlying cooperative work during food gathering (see earlier discussion). When an experienced worker forages on a given flower species, for example, it leaves the colony and flies toward its targeted location for a certain amount of time, without interrupting its flight even when alternative flowers of the same species might be within reach. Once this motor program is extinguished, it begins searching for the flowers it recognizes according to their odors, colors, and shapes and inspects them by means of specific motor commands that allow it to efficiently find and collect the offered nectar (von Frisch, 1967). Meanwhile, it adjusts its estimate of how much nectar ought to be collected (Núñez, 1966). Finally, it initiates its return flight to the hive. Although exaggeratedly simplistic, this scheme illustrates an intriguing feature of the honeybee foraging strategy: each animal leaves the colony with a large – and diverse – amount of information, which is used in context-specific ways and involves expected outcomes of particular behaviors. The contexts are defined by both time and space, a fact that becomes strikingly evident when bees forage on multiple locations throughout the same day. Furthermore, there is a cross talk between navigation and collective foraging (see earlier discussion), which involves specific responses to numerous features and dynamic components of the animal's sensory world. As we shall see next, the repertoire of navigational performances in honeybees is far from simple. Together with complex modulatory processes, learning is at the heart of the animals' navigation skills.

1.25.3.3 Navigation in Honeybees

Foraging honeybees usually follow straight flight trajectories between specific locations and the hive (Beutler, 1954; von Frisch, 1967). If they are caught at the moment they depart from the hive and then released at a different spot in the field, they fly in the direction they would have taken if they had not been moved to the release site, meaning that they fly in the correct compass direction but along a false route relative to the goal they were originally traveling to. They perform in a similar manner when caught at the beginning of their homeward flight. Once again, they fly in the predisplacement compass direction which might have connected their foraging location and the hive, but along a false route with respect to the actual location of the colony (Wolf, 1927; Menzel et al., 2005). The bees' flown distances and compass bearings in this type of experiment resemble the global vectors observed in desert ants (see the section titled 'Navigation in desert ants'), supporting the view that honeybees also use vector memories that develop through their regular flights. Furthermore, when bees arriving at a foraging spot are held captive for several hours, they subsequently fly farther outward from the hive along the same hive-target direction (Dyer et al., 2002). After being trained along a fixed route, therefore, vector memories will reliably guide honeybees back to the hive and toward specific field locations; unless they have been artificially displaced.

In fact, navigation research in flying hymenoptera has long been based on displacement experiments and the analysis of the animals' homing abilities (Tinbergen and Kruyt, 1938; Thorpe, 1950; von Frisch, 1967; Tinbergen, 1972; Menzel et al., 2000, 2005). If navigating bees only rely on global vectors and random searches, displaced foragers might have trouble rapidly finding their way back to the colony. They do return home when released at a new, unexpected location, however, and they do it reliably and relatively fast when released within the range of approximately 1 km from the hive (Capaldi and Dyer, 1999; Menzel et al., 2000). Consider the following experiment: One group of bees was trained to forage on a stationary feeder placed 300 m away from the hive, and another group was trained to forage on a close feeder that rotated around the hive at a constant radius of only 10 m. Hence, the foragers from the latter group had not experienced a flight vector connecting the hive and a fixed, distant foraging location. However, despite lacking this experience, they returned home equally well from various possible directions and as quickly as the animals from the former group, which had experienced a predisplacement route training (Menzel et al., 2000). Furthermore, the results of this experiment could not be explained by reference to local navigation strategies, due to the lack of landmarks in the vicinity of the hive and the actual distance to the different released sites (Menzel et al., 2000). This indicates that successful homing in honeybees does not necessarily depend on a random, time-consuming search strategy. Recently, radar traces of the full flight

trajectories of displaced honeybees revealed that the last phase of homing is eventually accomplished by straight, goal-directed flights toward the hive (Menzel et al., 2005), supporting the view that honeybees are able to store and retrieve allocentric cues that help in defining compass directions in the field (Menzel et al., 1998).

The results described, in addition, pose the question of how the forager's working memory is organized and what role its content actually plays during navigation. Path integration is the subject of computational errors (Benhamou et al., 1990) and controls navigation as long as the animal combines it with multiple environmental cues. It is therefore reasonable to ask how landmarks provide honeybees with a basis for accurate homing. The complexity underlying such a strategy still remains open. How does it rely on guidance? How many configurations of landmarks can be processed and stored? How much does the animal perceive about these configurations, and how does it relate them? Are sequentially learned configurations generalized in such a way that they can be categorized, or counted, or even embedded into a more general, combined representation of space (Menzel et al., 2006; *see also* Chapter 1.12)? These questions are also at the heart of a long-lasting controversy (Wehner and Menzel, 1990), namely, whether navigating insects have at their disposal only minimal cognitive modules enabling them to store and retrieve ordered sequences of context-dependent actions (Wehner, 1999), or whether they also store and retrieve relations among points, lines, and surfaces somehow embedded in an internal representation of space (Menzel et al., 2006; *see also* Chapter 1.12 for a detailed account of this issue). Answering these questions, however, would only be possible after revealing the mechanisms underlying what we now merely label as specific responses to specific configurations of external signals, such as the response of a navigating bee to a familiar visual scene. Consider the term snapshot (Cartwright and Collett, 1983), for example; it denotes an insect's memory of visual landmarks, and it helps in formulating hypotheses based on matching algorithms and behavioral data, but its neurobiological basis is yet to be established. Similarly, we simply do not know how multiple and complex procedures might be combined in a common spatial memory store.

One of the reasons that led to differences in conceptualizing navigation in flying insects lies in the fact that most experiments were performed with animals trained along fixed, predisplacement routes and subsequently observed only during their initial postdisplacement flight paths. Most likely, only the motor routines based on the actual content of the animals' working memory can be revealed in this manner. The bees' exploration of the environment, however, does not begin with flights along fixed routes, which develop relatively late in the animals' foraging careers, and it is the spatial memory that develops during the bees' exploratory flights that might supply the animals with information for successful homing when vector memories fail. Therefore, an important aspect related to the questions listed earlier might be at stake – how spatial behavior develops.

Honeybees begin foraging only after executing a series of exploratory flights of increasing lengths (Becker, 1958; Vollbehr, 1975; Winston, 1987; Capaldi and Dyer, 1999), normally on several consecutive days (von Frisch, 1967). Using harmonic radar, Capaldi et al. (2000) showed that when honeybees are engaged in these exploratory flights, they keep the trip duration constant, but fly faster with increased experience of the terrain, so that the later flights cover a larger area than the earlier flights. Each flight, in addition, is typically restricted to a well-defined, narrow sector around the hive, and changes in this respect appear to be related to the number of previous flights. Taken together, these results indicate that early flights provide honeybees with repeated opportunities to become exposed to different landscape features (including the hive's position) from different viewpoints, supporting the view that they may store landscape information in a progressive fashion (Capaldi et al., 2000). At the individual level, however, the ontogeny of these flights remains a mystery, and the question of how the animals use information available throughout successive flights is still unanswered.

Tracing the full flight trajectories of free-flying bees allows evaluation of the complexity of the animals' spatial memory, and this is now possible using harmonic radar techniques (Riley et al., 1996). Menzel (2005) and colleagues recorded more than 200 flight trajectories in this manner (Menzel et al., 2005) and analyzed the flight paths of three different groups of animals: (1) honeybees that had been trained to forage on a stationary feeder located 200 m east of the hive, thereby repeatedly following a well-defined predisplacement route; we shall call them SF-bees; (2) honeybees trained to forage on a feeder that circled around the hive within a distance of 10 m, thereby experiencing no route prior to

displacement; here we call them VF-bees; and (3) honeybees that lacked training and had closely followed a waggle dance for the feeder placed 200 m east of the hive; we shall call these bees R-bees. Three phases of navigation can be distinguished among these groups of honeybees (Menzel et al., 2005): (1) vector flights, (2) circuitous flights, and (3) straight homeward flights. Vector flights were apparent in the SF- and R-bees, but not in VF-bees. Those from SF-bees showed compass directions and distances that matched the predisplacement route, and those from R-bees matched the spatial information that the waggle dances conveyed to a human observer (Riley et al., 2005). Hence, it follows that, when accessible, route memories are invariably applied first, and vector flights are based on directions and distances from these memories. The circuitous flights showed multiple returns to the release site and to the end of the vector flights in the case of SF- and R-bees. During this phase, the flight speed was significantly lower than during phases of straight flights. These flights were also considerably longer for the SF- and VF-bees (carrying full crops) and shorter for R-bees (captured before getting in contact with any sugar reward), suggesting that this type of motion might not only underlie a process of reorientation (necessary after displacement), but also some sort of exploratory behavior (Menzel et al., 2005).

The honeybees from all these three groups returned to the hive by means of fast, straight homeward flights, and a detailed analysis of the straightness of these flights led to a clear distinction between the second and third navigation phases, thereby revealing the field locations where the straight homeward flights began (Menzel et al., 2005). They originated along different directions relative to the hive's position and usually began far outside a radius of 60 m, where the animals might have used visual cues to find their way back to the colony by means of aiming or guidance or both. Furthermore, they also began at locations with conspicuous, artificial landmarks, and when these landmarks were either displaced or removed, the animals were equally successful during homing. The most consistent hypothesis that accounts for these homing performances is that the ground structure itself provided the displaced honeybees with reliable information to find their way back to the hive (Menzel et al., 2000, 2005). Most significant is this: A third of the SF-bees made straight and fast flights directed not only to the hive but also first to the feeder and then to the hive (**Figure 2**). This latter result fits well with a

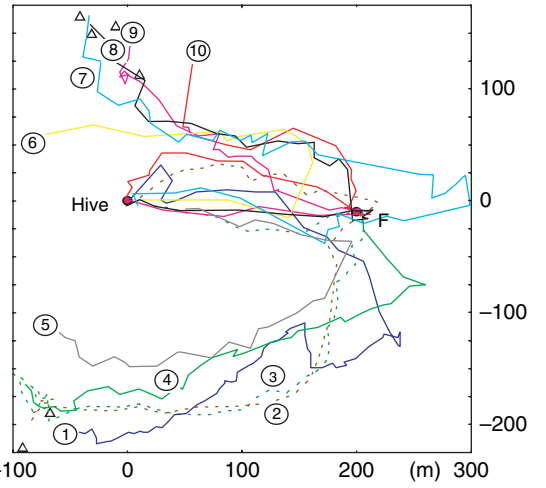


Figure 2 The homing flights via the feeder. Ten SF-bees (of the 29 bees tested under similar conditions) performed their homing flights via the feeder. Bees released south of the hive are shown by flight paths 1–5, and those released north of the hive are indicated by flight paths 6–10. The bee from flight path 4 landed at the feeder and flew to the hive after filling its crop. All bees were tested with the normal arrangement of tents under sunny weather conditions (for details see Menzel R, Greggers U, Smith A, et al. (2005) Honeybees navigate according to a map-like spatial memory. *Proc. Natl. Acad. Sci. USA* 102: 3040–3045).

topological representation of the environment (Trullier et al., 1997) and can also be explained by two mutually related hypotheses. First, homing bees might be able to integrate at least two vector memories. Assume the following premises: (I) they are able to associate vector memories defining homeward flights with specific configurations of external signals, and (II) when exposed to these signals, the corresponding vector memories can be recollected from a memory store. Next, if two of these memories are simultaneously recollected and transferred to the animal's active working memory, they might be combined to steer a seemingly new flight trajectory. The second hypothesis can be thought of as a more complex form of the first one. It assumes that the bees' orientation flights, together with their initial foraging excursions, lead to a memory of a network of several homeward vectors connecting specific distributions of external signals, including the hive's location. Such a process is believed to be possible in mammals and birds (Gallistel, 1989; O'Keefe and Nadel, 1978).

These concepts are closely interconnected to several issues about the honeybee dance communication

system, somehow embedded in the following question: Do dancers and followers have analogous memories? When von Frisch (1967) compelled honeybees to fly a two-legged detour path to reach an artificial feeder, the trained animals indicated in their dances the direction of a straight line toward the goal. They might have computed this compass direction from the two legs of the detour, but they also indicated the actual flown distance, and not the distance of the segment connecting the feeder and the hive. Thus the bees encoded in their dances the direction of a virtual flight vector, but not its length (von Frisch, 1967). This poses the question whether it is the outbound or the inbound flight or both that provides the dancer with the spatial information that is finally conveyed to the human observer. If spatial information available during the homeward flight (a directional bearing, for example) can successfully be incorporated into the dancer's maneuvers, the waggle dance might also be capable of conveying information that the dancer (and probably also a follower) has already linked to a specific spot in the field. It follows, therefore, that the efficiency of the dance communication system would greatly depend on the way in which both dancers and followers acquire, store, and retrieve navigational memories.

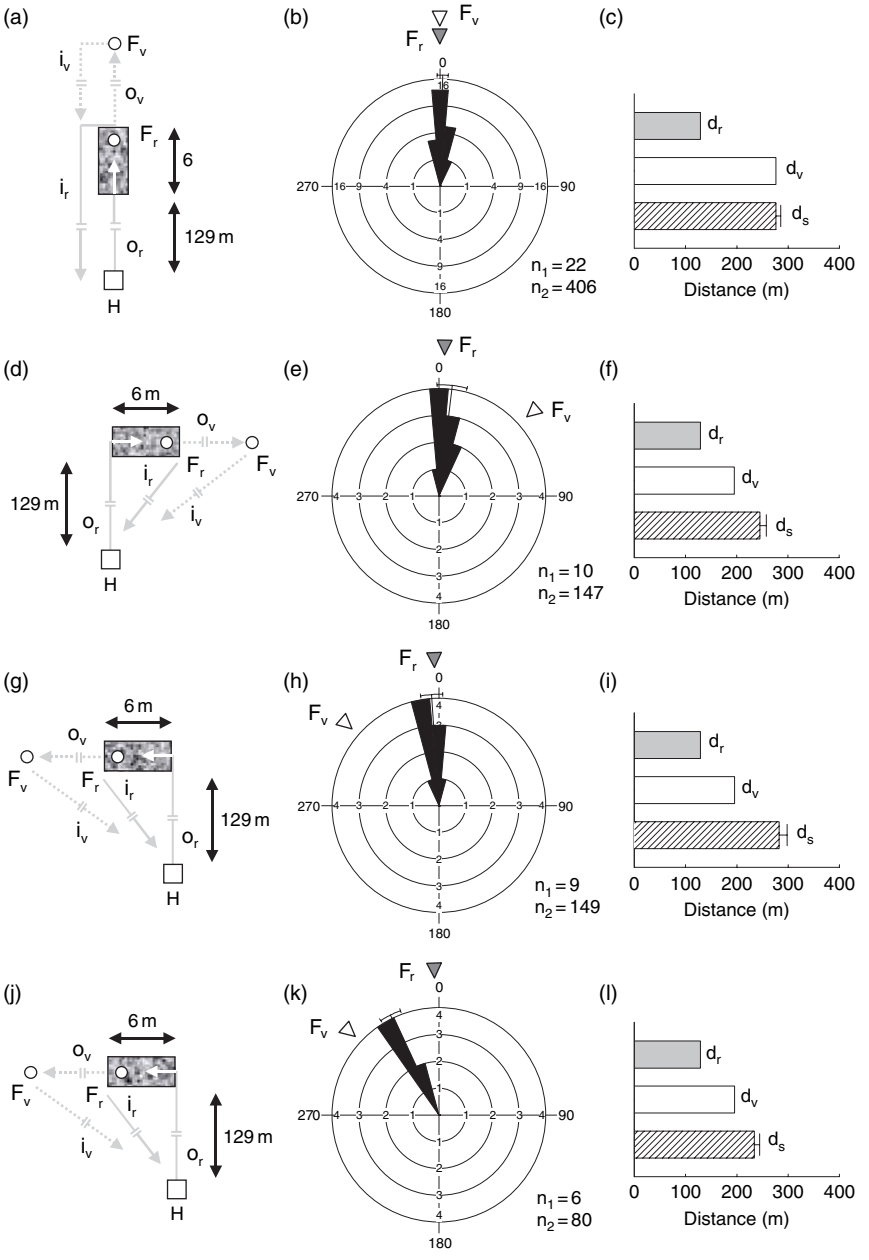
Behavioral studies of self-induced optic flow in honeybees (e.g., Srinivasan et al., 1997) take advantage of the following fact: Flying a short distance close to a surface gives the same integrated optic flow as flying a longer distance further from the surface. As a result, when honeybees fly through narrow tunnels with visually textured walls, they experience a subjectively flown distance that is greater than that actually flown (Srinivasan et al., 1996), also indicating a longer distance in their dances (Srinivasan et al., 2000). This allows manipulation of a bee's navigational experience of a subjective flight path (De Marco and Menzel, 2005). Honeybees perform longer waggle phases when they fly through a visually patterned tunnel on their outbound flight (**Figure 3(a)–(c)**). Thus, when the tunnel is set perpendicular to the straight line connecting its entrance and that of the hive, a mismatch arises between the animals' estimate of the goal's location (derived from path integration information from the outbound flight) and its actual location in the field. Under such conditions, the bees' waggle dances indicate a direction close to that of the straight line connecting the hive and the actual goal's location (**Figure 3(d)–(i)**), and the virtual detour has no significant effect on the duration of the bees' homeward flights, indicating that they fly directly back to the hive after leaving the tunnel through its far end. Moreover, path integration coordinates appear to be more strongly weighted in the dance maneuvers only with increasing experience of the terrain (**Figure 3(j)–(l)**), thus supporting previous interpretations (Otto, 1959; Edrich and Scheske, 1988) of the relationship between information available on-site and the encoding of direction in the dance. These results indicate that (1) a discrepancy between subjective measures of distances and directions and path integration coordinates already linked to visual scenes has no significant effect on the triggering of the waggle dance, and (2) the process of encoding spatial information in the dance involves detecting and processing such a discrepancy (De Marco and Menzel, 2005). It is not yet clear to what degree honeybees might refer in their dances to the inbound component of their journeys, or whether they embed the encoding spatial information in the dance into their maplike spatial memory.

1.25.3.3.1 Memory structure

It appears that honeybees develop spatial memories in three different contexts: (1) during their initial orientation flights, (2) while flying repeatedly from and to a specific field location, and (3) while following dances. We refer to these memories as (1) the general landscape memory, (2) the route memory, and (3) the dance memory, respectively. Note that the term general landscape memory makes no assumptions about the structure of the spatial information accessible through it, and that route memories may involve vector memories as well as procedures based on sequences of context-dependent actions. This typology, therefore, denotes processes not yet understood, but accounts for predictable actions. These memories might have different properties. Route memories provide information about directions and distances, and the same may be true for a dance memory, although the extent to which the latter may be combined with spatial information accessible from the bees' memory store remains an open question. Honeybees seem to transfer these two memory forms into their active working memory and apply them first. Once applied, however, they lose their influence on behavior. The directional component of these memories, in addition, is susceptible to updates according to changes in the animal's motivation. When at least two route memories are accessible, it becomes feasible to recognize that they have been linked to landmark-based information.

This led to the concept that honeybees use their route memories to estimate the sun's azimuth (von Frisch, 1967; Dyer and Gould, 1981), and that they may integrate at least two of them under specific circumstances (Menzel et al., 1998, 2005). The general landscape memory might be thought of as a structure of several recognizable locations within the range of the animals' orientation flights. It might arise through the integration of information provided by two or more route memories (Menzel et al., 2005) or by a process by which the bees innately store specific distributions of external signals and assign them specific identities based on idiothetic and allothetic cues. According to these hypotheses, honeybees may use their general landscape memory only when their active working memory has no access to route or dance memories.

The concept of multiple memories hierarchically organized is a generally accepted mind-set in neurosciences. Implicit and explicit knowledge, or



declarative and nondeclarative knowledge, develop from the various learning strategies, which involve various brain structures in mammals, including humans (Cohen and Squire, 1980; Packard and McGaugh, 1996; Schroeder et al., 2002; Chang and Gold, 2003). Navigation in mice and rats, intensively studied with respect to the role of the hippocampus and striatum, is actually embedded in a convincing theoretical framework, whereas hippocampal place cells are responsible for orientation based on specific distribution of signals and sequences of experiences that help in defining geometric relations among landmarks, and the striatum is responsible for those forms of learning based on signals sent by the goal (O'Keefe and Nadel, 1978; Moser and Paulsen, 2001; McNaughton et al., 2006; Witter and Moser, 2006). It might be interesting to evaluate whether and how the seemingly different navigational memories described earlier rely on the various neuronal structures in the bee brain. What can be behaviorally tested in the near future is whether dance memories are coupled to the general landscape memory.

1.25.3.4 Insect Migrations

Several insect orders exhibit far-distance movements referred to as migrations (Drake and Gatehouse, 1995). Populations of butterflies, moths, dragonflies, and locusts are seasonally engaged in far-distance migrations (e.g., Williams, 1958; Johnson, 1969; Holland et al., 2006). Costs and adaptations have long been addressed in migrating insects (e.g., Rankin and Burchsted, 1992), but the selective forces behind these movements are not yet fully understood. The distribution of offspring across a range of areas and conditions favorable for future reproduction might have played an important role in the evolutionary development of these movements (Wilson, 1995; Holland et al., 2006). At least two distinctive features of these far-distance movements distinguish them from the regular excursions of the central place (Orians and Pearson, 1979) foraging hymenopterans (i.e., bees, wasps, and ants). First, return migration has yet to be documented in insects (Holland et al., 2006), meaning that with a few exceptions (e.g., Urquhart and Urquhart, 1979), migrating individuals do not perform round-trip journeys that bring them into the areas from which they previously departed (Holland et al., 2006). In monarch butterflies, for example, several generations are produced during their northward migrations (Brower, 1995, 1996). Second, although migrating insects compensate for wind drift and maintain a heading using the sun compass (Srygley and Oliveira, 2001; Mouritsen

Figure 3 Experimental layout and results of an investigation of the encoding of spatial information in the waggle dance. A visually patterned tunnel was used to create a virtual detour. By compelling the bees to fly through such a tunnel, set up outdoors in various configurations, it is possible to add a virtual distance to the journey from the hive to the feeder – either straight ahead or to the right or left. Bees were trained to forage on a feeder placed at the far end of a 6-m-long, 30-cm-wide, and 30-cm-high tunnel. The tunnel's entrance was located 129 m away from the hive, and its walls and floor were decorated with a random visual pattern. (a) Experimental arrangements first had the tunnel oriented at 0° with respect to the direct line connecting its near entrance and the hive (h). The bees flew through the tunnel during their outbound flights (o_r) but not during their inbound flights (i_r). F_r and F_v correspond to the real and the virtual location of the feeder (white circle), respectively; whereas o_v and i_v correspond to the virtual outbound and inbound flights, respectively, as derived from the overestimated distance flown inside the tunnel. (b) Distribution of the individual mean directions signaled in the waggle dances recorded in the tunnel experiment described in (a), mean vector direction $\mu = 1.33°$, $r = 0.99$, $P < 0.001$, n_1 (number of animals analyzed) = 22, n_2 (number of waggle-runs analyzed) = 406. The frequencies within 10° class ranges are shown as the areas of the dark wedges. The dark spoke and segment indicate the mean vector μ and 95% confidence interval, respectively. The gray and white arrows indicate the directions toward the real (F_r) and the virtual (F_v) feeders shown in (a), respectively. (c) Shown are the flown distance (mean ± SE) signaled in the waggle dances recorded in the tunnel experiment described in (a) (d_s, striped bar), the distance to the virtual feeder (d_v, white bar, in this case equivalent to the signaled distance), and the real distance from the hive to the food site (d_r, gray bar). (d–f) Experimental arrangements and results as in (a–c) with the tunnel rotated 90° to the right. The distance flown inside the tunnel oriented at 0° (c) was used to compute the location to be signaled (F_v: direction and d_v: distance) if the global vector computed by the path integration of the outbound flight provides the dancers with the spatial information encoded in the waggle dance. In (e), mean vector direction $\mu = 6.77°$, $r = 0.98$, $P < 0.001$, $n_1 = 10$, $n_2 = 147$. (g–i) Experimental arrangements and results as in (d–f) with the tunnel rotated 90° to the left. In (h), mean vector direction $\mu = 356.1°$, $r = 0.99$, $P < 0.001$, $n_1 = 9$, $n_2 = 149$. (j–l) Experimental arrangements and results as in (g–i), obtained with the experienced bees. In (k), mean vector direction $\mu = 333.99°$, $r = 0.99$, $P < 0.001$, $n_1 = 6$, $n_2 = 80$. The reader will find a detailed description of this experiment in De Marco RJ and Menzel R (2005) Encoding spatial information in the waggle dance. *J. Exp. Biol.* 208: 3885–3894.

and Frost, 2002) and possibly also a magnetic compass (Etheredge et al., 1999), their flight routes depend strongly on atmospheric conditions and seasonal wind patterns (e.g., Rainey, 1951, 1976; Kanz, 1977; Drake and Farrow, 1988; Gatehouse, 1997; Chapman et al., 2002). It thus appears that insect migration is basically controlled by innate mechanisms and does not rely on learning, although the genetic control of flight direction in migrating insects has yet to be confirmed (Holland et al., 2006).

1.25.4 Conclusions and Future Prospects

We have argued that a distinction between instinctive and learned behaviors, a fundamental issue in behavioral research, is not possible without thoughtfully considering the interplay between an animal's possible phylogenetic boundaries and the sources of the external signals that belong to its specific sensory world, simply because the effects of learning on a subject's performance will always be superimposed onto those of its phylogenetic boundaries. We also claimed that it is in the context of communication and navigation that learning transcends elementary forms of association in particularly clear ways. Communication and navigation in insects have been extensively studied on the sensory processing level, but the structure and content of the spatial knowledge underlying such phenomena have yet to be addressed. This might be particularly feasible in honeybees, due to their extensive behavioral repertoire, which also seems to involve decisions, and their small, experimentally accessible brains, which allow the study of system-level neural correlates of learning and memory. In honeybees, in addition, these two behavioral domains appear strictly related to each other via the famous waggle dance, although their relation is not fully understood. We have explored new findings (Menzel et al., 2005) indicating that the spatial knowledge used by honeybees to navigate within the range of their orientation flights is much more complex than hitherto thought. Several interacting – and probably competing – memory systems seem to be at work. These findings also raise questions about the process of encoding and decoding information in the waggle dance (De Marco and Menzel, 2005). We reviewed published data (von Frisch, 1968) and recent evidence (Biesmeijer and Seeley, 2005) suggesting that the spatial knowledge available to followers is also involved in dance communication and that learning might be at the heart of this impressive communicating system. The flight paths of navigating bees can now be traced with radar techniques. Mechanical models of dancing bees and virtual environments allowing navigation experiments under controlled experimental situations can also be developed. Thus tools are available to tackle these questions.

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1.26 Spatial Learning in Fish

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1.26.1 Introduction

Fish represent the largest and most diverse vertebrate radiation. Members of this taxonomic group have inhabited the earth for more than 500 million years, occupying an immense range of aquatic habitats and ecological niches and achieving an enormous diversity of morphological and functional adaptations. The prevailing views on vertebrate brain and behavior evolution have largely considered fish as the most primitive and least evolved vertebrate group. According to these rooted evolutionary notions that dominated neuroscience virtually to the present, vertebrate brain and behavior evolution occurred in successive stages of increasing complexity and advancement (fishes, amphibians, reptiles and birds, and finally mammals, to reach the superior cerebral and cognitive level of humans). In this context, fishes were thought to have developed relatively simple neural circuits allowing only elemental forms of behavior and extremely limited cognitive capabilities. Consequently, their behavior was considered as essentially reflex or instinctive. However, during the last few years a wealth of new comparative developmental, neuroanatomical, functional, and behavioral evidence has strongly contradicted this anthropocentric and misleading view, showing that vertebrate brain and behavior evolution has been far more conservative than previously thought. The new evidence shows that fishes exhibit a wide assortment of highly sophisticated behavioral and cognitive capabilities (Rodríguez et al., 2006), indicating that these vertebrate groups share with land vertebrates (including birds and mammals) a number of brain and behavioral organization features (for reviews, see Nieuwenhuys et al., 1998; Broglio et al., 2005; Butler and Hodos, 2005; Striedter, 2005; Salas et al., 2006). We review here the evidence available on spatial learning and memory capabilities in fish and on their neural basis. For this review, we concentrate mainly on ray-finned fishes and especially the teleost fish, because considerably more research has been conducted on this taxonomic group, which makes up nearly half of all vertebrate species.

1.26.2 Spatial Cognition in Fish: Observations in the Natural Environment

Naturalistic studies show that fishes, similar to mammals and birds, possess impressive spatial abilities for orienting, piloting, and navigating (*See also* Chapters 1.21, 1.25). Although traditionally research in this field has been focused mainly on the description of the innate fixed patterns of behavior and on the study of sensory and ecological factors involved in navigation, a number of naturalistic studies suggest the possibility that fish spatial behavior is a flexible and adaptive process that involves a variety of cognitive phenomena and diverse learning and memory mechanisms (for reviews, see Dodson, 1988; Kiefer and Colgan, 1992; Quinn, 1992; Dittman and Quinn, 1996; Odling-Smee and Braithwaite,

2003). For example, many fish species are territorial and remain within a restricted home range, guarding sites and resources from competitors (Hallacher, 1984; Matthews, 1990a; Kroon et al., 2000), whereas others perform intercontinental migrations (Quinn and Dittman 1990; Dittman and Quinn, 1996). Foraging, reproduction, and predator avoidance require accurate knowledge of the spatial distribution of resources, mates, and refuge areas obtained through spatial learning and memory and based on well-developed orientation and navigation capabilities (Dodson, 1988; Brown and Laland, 2006; Kelly and Magurran, 2006; Odling-Smee et al., 2006; Warburton, 2006; Witte, 2006).

Some fish species accomplish large-scale navigation, including transoceanic migrations, and spatial learning and memory could play an important role in this ability (for a review, see Quinn, 1982; Dodson, 1988). One of the best-studied long-distance migratory fish is the salmon. Salmon return to their natal stream for reproduction after long foraging migrations in the sea, and during these migrations, they travel through diverse habitats such as oceans, lakes, and rivers. The precision of the salmon homing ability is remarkable, with approximately 95% of the ocean survivors returning to their natal streams (Quinn and Dittman, 1990; Dittman and Quinn, 1996). A variety of navigational mechanisms underlie salmon migration and homing. Salmon navigation in the open ocean seems to be based on the use of celestial and magnetic compasses as well as on olfactory cues and pheromone tracking (Quinn and Dittman, 1990; Dittman and Quinn, 1996). Navigation based on odor memories associated with their natal-stream sites seems to be essential during the final freshwater phase of the migration (Scholtz et al., 1976; Hasler and Scholtz, 1983; Hansen et al., 1993; Unwin and Quinn, 1993; Pascual and Quinn, 1994; Heard, 1996; Hard and Heard, 1999). Home-stream odor imprinting appears to occur at the time of peak thyroid hormone levels during the smolt transformation process that prepares salmon for ocean navigation (Morin and Doving, 1992; Hasler and Scholtz, 1983). However, odor learning also seems to take place many times during migration, and thus salmon can use a sequence of learned odors to guide the homeward journey (Quinn, 1985; Hansen et al., 1987; Dittman and Quinn, 1996; Heard, 1996).

Other interesting examples suggesting well-developed spatial cognition capabilities in fish are provided by experimental studies conducted in natural environments. For example, studies on homing behavior showed that relocated intertidal rockpool fishes are able to accurately return to familiar hiding places even after 6 months of absence (Aronson, 1971; Green, 1971; Griffiths, 2003). The benthic fish *Ulvaria subbifurcata*, which lives in small home ranges, can home even after displacements of several hundred meters (Green and Fisher, 1977). Furthermore, this work showed that fish were significantly well oriented in the homeward direction at the release time, suggesting that they were able to determine their actual location with respect to home. Some sedentary and territorial rockfishes of different species, displaced from their home sites, navigate back home from long distances, in some cases even more than 20 km away (Carlson and Haight, 1972; Hallacher, 1984; Quinn and Ogden, 1984; Markevich, 1988; Matthews, 1990a,b).

Fish discover and remember the location of profitable food patches and prey location by exploration and individual sampling as well as by observing other foragers (Hart, 1986; Pitcher and Magurran, 1983; Pitcher and House, 1987; Hart, 1993; Warburton, 2006). Learning and remembering the location of particular food patches and mapping their status and renewal rates, as well as identifying and remembering the predation risk associated with different locations, improve fish foraging efficiency and survival probabilities. For example, butterflyfishes (*Chaetodontidae*) follow predictable paths as they swim from one food patch to another within their territories. When a coral outcrop is removed, the fish look for it in its former location before resuming their route along the reef (Reese, 1989). For these displacements, Reese (1989) suggested that butterflyfishes use a system of spatially learned route-specific landmarks, possibly in conjunction with sun compass orientation, and a cognitive map of their territories. Brown surgeon fish (*Acanthurus nigrofuscus*) undergo daily migrations of up to 1.5 km to feeding and spawning sites apparently following a sequence of landmarks (Mazeroll and Montgomery, 1998). Interestingly, the migrating brown surgeon fish changed direction in accordance with the position of experimentally displaced landmarks. The reliance of the migrating brown surgeon fish on particular landmarks could be reduced by moving the landmarks more than 6 m from their original location; nonetheless, the animals still accurately navigate to the feeding or spawning sites after key landmarks had been manipulated, suggesting that there were redundancies in the cues used.

Social transmission of foraging routes provides other interesting examples of the role of learning and cognition in fish spatial behavior (Hobson, 1972; Ogden and Ehrlich, 1977; Gladfelter, 1979;

MacFarland, 1980; Mainardi, 1980; Helfman, 1981; Helfman et al., 1982; Helfman and Schultz, 1984). For example, French grunts (*Haemulon flavolineatum*) introduced to new schooling sites and initially allowed to follow residents in their displacements, rapidly learn to use the novel foraging routes even in absence of the residents (Helfman et al., 1982; Helfman and Schultz, 1984). Laland and Williams (1997, 1998) reported that naïve guppies could learn a route to a foraging patch by observing and following experienced fish. When the experienced fish were removed, the observers continued using the same routes even when alternative itineraries were available. Warner (1988, 1990) reported culturally transmitted migratory traditions in bluehead wreasse (*Thalassoma bifasciatum*). These coral reef fish establish mating-site locations that remain constant over several generations. The experimental replacement of entire local populations led to the use of new sites, which remained constant over subsequent generations.

Field studies and experiments in natural environments provide a broad range of suggestive observations and raise interesting insights into the biological and ecological significance of fish spatial cognition capabilities. Frequently, however, given the enormous difficulty of defining, isolating, and controlling important variables in the field, these studies do not provide clear-cut conclusions on the nature of the cues and spatial strategies being used by the fish. Thus, controlled laboratory experiments are called for, as they can overcome some of these difficulties and provide optimal conditions to reveal the mechanisms that fish use for orientation and navigation.

1.26.3 Exploration and Environment Investigation

Fish apply systematic exploration in unfamiliar environments in order to acquire spatial knowledge. When introduced into a novel environment, most fish display an intensive exploratory activity, which progressively decreases. This decrease could correspond to a state in which the processing of the initial spatial information has been completed (Wilz and Bolton, 1971; Poucet et al., 1986; Thinus-Blanc et al., 1987). For example, when introduced into a novel environment, goldfish engage in an organized and systematic pattern of exploration, avoiding previously visited locations (Kleerekoper et al., 1974; Rodríguez, 1996). After performing a few ample initial turns near the edges of the tank, the fish thoroughly explore a particular area before moving to the next one, in a rather systematic way, thus exploring the entire tank (**Figure 1**). This pattern of exploration, area by area, which is never replaced by random exploration, requires some degree of spatial memory. According to this view, through its exploration behavior, the goldfish becomes familiar with a new environment faster when salient visual cues reduce the spatial (geometric) ambiguity of the environment (Warburton, 1990). A similar pattern of exploratory activity has also been observed in fish foraging in their natural environment. The planktivorous reef fish, the stout-body chromis (*Chromis chrysurus*), use a local search strategy consisting in swimming in a circuitous and stereotypic pattern over a specific foraging region, completing the search before moving to the next foraging area (Noda et al., 1994). The use of such an organized search pattern prevents revisiting depleted foraging areas and provides the basis for exploring and mapping unfamiliar areas.

In addition, the rate of activity of different species of fish correlates inversely with the degree of environmental novelty. Moreover, the fish show increases in exploratory activity in response to changes in familiar environments, indicating that these animals detect environmental modifications, be it in the location of the objects or in the identity of the objects themselves (Breder and Halpern, 1946; Breder, 1950; Welker and Welker, 1958; Russell, 1967; Kleerekoper et al., 1974). Blind cave fish (*Anoptichthys jordani*) orient and navigate accurately in the environment and can find a well-known place in the tank, even when that place is not directly accessible (Campenhausen et al., 1981). Interestingly, the swimming behavior of the blind cave fish differs in familiar and unfamiliar environments. When introduced into an unfamiliar environment, and also after alterations of a well-known one, these fish swim through it for several hours, exploring the surfaces of the walls and objects, using their lateral line to sense self-induced perturbations in the surrounding flow field. For this activity, fish increase their swimming speed, supposedly to optimize the stimulation of the organ of the lateral line. Campenhausen and coworkers suggested that during this high-speed swimming phase blind cave fish build a memory representation of the environment (i.e., an internal map). Once the representation has been elaborated, the fish can rely on this internal map to navigate and therefore can reduce the swimming speed below the optimal required by the lateral line organ,

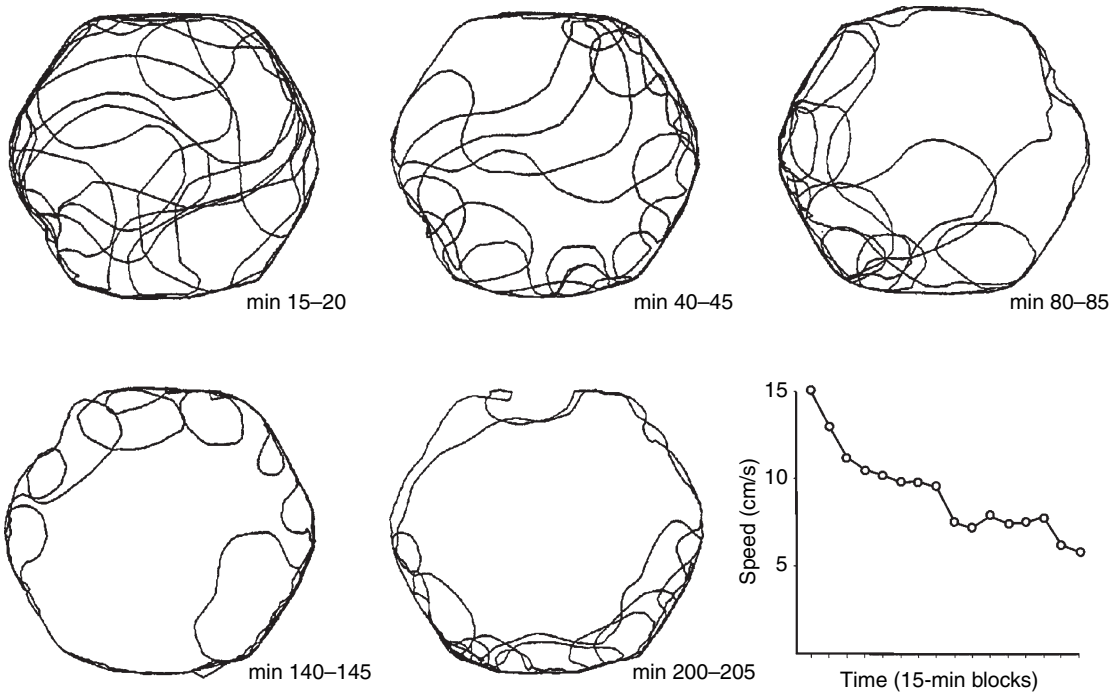


Figure 1 Exploratory behavior in goldfish. Examples of the exploratory pattern of a goldfish during a 4-h session of free exploration in a large open field. Each figure shows the fish trajectories during 5-min intervals throughout the session. Note the organized and systematic pattern of exploration area by area. The plot in the bottom right shows the mean swimming speed during the entire session of exploration in consecutive 15-min blocks.

without observable deficits in avoiding obstacles (Campenhausen et al., 1981; Teyke, 1989). Note that accurate navigation at speeds that are suboptimal for lateral-line organ stimulation implies that fish are using the internal map via feedback from senses other than those primarily used for map development (Demski and Beaver, 2001). Some additional evidence supports this hypothesis. For example, blind cave fish react by increasing swimming speed when changes are introduced in a familiar environment, such as the removal or the displacement of a familiar cue or the introduction of new objects in a well-known tank (Campenhausen et al., 1981; Teyke, 1985; Burt de Perera, 2004b). Teyke (1985) showed that the duration of the exploratory activity of the blind cave fish, *Anoptichthys jordani*, depends on the shape of the experimental tank, with bilaterally symmetrical tanks explored during longer periods of time than asymmetrical tanks or tanks in which objects have been introduced near the walls in asymmetrical positions (Teyke, 1985). Burt de Perera (2004a) showed that blind cave fish, *Astyanax fasciatus*, are able to remember the order of a landmark sequence. The ability to represent the order in which a series of places are spatially linked could be a mechanism that allows these animals to orient beyond the limits of their perceptual range. In addition, Teyke (1989) obtained evidence suggesting that the elaboration of these hypothetical internal maps by blind cave fish is a memory phenomenon that occurs in two phases, an initial volatile phase (short-term memory) and a resistant phase after consolidation (long-term memory). In fact, Teyke (1989) showed that if the fish is anesthetized by cooling only 4 h after the exploration of a new environment, when the animal recovers from the anesthesia it explores that environment again as if it were a completely unfamiliar one. However, a reactivation of the exploration is not observed when the anesthesia is produced 6–30 h after the habituation to the environment.

1.26.4 Spatial Learning in Fish: Cues and Strategies

Fish use diverse sources of spatial information, such as self-generated movement cues from different sensory modalities, directional cues that polarize the

environment, or positional landmarks that enable inferring the place locations relative to distances, angles, and the geometry of objects within an array (Thinus-Blanc, 1996; Burgess et al., 1999; Jacobs, 2003).

Teleost fish learn spatial tasks using visual cues and landmarks (Huntingford and Wright, 1989; Rodríguez et al., 1994; Girvan and Braithwaite, 1998; López et al., 1999; Hughes and Blight, 2000), as well as using nonvisual senses, such as olfaction (Quinn, 1985; Hansen et al., 1987; Dittman and Quinn, 1996; Heard, 1996), audition (Tolimieri et al., 2000; Simpson et al., 2004, 2005), the lateral line system (Campenhausen et al., 1981; Teyke, 1985, 1989), or electrolocation (Cain et al., 1994; Cain, 1995). Fish also use a variety of sources of directional information to orient and navigate, for example, sun position (Schwassmann and Braemer, 1961; Goodyear and Ferguson, 1969; Goodyear, 1970; Hasler, 1971; Loyacano et al., 1971; Goodyear and Bennet, 1979), polarized light (Dill, 1971; Davitz and McKaye, 1978; Hawryshyn et al., 1990), the earth's magnetic field (Kalmijn, 1978; Quinn, 1980; Quinn and Brannon, 1982), or water flow direction (Jonsson et al., 1994; Kaya and Jeanes, 1995; Smith and Smith, 1998; Girvan and Braithwaite, 2000; Braithwaite and Girvan, 2003; Hunter et al., 2004).

Fish learn and remember the location of profitable food patches. Pitcher and Magurran (1983) showed that goldfish (*Carassius auratus*) remember the spatial position of food patches in a tank and are able to shift to a different foraging strategy when the location of the feeders has been changed. The spined sticklebacks (*Gasterosteus aculeatus*) remember which of two patches were baited with food during training for at least 8 days (Milinski, 1994). Atlantic salmon (*Salmo salar*) trained in an artificial stream remembered the location of two feeding sites in the stream and were able to shift between these two alternative sites to continue feeding (Gotceitas and Godin, 1992).

A number of pioneering studies provide notable evidence on fish spatial learning and memory capabilities. For example, Aronson (1951, 1971) reported that gobiid fishes learn and remember the geographic features of their environment. The tide-pool-dwelling gobiid fish *Bathygobius soporator* leap with surprising accuracy from one pool to another, jumping over ledges and other obstacles. Although neighboring pools are not visible at the onset of the leap, the fish rarely, if ever, jump onto dry land, suggesting that these animals build a precise memory representation of the local topography during high tide. More recently, it has been reported that black-eye gobies (*Coryphopterus nicholsi*) increase their accuracy in escaping to their burrows if they previously had the opportunity to become familiar with the environment (Markel, 1994). Animals that were allowed to spend additional exploration time in the test tank were quicker to find the burrow. In addition, when the burrow position was shifted to a new location, the more experienced animals took longer than the less experienced fish to find the shifted burrow, suggesting that they have learned the spatial location of the burrow.

Goldfish use landmarks as direct cues or beacons but also are able to learn more complex spatial relationships and to use visual cues as indirect spatial reference points (Warburton, 1990), and they can also learn to swim in a constant direction relative to visual cues, even when their approach to the goal is from the opposite direction (Ingle and Sahagian, 1973). These abilities could reflect the capability of fish to discriminate spatial relationships in the environment independently of a body-centered reference system. Goldfish are able to use the visual angle of a familiar landmark to locate a food source (Douglas, 1996). Goldfish trained to search for food buried 20 cm from a landmark still searched in the correct location when the food was absent. Halving either the width or height of the landmark resulted in searches significantly closer to the landmark, suggesting that these animals determined their position relative to the landmark using their horizontal and vertical visual angles. In addition, fish use route-based orientation strategies, that is, a learned sequence of cues or an algorithm based on a sequence of turns (Braithwaite, 1998).

Roitblat et al. (1982) showed that Siamese fighting fish need fewer trials to learn a T-maze task when it is compatible with a win-shift strategy (i.e., to avoid revisiting the previously depleted maze arms) than in another task compatible with a win-stay strategy (to visit previously visited maze arms repeatedly). Mammals and birds typically use a win-shift strategy when solving T-maze tasks (Dember and Fowler, 1958; Uster et al., 1976; Olton et al., 1977; Withem, 1977; Kamil, 1978; Olton et al., 1981). More recently, it has been reported that spined sticklebacks (*Spinachia spinachia*) and corkwing wrase (*Crenilabrus melops*) can readily use visual cues to locate food sources in a radial maze and adequately track renewal frequencies and site productivity. These animals distinguished between renewable food sources differing in productivity, preferentially visiting those

containing more food. Fish showed preferentially win-shift strategies, avoiding recently visited locations, but switched from win-shift to win-stay strategies when appropriate (Hughes and Blight, 2000).

Roitblat et al. (1982) analyzed features of the Siamese fighting fish's (*Betta splendens*) spatial memory strategies using an aquatic version of a procedure widely used to test spatial memory in mammals, the Olton eight-arm radial maze (Olton, 1979). In this procedure, the optimal performance is achieved when the animals visit each arm only once, as the previously visited arms have already been depleted of the reinforcer. This study showed that although the performance of Siamese fighting fish in this task is characterized by a strong algorithmic component, it also involves some measure of spatial memory. The fish showed a strong tendency to choose the adjacent maze arms, turning in a consistent direction, either clockwise or counter-clockwise. This algorithmic behavior plays an important role in the sequential choice of the arms. However, the algorithmic component alone does not completely explain the performance of the *Betta splendens* in this radial arm maze task, and memory of the recently visited places is necessary to explain the fish's accurate behavior. Roitblat et al. (1982) suggested that the algorithmic component of the fish behavior in these training conditions could represent a choice strategy more than memory limitations. The fish could be using a list of the previously visited and already depleted arms or a list of the baited arms (probably including three or four items). That is, although the fish may be able to remember more information, in the Olton task they use the apparently rigid, but highly efficient, algorithmic search pattern, shadowing the use of spatial memory. In a follow-up experiment, the same researchers confirmed the participation of short-term memory in the Siamese fighting fish's performance in the radial arm maze procedure. They used a standard procedure consisting of confining the animals in the maze's central platform between choices 4 and 5 during periods of 0 s (no confinement), 30 s, and 5 min. This procedure assesses the memory component relative to the algorithm component in the eight-arm maze performance: Because the accuracy of the algorithm-based performance depends on a continuous chain of choices, any interference would restart the algorithm, increasing the number of errors after the interruption. Thus, it can be expected that the interruption by itself, independently of the confinement time, will produce greater deleterious effects if the performance only depends on a strategy based on algorithms. On the contrary, if the performance has a memory component, the effects of the interruption *per se* will be smaller, and they will depend on the duration of the confinement period. The results of this test showed that the performance accuracy depended on the duration of the confinement period as it deteriorated progressively with increasing durations. This indicates that the fish retain information on their previous choices for some time during the confinement period (spatial working memory). More recently, Hughes and Blight (1999) provided converging evidence as they observed that 15-spined sticklebaks and corkwing wrasse adopted an algorithmic strategy to solve an eight-arm maze task consisting of visiting every third arm in absence of spatial cues, but in the presence of visual cues they used spatial memory. The imposition of a delay within trials reset the algorithm, decreasing foraging behavior efficiency, but the delay in itself had no effects on performance if spatial cues could be used.

1.26.5 Separating Egocentric and Allocentric Navigation

A growing amount of evidence indicates that fish, like land vertebrates, are able to orient and navigate using a variety of cognitive mechanisms based on distinct spatial learning and memory systems (*See also* Chapters 1.20, 1.21). Fish use multiple, parallel spatial learning and memory systems to orient and navigate that show distinctive properties and depend on separate neural substrata. These multiple spatial orientation mechanisms range from simple reflex egocentric orientation mechanisms and stimulus–response simple associations to allocentric, map-like representations of the environment.

Several experiments provide direct evidence on the use of multiple spatial learning strategies in fish. For example, Rodríguez et al. (1994) aimed to ascertain whether goldfish are able to use egocentric (turn) or extramaze visual cue–based navigation strategies. The fish were trained to locate a baited feeder in a four-arm maze surrounded by an array of widely distributed distal visual cues, in one of the three following tasks (see **Figure 2(a)**):

1. an egocentric task, in which two opposite start arms were randomly used (50% each), but for all trials, the goal arm was determined by a fixed-turn

relative to the start arm (e.g., always right). As the extramaze cues were irrelevant for solving the task, this procedure made it possible to determine whether or not the fish could choose the correct arm on the basis of a particular turn response.

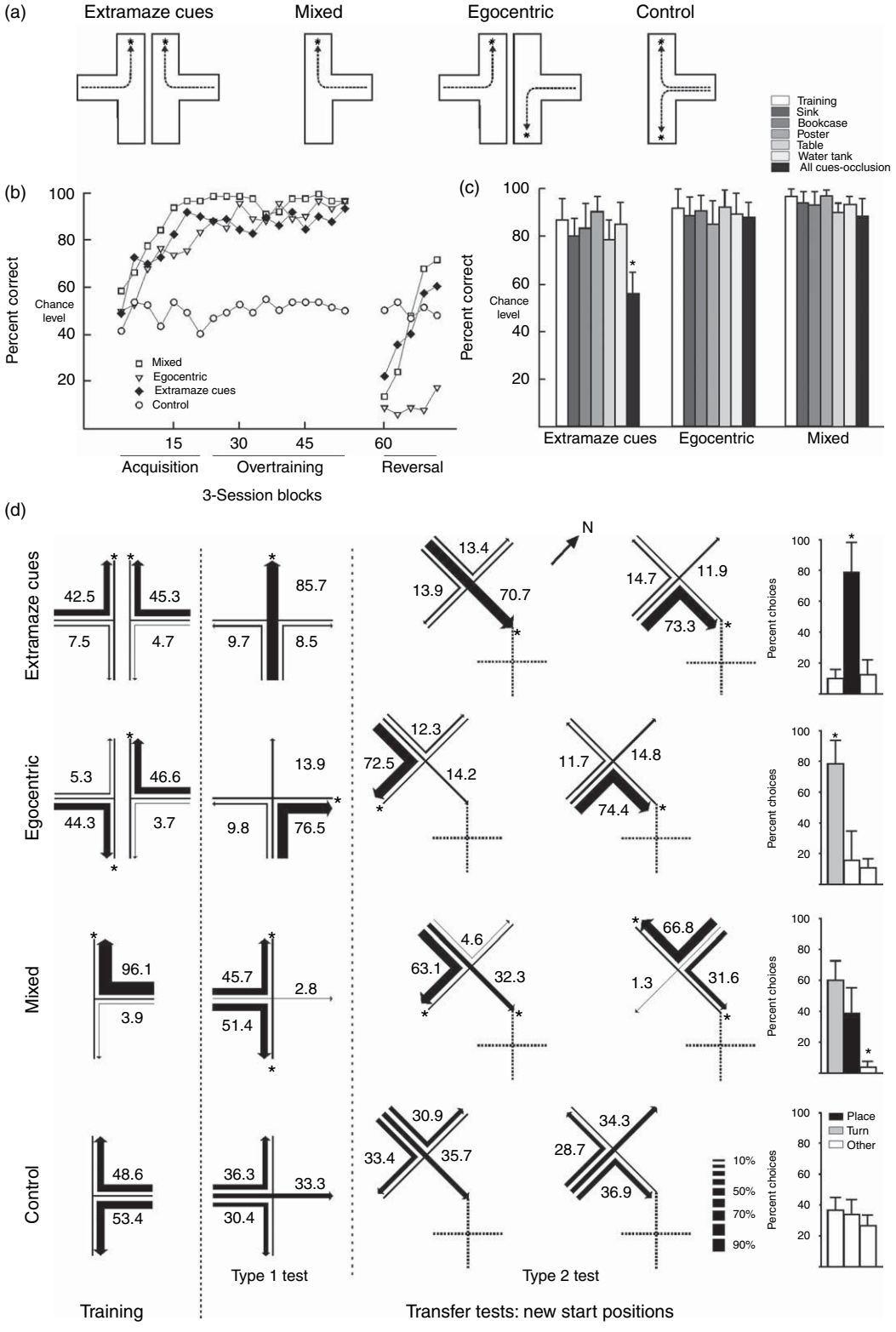
2. an extramaze visual cue task, in which two opposite start points were also randomly used (50% each), but the fish were always rewarded in the arm with the end located in a determined, stable place in the room. This procedure was used to determine whether or not the fish could learn to go to a rewarded place solely on the basis of the information provided by widely distributed (extramaze) cues. No fixed-turn direction was relevant to task solution, because, depending on the location of the start-box, the subjects were required to make a left- or a right-hand turn.

3. a mixed egocentric–extramaze visual cue task, in which the fish, in contrast to the procedures described previously, always left from the same start box and were rewarded exclusively in a goal arm ending in another constant place. This task thus allowed selecting the correct arm on the basis of a specific turn direction and/or extramaze cues.

The animals in all the groups quickly learned to reach the goal (**Figure 2(b)**). Interestingly, although the fish in the different groups achieved similar levels of performance, the transfer and probe tests revealed that these animals were using very different spatial strategies.

The test trials revealed that the goldfish trained in the extramaze visual cue procedure navigate directly to the rewarded place regardless of start position and swimming direction, even during the trials when the maze was displaced and they were required to start from previously unvisited locations (**Figure 2(d)**). In addition, these animals were able to accurately reach the goal when the extramaze cues were individually removed but not when all of them were occluded by a curtain that surrounded the maze (**Figure 2(c)**). The fish trained in the egocentric task also performed with accuracy, although they used a very different strategy. These animals mainly chose the arm corresponding with the fixed 90° turn learned during training, independently of the starting point and maze location (**Figure 2(d)**). Moreover, the performance of these animals was not affected by removing any one of the extramaze cues or all of them simultaneously. Thus, the choices of these animals were purely egocentric, and the environmental information was not taken into account for selecting the arm. Moreover, the results of the ego-allocentric group indicate that fish are also able to use both types of strategies (body-centered orientation and extramaze visual cue–based navigation) simultaneously, to solve spatial tasks, and use one or the other according to task requirements (**Figure 2(d)**). The use of both types of strategy simultaneously could explain the tendency of this group to perform more accurately and steadily than the other groups.

López et al. (2000a) also obtained evidence on the simultaneous and cooperative use of multiple spatial learning strategies in fish. In this study, goldfish were trained in a mixed place-cue procedure to find food at the end of a maze arm that occupied a constant room location, and that was, in addition, signaled by a distinct intramaze visual cue (**Figure 3**). That is, the fish were trained in a test environment where they could simultaneously rely on the spatial information provided by the extramaze distal landmarks and on a single intramaze cue that directly indicated the location of food. Subsequent probe trials were conducted in which the two sources of spatial information were set in conflict (the local cues were switched between sides; **Figure 3(a)**) or one of the two sources, the extramaze distal cues or the intramaze proximal cue, were removed (**Figure 3(b,c)**). When the intramaze visual cue was eliminated, the goldfish were still able to locate the goal using a place strategy based on the information provided by the extramaze visual cues (**Figure 3(c)**). Conversely, when the use of the extramaze distal visual cues was precluded by means of a curtain that surrounded the maze, the animals relied exclusively on the intramaze visual cue signaling the goal during training to accurately reach the goal (**Figure 3(b)**). These results indicate that goldfish implement place and guidance strategies simultaneously to solve spatial problems, and that they switch from one strategy to the other depending on the available information. Convergent results were obtained using a different apparatus and experimental procedure (Salas et al., 1996b; López et al., 1999; see **Figure 4**). Goldfish trained in a spatial constancy task or in a cue task to locate a goal in a small, stimulus-controlled enclosure, where only proximal visual cues were available, implemented guidance (orientation) or spatial relational strategies according to task demands. Whereas the performance of the fish in the cue task was dramatically impaired when the cue associated directly with the goal was removed (López et al., 1999), goldfish in the spatial constancy task navigated accurately to the goal from different start locations regardless of route direction and response requirements (Salas et al., 1996b) and despite the partial deletion of any subset of visual cues (López et al.,


(a)
Extramaze cues
Mixed
Egocentric
Control
Training
Sink
Bookcase
Poster
Table
Water tank
All cues-occlusion
(b)
Percent correct
Chance level
Mixed
Egocentric
Extramaze cues
Control
Acquisition
Overtraining
Reversal
3-Session blocks
(c)
Percent correct
Chance level
Extramaze cues
Egocentric
Mixed
(d)
N
Extramaze cues
Egocentric
Mixed
Control
42.5
45.3
7.5
4.7
85.7
9.7
8.5
13.4
13.9
70.7
14.7
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51.4
4.6
63.1
32.3
66.8
1.3
31.6
48.6
53.4
36.3
33.3
30.4
30.9
33.4
35.7
34.3
28.7
36.9
10%
50%
70%
90%
Percent choices
Place
Turn
Other
Type 1 test
Type 2 test
Training
Transfer tests: new start positions

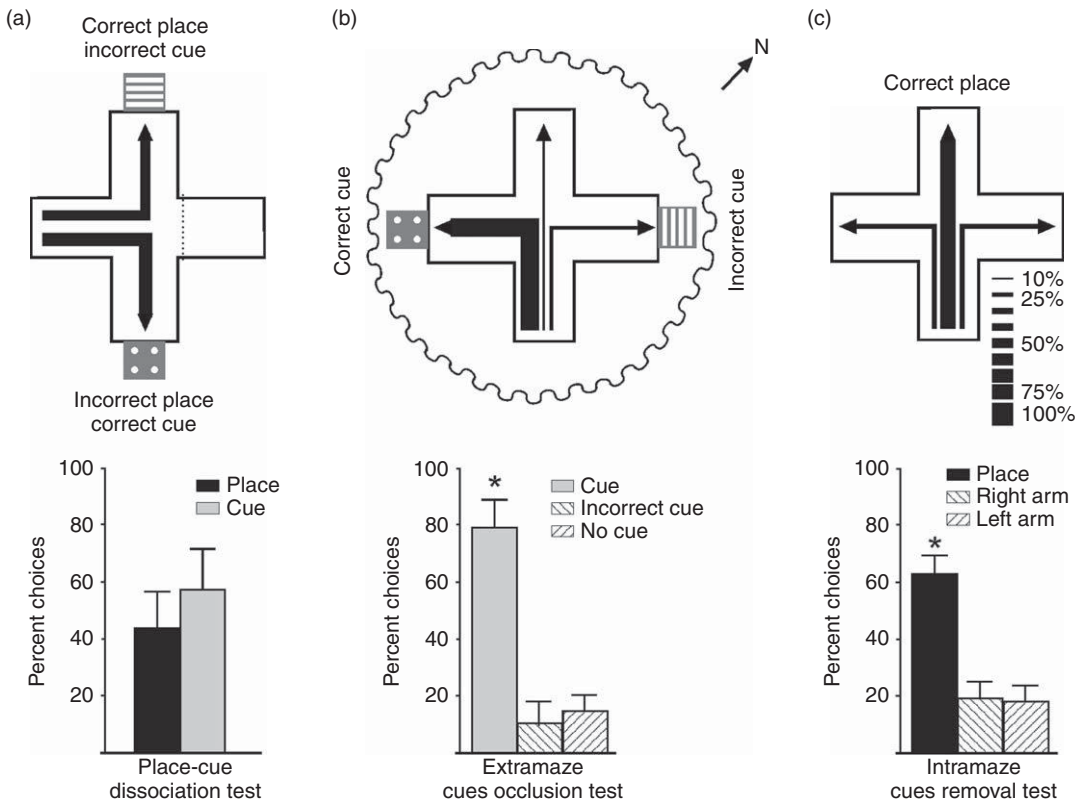


Figure 3 Simultaneous use of multiple spatial learning strategies in goldfish trained in a mixed place-cue procedure in a plus maze. In this experiment, the goal was in the arm end situated in a constant location in the room, and was, in addition, signaled by an intramaze visual cue. The figure shows the results of three different probe tests conducted following learning: (a) place-cue dissociation test; (b) extramaze cues occlusion test; (c) intramaze cues removal test. In the upper diagrams, the arrows show the trajectories chosen during each test from the start position, and their relative thickness denotes the percentage of times that each choice was made. The histograms show the mean percentage of choices to the various arms. Asterisks denote significant differences. Modified from López JC, Bingman VP, Rodríguez F, Gómez Y, and Salas C (2000a) Dissociation of place and cue learning by telencephalic ablation in goldfish. *Behav. Neurosci.* 114: 687–699.

Figure 2 Spatial learning strategies used by goldfish to find a goal in a plus arm maze. (a) Schematic diagrams of the four training procedures. The arrows mark the most appropriate trajectory from the start to the goal (asterisks) for each group. Note that for the extramaze cues and egocentric tasks two opposite start positions (50% each) were employed (see text for more details). The fish in the control group left from a start box always situated in a fixed place of the room. However, their training involved two possible correct goals, which were randomly assigned across trials. This group was used to ascertain whether or not the goal was reached by attending to odor or other uncontrolled variables. (b) Mean percentage of correct choices of goldfish trained in the four different experimental conditions, during acquisition, overtraining, and reversal of the task. (c) Percentage of correct choices when one salient cue was hidden or removed from the room and when all distal cues were removed by curtains surrounding the maze. The percentage of correct choices during training is also shown for comparison. (d) Trajectories chosen by the animals in the different groups during training and transfer trials. Once the animals learned their task, transfer tests were run to elucidate whether the animals of the different groups solved their respective tasks on the basis of turn or place strategies. In the type 1 transfer tests, the maze remained in its usual position but the animals were released from a novel start position. In the type 2 tests, the maze was displaced in the room in such a way that the end of one arm was located in the same place where the fish was rewarded during training trials, but the start positions were different from those used during training. The numbers and the relative thickness of the arrows denote the percentage of times that a particular choice was made. The dashed lines indicate the position of the maze before it was displaced for type 2 tests, and the asterisks mark the goal location. The histograms on the right show the accumulated mean percentage of choices for both types of transfer tests. Note that the animals in the allocentric group consistently chose the arm with the end at the place rewarded during training trials. In contrast, the arm most frequently chosen by the fish in the egocentric group was the one coinciding with the learned turn, independently of the location of the start arm. Asterisks on the histograms denote significant differences. Modified from Rodríguez F, Durán E, Vargas J, Torres B, and Salas C (1994) Performance of goldfish trained in allocentric and egocentric maze procedures suggests the presence of a cognitive mapping system in fishes. *Anim. Learn. Behav.* 22: 409–420.

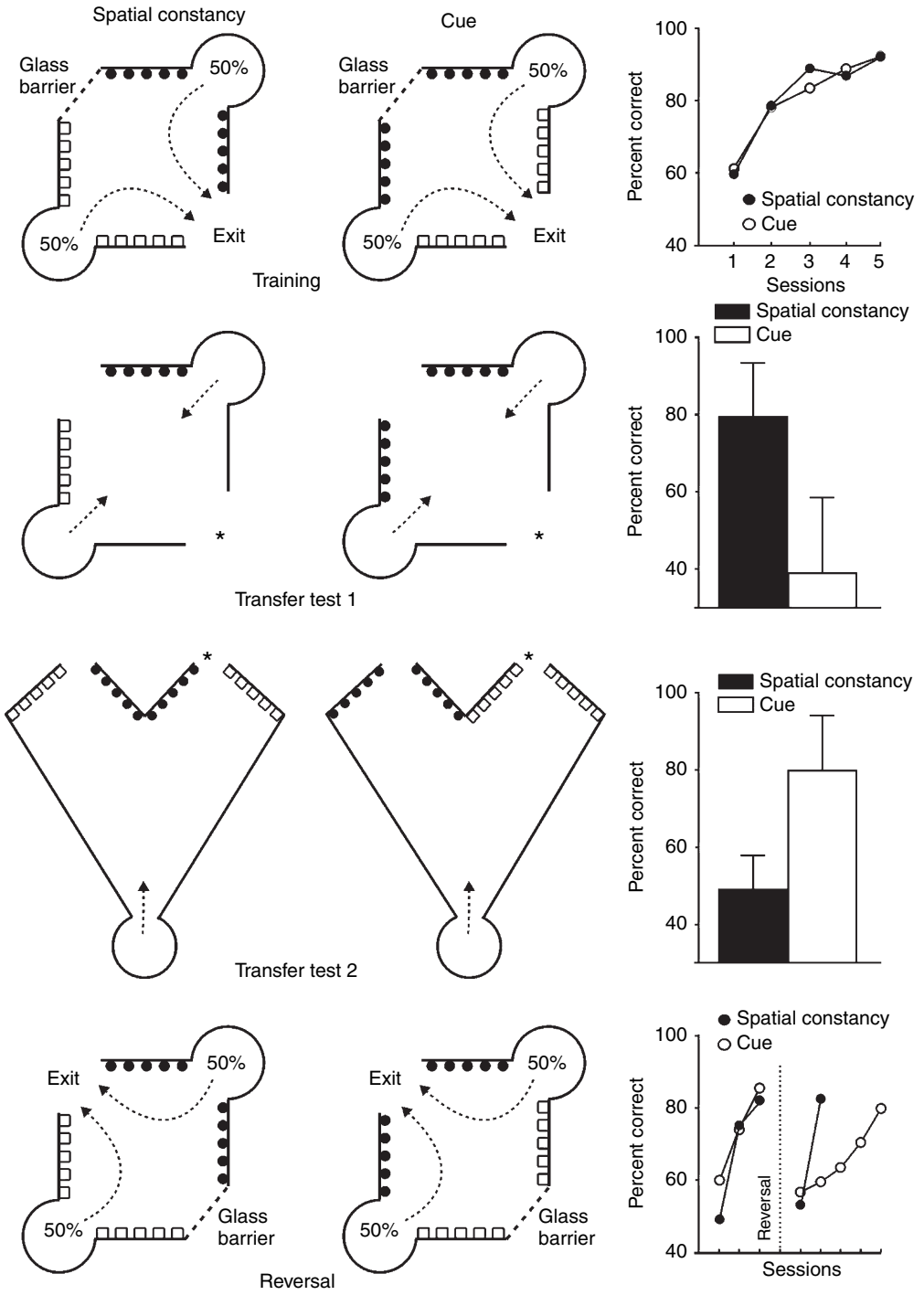


Figure 4 Multiple spatial learning strategies in goldfish trained in a spatial constancy or a cue task to locate a goal in a small, stimulus-controlled enclosure. The access from the start compartments, the distribution of the experimental visual cues (black circles and hollow squares), the position of the glass barrier, and the location of the goal (asterisk) are shown for the two experimental training conditions, transfer tests, and task reversal. The numbers indicate the percentage of trials initiated from each starting compartment. The arrows show the most efficient routes to the goal. The figures on the right show the percentage of correct responses during each experimental situation. Asterisks denote significant differences. Modified from López JC, Broglio C, Rodríguez F, Thinus-Blanc C, and Salas C (1999) Multiple spatial learning strategies in goldfish (*Carassius auratus*). *Anim. Cogn.* 2: 109–120.

1999). In fact, one might expect that the use of multiple orientation systems would increase navigational efficiency in an environment with redundant cues. The cooperative use of different spatial strategies has been described previously in other vertebrate groups (Schenk and Morris, 1985; Whishaw and Mittleman, 1986; Able, 1991).

1.26.6 Map-like Memory Representations of the Environmental Space

In the preceding sections, we mentioned the possibility that, in addition to using egocentrically referenced mechanisms for orienting, fish may also use allocentric spatial representations. In the present section, we discuss this point in more detail. Although this has been one of the most controversial topics in animal spatial cognition research, thorough studies carried out during the last few decades indicate that mammals, birds, and reptiles can use allocentric representations of space for navigation (O'Keefe and Nadel, 1978; Eichenbaum et al., 1990; Sherry and Duff, 1996; Bingman et al., 1998; Burgess et al., 1999; López et al., 2000b, 2001; Salas et al., 2003; Chapters 1.20, 1.22, 1.25). These allocentric representations, defined as map-like, world-centered cognitive representations (i.e., cognitive maps; Tolman, 1948; O'Keefe and Nadel, 1978), are the result of processing and encoding the properties and features of the environment in terms of the spatial relationships between multiple cues. These memory representations allow the subject to represent itself within a stable spatial framework and navigate accurately and flexibly within it, regardless of its own actual position and local view. These map-like spatial memory representations, which can be considered true relational memories, show some of the properties that distinguish the human declarative or episodic memory (Clayton and Dickinson, 1998; Eichenbaum, 2000; Chapters 1.22, 1.23). In comparative psychology and neuroscience, relational and allocentric spatial memories have long been considered attributes that distinctly characterize humans and other mammals, as these high-order cognitive capabilities are assumed to require complex associational brain structures, in particular the hippocampus and the six-layered neocortex. However, the results of some experiments indicate that teleost fish can implement allocentric, map-like spatial representations to navigate, based on processing multiple, reciprocal associations among different environmental features (for reviews, see Broglio et al., 2003; Salas et al., 2003).

For example, the results from the above-cited study by Rodríguez and coworkers (1994) showed that goldfish trained in an allocentric procedure were able to navigate directly to the rewarded place independently of start position and swimming direction, since they were able to reach the goal even when released from novel start arms (**Figure 2(d)**; transfer test, type 1) and from previously unvisited room locations (**Figure 2(d)**; transfer test, type 2). In fact, these animals chose spontaneously the most direct trajectory to the goal place from different, never experienced start locations, establishing new routes without a history of previous training (see trajectories in **Figure 2(d)**) The results of the probe and transfer trials are especially significant because they can rule out the possibility that these fish could be using guidance or other egocentric referenced orientation mechanisms, by relying on a particular landmark or in a direction sense, for example, approaching or avoiding a sensory cue, be it visual, auditory, odor, polarized light, or geomagnetic gradients. These results show that goldfish spontaneously use the most appropriate of the available routes from previously unvisited places, using shortcuts or detours, as convenient, although the new trajectories imply new, never experienced, egocentric relations to landmarks. These data provide solid evidence of the fish's capacity to represent spatial relationships in the environment independently of a body-centered reference system, that is, to perform place responses by using allocentric frames of reference.

Another important property proposed for map-like, relational spatial memory representations is the storage of redundant information on the encoded cues and environmental features. This property implies that the disappearance of any subset of environmental cues will not impair accurate navigation, because the animals can rely on the remaining cues (Suzuki et al., 1980; Morris, 1981; Mazmanian and Roberts, 1983). The study by Rodríguez et al. (1994) provided interesting evidence in this regard (**Figure 2(c)**): On the one hand, the performance of the fish trained in the allocentric task did not deteriorate when the most salient visual cues were individually removed or hidden, and on the other hand, when all the cues were simultaneously removed, performance became as poor as that of the control fish, indicating that the performance of the allocentric group was based on the knowledge of the relationships among the goal

location and many environmental cues. These data agree with the above-mentioned requirement for cognitive mapping, concerning the encoding of redundant information, as it was not impaired by the removal or occlusion of any one of the component elements of the landmark array, that is, none of those cues, taken individually, was essential to locate the goal.

Recently, Schluessel and Bleckmann (2005), using behavioral procedures that closely match those used in the above-cited study by Rodríguez et al. (1994), have obtained results that indicate that elasmobranchs, a sister group of actinopterygian and sarcopterygian vertebrates, can also use allocentric strategies for navigation. Rays (*Potamotrygon motoro*), in addition to using egocentric strategies, use the information provided by the extramaze visual cues to reach the goal using novel routes starting from unfamiliar locations, suggesting that the spatial mapping could be a widely extended cognitive capability in vertebrates.

Further studies have provided direct evidence of additional characteristics of the allocentric strategies in fish. For example, a number of recent studies indicate that besides using landmarks to build map-like spatial representations, fish can use the geometry or the shape of the environmental boundaries (Broglio et al., 2000; Sovrano et al., 2003; Vargas et al., 2004; Sovrano et al., 2007). In fact, these studies showed that fish match birds, rodents, monkeys, and humans in the capability to encode geometric spatial information for orientation and navigation (Tinklepaugh, 1932; Cheng, 1986; Vallortigara et al., 1990; Hermer and Spelke, 1994; Kelly et al., 1998; Gouteaux et al., 2001; Gouteaux and Spelke, 2001). These results are specially relevant, because the capability to encode and use the geometrical features of the environment for allocentric navigation reflects the knowledge of the spatial features as a whole, that is, the metric and geometrical relationships among the constituent elements (Cheng and Gallistel, 1984; Cheng, 1986; Hughey and Koppenaal, 1987; Gallistel, 1990; Cheng and Sherry, 1992; Cheng, 1994; Hermer and Spelke, 1994; Kamil and Jones, 1997).

Lopez et al. (1999) showed that goldfish trained in a spatial constancy task were impaired when a spatial modification was introduced in the experimental apparatus that altered the global shape (geometry) and the topography of the apparatus, although it left unchanged the local visual and geometric features of the areas corresponding to each of the doors. In contrast, the performance of the fishes trained in a cue task using the same apparatus was unimpaired by the global topographical alteration if the local cues were maintained (López et al., 1999, see **Figure 4**). In addition, recent experimental studies, directly addressing this issue in fish, demonstrated that fish use the geometry of the surroundings for spatial orientation. For example, Sovrano et al. (2003) showed that, when disoriented in a closed rectangular tank, redtail splitfin (*Xenotoca eiseni*) are able to reorient according to the shape of the environment and to combine geometric and nongeometric information of the environment such as the color of the walls or the features provided by the visual cues. Fish encode geometric information even when distinct feature information is sufficient to solve the task. In addition, Vargas et al. (2004) showed that goldfish locate a place in an environment by encoding the goal location with respect to the geometrical features of the experimental space, even in the absence of feature information. Goldfish trained in a symmetrical, rectangular apparatus, and in the absence of additional visual cues, made systematic rotational errors by confusing geometrically equivalent places (**Figure 5(a)**). In the test trials in which the geometric features were modified by means of replacing the rectangular apparatus used during training by a square one, the performance was significantly impaired, indicating that the fish used no other source of spatial information but the geometry of the rectangular arena (**Figure 5(a)**). In addition, the results of this study indicate that goldfish encode geometric and nongeometric information simultaneously when the environment provides both types of information and use one or the other according to task requirements. The dissociation tests showed that goldfish encoded the geometric information even when information provided by distinct visual cues was sufficient to solve the task, and that the fish's representation of the spatial environment was flexible and resistant to losses of redundant but relevant spatial information (**Figure 5(b)**, geometry test and feature test). However, the performance was disrupted when the metric and topological relationships among the composing elements were altered (**Figure 5(b)**, dissociation test). In fact, whereas the fish were able to solve the task successfully by using either the geometrical (**Figure 5(b)** geometry test) or the featural cues (**Figure 5(b)**, feature test), they chose at random between the possible exits when the information provided by both types of cues was dissociated and made contradictory (**Figure 5(b)**, dissociation test). Furthermore, this pattern of results suggests that goldfish elaborate a complex representation of the environment, in which the different elements and properties of the environmental space (geometry and featural information) are not only simultaneously

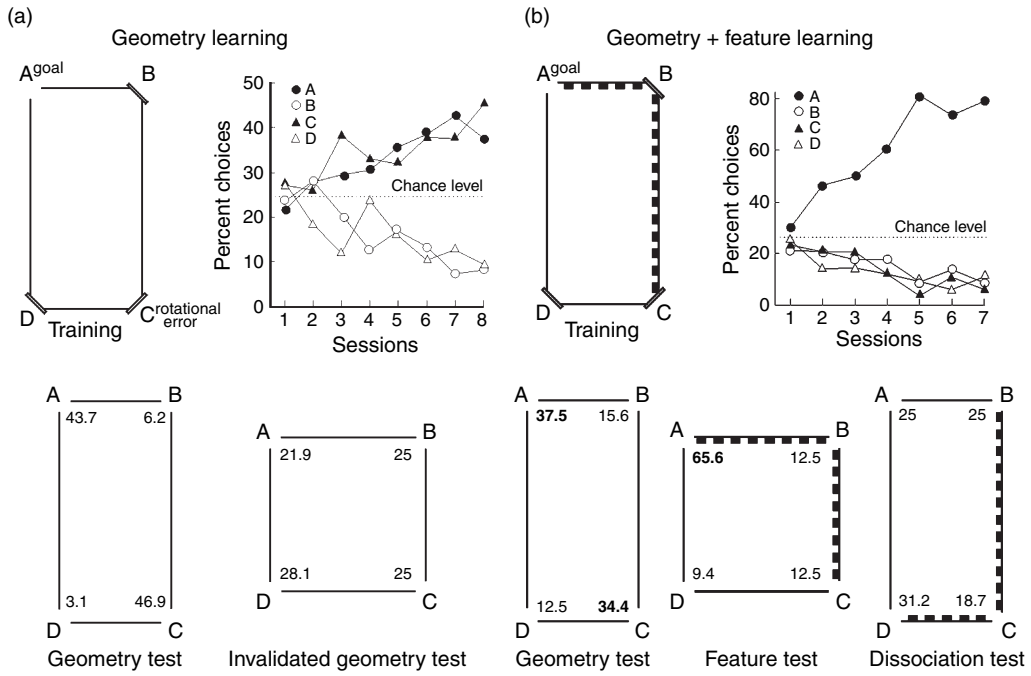


Figure 5 Encoding of geometric and featural spatial information by goldfish. (a) Geometry task. Fish were trained to find the exit door (goal) placed in a corner (A) of a rectangular arena that had three identical blocked openings in the other three corners (B, C, and D). Because of the geometric properties of the apparatus, the correct corner was indistinguishable from the diagonally opposite corner (rotational error). The curves show the percentage of choices for the four corners during training. The diagrams in the bottom show the percentage of choices (numbers) for each corner during the two different probe tests conducted for this group. (b) Geometry + feature task. Fish were trained in the same rectangular box but in which additional feature information was provided by alternate dark grey and white vertical stripes on two walls. The curves show the percentage of choices for the four corners during training. The diagrams on the bottom show the percentage of choices (numbers) for each corner during the three different probe tests conducted for this group. Modified from Vargas JP, Lopez JC, Salas C, and Thinus-Blanc C (2004) Encoding of geometric and featural spatial information by Goldfish (*Carassius auratus*). *J. Comp. Psychol.* 118: 206–216.

encoded but also are probably integrated in a single configuration, a relational, map-like representation (O'Keefe and Nadel, 1978; Eichenbaum et al., 1990; Poucet, 1993; Eichenbaum et al., 1994; Thinus-Blanc, 1996).

These data show that some significant features of the spatial capabilities of fish resemble those of reptiles, birds, and mammals, including primates (O'Keefe and Nadel, 1978; Schenk and Morris, 1985; Whishaw and Mittleman, 1986; Sherry and Vaccarino, 1989; Whishaw, 1989; Sherry and Duff, 1996; Bingman et al., 1998; López et al., 2000c, 2001) and suggest that spatial cognition is organized in a similar way in every vertebrate radiation. Such striking cognition similarities in vertebrate groups that diverged millions of years ago from a common evolutionary ancestor suggest the possibility that these multiple spatial memory systems may be a primitive feature in vertebrates, that is, that all of them could have inherited some common features in the behavioral and neural organization, maintained with little modification through phylogenetic branching. However, if the cognitive capabilities observed in fish and amniotes really have a common evolutionary origin, then they must stem from a conserved neural basis. This possibility is discussed in the next section.

1.26.7 Neural Basis of Spatial Cognition in Teleost Fish

In the preceding sections, we reviewed evidence suggesting that spatial orientation and navigation in teleost fish, as in land vertebrates, involves a variety of cognitive, perceptive, and motor mechanisms. We now summarize experimental evidence showing that these spatial cognition processes and memory systems are based on separate brain substrata and outline the central question of whether they are supported by neural centers and circuits equivalent to those that underline spatial cognition in land vertebrates.

Comparative evidence shows that separate brain systems contribute in different ways to spatial orientation and navigation in vertebrates, such that sensorimotor information is processed and integrated by a number of brain mechanisms, and the information about environmental space and the position of the body and body parts in space is coded and translated into a series of coordinate systems, from the receptive surfaces to head-centered and body-centered coordinates and to allocentric, world-centered coordinate systems. For example, in mammals, perception and action based on egocentric frames of spatial reference depend on brain circuits that involve the superior colliculus, cerebellum, basal ganglia, and parietal and frontal motor cortical areas (Stein and Meredith, 1993; Burgess et al., 1999). However, the use of allocentric frames of reference for navigation, based on the encoding of the reciprocal spatial relationships between the goal and multiple sensory and spatial features, depends on other neural systems, mainly the hippocampal formation and associated brain circuits (O'Keefe and Nadel, 1978; Burgess et al., 1999; Chapters 1.21, 1.33).

Unfortunately, the psychological and neurobiological research concerning the neural basis of learning and memory in fish has been largely limited by the misleading, but very common and deeply rooted, idea that vertebrate evolution occurred in successive stages of increasing complexity and advancement in brain organization and cognitive capabilities, following a lineal progression from inferior to superior forms (i.e., fishes, amphibians, reptiles, birds, and mammals; *Scala naturae*; Hodos and Campbell, 1969; Deacon, 1990; Hodos and Campbell, 1990; Preuss, 1995; Butler and Hodos, 2005). Thus, the classical theories on vertebrate brain and behavior evolution propose that fishes, the most primitive and least evolved vertebrate group, have developed relatively simple neural circuits. According to these theories, the forebrain of fishes was thought to consist mainly of a subpallium (paleostriatum) and a very reduced and primitive pallium (paleocortex), both entirely dominated by olfactory inputs and consequently allowing only elemental forms of behavior and learning capabilities. In fact, these theories considered that neural structures equivalent to the hippocampus and the neocortex were completely absent in the fish telencephalon. All these telencephalic structures (the neoencephalon) were thought to have evolved later, in supposedly more recent and complex vertebrate groups. However, by the end of the twentieth century, a fast-growing amount of comparative developmental, neuroanatomical, and functional evidence had led to an entirely different understanding of vertebrate brain and behavior evolution, as the traditional, anthropocentric view of linear brain evolution was consistently contradicted by the data: Vertebrates have not evolved linearly, but the lineage initiated in a remote common ancestor branched in different radiations, which evolved independently, and increases in brain size and complexity occur in every one of these branches. Furthermore, this new evidence reveals that the evolution of the vertebrate brain has likely been more conservative than previously thought and that the extant vertebrates share some inherited features of brain and behavior organization. Thus, although showing conspicuous morphological and cytoarchitectural differences, their brains can be considered as variations of a common vertebrate plan (Wiley, 1981; Northcutt, 1995; Nieuwenhuys et al., 1998; Butler and Hodos, 2005). The comparative evidence reveals not only that the central nervous system of vertebrates is organized in homologous main subdivisions (i.e., telencephalon, diencephalon, mesencephalon, rhombencephalon, and spinal cord) but also that the telencephalon of every vertebrate group, including fish, consists of equivalent pallial and subpallial zones. Moreover, we now know that the olfactory areas represent only a limited portion of the fish telencephalic pallium and that the main pallial subdivisions in the actinopterygian fish telencephalon are likely to be homologous to the main pallial subdivisions of tetrapods (Braford, 1995; Northcutt, 1995; Wulliman and Rink, 2002; Butler and Hodos, 2005).

1.26.8 Teleost Fish Telencephalon and Spatial Cognition

The results of the initial studies on the neural basis of behavior in teleost fish seemed consistent with the theories on vertebrate brain evolution prevailing at the time. For example, early studies reported that ablation of the entire telencephalon in teleost fish produced few (if any) deleterious effects on fish behavior, that gross sensory or motor deficits were not apparent, and that the motivation of the ablated animals appeared to remain at normal levels (see, e.g., Polimanti, 1913; Nolte, 1932; Janzen, 1933; Hosch, 1936; Hale, 1956; Savage, 1969b). However, more careful analyses revealed that telencephalon ablation causes profound learning and memory deficits in fishes as well as significant alterations in emotional and social behavior (Aronson, 1970; Hollis and

Overmier, 1978; de Bruin, 1980; Davis and Kassel, 1983; for revisions, see Savage 1980; Overmier and Hollis 1983, 1990). These studies showed that whereas telencephalon ablation in fish does not impair simple instrumental learning and classical conditioning (Overmier and Curnow, 1969; Flood and Overmier, 1971; Frank et al., 1972; Overmier and Savage, 1974; Farr and Savage, 1978; Hollis and Overmier, 1982), it does produce severe impairments in other learning instances, such as avoidance learning (Hainsworth et al., 1967; Savage, 1968; Overmier and Flood, 1969; Savage, 1969a), as well as instrumental learning when there is a delay between the response and the reward (Savage and Swingland, 1969; Overmier and Patten, 1982).

Interestingly, the involvement of the teleost fish telencephalon in spatial cognition was also already suggested by early ablation studies, although the initial reports were often contradictory. Thus, no deficits, impairments, or even improvement in spatial learning have been observed following telencephalon ablation in fish trained in alleys and mazes (Hosch, 1936; Zunini, 1954; Hale, 1956; Warren, 1961; Ingle, 1965; Frank et al., 1972; Flood et al., 1976; Farr and Savage, 1978). The inconsistency of the reports could be attributed to the fact that these experiments were not specifically aimed to analyze spatial cognition and therefore did not include a precise definition of the spatial requirements of the tasks nor adequate control tests, which are essential for identifying the effects of ablation on spatial performance. Moreover, spatial learning and memory and spatial cognition frequently involve somewhat confusing concepts that include a mixture of still poorly characterized perceptive and cognitive processes actually based in separate neural substrata.

More recent studies, designed specifically to analyze the nature of the spatial cognition deficits produced by telencephalic lesions, have provided strong evidence for the central role of the telencephalon in spatial cognition in teleosts. The data from the more recent ablation studies show that the teleost telencephalon is necessary for some specific spatial learning and memory functions, as it contains essential components of the neural network that underlies map-like spatial memories in fish (Rodríguez et al., 1994; Salas et al., 1996a, 1996b). For example, Salas et al. (1996b) trained goldfish in place (allocentric), turn (egocentric), or mixed place–turn procedures in a four-arm maze. After the animals had mastered their task, they were subjected to complete telencephalic ablation or sham operations. The results showed that the ablation dramatically and irreversibly impaired the ability of the fish trained in the place procedure to recognize goal location during postsurgery training (**Figure 6**). In contrast, telencephalon ablation did not alter the performance of the animals using egocentric orientation strategies. Interestingly, the lesion appeared to produce no deficit in the fish trained in the mixed place–turn procedure, as during postsurgery training their performance remained at the previous levels. However, the transfer trials revealed that the telencephalon ablation did produce a remarkable spatial cognition impairment: Whereas before ablation these animals used place (allocentric) or turn (egocentric) strategies in a flexible and cooperative way according to the experimental conditions, after surgery, their performance was based exclusively on turn responses (**Figure 6**).

Consistent results were obtained in an experiment in which intact and telencephalon-ablated goldfish were trained in a mixed place–cue procedure (López et al., 2000a). Sham-operated and telencephalon-ablated goldfish were trained in a test environment where they could simultaneously rely on the spatial information provided by a number of extramaze distal landmarks on the periphery of the experimental room and on an intramaze single cue that directly indicated the location of food. Paradoxically, telencephalon-ablated goldfish showed better performance during training relative to sham-operated animals (see **Figure 7(a)**). However, the results of the probe tests designed to examine the relative importance of the two sources of information (extra- vs. intramaze cues) revealed that these animals suffered a profound spatial learning deficit: their performance was based exclusively on egocentric strategies (**Figure 7(b)**). In the place–cue dissociation tests, in which the two sources of information were set in conflict (the place and the cue responses were incompatible), the control fish did not have a significant preference for either the cue or the place responses. In contrast, the performance of the telencephalon-ablated fish was notably biased in these test trials; they showed a significant preference for the arm containing the cue that signaled the goal during training. In the extramaze occlusion tests, when the maze was completely surrounded by curtains precluding the use of the extramaze visual cues, both telencephalic and control fish showed a strong preference for the arm containing the cue associated with the goal during training. However, in the intramaze cue-removal test, whereas the control goldfish consistently chose the arm placed at the location of the room where they were rewarded during training, the

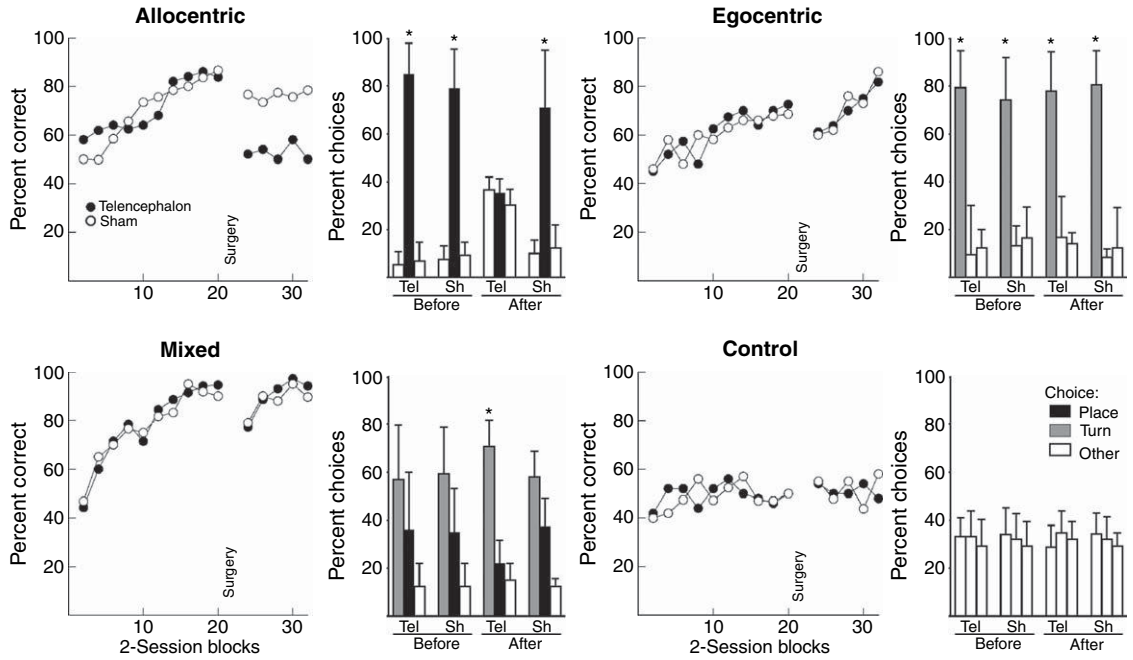


Figure 6 Spatial learning deficits after telencephalic ablation in goldfish trained in egocentric and allocentric maze procedures. The curves show the mean percentage of correct choices of telencephalon-ablated and sham-operated goldfish trained in different spatial procedures as described in **Figure 2**. The bars show the percentage of choices during the transfer tests conducted before and after surgery for each experimental group. Asterisks denote significant differences. Modified from Salas C, Rodríguez F, Vargas JP, Durán E, and Torres B (1996b) Spatial learning and memory deficits after telencephalic ablation in goldfish trained in place and turn maze procedures. *Behav. Neurosci.* 110: 965–980.

telencephalon-ablated fish chose at random between the maze arms, indicating that these animals were not able to use the array extramaze cues as a source of useful spatial information. These results indicate that although both groups learned the task, the sham-operated and the telencephalon-ablated goldfish differed in their capacity to use complementary navigational strategies; the control animals used both place and cue strategies, but the telencephalon-ablated animals solved the task exclusively on the basis of a cue strategy. Additional experiments using different behavioral procedures provided converging evidence on the role of the teleost fish telencephalon in spatial cognition. Thus, telencephalon ablation disrupted the postsurgery performance (Salas et al., 1996b) and reversal learning (López et al., 2000b) of goldfish trained in a spatial constancy task but did not produce any observable deficit in a cue procedure (Salas et al., 1996b). In summary, the place memory impairments observed in these experiments strongly suggest that forebrain ablation in fish produces a selective but severe disruption in a telencephalon-dependent spatial memory system, which sustains the use of allocentric, relational map-like representations of space.

In mammals, birds, and reptiles, map-like or relational spatial memories depend on the hippocampus and associated structures (O'Keefe and Nadel, 1978; Sherry and Duff, 1996; Bingman et al., 1998; Burgess et al., 1999; Rodríguez et al., 2002a, b; López et al., 2003a, b; Chapters 1.21, 1.22, 1.33). Similarly, compelling evidence suggests that specific areas and circuits of the teleost forebrain, in particular the lateral pallium, proposed as homologous with the hippocampus of land vertebrates, provide an essential neural substratum for cognitive mapping abilities in fish (Salas et al., 1996a, b; López et al., 2000a, b; Rodríguez et al., 2002b; Salas et al., 2003, Broglio et al., 2005; Salas et al., 2006).

1.26.9 Telencephalic Hippocampal Pallium and Map-like Memories in Teleost Fish

The telencephalon of ray-finned fishes presents morphological features that are unique among vertebrates; for instance, it consists of solid telencephalic hemispheres separated by a single ventricular cavity, instead of hemispheres with internal ventricles.

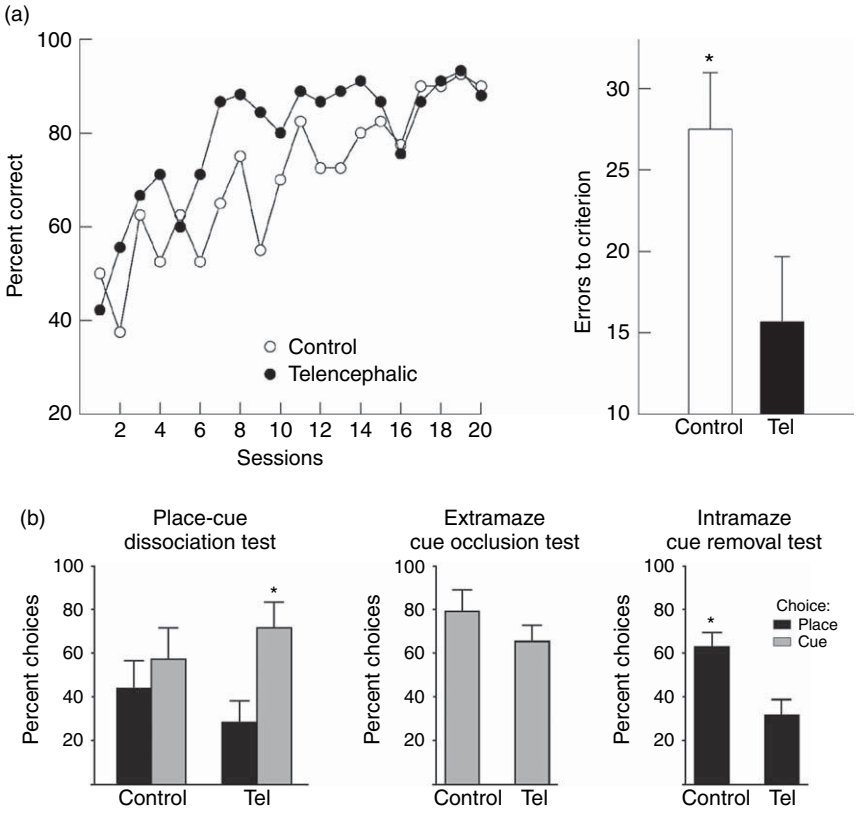


Figure 7 Spatial learning deficits by telencephalic ablation in goldfish trained in a mixed place–cue arm-maze procedure. (a) Percentage of correct choices and errors to criterion of sham-operated and telencephalon-ablated goldfish trained in a mixed place–cue procedure as described in **Figure 3**. (b) Mean percentage of choices in the three different probe tests conducted following learning of the task by both groups. Asterisks denote significant differences. Modified from López JC, Bingman VP, Rodríguez F, Gómez Y, and Salas C (2000a) Dissociation of place and cue learning by telencephalic ablation in goldfish. *Behav. Neurosci.* 114: 687–699.

Ray-finned fishes represent a particular case because their telencephalon develops by a process of eversion or bending outward of the embryonic prosencephalic alar plate, contrasting with the evagination that characterizes the telencephalic development of every other vertebrate group (Nieuwenhuys, 1963; Northcutt and Braford, 1980; Braford, 1995; Northcutt, 1995, 2006; Striedter and Northcutt, 2006). This developmental peculiarity implies that the medial-to-lateral topography of the pallial areas observed in the vertebrates with evaginated telencephalon is reversed in ray-finned fishes. In fact, the ray-finned fish's lateral telencephalic pallium (i.e., the embryonary distal pallium) is considered homologous to the medial pallium or hippocampus of the tetrapods (Northcutt and Braford, 1980; Nieuwenhuys and Meek, 1990; Braford, 1995; Northcutt, 1995; Butler, 2000; Northcutt, 2006; see **Figure 8**). This hypothesis of homology of hippocampus is supported by the neuroanatomical and developmental data (Northcutt, 1995; Wulliman and Mueller, 2004; Northcutt, 2006). Like the hippocampus, the lateral pallium is characterized by widespread reciprocal connections with other pallial areas, as well as with the contralateral lateral pallium, by means of commissural projections. In addition, the lateral pallium is reciprocally connected with the ventral nucleus of the area ventralis (Vv) considered homologous to the septal nucleus (Butler and Hodos, 2005), from which it receives a cholinergic input. The extratelencephalic pattern of connectivity of the lateral pallium is also similar to that of the hippocampus, as it projects to the preoptic area and other diencephalic regions and receives inputs from the preoptic area, the locus coeruleus, and the superior raphe. Particularly, the ventral subdivision of the lateral pallium (Dlv) is the most likely candidate as the specific homolog of the

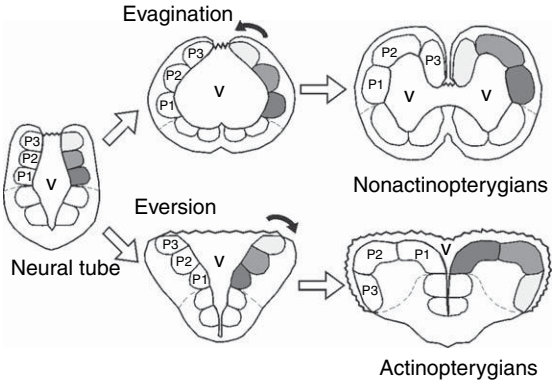


Figure 8 Schematic representation of the process of evagination and inversion that occurs in the telencephalon of nonactinopterygian vertebrates during embryonic development compared with the process of eversion or bending outward that occurs in actinopterygians. P1, P2, and P3 correspond to the three main subdivisions of the pallium. V, ventricle.

tetrapod medial pallium; it occupies the most distal topological position in the pallium and has extensive interconnections with the likely homologs of the septal nuclei and preoptic area. The hypothesis of homology concerning the teleost fish Dlv region and the hippocampus is also supported by the distribution pattern of several histochemical and molecular markers. For example, the dopamine receptor subtype D1B, which is characteristic of the mammalian hippocampus, is selectively expressed in the Dlv subdivision of the teleost pallium (Kapsimali et al., 2000), and this area, like the mammalian dentate gyrus, shows neurogenesis and migration of interneurons (Grandel et al., 2006).

Functional evidence also supports the hypothesis of homology between the lateral pallium of teleost fish and the hippocampus, as the ventral lateral pallium of teleost fish, like the amniote hippocampus, is selectively involved in spatial cognition (Rodríguez et al., 2002b; Broglio et al., 2005). Extensive lesions to the lateral pallium produce a dramatic impairment in place memory in goldfish trained in a plus maze surrounded by widely distributed distal visual cues (Rodriguez et al., 2002b; see **Figure 9(a)**). Following surgery, lateral pallium–lesioned fish showed a severe and permanent impairment to reaching well-trained goal locations. In addition, these animals were not able to locate the goal from new starting places during transfer tests in which reaching the goal involved the use of novel routes. The spatial learning and memory deficits following lesions restricted to the lateral pallium in goldfish are as severe as those produced by the ablation of the entire telencephalon (Salas et al., 1996b; López et al., 2000a; Rodríguez et al., 2002a,b). In addition, the involvement of the lateral pallium in spatial cognition is highly selective, as the damage to this area does not disrupt cue learning or other egocentrically referenced spatial behaviors (Salas et al., 1996a,b; López et al., 2000a; Rodríguez et al., 2002b; see **Figure 9(b)**). These results indicate that, like the hippocampal pallium of land vertebrates, the lateral pallium of teleost fish is selectively involved in the use of map-like or relational spatial memory representations for allocentric navigation, but it is not critical for simple stimulus–response associations or cue learning. In contrast, the medial and dorsal pallium lesions do not produce any observable impairment in spatial memory in goldfish (Rodríguez et al., 2002b; see **Figure 9**). In contrast, the teleost fish medial pallium, likely homologous to the pallial amygdala of amniotes (Northcutt and Braford, 1980; Northcutt, 1995; Wulliman and Mueller 2004; Yamamoto et al., 2007), seems to be involved in emotional behavior (Portavella et al., 2004a,b; Broglio et al., 2005; Salas et al., 2006), and the dorsal pallium, which contains several multisensory and motor representations, appears to be the homolog of the amniote dorsal cortex or isocortex (Northcutt, 1995; Prechtl et al., 1998; Saidel et al., 2001; Northcutt, 2006; Yamamoto et al., 2007).

The involvement of the lateral telencephalic pallium of teleost fish in spatial cognition is suggested also by studies using complementary techniques to reveal neural activity. Vargas et al. (2000) showed that training goldfish in a spatial task induces a significant and selective spatial learning-related increase in the transcription activity (protein synthesis) of the neurons in the lateral pallium, evaluated by means of a silver stain with high affinity for the argyrophilic proteins of the nucleolar organizing region (AgNORs). The size of the AgNORs in the cell nucleus indicates the level of transcriptive activity of rDNA, and heightened rDNA activity indicates increases in protein synthesis (Davis and Squire, 1984; Crocker and Nar, 1987; Dámaso et al., 1988; Lafarga et al., 1991; Underwood, 1992; González-Pardo et al., 1994). Vargas et al. (2000) showed that the size of the AgNORs in the neurons of the ventral part of the goldfish telencephalic lateral pallium (Dlv region) increased significantly and selectively after learning a spatial constancy task, compared with the same area of the brain in the animals of the control group (trained in a noncontingent procedure) or with

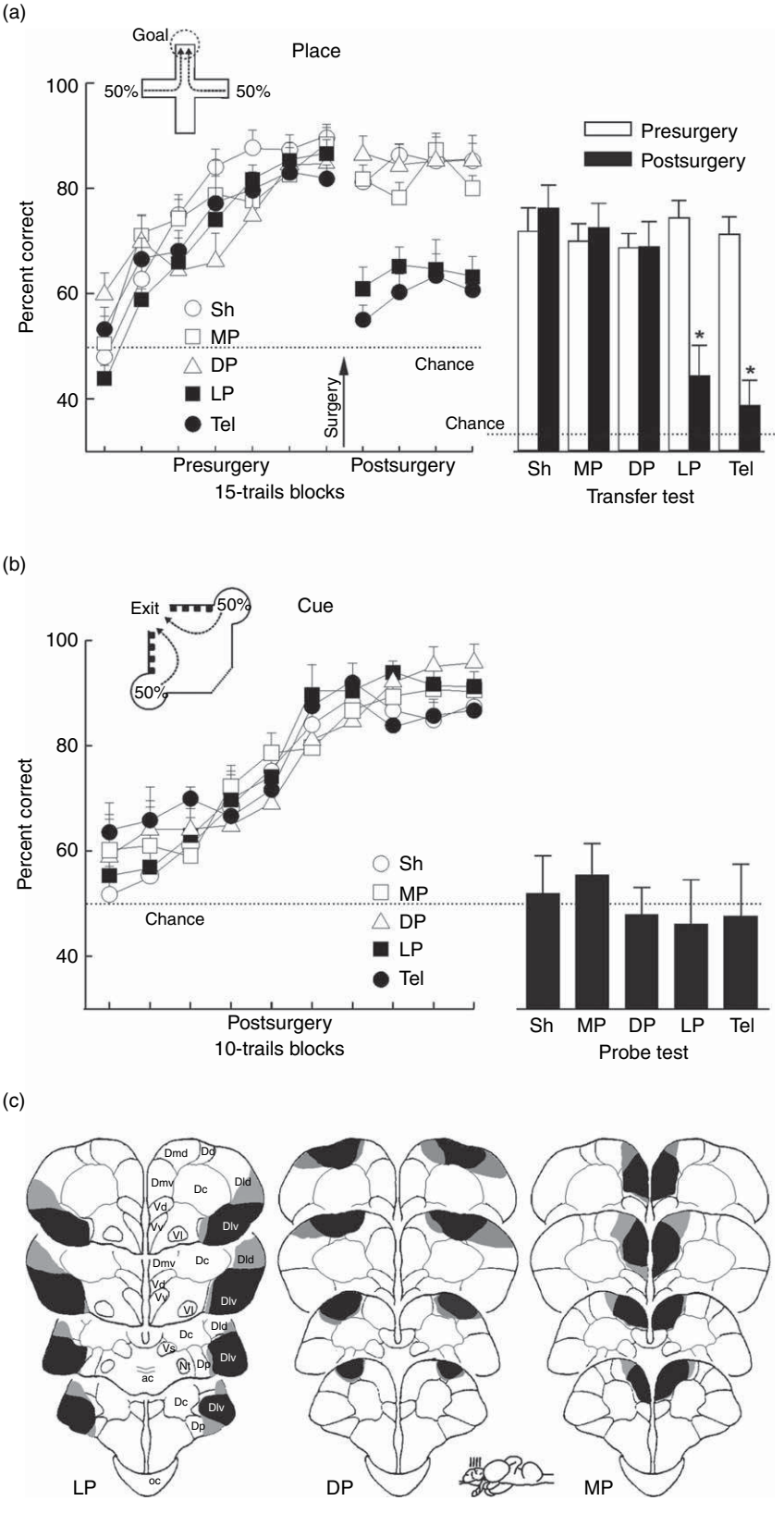
the neurons of the medial pallium of both groups (see **Figure 10**). An interesting result is also provided by Carneiro et al. (2001), who showed spatial cognition-related sex differences in the lateral pallium of Azorean rock-pool blennies (*Parablennius parvicornis*). In this species, males establish nest sites and remain in their nest area during the entire breeding season. In contrast, females need to move relatively long distances in order to visit different nests and spawn with males. Interestingly, Carneiro et al. (2001) found that female blennies have a larger lateral pallium compared to males. These authors related the greater size of the female blennies' lateral pallium with the increased spatial cognition demand on females, which need to displace greater distances than males and need to remember the nest location of different males.

Recently, Saito and Watanabe (2004, 2006) reported results apparently contradictory to this view, as they found that not lateral but, rather, medial pallium lesions produced spatial memory deficits in goldfish tested in a hole-board analog task. However, these experiments lack an adequate set of test trials to identify the spatial strategies used by the animals; therefore, the possible deficits observed in these experiments cannot have been clearly defined. In fact, the performance deficits observed in the medial pallium-lesioned animals in these studies could be better explained by nonspatial impairments, such as motivation, attention, and general activity alterations (Davis and Kassel, 1983; Riedel, 1998). Furthermore, the lateral pallium lesions in the experiment by Saito and Watanabe do not include the ventral portion of the lateral pallium but affect mostly the pallial region extending between the lateral and the medial pallium, which is most likely homologous with the dorsal cortex of land vertebrates (Northcutt, 1995; Pretchtl et al., 1998; Butler, 2000; Saidel et al., 2001; Northcutt, 2006; Yamamoto et al., 2007). In fact, it has not been possible to replicate the results of Saito and Watanabe in experiments when care was taken to lesion the medial pallium and the ventral part of lateral pallium selectively (Durán, 2004; Rodríguez et al., 2005). Durán (2004) trained lateral and medial pallium–lesioned goldfish to locate one baited feeder (goal) within a 25-feeder matrix surrounded by an array of intramaze visual cues, in a procedure analogous to the hole-board task used with rats and similar to the procedure and apparatus used by Saito and Watanabe. In this experiment, transfer and probe tests were performed to analyze carefully and identify the spatial strategies used by the animals. The results of this work are consistent with previous lesion studies and do not support Saito and Watanabe's conclusions. The lateral pallium–lesioned goldfish, as well as the medial pallium–lesioned and the control animals learned to solve the task with accuracy. However, the test trials when the different visual cues were independently removed showed that the lateral pallium–but not the medial pallium–lesioned fish suffered a spatial learning impairment. Thus, the lateral pallium–lesioned animals, but not the medial pallium–lesioned and the control fish, failed to reach the goal when the particular subset of visual cues situated in the proximity of the goal was excluded. These results reveal that the lateral pallium animals relied on a guidance strategy to solve the task (i.e., they learned to approach a particular visual cue that they associated with the goal), suggesting that these animals lack the capacity to encode the goal location relative to multiple environmental features in a map-like representation (place learning). The probe tests also showed that, in contrast, the medial pallium–lesioned goldfish are able to navigate readily to the goal independently of the removal of any particular subset of visual cues. These data clearly indicate that the lateral pallium of teleost fish, but not the medial pallium, provides the neural substratum for the ability of fish to use allocentric, relational representations of the environment.

1.26.10 Neural Mechanisms for Egocentrically Referenced Spatial Orientation

Fish spatial cognition also involves a variety of egocentrically referenced perceptive and motor mechanisms based on the function of different neural centers and circuits, for example, the reticulospinal and vestibular circuits, the cerebellum, and the optic tectum.

The optic tectum is a crucial center for the generation of egocentrically referenced actions in space. The anatomical and functional organization of the optic tectum (superior colliculus in mammals) appears to be quite well conserved in vertebrates (Vanegas, 1984; Stein and Meredith, 1993). The teleost optic tectum is a crucial center for sensorimotor integration and for the generation of egocentrically referenced actions in space. As in other vertebrates, the optic tectum of teleost fish presents a spatially ordered motor map in the deep tectal layers in correspondence with the retinotopic visual map in the superficial layers (Salas et al., 1997; Sparks, 2002). For example,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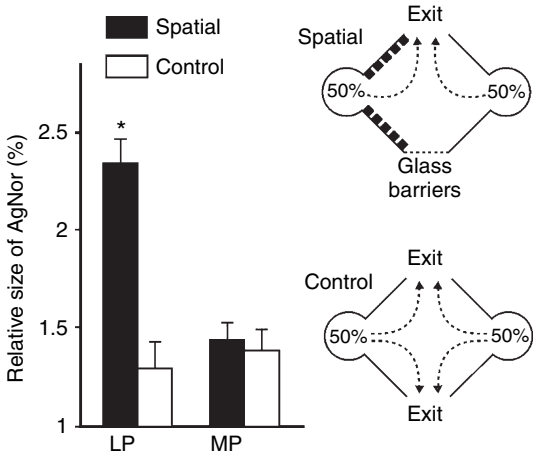

Figure 10 Spatial learning induced a significant increase in protein synthesis in the neurons of the lateral pallium of goldfish trained in a spatial task. The figure shows the size of the nucleolar organizing regions (NORs) relative to the size of the nucleus in lateral (LP) and medial (MP) pallium neurons of goldfish trained in a spatial or a control task as described in the diagrams. Asterisks denote significant differences. Modified from Vargas JP, Rodríguez F, López JC, Arias JL, and Salas C (2000) Spatial learning-induced increase in the argyrophilic nucleolar organizer region of dorsolateral telencephalic neurons in goldfish. *Brain Res.* 865: 77–84.

electrical microstimulation in the optic tectum elicited egocentrically referenced coordinated eye and body movements and postural adjustments in teleost fish (Meyer et al., 1970; Demski, 1983; Al-Akel et al., 1986; Salas et al., 1995, 1997; Herrero et al., 1998; see **Figure 11**). The optic tectum provides common body-centered frames of reference for multisensory integration and for sensorimotor transformation, based in their specialized intrinsic circuits and in their profuse connectivity with other motor and sensorial centers (Vanegas, 1984; Stein and Meredith, 1993; Salas et al., 1997), and probably participates in a neural interface for transforming the tectal information, coded in spatial coordinates, into a temporal signal in separate brainstem generators in the reticular premotor centers (Torres et al., 2002, 2005).

However, the distinction between only two main types of reference frames (egocentric vs. allocentric) is probably too restrictive. For example, both the egocentric and allocentric reference frames require a geocentric frame based on the invariant direction of the gravity forces (Trevarthen, 1968; Paillard, 1991; Berthoz, 1999). The vestibular information is essential in orientation and perceptual and motor stabilization, by providing an egocentric reference frame for orientation (Paillard, 1991), and is also likely to have a role in navigation based on inertial information (Mittelstaedt and Glasauer, 1991). In addition, different egocentrically referenced orientation mechanisms can interact to provide integrated behavioral outputs. For example, in the dorsal light response (DLR), the incident angle of the light, in addition to gravity, determines the postural position of fish (Holst, 1950). When the light angle of incidence is not completely vertical, fish assume a tilted posture, with the dorsal body area oriented somewhat toward the light source. The exact angle of deviation from the vertical position depends on light incidence angle and light intensity. Thus, this relatively simple reflex response involves an interaction between the visual and vestibular sensory inputs. Lesion studies in goldfish indicated that this response is abolished after lateral valvula cerebelli or pretectal nuclei lesions, but not after optic tectum lesions (Watanabe et al., 1989; Yanagihara et al., 1993b,c). The visual information is relayed to the cerebellum by the pretectal nuclei, which project to the valvula cerebelli directly, not via the optic tectum. In addition, the lateral valvula cerebelli receives vestibular and lateral line inputs indirectly, via the eminentia granularis and the medial nucleus of the octavolateralis column (Yanagihara et al., 1993a). As in land vertebrates, even these relatively simple and apparently mechanical fish reflexes are submitted to learning and memory modulation and undergo plastic modifications, adjustments, and recalibrations, with the cerebellum playing a central role in such plasticity (Löwenstein, 1932; Holst, 1935; Paul and Roberts,

Figure 9 Spatial memory deficits after lateral pallium lesion in goldfish. (a) Left, mean percentage of correct choices during pre- and postsurgery training in an arm-maze place task by goldfish with different pallial lesions or sham-operated goldfish. Right, mean percentage of correct choices during pre- and postsurgery transfer trials in which new start positions were used (see **Figure 2**). (b) Left, mean percentage of correct responses during postsurgery training in a cue task as described in **Figure 4**. Right, percentage of correct responses in the cue removal probe test. (c) Schematic representation of the largest (grey shading) and smallest (black shading) extent of the lateral (LP), dorsal (DP), and medial (MP) pallium lesions in goldfish, reconstructed in coronal sections. Asterisks denote significant differences. Modified from Rodríguez F, López JC, Vargas JP, Gómez Y, Broglio C, and Salas C (2002b) Conservation of spatial memory function in the pallial forebrain of amniotes and ray-finned fishes. *J. Neurosci.* 22: 2894–2903.

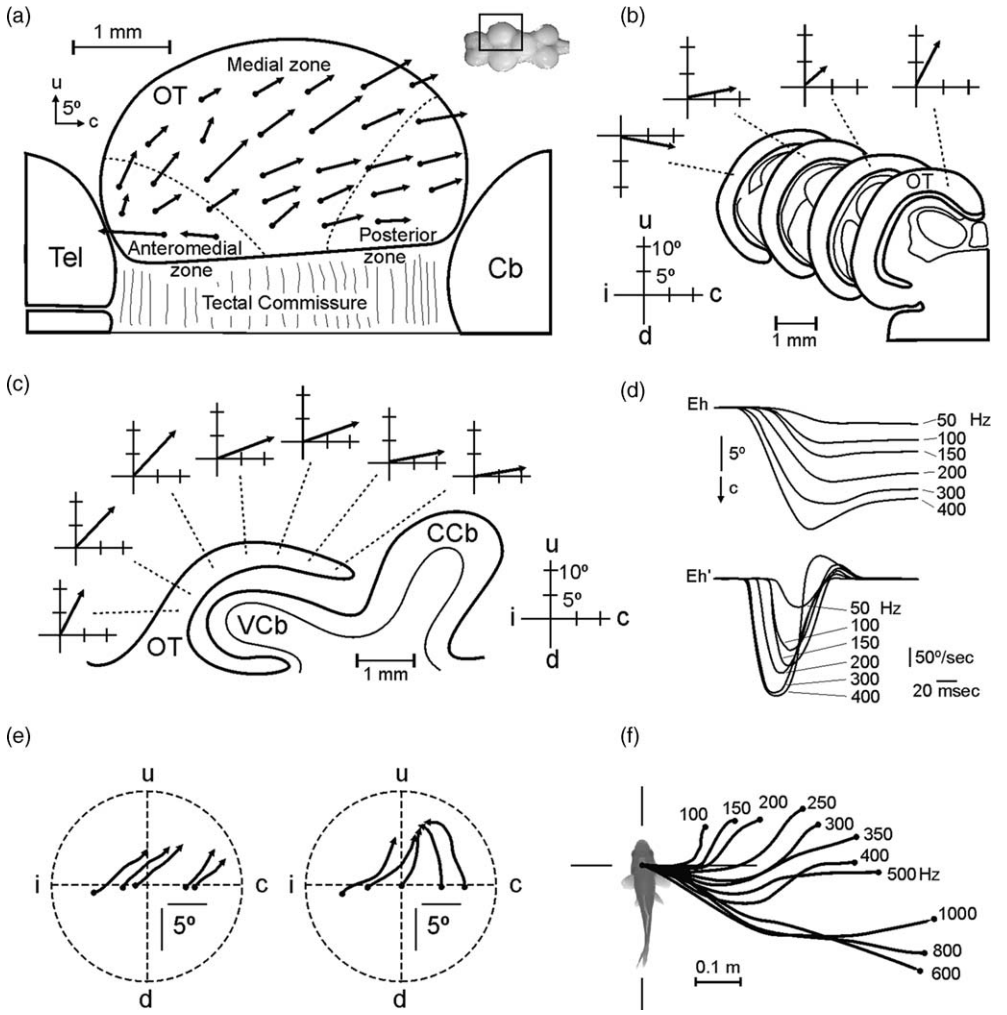

Figure 11 Focal electrical stimulation in the optic tectum of goldfish elicits coordinated eye and body movements, revealing that the optic tectum of teleost fish is a crucial center for the generation of egocentrically referenced actions in space. The amplitude and direction of eye movement vectors depend on the stimulation site within the tectum (a). Varying the stimulation site in the medial-lateral axis produces an increase in the vertical component (b), whereas varying the stimulation site in the rostrocaudal axis produces a systematic change in the amplitude of the horizontal component of the saccade (**c**). Varying the stimulation parameters (v.g. frequency) produces systematic changes in the metric and kinetic of the evoked orientation responses (d). Stimulating anatomically separated tectal areas evokes different types of eye movements (e). The electrical microstimulation of the optic tectum in free-swimming fish elicits body movements (f). Evoked movements consist of complete orientation responses including coordinated movements of the axial musculature, fins, and eyes, which closely resemble the natural responses. The direction and amplitude of the orienting responses depend on both the tectal stimulation site and the stimulus parameters. Abbreviations: Cb, cerebellum; CCb, corpus cerebellum; Eh, horizontal component of eye position; Eh′, eye velocity trace; OT, optic tectum; St, electrode for microstimulation; Tel, telencephalon; VCb, valvula cerebellum; d, u, i, c, downward, upward, ipsiversive, and contraversive direction of evoked eye saccade, respectively. Modified from Herrero L, Rodríguez F, Salas C, and Torres B (1998) Tail and eye movements evoked by electrical microstimulation of the optic tectum in goldfish. *Exp. Brain Res.* 120: 291–305; Salas C, Herrero L, Rodríguez F, and Torres B (1997) Tectal codification of eye movements in goldfish studied by electrical microstimulation. *Neuroscience* 78: 271–288.

1979; Burt and Flohr, 1988; Ott and Platt, 1988; Burt and Flohr, 1991; Pastor et al., 1994; Li et al., 1995; McElligott et al., 1998; Straka et al., 2006). However, like the mammalian cerebellum, the teleost fish cerebellum is likely not only an essential center for motor coordination and adjustment but is also involved in learning and memory and in spatial cognition. For example, the goldfish cerebellum is involved in eye blink–like classical conditioning (Rodríguez et al., 2005; Salas et al., 2006). In addition,

cerebellum lesions produce profound spatial cognition deficits in goldfish (Durán, 2004; Rodríguez et al., 2005, 2006). In this study, when goldfish were required to learn the location of a baited feeder within a 25-feeder matrix surrounded by a stable array of visual cues, the cerebellum-lesioned animals never reached the level of accuracy of the control and sham-operated animals. This could be due to the fact that their search pattern is stereotyped and inefficient. The results of test trials showed that the performance of the cerebellum-lesioned fish depends on approaching a particular subset of cues, suggesting that they are unable to use the entire array. These data indicate that these animals are impaired in their ability to generate or use map-like representations of the environment. Interestingly, the cerebellum lesions also impair orientation based on egocentric frames of reference. Durán (2004) showed that whereas telencephalic lesions disrupt goldfish performance in a spatial constancy task but spare cue orientation learning (see also Salas et al., 1996b), cerebellum lesions are equally disruptive in both tasks. These results indicate that the teleost cerebellum is also involved in the association of motor responses with single landmarks and in other egocentric orientation mechanisms. Remarkably, whereas the effects of cerebellum lesions in goldfish are profound and widespread in spatial learning, they do not produce observable sensorimotor impairments or deficits in posture, swimming ability, or obstacle avoidance, indicating that also in teleost fish the role of the cerebellum goes far beyond just motor-control modulation.

1.26.11 Concluding Remarks

The data presented here show that the complexity and plasticity of spatial behavior in fish parallels that of mammals and birds. Moreover, as in land vertebrates, spatial behavior in fish depends on a variety of learning and memory mechanisms and cognitive processes, supported by particular brain circuits. The fish's lateral pallium, likely homologous to the hippocampus of amniotes, is essential for simultaneously processing and encoding spatial information from multiple sources and forming map-like or relational representations of the environment. Also as in land vertebrates, other brain circuits, involving, for example, the optic tectum or the cerebellum, underlie egocentrically referenced spatial orientation. The notable similarity observed in the spatial cognition capabilities and their neural substrates in groups that diverged millions of years ago suggest that some features of these spatial learning and memory systems and their neural basis might be a primitive feature in vertebrates, conserved through evolution.

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1.27 Reconsolidation in Invertebrates

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1.27.1 Introduction

1.27.1.1 Memory Consolidation after Training and Retrieval

Formation of long-term memories (LTMs) can be disturbed during a discrete time window after learning with amnestic agents. It has been therefore concluded that LTMs undergo a labile phase of memory fixation, which is termed consolidation, and that an LTM, once consolidated, is stable (Dudai, 2004). Nevertheless, it has been known since the late 1960s that retrieving a memory in combination with the application of a consolidation inhibitor disturbs memory retention in a later memory test (Misanin et al., 1968; *See* Chapter 1.24) (**Figure 1(a)**). This observation of a retrieval-dependent amnesia first resulted in the theory that memories do not undergo a consolidation process but, rather, exist in an active or an inactive state (Lewis, 1979). In the active state, memories are vulnerable to inhibitors; in the inactive state, inhibitors have no effect. Memory retrieval transfers an inactive memory into an active memory, which makes the memories vulnerable again. Some years ago Nader and colleagues (2000) took on the issue of retrieval-dependent amnesia. They interpreted their findings in combining the consolidation theory with the hypothesis of active and

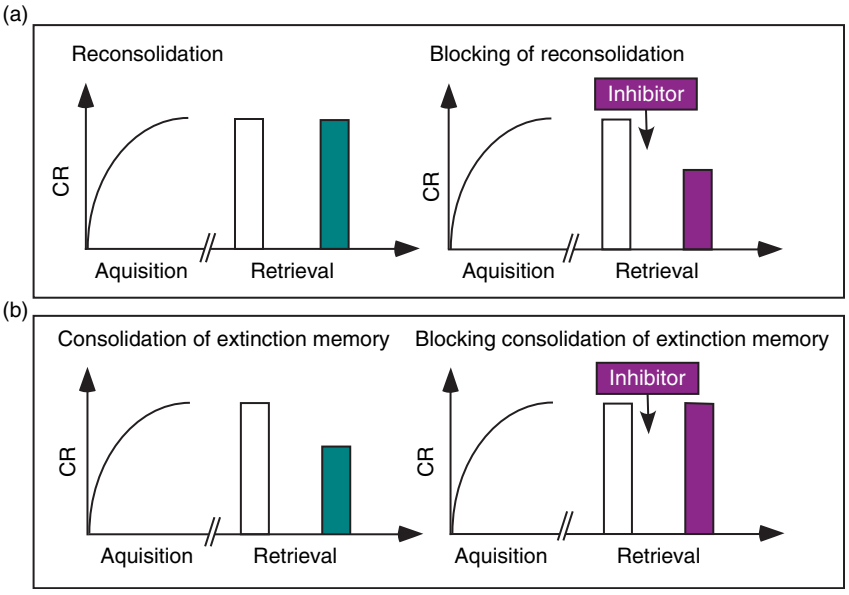

Figure 1 Consolidation processes following memory retrieval. Schematic diagram of the behavioral outcome of consolidation processes following memory retrieval. (a) Reconsolidation. The combination of memory retrieval and the application of an inhibitor of consolidation leads to a decrease of the conditioned response (CR) (right) in comparison to animals that are retrieved but not treated with the inhibitor (left). (b) Consolidation of an extinction memory. The CR is decreased after memory retrieval (left). This reduction of the CR is blocked by application of an inhibitor of consolidation after the first memory retrieval. Blocking is visible at the second retrieval trial (which is a retention test for the memory following memory retrieval) (right).

inactive memories (Nader, 2003). They proposed that retrieval makes the consolidated memory labile and that a second consolidation round is required to stabilize the retrieved memory. This second round of consolidation is targeted by the inhibitor, resulting in a decrease of the conditioned response (CR) at a later memory test. Accordingly, the process in question is called reconsolidation (Spear, 1973; Przybyslawski and Sara, 1997; Sara, 2000; Nader, 2003; Dudai and Eisenberg, 2004; *See also* Chapter 1.24). Meanwhile, an extensive study began of the neuronal and molecular basis of reconsolidation in vertebrates and invertebrates, and it is under dispute whether this hypothesis holds true. Several other hypotheses have been put forward to explain this phenomenon either based on the consolidation theory (Dudai and Eisenberg, 2004; Alberini, 2005; Eisenhardt and Menzel, 2007) or based on the assumption that the retrieval rather then the memory itself is affected (Riccio et al., 2002).

Sometimes memory retrieval leads to a decrease of the CR. This phenomenon is termed extinction (Pavlov, 1927). When a consolidation inhibitor like a protein synthesis inhibitor is applied around the time point of memory extinction, the CR increases in a later memory test (**Figure 1(b)**). It has therefore been concluded that the extinction memory undergoes a consolidation process that is blocked by the application of the inhibitor (Berman and Dudai, 2001; Vianna et al., 2001, 2003; Pedreira and Maldonado, 2003; Power et al., 2006). Accordingly, retrieving a memory induces two processes that result in contrasting behavioral phenomena – reconsolidation and consolidation of an extinction memory.

Meanwhile, reconsolidation has been described in several invertebrate model organisms, namely, four gastropod species, *Hermissenda* (Child et al., 2003), *Lymnaea stagnalis* (Sangha et al., 2003a; Kemenes et al., 2006), *Helix lucorum* (Gainutdinova et al., 2005), and *Limax flavus* (Sekiguchi et al., 1997); the crab, *Chasmagnathus granulatus* (Pedreira et al., 2002); and the honeybee, *Apis mellifera* (Stollhoff et al., 2005). Findings in each of these organisms contributed to the study of the reconsolidation phenomenon. Many of the results known from vertebrates have also been found in invertebrates, demonstrating generalities and differences in the reconsolidation phenomenon and its underlying mechanisms. To illustrate the generalities and differences, we present experiments on reconsolidation in gastropods, the crab *Chasmagnathus*, and the honeybee, *Apis mellifera.*

1.27.2 Studies on Reconsolidation in Invertebrates

1.27.2.1 The Terrestrial Slug *Limax flavus*

1.27.2.1.1 Memory for aversive odor-taste conditioning is cooling sensitive

The slug *Limax flavus* (**Figure 2(a)**) is native to Europe but has been imported throughout the world. To study memory formation in *Limax flavus*, an odor-avoiding paradigm is used (Yamada et al., 1992). A starved animal is placed in a box lined with carrot juice–moistened filter paper. Carrot juice is used as the CS. After a 2-min exposure, the animal is immediately transferred to another box with bitter-tasting quinidine sulfate, which presents the unconditioned stimulus (US). For memory testing a slug is placed into the center of a three-chambered apparatus (**Figure 2(b)**). The walls of the center chamber are perforated to allow odor sensing. The two side chambers contain moistened filter-paper with the trained carrot juice or frog chow, which is normally fed to the snails.

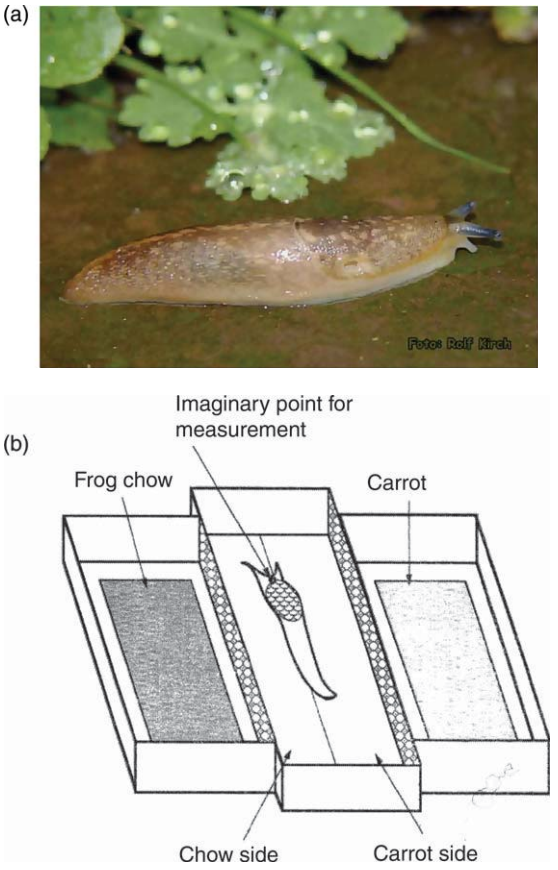

Figure 2 The slug *Limax flavus*: The odor-avoidance paradigm. (a) The yellow garden slug *Limax flavus*. Photo provided by Rolf Kirch. (b) The three-part test chamber: Carrot and frog chow are placed in opposite side chambers. The walls of the center chamber are perforated. A center line divides the room into a chow side and a carrot side. Individual slugs, marked with a dot on the head, which is used for measurement, are placed into the center chamber; the time the slug's head spent on the carrot side is recorded during three testing trials. Adapted from Figure 1 in Yamada A, Sekiguchi T, Suzuki H, and Mizukami A (1992) Behavioral analysis of internal memory states using cooling-induced retrograde amnesia in *Limax flavus*. *J. Neurosci.* 12: 729–735.

After a single CS–US pairing, slugs show an avoidance behavior for the trained carrot odor. Cooling the animal within 1 min after training induces retrograde amnesia, but later cooling has no effect (Yamada et al., 1992).

1.27.2.1.2 Reconsolidation in Limax flavus

The first study that found the reconsolidation phenomenon in an invertebrate was done in *Limax flavus*. In this study the authors focused on the temporal evolution of a memory (Sekiguchi et al., 1997). Although the authors did not term their findings reconsolidation, they revealed retrieval-dependent amnesia when trained snails were exposed to the CS and were cooled immediately afterward.

The combination of memory retrieval and cooling was applied at different time points after training, and the resulting memory was tested 1 day later. It turned out that by memory retrieval, a cooling-sensitive process can be induced until 3 days after training (**Figure 3**). After 3 days the memory becomes insensitive to retrieval-dependent amnesia. Nevertheless, when an additional CS–US pairing was presented before combining memory retrieval and cooling, a retrieval-dependent amnesia occurred even though the initial training had been applied more than 3 days before (**Figure 4**).

Interestingly, this induced susceptibility for retrieval-dependent amnesia followed the same temporal gradient as occurred after initial training, and it was supposed that the additional CS–US pairing results in a new memory with the same temporal gradient as the initial memory. To test this, a second-order conditioning trial, where a CS (CS 2) is paired with the formerly reinforced CS (CS 1), was presented instead of the additional CS–US pairing. The presentation of the CS1 in combination with cooling after the second-order conditioning resulted in a retrieval-dependent amnesia for the initial memory. But the presentation of the CS 2 in combination with cooling leads to retrieval-dependent amnesia for the initial memory and the

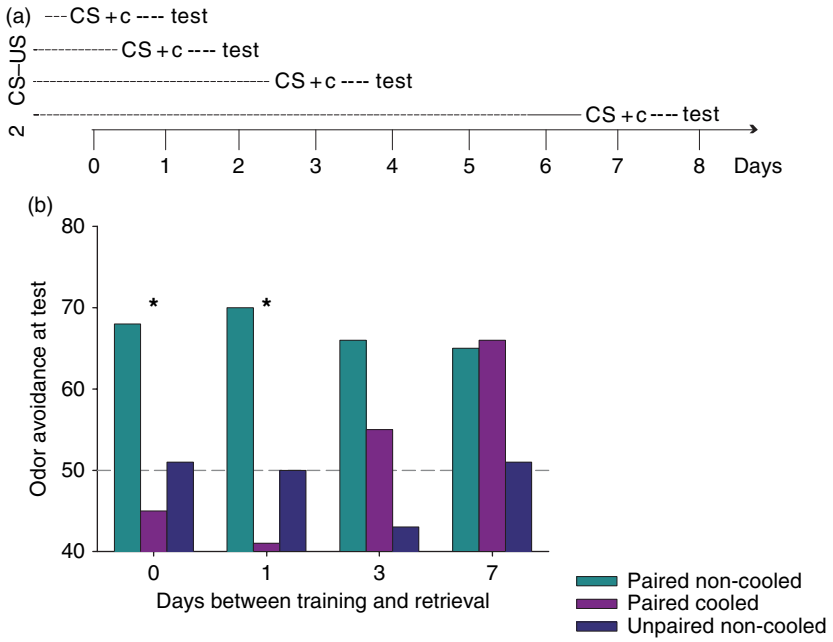

Figure 3 The slug *Limax flavus*: Retrieval-induced amnesia is dependent on the age of the memory (a) experimental schedule: Animals were trained to avoid an odor on day 0 by pairing the odor (conditioned stimulus, CS) with quinidine sulfate (unconditioned stimulus, US). Animals were divided into four experimental groups. For each group the memory was retrieved once by CS presentation at different time points (on day 0, 1, 3, or 7) followed immediately by a cooling procedure (CS + c). The slugs were tested 24 hours later in the three-chambered apparatus. Their odor avoidance behavior in the test was measured; the means are presented in (b) (paired cooled, violet bars). An additional group of slugs was trained, but not retrieved and cooled (paired noncooled, green bars). A second control group received unpaired presentation of the CS–US on day 0, but was not retrieved and cooled (unpaired noncooled, blue bars). The odor avoidance behavior of the experimental group (violet bars) is significantly decreased (indicated by an asterisk) in comparison to the trained noncooled group if the memory was retrieved > 3 days after training. Afterward, the reconsolidation phenomenon is not visible. The gray dashed line at 50% indicates no odor preference. Adapted from Figure 1 of Sekiguchi T, Yamada A, and Suzuki H (1997) Reactivation-dependent changes in memory states in the terrestrial slug *Limax flavus*. *Learn. Mem.* 4: 356–364.

second-order conditioned memory. Sekiguchi et al. (1997) concluded that the CS 1 in the second-order conditioning trial activates the initial memory. Accordingly, an additional CS–US pairing should also activate the initial memory rather than resulting in the formation of a new memory. According to this conclusion, Sekiguchi et al. (1997) stated that a memory becomes inactive after it matures and can no longer be inhibited by cooling. When the memory is retrieved, it becomes active again but might still be insensitive to cooling, depending on its age. Only additional CS–US pairings or second-order conditioning trials activate the initial memory that was insensitive to cooling, pushing it from a cooling-insensitive state back to a cooling-sensitive state. This model is based on Lewis's theory of activated and inactivated memories (1979) (see section titled "Memory Consolidation after Training and Retrieval") but extends beyond it. In contrast to Lewis (1979), Sekiguchi et al. (1997) posed an active and an inactive memory state, but in addition, showed a cooling-sensitive and a cooling-insensitive state. Accordingly, although memories are retrieved and are thus activated, they are not necessarily cooling sensitive. Only additional CS–US pairing or second-order conditioning leads to a memory that gets cooling sensitive by retrieval 24 h later. Interestingly, the findings by Sekiguchi et al. (1997) cannot be easily explained by the reconsolidation theory (Nader, 2003). The reconsolidation theory proposes a direct reactivation of a consolidated memory by a retrieval trial. Here, instead, the additional CS–US pairing enables a memory to be reactivated 24 h later.

1.27.2.2 The Pond Snail *Lymnaea stagnalis*

To study the reconsolidation phenomenon in the snail *Lymnaea stagnalis*, two learning paradigms have been used: the operant aerial respiration paradigm and the classical chemosensory conditioning paradigm.

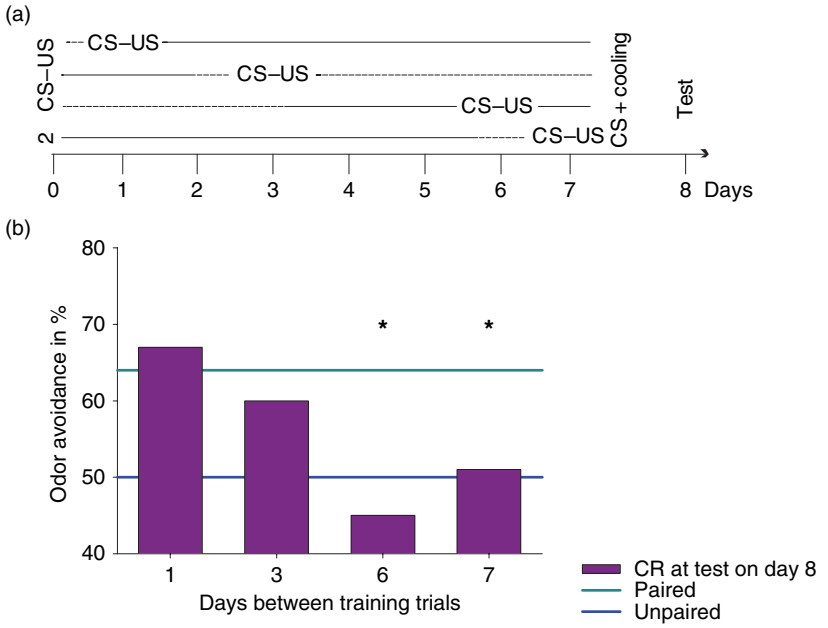

Figure 4 The slug *Limax flavus*: Additional training reactivates the memory. (a) Experimental schedule. Slugs received two conditioned stimulus (CS)–unconditioned stimulus (US) pairings on day 0. Afterward the animals were divided into four subgroups. Additional CS–US pairings were applied at varied time points (1, 3, 6, 7 days) after training. Memory was retrieved by CS presentation on day 7, and slugs were immediately cooled afterward. (b) Memory avoidance behavior was tested on day 8. Retrieval-induced amnesia is only detectable if an additional training was applied on day 6 and day 7. Significant difference in response between experimental group (bars) and paired control group are indicated by asterisks. The means of paired and unpaired control groups are presented as a blue or a green line, respectively. Adapted from Figure 2 in Sekiguchi T, Yamada A, and Suzuki H (1997) Reactivation-dependent changes in memory states in the terrestrial slug *Limax flavus*. *Learn. Mem.* 4: 356–364.

1.27.2.2.1 Lymnaea stagnalis: *The operant aerial respiration paradigm*

Lymnaea stagnalis is an aquatic pulmonate snail (**Figure 5**). It is a bimodal breather and can breathe via its skin (cutaneous respiration) or through a simple lung (aerial respiration). When the animal stays in stagnant water where the oxygen content is low, it becomes hypoxic. Then the snail comes to the water surface for aerial respiration. It opens and closes its respiratory orifice, the pneumostome, and breathes through the lung (**Figure 6(a)**). This behavior by the snail is used in the operant aerial respiration paradigm (Lukowiak et al., 1996). Here the snails are put in beakers of water, which is made hypoxic by bubbling N_2 through it. When the animal attempts to open its pneumostome as a reaction to the hypoxic water, it receives a gentle tactile stimulus to the pneumostome area, reducing its aerial respiration, without affecting cutaneous respiration. The number of openings is recorded during training periods and retention tests. Learning takes place if the number of attempted pneumostome openings is significantly decreased between the first and the last training trial. It is important to note that in this paradigm memory retrieval and retention tests consist of the same procedure as the training sessions. They are only designated differently for the reader's convenience.

Figure 5 The snail *Lymnaea stagnalis* The snail *Lymnaea stagnalis* sinks from the water surface to the ground of the pond. Photo by Kathrin Spöcker.

1.27.2.2.2 A long-term memory for the tactile stimulus is already formed after 4 h

Memory for the tactile stimulation of the pneumostome is defined by two criteria: (1) the number of

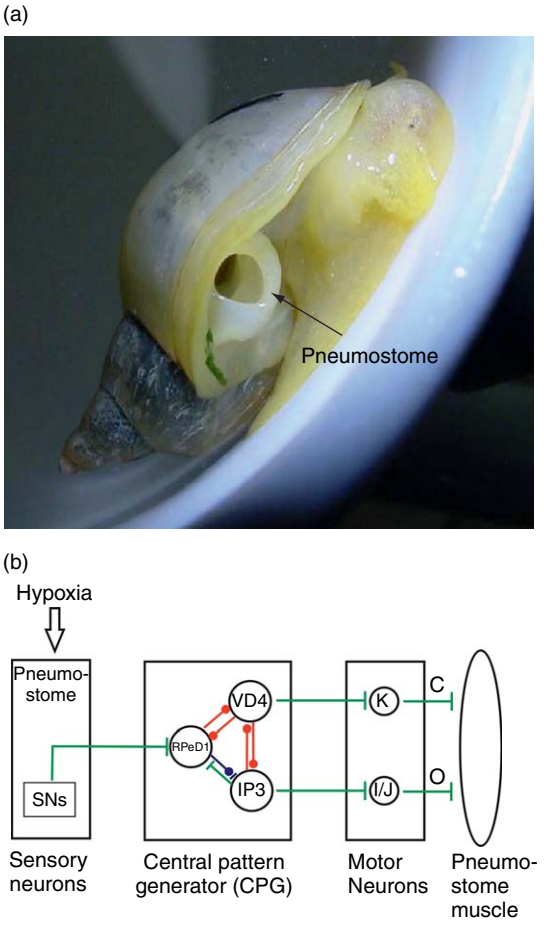

Figure 6 The snail *Lymnaea stagnalis*: Neuronal network underlying respiratory behavior. (a) *Lymnaea stagnalis* with opened pneumostome (arrow). From Lukowiak K, Sangha S, Scheibenstock A, et al. (2003) A molluscan model system in the search for the engram. *J. Physiol.* 69–76. (b) Schematic drawing of the central pattern generator (CPG). A chemosensory stimulus (here hypoxia) activates sensory neurons (SNs) in the pneumostome area, which in turn provide excitatory input (green line) to the right pedal dorsal 1 interneuron (RPeD1). Once stimulated, RPeD1 activates the input3 interneuron (IP3) via a biphasic effect (inhibition followed by excitation) (blue line) and inhibits visceral dorsal 4 interneuron (VD4) (red line). IP3 in turn excites both RPeD1 and the I/J motor neurons involved in pneumostome openings (O). IP3 also produces an inhibitory effect on VD4, and after release from this inhibition, VD4 fires, resulting in pneumostome closure (C). Tactile stimulation of the pneumostome area evokes closure of the pneumostome, and the aerial respiratory behavior stops. Adapted from Figure 1 in Sangha S, Varshney N, Fras M, et al. (2004) Memory, reconsolidation and extinction in *Lymnaea* require the soma of RPeD1. *Adv. Exp. Med. Biol.* 551: 311–318. Syed NI Winlow W (1991) Coordination of locomotor and cardiorespiratory networks of *Lymnaea stagnalis* by a pair of identified interneurones. J. Exp. Biol. 158: 37–62.

pneumostome openings is significantly reduced between the memory test and the first training session, and (2) the number of pneumostome openings at the memory test is not significantly different from the last training session (Lukowiak et al., 1996). Memory persists for at least 4 weeks if a spaced training protocol is used (Lukowiak et al., 1998). Within 1 h after the last training session, the consolidation process is susceptible for amnesic treatment. Memory that lasts beyond 4 h is considered long-term memory (LTM) because its consolidation depends on protein synthesis and RNA synthesis (Sangha et al., 2003b).

1.27.2.2.3 The neuronal network underlying the aerial respiration paradigm

One advantage of the aerial respiration paradigm is that the circuit of neurons controlling this behavior is known. Rhythmic behaviors like respiration and feeding are often controlled by neurons known as central pattern generators (CPGs). The respiratory CPG in *Lymnaea* consists of three neurons, named right pedal dorsal 1 (RPeD1), input 3 (IP3), and visceraldorsal 4 (VD4) interneurons. The latter two provide synaptic inputs to identified motor neurons, which mediate the opening movement (expiration) and the closing movement (inspiration) of the pneumostome (Syed et al., 1991; Syed and Winlow, 1991). The third neuron, RPeD1, receives excitatory chemosensory and mechanosensory input from the pneumostome area, which in turn initiates the CPG activity, and hence the respiratory behavior (**Figure 6(b)**).

Procedural memories are thought to be stored within the same network that mediates behavior (Milner et al., 1998), and therefore learning-induced changes were believed to be stored in the CPG. Indeed, neural correlates of learning and memory have been found in RPeD1 (Spencer et al., 1999). Removing the soma of RPeD1 by poking it gently with a glass microelectrode does not alter aerial respiratory behavior in a hypoxic environment, and the remaining neurite is electrophysiologically functional for at least 10 days after ablation (Scheibenstock et al., 2002). This preparation allows analyzing the function of RPeD1 in LTM formation in the aerial respiration paradigm. Snails with a soma-less RPeD1 are able to learn, but an LTM is not formed. Nevertheless, when RPeD1's soma is ablated after LTM consolidation has occurred, LTM can still be accessed. Accordingly, the soma of RPeD1 is a site of LTM consolidation but is not needed for memory retrieval (Scheibenstock et al., 2002).

1.27.2.2.4 Reconsolidation in the aerial respiration paradigm

To test for a possible reconsolidation process, snails are cooled in 4°C cold water immediately after memory retrieval. The memory of the control group is not retrieved, but the animal is also cooled. Memory retention is tested 4 hours after memory retrieval. The retrieved and cooled animals do not demonstrate memory. In contrast, the control group, which was cooled but was not retrieved, shows a memory for the tactile stimulus. Therefore, the reconsolidation process induced by memory retrieval is disturbed by the cooling procedure (Sangha et al., 2003a).

Similar results are obtained with the systemic application of the RNA synthesis-inhibitor, actinomycin D, after memory retrieval (**Figure 7(a)**). Only animals receiving the retrieval in conjunction with the actinomycin D treatment do not show memory in a test 4 hours later. Comparable to the results with this RNA inhibitor, RPeD1 soma ablation after retrieval blocks memory retention at the later test (**Figure 7(b)**). The memory remains intact in RPeD1 soma-ablated snails that were not retrieved before the ablation. Accordingly, the soma of RPeD1 is required for reconsolidation.

These experiments demonstrate that a single neuron is necessary for consolidation and reconsolidation. Accordingly, in *Lymnaea* both processes take place not only in the same structure but also in the same neuron (Sangha et al., 2003a, 2004). Interestingly, vertebrate studies on this issue reach contradicting results. Some demonstrate that consolidation and

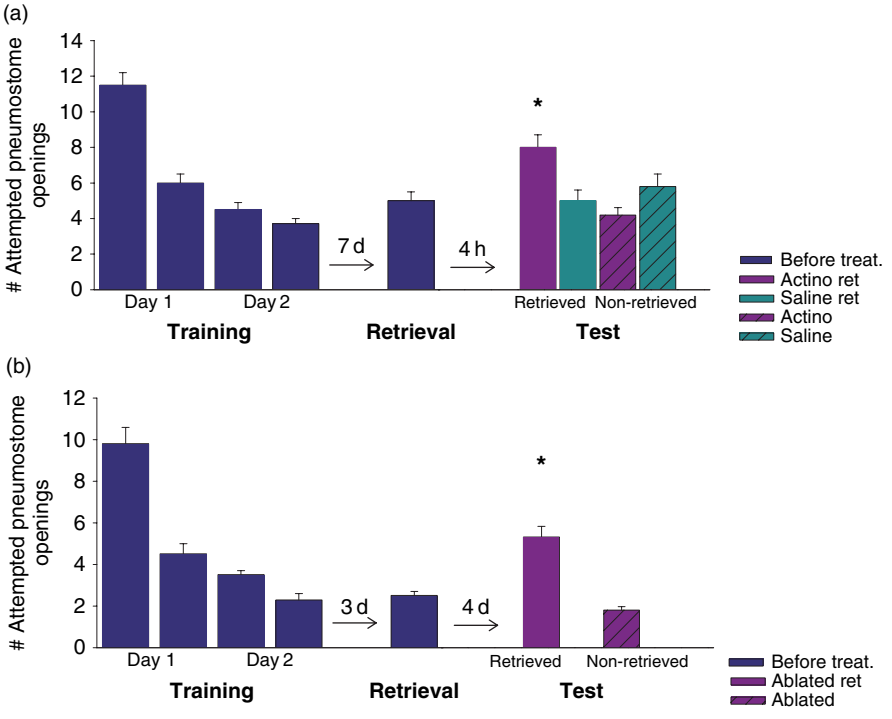

Figure 7 *Lymnaea stagnalis*: Reconsolidation is RNA syntheses dependent and takes place in a single neuron. (a) Training consisted of 4 45-min sessions in N_2-enriched hypoxic water. Sessions were dispersed over 2 days. On a single day the intertraining interval was 1 h. The memory was retrieved 7 days after training, and snails were injected with actinomycin D (Actino ret, violet bar) or saline (Saline ret, green bar) immediately after retrieval. The control groups (striped bars) received the injection (Actino ret, striped violet bar; Saline, striped green bar), but not the retrieval session. Memory retention was tested 4 h later. The retrieved and actinomycin D–injected animals (Actino ret) did not demonstrate memory. In contrast, the control group, which received the actinomycin D but was not retrieved, showed a memory for the conditioned response. (b) Operant training was administered as described above. Three days later half of the animals received the retrieval session immediately followed by the ablation of the soma of RPeD1 (Ablated ret, violet bar), whereas the other half was not reminded but underwent the ablation procedure (Ablated, striped violet bar). Animals were tested 4 days later. Memory was not observed in retrieved and ablated snails, whereas ablated snails that did not receive the retrieval procedure showed memory. Adapted from Figures 3 and 4 in Sangha S, Scheibenstock A, and Lukowiak K (2003a) Reconsolidation of a long-term memory in *Lymnaea* requires new protein and RNA synthesis and the soma of right pedal dorsal 1. *J. Neurosci.* 23: 8034–8040.

reconsolidation take place in different brain structures (Tronel and Sara, 2002; Salinska et al., 2004), whereas others find that reconsolidation can be disrupted with protein synthesis inhibitors targeting the same brain structure as in consolidation experiments (Rose and Benjamin, 1979; Nader et al., 2000; Debiec et al., 2002; Koh and Bernstein, 2003).

1.27.2.2.5 Lymnaea stagnalis: *The classical chemosensory conditioning paradigm*

The second paradigm used in *Lymnaea* is an appetitive classical conditioning based on its feeding behavior. Feeding behavior consists of a series of rasps that can be divided into three phases: (1) protraction phase, in which the animal opens the mouth and the radula, a toothed chitinous tissue, is extended to contact the food; (2) retraction phase, when the food substrate is grazed and lifted into the mouth; and (3) the swallow phase, during which the mouth is closed and the food is swallowed (Rose and Benjamin, 1979; Benjamin et al., 2000; Elliott and Susswein, 2002). Similar to the respiratory behavior described earlier, a known network driven by a CPG controls this behavior (Benjamin et al., 2000: *See also* Chapter 1.30).

Using sucrose as a stimulus increases the feeding behavior in terms of the number of rasps (Kemenes et al., 1986; *See also* Chapter 4.09). Therefore, sucrose is used as a US in an appetitive classical conditioning paradigm. Here food-deprived snails are put into a Petri dish with water and are allowed to acclimatize. Amyl acetate, used as the CS, and the sucrose solution are added to the Petri dish, one after another and the animals are exposed to the CS–US mixture for 10 s. Afterward, the trained snails are transferred to their home tanks. To test the taste memory, the animals are put back into the Petri dish, the CS (amyl acetate) is added after a resting period, and the number of rasps is counted. Following a single paired CS–US presentation, animals show significant higher feeding response to amyl acetate compared with their own naïve response and with that of control groups (unpaired, CS alone, US alone) (Fulton et al., 2005).

1.27.2.2.6 *A consolidated long-term memory in the appetitive chemosensory conditioning paradigm is formed after 5 hours*

An LTM is formed about the amyl acetate-sucrose association that lasts at least 19 days (Alexander et al., 1984). Applying the widely used protein synthesis-inhibitor anisomycin at different time points after single-trial training demonstrates a protein-dependent consolidation phase 10 min after training, whereas later tested time points (1–6 hours) no longer reveal any sensitivity to the inhibitor. An impairment of memory retention can be seen already 5 h after training and injection. Therefore, memory retention 5 h after learning is protein synthesis dependent (Fulton et al., 2005).

1.27.2.2.7 *Reconsolidation in the appetitive chemosensory conditioning paradigm*

A study by Kemenes et al. (2006) asked if the age of a memory has an influence on the molecular mechanisms that underlie a reconsolidation process. This study distinguishes between fresh and old memories. Protein synthesis shortly after conditioning (between 10 min and 1 h) is required for the formation for a memory expressed more than 5 h after training (Fulton et al., 2005). Therefore, a 6-h memory was considered to be a freshly consolidated memory, whereas a 24-h memory was regarded as an old consolidated memory. To demonstrate the reconsolidation phenomenon, the animals receive a CS presentation to retrieve the memory 6 or 24 h after conditioning, followed by an injection of the protein synthesis inhibitor anisomycin. At a retention test 18 h after the injection, the CR (number of rasps) of the anisomycin-injected and retrieved animals was reduced in comparison to the control groups. This demonstrates that protein synthesis is required for reconsolidation when a 6-h or a 24-h memory is retrieved (Kemenes et al., 2006).

A follow-up experiment directly measured the retrieval-induced activation of cAMP-dependent protein kinase A (PKA) in the cerebral ganglia. The cerebral ganglia were chosen because they are known to be a site for neuronal plasticity (Straub et al., 2004). Again the study differentiated between fresh and old memories (Kemenes et al., 2006). Interestingly, the PKA activity was increased in the group with trained animals subjected to a CS presentation 6 h after training in comparison to nonretrieved or unpaired trained control groups. This retrieval-induced increase of PKA activity was not found if the memory was retrieved 24 h after CS–US pairing. A PKA inhibitor was used to verify that retrieval-induced PKA activity is dependent on the age of the memory. Animals showed a reduced CR at the retention test if a freshly consolidated memory was retrieved followed by an injection of the PKA inhibitor. If the memory was

retrieved 24 h after training, the application of the PKA inhibitor did not lead to a reduced CR (**Figure 8**).

This confirmed the biochemical data. Accordingly, retrieval of a consolidated memory induces a protein synthesis–dependent reconsolidation process, but only reconsolidation of freshly consolidated memories requires the activation of PKA. It can be concluded that the molecular requirements for reconsolidation depend on the age or the maturation of the retrieved memory (Kemenes et al., 2006).

Reconsolidation's dependency on the age of the retrieved memory has been reported before, although on a different timescale. Rats trained in an inhibitory avoidance task, show the reconsolidation phenomenon only when a recent memory has been retrieved in combination with the application of a protein synthesis-inhibitor. When the memory for the inhibitory avoidance task is 14 days old or older, a decrease of the CR can no longer be observed after combining memory retrieval with a systemic application of a protein synthesis inhibitor (Milekic and Alberini, 2002). Similar results have been demonstrated by other authors in several paradigms in vertebrates (e.g., Eisenberg and Dudai, 2004; Suzuki et al., 2004; Frankland et al., 2006; Power et al., 2006). Nevertheless, contrasting findings have also been reported (Nader et al., 2000; Debiec et al., 2002). Debiec et al. (2002) demonstrated in rats that retrieval of a 45-day-old memory for contextual fear conditioning, in combination with protein synthesis inhibition in the hippocampus, still results in the reconsolidation phenomenon (Debiec et al., 2002). Taken together the reason for different temporal requirements for reconsolidation remains unknown.

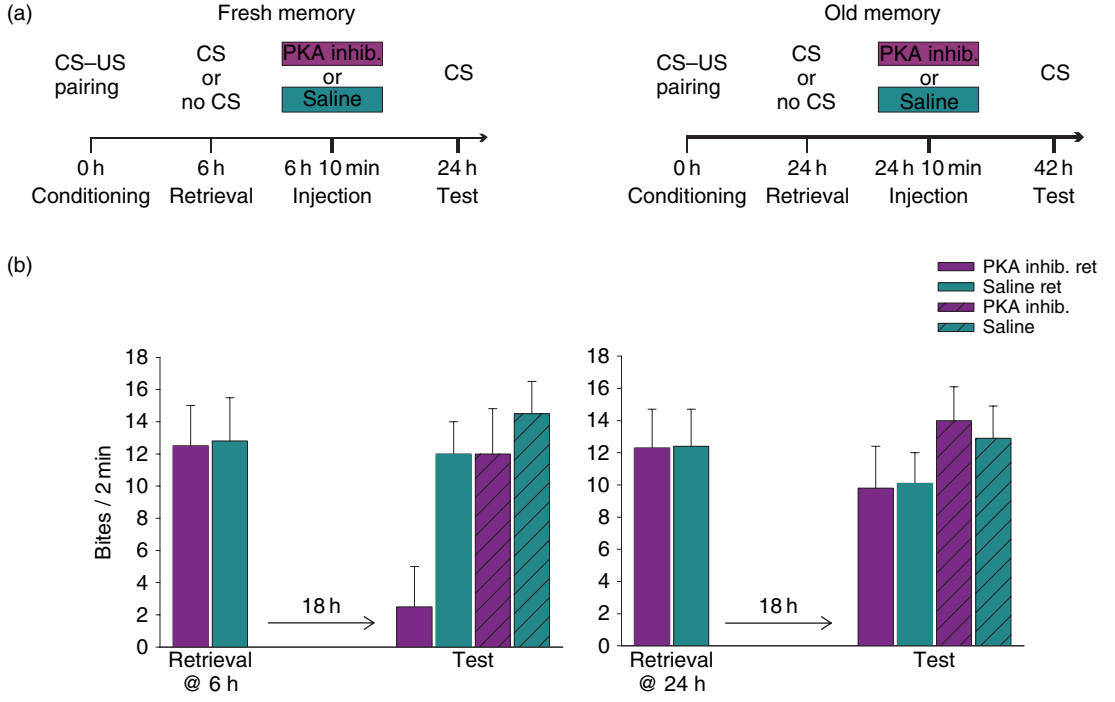

Figure 8 *Lymnaea stagnalis*: Reconsolidation in an appetitive conditioning paradigm. (a) The experimental scheme. Food-deprived snails were put in a Petri dish with water. Amyl acetate, as conditioned stimulus (CS), was added to a final concentration of 0.004%. After 15 s, a the sucrose solution was added and the animals remained in this CS + unconditioned stimulus (US) mixture for an additional 105 s. After this single CS–US pairing, the animals were rinsed. Animals were divided into two groups, and half of the animals received a CS presentation to retrieve the memory 6 h (fresh memory, left panel) or 24 (old memory, right panel) hours after conditioning. The other half received no CS stimulation. Ten minutes later, all animals were injected with PKA inhibitor (violet) or saline (green). All animals were tested 18 h after injection for their response to the CS in a 2-min test. (b) Animals that were retrieved at 6 h after training followed by an injection of the PKA inhibitor (PKA Inhib. ret, violet bars) show reduced conditioned response (number of rasps) during testing in comparison to retrieved and saline-injected (Saline ret, green bars) animals or to the nonretrieved groups (striped bars) (left panel). This significant reduction of CR of the retrieved and inhibited group is not observable if the memory is retrieved 24 h after training (right panel). Adapted from Figures 1 and 3 in Kemenes G, Kemenes I, Michel M, Papp A, and Muller U (2006) Phase-dependent molecular requirements for memory reconsolidation: Differential roles for protein synthesis and protein kinase A activity. *J. Neurosci.* 26: 6298–6302.

But the study in *Lymnaea stagnalis* illustrates that differences in the molecular events induced by memory retrieval might play a role.

1.27.2.3 The Terrestrial Snail *Helix lucorum*

1.27.2.3.1 Context memory in Helix lucorum *is protein synthesis dependent*

Helix lucorum is an edible snail, sometimes called escargot turc (**Figure 9(a)**). It is found in central Italy, from Yugoslavia through the Crimea to Turkey, and around the Black Sea. *Helix lucorum* is trained in an aversive conditioning paradigm, where it learns to associate the context, a rotating ball, with an electric shock (Gainutdinova et al., 2005). To do so, the animals receive an electric shock to the dorsal surface of their foot after they are fixed at their shell and placed on a ball, which rotates in water (**Figure 9(b)**). To test for the acquired context memory, the animals are placed onto the ball (the reinforced context) again or on a flat glass plate (the nonreinforced context), and a tactile stimulus is applied to the skin in both contexts. The defensive tentacle withdrawal amplitude is measured. A significantly different withdrawal amplitude between the reinforced and the nonreinforced context indicates an associative memory for the context.

To study memory consolidation, animals are trained over 5 days and receive an injection of anisomycin or saline every day after the first shock. Memory is tested 2 days after the last training day (**Figure 10**). Saline-injected snails show a significantly higher response to tactile stimulation in the trained context compared to the nonreinforced context. Therefore, the animals are able to differentiate between the context in which they get a sensitizing shock and the control context. Anisomycin-injected snails show no significant difference in the withdrawal amplitude between the two contexts. This indicates that the formation of a context memory depends on protein synthesis (Gainutdinova et al., 2005).

1.27.2.3.2 Reconsolidation in Helix lucorum

The training protocol used in the reconsolidation experiments is the same as in the consolidation experiment (see previous section). To remind the animals of the training situation, they are placed in the reinforced context, and neither a shock nor a tactile stimulus for testing is delivered. Anisomycin or saline are applied immediately after the reminding procedure. Testing the animals the next day does not reveal context memory in reminded and anisomycin-injected snails (**Figure 11**). In contrast, animals treated with saline show context memory. If the reminding process is omitted, the injection of anisomycin does not affect the context memory. Therefore, the reminder procedure induces a protein synthesis–dependent process, and hence reconsolidation. If in the reminding situation (the reexposure to the context) the reinforcing stimulus (the shock) is applied in combination with a protein synthesis inhibitor, the reconsolidation

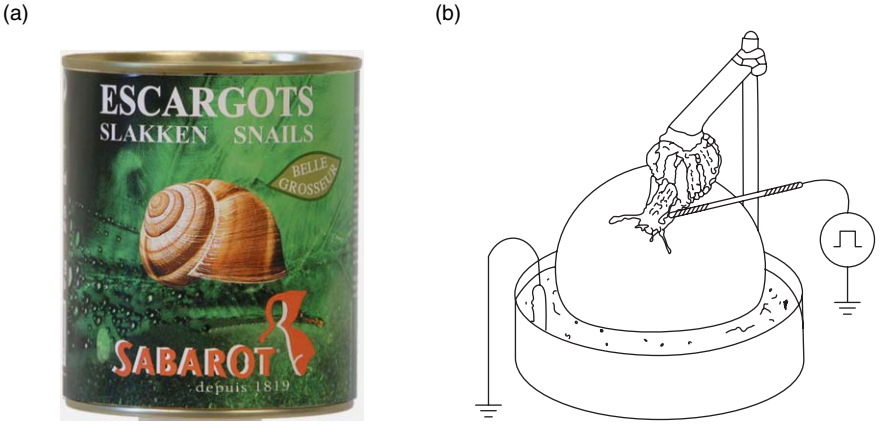

Figure 9 *Helix lucorum*: Aversive context learning. (a) *Helix lucorum* is sometimes called escargot turc. (b) The training apparatus. A snail is fixed at its shell and placed on a ball in a manner that allows crawling. The ball rotates freely in water and is laced with stainless steel wire to complete an electrical circuit between the animal's foot and a carbon electrode placed in the water. Electric shock is delivered through a macroelectrode applied manually to the dorsal surface of the foot. Adapted from Figure 1 in Gainutdinova TH, Tagirova RR, Ismailova AI, et al. (2005) Reconsolidation of a context long-term memory in the terrestrial snail requires protein synthesis. *Learn. Mem.* 12: 620–625.

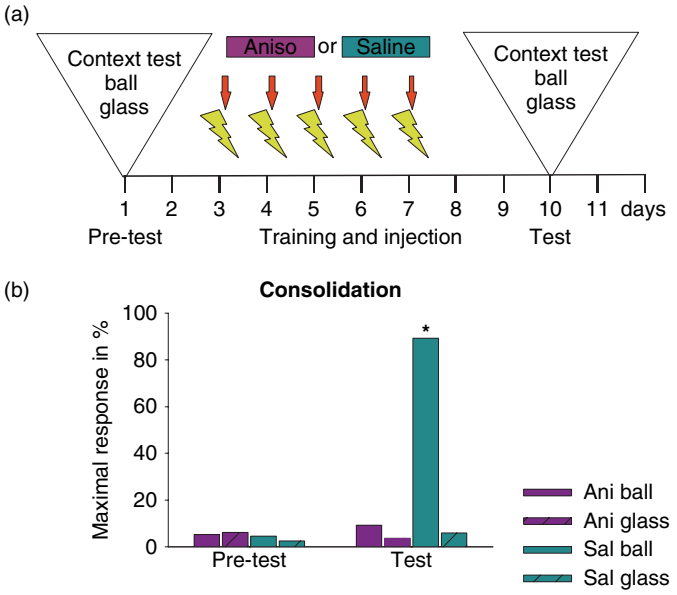

Figure 10 *Helix lucorum*: Memory consolidation. (a) Protocol of context conditioning. Snails received a pretest. Two days later all animals received five electrical shocks (flash) per day with a 20-min interval for 5 days. Every day, 3–5 min after the first shock, they were injected (arrow) with anisomycin (aniso, violet) or saline (green). A test was applied 3 days later. (b) Average of withdrawal response in the two contexts, reinforced context ball (plain) and nonreinforced context flat glass (striped). No difference in response to test stimulus was found in anisomycin-injected groups (Ani, violet bar), whereas the control group (Sal, green bar) showed context memory. Adapted from Figure 3 in Gainutdinova TH, Tagirova RR, Ismailova AI, et al. (2005) Reconsolidation of a context long-term memory in the terrestrial snail requires protein synthesis. *Learn. Mem.* 12: 620–625.

phenomenon is not visible (e.g., the animal can discriminate the two contexts) (Gainutdinova et al., 2005). This finding resembles a result in the crab *Chasmagnathus granulatus* (described in the next section), showing that reconsolidation can only be induced when the reminder is not accompanied by the reinforcer.

1.27.2.4 The Crab *Chasmagnathus granulatus*

1.27.2.4.1 Learning about a visual danger stimulus (VDA) leads to an associative and a nonassociative memory component

Chasmagnathus granulatus is a semiterrestrial crab that lives at the coast of South Brazil, Uruguay, and Argentina. Memory formation is studied on adult, male crabs (**Figure 12(a)**). They are caught in their natural habitat and transferred to the laboratory, where they are tested in an aversive learning paradigm. In this paradigm a danger stimulus (an opaque screen termed the visual danger stimulus = VDS) is passed over an animal that sits in a plastic container (**Figure 12(b)**).

The movement of this danger stimulus leads to an escape response that declines with further movements of the stimulus (Brunner and Maldonado, 1988). This decrease in the escape response has been initially assigned to habituation (Brunner and Maldonado, 1988). Depending on the training protocol, the decline of the escape response to the presentation of the VDS persists for several days (Lozada et al., 1990; Pedreira et al., 1995, 1998). Accordingly, a long-term memory about the VDS has been formed, which is termed long-term habituation (LTH). It consists of an associative component and a nonassociative component (Hermitte et al., 1999). The associative component is based on an association between the context and the signal (the VDS), and the resulting memory is termed context-signal memory (CSM) (Hermitte et al., 1999;Tomsic et al., 1998).

It is only induced by spaced training, depends on protein synthesis (**Figure 13(a)**), and lasts at least 5 days (Pedreira et al., 1995). The nonassociative component depends on the invariance of the VDS and is independent of the context of training. It is termed signal memory (SM). Massed training is sufficient to induce this nonassociative component of

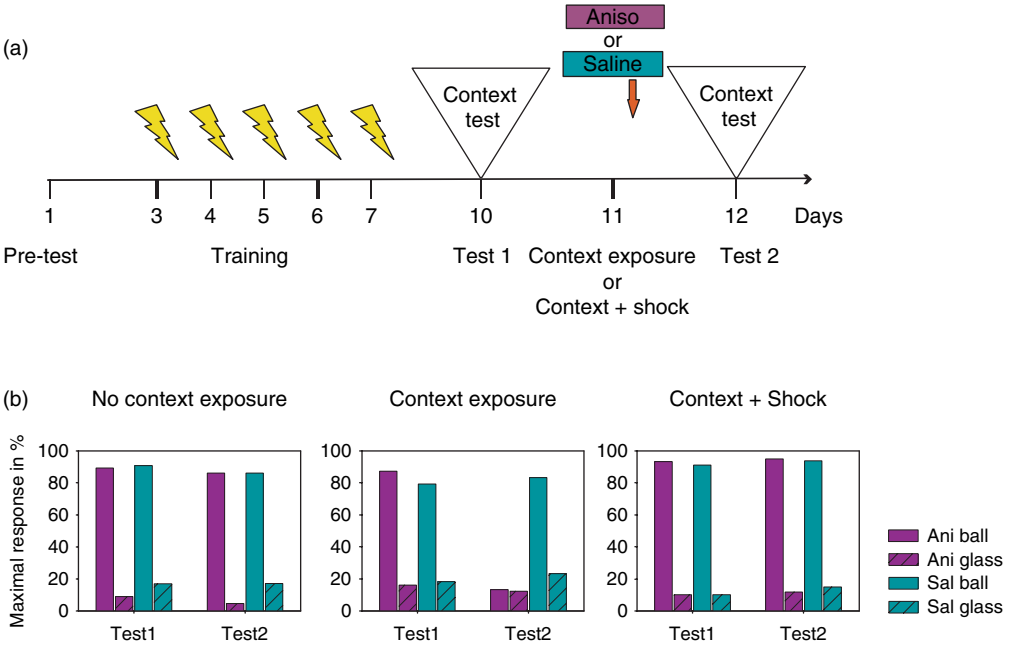

Figure 11 *Helix lucorum:* Reconsolidation. (a) Protocol of reminded context conditioning. Snails received a pretest on day 1 and were trained with five electrical shocks (flash) per day over 5 days. Three days later they received the test stimulus (test 1) to control if the animals had learned. Next day snails were reminded of training by placing them in the same context (context exposure, b middle panel) for 20 min or administering an electrical shock during the exposure (context + shock, b right panel), or as a control group received no context exposure (b left panel). All groups were injected (arrow) immediately afterward with anisomycin (aniso, violet) or saline (green). Next day all groups were tested again (Test 2) in both contexts. (b) Average response of withdrawal response in the two contexts, reinforced context ball (plain bars) and nonreinforced context glass (striped bars) before treatment (Test 1) and afterward (Test 2). In all experiments the context memory was intact in test 1. When the context exposure is omitted, injection of anisomycin (Ani, violet bars) or saline (Sal, green bars) has no influence on the context memory in test 2 (left panel: no context exposure). But reminded and anisomycin-treated snails show no context memory on test 2 to the trained context (ball), in contrast to the reminded saline-injected snails (middle panel: context exposure). If the reinforcer (shock) is presented during context exposure, injecting anisomycin does not lead to a loss of context memory (right panel: context + shock). Adapted from Figures 1, 2, and 4 in Gainutdinova TH, Tagirova RR, Ismailova AI, et al. (2005) Reconsolidation of a context long-term memory in the terrestrial snail requires protein synthesis. *Learn. Mem.* 12: 620–625.

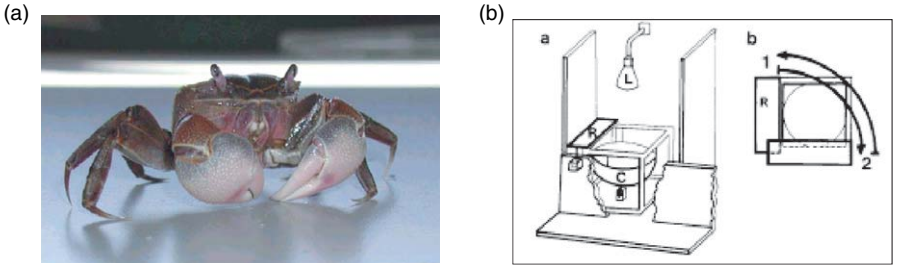

Figure 12 The crab *Chasmagnathus granulatus*: Learning about a visual danger stimulus (VDA). (a) The crab *Chasmagnathus granulatus*. (b) Left: The actometer, one of 40 units of the apparatus in which the crabs are trained. C, plastic container; R, rectangular screen used for the visual danger stimulus; M, motor; L, lamp; P, pizoelectric transducer; Right: Movement of the screen during a trial cycle (from 1 to 2 and vice versa). From Beron de Astarda M and Maldonado H (1999) Two related forms of long-term habituation in the crab *Chasmagnathus* are differentially affected by scopolamine. *Pharmacol. Biochem. Behav.* 63: 109–118.

the LTH (Pedreira et al., 1998; Hermitte et al., 1999) (**Figure 13(b)**).

It is important to note that memory for habituation can only be tested if the VDS is presented to the trained animals. Only then do the crabs show the learned freezing response. This means that retrieval of the initial memory and retraining occur in the same test session. Accordingly, when the usual series

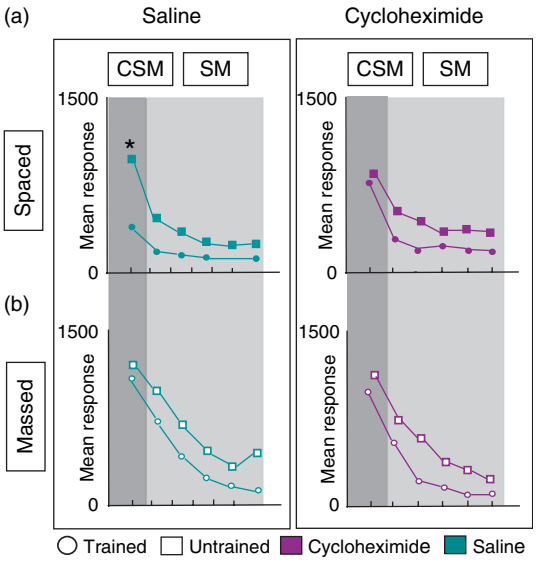

Figure 13 The crab *Chasmagnathus granulatus*: The induction of the context-signal memory (CSM) depends on the training protocol and is inhibited by a protein synthesis inhibitor. Crabs were systemically injected with 15 μg Cycloheximide (Cycloheximide; violet) or saline (Saline; green) immediately after spaced training (spaced, filled symbols) or massed training (massed, open symbols). Memory retention was tested 24 h later with six trials with the same intertrial interval that was used during training. Conditioned responses (mean response) at each test trial are shown here. The first test uncovers memory retention of the context-signal memory (CSM; dark gray); five following tests show memory retention of the signal memory (SM, light gray) and include a retraining phase. In each of the experiments, a trained group (trained, circles) is compared with an untrained control group (untrained, squares). (a) Memory retention after spaced training (15 trials; intertrial interval 171 s). The CSM is induced in the trained group in comparison to the untrained group (saline) and CSM but not SM depend on protein-synthesis (Cycloheximide). (b) Memory retention after massed training (300 training trials without intertrial interval). The CSM is not induced in the trained group in comparison to the untrained group (saline) and CSM and SM do not depend on protein synthesis (Cycloheximide). Adapted from Figure 3 in Hermitte G, Pedreira ME, Tomsic D, and Maldonado H (1999) Context shift and protein synthesis inhibition disrupt long-term habituation after spaced, but not massed, training in the crab *Chasmagnathus*. *Neurobiol. Learn. Mem.* 71: 34–49.

of six memory tests is applied, only memory retention at the first memory test resembles the memory for the initial training, and only this memory is based on the associative component of the LTM, the context-signal memory (CSM). Further memory tests in the same test session resemble a retraining phase (Pedreira et al., 1998).

1.27.2.4.2 Reconsolidation of the CSM

CSM retention can be observed 24 h after spaced training when the crabs are exposed to the VDS in the training context. When the crabs are exposed to the training context without the presentation of the VDS, a conditioned response is not elicited (Pedreira et al., 2002). Nevertheless, this exposure to the training context has an impact on subsequent memory retention. Namely, the combination of a 5-min context exposure 24 h after training with an injection of protein synthesis inhibitor leads to the inhibition of CSM memory retention 1 day later (Pedreira et al., 2002) (**Figure 14(a)**). The reconsolidation phenomenon has been induced accordingly.

1.27.2.4.3 The duration of the reexposure defined by its offset is critical for reconsolidation to occur

The reconsolidation phenomenon is only induced when reexposure to the training context endures for less then 40 min. A longer reexposure to the training context leads to a new, context–no signal association, and hence extinction learning. This extinction learning results in an extinction memory that depends on protein synthesis (Pedreira and Maldonado, 2003) (**Figure 14(b)**). Also in studies on vertebrates, the reconsolidation phenomenon becomes apparent when extinction is weak (Eisenberg et al., 2003; Suzuki et al., 2004; Power et al., 2006). On the basis of these findings, Nader (2003) and Dudai (2004) proposed the hypothesis of trace dominance. They interpreted these results as indicating a competition between two consolidation processes, the consolidation of extinction memory and the consolidation process underlying the reconsolidation phenomenon (Eisenberg et al., 2003; Nader, 2003). However, in the meantime, two studies, one in the honeybee and one in rats, reveal that the hypothesis of trace dominance is only half of the truth (see "The Honeybee *Apis mellifera*").

In crabs, the duration of the reexposure of the training context is critical for the consolidation process induced by the reminder. Accordingly, it has been found that the offset of the reminder stimulus without the appearance of the VDS is critical for either reconsolidation or consolidation of the extinction memory to occur (Pedreira et al., 2004). These data suggest that reconsolidation or the consolidation of an extinction memory occurred only when the nonoccurrence of the reinforcement (here the VDS) is irreversible due to the termination of the reminder (Pedreira et al., 2004). Accordingly, Pedreira et al. (2004) concluded

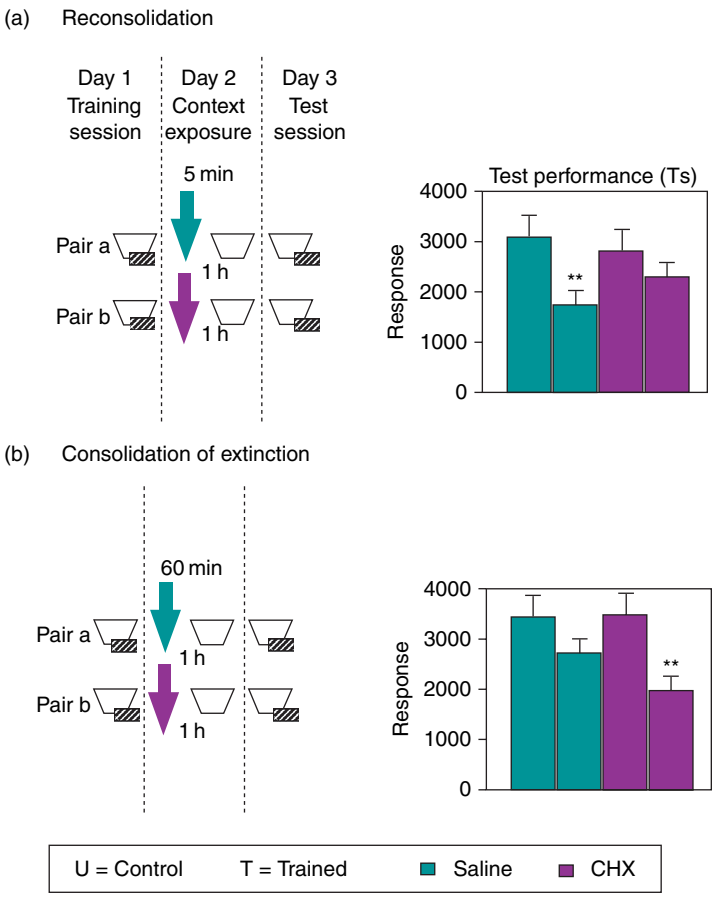

Figure 14 The crab *Chasmagnathus granulatus*: Reconsolidation and consolidation of extinction memory depend on the duration of the reexposure to the training context. Day 1: Training with 15 9-s presentations of the visual danger stimulus (striped bar), separated by 3 min; Day 2: Systemic injection (arrow) of Cycloheximid (CYX; violet arrow and bars) or saline (green arrow and bars) 1 h prior to reexposure to the training context trapeze for either 5 min (reconsolidation) or 60 min (consolidation of extinction); Day 3: Memory retention test: a single 9-s VDS presentation (striped bar). A significant difference between the untrained group (U) and the trained group (T) (U > T) at the memory retention test indicates memory retention. The trapeze stands for the container where a crab is placed during each of the experimental phases. Adapted from Figure 2 in Pedreira ME and Maldonado H (2003) Protein synthesis subserves reconsolidation or extinction depending on reminder duration. *Neuron* 38: 863–869.

that the mismatch between what is expected and what actually occurs triggers memory reconsolidation or extinction. Interestingly, a similar finding has been recently reported in an aversive paradigm in the gastropod *Helix lucorum*, namely, that the reconsolidation phenomenon is not visible when in the reminding situation (the reexposure to a context) the reinforcing stimulus (a shock) is applied in combination with a protein synthesis inhibitor (Gainutdinova et al., 2005) (see "The Terrestrial Snail *Helix lucorum*"). This is in contrast to findings in *Limax flavus* (see "The Terrestrial Slug *Limax flavus*") and in rats. In *Limax flavus* amnesia can be induced by retrieval with the CS alone or with an additional CS–US pairing followed by a cooling procedure (Sekiguchi et al., 1997). A similar result has been reported in an aversive conditioning paradigm in rats (Duvarci and Nader, 2004), namely, that the injection of anisomycin in selected brain regions after retrieval with and without reinforcement inhibits reconsolidation (Nader et al., 2000; Duvarci and Nader, 2004). The reason for this contradiction is not known. One could assume that the type of paradigm used in the different experiments is critical. In context conditioning (used in *Chasmagnathus granulatus* and *Helix lucorum*) memory retrieval that is accompanied by the reinforcement does not lead to reconsolidation. In contrast, the reconsolidation phenomenon is detectable after retrieval with or without reinforcing stimulus in cue-dependent conditioning (used in *Limax flavus* and rats).

1.27.2.4.4 CSM consolidation and reconsolidation share molecular mechanisms

Several molecules have been identified that are involved in the consolidation of the associative CSM (Romano et al., 2006). Presently it is not known if this holds true for reconsolidation and if the same molecular mechanisms underlie both the consolidation of the CSM and the reconsolidation. Growing evidence in vertebrates demonstrates that reconsolidation is not a faithful recapitulation of consolidation, but each process is mediated by distinct intracellular signaling cascades, which might overlap (Kelly et al., 2003; Lee et al., 2004). Findings in *Chasmagnathus* are in accordance with this in demonstrating that molecular mechanisms are shared by both consolidation processes, but differences between both processes have not yet been demonstrated.

Consolidation of the CSM and reconsolidation are sensitive to inhibitors of translation. In accordance with these results, a transcription factor, the nuclear factor κB (NF-κB), (*See* Chapter 4.28), has been found to be involved in the consolidation of the CSM and in reconsolidation (Freudenthal et al., 1998; Freudenthal and Romano, 2000; Merlo et al., 2002). After training, NF-κB is activated in two phases, directly and 6 h after spaced training (Freudenthal and Romano, 2000). This parallels activity of the cAMP-dependent protein-kinase A (PKA) after spaced training (Locatelli et al., 2002; Locatelli and Romano, 2005), suggesting a causal relationship between these processes, which nevertheless remains to be demonstrated. Also, reconsolidation depends on NF-κB activity during a 5-min reexposure to the trained context (Merlo et al., 2005), but it is unknown whether NF-κB is activated 6 h after memory retrieval as it is after training, and whether PKA plays a role in reconsolidation.

Are the same transmitters and receptors involved in consolidation and reconsolidation? Application of the *N*-methyl-D-aspartate (NMDA)-receptor antagonists during training up to 4 h after training inhibits the formation of CSM (Troncoso and Maldonado, 2002). This holds true for reconsolidation (Pedreira et al., 2002). Interestingly, the involvement of NMDA receptors in reconsolidation processes (and consolidation processes) has also been demonstrated in appetitive and aversive paradigms in several studies on vertebrates (Przybyslawski and Sara, 1997; Przybyslawski et al., 1999; Torras-Garcia et al., 2005; Akirav and Maroun, 2006; Lee et al., 2006). Accordingly, the involvement of the NMDA receptor might be an evolutionarily conserved feature of the reconsolidation processes.

The endogenous peptide angiotensin II, which plays a role in regulating fluid homeostasis and is present in higher amounts after water shortage in the crab brain, facilitates the consolidation of CSM (Delorenzi and Maldonado, 1999; Delorenzi et al., 2000; Frenkel et al., 2002). This memory enhancement is mediated by NF-κB that is activated by angiotensin II following water shortage (Frenkel et al., 2002). Angiotensin II is also involved in reconsolidation because its inhibition leads to retrieval-induced amnesia (Frenkel et al., 2005).

1.27.2.5 The Honeybee *Apis mellifera*

1.27.2.5.1 The appetitive, olfactory conditioning of the proboscis extension response

The honeybee is one of the oldest – if not the oldest – invertebrate models in learning and memory research. Its pronounced learning ability is used in a classical, Pavlovian conditioning paradigm in the laboratory, the olfactory conditioning of the proboscis extension response, where honeybee learning and memory formation are studied in single, restrained bees (**Figure 15(a)**) (*See also* Chapters 1.29 and 4.06).

Honeybees reflexively extend their proboscis when their antennae are touched with a drop of sucrose solution. This response to sucrose solution is called the proboscis extension response (PER) (**Figure 15(b)**). When the presentation of an odor precedes the sucrose reward (**Figure 15(c)**), honeybees learn to associate the odorant (CS) with the sucrose reward (US) (Menzel et al., 1974; Bitterman et al., 1983). After an association has formed, the odor alone elicits the proboscis extension. This reaction toward the odor is the CR. The CR can be elicited by the learned odor immediately after the acquisition and up to several days later, indicating the formation of short as well as long-lasting memories for this odor-sucrose association (Menzel, 1999; Eisenhardt, 2006).

1.27.2.5.2 A consolidated LTM is formed after three CS–US pairings

Three CS–US pairings are sufficient to induce a consolidated LTM. Two LTMs have been characterized so far by means of their sensitivity to systemically injected inhibitors. One and 2 days after three CS–US pairings, an LTM has been formed depending on translation during acquisition (Friedrich et al., 2004;

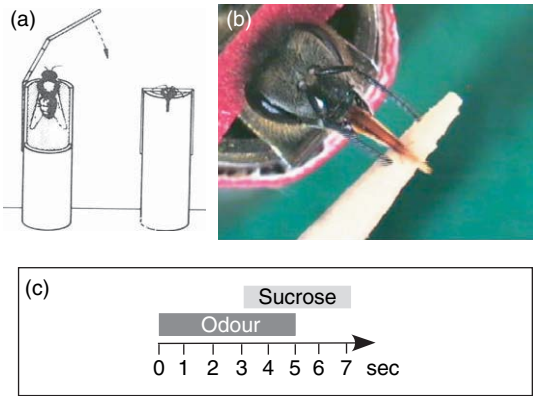

Figure 15 The honeybee *Apis mellifera*: Olfactory conditioning of the proboscis extension response. (a) Single honeybees are restrained in small tubes to condition the proboscis extension response (PER). Adapted from Bitterman ME, Menzel, R, Fietz, A, and Schäfer S (1983) Classical conditioning of proboscis extension in honeybees (*Apis mellifera*). *J. Comp. Psychol.* 97: 107–119. (b) Honeybees reflexively elicit their proboscis when the antennae and the proboscis are touched with sugar water. Here a toothpick that is moistened with sucrose solution is used to touch the honeybee's proboscis. After the PER is elicited, the honeybee licks sucrose solution from the toothpick. (c) Schematic diagram of the olfactory conditioning of the PER. An odor (conditioned stimulus) is presented for 5 s; after 3 s the PER is elicited with sucrose solution (unconditioned stimulus), and the honeybee is allowed to lick the sucrose solution for 4 s.

Stollhoff et al., 2005) (**Figure 16(a)**). A second long-lasting memory, formed 3–4 days after three CS–US pairings, depends on translation, but also on transcription. Inhibition of transcription and translation during acquisition, 1 h and 6 h after acquisition, results in an inhibition of this LTM (translation, 6 h: Eisenhardt & Stollhoff, unpublished data; Grünbaum and Müller, 1998; Wüstenberg et al., 1998; Friedrich et al., 2004). Thus, the activation of both processes for at least 6 h after acquisition is necessary for the formation of this later LTM. Interestingly, both LTMs are formed in parallel. They are induced by two different mechanisms that both depend on the cAMP-dependent protein kinase A (PKA) (Fiala et al., 1999; Müller, 2000; Friedrich et al., 2004).

1.27.2.5.3 Retrieval of a consolidated olfactory LTM

When a consolidated LTM is retrieved 1 day after training, two different consolidation processes can be induced, and the consolidation of an extinction memory and reconsolidation (Stollhoff et al., 2005). A series of experiments with one, two, and five retrieval trials that were applied 1 day after the initial training revealed that the resulting memory is dependent on the number of retrieval trials. Memory retention was

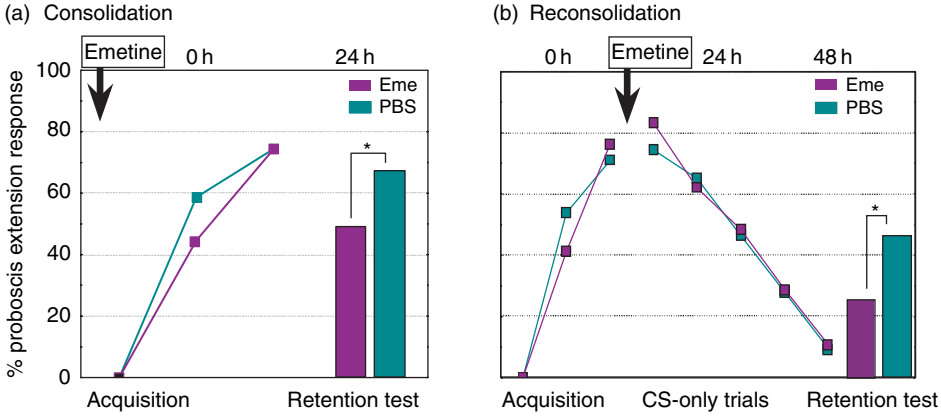

Figure 16 The honeybee *Apis mellifera*: Consolidation and reconsolidation. (a) Consolidation in the honeybee. 0 h: Emetine or PBS were systemically injected 30 min before honeybees were conditioned with three trials with an intertrial interval of 10 min (acquisition); 24 h: Memory retention was tested 1 day after acquisition by one 5-s presentation of the conditioned odor (CS). Eme: Animals that were injected with emetine; PBS: Control group; Animals that were injected with PBS. (b) Reconsolidation in the honeybee occurs after extinction. 0 h: Honeybees were conditioned with three trials with an intertrial interval of 10 min (acquisition); Emetine or PBS were systemically injected 30 min before the initial memory was retrieved with five conditioned stimulus (CS)-only trials with an intertrial interval of 10 min (CS-only trials); 48 h: memory retention was tested with one CS-only trial Eme: Emetine; PBS: Control group injected with saline. Adapted from Stollhoff N, Menzel R, and Eisenhardt D (2005) Spontaneous recovery from extinction depends on the reconsolidation of the acquisition memory in an appetitive learning paradigm in the honeybee (*Apis mellifera*). *J. Neurosci.* 25: 4485–4492.

tested 24 h after the initial memory retrieval. It turned out that one retrieval trial induced neither an extinction memory nor a reconsolidation phenomenon. Two retrieval trials led to significant extinction, and application of protein synthesis inhibitor before this memory retrieval resulted in an inhibition of the extinction memory. Five retrieval trials also induced significant extinction and spontaneous recovery of the CR (**Figure 16(b)**). After the application of the protein synthesis inhibitor, the amount of spontaneous recovery from extinction was reduced, but inhibition of the extinction memory was not observed. Hence, reconsolidation was induced after five retrieval trials (**Figure 16(b)**). Accordingly, although extinction was induced, the reconsolidation phenomenon was observed (Stollhoff et al., 2005).

These data contrast to those gathered in studies of the crab *Chasmagnathus* and in vertebrates (Eisenberg et al., 2003; Pedreira and Maldonado, 2003; Suzuki et al., 2004). Reconsolidation was induced in these studies only when extinction did not take place, and it was concluded that extinction is a boundary condition for reconsolidation (Nader, 2003).

The reason for this contradiction remains unclear, but only recently a study in vertebrates revealed a similar phenomenon, namely, the occurrence of reconsolidation after extinction (Duvarci et al., 2006). Hence extinction is not always a boundary condition for reconsolidation.

Spontaneous recovery from extinction after five retrieval trials in the honeybee is not complete (**Figure 16(b)**). From that it was concluded that after five retrieval trials, an extinction memory has been found, although spontaneous recovery occurred. Because this spontaneous recovery is based on reconsolidation, it seems likely that consolidation of the extinction memory and reconsolidation are two parallel processes (Stollhoff et al., 2005). What might be the mechanisms generating two parallel memory traces? A new hypothesis was put forward to explain the existence of these two memory traces (Eisenhardt and Menzel, 2007). This internal reinforcement hypothesis is substantially different from the original reconsolidation hypothesis (Nader, 2003) because it proposes that memory retrieval initiates new learning rather then activating and destabilizing the original CS–US memory trace. The hypothesis is based on the assumption that mechanisms underlying the reconsolidation phenomenon can be ascribed to basic neuronal mechanisms that are found in both vertebrates and invertebrates. The central tenet refers to the properties of reinforcement-predicting neurons, such as the dopamine neurons of the ventral tegmentum (Schultz, 2006) and the VUMmx1 neuron in the honeybee (Hammer, 1993). Neurons with comparable properties are assumed to exist also in the fruit fly *Drosophila melanogaster* (Riemensperger et al., 2005) and the molluscs (e.g., Aplysia et al., 1984). The internal reinforcement hypothesis proposes that two learning processes take place when memory is retrieved: one based on the lack of reinforcement (extinction learning) and one based on the internal existence of the reinforcement, which is activated by the CS (reminder learning) (Eisenhardt and Menzel, 2007). This internal existence of the reinforcement is proposed to be due to neuronal activity that is induced by memory retrieval, like the activity that can be observed in the honeybee's Vum_{mx1} neuron (Hammer, 1993). When retrieving a memory, the presentation of the CS and the activation of these neurons at the same time should then result in excitatory learning comparable to physical CS–US pairing. This form of learning should lead to a consolidation process underlying the reconsolidation phenomenon. Accordingly, reminder learning and extinction learning induce the formation of two memories that are consolidated in parallel and together constitute controlling behavior.

1.27.3 Conclusion

Several invertebrate species are currently used to study the reconsolidation phenomenon: the gastropods *Helix lucorum*, *Limax flavus*, *Lymnaea stagnalis*, the crab *Chasmagnathus granulatus*, and the honeybee, *Apis mellifera*. Findings from each of these organisms contribute to elucidate the reconsolidation phenomenon and its underlying mechanisms.

The slug *Helix lucorum* was the first invertebrate for which the reconsolidation phenomenon was demonstrated (Sekiguchi et al., 1997). In addition, the study of an odor-avoidance paradigm revealed that the age of the retrieved memory is important for the reconsolidation process to occur. It is only when fresh memories are retrieved that reconsolidation occurs. When retrieving an old memory, reconsolidation occurs only when either an additional training trial or a second-order conditioning was applied shortly before (Sekiguchi et al., 1997).

Furthermore, a study of an appetitive classical conditioning paradigm in the snail *Lymnaea stagnalis*

revealed that the age of the retrieved memory is important for the reconsolidation process. Retrieval of fresh and old memories induces a reconsolidation process that depends on protein synthesis. But only retrieval of a fresh memory induces a reconsolidation process that depends on the cAMP-dependent PKA (Kemenes et al., 2006). Accordingly, the molecular mechanisms underlying the reconsolidation processes depend on the age of the retrieved memory (*See* Chapter 4.09).

The neuronal network underlying reconsolidation in an aversive, operant conditioning paradigm has been studied in *Lymnaea stagnalis*. It has been demonstrated that only a single neuron is necessary for reconsolidation. In this paradigm, the reconsolidation phenomenon has been uncovered by cooling and using a transcription inhibitor. In accordance with this, reconsolidation is not induced when the nucleus of the relevant neuron has been ablated. Nevertheless, the nucleus is not needed to retrieve the inhibited reconsolidated memory (Sangha et al., 2003a).

In an aversive context conditioning paradigm in the snail *Helix lucorum*, the exposure to a context as a reminder elicits the reconsolidation phenomenon (Gainutdinova et al., 2005). But if the reinforcer is presented during the exposure to the reminder, reconsolidation cannot be observed.

A similar result has been found in the crab *Chasmagnathus granulatus*. Work on an aversive classical conditioning paradigm revealed that the duration of the CS presentation when retrieving a consolidated memory is critical for the induction of the reconsolidation phenomenon: Only a short exposure to the CS results in reconsolidation, whereas a long exposure leads to extinction and the consolidation of an extinction memory (Pedreira and Maldonado, 2003). Accordingly, it is only when the CS exposure is terminated without the occurrence of the US that reconsolidation and the consolidation of an extinction memory occur (Pedreira et al., 2004). In contrast to the findings in *Chasmagnathus*, a study on an appetitive classical conditioning paradigm in the honeybee demonstrates that – although extinction was induced – reconsolidation occurs after many CS presentations (Stollhoff et al., 2005). These results do not support the hypothesis of trace dominance that derives from studies in *Chasmagnathus* and vertebrates stating that extinction is a boundary condition for reconsolidation. It rather suggests that reconsolidation and the consolidation of an extinction memory are two parallel processes.

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1.28 Behavioral Analysis of Learning and Memory in *Drosophila*

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Associative learning is supposed to come about through changes in neurons, and it is believed that memory-guided behavior relies on these changes (Lechner and Byrne, 1998; Martin et al., 2000; Cooke and Bliss, 2006). Thus, in terms of physiology, past experiences would leave traces in terms of altered properties of neuronal circuits (*See* Chapters 1.02, 1.04, 1.33, 1.34). Here, we discuss whether it is possible to assign such changes to specific cells in the brain and, in this sense, to localize those memory traces underlying conditioned behavioral modifications. What could be the criteria for an accomplished localization of such a memory trace? As argued elsewhere (Gerber et al., 2004), if a certain set of cells were said to be the site of a memory trace, one should be able to show that:

1. Neuronal plasticity occurs in these cells and is sufficient for memory.
2. The neuronal plasticity in these cells is necessary for memory.
3. Memory cannot be expressed if these cells cannot provide output during test.
4. Memory cannot be established if these cells do not receive input during training.

If these criteria are met, it must be concluded that the group of cells in question is the one and only site of the memory trace under investigation. Clearly, neither of these criteria alone is sufficient to draw this conclusion; in particular, despite claims to the contrary (e.g., Yu et al., 2004, 2005; Liu and Davis, 2007), the mere observation of altered neuronal activity after some training regime in imaging experiments, albeit indispensable for the argument, is in itself not sufficient to localize a memory trace. This is because such data allow concluding that some memory trace must be residing between the stimulation site (i.e., the sensory level in Yu et al., 2004, 2005; Liu and Davis, 2007) and the site of the measurement, but more specific conclusions cannot be drawn.

In *Drosophila*, three different learning paradigms have been used in efforts to localize memory traces according to these criteria (Zars et al., 2000a,b; Liu et al., 2006). We will go through the available evidence, restricting ourselves to short-term (3–30 min) memory throughout, unless explicitly stated otherwise. *See* Chapter 4.07 for additional information on mechanisms of olfactory memory in *Drosophila*.

1.28.1 Neural Plasticity?

Criteria (1) and (2) rely on the ability to measure the plasticity of candidate neurons during the learning

process, as well as on the manipulation of these neurons *in vivo*. In *Drosophila*, no method is available to directly measure neural plasticity *in vivo* in adult central brain neurons, despite impressive advances in physiological techniques (Fiala et al., 2002; Ng et al., 2002; Hallem et al., 2004; Wang et al., 2004; Wilson et al., 2004). In a strict sense, this makes it difficult to test for criteria (1) and (2). Actually, the only way to directly observe neural plasticity in flies to date is to study the larval neuromuscular junction (Koh et al., 2000; Schuster, 2006). Being left with inferences from larval motor neuron-to-muscle synapses to central brain neurons in adults, however, is unsatisfying. Therefore, as a second best approach, genetic intervention was used to locally manipulate molecular components underlying neural plasticity. The process of choice was the cAMP/PKA signaling cascade. It had been shown to be required for learning as well as for neuronal plasticity in various paradigms throughout the animal kingdom (Byrne et al., 1991; Davis et al., 1995; Renger et al., 2000; Davis, 2005; Hawkins et al., 2006). More specifically, *in vitro* studies in *Aplysia* suggest that the type I adenylyl cyclase (in flies encoded by the *rutabaga* gene, *rut*) might act as a timing-specific molecular coincidence detector for the stimuli to be associated (Abrams et al., 1998). Hence, by an admittedly indirect argument, one is inclined to attribute an impaired or restored capacity to learn, caused by a genetic manipulation of the cAMP cascade in specific brain regions, to impaired or restored neural plasticity in that region (Zars et al., 2000a,b; McGuire et al., 2003; Mao et al., 2004; Liu et al., 2006).

As will be discussed later, such local *rut* rescues were successful for all three learning paradigms covered here (**Figure 1**). We therefore tentatively assume that indeed a lack of *rut* impairs neuronal plasticity and that a local rescue of *rut* does rescue

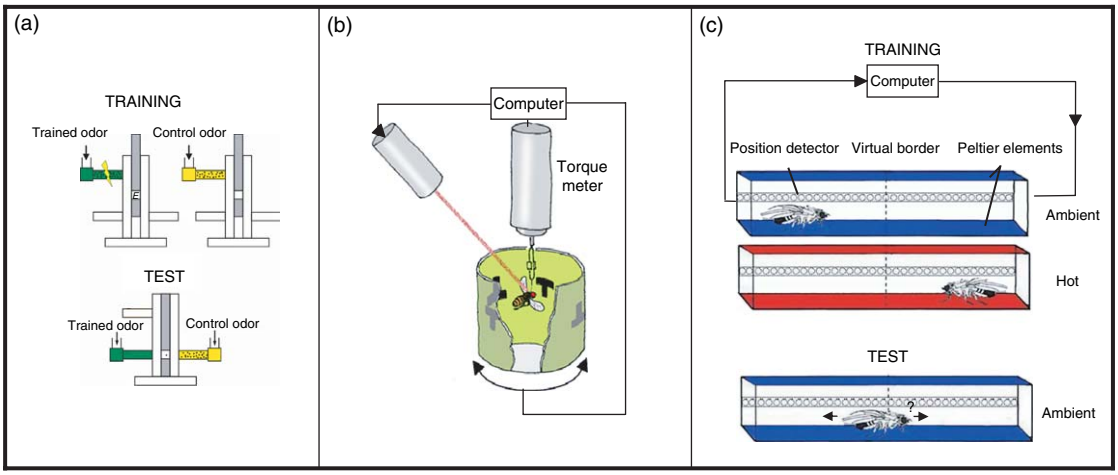


Figure 1 Behavioral procedures for measuring associative learning and memory. In all three cases flies are tested for memory by choosing between two conditions, of which they previously experienced one together with the reinforcer. In all cases, the *rutabaga* adenylyl cyclase is necessary for learning/memory, presumably via its role in synaptic plasticity. (a) Odor discrimination learning. Two experimental groups are run that undergo reciprocal training: One group of flies is exposed to odor A (green) and the reinforcer (in this case electric shock, but sucrose as appetitive reinforcer works as well). Next, the flies are exposed to odor B (yellow) without reinforcer. For testing, flies are shaken into the elevator (grey) and placed between two tubes simultaneously presenting the odors A and B; then flies on either side are counted and the difference in the number of flies on the control versus the trained side is calculated. For the other group of flies, the shock is presented with the respective other odor. Performance indices then present the mean of the odor choice scores in these reciprocally trained groups. (b) Visual pattern memory in the flight simulator. Tethered flies are suspended at a torque meter such that their yaw torque drives the angular velocity of a rotating panorama surrounding the fly. The wall of the panorama is decorated by two alternating patterns spaced by 90°. Flies can choose their direction of flight with respect to the patterns. Flies that are heated by a laser beam while heading toward one of the patterns subsequently avoid that pattern. The paradigm also uses a reciprocal training design; performance indices are calculated as in (a). (c) Heat box learning. Flies walk in a dark alley, and their position is monitored. During training they are heated if they enter one half of the chamber, some flies on the left side, some on the right side. During the memory test, flies still avoid the side where they had previously been heated. Again, the performance index is calculated as in (a).

this plasticity in the respective neurons. Clearly, the scope of conclusions thus is limited to those kinds of neuronal plasticity that involve *rut* and that are impaired in the available *rut* mutants. Still, as criteria (3) and (4) do not rely on any knowledge of the biochemistry underlying memory, they can serve to independently validate claims of localization of a memory trace.

1.28.2 Olfactory Learning

In odor discrimination learning (**Figure 1**), flies receive one odor together with an electric shock, and later another odor without shock. In a subsequent binary choice test, flies avoid the previously punished odor (Tully and Quinn, 1985). A similar paradigm can be used with sugar instead of electric shock, making flies approach the trained odor (Tempel et al., 1983; Schwaerzel et al., 2003). Within this section, however, we refer to odor-shock learning throughout unless explicitly stated otherwise.

Odors are detected (reviews by Stocker, 1994, 2001; Hallem et al., 2006) by receptor neurons, each expressing a single functional receptor gene of the *Or* gene family; receptor neurons expressing the same *Or* gene converge to one so-called glomerulus in the antennal lobe. Hence, the glomeruli represent the fly's primary odor qualities. The glomerular patterns of activity are shaped in addition by local interneurons. Projection neurons then relay the primary odor qualities to two sites, the lateral horn, a presumed premotor center, and the mushroom body calyx. Output from the mushroom bodies projects to a variety of target regions, including premotor areas. Thus, the mushroom bodies constitute a side branch of the olfactory pathway.

A memory trace underlying conditioned odor avoidance was proposed to be localized within the mushroom body neurons, also called Kenyon cells (reviews by Heisenberg, 2003; Gerber et al., 2004): Whenever the activation of a pattern of Kenyon cells elicited by a given odor coincides with a modulatory reinforcement signal impinging onto the Kenyon cells, future output from these activated Kenyon cells onto mushroom body output neurons is suggested to be strengthened. This facilitated output is thought to subsequently mediate conditioned behavior in response to the odor alone. The following data are the basis for this working model.

1.28.2.1 Sufficiency of Neuronal Plasticity in the Mushroom Bodies

In *rut* mutants, local transgenic expression of an intact *rut* cDNA can fully rescue the learning impairment (Zars et al., 2000a); notably, such rescue requires *rut* expression in the γ- and α/β-lobes of the mushroom bodies (GAL4 driver lines mb247, c772, 30y, 238y, and H24; for the GAL4/UAS technique, see Brand and Perrimon, 1993). A partial rescue is observed with the driver line 201y. In contrast, three drivers not expressing in the mushroom body γ-lobes (c232, 189y, and 17d) do not rescue (Zars et al., 2000a) (concerning H24, see also supplementary material provided with McGuire et al., 2003). The driver line mb247 also rescues sugar reward learning (Schwaerzel et al., 2003). Furthermore, Davis and colleagues (McGuire et al., 2003; Mao et al., 2004) showed that providing the *rut* cDNA during adulthood is sufficient for rescuing short-term memory. These results argue that the mushroom bodies are a site at which restoring neuronal plasticity is sufficient to restore olfactory learning, and that criterion (1) for the localization of a memory trace to the mushroom bodies is met. It needs to be emphasized that neuronal plasticity related to the *rut* adenylyl cyclase (rut-AC) can account for only about 50% of memory in conditioned odor discrimination; under the assumption that the used *rut* mutants completely lack the type I adenylate cyclase function required in this task, this would imply a distinct *rut* independent memory trace.

1.28.2.2 Necessity of Neuronal Plasticity in the Mushroom Bodies

Transgenic mushroom body expression of a dominant negative $G\alpha_s$ protein subunit ($G\alpha_s^*$), which constitutively activates the type I adenylyl cyclase, completely abolishes learning (Connolly et al., 1996) (driver lines 238y, c309, and c747). The driver line 201y that provides a partial rescue also inhibits partially (about 50%) when driving $G\alpha_s^*$ expression. Two lines with expression outside of the mushroom bodies (c232 and ok348) were normal. Under the plausible assumption that a constitutively activated cyclase prevents regulation of cAMP levels, and hence regulation of neuronal efficacy, this argues that plasticity within the mushroom body Kenyon cells is necessary for memory trace formation and that criterion (2) for the localization of a memory

trace to these cells is met. Strikingly, expression of the $G\alpha_s^*$ transgene in the mushroom bodies completely blocks 3-min memory in odor-shock learning, but *rut* mutants show only a 50% reduction, suggesting that any potentially *rut*-independent memory trace is also blocked by $G\alpha_s^*$ and is also located in the Kenyon cells.

In contrast, odor-sugar learning is only partially suppressed by $G\alpha_s^*$ expression in the mushroom bodies (A. Thum and H. Tanimoto, personal communication), suggesting that the memory trace in the Kenyon cells is not the only one underlying the odor-reward association. Indeed, a second memory trace may be located in projection neurons connecting the antennal lobe to the calyx and lateral protocerebrum (Thum, 2006). Whether these two traces are functionally redundant remains to be investigated; that is, do they differ in terms of the kinds of behavior supported, in their role during different memory phases, or the specificity of their 'content' (see Menzel, 2001 for such analyses in the honeybee; *See also* Chapter 1.29)?

1.28.2.3 Blocking Mushroom Body Output during Test

In flies it is possible to within minutes reversibly turn off the output of chemical synapses using a dominant negative, temperature-sensitive dynamin transgene, Shi^{ts} (Kitamoto, 2002). If Shi^{ts} is expressed in the mushroom bodies, flies can be trained with mushroom body output enabled, but tested with mushroom body output blocked. Three different laboratories have independently found that flies do not express odor memory if the temperature is raised to the off-condition 15 min prior to the test (driver lines c739, c747, c309, mb247, and c772) (Dubnau et al., 2001; McGuire et al., 2001; Schwaerzel et al., 2002; see Krashes et al., 2007, for a specific role of output from the $\alpha'\beta'$ lobes for stabilizing short- into longer-term memory). Apparently, criterion (3) for the localization of memory traces of odor-shock and odor-sugar learning to the Kenyon cells is met. Because the Shi^{ts} method is unspecific with regard to the biochemical mechanism(s) of memory trace formation, this supports the conclusions discussed earlier and, moreover, indicates that any *rut*-independent memory trace is unlikely to be located downstream of the Kenyon cell output synapses.

1.28.2.4 Blocking Input to the Mushroom Body during Training

Olfactory input to the mushroom bodies is carried by the uniglomerular projection neurons from the antennal lobes. If, by means of Shi^{ts} expression, synaptic output during training is blocked in approximately 60% of these projection neurons (driver line GH 146; Stocker et al., 1997), flies do not show any sign of memory during subsequent test (Schwaerzel, 2003), suggesting that criterion (4) for the localization of a memory trace to the mushroom bodies is met as well. However, the projection neurons provide input not only to the mushroom bodies but also to the lateral protocerebrum (Yasuyama et al., 2003) (and presumably even the antennal lobe; Ng et al., 2002). Therefore, this conclusion needs to be taken with caution, as we are still lacking appropriate tools to specifically block mushroom body input.

Concerning reinforcer-related input to the mushroom bodies, Riemensperger et al. (2005) showed that dopaminergic neurons, which impinge onto the mushroom body lobes, are activated by electric shock. Concerning behavior, Schwaerzel et al. (2003) found that for aversive learning dopamine (driver line TH-Gal4) but not octopamine signaling is necessary, and that in turn for appetitive learning, octopamine but not dopamine signaling is necessary. Furthermore, as shown in the *Drosophila* larva by using the 'remote control' of neuronal activation with a transgenically expressed light-gated ion channel (channelrhodopsin; Schroll et al., 2006), the respective catecholaminergic neurons also are sufficient to substitute for reinforcement in appetitive and aversive training, respectively. In the adult fly, both dopamine and octopamine signaling were shown to be specifically required during training, but not during test (Schwaerzel et al., 2003). The Kenyon cells clearly express octopamine and dopamine receptors and are targets of dopamine- and octopamine-immunoreactive neurons (Han et al., 1996, 1998; Friggi-Grelin et al., 2003; Kim et al., 2003; Strausfeld et al., 2003); notably, the antennal lobe receives strikingly little if any dopaminergic innervation (Riemensperger et al., 2005). However, whether specifically those octopaminergic and/or dopaminergic neurons that carry the reinforcing information do indeed directly impinge onto the mushroom bodies is at present unknown. Interestingly, using Ca^{++} imaging, Riemensperger et al. (2005) have reported that dopaminergic neurons innervating the mushroom

body lobes acquire increased responsiveness specifically to the learned odor, suggesting that training confers the reinforcing function of the shock to the odor. Thus, in keeping with the seminal work on reinforcement processing in bees (Hammer, 1997; Menzel, 2001; see also Schultz, 2006, for analogous results concerning dopaminergic neurons in monkeys), it seems likely that (i) also in flies (and fly larvae), signaling via aminergic neurons carries a prediction error–like internal reinforcement signal, (ii) these neurons impinge onto the mushroom bodies, and (iii) these neurons act during acquisition to induce, but not during retention to express, olfactory memory traces.

1.28.2.5 A Memory Trace Downstream of the Mushroom Bodies?

Three different laboratories found that blocking Kenyon cell output during odor-shock training (in the driver lines c739, c747, c309, mb247, and c772) leaves performance during test intact (Dubnau et al., 2001; McGuire et al., 2002; Schwaerzel et al., 2003; but see Krashes et al., 2007). Thus, for learning to occur, olfactory information needs to enter but does not need to leave the Kenyon cells. In fact, any kind of plasticity underlying learning, be it *rut* dependent or *rut* independent, thus seems to occur upstream of mushroom body output and, as argued in the previous paragraph, likely downstream of projection neuron output.

1.28.2.6 A Memory Trace in the Projection Neurons and/or Antennal Lobes?

Concerning an additional memory trace established in the projection neurons (for review of the work in honeybees, see Menzel, 2001), Yu et al. (2004) reported that two identified glomeruli (D and VA1) in the *Drosophila* antennal lobe are more strongly activated by the odor after training than before. This effect is stimulus specific, in that training with either of two different odors (3-octanol or 4-methylcyclohexanol) leads to the recruitment of either of the two glomeruli; it is also associative, as it is seen after odor-shock training, but not after a reversed order of stimulus presentation (i.e., shock-odor training); it is cell specific in that neither olfactory receptor neurons nor local antennal lobe interneurons show such recruitment; and it is specific for very short term memory, as after 7 min the effect has waned. Thus, there must be a memory trace somewhere between olfactory input and the projection neurons. As neither the olfactory sensory neurons nor the local interneurons, which are canonically acknowledged as inputs to the projection neurons, show any training-induced change, Yu et al. (2004) are inclined to conclude that a memory trace for very short term memory is located within the projection neurons themselves.

However, some caveats may warrant an alternative explanation. First, in naïve flies, neither of the odors induces excitation in either of these glomeruli (Wang et al., 2003; Yu et al., 2006); rather, olfactory sensory neurons innervating VA1 likely respond by inhibition (Hallem and Carlson, 2006), so that a specific explanation is required as to how an inhibited projection neuron could be the site of coincidence detection of odor and shock. Second, the antennal lobe is receiving little if any dopaminergic input (Riemensperger et al., 2005), so that one may need to invoke a dopamine-independent internal reinforcement signal to induce a memory trace in the antennal lobe. Third, $G\alpha_s^*$ expression in the mushroom bodies completely blocks learning (Connolly et al., 1996), and fourth, attempts to rescue aversive learning using *rut*-AC expression in the projection neurons have failed (H. Tanimoto, personal communication; A. Keene and S. Waddell, personal communication). The latter two arguments suggest that in electric shock learning any projection-neuron memory trace in itself would be insufficient for behavior control. Fifth, blocking projection neuron output during training completely abolishes learning (Schwaerzel, 2003), arguing against a cell-autonomous memory trace within the projection neurons. Finally, blocking mushroom body output during test completely blocks expression of memory (Dubnau et al., 2001; McGuire et al., 2001; Schwaerzel et al., 2002), which is difficult to reconcile with a memory trace located in the projection neurons because such a trace could be read-out directly via the lateral protocerebrum, independently of the mushroom bodies.

These caveats may prompt the question of whether the recruitment of antennal lobe glomeruli may be explained without reference to an antennal-lobe memory trace. Could it be that the two glomeruli are activated by a memory trace located elsewhere? It was shown in honeybees that mushroom body output neurons feed back onto the antennal lobe (Rybak and Menzel, 1993; loc. cit. Figure 4b, 16; Kirschner et al., 2006); thus, at the moment of test the learned odor may activate its memory trace in the mushroom body, which by virtue of this feedback loop could disinhibit antennal

lobe glomeruli to indicate the odors' behavioral relevance. Such a scenario predicts that glomerular recruitment would not be inducible if projection neuron output was blocked during training and would not be observed if it were blocked during test.

1.28.2.7 A Memory Trace in the DPM Neurons?

In a seminal discovery, Waddell et al. (2000) found that a single pair of identified, dorsal paired medial neurons (DPM neurons) are expressing the *amnesiac* gene product, the lack of which leads to an unusually fast decay of memory. That is, retention is more or less normal right after training but has completely waned already after 60 min. Restoring *amnesiac* function in these cells is sufficient to overcome this defect. However, given that output from the DPM neurons is dispensable at the moment of test (Keene et al., 2004, 2006; Yu et al., 2004). by our criteria they cannot be the site of a memory trace underlying retention (criterion [3]).

In summary, concerning short-term memory after odor-shock training, the rut^{2080}-dependent memory trace can be assigned to the Kenyon cells with reasonable confidence because this hypothesis accommodates all available experimental data.

1.28.3 Learned Visual Pattern Preference

Visual pattern learning at the flight simulator (**Figure 1**) involves an association between the heat of a laser beam and a specific visual feature of a landmark in the panorama (Ernst and Heisenberg, 1999; Tang et al., 2004). Landmark features so far have been found to fall into five classes (parameters): the height of the pattern in the panorama, its size, its color, the vertical distance between its components, and the orientation of its contour. Flies can be conditioned to discriminate landmarks according to any of these parameters if the landmarks have different values for them (how high? how big? etc.). This kind of learning, at the very least concerning pattern height, clearly is independent of the mushroom bodies, as their ablation leaves this task unaffected (Wolf et al., 1998). Until recently it had remained elusive which parts of the brain might house the corresponding memory traces.

Genetically, visual pattern recognition requires *rut* (Liu et al., 2006), allowing the application of a similar mapping strategy as in the case of olfactory memory. A total of 27 driver lines with different expression profiles were used to locally supply *rut*-AC to the brain of the rut^{2080} mutant. Seven of them restored the learning defect. What distinguishes the expression patterns in these seven lines from those of the 20 nonrescuing lines seems to be a group of about 15 neurons on either side of the protocerebrum (the so-called F5 neurons), forming a sharp horizontal layer of fibers in the upper fan-shaped body. All flies with *rut*-AC in F5 neurons were able to learn to avoid flying toward a pattern previously combined with heat. No fly without *rut*-AC in F5 neurons was able to perform this learning task (Liu et al., 2006).

In these experiments the authors used pairs of patterns that the flies could possibly distinguish only by their height in the panorama. However, *rut*-AC expression in the F5 neurons failed to restore pattern learning when the patterns were distinguishable only by their size or only by the orientation of their contours. Hence, the rescuing effect of *rut*-AC in the F5 neurons was specific for the pattern parameter 'height.' Subsequently, two driver lines were investigated that showed transgene expression in a horizontal fiber layer of the fan-shaped body that was different from that of the F5 neurons. This layer is formed by two clusters of about 10 neurons each (F1 neurons). Strikingly, expression of *rut*-AC in these cells did not rescue the memory defect for height or size but did restore the memory for contour orientation. Both rescue effects, that for height and that for contour orientation, are adult specific, as was shown by temporal and regional expression of *rut*-AC using tub-$GAL80^{ts}$ (Liu et al., 2006), a temperature-sensitive silencer of GAL4 (McGuire et al., 2003).

These results argue that by criterion (1), two further memory traces can tentatively be localized, the memory trace for the pattern parameter height in F5 neurons and that for the parameter contour orientation in F1 neurons of the fan-shaped body. Moreover, *rut*-AC also seems to be necessary in these neurons (criterion [2]), as expression of $G\alpha_s^*$ in the F5 and F1 neurons specifically interferes with the corresponding pattern memories but leaves the memories for the other pattern parameters unaffected. Thus, these memory traces appear to be the only ones for the respective tasks. Regarding criteria (3, 4), we cannot make any conclusion, as using heat as the punishing stimulus in pattern learning may

interfere with Shi^{ts} function, at present precluding this kind of experimental strategy.

1.28.4 Heat Box

Historically, the first attempt at mapping a memory trace was conducted for place memory in the so-called heat box (Zars et al., 2000b). In this paradigm, single flies walk in a small dark box that can be quickly heated and cooled between 24 °C and 37 °C (**Figure 1**). The position of the fly in the box is monitored, and the fly is heated when it is located on one side of the box but not when located on the other. Flies quickly learn to avoid being heated and retain their side preference for some minutes even after place-dependent heating has ceased. Clearly, this kind of learning is independent of the mushroom bodies (Wolf et al., 1998).

By mutant analysis, it turned out that *rut* mutant flies show reduced acquisition and memory scores, allowing for local rescue experiments. Seventeen driver lines with transgene expression in various parts of the central nervous system were used to restore the *rut*-AC in the mutant. In four lines the learning/memory defect was rescued. All four lines had substantial, potentially overlapping expression in the median bundle and more subtle overlapping expression in the antennal lobes and ventral ganglion. Of five mushroom body expressing lines that rescue olfactory learning, four do not rescue heat-box learning (30y, 238y, 201y, and H24); one line (c772) rescues in both paradigms. A refined anatomical assessment of the expression patterns in rescuing and nonrescuing lines is still pending, but neither the mushroom bodies nor the central complex seem to contribute to heat-box learning. The most likely sites thus include the median bundle and/or the antennal lobes and the ventral ganglion. So far, experiments neither using $G\alpha_s^*$ nor tub-GAL80^{ts} have been performed. Moreover, as in the case of visual pattern learning, the use of Shi^{ts} is not readily possible. In all, exactly where the memory trace for place learning is localized remains poorly resolved.

1.28.5 Selectivity of Rescue Effects

In the studies reviewed here (**Figure 2**), 33 selected lines were tested in *rut* rescue experiments, 16 of them successfully. A total of 60 rescue experiments were performed. Of these, 18 were positive. Seventeen lines were tested in more than one memory task. Only two (c772 and c271) rescued in two tasks, and none of the 11 lines that were used in all three tasks rescued in all three of them. These numbers document the high local specificity of the rescue effects by GAL4 expression.

Taking into account that the used GAL4 lines typically express the transgene in much less than 1% of the brain neurons, one might wonder why that many positive results were obtained. It is likely that, in the studies on olfactory and visual memory, the choice of driver lines was biased toward the mushroom bodies and central complex, respectively, because previous studies on HU-ablation as well as structural brain mutants in which the mushroom bodies or the central complex were affected had pointed to these structures. In any event, the amazing selectivity of the rescue effects, even for different parts of the fan-shaped body housing traces for different visual parameter memories, is independent of such bias in the choice of lines.

1.28.6 Assessing Gene Expression Patterns

The most important caveat for memory mapping – and actually for any circuit analysis in the fly brain using the GAL4/UAS approach – is that the spatial expression patterns of driver lines are difficult to reliably assess (Ito et al., 2003; Saper and Swachenko, 2003). Neither for Shi^{ts} nor $G\alpha_s^*$ are any means available to directly detect the transgenically expressed effector proteins. Regarding the *rut-AC*, the *rut* mutant used for rescue experiments (rut^{2080}) most likely is no protein-null mutant (H. Tanimoto and T. Zars, personal communication). This leaves us with inferences from the expression patterns of reporter genes like GFP. However, this again is no reliable approach. First, the expression of the reporter may alter connectivity, as was shown for *tau* (Wittmann et al., 2001); second, with the same GAL4 driver line, the expression pattern of different effectors and – even the expression pattern for the same effector construct at different insertion sites (e.g., UAS-GFP on different chromosomes; A. Jenett and M. H., unpublished) – can be different. The construction of tagged transgenes and appropriate immunocytochemistry will be a major advance in this field, in particular when it comes to arguing about levels of transgene expression. At present, using multiple GAL4 lines for answering the same question seems the best way to deal with these concerns.

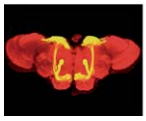
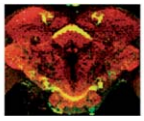
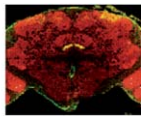
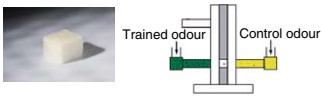
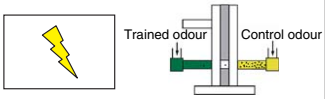
Expression pattern / Learning tasks	 Mushroom body	 Projection neurons	 F5 neurons	 F1 neurons	 Median bundle
	not sufficient	n.d.	not sufficient	n.d.	sufficient
 Trained odour Control odour	sufficient, not necessary	sufficient, not necessary	n.d.	n.d.	n.d.
 Trained odour Control odour	sufficient and necessary	not sufficient	n.d.	n.d.	n.d.
 Height	not sufficient	n.d.	sufficient and necessary	not sufficient	n.d.
 Contour orientation	n.d.	n.d.	not sufficient	sufficient and necessary	n.d.

Figure 2 Locally restoring the *rutabaga* adenylyl cyclase (*rut*-AC) rescues specific memory tasks. Top row: Five different groups of neurons (yellow in columns 2–5 and black in column 6). Leftmost column: Five learning paradigms requiring *rut*-AC (see **Figure 1** for further details). The matrix shows in which of the above neuronal groups *rut*-AC is sufficient and necessary to allow for the respective task. Odor–reward learning/memory can be rescued at either of two places.

1.28.7 Memory Mapping Reveals Functional Architecture

Contemplating the sites for olfactory and visual memory traces (**Figures 2, 3**), one notices interesting parallels and deviations. In both cases the memory trace is known from the behavioral analysis to be specific for a particular learned stimulus, whereas the neurons highlighted in the rescuing lines likely contribute to processing of many stimuli from the same sensory modality. In other words, the precise set of neurons housing a particular memory trace most likely is smaller than the group of neurons genetically highlighted in a certain driver line. Searching through more driver lines one might gradually approach the minimal set. Yet, at least in the mushroom bodies, the GAL4/UAS approach may fall short of reaching the resolution of individual memory traces: GAL4 expression patterns highlight groups of Kenyon cells that are genetically defined, whereas the sets representing given odors are functionally specified.

Odor quality appears to be coded in a combinatorial way such that the primary odor qualities constitute the coordinates of one multidimensional feature space. To accommodate a memory trace for a given odor quality within this feature space, the output of a subset from one common pool of Kenyon cells is strengthened.

In contrast, visual processing is parametrical in the sense that it is subdivided into several parameters (e.g., height and contour orientation). For visual memory, the values for each of these parameters need to be stored; consequently, each of these parameters has its own feature space employing distinct sets of neurons (e.g., F1 and F5 neurons for contour orientation and height, respectively); each of these feature spaces typically has a low dimensionality tailored toward storing parameter values. In short, olfaction employs one multidimensional feature space, whereas vision

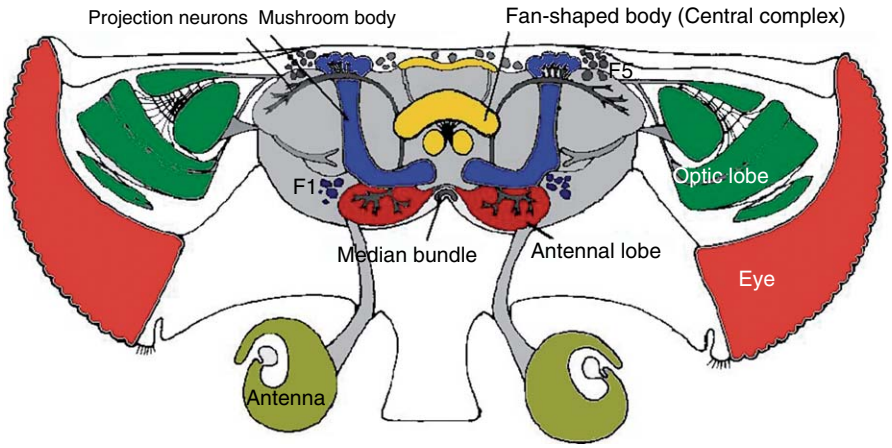


Figure 3 Schematic horizontal section of the *Drosophila* head showing some of the brain structures indicated in Figure 2. The Median bundle runs perpendicular to the plane of section. In the fan-shaped body, the horizontal strata of the F5 and F1 neurons lie about parallel to, but above and below, the plane of section.

appears to work in multiple, low-dimensional feature spaces. This example shows that beyond localizing the engram, memory mapping can elucidate the functional organization of the brain at the circuit level.

1.28.8 Conclusions

We have suggested a set of criteria for localizing a site of neuronal plasticity as the one and only trace (engram) underlying an associative memory. These criteria are that plasticity in a given group of cells be (i) sufficient and (ii) necessary for memory, and that memory be abolished if (iii) these cells could not output during test, and/or (iv) would not receive input during training. Regarding short-term memory for odor quality in aversive conditioning, the working hypothesis of the underlying memory trace being localized to Kenyon cells of the mushroom body is holding up well. Regarding visual pattern recognition, restoring the *rut*-AC is sufficient and presumably necessary in the F1 and F5 neurons of the fan-shaped body to restore memory for contour orientation and height, respectively. However, the containment of the engram in these neurons using temporally specific blockade of synaptic output has not yet been verified. For place learning in the heat box, the situation is less clear. Most likely, neither mushroom body nor central complex play a role; candidate structures are neurons in the median bundle, the antennal lobe, and the ventral ganglion.

These data altogether show that memory traces for different simple learning tasks reside in distinct parts of the brain, and that for any given learning task, the site of the underlying memory trace can be amazingly local. It should be interesting to see whether this kind of conclusion may hold up generally (i.e., also for memory traces in bees, snails, rabbits, mice, and humans). Keeping the neuronal changes of elemental memory traces locally confined may be a general operating principle of brains.

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1.29 Behavioral and Neural Analysis of Associate Learning in the Honeybee

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1.29.1 Introduction

French naturalist Georges-Louis Leclerc Buffon (1707–1788) became famous for the 36 volumes of his *Natural History*, an entire life's work, in which he covered subjects as diverse as the origin of the solar system, the fossilization processes, the classification of flora and fauna, and the origin of humans. Following a peculiar vision of animal intelligence, he expressed admiration for some creatures but fervently rejected others. Among the despised animals, an insect gathered his anger and devastating criticisms. It was neither an irritating mosquito nor a creeping cockroach. It was the honeybee. Buffon was impressed by the reproductive capabilities of a honeybee queen, which "produces thirty or forty thousand flies" (bees were indistinctly called bees and flies in his works), thus constituting "the largest known multiplication in the animal kingdom." This led him to conclude that "the most abject, vilest and smallest species are the most abundant ones" (Buffon, 1749a: 13–14). He went further and argued that "it is forceful to conclude that bees, taken individually, have less genius than a dog, a monkey and the vast majority of living animals; we shall also agree that they have less docility, less attachment and fewer feelings, in a word, fewer qualities relative to our own" (Buffon, 1749b: 93–94).

This animadversion contrasts with the admiration expressed by another famous scientist, who devoted his life to the study of honeybees. Karl von Frisch (1886–1982) became famous for the discovery of the honeybee dance, a ritualized behavior that allows a successful bee forager to inform other bees within the hive about the distance and direction of a profitable food source (von Frisch, 1967). This was not the only contribution made by von Frisch. He left us an amazingly rich and accurate body of evidence on honeybee behavior that spans studies on honeybee navigation, vision, olfaction, taste, and magnetic sensing, among other things (Frisch, 1967). Von Frisch liked to describe honeybees as a 'magic well' for discoveries in biology because the more that is drawn from it, the more there is to draw. Surprisingly, this fascination ended at a particular

point, in which, ironically, von Frisch could be said to approach some of Buffon's ideas. He expressed his view on the plasticity underlying honeybee behavior in the following way: "The brain of a bee is the size of a grass seed and is not made for thinking. The actions of bees are mainly governed by instinct" (Frisch, 1962, p. 78). Admittedly, von Frisch expressed this view in relation to communication behavior, but it is nevertheless striking that a tendency to dismiss the cognitive capacities of bees – and insects in general – has been perpetuated for centuries.

Despite this prolonged skepticism, in the last three decades honeybees have become a useful model for the study of learning and memory (Menzel and Erber, 1978; Menzel, 1985; Menzel et al., 1993). More recently, they have also acquired a new reputation in studies addressing higher-order cognitive capacities that have long seemed to exclusively belong to vertebrates such as monkeys, pigeons, or dolphins, which are known for their learning abilities. In this chapter, I analyze the contributions made by research on honeybee learning and memory that have facilitated this progress. I present findings and open questions that show the extent to which honeybees have increased our current understanding of cognitive processing at both the behavioral and the cellular levels, in hopes of underlining the power and potential of the honeybee in cognitive neurosciences. Chapter 4.06 provides a detailed description of the molecular mechanisms of bee learning.

1.29.2 Elemental and Nonelemental Forms of Associative Learning

Because this chapter intends to present the different levels of complexity that honeybees can reach in mastering different learning tasks, it is worth starting with operational definitions that allow discerning the simple from the complex. I focus on associative learning and introduce the distinction between elemental and nonelemental learning, which may be useful as a boundary between simple and complex forms of learning.

Associative learning is a capacity that is widespread among living animals and that allows extracting the logical structure of the world. It consists in establishing predictive relationships between contingent events in the environment so that uncertainty is reduced and adaptive behavior results from individual experience with such events. Two major forms of associative learning are usually recognized: In classical conditioning (Pavlov, 1927), animals learn to associate an originally neutral stimulus (conditioned stimulus, CS) with a biologically relevant stimulus (unconditioned stimulus, US); in operant conditioning (Skinner, 1938), they learn to associate their own behavior with a reinforcer. Both forms of learning, therefore, reliably predict reinforcement, either appetitive or aversive, and admit different levels of complexity. In their most simple version, both rely on the establishment of elemental links connecting two specific and unambiguous events in the animal's world. What has been learned for a given tone in terms of its outcome is valid for that tone but not necessarily for another stimulus, such as a light. The outcome of a given behavior, such as pressing a lever, is valid for that behavior but not for a different one such as pulling a chain (*See* Chapters 1.03, 1.07, 1.08). These forms of learning, which have been intensively studied by experimental psychologists, are also particularly interesting for neuroscientists interested in the neural bases of learning because they allow tracing to the level of neural circuits and single neurons the basis of associations underlying learning. Because these forms of learning rely on specific stimuli (e.g., a given CS and a given US), it is possible to study where and how in the central nervous system such stimuli are represented, where and how their neural pathways interact in order to facilitate association, and how experience modifies their respective neural representations. Both at the behavioral and neural level, these forms of learning have in common the univocal and unambiguous relationships established between events in the world. Because they can be characterized through specific links between unique events, simple forms of associative learning are termed elemental learning forms. Typical examples of elemental learning are absolute conditioning (A+), in which a single stimulus A is reinforced (+), and differential conditioning (A+ vs. B−), in which one stimulus, A, is reinforced (+), while another stimulus, B, is nonreinforced (−) (see **Table 1**). In the former, an animal has to learn to respond to A, which is unambiguously associated with reinforcement; in the latter, it has to learn to respond to A and not to B because both are unambiguously associated with reinforcement and with the absence of it, respectively.

However, other forms of associative learning are possible, in which unique links connecting specific events are useless because ambiguity characterizes the events under consideration (see **Table 1**). For instance, in the so-called patterning problems, animals have to learn to discriminate a stimulus

Table 1 Examples of elemental and nonelemental conditioning protocols[a]

Conditioning task	*Training*	*Processing*
Absolute conditioning	A+	Elemental
Differential conditioning	A+ vs. B−	Elemental
Feature positive discrimination	AB+ vs. B−	Elemental
Negative patterning	A+, B+ vs. AB–	Nonelemental
Biconditional discrimination	AB+, CD+ vs. AC–, BD–	Nonelemental

[a]In absolute conditioning, the subject has to learn to respond to stimulus A, which is unambiguously associated with reinforcement (+); in differential conditioning, the subject has to learn to respond to stimulus A and not to B; A is unambiguously associated with reinforcement (+), whereas B is unambiguously associated with the absence of reinforcement (−); in feature-positive discrimination, the subject has to learn to respond to the compound AB, which is reinforced (+), and not to B (−), which is nonreinforced; although B is ambiguous because it appears as often reinforced as nonreinforced, the fact that A is unambiguously associated with the reinforcement allows solving the problem. Simple links between a stimulus and reinforcement allow solving these three elemental problems. Elemental solutions cannot account for negative patterning solving, in which the subject has to learn to respond to the single stimuli A+ and B+ but not to their compound AB−, because elements are as often reinforced as nonreinforced. The same remark applies to biconditional discrimination, in which the subject has to learn to respond to the compounds AB+ and CD+ but not to AC− and BD−.

compound from its components, a task that is not necessarily trivial. Consider, for example, negative patterning, a problem in which an animal has to learn to discriminate two single components reinforced from their nonreinforced binary compound (A+, B+ vs. AB−). This situation is challenging because each element A and B appears as often reinforced as nonreinforced. Relying on elemental links between A (or B) and reinforcement (or absence of reinforcement) cannot solve this problem. Different strategies, such as treating the binary compound in a nonlinear form (i.e., as being different from the simple sum of A and B) have to be implemented to solve this kind of problem. A profuse literature has shown that some vertebrates can solve this kind of nonlinear processes and has put the accent on the nervous circuits and brain structures required for this kind of cognitive processing (Rudy and Sutherland, 1995; O'Reilly and Rudy, 2001; Bucci et al., 2002; Alvarado and Bachevalier, 2005; Moses et al., 2005; Borlikova et al., 2006; Jacobs, 2006; *See* Chapter 1.10).

Having introduced these two forms of learning, which define different levels of complexity in cognitive processing in a formalized and operational way, I present findings showing that it is possible to dissect and understand the basic mechanisms underlying these two levels of processing, using honeybees as a model system. I demonstrate that this insect exhibits elemental and nonelemental forms of learning that are relevant in its natural life and that are amenable to the laboratory, thus allowing controlled study and access to the underlying nervous system.

1.29.3 The Honeybee as a Natural Model for Studies on Learning and Memory

Several reasons warrant the use of the honeybee as a model for the study of learning abilities. In a natural context and despite their small size, honeybees exhibit an extremely rich behavioral repertoire (von Frisch, 1967). A social lifestyle is obligatory, and a single bee cannot survive very long separated from its mates. Outside of the hive, a bee travels over distances of several kilometers and visits hundreds of flowers in quick and efficient succession for gathering food (nectar and/or pollen). It also collects resin or water or roams for information-gathering purposes. Sensory capacities and motor performance are highly developed. Bees see the world in color (Menzel and Backhaus, 1991), perceive shapes and patterns (Srinivasan, 1994; Giurfa and Lehrer, 2001), and resolve movements with a high temporal resolution (Srinivasan et al., 1999). Their olfactory sense is able to distinguish a wide range of odors (Guerrieri et al., 2005b), and their mechanosensory perception is also extremely rich because of thousands of hair cells all around the body and proprioreceptors inside the body.

In a natural context, bees learn and memorize the local cues characterizing the places of interest, which are essentially the hive and the food sources (Menzel, 1985; Menzel et al., 1993). In the case of food sources, learning and memory are the very basis of floral constancy, a behavior exhibited by foragers that consists of foraging on a unique floral species as long as it offers profitable nectar and/or pollen reward (Grant,

1951). Only when such an offer becomes unprofitable will bees switch to a different species. Learning and memorizing the sensory cues of the exploited flower through their association with nectar and/or pollen reward is what allows a bee forager to track a particular species in the field. Similarly, learning abilities for landmark constellations and for celestial cues used in navigation (azimuthal position of the sun, polarized light pattern of the blue sky) ensure a safe return to the nest and enhance foraging efficiency (Collett et al., 2003).

Honeybees communicate information about important locations around the hive through ritualized body movements, called the waggle dance, a communication system that transmits information on the vector flown toward an attractive food source or nest site (von Frisch, 1967). Hive bees attending such a dance decode from the speed of dance movement the distance to the food source, and from the angle of the waggling phase relative to gravity, the flight direction relative to the sun. In this context, specific associations are built as dance followers learn to associate the odor of nectar brought by a dancer with the nectar that it regurgitates and passes them through trophallactic contacts (Farina et al., 2005, 2007; Gil and de Marco, 2005, 2006; *See* Chapter 1.25). Usually, many such dances occur in parallel within a colony. Individual and collective decision-making result from multiple and independent decisions without reference to full knowledge of all potential options available (Seeley, 1995).

The complexity and richness of the honeybee's natural life is therefore appealing in terms of the opportunities it offers for the study of natural learning and memory. Such an appeal would be, however, useless if these phenomena were not amenable to controlled laboratory conditions. However, several protocols have been developed to allow experimental access in terms of controlled training and testing conditions, thus underscoring the remarkable plasticity of this insect, which can learn even under restrictive (in terms of movement, for instance) or stressful (in terms of the aversive reinforcement experienced) conditions.

1.29.4 Experimental Access to Learning and Memory in Honeybees

Honeybees can be easily trained individually to solve different kinds of discrimination problems (von Frisch, 1967). Different from the en-masse training commonly used in other insects (e.g., *Drosophila*; Tully and Quinn, 1985), which does not always allow controlling the exact experience of the experimental subjects, different experimental protocols have been implemented to study learning and memory in honeybees at the individual level. This individual approach is important because learning and memory result from individual experience and because a neurobiological approach can then be undertaken and correlated with individual learning and memory scores only if such scores have been recorded in a precise way. Three main protocols developed to study honeybee learning and memory can be mentioned here: (1) conditioning of the approach flight toward a visual target in free-flying bees, (2) olfactory conditioning of the proboscis extension reflex in harnessed bees, and (3) olfactory conditioning of the sting extension reflex in harnessed bees. The first two protocols exploit the appetitive context of food search, as in both cases bees are rewarded with sucrose solution as an equivalent of nectar. The third protocol represents a case of aversive learning, as bees learn to associate odorants paired with the noxious reinforcement of an electric shock. In all three cases, and with different possible modifications derived from particular experimental needs, the basic experimental design comprises an acquisition or training phase in which the bees experience a particular stimulus or perform a given task that is reinforced, and a test or retrieval phase without reinforcement in which the bees are presented with the trained situation in order to assess the memory created by training. Eventually, novel stimuli can be presented in the test phase together with the trained stimulus in order to study generalization and discrimination capabilities. Transfer to novel stimuli (i.e., in absence of the trained stimulus) can also be tested to characterize the flexibility of the bee's choice (see below).

1.29.4.1 Conditioning of the Approach Flight Toward a Visual Target in Free-Flying Bees

Free-flying honeybees can be conditioned to visual stimuli such as colors, shapes and patterns, and depth and motion contrast, among others (von Frisch, 1914; Wehner, 1981; Giurfa and Menzel, 1997; Lehrer, 1997; Giurfa and Lehrer, 2001). In such a protocol, each bee is individually marked by means of a color spot on the thorax or the abdomen so that individual performance can be recorded. The marked bee is

generally displaced by the experimenter toward the training/test place, where it is rewarded with sucrose solution to promote its regular return (**Figure 1(a)**). Such pretraining is provided without presenting the training stimuli in order to avoid uncontrolled learning. When the bee starts visiting the experimental place actively (i.e., nondisplaced to it by the experimenter), the training stimuli are presented and the choice of the appropriate visual target reinforced with sucrose solution. As pointed out above, bees have to be trained and tested individually to achieve a precise control of the experience of each subject when it enters into a particular test. It is also important to control the distance at which a choice is made because orientation and choice are mediated by different visual cues at different distances or angles subtended by the target (Giurfa and Menzel, 1997; Giurfa and Lehrer, 2001). The time between visits to the experimental place is also an important variable to be recorded, because it reflects the appetitive motivation of the bee (Núñez, 1982), and thus its motivation to learn. For a food source approximately 100 m from the hive, motivated bees take between 3 and 10 min between foraging bouts. Longer intervals reflect a lower appetitive motivation and thus unreliable data.

Several behaviors can be used to quantify the bees' choice in these experiments. Touches (i.e., the flights

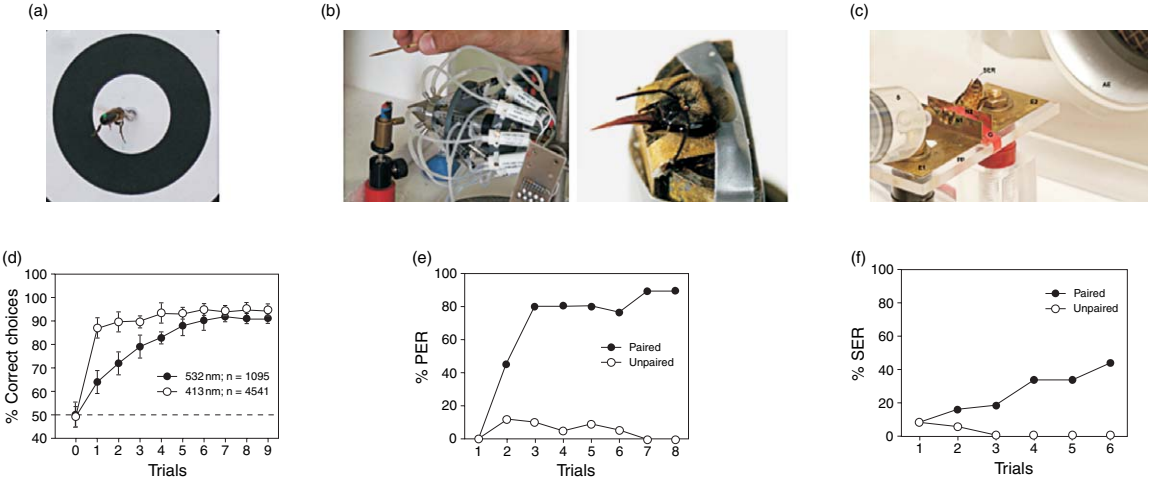

Figure 1 Experimental protocols for the study of learning and memory in honeybees. (a) Visual appetitive conditioning of free-flying bees. A bee marked with a green spot on the abdomen is trained to collect sugar solution in the middle of a ring pattern. (b) Olfactory appetitive conditioning of harnessed bees. The left panel shows a bee immobilized in a metal tube facing an olfactory stimulation device controlled by a computer. The toothpick soaked in sucrose solution held by the experimenter allows delivering reward to the antennae and mouthparts. The right panel shows the proboscis extension reflex (PER) of a trained bee to an odorant previously paired with sucrose solution. (c) Olfactory aversive conditioning of harnessed bees. A bee is immobilized by an elastic girdle (G) between two metal plates (E1, E2) on a plexiglas plate (pp) through which a mild electric shock is delivered. Odorant presentation is achieved by a syringe (S), and olfactory contamination is avoided by an air extractor (AE) placed behind the bee. Bees learn to extend the sting (sting extension reflex, SER) to the odorant aversively reinforced. (d) Acquisition curves for bees trained with colors in dual-choice experiments. Adapted from Figure 4 in Menzel R (1967) Untersuchungen zum Erlernen von Spektralfarben durch die Honigbiene (*Apis mellifica*) *Z. Vergl. Physiol.* 56: 22–62. The curves depict the percentage of correct choices along conditioning for two wavelengths, 413 nm (human violet) and 532 nm (human green). Trial 0 constitutes a spontaneous-choice test in which bees freely choose between the color that will be trained and an alternative. Although bees reached comparable levels of correct choices at the end of training, they learned 413 nm faster than 532 nm. n, number of choices recorded. (e) Acquisition curves for bees trained to associate an odorant with sucrose solution. Adapted from Figure 2 in Bitterman ME, Menzel R, Fietz A, and Schäfer S (1983) Classical conditioning of proboscis extension in honeybees (*Apis mellifera*) *J. Comp. Psychol.* 97: 107–119. The curves depict the percentage of PER along conditioning trials for two groups of bees, one trained with explicitly paired presentations of odorant and reward (paired, black dots), and another trained with explicitly unpaired presentations of odorant and reward (unpaired, white dots). Only the paired group learned the odorant–reward association, thus showing that learning has an associative basis and is not merely a result of experience with the stimuli independently of their temporal relation. (f) Acquisition curves for bees trained to associate an odorant with an electric shock. Adapted from Figure 2 in Vergoz V, Roussel E, Sandoz JC, and Giurfa M (2007a) Aversive learning in honeybees revealed by the olfactory conditioning of the sting extension reflex. *PLoS ONE* Mar 14 2: e288. The curves depict the percentage of SER along conditioning trials for two groups of bees, one paired (black dots) and another unpaired (white dots). Only the paired group learned the odorant–reward association, thus showing that learning has an associative basis.

toward a target that end with a contact of the bee's antennae or legs with the stimulus surface) and landings on a given stimulus are usually recorded to this end. The associations built in these contexts can be either operant, classical, or both; that is, they may link visual stimuli (CS) and reward (US), the response of the animal (e.g., landing) and the US, or both. The experimental framework is nevertheless mainly operant, as the bee's behavior is determinant for obtaining or not the sucrose reinforcement.

1.29.5 Olfactory Conditioning of the Proboscis Extension Reflex in Harnessed Bees

Apart from visual stimuli, honeybees can be conditioned to olfactory stimuli (Takeda, 1961; Bittermann et al., 1983). In this type of protocol, each bee is restrained in an individual harness such that it can only freely move its antennae and mouth parts (mandibles and proboscis) (**Figure 1(b)**). The antennae are the bees' main chemosensory organs. When the antennae of a hungry bee are touched with sucrose solution, the animal reflexively extends its proboscis to reach out to and suck the sucrose (proboscis extension reflex, PER). Neutral odorants blown to the antennae do not release such a reflex in naive animals. If, however, an odorant is presented immediately before sucrose solution (forward pairing), an association is formed that enables the odorant to release the PER in a following test (**Figure 1(b)**) This effect is clearly associative and constitutes a case of classical conditioning (Bittermann et al., 1983); that is, the odorant can be viewed as the conditioned stimulus (CS) and the sucrose solution as the rewarding, unconditioned stimulus (US). Within this framework, bees learn to associate the odorant with the sucrose reward.

As in any learning protocol, it is important to ensure the appropriate appetitive motivation of the experimental subjects. Immobilized bees in the laboratory therefore have to be starved prior to conditioning. A period of 2–3 h or a whole night is usually used as a starvation period in which bees have to be kept in a calm, darkened, humid environment. In olfactory PER conditioning, the response recorded is the extension of the proboscis, which is a dichotomous response (1 or 0). The duration of PER can also be recorded in order to provide a continuous, instead of a discrete, measure of acquisition (Hosler and Smith, 2000). To quantify learning, responses to the CS (the odorant) have to be measured before US delivery in each acquisition trial. In quantifying responses to the US, it is also important to control for the presence of the unconditioned reaction, and thus for the maintenance of the appetitive motivation of the bee throughout the experiment. A useful practice is to check the integrity of PER before and after the experiment by touching the antennae with sucrose solution. Animals not exhibiting PER in these control assays should not be included in the experimental analyses, as negative responses during acquisition and/or retrieval can be caused by sensory-motor deficits instead of learning and/or memory deficits.

1.29.5.1 Olfactory Conditioning of the Sting Extension Reflex in Harnessed Bees

Contrary to the previous protocols, this new form of conditioning (Vergoz et al., 2007a) offers the opportunity to study aversive instead of appetitive learning in honeybees. In this case, each bee is restrained in an individual harness such that it builds a bridge between two metallic plates through which an electric shock can be delivered (**Figure 1(c)**). Bees stimulated in this way exhibit an unconditioned, defensive reaction, which is the extension of the sting (sting extension reflex, SER) (Núñez et al., 1997). Using odorants paired with electric shocks, it is possible to condition the SER so that bees learn to extend their sting in response to the odorants previously punished (Vergoz et al., 2007a). Because no appetitive responses are involved in this protocol, true aversive learning can be characterized in this way.

As in appetitive olfactory conditioning of PER, the experimenter controls the stimulus contingency and can therefore vary the interstimulus interval and/or the intertrial interval in order to study the impact of these variations on aversive olfactory memory. Responses recorded are also dichotomous (1 or 0), but continuous measures can be obtained by recording SER duration. To quantify learning, responses to the CS (the odorant) have to be measured before shock delivery in each acquisition trial. Quantifying responses to the shock is also important to control for the presence of the unconditioned reaction, and thus for the aversive motivation of the bee throughout the experiment. As for PER conditioning, the integrity of SER has to be checked before and after the experiment to ensure the use of reliable data for the experimental analyses.

1.29.6 Accessibility of the Central Nervous System

The brain of a honeybee has a volume of approximately 1 mm^3 and contains around 960 000 neurons (Figure 2). Despite this apparent simplicity, the bee brain is capable of supporting learning and memory under simplified and restrictive conditions such as those described above. Accessing it in order to understand how neural architecture relates to cognitive processing is therefore possible, and several approaches can be employed to this end.

Although the free-flying visual conditioning protocol offers the obvious advantage of keeping the bee free, and thus allows visualizing the richness of its natural learning abilities, it is not helpful to uncover the neural bases of such abilities. Appetitive and aversive olfactory PER and SER conditioning, respectively, have the advantage of being controlled learning protocols precluding the bees' movement so that they can be easily combined with physiological approaches allowing *in vivo* the study of cellular and molecular substrates of learning and memory. This is a considerable advantage offered by bees and other invertebrate models with respect to some vertebrates, namely the possibility of an on-line access to the nervous system of a nonanesthetized animal while it learns and memorizes. It is possible to expose the bee brain through a small window cut in the cuticle of the head and to employ several invasive methods to study the bases of learning and memory. Physiological correlates of these different forms of olfactory conditioning can be found at different levels, ranging from the molecular and pharmacological levels to single identified neurons and neuronal ensembles whose activity can be visualized using electrophysiological or optophysiological techniques (Menzel, 1999, 2001). Neuropharmacological approaches based on the injection of agonists or antagonists of neurotransmitters or receptors into the brain can also be employed. Furthermore, RNAi can also be injected into the bee brain to characterize the role of certain receptor molecules functionally (Farooqui et al., 2003).

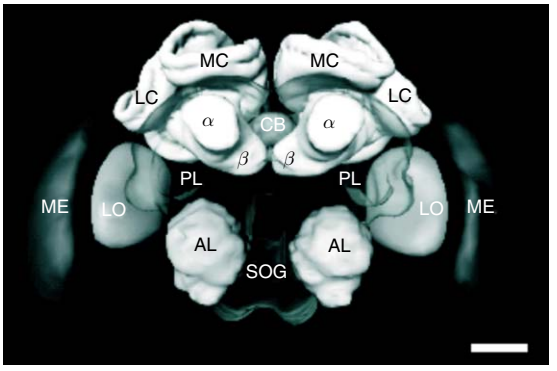

Figure 2 Three-dimensional reconstruction of a honeybee brain in frontal view based on confocal microscopy. Different neuropiles are indicated: ME, medulla, together with the lamina (not shown); LO, lobula; together with the lamina (not shown), both constitute the visual lobes in which visual processing occurs; AL, antennal lobe, the primary olfactory neuropile; PL, protocerebral lobe (lateral horn), a neuropile whose function is unclear; SOG, subesophageal ganglion, a region of the brain related to gustatory input; CB, a region of the brain related to motor responses; the two prominent, lighter symmetric structures in the middle of the brain are the mushroom bodies. Each mushroom body consists of two subunits, the calyces, lateral (LC) and median (MC) that constitute the input region of the mushroom bodies. Two lobes α and β constitute their output. Bar = 200 μm. From Malun D, Giurfa M, Galizia CG, et al. (2002) Hydroxyurea-induced partial mushroom body ablation does not affect acquisition and retention of olfactory differential conditioning in honeybees. *J. Neurobiol.* 53: 343–360.

Experimental access to the bee brain has characterized its basic architectural principles (Menzel and Giurfa, 2001). It comprises (1) dedicated neuropiles (i.e., brain regions devoted to the processing of specific sensory information [vision, olfaction, etc.]), (2) dedicated neurons (i.e., neurons that can be recurrently identified from bee to bee and within the same bee because of their unique morphology and because they accomplish specific functions in sensory-motor routines), and (3) higher-order integration centers (i.e., centers in which different sensory pathways converge such that multimodal integration takes place in them). Examples of these elements are provided when discussing the neural substrates of elemental and nonelemental learning in bees.

1.29.7 Elemental Appetitive Learning in Bees

Having described the main protocols used for the study of learning in honeybees, in this section I outline the main findings that have characterized elemental forms of learning at the behavioral and the cellular levels.

1.29.7.1 Elemental Color Learning and Memory in Free-Flying Honeybees

The first pioneering study on honeybee learning and memory that used controlled protocols for

characterizing individual acquisition and retention employed colors as rewarding stimuli (Menzel, 1967). Free-flying bees were trained to choose a rewarded monochromatic light and were then presented in dual-choice situations with the rewarded light vs. an alternative color. This study reported acquisition curves for different wavelengths and showed that bees learned all wavelengths after few (generally three) learning trials (**Figure 1(d)**). Performance was independent of the alternative, nonrewarded wavelength presented in the test. Moreover, some wavelengths, particularly 413 nm, were learned faster than others, especially after a first acquisition trial (Menzel, 1967). This result argued in favor of innate biases in color learning, probably reflecting the intrinsic biological relevance of the color signals that are learned faster (Menzel, 1985). Indeed, color-naïve honeybees in their first foraging flight prefer these colors, which experienced bees learn faster (Giurfa et al., 1995), and preliminary findings indicate that these colors could correspond to floral colors that are highly associated with a profitable nectar reward (Giurfa et al., 1995).

Menzel's (1968) experiments determined that one learning trial leads to a memory trace that fades a few days after learning if the animal is not allowed to learn anything else during this time, but three learning trials lead to a lifelong color memory. This was the very basis for discovering the existence of different memory phases in honeybees, some of which are short-term memories susceptible to interferences from additional color trials, and others are long-term memories that are resistant to such interferences. Short-term memories that allow keeping memory active during shorter periods of time are dominated by nonassociative processes such as sensitization. It was shown that at intervals of 24 h, memory formation is not protein-synthesis dependent, thus leading to the conclusion that long-term visual memories were not dependent on protein synthesis (Wittstock and Menzel, 1994). However, this conclusion is only valid for early components of long-term memory, as bees in these experiments were only tested 24 h after conditioning. Results from olfactory conditioning (see below) have shown that olfactory memories older than 4 days are indeed dependent on protein synthesis (Wüstenberg et al., 1998). A similar dependence on color memory is expected, given the parallels in dynamics between olfactory and visual memories. However, the demonstration that later components of long-term color memory do indeed depend on protein synthesis is still pending.

Color conditioning of free-flying bees was used to characterize other elemental learning phenomena such as overshadowing, the fact that after learning a color compound a bee responds significantly more to one color at the expense of the other (Couvillon and Bittermann, 1980; Couvillon et al., 1983), and blocking, the fact that after learning a single color and being then trained with a color compound made of the previously rewarded color and a new color, a bee may not learn the new color despite its close association with reward in the second training phase (Couvillon et al., 1997). Often, studies addressing these phenomena did not consider important stimulus characteristics that could bias performance, such as chromatic salience and detectability. They nevertheless had the merit of underlining that general principles of learning studied in vertebrates could be also found in honeybees.

In the 1980s and 1990s, visual learning was mainly used as a tool to answer questions on orientation close to the goal and visual perception and discrimination. The questions raised by these studies (see Lehrer, 1997; Srinivasan and Zhang, 1997, for reviews) focused on visual capabilities such as visual spatial resolution, shape discrimination, orientation detection, and movement perception and parallax, among others, and were not concerned by learning itself. Not surprisingly, none of these investigations quantified individual acquisition performance in order to present acquisition curves. This is a critical point because the visual strategies used by bees to solve a visual discrimination may be affected by the amount of accumulated experience at the moment of a test (Giurfa et al., 2003; Stach and Giurfa, 2005).

Because of obvious limitations, color learning in honeybees was never amenable at the cellular level because of the free-flying activity of the bees under study. Stages of central color processing such as color opponent neurons (Kien and Menzel, 1977; Yang et al., 2004) are known in the bee brain (Menzel and Backhaus, 1991), but there is no evidence on the possible interactions between the known neural elements of the color processing circuit and a reward-processing pathway. Recently, a protocol for color conditioning of the proboscis extension reflex has been proposed (Hori et al., 2006), based on pioneer findings by Kuwabara (1957). This protocol consists of training harnessed bees to extend the proboscis to color signals paired with sucrose solution.

1.29.7.2 Elemental Olfactory Learning and Memory in Harnessed Bees

The first study on olfactory PER conditioning was conducted by Takeda (1961) and was inspired by Kuwabara (1957), who reported PER conditioning using colors as CS. Olfactory PER conditioning was first used to assess olfactory discrimination capabilities in bees. These were typically trained with one rewarded odor and then tested for their choice of different odors, which differed from the rewarded one in structure (Vareschi, 1971). Even now, the protocol continues to serve this purpose and has provided a description of a putative olfactory space for the honeybee (Guerrieri et al., 2005b). This space was established by quantifying similarity and difference relationships between several odorants through olfactory PER conditioning. However, the protocol turned out to be the most powerful tool to characterize elemental olfactory learning after Bitterman et al. (1983) characterized it as a case of classical conditioning in which bees learn a CS (odorant)–US (sucrose solution) association. Forward (CS precedes US) but not backward (US precedes CS) pairing results in acquisition and learning of the trained odorant (**Figure 1(e)**). The memory trace initiated by a single CS–US pairing follows a biphasic memory function similar to that found for color learning of free-flying bees. The initial high response level is dominated by a nonassociative sensitization component, because a single US alone also arouses the animal for a short period of time, leading to a transient increase in response to many stimuli, including the CS.

A single learning trial results in a medium-term memory that fades away after a few days, whereas three learning trials lead to a stable long-term memory that is resistant to different forms of interference. Olfactory memory and its different phases (short-term, early and long; medium-term, early and long; and long-term, early and long; see Menzel, 1999) have been accurately described in terms of their dynamics (Menzel, 1999). The molecular bases of these phases are currently either known or being explored (Menzel, 2001), but this subject will not be reviewed here. The fact that similar dynamics underlie color and olfactory memory can be related to the natural lifestyle of the honeybee. Indeed, olfactory memory phases correspond to the temporal dynamics of foraging activities in the field (Menzel, 1999), such that early components of memory can be related to the fast succession of experiences that a bee gathers while foraging within a patch or when moving between close patches, whereas medium-term memory corresponds, because of its intrinsic dynamic, to the intervals occurring between foraging bouts. Long-term memory relates to foraging bouts that are spaced in time and that may occur in different days or after long-interrupted bouts (Menzel, 1999).

As in other classical (Pavlovian) protocols, olfactory memory acquired through PER conditioning is dependent on variables such as the kind of CS, US intensity (i.e., the amount and/or quality of sucrose solution received during conditioning), the number of conditioning trials, and the intertrial interval; Menzel et al., 2001). Trial spacing is the dominant factor for both acquisition and retention. Generally, massed trials (i.e., trials succeeding each other in a fast sequence) lead to impaired memory performance compared with spaced trials (i.e., trials separated in time). Longer-intertrial intervals lead to better acquisition and higher retention. Several studies on olfactory memory dynamics (reviewed in Menzel, 1999) showed that memories in bees pass through an early consolidation phase and that memories are fragile before consolidation is completed. Transfer from short-term memory to long-term memory via medium-term memory is not a purely sequential process but also includes parallel processes (Menzel, 1999). As in color learning, olfactory short-term memories are dominated by a nonassociative sensitization component, and long-term memory at its latest phase is protein synthesis dependent (Wüstenberg et al., 1998). The main conclusion arising from studies on honeybee olfactory memory (Menzel, 1999, 2001) is that behavioral performance reflecting memory storage and retrieval is guided by multiple and discrete memory traces rather than by a single, continuously decaying memory trace.

Olfactory conditioning of PER allowed studying associative phenomena such as overshadowing (Smith, 1998), blocking (Smith and Cobey, 1994; Gerber and Ullrich, 1999; Hosler and Smith, 2000; Guerrieri et al., 2005a), and other forms of compound conditioning (e.g., sensory preconditioning; Müller et al., 2000; backward blocking, Blaser et al., 2004) in a more controlled way. In some cases, clear effects were found (e.g., overshadowing, sensory preconditioning), whereas in others (e.g., blocking), the responses were rather inconsistent (Guerrieri et al., 2005a).

1.29.7.3 Cellular Bases of Appetitive Olfactory Proboscis Extension Reflex Conditioning

Apart from behavioral studies, the significant advance yielded by olfactory PER conditioning was

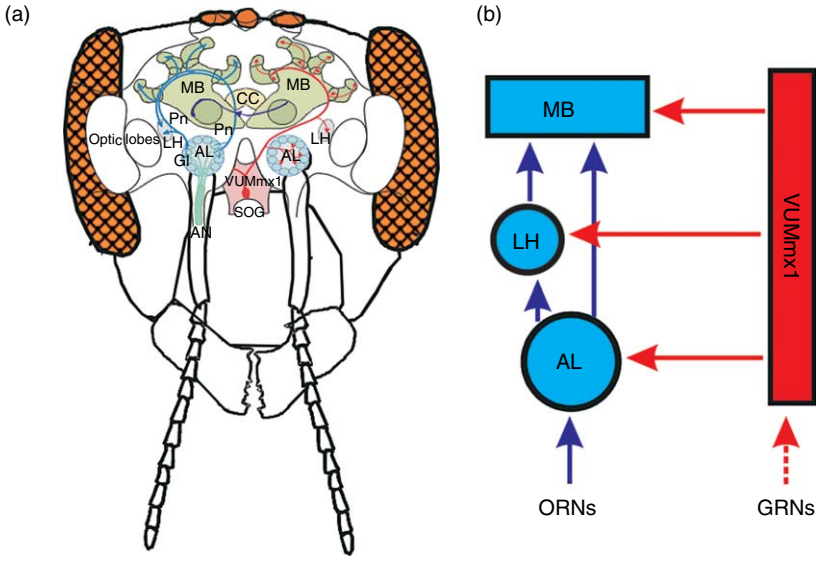

Figure 3 CS–US associations in the honeybee brain. (a) Scheme of a frontal view of the bee brain showing the olfactory (CS, in blue on the left) and gustatory (US, in red on the right) central pathways. The CS pathway: olfactory sensory neurons send information to the brain via the antennal nerve (AN). In the antennal lobe (AL), these neurons synapse at the level of glomeruli (Gl) onto local interneurons (not shown) and projection neurons (Pn) conveying the olfactory information to higher-order centers, the lateral horn (LH) and the mushroom bodies (MB). MBs are interconnected through commissural tracts (in violet).The US pathway: this circuit is partially represented by the VUMmx1 neuron, which converges with the CS pathway at three main sites: the AL, the LH, and the MB. CC, central complex, SOG, subesophageal ganglion. (b) Localization and distribution of CS–US associations in the bee brain. ORNs, olfactory receptor neurons; GRNs, gustatory receptor neurons. The dashed line between GRNs and VUMmx1 indicates that this part of the circuit is actually unknown. Adapted from a scheme provided courtesy of B. Gruenewald.

the possibility of tracing CS and US pathways in the honeybee brain and integratively studying the neural circuits underlying elemental associative learning. Odorants are processed in a neural pathway (the CS processing pathway) characterized by different processing stages (**Figure 3(a)**). Olfactory perception starts at the level of the antennae, where olfactory receptor neurons are located within specialized hairs called sensilla. Sensory neurons endowed with molecular olfactory receptors convey information on odorants to the antennal lobe. This structure is a good example of dedicated neuropile (see above), as it is the primary olfactory center of the bee brain. Antennal lobes are made up of globular structures called glomeruli, which are synaptic interaction sites between olfactory receptors, with local inhibitory interneurons connecting glomeruli laterally and projection neurons conveying processed olfactory information to higher-order centers such as the lateral horn or the mushroom bodies. The latter are higher-order integration centers, as they receive input from visual and mechanosensory pathways apart from the olfactory pathway.

Optophysiological studies based on recording calcium activity at the level of the antennal lobe following olfactory stimulation have shown that in naïve (i.e., nontrained) honeybees, odors are represented in terms of glomerular activity patterns (Joerges et al., 1997; Galizia and Menzel, 2000). Activity patterns for a given odor are symmetric between brain hemispheres and are conserved between individuals (Galizia et al., 1998, 1999). The pattern of active glomeruli tells the brain the identity of the odorant processed. When two odorants are presented in a mixture, the glomerular representation resembles the sum of the responses to the components or the response of the strongest component (Deisig et al., 2006). As more components are added, the picture changes and inhibitory interactions become apparent (Joerges et al., 1997). This across-fiber pattern coding is maintained upstream (Faber and Menzel, 2001), but there are differences in odor coding between the antennal lobes and the mushroom bodies. Indeed, odors evoke combinatorial activity patterns also at the level of the calyces, the input region of the mushroom bodies, but these are

substantially sparser (Szyszka et al., 2005). Moreover, the Kenyon cells, the neurons that constitute the mushroom bodies, exhibit a temporal sharpening of responses in response to odorants.

Although some parts of the CS pathway are still superficially characterized (e.g., the lateral horn), and although we are only starting to understand the dynamic aspects of odorant processing in several of the stages of this pathway (Szyszka et al., 2005), an integrative view of the CS circuit is already available. How does learning modify neural activity in this circuit? Faber et al. (1999) found that olfactory differential conditioning induces an increase in the intensity of the glomerular activation pattern for a rewarded odorant but not a qualitative change in its global nature. The nonrewarded odorant induced no change in glomerular activity. This conclusion has been recently challenged by experiments that showed no change in activity in a subpopulation of uniglomerular output neurons (projection neurons) after different elemental olfactory conditioning protocols (Peele et al., 2006). How olfactory representations are modified by associative learning therefore remains to be determined. In doing this, changes at the different stages of the olfactory pathway have to be studied at different intervals after conditioning. Moreover, the effect of different conditioning protocols of varying complexity should also be considered as a possible determinant of the olfactory representation achieved.

In the case of the US processing pathway, our knowledge is only partial, at least in neuroanatomical terms, as it has thus far been restricted to a unique neuron that is necessary and sufficient to substitute for the sucrose reward in the honeybee brain. This neuron, which constitutes a good example of dedicated neuron (see above), is called VUMmx1 (ventral unpaired median neuron of the maxillary neuromere 1) and responds with long-lasting spike activity to sucrose solution delivered both at the antennae and the proboscis (Hammer, 1993). The dendrites of VUMmx1 arborize symmetrically in the brain and converge with the olfactory pathway at three sites: the antennal lobes; the calyces, which are the olfactory input areas of the mushroom bodies; and the lateral horns (**Figure 3(a)**). This convergence is particularly remarkable in the case of a neuron coding for sucrose solution because it provides the structural basis for CS–US associations. That VUMmx1 indeed constitutes the neural representation of the US in olfactory PER conditioning was shown through an elegant substitution experiment conducted by Hammer (1993). He showed that behavioral learning of an olfactory stimulus can be induced by substituting the sucrose reward in PER conditioning with an artificial depolarization of VUMmx1 immediately after olfactory stimulation (forward pairing). If depolarization, and thus spike activity, precedes olfactory stimulation (backward pairing), no learning was observed. The same forward–backward effect was seen when sucrose was used as the reward under similar experimental conditions. These results showed that VUMmx1 constitutes the neural correlate of the US in associative olfactory learning.

This conclusion was reaffirmed by neuropharmacological experiments aimed at discovering the neurotransmitter acting as appetitive reinforcement in olfactory PER conditioning. As VUMmx1 belongs to a group of octopamine-immunoreactive neurons (Kreissl et al., 1994), it was hypothesized that octopamine, a biogenic amine usually associated with increased levels of arousal and behavioral facilitation in invertebrates (Libersat and Pflüger, 2004; Huber, 2005), acts as the neurotransmitter necessary and sufficient to substitute for the sucrose reward (Hammer and Menzel, 1998). Indeed, pairing an odorant with injections of octopamine as a substitute for sucrose into the mushroom bodies or the antennal lobes (but not the lateral horn lobe) produced a lasting, learning-dependent enhancement of proboscis extension (Hammer and Menzel, 1998). Thus, octopamine signaling via VUMmx1 is sufficient to substitute for sugar reinforcement in honeybees. This conclusion was confirmed by silencing octopaminergic receptor expression in the honeybee antennal lobe using double-stranded RNA (Farooqui et al., 2003). This treatment inhibited olfactory acquisition and recall but did not disrupt odorant discrimination. This result underscores that appetitive reinforcer function in the invertebrate brain is subserved by specific neurons and associated biogenic amines (here octopamine), which act as value systems in associative learning phenomena (i.e., as systems allowing ordering, prioritizing and assigning good or bad labels to odorants; Giurfa, 2006).

We still lack a more integrative view of the US pathway. Although gustatory receptor neurons tuned to sucrose have been located on specialized sensilla on the antennae, mouth parts, and tarsi (Whitehead and Larsen, 1976; Whitehead, 1978; Haupt, 2004; de Brito Sanchez et al., 2005), less is known about the circuit allowing these receptors to convey US information to the central level, and more specifically to VUMmx1. This circuit is probably localized in the subesophageal ganglion, which is the first synaptic

relay in the gustatory pathway (Altman and Kien, 1987). Despite this incomplete view of the US pathway, an important principle of classical conditioning was verified by studying VUMmx1 activity, namely, stimulus substitution. Classical conditioning relies on a CS acquiring the capacity of replacing the US as it becomes a reliable predictor of reinforcement. This was evident in recordings of VUMmx1 activity after olfactory conditioning (Hammer, 1993). After training a bee to discriminate a rewarding (CS1) from a nonrewarded odorant (CS2), it was found that VUMmx1 fired to CS1 and not to CS2 (Hammer, 1993). Thus, CS1, the odorant that reliably predicted the US, acquired the capacity of activating VUMmx1. At the same time, VUMmx1 continues to respond to the US when it is presented unexpectedly (i.e., not preceded by a predictive odorant; Menzel and Giurfa, 2001). Thus, the VUMmx1 neuron has the characteristic properties of a system that provides reinforcement prediction error information that is critical to associative learning (e.g., Schultz and Dickinson, 2000). In other words, it provides information on the discrepancy between the expected and delivered values of a reinforcing event (the prediction error), which determines the effective reinforcement value of that event (Rescorla and Wagner, 1972).

The picture emerging from these and other studies is one in which elemental, associative, olfactory learning can be accurately characterized at both the behavioral and cellular levels in order to understand the mechanisms of this simple form of learning. The honeybee offers the unique chance of dissecting elemental olfactory learning and identifying its building blocks. From this dissection, it appears that learning relies on distributed, but localized, interactions between CS and US pathways throughout the brain (**Figure 3(b)**). Distribution is reflected by the fact that interactions between these pathways occur in at least three different regions of the brain, the antennal lobes, the mushroom bodies, and the lateral horns (see **Figure 3(b)**). Localization is reflected by the fact that these interactions are spatially delimited. Redundancy could also be designated as a principle because of the repetition of synaptic interactions between CS and US pathways, but so far it remains unknown whether the interactions occurring at one of these three sites are equivalent to those occurring in another site (i.e., whether different memory contents are formed and stored in the antennal lobes, mushroom bodies, and lateral horns). Instead, it appears that these different brain structures intervene in different forms of learning (see below) so that the concept of redundancy would not be appropriated.

1.29.8 Elemental Aversive Learning in Bees

The previous sections underline that for almost a century research on honeybees has made significant contributions to our general understanding of learning and memory but that such an understanding is restricted to appetitive learning. As mentioned above, olfactory conditioning of PER has been used for 45 years (beginning in 1961; Takeda, 1961) as the only tool to access the neural and molecular bases of learning in honeybees.

Recently, aversive learning could be studied in honeybees in such a way that both behavioral and neural levels were made accessible to experimentation (Vergoz et al., 2007a). Pairing an odorant with an electric shock resulted in associative learning, in which bees learned to extend their sting (SER) in response to the odorant previously punished (**Figure 1(f)**). They were also able to learn to master appetitive and aversive associations simultaneously and exhibited the appropriate response, PER or SER, to the appropriate odorant. Moreover, neuropharmacological experiments addressed the question of modularity of appetitive and aversive learning and the possible dependency of this modularity on two different biogenic amines subserving appetitive and aversive reinforcement. Indeed, although octopamine has been shown to substitute for appetitive reinforcement (Hammer and Menzel, 1998; see above), it was found that blocking of dopaminergic, but not octopaminergic, receptors suppresses aversive olfactory learning. Thus, octopamine and dopamine subserve appetitive and aversive reinforcement in the honeybee, respectively. Again, this finding emphasizes the conclusion that dedicated biogenic amines act as value systems in the invertebrate brain and that they fulfill different reinforcing roles in different forms of learning.

This finding brought the honeybee closer to other insect models such as the fruit fly *Drosophila melanogaster* and the cricket *Gryllus bimaculatus*. In crickets, pharmacological experiments showed that octopamine and dopamine subserve the appetitive and aversive reinforcing functions, respectively (Unoki et al., 2005). In fruit flies, the same result was previously found using mutants with inactivated dopaminergic or octopaminergic neurons (Schwaerzel et al., 2003). Recently,

Schroll et al. (2006) showed that octopamine and dopamine are necessary and sufficient to substitute for appetitive and aversive reinforcement in *Drosophila* larvae. Furthermore, neurons capable of mediating and predicting aversive reinforcement have been found in the *Drosophila* brain (Riemensperger et al., 2005; *See* Chapter 1.28). These neurons may be a general feature of the insect brain, and dopamine may underlie other forms of aversive learning involving stimuli of different sensory modalities (e.g., visual stimuli associated with aversive gustatory reinforcements; Unoki et al., 2006). Interestingly, dopaminergic neurons in the fly brain exhibit the same functional principle as the VUMmx1 neuron in the bee brain, namely, stimulus substitution. Here too, dopaminergic neurons did not respond to the odorant used for aversive conditioning before conditioning and acquired this property after conditioning (Riemensperger et al., 2005). Similarly to the VUMmx1 neuron, dopaminergic neurons in the fly brain provide, therefore, reinforcement prediction error information that is critical to associative learning.

The study of aversive learning in honeybees is just starting. As this form of conditioning uses odorants as CS, it is possible to ask how odorant representations are modified by aversive experiences compared with appetitive experiences. An important goal is to identify dopaminergic neurons in the bee brain whose morphology and functional properties make them candidates for integrating the aversive US pathway. Furthermore, characterizing aversive olfactory memory at both the cellular and molecular levels is crucial for a comparative analysis of appetitive and aversive learning and memory in bees, which can now be performed. The usefulness of the aversive learning protocol has been demonstrated by the finding that green mandibular pheromone, the pheromone that is responsible for social dominance and control exerted by the queen within the hive, inhibits selectively olfactory aversive learning in young bees (Yugoz et al., 2007b). In this way, young bees, which have to stay closer to the queen are prevented from forming any aversive experience that could result from such an important bond. This fascinating result could only be accessed thanks to the aversive learning protocol, as no significant effect of the pheromone was found in the case of the appetitive olfactory learning.

1.29.9 Nonelemental Learning in Bees

Elemental appetitive and aversive learning, as discussed above, rely on the establishment of elemental associative links connecting two specific and unambiguous events in the bee's world. What has been learned for a given color in terms of its outcome is valid for that color but not for a different one. The sucrose reward that follows a given behavior, such as contacting a given surface with the left antenna, is valid for that behavior but not for a different one such as contacting the same surface with the right antenna. However, in the forms of associative learning that we discuss here, unique links connecting specific events are not useful because the events under consideration are ambiguous in terms of their outcome. A typical case introduced above is negative patterning, in which the subject needs to learn to discriminate a nonreinforced compound from its components (A+, B+, AB−). This problem does not admit elemental solutions because the animal has to learn that AB is necessarily different from the linear sum of A and B. In biconditional discrimination, the subject has to learn to respond to the compounds AB and CD and not to the compounds AC and BD (AB+, CD+, AC−, BD−). As in negative patterning, each element, A, B, C, and D, appears as often reinforced as nonreinforced, so that it is impossible to rely on the associative strength of a given stimulus to solve the task. These examples show that more elaborated computational strategies are necessary in the case of nonelemental discrimination problems.

Treating compound stimuli as entities different from the simple sum of their components (e.g., $AB = X \neq A + B$) constitutes the basis of the configural learning theory proposed to account for the solving of these nonlinear discrimination problems (Pearce, 1994). For this account, animals trained with AB can respond to A or B only to a low extent. Another theory, the unique-cue theory, proposes that a mixture is processed as the lineal sum of its components plus a stimulus (u) that is unique to the joint presentation of the elements in the mixture (e.g., $AB = A + B + u$) (Whitlow and Wagner, 1972). The unique cue is what gives a unique signature to a compound, which differentiates it from the linear sum of its components. For this account, animals trained with AB can respond to A or B to a relatively high extent.

Probably because of their inherent complexity, these problems have been rarely studied in invertebrates. However, several recent studies have addressed the issue of elemental vs. nonelemental learning in honeybees, using visual conditioning of free-flying animals and olfactory PER conditioning (Giurfa, 2003). In both experimental protocols, bees were shown to solve a biconditional discrimination (AB+, CD+, AC−, BD−). In the visual modality, free-flying

bees had to discriminate complex patterns that were arranged to fulfill the principles of this discrimination problem (Schubert et al., 2005). In the olfactory modality, olfactory compounds were used (Hellstern et al., 1995; Chandra and Smith, 1998), and bees learned to respond appropriately to each compound, independent of the ambiguity inherent to the components. This capacity demonstrates that under certain circumstances both visual and olfactory compounds are learned as entities different from the simple sum of their components.

This conclusion is underlined by studies showing that bees can solve a negative patterning discrimination (A+, B+, AB−) both in the visual (Schubert et al., 2005) and the olfactory (Deisig et al., 2001, 2002, 2003) modality. Solving this problem is possible if the compound AB is treated as being different from the simple sum of its elements. In the case of olfactory compound learning, experiments were conceived to discern between the two nonelemental theories mentioned above, the configural and the unique-cue theory. It was shown (Deisig et al., 2003) that the bees' performance was consistent with the unique-cue theory (i.e., when bees perceive an olfactory compound they detect the presence of the components in it, but they also assign a unique identity to the compound, resulting from the interaction of its components).

Another study used an original protocol, side-specific olfactory PER conditioning, which posed a nonlinear discrimination problem (Sandoz and Menzel, 2001). In this case, a thin plastic wall separated the honeybee's antennae during olfactory stimulation (**Figure 4(a)**). Bees were differentially conditioned using two odors (A and B). When odorants were delivered to one antenna, the contingency was A+ vs. B−, but it was reversed (A− vs. B+) when they were delivered to other antenna. This discrimination resembles a form of contextual learning, as the context of each antennal side (left vs. right) determines the contingency of the stimuli. Bees learned to respond appropriately to the rewarded odor and to inhibit their reaction to the nonrewarded odor on each side (Sandoz and Menzel, 2001). They thus solved this side-specific, nonelemental discrimination and remembered the contingencies learned 24 h later (**Figure 4(b)**). In this case, insight into the neural bases of this type of nonelemental problem solving was obtained by combining this protocol with *in vivo* calcium imaging recordings of glomerular activity at the level of both antennal lobes (**Figure 4(c)**). It was found that in naïve bees, odor response patterns were highly symmetrical (i.e., before conditioning, the same odorant elicited the same activation pattern in both antennal lobes). In conditioned bees, topical differences between sides were found. After side-specific conditioning, the left and right representations of the same odorant became slightly different, thus allowing differentiation between sides (Sandoz et al., 2003). Thus, this form of nonelemental learning resulted in a decorrelation of the representations of the conditioning odors between sides (**Figure 4(d)**). This result emphasizes that bees may form odor/side associations of the type AS1+/AS2− and BS1−/BS2+ (S1: side 1, S2: side 2). It is thus conceivable that structures situated upstream of the antennal lobes (e.g., the mushroom bodies or the lateral horn) are crucial for decoding differences in neural representations, such as those generated in side-specific conditioning.

Komischke et al. (2003) showed that bilateral olfactory input is required for solving a negative patterning discrimination. Given that the olfactory circuit remains practically unconnected between hemispheres to the mushroom bodies, this result suggests that the reading of a unique cue, arising from odorant interaction within the mixture, occurs upstream of the antennal lobes (i.e., at the level of the mushroom bodies). Komischke et al. (2005) used mushroom body–ablated honeybees to determine whether intact mushroom bodies are necessary to solve nonelemental olfactory discriminations. Bees were treated with hydroxyurea, which partially or totally removes the calyces (the input region to the mushroom bodies) (Malun, 1998). In previous studies, Scheiner et al. (2001) and Malun et al. (2002) showed that such ablations do not affect elemental forms of learning. Scheiner et al. (2001) showed that tactile learning, a form of elemental learning in which bees learn to associate an object within the range of one antenna with sucrose solution and discriminate it from an object presented to the opposite side, was unaffected in ablated bees. Malun et al. (2002) studied olfactory learning and showed that the presence of ablations did not impair acquisition of an elemental olfactory discrimination in which one odor was rewarded and another odor was nonrewarded (A+ vs. B−).

In the experiments of Komischke et al. (2005), bees with unilateral lesions of the mushroom bodies (a median calyx was usually absent) were trained in different olfactory discrimination problems. When odorants were delivered in a side-specific manner, bees with mushroom body lesions could not solve an

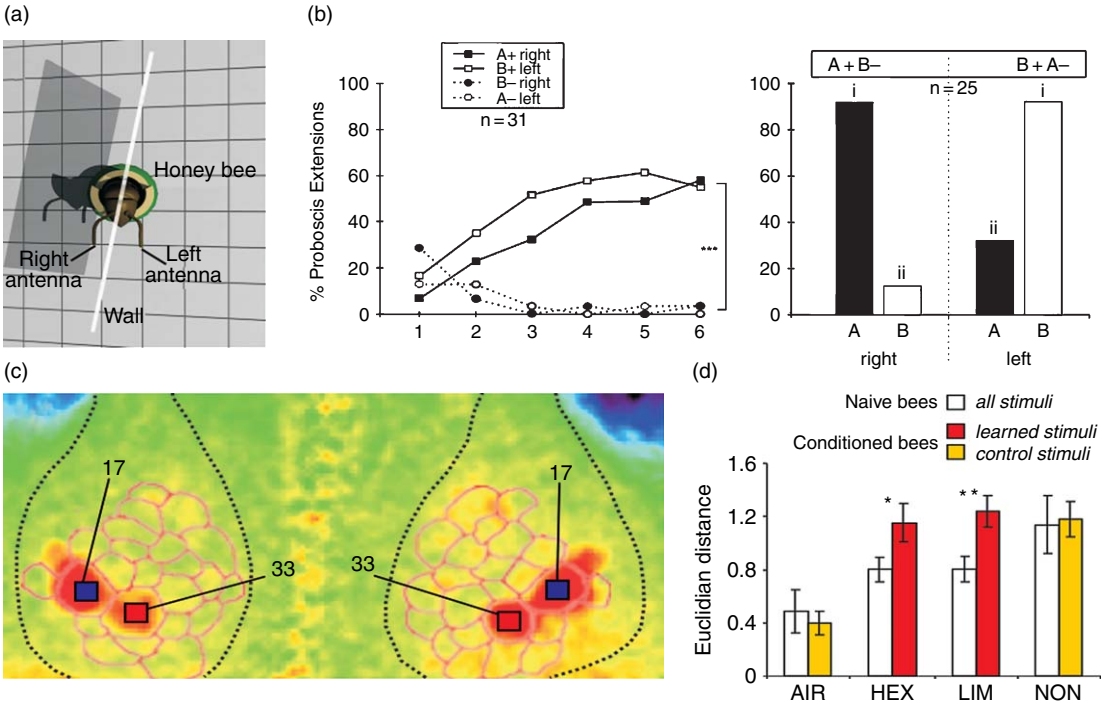

Figure 4 Side-specific olfactory conditioning of harnessed bees. (a) In this protocol, a harnessed bee is conditioned to discriminate two odorants A and B, which, depending on the presentation side (the antenna to which they are delivered), have different contingencies. To this end, the left and right antennae are separated by a thin wall glued along the bee body. Bees have to learn that when odorants are delivered to the right antenna, the contingency is A+ vs. B−, but it is A− vs. B+ if they are delivered to the left antenna. (b) Bees learn to solve this nonelemental double discrimination. The acquisition curves display the responses (% PER) to the rewarded odorants (A+ right and B+ left) and to the nonrewarded odorants (B− right and A− left). At the end of training, bees discriminate the rewarding from the nonrewarding stimuli. Twenty-four hours later, they still remember the appropriate contingencies on the appropriate side (histogram); n, number of bees trained or tested. (c) Simultaneous calcium imaging recording of both antennal lobes (delimited by the dashed lines). In this case, the response of naïve (nontrained) bees to Nonanol is shown. Glomeruli 17 and 33 are activated by this odor, and this activation is symmetric between sides. (d) The effect of side-specific conditioning on odor representation. The perceptual distance between the left and right representations of the same odorant was measured in a putative olfactory space, calculated for the honeybee. Odor representations differing perceptually are separated by a larger Euclidian distance in that space. For Hexanol and Limonene, the two odors used in the side-specific conditioning, the distance between left and right representations increased significantly as a consequence of training (red bars), thus showing that left and right representations of the same odorant became more different. For a control odorant, Nonanol, and for a clean-air control, the responses on the right and left antennal lobes were the same. Adapted from Sandoz JC and Menzel R (2001) Side-specificity of olfactory learning in the honeybee: Generalization between odors and sides. *Learn. Mem.* 8: 286–294 and Sandoz JC, Galizia CG, and Menzel R (2003) Side-specific olfactory conditioning leads to more specific odor representations between sides but not within sides in the honeybee antennal lobes. *Neuroscience* 120: 1137–1148; courtesy of J.C. Sandoz.

unambiguous double discrimination (Problem 1: A+ vs. B− on one antenna, C+ vs. D− on the other; A + B−/C + D−), even though each of the four odorants had an unambiguous outcome. When confronted with the ambiguous side-specific discrimination (Problem 2: A + vs. B− on one antenna, A− vs. B+ on the other; see above and **Figure 4**), bees were also impaired because they could only learn the discrimination proposed to their intact brain side. Nonablated bees could master both side-specific discriminations. When odorants were delivered simultaneously to both antennae (Problem 3: A + B−C + D−), ablated bees learned slower than normal bees.

Thus, in all three cases, the unilateral loss of a median calyx affected olfactory learning (Komischke et al., 2005). It was proposed that mushroom bodies are required for solving nonelemental discriminations but also elemental tasks whose complexity is enhanced by virtue of the number of stimuli involved (Problems 1 and 3: 4 stimuli). To solve an A + B−/A−B+ discrimination, information exchange between brain

hemispheres has to be inhibited such that A on the right side is not generalized to A on the left side, and vice versa. Mushroom body ablations could have an effect on this inhibition; in normal bees conditioning would result in inhibition of interhemispheric transfer. Ablations would restore the transfer from the intact side, thus creating confusion on the ablated side.

Cumulative experience seems to play a critical role for adopting elemental or nonelemental learning strategies (Giurfa et al., 2003). When free-flying bees are trained to fly into a Y-maze to collect sucrose solution on a rewarded visual target presented in one of the arms of the maze, the strategy underlying the choice of visual compounds changes along training. Bees were trained with color stimuli that were color disks violet (V), green (G), or yellow (Y), which were of equal salience for honeybees. Training followed an A+, BC+ design, followed by an AC vs. BC test. Training consisted of six (three A+ and three BC+), 20 (10 A+ and 10 BC+), or 40 (20 A+ and 20 BC+) acquisition trials, thus increasing the amount of experience on the same problem. Elemental models of compound processing predict that in the test (AC vs. BC), a preference for the nontrained stimulus AC should occur, whereas configural models predict a preference for the trained stimulus BC (Giurfa et al., 2003). After six training trials, bees favored an elemental strategy and preferred AC to BC during the tests. Increasing the number of training trials resulted in an increase of the choice of BC. Thus, short training favored processing the compound as the sum of its elements (elemental theory), whereas long training favored its processing as being different from the sum of its elements (configural theory). It was also observed that the change in stimulus processing was influenced by stimulus similarity. Color similarity favored configural processing with increasing experience (Giurfa et al., 2003), a result that was consistent with the results of honeybee olfactory compound conditioning (Deisig et al., 2002). Further factors favoring nonelemental compound processing and learning could be the spatial and temporal proximity of elements and the animals' previous experience.

There is, however, a limitation in these studies that has to be overcome in future research, namely that all compound stimuli used were of the same modality, either visual or olfactory. It would be important to verify that similar rules apply for intermodal compounds. This goal is particularly important in the context of searching for the neural substrates of nonelemental learning forms. If mushroom bodies, which are multimodal sensory integration structures, are indeed an important center for achieving nonlinear processing, affecting their normal function could have more dramatic consequences in the case of bimodal than unimodal compounds. In studying nonelemental learning with stimuli from different modalities, one should guarantee comparable salience between stimuli, because differences at this level may lead to overshadowing or blocking.

1.29.10 Positive Transfer of Learning in Honeybees

In this section, I focus on problem solving in which animals respond in an adaptive manner to novel stimuli that they have never encountered before and that do not predict a specific outcome *per se* based on the animals' past experience. Such a positive transfer of learning (Robertson, 2001) is therefore different from elemental forms of learning, which link known stimuli or actions to specific reinforcers. In the cases considered in this section, the response can attain levels in which it becomes independent of the physical nature of the stimuli presented so that it acts as a rule guiding the animal's behavior (like relational rules such as 'on top of' or 'larger than,' which can be applied irrespective of the similarity of the stimuli considered).

1.29.10.1 Categorization of Visual Stimuli

Positive transfer of learning is a distinctive characteristic of categorization performance. Categorization refers to the classification of perceptual input into defined functional groups (Harnard, 1987). It can be defined as the ability to group distinguishable objects or events on the basis of a common feature or set of features, and therefore to respond similarly to them (Troje et al., 1999; Delius et al., 2000; Zentall et al., 2002; *See* Chapter 1.08). Categorization deals, therefore, with the extraction of these defining features from objects of the subject's environment. A typical categorization experiment trains an animal to extract the basic attributes of a category and then tests it with novel stimuli that were never encountered before and that may or may not present the attributes of the category learned. If the animal chooses the novel stimuli based on these attributes, it classifies them as belonging to the category and therefore exhibits positive transfer of learning.

Using this basic design in which procedural modifications can be introduced, several studies have recently shown the ability of visual categorization in free-flying honeybees trained to discriminate different patterns and shapes. For instance, van Hateren et al. (1990) trained bees to discriminate two given gratings presented vertically and differently oriented (e.g., 45° vs. 135°) by rewarding one of these gratings with sucrose solution and not rewarding the other. Each bee was trained with a changing succession of pairs of different gratings, one of which was always rewarded, while the other was not. Despite the difference in pattern quality, all the rewarded patterns had the same edge orientation, and all the nonrewarded patterns also had a common orientation, perpendicular to the rewarded one. Under these circumstances, the bees had to extract and learn the orientation that was common to all rewarded patterns to solve the task. This was the only cue predicting reward delivery. In the tests, bees were presented with novel patterns, to which they had never been exposed, and that were all nonrewarded, but that exhibited the same stripe orientations as the rewarding and nonrewarding patterns employed during the training. In such transfer tests, bees chose the appropriate orientation despite the novelty of the structural details of the stimuli. Thus, bees could categorize visual stimuli on the basis of their global orientation.

They can also categorize visual patterns based on their bilateral symmetry. When trained with a succession of changing patterns to discriminate bilateral symmetry from asymmetry, bees learn to extract this information from very different figures and transfer it to novel symmetrical and asymmetrical patterns (Giurfa et al., 1996). Similar conclusions apply to other visual features such as radial symmetry, concentric pattern organization and pattern disruption (see Benard et al., 2006 for review), and even photographs belonging to a given class (e.g., radial flower, landscape, plant stem) (Zhang et al., 2004).

How could bees appropriately classify different photographs of radial flowers if these vary in color, size, dissection, and so on? An explanation is provided by Stach et al. (2004), who expanded the demonstration that bees can categorize visual stimuli based on their global orientation to show that different coexisting orientations can be considered at a time and integrated into a global stimulus representation that is the basis for the category (Stach et al., 2004). Thus, a radial flower would be, in fact, the conjunction of five or more radiating edges. Besides focusing on a single orientation, honeybees were shown to assemble different features to build a generic pattern representation, which could be used to respond appropriately to novel stimuli sharing this basic layout. Honeybees trained with a series of complex patterns sharing a common layout comprising four edge orientations remembered these orientations simultaneously in their appropriate positions and transferred their response to novel stimuli that preserved the trained layout (**Figure 5**). Honeybees also transferred their response to patterns with fewer correct orientations, depending on their match with the trained layout. These results show that honeybees extract regularities in their visual environment and establish correspondences among correlated features such that they generate a large set of object descriptions from a finite set of elements.

Thus, honeybees show positive transfer of learning from a trained to a novel set of stimuli, and their performance is consistent with the definition of categorization. Visual stimulus categorization is not, therefore, a prerogative of certain vertebrates. However, this result might not be surprising because it admits an elemental learning interpretation. To explain this interpretation, the possible neural mechanisms underlying categorization should be considered. If we admit that visual stimuli are categorized on the basis of specific features such as orientation, the neural implementation of category recognition could be relatively simple. The feature(s) allowing stimulus classification would activate specific neuronal detectors in the optic lobes, the visual areas of the bee brain. Examples of such feature detectors are the orientation detectors whose orientation and tuning have been already characterized by means of electrophysiological recordings in the honeybee optic lobes (Yang and Maddess, 1997). Thus, responding to different gratings with a common orientation of, say, 60° is simple because all these gratings will elicit the same neural activation in the same set of orientation detectors despite their different structural quality. In the case of category learning, the activation of an additional neural element is needed. This type of element would be a reinforcement neuron equivalent to VUMmx1 (Hammer, 1993; see above) but contacting the visual circuits at its relevant processing stages. Other VUM neurons whose function is still unknown are present in the bee brain (Schroter et al., 2006). It could be conceived that one of them (or more than one) acts as the neural basis of reinforcement in associative visual learning. Category learning could thus be reduced to the progressive reinforcement of an associative neural

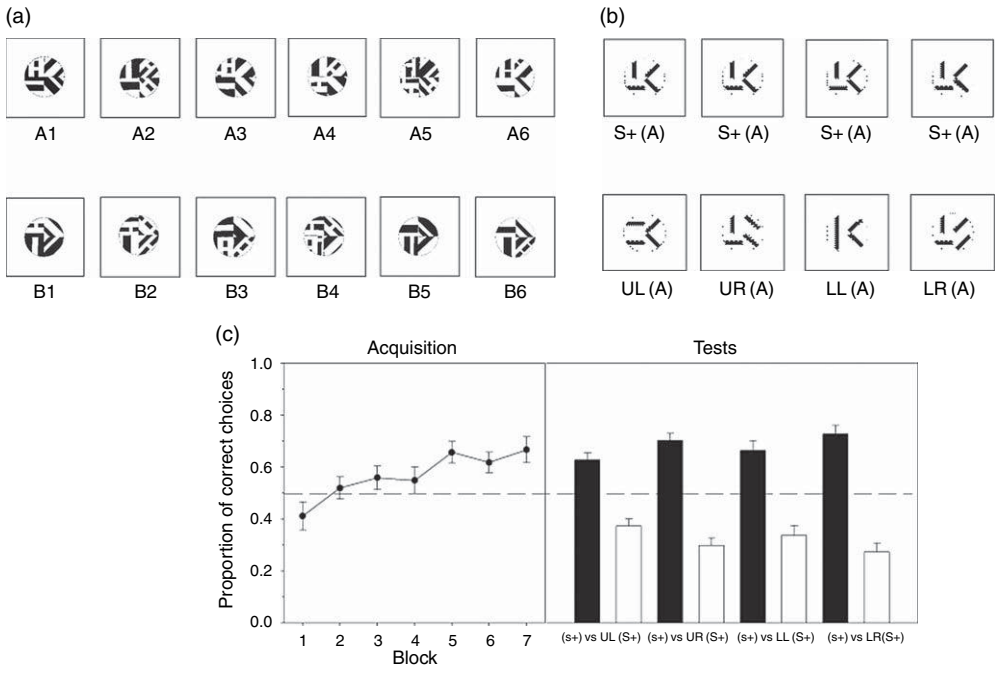

Figure 5 Categorization of visual patterns based on sets of multiple features. (a) Training stimuli used in Stach et al.'s experiments (2004). Bees were trained to discriminate A from B patterns during a random succession of A vs. B patterns. A patterns (A1–A6) differed from each other but shared a common layout defined by the spatial arrangement of orientations in the four quadrants. B patterns (B1–B6) shared a common layout perpendicular to that of A patterns. (b) Test stimuli used to determine whether or not bees extract the simplified layout of four bars from the rewarded A patterns. The four test pairs shown correspond to the honeybees trained with A patterns. Equivalent tests were performed with the honeybees trained with B patterns (not shown). S+, simplified layout of the rewarded A patterns; UL, upper-left bar rotated; UR, upper-right bar rotated; LL, lower-left bar rotated; LR, lower-right bar rotated. (c) Left panel: acquisition curve showing the pooled performance of bees rewarded on A and B patterns. The proportion of correct choices along seven blocks of six consecutive visits is shown. Bees learned to discriminate the rewarding patterns (A or B) used for the training and significantly improved their correct choices along training. Right panel: proportion of correct choices in the tests with the novel patterns. Bees always preferred the simplified layout of the training patterns previously rewarded (S+) to any variant in which one bar was rotated, thus showing that they were using the four bars in their appropriate spatial locations and orientations. Adapted from Stach S, Benard J, and Giurfa M (2004) Local-feature assembling in visual pattern recognition and generalization in honeybees. *Nature* 429: 758–761.

circuit relating visual-coding and reinforcement-coding neurons, similar to that underlying simple associative (e.g., Pavlovian) conditioning. From this perspective, even if categorization is viewed as a nonelemental learning form because it involves positive transfer of learning, it may simply rely on elemental links between CS and US.

1.29.10.2 Rule Learning

This argument is not applicable to rule learning in which positive transfer occurs independently of the physical nature of the stimuli considered. In this case, the animal learns relations between objects and not the objects themselves. Typical examples are the so-called rules of sameness and difference. These rules are demonstrated through the protocols of delayed matching to sample (DMTS) and delayed nonmatching to sample (DNMTS), respectively. In DMTS, animals are presented with a sample and then with a set of stimuli, one of which is identical to the sample and is reinforced. Since the sample is regularly changed, animals must learn the sameness rule, that is, always choose what is shown to you (the sample), independent of what else is shown to you. In DNMTS, the animal has to learn the opposite, that is, always choose the opposite of what is shown to you (the sample). Honeybees foraging in a Y-maze learn both rules (Giurfa et al., 2001). Bees were trained in a DMTS problem in which they were presented with a changing nonrewarded sample (i.e., one of two different color disks or one of two different black-and-white gratings,

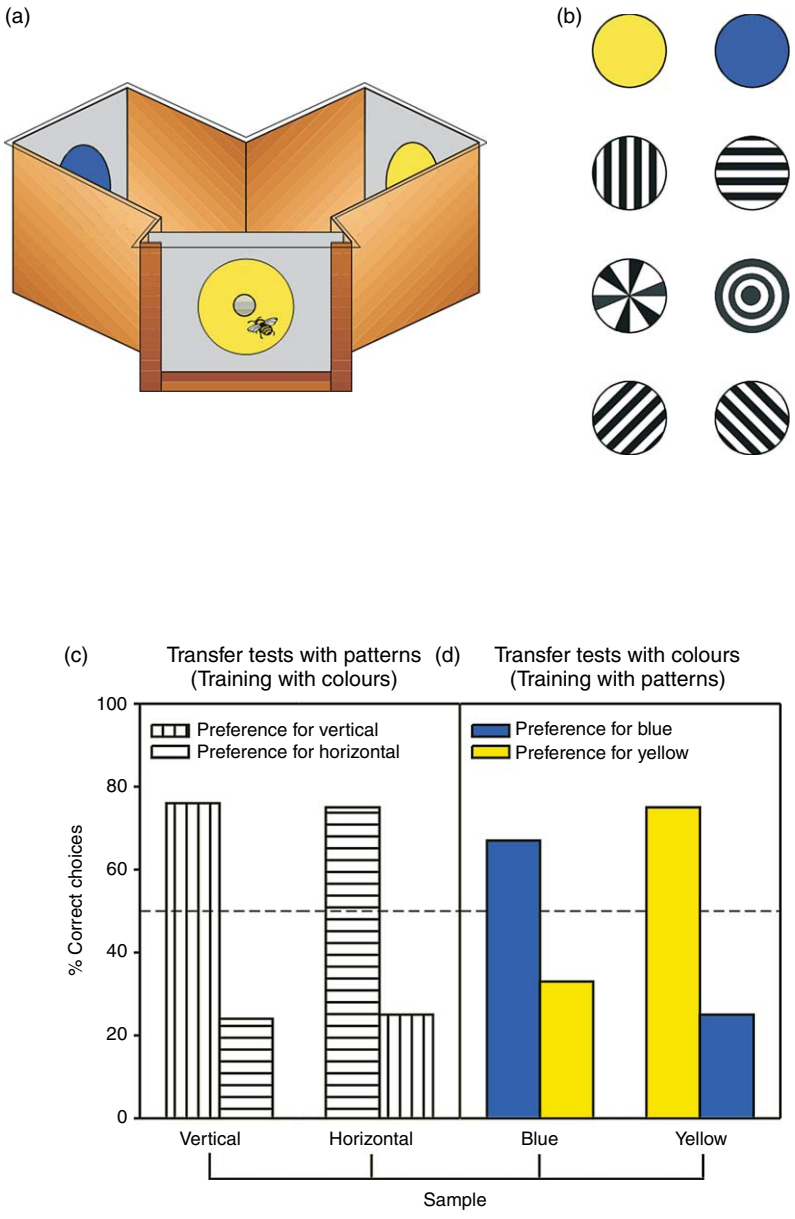

Figure 6 Rule learning in honeybees. Honeybees trained to collect sugar solution in a Y-maze (a) on a series of different patterns (b) learn a rule of sameness. Learning and transfer performance of bees in a delayed matching-to-sample task in which they were trained to colors (Experiment 1) or to black-and-white, vertical and horizontal gratings (Experiment 2). (c, d) Transfer tests with novel stimuli. (c) In Experiment 1, bees trained on the colors were tested on the gratings. (d) In Experiment 2, bees trained on the gratings were tested on the colors. In both cases, bees chose the novel stimuli corresponding to the sample, although they had no experience with such test stimuli. n denotes number of choices evaluated. Adapted from Giurfa M, Zhang S, Jenett A, Menzel R, and Srinivasan MV (2001) The concepts of 'sameness' and 'difference' in an insect. *Nature* 410: 930–933.

vertical or horizontal) at the entrance of a maze (Figure 6). The bees were rewarded only if they chose the stimulus identical to the sample once within the maze. Bees trained with colors and presented in transfer tests with black-and-white gratings that they had not experienced before solved the problem and chose the grating identical to the sample at the entrance of the maze. Similarly, bees trained with the gratings and tested with colors in transfer tests also solved the problem and chose the novel color corresponding to that of the sample grating at the maze entrance. Transfer was not limited to different kinds

of modalities (pattern vs. color) within the visual domain, but it could also operate between drastically different domains such as olfaction and vision (Giurfa et al., 2001). Furthermore, bees also mastered a DNMTS task, thus showing that they learn a rule of difference between stimuli (Giurfa et al., 2001). These results document that bees learn rules relating stimuli in their environment. The capacity of honeybees to solve a DMTS task has recently been verified and studied with respect to the working memory underlying it (Zhang et al., 2004, 2005). It was found that the working memory for the sample underlying the solving of DMTS lasts approximately 5 s (Zhang et al., 2005) and coincides with the duration of other visual and olfactory short-term memories characterized in simpler forms of associative learning in honeybees (Menzel, 1999; see above). Moreover, bees trained in a DMTS task can learn to pay attention to one of two different samples presented successively in a flight tunnel (either to the first or to the second) and can transfer the learning of this sequence weight to novel samples (Zhang et al., 2005).

Despite the honeybees' evident capacity to solve relational problems such as the DMTS or the DNMTS tasks, such capacities are not unlimited. In some cases, biological constraints may impede the solving of a particular problem for which rule extraction is necessary. It is therefore interesting to focus on a different example of rule learning that bees could not master, the transitive inference problem (Benard and Giurfa, 2004). In this problem, animals have to learn a transitive rule (i.e., A > B, B > C, then A > C). Preference for A over C in this context can be explained by two strategies: (1) deductive reasoning (Fersen et al., 1990), in which the experimental subjects construct and manipulate a unitary and linear representation of the implicit hierarchy A > B > C, or (2) responding as a function of reinforced and not reinforced experiences (Terrace and McGonigle, 1994), in which case animals choose among stimuli based on their associative strength (i.e., on the effective number of reinforced and nonreinforced experiences with the stimuli; A is always reinforced, and B is always nonreinforced).

To determine whether bees learn a transitive rule, they were trained using five different visual stimuli A, B, C, D, and E in a multiple discrimination task: A+ vs. B−, B+ vs. C−, C+ vs. D−, D+ vs. E− (Benard and Giurfa, 2004). Training involved overlapping of adjacent premise pairs (A > B, B > C, C > D, D > E), which underlie a linear hierarchy A > B > C > D > E. After training, bees were tested with B vs. D, a nonadjacent pair of stimuli that were never explicitly trained together. In theory, B and D have equivalent associative strengths because they are, in principle, equally associated with reinforcement or absence of it during training. Thus, if bees were guided by the stimulus's associative strength, they should choose randomly between B and D. If, however, bees used a transitive rule, they should prefer B to D. Honeybees learned the premise pairs as long as these were trained as uninterrupted, consecutive blocks of trials (Benard and Giurfa, 2004). But if shorter and interspersed blocks of trials were used, such that bees had to master all pairs practically simultaneously, performance collapsed, and bees did not learn the premise pairs. The bees' choice was significantly influenced by their experience with the last pair of stimuli (D+ vs. E−), such that they preferred D and avoided E. In the tests, no preference for B over D was found. Although this result agrees with an evaluation of stimuli in terms of their associative strength (see above), during training bees visited B more when it was rewarding than D, such that a preference for B should have been expected only if the associative strength were guiding the bees' choices. It was then concluded that bees do not establish transitive inferences between stimuli but, rather, guide their choices by the joint action of a recency effect (preference for the last rewarded stimulus, D) and by an evaluation of the associative strength of the stimuli (in which case preference for B should be evident). As the former supports choosing D while the latter supports choosing B, choosing B and D equally in the tests could be explained (Benard and Giurfa, 2004). At any rate, memory constraints (in this case, that simultaneous mastering of the different premise pairs was not possible and that the last excitatory memory seems to predominate over previous memories) impeded learning the transitive rule. Recently, Chen (2006) demonstrated that failure to master several consecutive visual discriminations is a result of the response competition occurring when animals are tested. This may explain why bees in the transitive inference protocol were unable to master the successive short blocks of training with different premise pairs.

1.29.11 Distributed Cognition in Honeybees

So far, we have concentrated on individual cognitive capabilities, but bees live in societies and therefore face problems that require coordination, task sharing, and collective decision making. From this perspective, it is

legitimate to ask whether collective behaviors reflect or even surpass individual plasticity, because of, for instance, the possible additive effect of individual cognitive capacities.

This question has been the subject of debate in the case of social insects in which colonies were considered superorganisms (Southwick, 1983; Seeley, 1989). It has been argued that the superorganism protects and constitutes itself from colony recognition systems based on cuticular hydrocarbons that are transferred between individuals within the colony, thus obscuring, in theory, individual identity. The metaphor of the superorganism may be in a sense misleading because an individually behaving organism is made from cells and structures that are tightly interconnected by complex neuronal, circulatory, and regulatory networks and has a central brain that commands and produces behavior. The superorganism, on the other hand, is made up of individuals that may be interconnected by complex chemical interactions but that are rather autonomous and can hardly be compared to constituent cells. The essential difference, however, is that although an insect colony produces collective behavior, it does not have a central brain to command and control such behavior. On the contrary, studies on collective decision making in social insects show that collective behavioral patterns can arise from simple interactions between individuals, without any central control and without memory (Theraulaz et al., 2003).

From this point of view, the sophisticated cognitive capacities that honeybees exhibit in individual tests are not required for the close coordination of the social group. Differences in individual thresholds for reacting to environmental sensory stimuli seem to be a critical factor for the emergence of collective behaviors based on task partitioning. This may account, for instance, for the collective behavior of nest choice by a honeybee swarm. Group decision making in honeybee swarms has been studied (see Seeley and Vischer, 2004) to determine the rules underlying collective choice. It was found that the essence of a swarm′s decision making relies on sensing a quorum (a sufficient number of scouts) at one of the nest sites rather than sensing a consensus (agreement of dancing scouts) at the swarm cluster. By this quorum-sensing hypothesis, a scout bee votes for a site by spending time at it. Somehow the scouts act and interact so that their numbers rise faster at superior sites, and somehow the bees at each site monitor their numbers there so that they know whether they have reached the threshold number (quorum) and can proceed to initiating the swarm′s move to this site. Exactly how scout bees sense a quorum remains an enigma (Seeley and Visscher, 2004). They may use visual, olfactory, or even tactile information to assess the number of fellow scouts at a site. But the complex migration pattern involving the coordinate displacement of thousands of bees does not require sophisticated mechanisms such as dance comparisons or verifying the reliability of information conveyed by a hive mate. In short, the bees appear to begin preparations for liftoff as soon as enough of the scout bees, but not all of them, have approved of one of the potential nest sites.

The interesting conclusion emerging from studies on social insect collective behavior is that individuals, who may be viewed as extremely sophisticated at the cognitive level when performing some individual tasks, appear to be automatons with limited cognitive capacities when performing collective tasks. This difference may seem puzzling and could be the result of cognitive richness being lost or at least temporally inhibited in a social context. However, a possible explanation is that in an individual and in a social context, the animal will adopt the behavioral strategies leading to adaptive solutions, either boosting or sacrificing what researchers would view as cognitive sophistication. Whenever simple behaviors can lead to adaptive solutions, they are adopted. When, on the contrary, cognitive abilities are required, they are used. The critical question in this context is therefore what determines the adoption of one or the other level of cognitive complexity? Which factors are responsible for the fact that an ant or a bee that can learn and memorize several cues while foraging, solve complex discriminations, and generate novel behaviors leading to adaptive solutions behaves like an automaton following a reduced set of repetitive patterns and simple rules in a social context? Which physiological changes, if any, determine the passage from one state to the other? Do social regulation pheromones intervene in the expression or inhibition of behavioral autonomy in a social context by acting on neurotransmitter levels in the insect nervous system? Do social pheromones determine changes in immediate early gene (IEG) expression in the brain affecting cognitive processing? So far, we have no answers to these questions, but they can be approached experimentally. Studying whether or not individual learning and memory are modified by exposure to social pheromones or by chemosensory cues within a group and whether or not biogenic amine and neurotransmitter levels and IEG expression are changed in the presence of a group of cospecifics are just some of the questions that need to be considered.

1.29.12 Conclusion

This chapter underlines the enormous richness of experience-dependent behavior in honeybees, its high flexibility, and the fact that it is possible to formalize and characterize in controlled laboratory protocols some forms of cognitive processing. Adopting rigorous definitions from elemental and nonelemental learning is useful to determine the extent to which honeybees can go beyond simple forms of associative learning. This type of experimental approach is possible, as illustrated by the numerous examples reviewed here, which have made it possible to appreciate the sophistication of cognitive processing in a honeybee, which, as most insects, was traditionally considered as being limited in terms of its cognitive capabilities.

Contrary to simple forms of associative learning for which specific neural circuits have been identified, more work is needed to relate complex problem solving to neural structures of the honeybee brain. The existing evidence points toward the mushroom bodies, a central structure in the insect brain that has been repeatedly associated with learning and memory capabilities (Menzel, 1999). It has been shown that some elemental discriminations can be achieved without the mushroom bodies (Malun et al., 2002), but this does not seem to be so obvious in the case of nonelemental discriminations. Although specific substrates or circuits for complex problem solving in the bee brain are still unknown, it is possible to be optimistic with respect to their future identification. In this case, what is delaying our understanding of cognitive brain processing is not the technical level but, rather, that up to now researchers have not dared to raise questions on complex cognitive processing in an insect.

What are the specific limitations of the bee brain when compared with larger brains, and what might the structural/functional basis be? To address this question, one would need to know more about its deficiencies, an area that has so far been investigated very little (but see Benard and Giurfa, 2004). Because of obvious limitation in space, we have not discussed the role of different forms of learning in natural contexts such as navigation and communication. These contexts also offer promising frameworks for the study of cognitive processing. Questions such as the nature of space representation and the flexibility of communication strategies are important to characterize the potential of the bee brain. They need to be related, when possible, to underlying neural circuits and structures, a task that has been impossible until now.

Studies on honeybee behavior allow researchers to be optimistic in view of these questions. Moreover, as learning in honeybees can be compared to that of vertebrates in many senses, the honeybee may serve as a model system for understanding intermediate levels of complexity of cognitive functions and their neural substrates.

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1.30 Behavioral and Circuit Analysis of Learning and Memory in Mollusks

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1.30.1 Introduction

The major advantage of using gastropod mollusks for investigating neural mechanisms underlying learning and memory is that behavioral studies can be linked to circuit analysis by taking advantage of the ability to identify individual neurons with known electrical properties and synaptic connectivity (Benjamin et al., 2005). Simple forms of associative learning such as classical and operant conditioning and nonassociative forms such as habituation and sensitization have been extensively investigated. The use of a wide variety of learning paradigms in a number of different gastropod species allows comparisons to be made of underlying neural mechanisms involved in memory formation in different types of learning (e.g., associative versus nonassociative, classical versus operant conditioning). Important progress has been made in understanding the mechanisms of synaptic plasticity in gastropod memory formation, but nonsynaptic (cellular) mechanisms such as changes in excitability also have been increasingly recognized as contributing to memory formation. These data on gastropod molluscs are of general importance in understanding the mechanisms of memory formation in the brain.

1.30.2 Model Circuits

A brief review of the four molluscan circuits most useful for learning and memory studies will be given next because the synaptic and cellular changes that underlie memory formation are located within these

circuits. Two general types of circuit will be described: defensive reflexes in *Aplysia* and *Hermissenda* and the more complex central pattern generator (CPG)-mediated feeding and respiratory circuits of *Lymnaea*.

1.30.2.1 *Aplysia* Gill-Siphon Defensive Withdrawal Reflex

A brief tactile stimulus applied to the siphon of *Aplysia* elicits gill and siphon withdrawal into the protective mantle cavity (**Figure 1(ai)**). The withdrawal responses in these two organs are mediated by identified sensory and motor neurons located in the abdominal ganglia (**Figure 1(aii)**), and because the strength of these responses is modified by experience, they have been extensively used to study the mechanisms underlying both associative and nonassociative forms of learning (Hawkins et al., 2006). Early studies of habituation focused on gill withdrawal plasticity, but the ease of identifying siphon responses in the live animal and the identification of a distinct set of siphon motor neurons (the LFS cells, **Figure 1(aii)**) has meant that the siphon circuit has been used mainly for more recent studies of sensitization and classical conditioning (Antonov et al., 2003). A variety of centrally located siphon sensory neurons and excitatory and inhibitory interneurons provide synaptic input to the LFS motor neurons but the direct LE mechanosensory to LFS monosynaptic pathway (**Figure 1(aii)**) has been the main focus of attention for learning studies, although sensorimotor synapses in the pleural and pedal ganglia also have been used. Monosynaptic excitatory connections from the LE to LFS motor neurons have been estimated to mediate about one-third of the siphon reflex response that corresponds to siphon flaring in the intact animal. The remainder of the response is mediated by peripheral motor neurons (Hawkins et al., 2006).

For habituation studies, sequences of weak touch stimuli are applied to the siphon, and the strength of the gill/siphon withdrawal reflex is measured either mechanically or by using a photocell to record displacement. Electrical shocks are applied to the tail or neck for sensitization of the siphon reflex, and repeated shocks (unconditioned stimulus, US) are paired with siphon touch (conditioned stimulus, CS) for behavioral studies of aversive classical conditioning. The detailed pathway by which the electrical stimuli influence the gill-siphon circuitry is not known for certain, although it may recruit the serotonergic cerebral CB1 neurons that have projections to the abdominal ganglion (Mackey et al., 1989).

1.30.2.2 *Hermissenda* Statocyst-Mediated Behaviors

High-speed rotation or orbital shaking elicits two types of behavioral response in *Hermissenda:* foot shortening (**Figure 1(bi)**) and inhibition of the normally positive phototactic response, indicated by a reduced forward locomotion toward light (Crow, 2004). The type of stimuli used to elicit this response in the laboratory mimics the natural response to mechanical turbulence caused by wave action that results in the animal clinging to the substrate for protection. The sensory response is mediated by a pair of gravity detector organs called the statocysts that have hair cell receptors analogous in function to the vestibular hair cells of vertebrates. In *Hermissenda*, depolarization of the hair cells by rotation generates responses in interneurons in the central ganglia that excite foot motor neurons responsible for foot shortening and inhibit ciliary motor neurons to inhibit forward locomotion (**Figure 1(bii)**). The interneurons involved in the two behavioral responses are different, giving independent pathways for the control of the two unconditioned responses.

Rotation of the body is used as the aversive US in behavioral associative conditioning studies, and a light flash is used as the CS. Repeated pairing of these two stimuli over several days leads to a reduction of the phototactic response to the CS and foot shortening similar to the previously described response to the US. Photoreceptors in the eye respond to the light CS, and these cells have a variety of excitatory and inhibitory synaptic connections with sensory receptors and interneurons of the US statocyst pathway. However, these synaptic connections are ineffective in eliciting unconditioned responses in naive animals (Crow, 2004).

1.30.2.3 *Lymnaea* Feeding

Feeding in *Lymnaea* consists of a sequence of repetitive movements called rasps. During each rasp, the mouth opens (**Figure 1(ci)**), and a toothed radula is scraped forward over the food substrate (protraction phase). Food is then lifted into the mouth (retraction phase), which closes while the food is being swallowed (swallow phase), and the sequence is repeated. Rhythmic

movements of the feeding muscles are driven by a network of motor neurons (B1 to B10) that, in turn, are driven by synaptic inputs from a feeding CPG network of interneurons (**Figure 1(cii)**). Each phase of the feeding rhythm is generated by one of three main types of CPG interneurons, N1 (protraction), N2 (retraction), N3 (swallow), providing sequences of excitatory and inhibitory synaptic inputs to motor neurons active in different phases of the feeding rhythm (Benjamin and Elliott, 1989). CPG-driven rhythmic electrical activity can be recorded in the feeding network even in the absence of feeding muscles, and this is called fictive feeding. Activity in the motor neurons and CPG neurons is modulated by identified higher-order interneurons (**Figure 1(cii)**), such as the cerebral giant cells (CGCs) and cerebral ventral 1 cells (CV1s). These higher-order neurons have been the major focus of learning and memory studies (Benjamin et al., 2000). The CGCs act as gating neurons in the feeding circuit. Increased CGC spiking activity during feeding facilitates feeding responses to food. The CV1 cells are members of a larger population of neurons called the cerebrobuccal interneurons (CBIs) (**Figure 1(cii)**) that are commandlike neurons involved in the activation of feeding.

Sucrose is an effective chemical stimulus for feeding and is therefore used as the US for reward conditioning. At the cellular level, sucrose applied to the lips in semi-intact preparations induces fictive feeding in motor neurons and interneurons of the feeding network. The CPG neurons are activated in the sucrose-driven fictive feeding rhythm, along with the modulatory CGC and CV1 cells.

The CS used for reward conditioning in *Lymnaea* is either a chemical (amyl acetate) or a tactile stimulus (a gentle brushstroke applied to the lips). The chemical CS was previously thought to have no effect on feeding ('neutral stimulus'), either at the behavioral or electrophysiological levels, but has recently been shown to have stimulatory or inhibitory effects in naive animals, depending on concentration (Straub et al., 2006). Touch to the lips, monitored either at the behavioral or the electrophysiological level, cannot initiate or maintain feeding, but nevertheless touch produced a complex sequence of inhibitory and excitatory synaptic inputs on all neurons of the feeding network (Staras et al., 1999b).

1.30.2.4 *Lymnaea* Respiration

The aquatic pulmonate snail *Lymnaea* can breathe either through the skin or through a simple lung. As their name implies, *Lymnaea stagnalis* populations often live in stagnant water, and when the environment becomes hypoxic, the snails float to the surface and perform rhythmic opening and closing movements of their pulmonary opening, the pneumostome (**Figure 1(di)**). This aerial respiration behavior is controlled by a respiratory CPG, the three main components of which are the right pedal dorsal 1 (RPeD1), input 3 (IP3), and visceral dorsal 4 (VD4) interneurons (**Figure 1(dii)**). These components provide synaptic inputs to identified motor neurons (I and J, opener, and K, closer) innervating pneumostome opener and closer muscles (**Figure 1(dii)**). The chemosensory stimulus (hypoxia) that triggers pneumostome opening first activates sensory cells in the pneumostome–osphradial area, which in turn provides excitatory afferent inputs to RPeD1. Through its synaptic connections with the other members of the CPG network, activation of RPeD1 initiates CPG activity, which underlies the respiratory rhythm. Tactile stimulation of the pneumostome area evokes pneumostome closure and stops aerial respiratory behavior. This type of tactile stimulation is used as the positive punishment in behavioral operant conditioning studies (Lukowiak et al., 2003).

1.30.3 Nonassociative Learning: Habituation and Sensitization in the Gill-Siphon Withdrawal Reflex

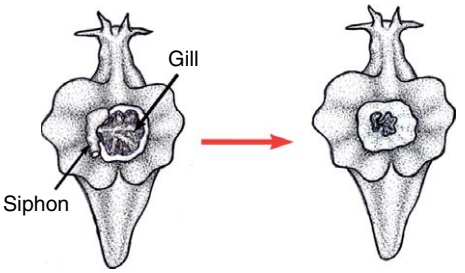
The gill-siphon withdrawal reflex of *Aplysia* shows both habituation and sensitization (Kandel, 1976). When a weak touch (usually by a calibrated water jet) is applied repeatedly to the siphon or adjacent mantle skin at intervals of between 30 s and 3 min, the gill withdrawal response habituates (decrements) to about 30% of control values after 10–15 trials. This short-term habituation lasts for several hours, but if these trials (10 per day) are repeated over 4 days then a long-term habituation of the gill withdrawal response is induced that lasts for up to 3 weeks. If, prior to touch, a single strong noxious stimulus, such as an electric shock, is applied to the tail or neck, the subsequent touch-evoked response is enhanced or sensitized for a few hours. Long-term sensitization can be induced by the application of multiple shocks. Applying four shocks a day to the head for 4 days significantly increased the duration of withdrawal so that it was greater than in controls up to 1 week after training. Both of

these nonassociative phenomena are central processes that involve changes in synaptic strength at the sensory level to motor synapses that mediate the normal gill-siphon withdrawal reflex.

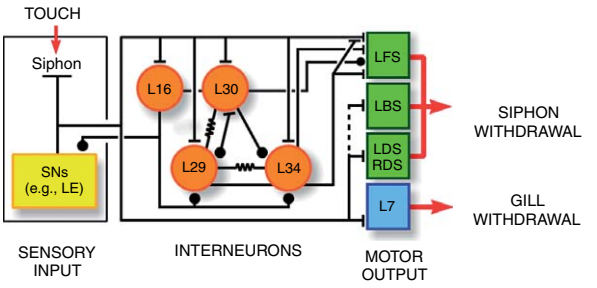
1.30.3.1 Habituation

Behavioral habituation was found to be paralleled by suppression of neurotransmitter release from the presynaptic terminals of the touch-sensitive

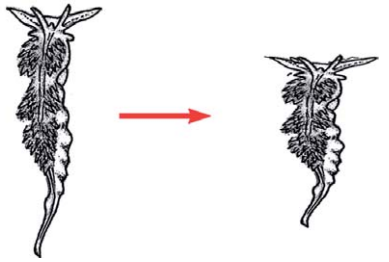
(ai) *Aplysia* defensive reflexes



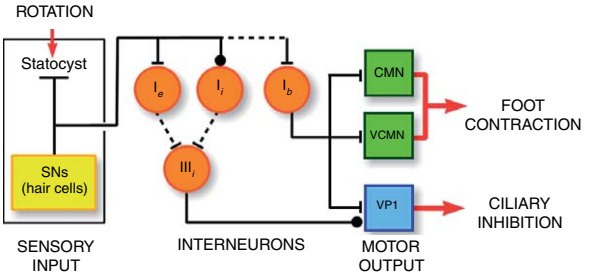
(aii) *Aplysia* siphon and gill withdrawal networks



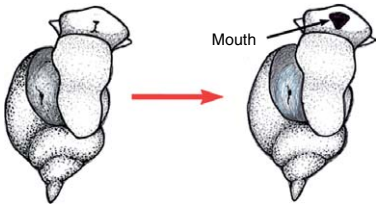
(bi) *Hermissenda* defensive reflexes



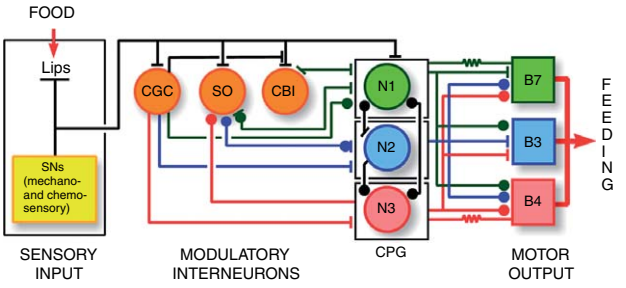
(bii) *Hermissenda* foot contraction and ciliary inhibition networks



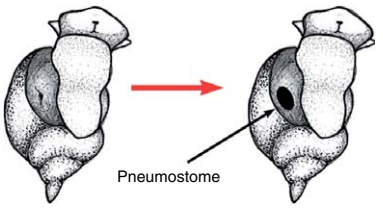
(ci) *Lymnaea* feeding behavior



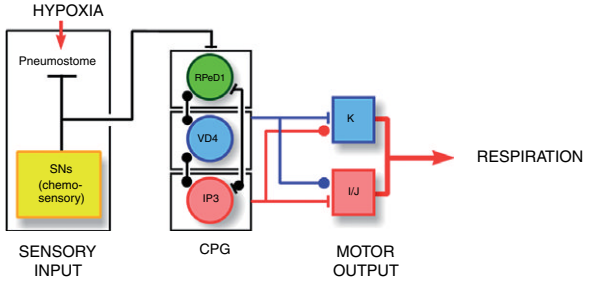
(cii) *Lymnaea* feeding network



(di) *Lymnaea* aerial respiration



(dii) *Lymnaea* respiratory network



mechanosensory neurons resulting in homosynaptic depression (HSD). A consequent reduction in the size of the motor neuron excitatory postsynaptic potential (EPSP) is followed by a reduction in the activation of the motor neurons, which consequently fires less. Both calcium-dependent and -independent mechanisms have been suggested to underlie HSD, the cellular mechanism of habituation.

There appears to be a consensus that short-term habituation (STH) involved purely presynaptic mechanisms (Armitage and Siegelbaum, 1998; Ezzeddine and Glanzman, 2003), but recent work by Ezzeddine and Glanzman (2003) suggests that long-term habituation (LTH) may involve postsynaptic mechanisms as well. In a behavioral preparation, these authors showed that induction of LTH depended on the activation of specific types of glutamate receptors presumed to be located on the postsynaptic motor neurons. LTH was blocked either in the presence of the *N*-methyl-D-aspartate (NMDA) antagonist 2-amino-5-phosphonopentanoic acid (APV) or the alpha-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA) antagonist 6,7-dinitroquinoxaline-2,3-dione (DNQX) (Ezzeddine and Glanzman, 2003). DNQX had no effects on STH, as shown in earlier experiments (Armitage and Siegelbaum, 1998).

1.30.3.2 Sensitization

Sensitizing stimuli have the opposite effect of habituation, causing an increase in transmitter release (reviewed in Carew and Sahley, 1986). Sensitizing stimuli cause the release of the transmitter serotonin (5-hydroxytryptamine, 5-HT) from facilitatory interneurons onto the sensory neuron terminals of the mechanosensory neurons, and this transmitter acts presynaptically to facilitate synaptic transmission at the sensorimotor junction. This causes the motor neurons to fire more after sensitization, thereby increasing the strength of the withdrawal reflex. This type of heterosynaptic facilitation can be mimicked in reduced preparations and in a simplified cell culture system if tail shock is replaced by brief applications of 5-HT to the sensory neurons. The mechanisms underlying presynaptic facilitation involve two types of synaptic mechanisms that result in an increase in transmitter release (reviewed in Hawkins et al., 2006). The first synaptic mechanism involves an increase in the duration of the spike in the sensory neuron. A second synaptic mechanism is independent of spike broadening and is thought to involve vesicle mobilization. A nonsynaptic mechanism increases the excitability of the sensory neurons, making it more likely that the

Figure 1 Molluscan behaviors and underlying neural circuitries most widely used in studies of the cellular mechanisms of learning and memory. (ai) Touch-evoked withdrawal reflex of the gill and siphon in *Aplysia californica* (drawing by Dr. I. Kemenes). (aii) Sensory neurons (SNs, yellow square) activated by touch to the siphon provide direct excitatory synaptic inputs to motor neurons innervating the gill and siphon (green and blue squares, respectively). In addition, there are indirect excitatory and inhibitory connections from the sensory to the motor neurons, which are mediated by a set of interneurons (orange circles) interconnected by both chemical and electrical synapses. Touch can normally only evoke a weak contraction of the siphon and gill, but the reflex becomes stronger after sensitization and classical conditioning (see **Figure 2**). (bi) Foot contraction and ciliary inhibition evoked by rotation in *Hermissenda crassicornis* (drawing by Dr. I. Kemenes). (bii) Sensory cells activated by rotation provide polysynaptic excitatory and inhibitory inputs to motor neurons responsible for foot contraction (green squares) and ciliary inhibition (blue square). Dashed lines represent polysynaptic connections with potential interneurons not yet identified. When a visual input is repeatedly paired with rotation, it will become effective in evoking both foot contraction and ciliary inhibition (see **Figure 3**). (ci) Feeding behavior in *Lymnaea stagnalis* (drawing by Dr. I. Kemenes). (cii) Chemosensory neurons located in the lip structures detect the presence of food or chemostimulants, such as sucrose. Excitatory inputs from the sensory pathways are distributed in parallel to modulatory interneurons (orange circles) and also to central pattern generators (CPG) neurons (green, blue, and pink circles). The cerebral giant cells (CGCs) may presynaptically modulate certain types of chemosensory inputs to the cerebrobuccal interneurons (CBIs) (see **Figure 4**). The complex synaptic connectivity between the modulatory and N1 interneurons leads to activation of the whole CPG, with the protraction (N1), rasp (N2), and swallow (N3) phases of the feeding cycle following in sequence due to the synaptic connectivity within the CPG and their intrinsic properties. Several subtypes of each of the N cells have been characterized, but because of the complexity of their synaptic connections with modulatory interneurons and motor neurons, only a generalized representative of each N-type is shown. The rhythmic pattern generated by the CPG drives protraction, rasp, and swallow phase motor neurons, such as B7 (green squares), B3 (blue squares), and B4 (pink squares), leading to sequences of muscular activity and feeding movements executed by the mouth (shown in drawing), the radula, and the buccal mass. Reward classical conditioning leads to an enhanced activation of the feeding network by tactile or chemically (see **Figure 4**) conditioned stimuli. (di) Hypoxia-evoked opening of the pneumostome in *Lymnaea stagnalis* (drawing by Dr. I. Kemenes). (dii) The motor neurons are excited or inhibited by a three-neuron CPG network activated by chemosensory inputs. Operant conditioning leads to the suppression of pneumostome opening and aerial respiration (see text). Dots indicate inhibitory chemical synapses, bars excitatory chemical synaptic connections, and resistor symbols electrotonic (electrical) synapses.

sensory neurons will respond to siphon touch after sensitization. These types of presynaptic mechanisms are produced by single brief applications of 5-HT (1 min) or single shocks to the tail, and they are believed to underlie behavioral short-term sensitization or short-term facilitation (STF) of synaptic transmission seen in reduced preparations and in cell culture.

Long term-facilitation (LTF) of synaptic responses in culture is induced by repeated and spaced application of 5-HT and lasts for as long as the cells survive in culture. Its behavioral equivalent, long-term sensitization, lasting for days or weeks, requires repetitive sensitizing stimuli lasting for 1 to 1.5 h. Recently, an intermediate-term memory (ITM) for sensitization and correlated intermediate-term synaptic facilitation (ITF) have been identified, lasting for up to 85 minutes (Ghirardi et al., 1995; Sutton and Carew, 2000).

There is a consensus that short-term sensitization and STF are due to purely presynaptic mechanisms (Glanzman, 2006; Hawkins et al., 2006) and until recently this was also thought to be the case for other forms of sensitization, such as ITM. However, important work by the Glanzman laboratory in culture and in reduced preparations (e.g., Lin and Glanzman, 1994; Li et al., 2005) and now confirmed by simultaneous behavioral and electrophysiological investigations by Antonov et al. in a more intact preparation (reviewed in Hawkins et al., 2006) have clearly shown that postsynaptic mechanisms are also involved in ITF.

A 10-minute application of serotonin in culture induced synaptic ITF, after a delay of about 5 minutes, and facilitated synaptic transmission for at least 50 minutes without decrement (reviewed in Glanzman, 2006). This ITF induced by serotonin application was found to depend on the activation of AMPA-like glutamate receptors in the motor neurons because it was blocked by DNQX. Prior injection of exocytotic inhibitors into the motor neurons blocked this facilitation, suggesting that 5-HT might stimulate the insertion of AMPA receptors into the motor neuron membrane. This trafficking of AMPA receptors is known to involve calcium/calmodulin-dependent protein kinase II (CaMKII) in vertebrate systems, so it is interesting that injecting inhibitors of this kinase into *Aplysia* motor neurons reduced synaptic ITF (see Hawkins et al., 2006). ITF also depends on the elevation of calcium levels in the motor neurons. Injection of BAPTA (1,2-bis(o-aminophenoxy)ethane-N,N,N′,N′-tetraacetic acid), a rapid chelator of intracellular calcium, blocked the delayed facilitation of the sensorimotor synapse *in vitro* (Li et al., 2005), induced by a 10-min application of 5-HT. 5-HT causes a release of intracellular calcium, mediated by both IP3 and ryanodine receptors (Li et al., 2005). ITF produced by 10-min applications of 5-HT also involves presynaptic mechanisms because injection of protein kinase C (PKC) inhibitors into the sensory neurons in culture also reduces the facilitatory effects of 5-HT on synaptic transmission (Hawkins et al., 2006).

In summary, ITF of sensorimotor synapses, a mechanism thought to underlie behavioral sensitization, involves both presynaptic (PKC-dependent) and postsynaptic (calcium- and CaMKII-dependent) mechanisms with CaMKII suggested to be linked to insertion of AMPA receptors in the postsynaptic membrane. Short-term sensitization involves only presynaptic mechanisms, mainly involving a cyclic adenosine monophosphate (cAMP)-dependent protein kinase A (PKA) pathway.

Long-term sensitization has also been investigated for changes in postsynaptic mechanisms. In a pioneering study, Cleary et al. (1998) showed that the biophysical properties of tail motor neurons were changed after behavioral long-term sensitization, resulting in hyperpolarization of the resting membrane potential and a decrease in spike threshold. This suggests that these postsynaptic neurons are a locus for memory and provides evidence for the importance of postsynaptic mechanisms in both long-term and intermediate-term sensitization (*See* Chapters 4.03 and 4.38 for additional details on the molecular mechanisms of short-, intermediate-, and long-term sensitization).

1.30.4 Associative Learning

1.30.4.1 Aversive Classical Conditioning of the *Aplysia* Gill-Siphon Withdrawal Reflex

Carew and colleagues (see Carew and Sahley, 1986) showed that the gill and siphon withdrawal reflex is subject to aversive classical conditioning as well as habituation and sensitization. In their associative conditioning paradigm, they paired a weak tactile stimulus (the CS) to the siphon with a strong electric shock to the tail (the US). After 15 trials, the CS came to elicit a stronger gill withdrawal than controls; the effect lasted for several days. Later they showed that differential classical conditioning also worked. A weak tactile stimulus applied to the siphon or mantle shelf was either paired with shock (CS+) or not paired (CS–) in the

same animal. Differences between the effects on gill withdrawal at the two sites were enhanced, even after a single trial. Cellular analysis of differential conditioning, using a reduced preparation, showed that the mechanism underlying classical conditioning of the gill withdrawal response was the elaboration of presynaptic facilitation that was previously shown to underlie sensitization, except that the effect of the pairing of CS and US produced an even greater facilitation of the withdrawal response. This effect was called activity-dependent presynaptic facilitation (ADPF) (Hawkins et al., 1983). Evidence for this mechanism was obtained in a reduced preparation where the effects of touch were mimicked by stimulation of two different sensory neurons that made excitatory synaptic connections with siphonal motor neurons. Differential conditioning produced a significantly greater enhancement of the sensorimotor synapse if weak sensory neuron spike activation (by current injection) was followed by the tail shock (CS+) than if the sensory neuron stimulation was unpaired (CS–) or if shock occurred alone (sensitization). This result supported the notion of ADPF because of its dependence on temporal pairing. Like sensitization, this mechanism was shown to be presynaptic and could also be mimicked by the application of serotonin. With a larger number of pairings, a longer-term form of synaptic plasticity is induced underpinned by changes in gene regulation and synaptic remodeling (*See* Chapter 4.10).

As was the case with sensitization, the early model for aversive classical conditioning was entirely presynaptic, but the discovery that sensorimotor synapses in culture or in reduced preparations exhibit NMDA-dependent long-term potentiation (LTP) (Glanzman, 1995; Roberts and Glanzman, 2003) led to the now generally accepted hypothesis that associative learning involves postsynaptic processes utilizing Hebbian-type LTP as well as presynaptic ADPF (**Figure 2(a)**). Use of the more intact preparation by Antonov et al. (2003) allowed classical conditioning of the behavioral siphonal withdrawal reflex to be correlated with synaptic plasticity. Pairing siphonal touch with tail shock induced a parallel associative enhancement of siphonal withdrawal and synaptic strength. Application of the NMDA receptor blocker APV to the ganglion or injection of the calcium chelator BAPTA into the siphonal motor neurons blocked conditioning, providing direct evidence that Hebbian LTP is part of the mechanism for behavioral classical conditioning. To compare the role of pre- and postsynaptic mechanisms in synaptic plasticity, Antonov et al. (2003) injected the peptide inhibitor of PKA (PKAi) into LE sensory neurons and BAPTA into LFS siphonal motor neurons. Both procedures reduced the pairing specific facilitation of

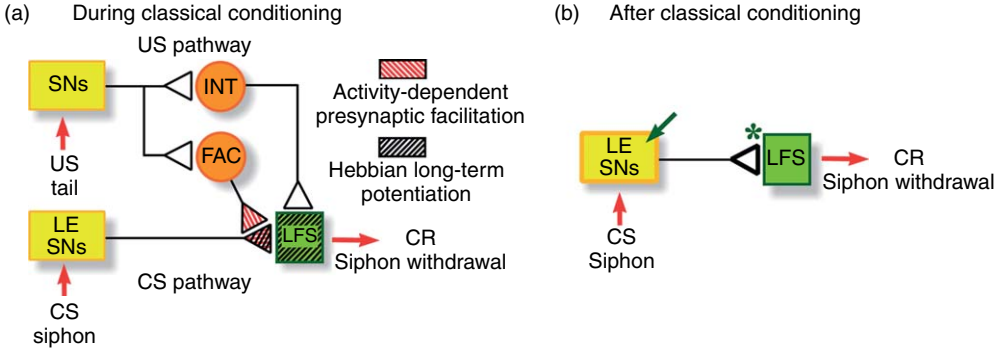


Figure 2 Aversive classical conditioning of the siphon withdrawal reflex in *Aplysia.* (a) During aversive classical conditioning, the conditioned stimulus (CS, touch to the siphon) weakly activates LE sensory neurons (SNs), which make monosynaptic connections onto LFS motor neurons. The unconditioned stimulus (US, electric shock to the tail) strongly activates tail sensory neurons. These sensory neurons excite both facilitatory interneurons (FAC) that produce presynaptic facilitation at the LE-to-LFS synapse and other types of interneurons (INT) that postsynaptically excite the LFS motor neurons. Thus, both the sensory neurons and the motor neurons are sites of CS and US pathway convergence and can act as coincidence detectors. The shading indicates neuronal elements that must be activated conjointly for the induction of either activity-dependent presynaptic facilitation (red) or Hebbian LTP (black) at the LE-LFS synapses (from Antonov I, Antonova I, Kandel ER, and Hawkins RD (2003) Activity-dependent presynaptic facilitation and hebbian LTP are both required and interact during classical conditioning in *Aplysia*. *Neuron* 37: 135–147; used with permission from Elsevier). (b) After aversive classical conditioning the synaptic connections of the LE sensory neurons with the LFS motor neurons are strengthened (synaptic plasticity, green asterisk and bold outline) and the excitability of the LE sensory neurons is increased (cellular plasticity, green arrow and bold outline). These changes lead to an increased siphon withdrawal response to the touch CS.

postsynaptic potentials (PSPs) in the motor neurons during conditioning. Thus pre- and postsynaptic mechanisms appear to be contributing (**Figure 2(a)**) to the plastic changes in synaptic strength between the sensory neurons and the motor neurons (**Figure 2(b)**). There also appears to be a retrograde signal involved, as well as orthograde, because injecting BAPTA into the motor neurons blocks the changes in the cellular membrane properties of the sensory neurons (increase in membrane resistance), so some of the changes occurring in the two sides of synapse following conditioning are coordinated. The nature of the retrograde signal is unknown, but it could be the diffusible gas nitric oxide (NO), as injecting the NO scavenger myoglobin into the sensory neuron blocks the facilitation of the PSP during conditioning (Hawkins et al., 2006). Whether both presynaptic and postsynaptic mechanisms are active together at all phases of synaptic facilitation is still an open question. On the basis of experiments where 5-HT is used to mimic classical conditioning, Roberts and Glanzman (2003) have speculated that the rapid-onset ADPF mechanism is responsible for short-term synaptic facilitation. Hebbian LTP has a longer onset and leads to more persistent synaptic plasticity for medium-term and perhaps long-term synaptic facilitation.

1.30.4.2 Aversive Classical Conditioning of *Hermissenda* Phototactic Behavior

Behavioral training in *Hermissenda* involves the pairing of the CS, a light flash, with the US, a mechanical perturbance such as rotation. This induces a short-term memory that can last for a few minutes (e.g., single trial) or long-term memory lasting for days and weeks, depending on the number of trials (e.g., 150 trials per day over 3 days gives a week-long memory). Neural correlates can be studied in semi-isolated central nervous system (CNS) preparations made from behaviorally trained animals, or single-trial *in vitro* conditioning can be induced by pairing a light flash with CNS application of 5-HT, a key transmitter in the US pathway. Conditioning in the *Hermissenda* system involves the development of two different behavioral responses to the CS, foot contraction and inhibition of ciliary locomotion (Crow, 2004). Both these conditioned responses are thought to develop independently due to the involvement of different components of the central circuit involved in the two behaviors (see earlier discussion). Functionally, there is a learning-induced 'transfer' of the ability to activate these circuits from the US to the CS. We will focus on the part of the circuit that underlies the conditioned inhibition of ciliary motor neurons, responsible for the cessation of forward locomotion in the intact animal, because most is known about how conditioning changes this circuit. After conditioning, the CS is able to activate the same interneuronal pathway that normally mediates the inhibition of ciliary motor neurons by the mechanical US in naive animals. Earlier, it was proposed that changes in the cellular properties of photoreceptor cells and the strength of synaptic connections between the two classes of photoreceptors' synaptic connections solely could account for the changes underlying behavioral conditioning (Goh and Alkon, 1984), but more recent identification of elements of the interneuronal circuit that forms an intermediate level of processing between sensory and ciliary motor neurons (Crow and Tian, 2002) has identified further sites of synaptic and nonsynaptic plasticity that are also involved in the conditioned response so that a more complex multisite model for associative conditioning in *Hermissenda* has emerged (Crow, 2004; Crow and Tian, 2006).

The electrical changes following conditioning are thought to be due to interactions between convergent CS (photic) and US (mechanical) synaptically mediated pathways that exist at various levels in the ciliary control network (**Figure 3(a)**). There are two types of synaptic connections between the CS and US pathways at the level of the sensory receptors. Reciprocal inhibitory monosynaptic connections occur between the statocyst hair cells and the B-type photoreceptors in the eye, and another unidirectional excitatory polysynaptic pathway, mediated by 5-HT, exists between the hair cells and the photoreceptors (**Figure 3(a)**). The presence of this US excitatory pathway mediated by 5-HT is of particular significance because this chemical is thought to underlie the ability of the US to induce changes in the CS photoreceptor pathway following conditioning. Conditioning induces cellular changes in both the type A and type B photoreceptors, which increase the firing of the cells in response to the CS. The CS evokes a larger receptor potential and an enhanced excitability in the B cells as tested by the response to a standard applied depolarizing pulse. An increase in membrane resistance contributes to this increase in spike activity, and a decrease in spike accommodation is also important in producing a sustained response to receptor depolarization in response to the CS. Conditioning reduces the peak amplitude of several types of potassium conductances in the B-type photoreceptors, including calcium-dependent and voltage-dependent types.

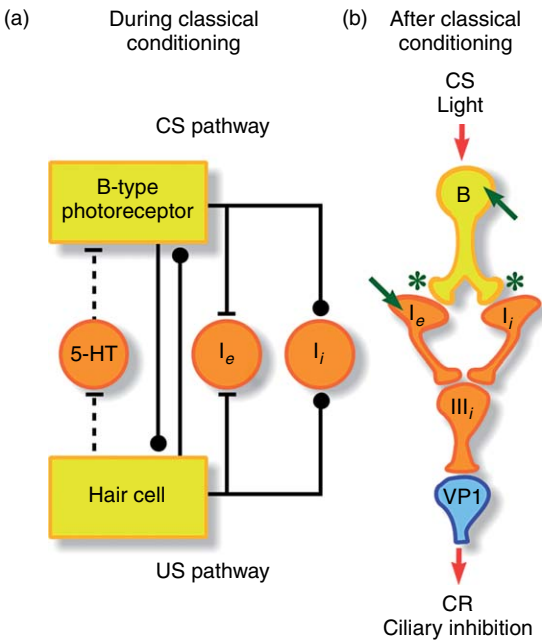


Figure 3 Aversive classical conditioning of the phototactic response in *Hermissenda*. (a) Sites of convergence between identified components of the CS and US pathways. Statocyst hair cells project have indirect excitatory synaptic connections with B-type photoreceptors through a proposed 5-HT-mediated interneuronal pathway. Hair cells and photoreceptors also have reciprocal inhibitory monosynaptic connections. Both hair cells and B-type photoreceptors form monosynaptic connections with both type I_e and type I_i interneurons (see also **Figure 1(b)**), which are therefore further sites of synaptic interactions between the CS and the US pathway (from Crow, 2004). Dots indicate inhibitory chemical synaptic connections and bar excitatory synaptic connections. (b) Components of the CS pathway involved in ciliary inhibition after classical conditioning. Changes in both cellular excitability (bold outlines, green arrow) and synaptic efficacy (bold outline, green asterisk) contribute to CS-elicited inhibition of ciliary locomotion. The net effect of cellular and synaptic plasticity is to increase the spike activity of type III_i inhibitory interneurons (see **Figure 1(b)**), during which light produces an inhibition of VP1 ciliary activating motor neurons (see **Figure 1(b)**) in conditioned animals. From Crow T (2004) Pavlovian conditioning of *Hermissenda*: Current cellular, molecular, and circuit perspectives. *Learn. Mem.* 11: 229–238; used with permission from Cold Spring Harbor Laboratory Press.

Importantly, these conductance changes can be mimicked by the application of 5-HT, providing evidence that the increased release of this transmitter via the hair cell polysynaptic pathway might be responsible for the changes in the intrinsic properties of the photoreceptors following conditioning. The molecular mechanisms underlying the effects of 5-HT on long-term memory are reviewed elsewhere (*See* Chapter 4.08).

The role of these convergent inputs in conditioning is best understood at the level of the primary sensory neurons, but convergent interactions may also be important at the level of the cerebropleural interneurons known as the I_e and I_i cells. Both cell types receive conjoint synaptic input from the photoreceptors and statocyst hair cells (**Figure 3(a)**). Photoreceptors and statocysts form monosynaptic excitatory connections with the I_e interneurons and monosynaptic inhibitory connections with the I_i interneurons.

Both I-cell types receive larger compound synaptic inputs in response to the CS in conditioned animals compared with controls (Crow and Tian, 2002). Larger EPSPs in the I_e cells increase spike activity, and larger inhibitory postsynaptic potentials (IPSPs) in the I_i cells reduce ongoing spike activity. This increase in the amplitude of the PSPs in the I-cells is due not only to increased CS-induced spike activity in the photoreceptors following conditioning but also to an increase in the strength of the B photoreceptor monosynaptic connections to both I-cell types (**Figure 3(b)**). In addition, the intrinsic excitability of the I_e interneurons also appears to be increased after conditioning, a third mechanism leading to the increased spike response of the I_e cells to the CS (**Figure 3(b)**). The mechanism increasing the excitability of the I_e cells has yet to be investigated.

Recording the VP1 ciliary motor neurons shows that light inhibits the tonic spike activity in conditioned animals compared with controls. Reduction of VP1 spiking reduces foot ciliary activity and inhibits forward locomotion. Facilitation of the synaptic connection between the B-type photoreceptors and type I_e interneurons in conjunction with intrinsic enhanced excitability in type B photoreceptors and type I_e interneurons would result in an increase in spike activity in the type III_i inhibitory interneurons and inhibition of the VP1 ciliary interneurons via the III_i-to-VP1 monosynaptic connection (**Figure 3(b)**). Thus, this combined set of synaptic and cellular changes located within the ciliary control circuit can elegantly account for the light-elicited inhibition of locomotion following associative conditioning. In the *Hermissenda* system, conditioning increases both the strength of already existing synapses by presynaptic mechanisms and postsynaptically by increasing the excitability of neurons in the circuit (Crow, 2004).

1.30.4.3 Aversive and Appetitive Conditioning of Chemosensory Responses in Terrestrial Slugs and Snails

Terrestrial slugs and snails show striking changes in the preference for food odors as a result of experience. Simply allowing the land snail *Achatina* to feed on a specific food, such as carrot, for 48 h leads to a preferential orientation of the snails toward odors of these foods, compared with the odors of a novel food, for up to 21 days after exposure to the food (Croll and Chase, 1980). Formation of food preferences depended on the consumption of food and does not occur by exposure to food odors alone, so an associative memory trace appears to have been formed between the odors and the consumption of the food, perhaps its nutritional value. Odor preferences have also been extensively investigated in the slug, *Limax*, but the emphasis in this mollusk has been on aversive classical conditioning. Slugs are attracted by odor to locomote toward a food source, such as carrot or potato, and when the food is located, they will evert their lips to taste the food and then consume the food with rhythmic feeding movements. If the food odors are paired with the bitter taste of quinidine, then the slug learns to avoid the food odor when compared with naive and unpaired groups of slugs that show no change in their food preference (Sahley et al., 1981a). A single pairing of a CS odor (carrot or potato) and the aversive US can reduce the time spent near the CS form by 80% in control groups to 20% for conditioned animals. The association of the food odor (CS) with the bitter taste (US) is an example of first-order classical conditioning, but the paradigm can also be extended to demonstrate second-order conditioning in the slug (Sahley et al., 1981b). Slugs are first presented with carrot/quinidine (phase 1) and then given the same number of pairings of two odors, potato and carrot (phase 2). To ensure that changes in the slug's preference for potato depended on it receiving both the phase 1 and phase 2 pairings, control groups were included. One control group received paired presentations of odor and quinidine in phase 1, but during phase 2, potato and carrot were unpaired. The other control group received unpaired carrot odor and quinidine in phase 1 but paired carrot and potato odor in phase 2. Slugs that received pairings during both phases of training displayed a reduced preference for potato odor when presented alone compared with slugs from the two control groups, indicating that second-order conditioning had occurred.

Another type of higher-order conditioning behavior shown by slugs is known as 'blocking' (*See* Chapter 1.18). Sahley et al. (1981b) showed that prior conditioning to one stimulus, carrot odor, reduced or blocked learning to a second stimulus, potato odor, when a compound odor consisting of both carrot and potato odors was subsequently paired with quinidine. In contrast, control groups of slugs without prior training to carrot learned to avoid both carrot odor and potato odor when the compound presentation of both odors was paired with quinidine.

As might be expected from such complex chemosensory behavior, *Limax* has an elaborate olfactory system for processing odor signals and the elaboration of memory traces. *Limax* and other terrestrial slugs and snails have two pairs of chemosensory tentacles located on the head, the 'superior' being responsible for sensing airborne odorous signals (Chase, 2002). At the tip of the tentacles is a large tentacle ganglion organized in glomeruli, and these structures contain primary olfactory neurons that are thought to code the airborne odor stimuli. These primary olfactory neurons project to a large CNS processing center, the procerebrum (PC), which is involved in odor discrimination as well as odor learning. Gelperin and Tank (1990) demonstrated the existence of synchronized neuronal oscillations in the PC of *Limax* that could be recorded as a local oscillatory field potential (LFP) with a frequency of about 0.7 Hz. This LFP is modulated by odor stimuli applied to the tentacle. As well as local potentials, waves of hyperpolarization and depolarization can be recorded that propagate continuously at 1 mm/s across the whole PC structure from the apical end of the PC to the basal end. The oscillatory activity is thought to be due to the bursting properties and inhibitory synaptic connections of the two types of neurons that form the PC (reviewed in Gelperin, 1999).

Evidence that the PC is involved on odor memories came from experiments where the fluorescent dye Lucifer yellow (LY) was used as a measure of neuronal activity (Kimura et al., 1998a). When LY was injected into the body cavity of intact *Limax* just after conditioning, many cells of the PC were stained. This was not observed in unpaired controls, so the incorporation of the dye was specific to the odor conditioning. The dye-stained clusters of neurons formed a characteristic beltlike structure transverse to the axis of wave propagation. If two different odors were used as the CS for aversive conditioning, then different clusters of cells showed dye labeling,

suggesting that memory traces for different odors were stored in distinct populations of PC cells. In related experiments (Kimura et al., 1998b), electrical activity in the PC was recorded following aversive odor conditioning using voltage-sensitive dyes. Presentation of an aversively conditioned odor to the tentacle modulated the spontaneous oscillatory activity. Initially a depolarization of the basal level of oscillation occurred in specific belt-shaped regions of the PC (cf. LY staining), and then a later phase of hyperpolarization occurred that covered a wider area. Different odors produced different patterns of spatial modulation of oscillatory activity. Spatial and temporal modulation was not observed when unpaired control odors were tested, suggesting that the electrical changes were specific to conditioning. It appears from this work that olfactory memories are stored as spatial and temporal activity patterns of oscillators that form a coherent network.

In more recent work an *in vitro* version of odor conditioning has been developed where it is possible to stimulate the olfactory organs with odors and record motor output (Inoue et al., 2006). In this preparation it was also possible to record a behavioral correlate of PC oscillation. After odor-aversion conditioning, shortening of mantle muscles was recorded *in vitro* that form part of the conditioned response in the whole animal. This was accompanied by increased activity in the parietal motor nerves that innervate the mantle. In a differential conditioning paradigm two attractive odors (carrot and cucumber) were used as CSs; one was paired with the US (CS+), and the other was applied alone (CS–). The US was an electrical stimulus applied to pedal and lip sensory nerves. Before conditioning, neither CS caused activity in the motor nerves innervating the mantle. After one pairing, the CS+ increased larger-amplitude discharges in the motor nerve but the CS– did not. In addition, LFPs were recorded from the PC during conditioning. Before conditioning, attractive odors caused little change in the PC oscillations, but after conditioning, the PC oscillation frequency doubled with increases in activity in the motor nerves. Only the aversively conditioned CS+ increased the frequency of PC oscillation with no effect from application of the CS–. Whether the changes in PC oscillation are due purely to learning-related events is still an open question, because natively aversive odors (say onion or garlic) also cause increases in PC oscillation in naive animals (Inoue et al., 2006) similar to those produced by the CS+ odors (say, cucumber) that are made aversive by conditioning.

1.30.4.4 Reward Classical Conditioning of *Lymnaea* and *Aplysia* Feeding Behavior

In the original successful formulation of chemical reward conditioning in *Lymnaea*, snails were subjected to a multi-trial chemical conditioning protocol (15 trials, 5 trials per day for 3 days) using amyl acetate as a neutral CS and sucrose as the US (Audesirk et al., 1982). Following training, the explicitly paired (CS-US) experimental group showed significantly greater feeding responses to amyl acetate over their own naive responses and all the standard control groups (random, explicitly unpaired, CS alone, US alone). As might be predicted for appetitive conditioning, both age and motivational state (hunger versus satiety) influenced learning. Both hungry and sated young snails could acquire the conditioned response, but in the latter group, its expression was only apparent when the animals were starved before testing. On the other hand, old snails could only acquire the conditioned response if they were maintained in a hungry state during training (Audesirk et al., 1982). The significance of motivational state became even more apparent when it was realized that if snails were starved long enough (for 5 days) before and throughout the experiment, even a single pairing of amyl acetate and sucrose resulted in long-term memory (LTM), which lasted for at least 19 days (Alexander et al., 1984). This is a remarkable example of single-trial learning, which is used now for analyses of the time course of the mechanisms underlying LTM formation.

An electrophysiological correlate of the conditioned response to amyl acetate was recorded as changes in the fictive feeding responses in motor neurons (Kemenes et al., 2002), but electrical activity following conditioning has also been recorded in other parts of the feeding system in attempts to localize sites of plasticity. The cell bodies of chemosensory neurons are located in lip epithelial tissue and project to the cerebral ganglia via the lip nerves, where they synapse with cerebral ganglion neurons like the CBIs (Straub et al., 2004). Extracellularly recorded spike responses to both the CS and US can be recorded in the lip nerves from naive animals, and these responses do not change after conditioning. In contrast, neuronal output from the cerebral

ganglia is significantly enhanced in response to the CS after conditioning (Straub et al., 2004). This indicates that chemical conditioning affects central but not peripheral processing of chemosensory information, with the cerebral ganglia being an important site of plasticity. The fibers that were recorded extracellularly to indicate cerebral plasticity were originating from the CBI interneurons, so their activation is particularly significant. Confirmation that the CBIs do increase their activity after conditioning was obtained by showing increases in feeding patterns to the CS in CV1 neurons, a specific CBI cell type. From this work, it is suggested that the synapses between the primary chemosensory neurons and the CBIs are increased in strength. Two other CS pathways are present in naive animals, but these are not affected by conditioning (Straub et al., 2006).

The current network model for chemical conditioning (**Figure 4(a), (b)**) includes nonsynaptic plasticity. The CGCs are persistently depolarized by about 10 mV after behavioral conditioning. This indirectly increases the strength of postsynaptic responses to CGC stimulation by a process that involves an increase in intracellular calcium concentration (Kemenes et al., 2006). The local target for CGC depolarization is the CBI cells, and artificial depolarization of the CGCs in naive snails increases the response of the CBI cells to the CS, mimicking the effects of behavioral conditioning. It appears that the CGCs are increasing the strength of the CS-to-CBI synapse by presynaptic facilitation (**Figure 4(b)**). The onset of the CGC depolarization is between 16–24 h after training, and it persists for at least 14 days, as long as the behavioral memory trace is present. There is an early behavioral memory trace from 2 h after conditioning, so the CGCs cannot be involved in memory expression immediately after training. It is more likely that the CGCs are involved in the maintenance of the LTM after the trace has already been consolidated and encode information that is important for memory recall. Interestingly, the CV1 cells that show a persistent change in membrane potential after tactile conditioning (see later discussion) show no change after chemical conditioning. A variety of molecular pathways are involved in chemical conditioning in *Lymnaea*, and these are reviewed elsewhere (*See* Chapter 4.09).

Lymnaea can also be classically conditioned to a lip touch CS by repeatedly pairing a touch to the lip with food (5–15 trials over 3 days). This type of reward learning shares important characteristics with associative conditioning in vertebrates, such as

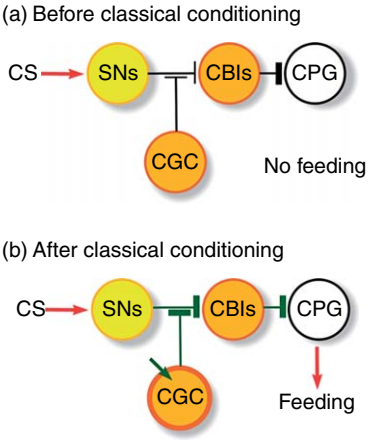


Figure 4 A cellular model for long-term memory after chemical classical conditioning in *Lymnaea*. (a) In naive animals, the excitatory connections between the sensory neurons (SNs) and the command-like cerebrobuccal interneurons (CBIs) are weak, and the chemical CS (amyl acetate) cannot activate feeding. The cerebral giant cells (CGCs) are at normal membrane potential (~65 mV), and the presynaptic modulatory input from the CGCs to the SNs is inactive or weak. (b) In conditioned animals, the CGC soma is depolarized by ~10 mV compared to naive animals, and this leads to an enhancement of the CGC presynaptic modulatory inputs to SNs and a strengthening of the SN-to-CBI excitatory synapse, which results in a feeding response to the CS. Black lines, inactive connections; green lines, active connections. Thin bars, weak chemical excitatory synapses; thicker bars, stronger/enhanced excitatory synapses. The thicker outline of the CGC and the green arrow in (b) indicate learning-induced nonsynaptic plasticity (membrane depolarization), which increases the strength of CGC-to-SN synaptic connections. CPG, central pattern generator.

stimulus generalization and discriminative learning, classical-operant interactions, and strong dependence on both external and internal background variables (Kemenes and Benjamin, 1989a,b, 1994).

Two approaches have been used to investigate the neural basis of tactile reward classical conditioning in *Lymnaea*. One approach was based on the development of an *in vitro* preparation where electrophysiological manipulation of neuronal pathways aims to mimic the behavioral conditioning paradigm (Kemenes et al., 1997). In this study a lip touch stimulus was paired with intracellular activation of the modulatory slow oscillator neuron, which can drive fictive feeding. After 6–10 pairings, presentation of the touch stimulus could activate a robust fictive feeding rhythm in feeding motor neurons.

A second approach used behavioral conditioning followed by electrophysiological analysis to record

changes in electrical activity that follows LTM formation. Using the lip touch behavioral training protocol, snails were subjected to 15 training trials over 3 days, and then these and control animals were dissected for electrophysiological analysis, starting on the day after the last training trial. Touching the lips of the intact snails from the experimental group after training induced a pattern of feeding movements significantly greater than controls. Similar significant differences were seen between experimental and control animals at the level of the electrophysiologically recorded fictive feeding pattern in motor neurons made from the same snails (Staras et al., 1998, 1999a).

The CPG-driven activity in the motor neurons depends ultimately on activity of neurons at all levels of the feeding network, so the conditioned fictive feeding recorded in the motor neurons is a systems level 'readout' of the memory trace in the whole feeding system. However, more detailed changes can also be recorded in different parts of the network (Staras et al., 1999a). One of these is the early EPSP that occurs in the B3 motor neuron before the onset of the fictive feeding pattern. The amplitude, but not the latency and duration of the EPSP, was significantly enhanced after conditioning. In sated snails the conditioned fictive feeding response to touch was lost, but the increase in the EPSP amplitude persisted. This suggests that there is unlikely to be a causal link between increases in amplitude in B3 and generation of the fictive feeding pattern.

Electrical correlates of tactile conditioning were also recorded at other levels within the feeding circuit, and these could all be potential sites of plasticity. That sites quite early in the CS pathway could be involved in conditioning was revealed by extracellularly recording mechanosensory fibers located in the connective between the cerebral and buccal ganglia. Tactile responses could be recorded in these fibers, and following conditioning, the number of spikes occurring early in this response increased, compared with controls (Staras et al., 1999a).

Interestingly, a correlate of tactile conditioning could also be recorded in the CPG network. A long-lasting sequence of inhibitory synaptic inputs that occurs in the N1 CPG interneurons in response to lip touch in naive animals changes to a strong sustained depolarizing synaptic input after *in vitro* conditioning, and this drives a sustained plateauing pattern in the N1 cell (Kemenes et al., 1997). This is an example of synaptic plasticity affecting an important CPG component of the feeding network.

One candidate for initiating CPG activity following conditioning is the CBI cell type known as CV1. This neuron is capable of driving a fictive feeding pattern via its connections with the N1M cells of the CPG network, and activity in these cells normally accompanies unconditioned feeding patterns stimulated by sucrose (Kemenes et al., 2001). After conditioning, the CV1 cells are significantly more active following touch in conditioned snails compared with controls, and they show the typical patterned activity seen with sucrose (US) application (Jones et al., 2003). More detailed experiments on the role of CV1 cells in tactile conditioning have revealed that nonsynaptic electrical changes play a role in memory (Jones et al., 2003). A long-lasting membrane depolarization of 11 mV on average was recorded in CV1s from conditioned compared with control snails that persisted for as long as the electrophysiological and behavioral memory trace. The depolarization makes the cells more responsive to the CS and can account for the activation of the feeding response after conditioning via the CV1 cell's strong excitatory synaptic connection with the CPG. The importance of this result is emphasized by experiments in which the membrane potential of the CV1 cells is manipulated to either reverse the effect of behavioral conditioning or to mimic the effects of conditioning in naive snails. These experiments showed that the persistent depolarization of the CV1 cells was both sufficient and necessary for the conditioned tactile response in the feeding network.

Like *Lymnaea*, the *Aplysia* feeding system has also been subjected to behavioral and electrophysiological analysis of reward conditioning using lip touch as the CS and food reinforcement (seaweed) as the US. An *in vitro* electrophysiological analogue of classical conditioning employed electrical stimulation of nerves to activate the CS and US pathways. Lesions of the En (esophageal nerve) blocked classical conditioning and suggested that this nerve mediates the effects of the US (Lechner et al., 2000). This nerve also contains dopamine (DA) immunofluorescent fibers, so it was interesting that the DA antagonist, methylergonovine, blocked the acquisition of the *in vitro* analogue of reward conditioning. Thus the behavioral, immunostaining, and pharmacological data are consistent with the hypothesis that DA located within nerve fibers in the En nerve mediates the actions of the US during conditioning.

Individual neurons of the *Aplysia* feeding circuit have been analyzed for changes occurring during conditioning. Buccal neurons B31/32 play a key

role in initiating buccal motor programs, and these neurons were significantly more depolarized by the CS after conditioning, although their intrinsic properties were unaffected. This suggests that there is greater excitation of the B31/B32 neurons after conditioning due to an enhancement of the CS pathway. Another neuron that plays a key role in expression of the buccal feeding program is B51. In preparations made from behaviorally trained animals, this cell type showed a greater number of plateau potentials compared with controls, and it was depolarized more by the CS. Neither the input resistance nor resting potential of the B51 was affected by conditioning, but another intrinsic property, the threshold for plateau initiation, was increased. This would make the cell less responsive to excitatory synaptic input, but nevertheless, the cell still showed more plateau potentials after conditioning, so some other unknown factor must overcome this diminished excitability (Baxter and Byrne, 2006). Similar results were obtained in both *in vivo* and *in vitro* conditioning. Recently, the CBI-2 cell type has been examined after *in vitro* conditioning and shown to increase its activity after conditioning. CBI-2 has the ability to initiate ingestive feeding motor programs, and this may contribute to the increased activation of the buccal B31/32 interneurons after conditioning (Mozzachiodi et al., 2003). No changes in the nonsynaptic cellular properties of the CBIs was reported, which contrasts with the results from *Lymnaea*, where the homologs of the CBIs, the CV1a cells, were shown to be persistently depolarized after tactile reward conditioning (Jones et al., 2003, see earlier discussion).

1.30.4.5 Reward Operant Conditioning of *Aplysia* Feeding Behavior

Brembs et al. (2002) developed a behavioral paradigm for operant conditioning using the consummatory (ingestive) phase of the feeding cycle as the operant. Contingent electrical stimulation of the En nerve that carries the US pathway from the esophagus, using implanted electrodes, was used to positively reinforce spontaneous extracellular recorded feeding bursts of activity monitored on the same nerve in freely moving animals. These extracellularly recorded bursts occurred at the same time as feeding ingestion movements and so acted as a direct monitor of the behavior. The number of spontaneous feeding bursts was increased compared with nonstimulated or yoked controls after 10 min of training immediately after the training period and when measured 24 h later. This indicates that operant conditioning was successful in producing both short-term and long-term memory. A cellular correlate of operant conditioning was monitored by intracellularly recording the B51 feeding interneuron in isolated buccal ganglia made from behaviorally trained animals. Cells from the contingent group showed a significant decrease in threshold for plateau formation and a significant increase in input resistance compared with cells from yoked controls. To test whether this was due to an intrinsic change in the B51 cell rather than changes in input originating from outside the cell, an analogue of conditioning was developed where B51 was grown in culture and electrically triggered burst of spikes paired with 6-s puffs of DA applied close to the isolated cell over a 10-min training period. Contingent application of these two stimuli produced a significant reduction in plateau threshold and a significant increase in input resistance compared with unpaired controls similar to that occurring in the previous intact buccal ganglion preparation (Brembs et al., 2002). These results indicate that intrinsic changes are induced in B51 by operant conditioning. How these changes in B51 contribute to the network and behavioral expression of the conditioned response are yet to be determined. Some of the molecular changes involving the effects of DA have been elucidated (reviewed in Baxter and Byrne, 2006).

1.30.4.6 Aversive Operant Conditioning of *Lymnaea* Breathing Behavior

The aerial respiratory behavior of *Lymnaea stagnalis* has been used to investigate the behavioral and neuronal mechanisms of operant conditioning (Lukowiak et al., 2003). Hypoxia triggers pneumostome opening, and this was used as the operant for behavioral conditioning. Tactile stimulation of the pneumostome area evokes pneumostome closure and stops aerial respiratory behavior. Animals were tested and trained in an artificially created hypoxic N_2-rich environment to increase the level of respiratory behavior. In the operantly trained group a tactile stimulus was applied to the pneumostome area each time aerial respiration was attempted by the animal. Suitable yoked and hypoxic control groups were also used. The number of openings, latency to first opening, and total breath durations were recorded in pre- and posttraining periods. Only the operantly

conditioned group showed significant changes between the pre- and posttraining behaviors, with significant reductions in openings and total breathing time and significant increases in the latency to first breath. It has since been demonstrated that a memory for this conditioned response could persist for at least 4 weeks when a spaced training procedure was used. Both intermediate memory (ITM) and LTM have been described based not only partly on the length of time the memory persists but also on the sensitivity to protein and mRNA synthesis blockers. Anisomycin prevents the formation of both ITM and LTM, whereas actinomycin-D only prevents LTM. Both reconsolidation and extinction have been studied following operant conditioning, and both have been shown to be dependent on new RNA and protein synthesis (Sangha et al., 2003a,b; *See* Chapters 1.24, 1.27). Extinction is viewed as a new type of associative memory that 'covers up' but does not replace the original memory. Thus, following extinction trials, a loss of memory at 2 h is followed by full 'spontaneous recovery' at 24 h (Sanga et al., 2003b).

Neural changes associated with this learned behavior have been identified in the isolated CNS derived from operantly conditioned animals. Specifically, spontaneous patterned activity in the IP3 interneuron, which is involved in pneumostome opening (see **Figure 1(dii)**), showed a significant reduction compared to activity in the IP3 neurons of brains derived from yoked controls. Furthermore, a higher percentage of RPeD1 CPG interneurons, which are important in the onset of the respiratory cycle, were silent in conditioned versus control preparations (Spencer et al., 1999). A reduction in the ability of the RPeD1 cells to induce IP3 activity was also observed. More direct evidence for the role of RPeD1 in memory formation came from somal ablation experiments. Removal of the soma 2 h prior to conditioning prevented LTM but had no effect on ITM, without affecting the ability of the snail to carry out respiratory behavior, suggesting that the RPeD1 soma was necessary for LTM formation (Scheibenstock et al., 2002). Removal of the RPeD1 soma 1 h after conditioning had no effect on LTM, indicating that the effects of soma ablation were not related to memory access or retrieval. Interestingly, both extinction and reconsolidation also require the presence of the soma of RPeD1, indicating that RPeD1 is involved in the formation of more than one type of memory trace (Sangha et al., 2003a,b).

1.30.5 Discussion

1.30.5.1 The Complexity of Molluscan Learning

Molluscan studies are focused on implicit forms of memory such as classical/operant conditioning and sensitization. Initially, simple forms of associative and nonassociative learning behavior were investigated. However, gastropod mollusks are capable of showing more complex types of associative learning behavior with features that are similar to those found in vertebrates (*See* Chapters 1.18, 1.36). For instance, differential conditioning has been described in a number of mollusks (Hawkins et al., 1983; Kemenes et al., 1989a; Jones et al., 2001; Inoue et al., 2006). In addition, second-order conditioning and blocking of aversive-odor conditioning has been demonstrated in *Limax* (Sahley et al., 1981b), and stimulus generalization, goal tracking, and context dependence (increased learning in a novel environment) were found in *Lymnaea* tactile conditioning (Kemenes and Benjamin, 1989a,b, 1994). The circuits underlying these behaviors are more complicated than those originally used for the study of reflexive defensive withdrawal responses and require the understanding of CPG and other interneuronal circuits mediating multimodality sensory responses. A key finding in these studies is that conditioning-induced synaptic and nonsynaptic changes occur at several sites within the same network (Benjamin et al., 2000; Crow, 2004; Baxter and Byrne, 2006; Kemenes et al., 2006; Straub et al., 2006). These include sensory neurons, modulatory and pattern-generating interneurons, and motor neurons. A future task will require us to understand how these various changes may be integrated to generate the final behavioral output.

1.30.5.2 Comparison of Nonsynaptic Electrical Mechanisms in Different Types of Molluscan Learning

It has long been recognized that changes in synaptic plasticity play a major role in molluscan learning and memory, but an increasing number of examples of nonsynaptic plasticity have been discovered that are known to be involved in circuits underlying behavioral learning. These include changes in input resistance, membrane potential, and threshold for plateau initiation. Examples of input resistance increases induced by conditioning occur in the B-type photoreceptor cells of *Hermissenda* (Crow, 2004). This leads to an increase in excitability of

the photoreceptors so that they fire more in response to the light CS. Similar input resistance increases have been described in the LE mechanoreceptors of the gill-siphon reflex of *Aplysia* following sensitization and classical conditioning (Hawkins et al., 2006). In both examples, a reduction in the size of intrinsic potassium channel conductances appears to be involved.

Persistent changes in membrane potential occur in whole body withdrawal interneurons in *Helix* (Gainutdinov et al., 1998) and in feeding command-like CV1 interneurons in *Lymnaea* (Jones et al., 2003). In both snails, the cells are depolarized following conditioning, and this lowers the threshold for firing in response to the CS, thus allowing the command cells to directly activate the motor circuits. The CGCs in *Lymnaea* also show persistent changes in membrane potential (Kemenes et al., 2006) after conditioning, but this does not lead to an increase in spiking responses to the CS. Instead, the 10-mV depolarization facilitates CS responses in the chemosensory pathway by increasing presynaptic release of transmitter at the sensory-to-command-neuron synapse. The CGCs are extrinsic to the feeding circuit, and so the change of membrane potential activates feeding responses indirectly by affecting command interneurons intrinsic to the circuit. Interestingly, the CGC's changes occur in chemically conditioned snails but not those subjected to tactile conditioning. For tactile conditioning the CV1 cells are depolarized but not the CGCs. The reason for this difference in the two types of classical reward conditioning is unclear but is probably linked to differences in the neural pathways activated by sucrose versus lip touch.

Changes in the threshold for plateau formation in the pattern generation occurs in the same *Aplysia* interneuron (B51) in both classical and operant conditioning of feeding, allowing comparisons to be made of the intrinsic changes occurring in B51 in the two different types of learning (Lorenzetti et al., 2006). The two types of conditioning have the opposite types of effect – increasing the plateau membrane potential threshold in classical conditioning and decreasing it in operant conditioning. This is despite the increase in overall plateauing frequency in both types of conditioning in the intact network. B51 is thought to have some type of 'decision-making role' in the feeding circuit but how the differential effects of conditioning on plateauing translate to differences in the type of conditioning used is unclear.

1.30.5.3 Comparison of Synaptic Mechanisms

Changes in synaptic strength have been described in several molluscan systems following training. In *Aplysia*, the gill-siphon withdrawal response is present in naive animals and is enhanced by learning (Hawkins et al., 2006). In contrast, *Hermissenda* phototactic conditioning causes a different response to the CS in naive animals compared with after conditioning, and so a new response has to be learned (Crow, 2004). There are weak synaptic responses to the photic CS-conditioned response in *Hermissenda*, but they are too ineffective in naive animals to generate a network inhibitory response in the ciliary motor neurons. It might have been expected that there might be formation of novel synapses or activation of 'silent synapses' in the circumstances where there is no behavioral response prior to conditioning, but this does not appear to be the case. In *Lymnaea* there are two alternative CS pathways, one excitatory and one inhibitory, that can be activated by the chemical CS in naive animals (Straub et al., 2006). At high concentrations the inhibitory effect is predominant on feeding behavior. Following reward conditioning, the CS becomes overall excitatory on feeding responses to high CS concentrations, but this is not due to any change in the preexisting pathways, but a previously ineffective excitatory pathway becomes predominant over the inhibitory pathway. These results from *Hermissenda* and *Lymnaea* make it clear that there are no simple rules so far elucidated that can predict whether new synapses will be formed or already-existing synapses strengthened following associative conditioning.

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1.31 Behavioral Analysis of Learning and Memory in Cephalopods

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1.31.1 An Historical Overview

The ancient civilizations of the Mediterranean, whose lives were closely linked to the sea, were clearly aware of the existence and beauty of cephalopods. Exquisite motifs and sketches of marine creatures were recurrent in the well-known Middle and Late Minoan pottery (approximately 2000–1000 BC) and also in the probably less famous Chiriquian art, typical of northwest Panama (approximately 300 BC). Both made extensive use of lozenge-shaped figures from which eight waving arms emerged. Independent of their realistic or stylized rendering, common characters such as dots, spots, and circles of various shades and colors convinced archeologists, anthropologists, and art historians that the figures were artistic representations of the same peculiar marine creature: the octopus.

This brings our attention to the fact that, among living organisms, cephalopods have been familiar to man since antiquity. In his *Historia Animalium*, Aristotle (fourth century BC) described the difference in occurrence between the inking response of cuttlefishes (concealment and fear) and that of squids and octopuses (fear only), together with the changing of the patterns of their skin to match the background and disguise themselves from predators and prey. He also mentioned the octopus's strong curiosity (or stupidity, to put it in his words), which made it an easy catch since a hand shaking under the water surface was sufficient to elicit its exploratory drive (Aristotle, 1910). Cephalopods' behavior even intrigued Darwin, who gave a detailed account of his observations of octopuses and cuttlefishes in the waters of the islands of Cape Verde (Darwin, 1870).

Many other anecdotal and popular reports are known from the classic literature (review in Cousteau and Diolé, 1973; see also Borrelli et al., 2006).

1.31.1.1 The Role of the Zoological Stations in Cephalopod Research

It is without doubt, however, that the scientific and systematic analysis of cephalopods' anatomy, physiology, and behavior started near the beginning of the twentieth century, mainly from initiatives of the Zoological Station of Naples (Stazione Zoologica di Napoli) and its outstanding guests.

The Stazione, with its strategic position on the seafront, fleet of fishing boats, and professional personnel, gave foreign scientists the unique opportunity to combine leisure and work as temporary guests of the institute. Since its foundation, octopuses were among its favorite research models; moreover, they figured as a symbol of the Stazione, as is clear from the decorations on the wrought-iron gates of the main entrance to the institute (**Figure 1**) and from the numerous old postcards of the Naples Aquarium, which often depicted them. This stemmed mainly from the the long-standing tradition of Neapolitan fishermen to catch live cephalopods, always considered a prime delicacy (for review, see Lo Bianco, 1909; Lane, 1960).

Figure 1 The gate of the main entrance to the Stazione Zoologica A. Dohrn, built during the final phase of the construction of the institute (1905–1910). It is embellished with motifs of traditional fishing tools, such as the fishing net (bottom) and the row of spears and tridents (top), the latter being a symbol of the Greek sea god Poseidon. Marine creatures, such as crabs and octopuses (detail on right), are carefully depicted by Neapolitan craftsmen.

During this period, the outstanding studies of Giuseppe Jatta (1896) and Adolf Naef (1923, 1928) on the anatomy, systematics, and ontogeny of cephalopods, conducted and published at the Stazione, formed the groundwork for further studies. In addition, the first observations of cephalopods living in captive conditions (in tanks at the Naples Aquarium) gave an idea of the complexity of their behavior and learning capabilities (e.g., Piéron, 1911; for review see Boycott, 1954). Dr. Ariane Droescher referred to a description by Anton Dohrn, who tried to avoid octopuses feeding on lobsters kept in adjacent tanks. Solid cement walls, raised several centimeters above the water surface, separated the aquarium's tanks. However, his attempts proved to be unsuccessful. In fact, as observed by Dohrn, the "same day, one of [the octopuses] climbed over the wall, attacked the unsuspecting crayfish and, after a short battle, tore him into two pieces! … The octopus [could] have seen that the crab [was] set by the keeper into the neighbouring tank, or he [could have] smelled the prey in the circulating water of the tanks. Anyhow, the event shows that the octopus [was] able to deduce from a sensual impression that there [was] a prey that he did not see, to conclude and, finally, to perform an air-jump in the right direction" (Salvini-Plawen, 1979, pp. 218–219).

Research on the biology and behavior of cephalopods was not confined to the Stazione but attracted the attention of and flourished in many other marine laboratories, both in Europe and the United States. As an example, a thorough description of the chromatic changes and behavioral repertoire of *Octopus vulgaris* was carried out by Cowdry (1911) at the Bermuda Biological Station for Research. Similar studies were conducted on *Loligo pealeii* by Williams (1909) at the Marine Biological Laboratory (Woods Hole, MA, USA) and by Stevenson (1934) at the Biological Station of St. Andrews (Scotland). Finally, the behavior and complexity of body patterning of *Sepia officinalis* was described by Bert (1867) at Arcachon (France), by Tinbergen (1939) at the Aquarium of the Zoological Station of Der Helder (The Netherlands), and finally by Holmes (1940) and Sanders and Young (1940), who carried out their studies at the Marine Biological Laboratory in Plymouth (UK).

1.31.1.2 A Research Effort Lasting Over a Century

In order to evaluate the research on cephalopods from the pioneering studies to the present day, we counted the number of publications cited in the Zoological

Record on a yearly basis for over a century, from 1900 to 2006 (**Figure 2**). Only publications concerning the anatomy and physiology of the central nervous system, the behavioral responses to learning paradigms, or living habits of cephalopods were considered pertinent and were accounted for. The number and distribution of papers over years clearly shows the prominent role achieved by the octopus in the study of learning and memory in cephalopods in the 15 years spanning from 1955 to 1970 (**Figure 2**).

1.31.1.2.1 The contribution of J. Z. Young

After the Second World War, the insight into the octopus rose mainly by the initiative of the British anatomist and zoologist John Zachary Young. On a yearly basis, JZ (as he was commonly called), together with a plethora of students and co-workers, was hosted by the Stazione Zoologica, where he systematically studied the anatomy and physiology of cephalopods' nervous system, increasing the knowledge on the behavior and learning capabilities of these animals. Many people were involved in these and related physiological studies: Brian B. Boycott, Francesco Ghiretti, Pasquale Graziadei, Nicolas J. Mackintosh, Hector Maldonado, John B. Messenger, William R.A. Muntz, Andrew Packard, Geoffrey D. Sanders, Norman S. Sutherland, and Martin J. Wells are but a few names.

The impressive bulk of knowledge gained over these years at the Stazione and in other laboratories around the world has confirmed the old view of cephalopod preparations as marine guinea pigs (A. Droescher, personal communication) or as primates of the sea (Kerstitch, 1988). In fact, Young became more and more conscious of the fact that the "brain of the octopus has already abundantly proved its value for the study of behaviour. It is perhaps the type most divergent from that of mammals that is really suitable for study of the learning process" (Young, 1971: p. vii).

Apart from a few other contributions (for review, see Boycott, 1954), when J. Z. Young and Brian

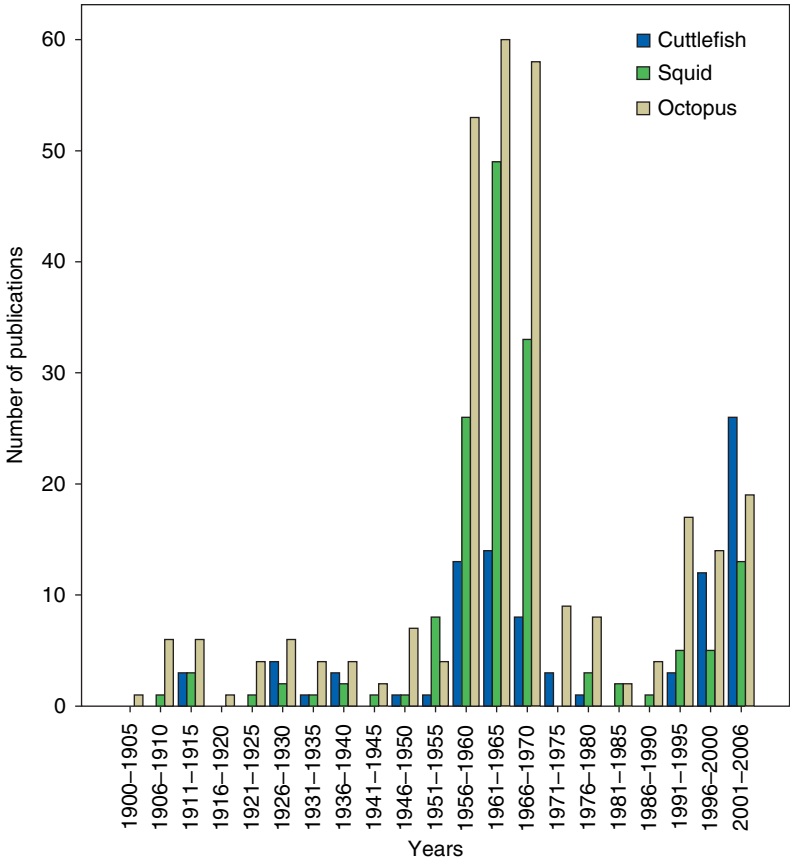


Figure 2 Number of publications per quinquennium on behavior, learning, and memory in cephalopods (cuttlefish, squid, and octopus) indexed in the Zoological Record from 1900 to 2006.

Boycott started their adventure with *Octopus vulgaris* at the Stazione Zoologica, two major works were available to them: a comprehensive overview of the brains of different cephalopod species (*Sepia officinalis*, *Sepiola robusta*, *Loligo vulgaris*, *Illex coindetii*, *Argonauta argo*, *O. vulgaris*, *Eledone moschata*, *Ocythoe tuberculata*; Thore, 1939) and an experimental study on the effects of the removal of certain lobes of the brain of *S. officinalis* on the behavior and learning of the cuttlefish (Sanders and Young, 1940).

Their aim was to study the learning capabilities of these animals by combining behavioral observations and lesions of the neural centers in order to disclose the functional organization of the cephalopod brain involved in the control of different behaviors; essentially, the predatory response.

O. vulgaris appeared to them as the ideal candidate for a series of reasons. First, because it was easy to maintain in aquaria. A tank of relatively reduced space ($30 \times 100 \times 40$ cm) with running seawater and a pair of bricks as shelter was (and still is) sufficient to make an octopus happy and at home. In this type of experimental setting, an initially scared, hiding, and pale octopus, as it commonly appears on the day it is captured becomes a tame, pet-like animal with time (Buytendijk, 1933; Hochner et al., 2006). Second, because of its natural curiosity. A few days in captivity are normally enough for the octopus to show its intrinsic attitude to attend to any object placed in its aquaria, which is largely a result of its voracity and exploratory drive toward natural (or artificial) objects. Third, because of its resilience to recover from massive brain surgery, contrary to what Young and coworkers experienced with *S. officinalis* (Sanders and Young, 1940).

The advantage of the octopus preparation became clear when Boycott introduced an efficient training technique. *O. vulgaris*'s hunting behavior was utilized as biological drive to teach animals to discriminate between stimuli that were positively and negatively reinforced. In the first experiments, octopuses were presented with crabs alone or associated with a white square; every attack on the latter was negatively reinforced (6–12 V AC). Several dozen trials were enough for the animals to learn the task and to respond correctly in quite a stable and predictive way.

In a series of subsequent experiments, it was demonstrated that octopuses were able to distinguish between different shapes by successive presentation of the two objects. The octopus was allowed to eat the crab when it attacked the positive figure, but an electric shock was delivered to the animal by a probe when it attacked the negative stimulus (Boycott and Young, 1956; for review, see Sanders, 1975; Hanlon and Messenger, 1996). Following this initial set of experiments, the protocol was improved by giving the animal a piece of anchovy as positive reinforcement instead of crabs, which could reduce the octopuses' predatory response because of satiety (Young, 1961).

1.31.1.3 The Breadth of the Studies on Octopus and Other Cephalopods

The versatility of this training protocol allowed a rapid growth of the field up to the 1960s (see also **Figure 2**). In fact, different research directions emerged from the original study by Boycott and Young (for review, see also Wells, 1965b).

For example, the first were centered on the study of the different sensorial capabilities (orientation in space, vision, chemotaxis, touch, and proprioception) and of the structures devoted to their control using different approaches (e.g., behavioral, ablation, and stimulation). Subsequent attention was focused on disclosing the role of the lobes of the brain (essentially the vertical and the peduncle lobes) in the learning and behavioral responses and in the motor coordination.

All these studies allowed Young and coworkers to produce a model of the brain of a learning (and behaving) octopus (Young, 1964; Clymer, 1973). In addition, these studies demonstrated that the animals were capable of sensitization, habituation, associative learning (passive avoidance, visual and tactile discrimination), and spatial learning (for a review, see Young, 1961; Sanders, 1975; Wells, 1978; Boyle, 1986; Boal, 1996; Hanlon and Messenger, 1996; Hochner et al., 2006). As clearly stated by Wells, "Octopuses can be taught to make a wide variety of tactile and visual discriminations. They learn rapidly under conditions that would lead to learning by mammals, and they achieve similar standards of accuracy of performance. This places them in a different category from many invertebrates, where research has tended to concentrate on demonstrating that the animals can learn at all. In the case of *Octopus* there is now no doubt that the species can learn, whatever definition of learning one cares to employ. One is free to pass on to considerations of *what* these animals can detect and learn about the world around them and what *we* can learn about the organisation of the cephalopod brain from their successes and failures" (Wells, 1965a, p. 115).

The analysis of the literature clearly shows what cephalopod workers (and modern neuroscientists) know well: The octopus, as a model, was almost (and suddenly) abandoned by the end of the 1960s (**Figure 2**).

This was mainly a result of:

- The relatively poor control by experimenters of the behavioral training procedures (Bitterman, 1966, 1975);
- The lack of appropriate tools to explore the neurophysiological properties of cells within certain lobes of the brain (e.g., amacrine cells in the vertical lobe; Young, 1985); and
- The inability of octopuses to pick up kinesthetic cues and the lack of proprioceptive feedback of the higher centers of the octopus brain (review in Wells, 1978).

Controlled handling, maintenance, and training procedures (Walker et al., 1970) and the thorough knowledge of the behavior and learning capabilities of cephalopods (Maldonado, 1963a; Packard, 1963; Messenger, 1968, 1977; Packard and Sanders, 1969, 1971; Packard and Hochberg, 1977; Hanlon, 1978; Hanlon and Messenger, 1988) have allowed subsequent workers to cope with the difficulties implicit in the training protocols and to produce renovated experimental approaches to the study of the behavioral biology of learning in these animals. This has led to a growing number of publications over the last 20 years (**Figure 2**) that are not necessarily focused on the octopus model but on different cephalopod species.

1.31.2 The Cephalopod Brain and Its Learning Capabilities

Dramatic evolutionary changes in the body plan and in the gross morphology of the nervous system (and of its relative organization) led to the origin and diversification of the phylum Mollusca (Kandel, 1979; Lee et al., 2003).

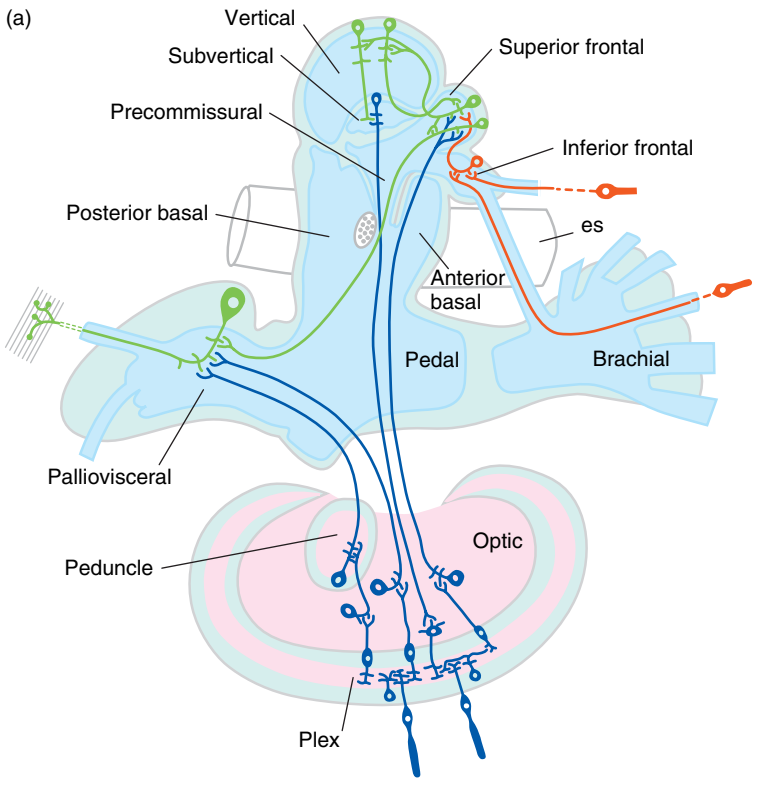
The nervous system, in particular, varies greatly in complexity and in the number of neurons among taxa (Bullock, 1965a,b,c,d). However, it is in cephalopods that this complexity reaches its highest degree within the phylum – a complexity that can be recognized at three levels. First, the brain size (relative to body weight) is comparable to that of vertebrate brains and positions cephalopods just below higher vertebrates (i.e., birds and mammals; Packard, 1972). Second, an average-sized octopus presents roughly 500 million neurons. More than one-third (roughly 200 million cells) are recruited to form its central nervous system (Young, 1963), a number that appears to be from 200 to 10 000 times higher when compared with the brains of other invertebrates (*Apis* and *Aplysia*, respectively). Finally, the degree of complexity of the nervous system is not only limited to the relative size and number of neurons within the brain but also stems from its neuroanatomical organization (for review, see Young, 1971; Budelmann, 1995; Williamson and Chrachri, 2004).

1.31.2.1 General Organization of the Brain

In cephalopods, the ganglia recruited to form the central nervous system may be considered homologous to the labial, buccal, cerebral, pedal, pleural, and visceral ganglia of gastropod mollusks. Contrary to what occurs in the typical molluscan design, in a cephalopod the ganglia are fused together and clustered around the most anterior part of the esophagus (for a review, see Bullock, 1965b; Budelmann, 1995). The agglomeration of the ganglia, which happened by the shortening of the connectives and commissures, form three almost distinct parts: the supra- and the subesophageal masses, and a pair of optic lobes that emerge laterally from the supraesophageal mass (one for each side positioned just behind the eyes).

The supraesophageal mass originated from the ancestral labial, buccal, and cerebral ganglia. The main lobes constituting it are the inferior, superior, and posterior buccal; the inferior frontal, subfrontal, and superior frontal; the vertical; and the basal lobes (**Figure 3**). Moreover, certain lobes of the supraesophageal mass (e.g., the optic and olfactory lobes) are of more recent neural formation. The subesophageal mass, instead, essentially derived from the paired pedal, pleural, and visceral ganglia that fused together to a different extent within and between cephalopod species (Nixon and Young, 2003). Again, neural masses of more recent origin were added to the ganglia listed above to form the brachial ganglion, mainly devoted to the control of the actions of arms and suckers.

Altogether, this provides cephalopods with the highest degree of centralization compared with any other mollusk and with the vast majority of other invertebrate phyla. Nevertheless, the nervous system keeps the basic invertebrate organization with layers of cell bodies distributed externally and with an inner neuropil.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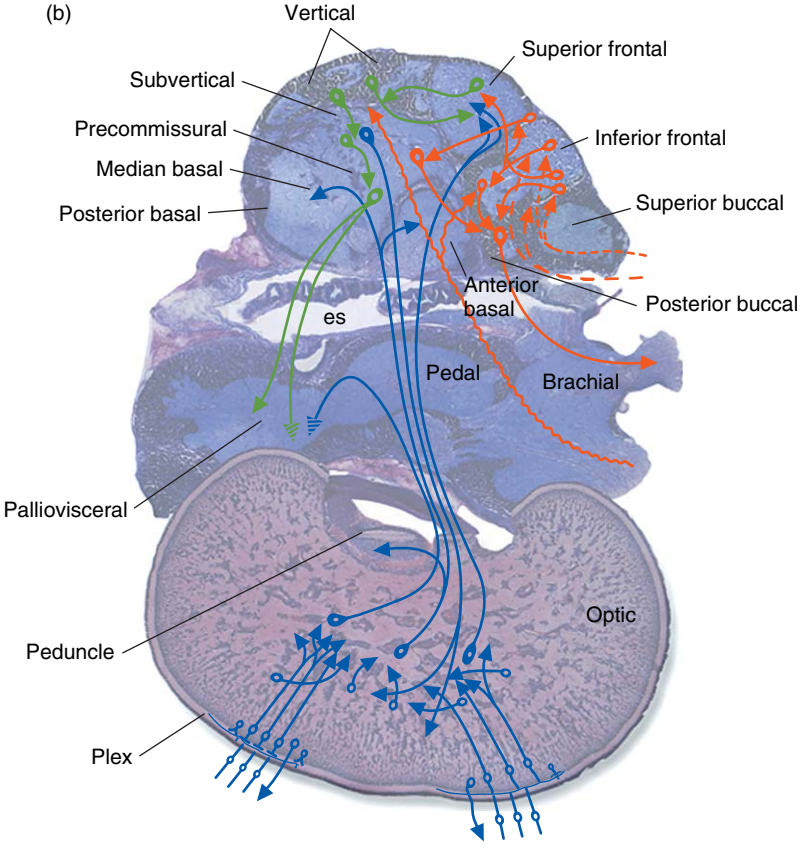
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Brachial
Palliovisceral
Peduncle
Optic
Plex

A thorough description of the gross morphology, neuroanatomy, and organization of the cephalopod brain is known for *O. vulgaris* (Young, 1971) and for decapods (Young, 1974, 1976, 1977, 1979; Messenger, 1979). (Reviews on this topic are available in Bullock, 1965b; Budelmann, 1995; Nixon and Young, 2003; but see also Budelmann et al., 1997). In addition, quantitative data on the relative size of the different brain lobes of various species of cephalopods is also available following the contributions of Wirz (1959) and Maddock and Young (1987). Information on the relative size of the lobes in hatchlings has been determined for several Mediterranean species by Frösch (1971; for review see also Nixon and Young, 2003).

Taken as a whole, these studies have shown that the Cephalopoda present a marked diversification of cerebrotypes that should correspond to differences in the habitats occupied within the marine environment (Nixon and Young, 2003; Borrelli, 2007). For example, the brachial and inferior frontal lobes are highly diverse between octopods and decapods, with those in the former being considerably larger than in the latter as a consequence of their benthic lifestyle (and tactile sensorial modality). On the other hand, the vertical lobe (as integrative center, see Sections 1.31.2.2, 1.31.2.3) shows greater variability among species both in relative size and gross morphology (Young, 1979; Maddock and Young, 1987; for a review, see Nixon and Young, 2003).

Finally, as mentioned above, the arms of an octopus contain about two-thirds of the some 500 million neurons in total. The arms can thus work rather autonomously (following a hierarchical functional control of the higher motor centers), as they can generate highly stereotyped movements (Altman, 1971; Sumbre et al., 2001, 2005, 2006).

Moreover, we now know the possible function of roughly 40 lobes within the cephalopod brain by stimulation experiments carried out in *S. officinalis* (Boycott, 1961) and *O. vulgaris* (Boycott and Young, unpublished data: cited in Young, 1963, 1971).

These studies have shown that the supraesophageal mass is responsible for sensory processing and analysis and control of behavior, and it also provides motor commands and coordination to the higher motor centers (i.e., basal lobes). The subesophageal mass, instead, provides the control of particular sets of effectors via intermediate and lower motor centers.

1.31.2.2 Neural Substrates of Behavior

The analysis of hundreds of lesion experiments conducted on octopuses (mostly), squids, and cuttlefishes (review in Sanders, 1975; Boyle, 1986) and of several dozen serial histological sections of cephalopod brains allowed Young and coworkers not only to describe the anatomy of the nervous system of these animals (for a review, see Nixon and Young, 2003) but also to unravel the circuit leading to their visual and tactile learning capabilities (**Figure 3**). In Young's view, learning and memory are achieved in cephalopods by "a series of matrices of intersecting axes, which find associations between the signals of input events and their consequences" (Young, 1991: p. 200).

1.31.2.2.1 Tactile information

During tactile processing (and learning), the decision to grasp or reject an object by an octopus is made on the basis of the interaction of a network made up by the following eight matrices:

- Lateral inferior frontal lobes
- Median inferior frontal lobe
- Subfrontal lobe
- Posterior buccal lobes
- Lateral superior frontal lobes
- Median superior frontal lobe
- Vertical lobe
- Subvertical lobe

The system, which corresponds to roughly six lobes of the supraesophageal mass, is tuned to take any object touched (i.e., tactile exploratory drive) unless pain signals are conveyed.

Figure 3 A schematic representation of the neural pathways for the visual (blue) and tactile (red) sensorimotor systems in the brains of *Sepia officinalis* (a) and *Octopus vulgaris* (b). Possible integrations (or shared pathways) between the two systems are indicated in green. In the supraesophageal mass, several matrices of the two systems overlap, particularly in *Octopus* (see text for details). The optic lobes (bottom) are depicted from horizontal sections. The reciprocal positions and sizes are not in scale. The supra- and subesophageal masses of the two species are drawn or photographed from a sagittal section: (*Sepia* (a) after Sanders FK and Young JZ (1940). Learning and other functions of the higher nervous centres of *Sepia*. *J. Neurophysiol.* 3: 501–526. *Octopus* (b); after Young JZ (1991) Computation in the learning system of cephalopods. *Biol. Bull.* 180: 200–208). Esophagus (es); plexiform zone (plex).

The interaction between the inferior frontal (positive signals) and the subfrontal (negative signals) plays the major role in decision making (i.e., take/reject) by the animal.

Finally, the inferior frontal system (in decapods, the posterior buccal and the lateral inferior frontal lobes; in octopods, the posterior buccal, the lateral and median inferior frontal, and the subfrontal lobes) is qualified as the main site of the long-term memory storage for tactile information, as roughly 60% of the total tactile learning capacity is thought to reside in this brain region (Young, 1983). The remaining quota is distributed between the superior frontal and vertical lobes (approximately 25%) and, to a limited extent, in areas of the subesophageal mass (the remaining 15%; Young, 1983; see also Budelmann and Young, 1985; for a review, see Wells, 1978; Young, 1991, 1995; Williamson, 1995; Williamson and Chrachri, 2004).

In decapods, the so-called inferior frontal lobe is more comparable to the posterior buccal lobe of *Octopus* than to the inferior frontal lobe *sensu stricto* (Young, 1971, 1979), which is lacking in cuttlefishes and squids. However, the distribution and interchange of fibers originating from the arms make the inferior frontal lobe of decapods appear close to the median inferior frontal lobe of *O. vulgaris* (see p. 350 and p. 314 of Young, 1971, 1979, respectively).

From a functional point of view, the fact that cuttlefishes and squids detect their prey visually and capture it with the arms or by ejection of the tentacles (depending on the prey species; see a review in Hanlon and Messenger, 1996) makes us assume that they have only a limited need and capacity for learning tactile information. Manipulatory activities related to feeding are considered largely programmed and based on reflexes (but see Halm et al., 2000, 2002, 2003), with some inhibitory pathways (i.e., reciprocal inhibition) from the small amacrine cells that are distributed among the large motor neurons of the buccal and subesophageal centers (Young, 1976, 1991), in close resemblance to the spinal cord of mammals (Young, 1995).

1.31.2.2.2 Visual information

Like the tactile learning system, visual stimuli are classified and processed behaviorally by a network composed of the following four matrices:

- Lateral superior frontal lobes
- Median superior frontal lobe
- Vertical lobe
- Subvertical lobe

The optic lobe also plays a major role in the visual learning system but was excluded by Young from the assemblage of matrices because of its location outside of the supraesophageal mass.

Again, like before, the system of matrices is tuned to promote the animal to attack the stimulus unless unpleasant feelings are perceived. At the level of the optic lobes, the cells from the retina reach the outer plexiform zone, where they make contact with a large number of cells (second-order visual cells; see **Figure 3**) that act as feature detectors. They constitute dendritic fields of various shapes and extensions that allow the recognition of "the relevant features of objects and scenes" (Young, 1995, p. 434) encountered by the animals during their everyday life (see also Deutsch, 1960; Sutherland, 1960; for a review, see Sanders, 1975; Hanlon and Messenger, 1996). The axons of the second-order cells "form columns proceeding to the center of the [optic] lobe, where they interact in an interweaving matrix of cells and fibers" (Young, 1991, p. 205).

Outputs of the neurons of the optic lobe proceed toward various areas of the brain (**Figure 3**). Some go directly to the magnocellular lobe, which is considered to act in situations where rapid escape reactions are needed (Young, 1971, 1973, 1991, 1995). Other fibers proceed to the peduncle and basal lobes, thus serving to regulate movements, and finally the third pathway goes toward the core of the visual matrices. It is at this level that the interaction between the lateral superior frontal (promoting the attack) and the median superior frontal and vertical lobes (inhibiting the attack) regulates the animal's behavior. Finally, according to Young, memory formation for visual experiences and their outcome take place within the optic lobes, with active participation of the supraesophageal centers (Young, 1991, 1995).

As is shown in **Figure 3**, the neural organization of the visual system of decapods has close affinities to that of octopods (Cajal, 1917; Sanders and Young, 1940; Young, 1973; for a review, see Nixon and Young, 2003; Williamson and Chrachri, 2004). Perhaps the system works in a similar way in cuttlefishes and squids, although it has not yet been extensively studied in the learning context, as has been done for octopuses (see a review in Young, 1991; Agin et al., 2006a).

1.31.2.3 How Computation in the Learning System Is Achieved

The idea promoted by Young and colleagues on the existence of multiple matrices in the central nervous

system, working in the control of behavioral responses, found its roots in the pioneering studies of Cajal (1917) and Sanders and Young (1940). The model proposed by Young was deduced essentially on the basis of morphological and experimental evidence (see the review in Young, 1961).

Following Young, the two systems work on similar principles. The information is processed through a series of matrices that allow signals of different types (and meanings) to interact to some extent with each other and to regulate subsequent behavior for attack/take and retreat/reject responses (**Figure 3**). In addition, the modulation between promotion and inhibition is tuned in order to facilitate exploratory behavior. According to Young, the systems are designed with a close similarity with complex nervous systems such as the mammalian hippocampus and neocortical centers (Young, 1995). This system seems to be limited in that a complete integration (transfer) between visual and tactile information is relegated only at the level of the effectors (Allen et al., 1986), although limited cross-modality has been shown in *O. digueti* at higher neural levels (Michels et al., 1987). Similar findings are reported for *O. vulgaris* by Robertson and Young (in preparation, cited by Michels et al., 1987) but unfortunately are unpublished as far as we know.

Wells (1978) published an alternative hypothesis worthy of mention based on the response of animals to an associative learning context. In his opinion, the sensorial inputs (visual system) reach the vertical lobe, where they are modulated on the basis of the effects of positive or negative signals (tactile system). Here the system is tuned to sensitize by raising or lowering the level of response on the basis of previous experiences. In this way, new information is added to the long-term cumulative experience, allowing short-term fluctuations and flexible and adaptive behavior (Wells, 1978).

The hierarchical control of motor patterns (described earlier) with lower (i.e., arms), intermediate (i.e., subesophageal), and higher (i.e., supraesophageal) motor centers (Boycott, 1961; Young, 1971, 1991) has been updated by experimental evidence (Plän, 1987). According to this view, different motor areas work in consensus, contributing to a more democratic concept of neuronal assembly. In other words, parallel central sensorimotor pathways cooperate synaptically to produce a given motor pattern (i.e., behavior; Plän, 1987), which appears to be similar to what has been shown in other invertebrates (for a review, see Getting, 1989; Leonard and Edstrom, 2004; Calabrese, 2007).

These models should be validated (hierarchical vs. democratic) in view of modern experimental approaches.

The compartmentalization (or certain modularity of the system) achieved by the multiple matrices may correspond well to the lifestyle adaptations of cephalopod species in different environments and niches (Nixon and Young 2003; **Figure 4**), as recently tested by combining ecological and neuroanatomical data (Borrelli, 2007).

As solicited in several occasions by Young himself (1985, 1995), a physiological investigation of the responses of the cells within the various regions of the brain is necessary to disclose the functional characteristics of the system. It is only relatively recently that attention has been focused on studies of this kind, starting from the pioneering studies on *S. officinalis* (Bullock and Budelmann, 1991) and *O. vulgaris* (Williamson and Budelmann, 1991) up to the latest findings of Hochner and coworkers (2003, 2006).

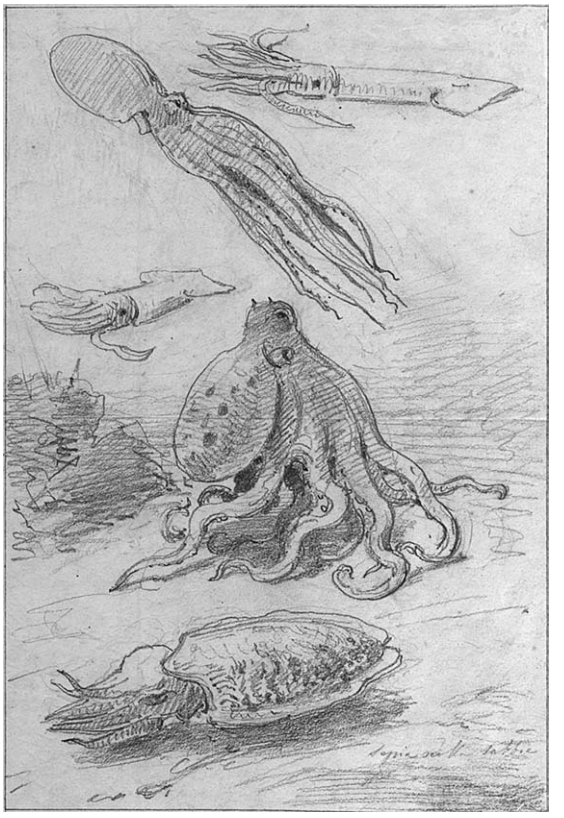

Figure 4 Old drawing by Comingio Merculiano (188?, unpublished) of octopuses (*Octopus vulgaris*), squids (probably *Loligo vulgaris*), and cuttlefishes (*Sepia officinalis*) expressing their different behavioral adaptations. Stazione Zoologica Archives, ASZN: Ua.I.506.

The most recent electrophysiological studies in the octopus confirm the view that convergent evolution has led to the selection of similar networks and synaptic plasticity in remote taxa (i.e., cephalopods and higher vertebrates), contributing to the production of complex behavior and learning capabilities (for review, see Hochner et al., 2006). A similar architecture and physiological connectivity of the vertical lobe system (i.e., median superior frontal and vertical lobes) of the octopus with the mammalian hippocampus, together with the large number of small neurons acting as interneurons, suggest a typical structure with high redundancy of connections working with en passant innervations. This makes it possible to create large-capacity memory associations (Hochner et al., 2003, 2006). However, the analogy between the octopus and mammalian systems is not complete, the major differences being in the morphological organization and biophysical characteristics (see a review in Bullock, 1965b; Williamson and Chrachri, 2004; Hochner et al., 2006).

Finally, it is important to underline that a system similar to *Octopus* has been found in *S. officinalis*, but with differences emerging at various levels (morphological, physiological, and behavioral; Agin et al., 2006a; Graindorge et al. 2006; B. Hochner, personal communication). The present results, although promising, must be taken with caution since they are preliminary at this stage.

Last, it is worth mentioning that a computer simulation model of the predatory response of *O. vulgaris* (attack behavior *sensu*: Maldonado, 1963a; Packard, 1963; for a review, see also Borrelli et al., 2006) has been developed (Clymer, 1973).

In the model, a mnemon (i.e., a visual feature with associated memory value resulting from experience; Young, 1965) is activated by a given visual input to a specific set of classifying cells and switched on/off on the basis of other inputs that depend on the taste–pain circuits. The output of these units (i.e., attack command) is summed up to produce an overall attack strength, in contrast to the opposite units (retreat command) that in a similar way build an overall retreat strength. These values (or strengths) are combined in the model and determine the final attack/retreat response (Clymer, 1973).

The model proposed by Clymer was based on the knowledge of *O. vulgaris*'s predatory behavior (also as a result of the discrimination experiments) as deduced by Young (1964, 1965) and Maldonado (1963b). Interestingly, the results produced from the model are comparable to those obtained from proper experiments with live animals, including the responses resulting from short- and long-term changes in behavior and interference on learning performance when spacing between trials is reduced in time (Clymer, 1973). In addition, the model has been recently reviewed and modified on the basis of the most advanced findings on neural networks and learning in simulated environments (Myers, 1992).

1.31.3 Learning in Cephalopods

Many reviews centered on the biology and learning capabilities of cephalopods have been published during the course of the last 50 years. An analysis of the literature indexed in both the Zoological Record and the Web of Science (from 1950 to 2007) selected roughly 100 reviews regarding the topic. Several other reference works (e.g., *The Mollusca* by Wilbur, 1983–1988) with chapters relevant to the subject must also be taken into consideration.

Over the last few decades, different workers have attempted to synthesize the knowledge on the behavioral biology of cephalopods and its flexibility (Packard and Hochberg, 1977; Hanlon, 1988; Mather, 1995, 2007; Boal, 1996; Messenger, 1996, 2001; Williamson and Chrachri, 2004; Borrelli et al., 2006; Hochner et al., 2006, to cite just a few). The most significant and comprehensive reviews published on cephalopod biology, learning, and memory are those by Young (1961), Sanders (1975), Wells (1978), Boyle (1986), and Hanlon and Messenger (1996).

In this chapter, we are deliberately not summarizing the information provided by the papers listed above, as this would necessarily result in redundancy. Our aim is simply to offer the reader with a general overview of what is known on the subject, which phenomena/mechanisms have been described and analyzed in detail, and which cephalopod species have been chosen as models for studies on the learning and memory capabilities of this taxon (**Table 1**). In the following pages, we focus our attention on the results and directions of the most recent advancements on the behavioral biology of learning and memory in cephalopods.

As thoroughly reviewed by Hanlon and Messenger (1996), various forms of learning (**Table 1**) have been demonstrated in cephalopods, from simple sensitization, to associative learning and problem solving, to more complex forms such as spatial and social learning and tool use. In essence, a large number of the entities proposed by Moore (2004) in his cladogram of learning processes have been shown in some of the 780 cephalopod species known to date. It is a pity that

Table 1 Breadth of the learning paradigms shown in cephalopods, by species[a]

	Habituation	*Sensitization*	*Classical conditioning*	*Instrumental conditioning*	*Avoidance learning*	*Spatial learning*	*Mazes and problem solving*	*Social learning*	*Perceptual processes in visual learning*
Sepia officinalis	X		√	√	X	X		X	
Loligo vulgaris									
Lolliguncula brevis	√		√						
Todarodes pacificus			√						
Octopus vulgaris	√	√	√	√	√	√	√	√	√
Octopus bimaculatus			√						
Octopus bimaculoides			√			X			√
Octopus cyanea			√	√					
Octopus joubini					√				
Octopus maorum					√				
Octopus maya			√				√		√
Enteroctopus dofleini			√						
Eledone moschata			√		√				

[a]Data are arranged following Hanlon RT and Messenger JB (1996) *Cephalopod Behaviour*. Cambridge: Cambridge University Press. The learning capabilities (√) by species are deduced from reviews (Sanders GD [1975] The cephalopods. In: Corning WC, Dyal JA, and Willows AOD [eds.] *Invertebrate Learning. Cephalopods and Echinoderms*, pp. 1–101. New York: Plenum Press, and Hanlon RT and Messenger JB [1996] *Cephalopod Behaviour*. Cambridge: Cambridge University Press), with the exception of more recent findings (X) that are also described in the text. It is important to underline that certain paradigms, such as discrimination learning, are here classified as classical conditioning, although they could also be considered as cases of operant (or Thorndikian) conditioning (see Moore, 2004).

learning (and behavior) studies are still restricted to a limited number of representatives (**Table 1**). In fact, a detailed description of the behavioral repertoire is available for only roughly 30 species (review in Hanlon and Messenger, 1996; Borrelli et al., 2006). Another problem inherent to the cephalopod literature is that some phenomena have been relegated to anecdotal accounts (e.g., tool use: Power, 1857; Pliny the Elder, 1961) and that experimentally controlled observations on such capabilities remain to be done.

1.31.3.1 Sensitization

Following our everyday practice with octopuses, the daily presentation of food increases the chance of the animal attacking, so that its predatory performance (measured as the time to attack the prey from its appearance in the tank) improves with time. This is a clear case of sensitization, similar to what was tested empirically in the past by Wells and coworkers, who showed that food could enhance the probability to attack the stimuli (as much as shock depressed it; Wells, 1967a, cited in Hanlon and Messenger, 1996), or as was demonstrated in conditions where chemotactic behavior was studied (Chase and Wells, 1986).

This phenomenon has recently been confirmed in our laboratory for *O. vulgaris* following the classic acclimatization phase to the experimental setting. The continuous administration of a reward (food) over days improves the predatory performance that reaches a steady-state level during consecutive attacks. However, it is difficult to rule out whether different speeds in the attack curves (when interindividual variability is evaluated) may be related to food preferences, novelty, or familiarity toward food items, or to individual differences in the capability to cope with contextual learning processes, and so on (see also Section 1.31.4.1).

Finally, it could be extremely interesting to explore whether the relative length of time in the laboratory has an effect on octopuses' performance in a learning paradigm, say, for example, on the individual preferences in a simultaneous visual discrimination task. This concept should not be underestimated, also considering that as the positive learning process (see Section 1.31.4.1) proceeds with time, differences between individuals may be reduced to a few clear-cut responses and other characteristics of the subjects may emerge. Such processes may be easily extended to other cephalopods, although significant differences between species are expected as a result of different lifestyles and adaptive capabilities (for a review, see Packard, 1972; Nixon and Young, 2003).

1.31.3.2 Various Forms of Associative Learning

A new set of learning protocols (or variations on the theme) has recently been developed successfully in cephalopods. We provide a few examples below.

Painting quinine on the carapax of prey items (crabs, shrimps) was sufficient to show simple and rapid taste aversion learning in *S. officinalis* (Darmaillacq et al., 2004). This produced significant shifts in prey choice that were retained over the long term (for at least 3 days; Darmaillacq et al., 2004).

Calvé (2005) showed that the cutout of a bird (predator) gliding over individual cuttlefishes elicited startling reactions of different intensities. The startling stimulus significantly affected the cuttlefishes' hunting behavior, although evidence suggests that the animals habituated to it (Calvé, 2005).

Plastic spheres were utilized in successive visual discrimination tasks in order to test whether classical conditioning could change the species-specific predatory (or hunting) behavior; the results suggest that autoshaping occurs in *S. officinalis* (Cole and Adamo, 2005).

All the cases mentioned above represent innovations in the practice of learning studies with *Sepia* and clear additions to the classic prawn-in-the-tube training procedure pioneered by Sanders and Young (1940) and modified by successive authors (Wells, 1962; Messenger, 1973; see also Chichery and Chichery, 1992a,b; **Figure 5**). Notwithstanding, this procedure promoted a large number of studies on associative learning in the cuttlefish and on the biological machinery involved (e.g., Agin et al., 2000, 2001, 2003; Bellanger et al., 1997, 1998, 2003, 2005; Halm et al., 2003). Recent findings in *S. officinalis* strongly support the view that learning not to attack prey trapped in a transparent tube (inhibitory learning) corresponds to associative learning (Agin et al., 2006b; Purdy et al., 2006).

The number of training protocols available for *Octopus* is traditionally greater than for other species, mainly as a result of the animal's behavioral flexibility and feasibility of experimental studies with this species (see also **Table 1**). Moreover, recent protocols such as passive avoidance, additional problem-solving tasks (e.g., jars with multiple openings, black boxes; Borrelli, 2007), and even habituation tests have extended the repertoire of training paradigms that may be utilized to find answers to the fundamental question of how and to what extent *O. vulgaris* is capable of learning to modify its behavioral response.

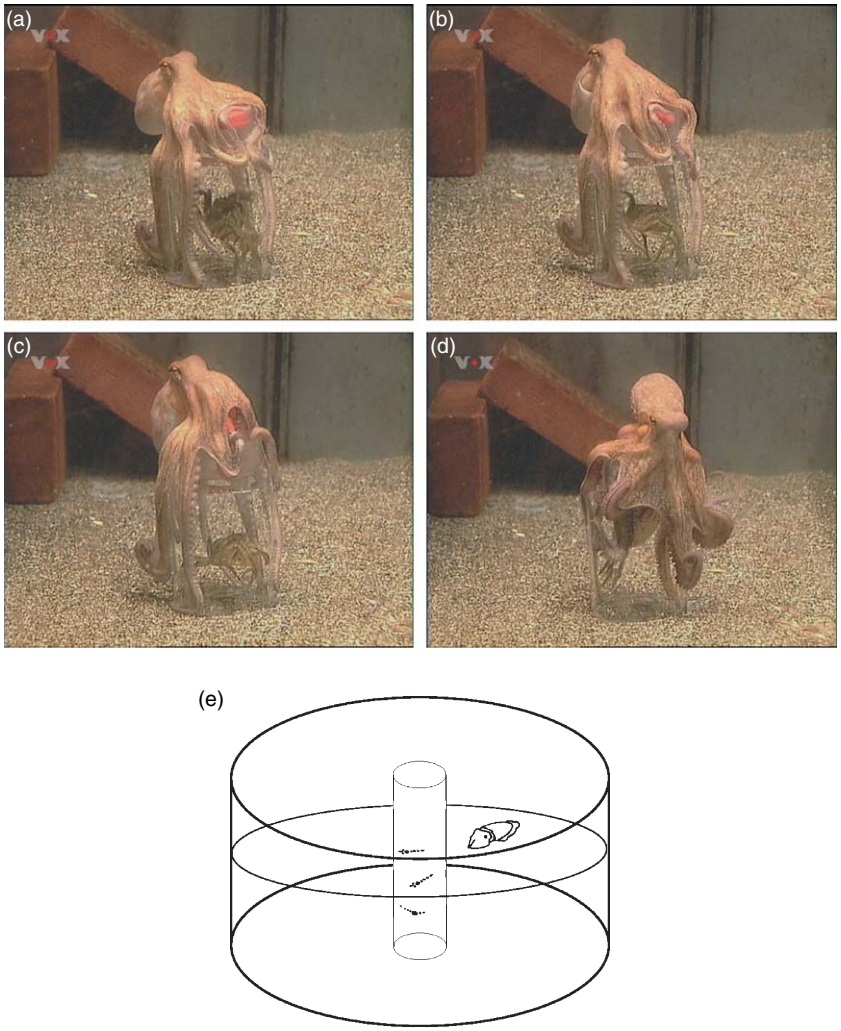

Figure 5 Two examples of classic tasks utilized to study learning and memory recall in *Octopus* and *Sepia*. (a)–(d) A sequence of frames taken from video recordings of the problem-solving experiment in *O. vulgaris* (classic jar). Courtesy of Mr. M. Schumacher, RS-Film. (e) A sketch of the prawn-in-the-tube protocol utilized with *S. officinalis* (after Wells MJ (1962) Early learning in *Sepia*. *Symp. Zool. Soc. Lond.* 8: 149–169).

1.31.3.3 Development of the Learning Capabilities

The prawn-in-the-tube protocol, which was and is still utilized to study associative learning in *S. officinalis*, disclosed important details on how cuttlefishes' behavioral plasticity changes during ontogeny. The analysis of this phenomenon made it possible to find significant correlations with the development (and maturation) of the neural circuitry, considered to play a role in the processing of behavioral responses and learned changes (Wells, 1962; Messenger, 1973; Chichery and Chichery, 1992a,b; Dickel et al., 1997, 1998, 2001; Agin et al., 2006a,c; for review see also Dickel et al., 2006).

In a similar way, the predatory efficiency of *Loligo* spp. on copepod swarms is reported to improve with age. It was found that the mastery of copepod capture develops progressively from the initial basic attack type up to more specialized strategies that effectively extend the range of capture to both longer and shorter distances, culminating in the adult-like prey capture behavior (Chen et al., 1996).

Similar changes of behavior with experience have also been described for octopuses; the optimization of the site and number of holes drilled in bivalve preys, for example, distinguishes juvenile from adult performance (see a review in Mather, 1995, 2007).

1.31.3.4 Spatial Learning

Spatial learning was originally tested in cephalopods using mazes, which led to a heated debate as to whether octopuses were capable or not of learning a detour (Bierens de Haan, 1926; Buytendijk, 1933; Schiller, 1949; Boycott, 1954; Wells, 1965a; but see Walker et al., 1970; Moriyama and Gunji, 1997).

During the last few decades, studies on the problem-solving abilities of these animals have been frequently confused and erroneously attributed to spatial learning processes (Piéron, 1911; Fiorito et al., 1990, 1998b; but for critiques, see Mather, 1995; Hanlon and Messenger, 1996).

Nevertheless, spatial learning *sensu stricto* has been shown in several species over the last few years. Apart from the pioneering studies of Mather (1991) on *O. vulgaris*, learning to orient and navigate in space is reported for *O. bimaculoides* (Boal et al., 2000a) and *S. officinalis* (Karson, 2003; Karson et al., 2003; Graindorge et al., 2006; Alves et al., 2007). However, our knowledge is probably underestimated relative to the large number of cephalopods that are known to cover short and/or long distances in space (e.g., Shevtsov, 1996; Sims et al., 2001; Arkhipkin and Middleton, 2002; Oosthuizen and Smale, 2003; Arkhipkin et al., 2004, 2006; Olyott et al., 2006; Smith et al., 2006; Watanabe et al., 2006; for a review, see also Hanlon and Messenger, 1996).

Such studies should be encouraged to increase our knowledge on the differences in the spatial capabilities among cephalopod species. In addition, this new avenue of research could facilitate comparative analysis to answer questions relative to the neural structures (i.e., the hippocampus and possibly the vertical lobe system; Graindorge et al., 2006) involved in the processing of such a sophisticated ability, as recently found in other animals (Jacobs, 2003; Frost and Mouritsen, 2006).

1.31.3.5 Other Learning Capabilities

Problem solving and social learning have mostly been studied in *O. vulgaris*. These learning paradigms have often been debated and criticized for not providing clear evidence of learning in these animals (Mather, 1995, 2007; Hanlon and Messenger, 1996; but see also Biederman and Davey, 1993; Suboski et al., 1993).

1.31.3.5.1 Problem solving

The task traditionally utilized to test the problem-solving capabilities of *O. vulgaris* (Fiorito et al., 1990), derived from the pioneering experiments by Piéron (1911), uses the well-known skills of octopuses with jars (Cousteau and Diolé, 1973). In the classic experiment, the animal is faced with a transparent glass jar, closed with a plug, containing a live prey. The octopus generally attacks the object at first glance, with the attack elicited by visual cues (the sight of the prey). The physical contact with the jar makes the animal switch to the tactile-driven exploration of the stimulus (**Figure 5**). While manipulating the jar, the octopus must solve two problems: the operandum (i.e., removing the plug by pulling), and the detour (i.e., the blind exploration of the inside of the jar with the arms to reach for the prey).

It was shown that octopuses spent significantly less time on the two motor components of the task with trials, thus suggesting that learning is involved in solving the problem (Fiorito et al., 1990, 1998b).

The switch between the two modalities (from visual to tactile) that controls and determines the octopus' performance in the task is not automatic and occurs at different instances in different animals. This further supports the view that learning of the task is required in order to sort out the motor programs (probably species specific) that are necessary to solve the paradigm. In fact, the pulling action, which is required by an animal dealing with the operandum, is already present in the species' behavioral repertoire, as the same technique is adopted by octopuses preying upon bivalves (McQuaid, 1994; Fiorito and Gherardi, 1999; Steer and Semmens, 2003). Moreover, the detour may be compared to the so-called speculative pounce (e.g., Yarnall, 1969), a common foraging strategy in which animals mostly use a tactile-driven manipulation and blind exploration of the sea bottom in search of edible items hidden under rocks or in crevices (for a review, see Hanlon and Messenger, 1996; Borrelli et al., 2006).

Different tasks (e.g., jars with multiple openings and boxes with drawers) have recently been designed to further explore *O. vulgaris*' problem-solving capabilities.

Similar objects could be used with other octopod species, facilitating comparative analysis of the taxon's behavioral flexibility. In addition, such tasks may provide more general information on the biological correlates involved in the two modalities (visual and tactile) that govern such behavioral responses.

1.31.3.5.2 Social learning

Fiorito and Scotto (1992) provided experimental evidence of social learning in *O. vulgaris*. They showed that naïve animals were able to discriminate and choose between two stimuli following observation

of trained conspecifics. The observer octopuses appeared to follow the model witnessed, and their choice seemed to be stable over time. The original work was replicated to test the role of the neural circuit considered to be involved in social learning (Fiorito and Chichery, 1995) and to study the attention and memory retrieval capabilities of this kind of learning after a pharmacological interference (Fiorito et al., 1998a).

The results of social learning in *O. vulgaris* were debated and criticized in a number of publications (e.g., Biederman and Davey, 1993; Hanlon and Messenger, 1996).

Future studies are required to reply to the many questions that remain open on this peculiar learning capability, since it is possible that certain other factors may act in favor of or against the outcome of the observation of the conspecific's behavior by naïve observers (e.g., reproductive drive, social dominance, territoriality; for review, see also Boal, 2006). This, at least, appears to be the case in *S. officinalis*, where social experience promotes (Warnke, 1994) or inhibits (Boal et al., 2000b) social facilitation in the feeding behavior of cuttlefishes, probably depending on the relative age of the animals.

1.31.4 Neglected Issues in the Study of Cephalopod Learning

As amply discussed above, most of our knowledge on the learning capabilities and memory recall in cephalopods derives from experiments conducted in the laboratory, the sole exception being the field studies on *O. vulgaris* carried out in the waters of Bermuda (Mather, 1991; Mather and O'Dor, 1991). Mather and colleagues demonstrated that octopuses acquire information and keep memories of their surroundings to navigate during foraging trips (i.e., spatial learning; Mather, 1991; see also Hanlon and Messenger, 1996; Forsythe and Hanlon, 1997). Moreover, *O. vulgaris* is capable of behavioral plasticity in lifestyle resulting from changes in foraging needs and predation pressure (Mather and O'Dor, 1991).

Despite the growing number of publications on the habits and adaptive responses of cephalopods in the wild (e.g., Smale and Buchan, 1981; Moynihan and Rodaniche, 1982; Roper and Hochberg, 1988; Mather and Mather, 1994; Hanlon et al., 1999), our inference on learning in cephalopods is still largely biased by laboratory evidence. Whether this is the result of the behavioral flexibility (neural and behavioral) of the cephalopod system to the new context (captive situation), or whether it corresponds to the animals' real needs in the wild, still remains an open question.

1.31.4.1 Effect of Acclimatization: Contextual Learning

Following capture, the animals are generally immediately brought by the fishermen to the laboratory, where they are exposed to the experimental setting (i.e., a novel environment). In the case of an octopus, the animal is thus constrained to a tank that represents a foraging area about 300 times smaller than that required by the animal in natural conditions. In addition, the scenery to which the animal is exposed is somewhat dull and uniform, independent of whether it is designed to provide an enriched or an impoverished environment (e.g., Dickel et al., 2000; Anderson and Wood, 2001; Poirier et al., 2004). We may therefore hypothesize that the new context has only a poor resemblance to the seascape the cephalopod had experienced until then.

This is true not only for octopuses but also for other cephalopod species. A rough analysis of the literature on cephalopods published over the last 10 years has provided exhaustive examples and confirms this view.

The animal must adapt to this novel environment, which is achieved in a variable amount of time and is generally referred as acclimatization. During the acclimatization phase, a common practice is to expose cephalopods to live prey (for exceptions, see, e.g., Boletzky and Hanlon, 1983; Boal, 1993; Koueta et al., 2006), not only to maintain the animals in captivity but also to test their recovery in motivation to attack (i.e., well-being; for a review, see Boyle, 1991). In addition, the animals are exposed to experiments that study their predatory behavior (attack/not attack or take/reject responses). Thus, they must be able to face the task and plastically adapt their species-specific predatory behavior to the new context. As already mentioned above, this phase takes a variable length of time and depends (from species to species) on the animals' previous experience, the individual variability resulting from biological and possibly ecological factors (differences in age, sex, maturity, etc.), and the common practice of experimenters, to cite a few examples.

It has been recently demonstrated that during the acclimatization phase, the animal is exposed to a positive learning process, which is a form of contextual learning. As described by Maldonado (1963a,b, 1964), at the beginning of acclimatization (or

training), the time spent by an octopus to attack the stimulus is relatively long, and the behavior (in terms of types of attack) is highly variable. However, as the animal becomes more and more accustomed to the experimental setting (or paradigm), its attacks on the stimulus become faster and faster, a process known as the positive learning process. Moreover, this reduces the types of attack curves to nearly a stereotype, that is, the full attack in the octopus (Maldonado, 1963a; Packard, 1963) and the tentacle attack in decapods (e.g., *S. officinalis*: Messenger, 1968; *Loligo vulgaris*: Neill and Cullen, 1974; *L. pealeii* and *L. plei*: Kier, 1982; *Illex illecebrosus*: Foyle and O'Dor, 1988).

In sum, during the positive learning process (*sensu* Maldonado, 1963a), behavioral syndromes are generally reduced to a few broad types representing populational differences (within a species). These differences are recognized to play important ecological and evolutionary roles, mainly during the adaptation to environmental changes (Sih et al., 2004a,b).

In addition, there is evidence in *O. vulgaris* that this phenomenon is directly linked to a more general form of contextual learning, as recently shown in other invertebrates (e.g., Tomsic et al., 1998; Liu et al., 1999; Haney and Lukowiak, 2001; Menzel, 2001; Law et al., 2004; Skow and Jakob, 2006; Zhang et al., 2006).

A series of factors may interfere in this process, such as:

- The time of day at which the experiment is conducted (which does not necessarily correspond to the animal's peak of activity in natural conditions);
- The feeding regime;
- The experimental setting to which the animal is exposed; and
- The prey types utilized in captivity that do not necessarily correspond to those fed upon in the wild.

Concerning this last point, for example, the animals' performance in the new context may be affected by individual dietary preferences derived from previous feeding habits (e.g., for *S. officinalis* Darmaillacq et al., 2006), which have also been shown to influence the performance in visual and tactile discrimination tasks during associative learning (e.g., for *O. vulgaris*: Messenger and Sanders, 1972; Bradley and Messenger, 1977).

1.31.4.2 Neophobia/Neophilia and the Shy–Bold Continuum

Another neglected issue in the study of learning in cephalopods is whether novelty may interfere with the animals' decision-making processes (Greenberg and Mettke-Hofmann, 2001). In other words, familiar objects or prey types should be preferred to novel ones, and the natural exploratory drive, connected to cephalopods' voracious appetite, should be reduced by the presence of novel stimuli.

In *O. vulgaris*, for example, the behavioral flexibility (in terms of learning capabilities) of this species corresponds to ecological plasticity, where opportunistic behaviors and reduced neophobia are exhibited (see also Section 1.31.2.3). *O. vulgaris* seems to show less feeding specialization and a higher versatility in foraging than other cephalopods. This appears to be related to the changes in the lifestyle during ontogeny (Hanlon and Messenger, 1996; Nixon and Mangold, 1998) and to the frequent horizontal and vertical migrations by the different age classes moving in the water column (e.g., Oosthuizen and Smale, 2003). Therefore, octopuses have to deal with different environments both at a small and a large time scale (seasons and life history, respectively) that expose them to potentially different degrees of complexity. Under such circumstances, a low neophobia is expected for *O. vulgaris*, as results from current experimental work.

On the other hand, ecological stereotypy may favor individuals (or species) that contrast novel situations by exhibiting less flexible behaviors. This should be investigated.

A consequence of the neophobia/neophilia behavioral types discussed above, at any level (from the individual to the species), leads to the shy–bold continuum (or in the cephalopod sense, to the approach–withdrawal axis *sensu*; Packard, 1963). This has been shown in several cephalopods: *S. officinalis* (Hanlon and Messenger, 1988; Calvé, 2005), *Euprymna tasmanica* (Sinn and Moltschaniwskyj, 2005), *O. vulgaris* (Packard, 1963; Borrelli, 2007), and *O. rubescens* (Mather and Anderson, 1993).

As described by Calvé (2005), cuttlefishes are classified as shy when they mostly remain inactive and when, following stimulation, respond by inking and jetting away. At the opposite end of the spectrum, bold animals appear active in the tank and interact more with humans (i.e., are tame; Buytendijk, 1993; Hochner et al., 2006). Of course, individuals will respond differently to the same tests on the basis of whether their behavioral traits tend more toward one or the other of the opposite shy–bold extremes of the axis.

Similar findings have been shown for *O. rubescens*, where individuals were classified into three major

behavioral components (activity, reactivity, avoidance; Mather and Anderson, 1993).

As already mentioned, the individual differences of behavioral traits in cephalopods confirm the phenomenon to be widespread among different taxa, ranging from invertebrates to vertebrates (e.g., Armitage, 1986; Kagan et al., 1988; Coleman and Wilson, 1998; López et al., 2005; Mettke-Hofmann et al., 2005a; for a review, see also Gosling, 2001; Sih et al., 2004b; Mettke-Hofmann et al., 2005b).

In planning future studies testing neophobia/neophilia in cephalopods, the importance of population differences (i.e., genetic polymorphism) and individual experience on environmental factors should be considered, as has been done in other animals. For example, it could be interesting to test how the response of individuals captured with different fishing methods in the wild would appear along the shy–bold continuum in laboratory conditions, taking advantage of what is known in the pumpkinseed sunfish (Coleman and Wilson, 1998). Moreover, Coleman and Wilson (1998) discovered that animals that behaved boldly in threatening contexts did not act necessarily the same when exposed to novel foods (i.e., foraging contexts). Would it not be intriguing to find similar – or even contrasting – results in cephalopods?

1.31.5 Memory in Cephalopods

Despite the considerable number of studies published on the extent of memory recall and on the effects of its impairment induced by experimental interference (for a review, see Sanders, 1975; Wells, 1978), very little is known on the ability of cephalopods to encode and retrieve information.

From the classic works of Sanders and Young (1940) and Schiller (1949), it was shown that cuttlefishes and octopuses are capable of short- and long-term memory, although differences emerged between the two species and among paradigms (**Table 2**).

It is astonishing that in many cases the memory trace was reported to last for a very long time (e.g., in octopus for weeks, according to Boal, 1991; Fiorito and Scotto, 1992; for months, according to Sanders,

Table 2 Summary of studies on the time course of memory recall in *Sepia officinalis* (*So*) and *Octopus vulgaris* (*Ov*)

Paradigm	*Species*	*Training*	*STM*	*MTM*	*ARM*	*LTM*	*PSD*	*Refs*
Prawn-in-the tube	*So*	1-t	20 m			48 h		6
Prawn-in-the tube	*So*	1-t	5 m					7
Prawn-in-the tube	*So*	1-t				24 h	+	8
Prawn-in-the tube	*So*	Spaced				24 h		6
Instrumental conditioning	*So*	Spaced				14 d		10
Avoidance	*So*	Spaced				3 d		9
Classical conditioning	*Ov*	Massed				2 d		2
Classical conditioning	*Ov*	Massed				2 d		4
Classical conditioning	*Ov*	Massed				24 h	–	11
Classical conditioning	*Ov*	Spaced				4 w		1
Classical conditioning	*Ov*	Spaced				16 w		3
Avoidance	*Ov*	Massed	1h	8 h		1 d		5
Avoidance	*Ov*	Massed			+	24 h	–	11

Different learning paradigms and training procedures with different intervals (massed vs. spaced) or length (single trial, 1-t vs. training to criterion) produce memories of variable time span. Moreover, the extent of the recall at each stage (STM, short term; MTM, medium term; LTM, long term) has not been systematically measured by each of the cited papers (see following, so a blank cell stands for missing data. Finally, only a handful of studies have addressed questions on whether long-term memory is anesthesia resistant (ARM; +, resistance) or protein synthesis dependent (PSD; + dependence; – no effect). Time units are as follows: minutes (m), hours (h), days (d), weeks (w). The references (Refs) included are: 1. Sutherland, 1957a, cited in Sanders GD (1975) The Cephalopods. In: Corning WC, Dyal JA, and Willows AOD (eds.) *Invertebrate Learning. Cephalopods and Echinoderms*, pp. 1–101. New York: Plenum Press; 2. Maldonado H (1968) Effect of electroconvulsive shock on memory in *Octopus vulgaris* Lamarck. *Z. Vgl. Physiol.* 59: 25–37; 3. Sanders, 1970b, cited in Sanders GD (1975) The Cephalopods. In: Corning WC, Dyal JA, and Willows AOD (eds.) *Invertebrate Learning. Cephalopods and Echinoderms*, pp. 1–101. New York: Plenum Press; 4. Wells MJ and Young JZ (1970) Single-session learning by octopuses. *J. Exp. Biol.* 53: 779–788; 5. Sanders GD and Barlow JJ (1971) Variations in retention performance during long-term memory formation. *Nature* 232: 203–204; 6. Messenger JB (1973) Learning in the cuttlefish *Sepia*. *Anim. Behav.* 21: 801–826; 7. Agin V, Dickel L, Chichery R, and Chichery MP (1998) Evidence for a specific short-term memory in the cuttlefish *Sepia*. *Behav. Process.* 43: 329–334; 8. Agin V, Chichery R, Maubert E, and Chichery MP (2003) Time-dependent effects of cycloheximide on long-term memory in the cuttlefish. *Pharmacol. Biochem. Behav.* 75: 141–146; 9. Darmaillacq A-S, Dickel L, Chichery MP, Agin V, and Chichery R (2004) Rapid taste aversion learning in adult cuttlefish *Sepia officinalis*. *Anim. Behav.* 68: 1291–1298; 10. Cole PD and Adamo SA (2005) Cuttlefish (*Sepia officinalis*: Cephalopoda) hunting behavior and associative learning. *Anim. Cogn.* 8: 27–30; 11. Zarrella, unpublished.

1970). Many unpublished observations carried out in our laboratory confirm this view. However, it was found that this remarkably long memory trace is not common to all individuals that learn a given task (Sanders, 1970). The reasons behind this surprising result of only certain individuals having a particularly long memory should be further investigated.

Unfortunately, the systematic analysis of the memory phases (**Table 2**), together with the time course of retention and memory consolidation (and perhaps reconsolidation), in cephalopods remains insufficient, especially when compared with the knowledge currently available for other taxa (for a review, see, e.g., McGaugh, 2000; Dudai, 2004).

Whether the memory recall observed in cephalopods corresponds to a more phylogenetically conserved consolidation mechanism is an issue that has been tested in *O. vulgaris* using several approaches (Maldonado, 1968, 1969; Zarrella, unpublished data). The data suggest that the establishment of long memory traces, to learn conditioned or associative responses, is maintained in *Octopus* following anesthetic treatments and protein synthesis inhibition (Zarrella, unpublished data) applied before or after training (massed intervals). In contrast, electroconvulsive shocks cause significant deficits in retention of previously learned paradigms (spaced intervals: Maldonado, 1968, 1969).

Moreover, protein synthesis inhibition was found to impair memory recall for the prawn-in-the-tube protocol in *S. officinalis* (Agin et al., 2003).

The above studies, although limited in number and species studied, show how promising it could be to test the role of the biological machinery in the establishment of long-term memories in cephalopods, especially when considering the contrasting evidence between species that has emerged so far.

1.31.6 Concluding Remarks

Whoever has had the chance to interact with a cephalopod, in the tank or at sea, remains struck by the richness of its behavioral repertoire, its distinct personality traits, and its penetrating gaze, which make it an extraordinary and fascinating creature. There is no doubt that cephalopods are learning animals, although it is difficult to give an objective view of the variety and extent of their learning capabilities.

What is still lacking in the field is more communication and exchange of ideas among cephalopod researchers and the focus on common aims or objectives that could strengthen the work in these animal models. The contribution to the knowledge of the behavioral biology in cephalopods should not be restricted to a handful of workers but be of interest to a greater number of scientists.

Through our behavioral analysis of learning and memory in cephalopods, we hope to have contributed to increasing the understanding and scientific interest and awareness of these animals.

As a last note, the study of cephalopods, despite the long historical tradition, suffers from the lack (or incomplete) availability of tools that are now available for other invertebrate models (such as *Apis*, *Aplysia*, *Caenorhabditis*, and *Drosophila*). The approach and direction of studies such as those pioneered and masterly conducted by Maldonado and coworkers on *Chasmagnathus* should be the example (see the review in Romano et al., 2006). The recent work on *Octopus*' genomics (Ogura et al., 2004; Choy et al., 2006) has contributed outstanding results that give us hope for the future.

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1.32 Behavioral Analysis of Learning and Memory in *C. elegans*

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1.32.1 Introduction

Caenorhabditis elegans was first developed as a model system by Sydney Brenner in the 1960s, with the goal of having a simple multicellular system in which to study the cellular and molecular mechanisms of development using modern genetic and molecular biological techniques. *C. elegans* is a 1-mm soil-dwelling nematode. In the laboratory it lives on a bacterial lawn of *Escherichia coli* in an agar-filled Petri dish. Brenner, along with John Sulston, who mapped the complete cell lineage of the organism, and Robert Horvitz, who elucidated the first programmed cell death mechanisms, won the Nobel Prize in Physiology or Medicine in 2002 for this work. For our purposes *C. elegans* is an excellent model for studying the behavioral, cellular, and molecular mechanisms involved in learning and memory.

Rankin et al. (1990) were the first to recognize *C. elegans* as a model for learning and memory when they showed that *C. elegans* is capable of both short- and long-term memory for the nonassociative form of learning known as habituation. Since 1990, many labs have investigated a variety of types (i.e., mechanosensory, chemosensory, and thermosensory) of both nonassociative (habituation) and associative learning (classical conditioning) in *C. elegans*. As a model of the cellular and molecular mechanisms of learning and memory *C. elegans* has many advantageous qualities. Composed of only 302 neurons, with only 5000 synaptic connections, all of which are identified, the *C. elegans* nervous system offers an opportunity to study a system in which all the neurons that mediate a learned behavior or memory can be identified (keep in mind that even the simplest mammals have nervous systems containing millions of neurons, between which are billions of synaptic connections). *C. elegans* can serve as a model of the mammalian nervous system because many of the elements that constitute the *C. elegans* nervous system are homologous to those of mammals, and these elements are incorporated into conserved pathways for many cellular functions, such as neurotransmission and intracellular signaling. *C. elegans* is an excellent subject for techniques that probe into cellular mechanisms because they have a short life cycle, they live in clonal populations, and their bodies are transparent. Because of this transparency, manipulations of the neural circuitry can easily be performed using techniques such as laser ablation. In addition, the worm's genome has been sequenced; there are thousands of identified mutants available for analysis, and there is a knockout consortium that accepts gene knockout requests at no cost. Finally, and most importantly for this chapter, *C. elegans* exhibits well-described and measurable behaviors, and these behaviors can change with experience.

It is absolutely crucial to understand the behavioral characteristics of learning and memory in *C. elegans* to properly elucidate the molecular mechanisms involved. The focus of this chapter is the behavioral analysis of the different types of learning and memory that have been discovered in *C. elegans*; however, we also briefly touch on how these behavioral studies have already begun to help elucidate cellular and molecular mechanisms of learning and memory. In the first section, we discuss how *C. elegans* changes with the experience of individual stimuli that it encounters in its environment, such as a mechanical tap or exposure to a certain chemical stimulus. The second section is an overview of how *C. elegans* learns to predict the presence of food using cues like chemical concentration and temperature. The third and final section reviews how *C. elegans* learns to avoid harmful stimuli such as pathogenic bacteria.

1.32.2 Learning about the Environment

During an organism's life, it is constantly bombarded with stimuli. To be successful, it needs to allocate energy, resources, and attention appropriately. One way to achieve this is by learning to ignore stimuli that are irrelevant, thereby avoiding the waste of energy caused by unnecessary responses; this is known as habituation. More objectively, it is a decrease in response to an irrelevant stimulus over repeated stimulation (Groves and Thompson, 1970). Habituation is considered a nonassociative form of learning. This phenomenon can be distinguished from decreases in response resulting from sensory adaptation or motor fatigue (or both) by the process of dishabituation. Dishabituation is achieved by following habituation training with a novel or noxious stimulus, such as electric shock used for dishabituation of the gill withdrawal response in *Aplysia* (Carew et al., 1971). If a habituated animal is given a dishabituating stimulus, its response to the initial stimulus will immediately increase above the habituated level. In contrast, sensory adaptation or motor fatigue will only recover after a period of rest.

1.32.2.1 Mechanical Stimuli

In its natural environment, *C. elegans* can sense mechanical forces that might include bumping into subterranean obstacles in their path of travel, bumping into other similarly sized organisms, or even vibration of the surrounding environment from a close encounter with a larger organism. In the laboratory, these stimuli can be mimicked by touching the anterior or posterior body with a hair or by a discreet tap to the side of the Petri dish in which they live. To study habituation, the latter of these stimuli was used because the frequency and intensity of the stimulus could be easily controlled and manipulated. *C. elegans* responds to this stimulus with a reversal, which is called the tap withdrawal response (Rankin et al., 1990). When this stimulus was repeated at a constant interstimulus interval (ISI) of 10 s, the magnitude of the response significantly decreased over a period of 40 stimuli (Rankin et al., 1990). An analysis across 60 trials showed that habituation consisted of an initial steep downward slope of decreasing response magnitude during the first 10–20 stimuli, followed by a relatively flat asymptotic stage for the remainder of the stimuli (Rankin and Broster, 1992). The response to tap was also measured at various time intervals (30 s, 10 min, 20 min, and 30 min) after the 60th stimulus, and it was observed that the response magnitude spontaneously recovered over time (Rankin and Broster, 1992). Dishabituation was observed by applying an electrical shock to the agar near the worm immediately after the taps; response magnitude to subsequent taps significantly increased (Rankin et al., 1990). Dishabituation indicates that the response decrement observed after repeated stimuli was in fact habituation and not a form of sensory adaptation or motor fatigue.

The most important procedural factor that affects habituation of the tap withdrawal response in *C. elegans* is the length of the ISI. Short ISIs (i.e., 10 s) produced steep slopes in the initial stage of habituation and very low asymptotic levels of response during the second stage, whereas long ISIs (i.e., 60 s) produced shallow slopes and high asymptotic levels (Rankin and Broster, 1992). Variations in ISI also affected the spontaneous recovery after habituation; spontaneous recovery was faster and more complete after short ISIs than after long ISIs (Rankin and Broster, 1992). This was an important finding because it provided an alternate and more elegant way to distinguish habituation from sensory adaptation and motor fatigue than dishabituation, which can be quite variable. The rate of recovery following sensory adaptation or motor fatigue is determined by the degree of decrement, and so greater decrement, which is observed at short ISIs, would take

longer to recover than long ISIs, and this is opposite to what is observed after habituation.

Rankin and Broster (1992) investigated the effects of habituation level, achievement of the asymptotic level, and number of missed stimuli before the recovery test on the rate of spontaneous recovery. To test the effects of habituation level, they set a criterion for the level of decrement *C. elegans* had to reach before recovery began, regardless of ISI. *C. elegans* stimulated at a 10 s ISI achieved the criterion level after fewer stimuli than those tapped at a 60 s ISI; however, the 10-s ISI group still showed faster and more complete spontaneous recovery than the 60-s ISI group, even though they were habituated to the same response level.

Although the level of habituation did not affect spontaneous recovery, whether *C. elegans* had reached the asymptotic stage of habituation was found to be an important factor. *C. elegans* that reached asymptote after only 8 stimuli recovered at the same ISI dependent rate as worms that had received 60 stimuli (Rankin and Broster, 1992). This result supports the idea that reaching the asymptotic level of habituation is an important factor in determining rate of spontaneous recovery, but the number of stimuli an animal experiences after reaching the asymptotic stage has very little effect on the spontaneous recovery of the behavior.

Finally, Rankin and Broster (1992) tested how the number of missed stimuli affected recovery. For example, at the 10-min recovery test worms habituated at a 10-s ISI would have missed 60 stimuli, whereas worms habituated at a 60-s ISI would only have missed 10 stimuli. Spontaneous recovery of *C. elegans* trained at a 10-s ISI still showed significantly greater recovery at 5 min (30 missed stimuli) compared to worms trained at a 60-s ISI after 30 min of recovery (also 30 missed stimuli; Rankin and Broster, 1992). Therefore, ISI length is a crucial factor for both habituation and spontaneous recovery from habituation in *C. elegans*, regardless of the habituated level, the number of stimuli (as long as asymptote is achieved), or the number of missed stimuli during recovery.

The purpose of studying *C. elegans* as a simple model of learning is to be able to more easily research questions involving the cellular and molecular mechanisms that mediate learning. These behavioral analyses have created a foundation that can now be used to conduct this investigation. In fact, one of the most important mechanistic hypotheses regarding habituation has been identified by these behavioral analyses: That is, that habituation is not a single process but, instead, habituation at different ISIs must be mediated at least in part by different mechanisms (Rankin and Broster, 1992). This hypothesis has been supported by the identification of mutations that selectively disrupt habituation at either short or long ISIs, but not both (*See* Chapter 4.04).

The neural circuit that mediates the tap withdrawal response has been described in detail (Wicks and Rankin, 1995). It consists of five sensory neurons and four pairs of command interneurons; the most likely site for plasticity has been identified as the chemical synapses between the mechanosensory neurons and the command interneurons within the circuit (Wicks and Rankin, 1997). The chemical synapses between these sensory neurons and interneurons, as well as some of the synapses between interneurons, are glutamatergic (Brockie and Maricq, 2006), suggesting a possible role for glutamate in habituation. This was confirmed by a study that found that mutants that lack a glutamate vesicular transporter (*eat-4*), which is important for loading vesicles ready for neurotransmission, showed abnormal habituation (Rankin and Wicks, 2000). Recently, dopamine has been shown to play a role in habituation because mutants with disrupted dopamine neurotransmission (*dop-1* and *cat-2*) show abnormal habituation (Sanyal et al., 2004). Together these results are of general interest because glutamate–dopamine interactions have been noted in other animal learning and are associated with human disorders that are usually associated with learning and memory deficits (Palomo et al., 2004; David et al., 2005). More research is necessary to fully understand the mechanisms of habituation; however, it is clear that glutamate, dopamine, and the synapses between the mechanosensory neurons and the command interneurons are important for habituation of the tap withdrawal response in *C. elegans*.

So far this section has focused on the short-term effects of habituation to a mechanosensory stimulus. Under the right conditions *C. elegans* can also show long-term memory for this mechanosensory habituation. This was first observed by Rankin et al. (1990) and then studied in more detail by Beck and Rankin (1995, 1997) using a protocol adapted from a study of long-term memory for habituation, in *Aplysia*. Beck and Rankin (1997) investigated the parameters necessary for long-term memory for habituation in *C. elegans*. They tested distributed training, which features a number of blocks of stimuli separated by hour-long rest periods, and massed training, in which the same number of stimuli is administered without

any rest periods (a longer single block). Distributed versus massed training appears to be a fundamental feature of memory because it has been shown to enhance long-term memory in many other training paradigms in a number of other organisms, such as habituation in *Aplysia* (Carew et al., 1972), classical conditioning in *Drosophila* (Tully et al., 1994), and memorization of lists of nonsense syllables in humans (Ebbinghaus 1885). The ISI within blocks was either 10 s or 60 s, and the number of taps varied between 40 (10 per block) and 60 (20 per block). Only distributed training at a 60-s ISI with either 40 (4 blocks of 10 taps) or 60 (3 blocks of 20 taps) stimuli was capable of reliable expression of memory 24 h after training. In general, more stimulation (60 stimuli) was better at producing long-term memory than less stimulation (40 stimuli). Beck and Rankin (1997) concluded that training protocols with a high total number of stimuli, long ISIs, and distributed training produced significant long-term memory of habituation in *C. elegans*.

One very important aspect that defines long-term memory and helps distinguish it from shorter forms is the fact that the mechanisms that store long-term memory are protein synthesis dependent. Long-term memory for habituation in *C. elegans* was found to be protein synthesis dependent by a set of heat shock experiments (Beck and Rankin, 1995). Heat shock is a technique that can block protein synthesis in the worm because at high temperatures (32 °C), all nonessential protein synthesis is stopped to give way to the synthesis of a set of heat shock proteins important for protection against cellular stress (Lindquist, 1986). Heat shock for the first 45 min during each of the 1-h rest periods of the long-term protocol blocked the formation of the long-term memory without disrupting the short-term effects of habituation (Beck and Rankin, 1995). Heat shock immediately prior to training or testing had no effect on long-term memory formation. This suggests that the protein synthesis that is taking place during the rest periods after each training block leads to consolidation of long-term memory for habituation of the tap withdrawal response in *C. elegans*. Interestingly, 15 min of heat shock exposure during either the first 15 min of the period or the second 15 min of the period is enough to disrupt the formation of long-term memory, whereas heat shock during the last 15 min of the rest period had no effect (Beck and Rankin, 1995). This suggests that the protein synthesis portion of the molecular mechanism responsible for the formation of long-term memory takes place during the first 30 min after each training block.

The consolidation of this long-term memory is not always permanent. If the memory is recalled by 10 reminder taps 24 h after the training, the memory becomes labile and must undergo consolidation again (Rose and Rankin, 2006); this is known as reconsolidation (Nader et al., 2000). By blocking protein synthesis immediately after the reminder, the memory is lost; nontrained worms showed similar responses to the trained worms 48 h after training (24 h after reminder). Protein synthesis blockade 24 h after training in the absence of a set of reminder taps has no effect on the long-term memory; trained animals had significantly lower magnitudes of response than nontrained animals.

Similar to short-term habituation, glutamate appears to be an important molecule for the consolidation of long-term memory. Mutants with defective glutamate neurotransmission (*eat-4* and *glr-1*) cannot form long-term memory for habituation, and pharmacological agents that disrupt glutamate neurotransmission block the formation of long-term memory (Rose et al., 2002, 2003). Also, pharmacological blockade of glutamate neurotransmission during 10 reminder taps 24 h after training blocks the reconsolidation of the long-term memory (Rose and Rankin, 2006). A subunit of an AMPA-like glutamate receptor (*glr-1*) appears to play an especially important role in the mechanism of long-term memory consolidation, as its expression, assessed by quantitative measurements of a GLR-1::GFP fusion construct using confocal imaging, was significantly decreased 24 h after the administration of the long-term memory protocol, which strongly correlates with the observed behavior (Rose et al., 2003). Similarly, GLR-1::GFP levels return to nontrained levels if reconsolidation is disrupted following a reminder 24 h after training (Rose and Rankin, 2006), again directly correlating with the behavior. This evidence supports the idea that the level of expression of these glutamate receptors mediates the level of response to tap, and a portion of the mechanism that mediates the long-term memory is a downregulation of expression of these receptors.

In summary, both short- and long-term habituation of the tap withdrawal response in *C. elegans* have been well characterized at the behavioral level. This behavioral analysis has been an important foundation on which to build a strong investigation for the mechanisms that mediate it, and a number of clues into this mechanism have been uncovered.

1.32.2.2 Chemical Stimuli

The first evidence that behavioral responses to chemical cues are also plastic in *C. elegans* was olfactory adaptation (Colbert and Bargmann, 1995). Usually, *C. elegans* exposed to a concentration gradient of a chemical (such as benzaldehyde and diacetyl) will move up the concentration gradient toward the chemical stimulus. This is known as chemotaxis and is usually measured by placing a group in a gradient and observing the fraction that migrates toward the stimulus. If *C. elegans* is exposed to a strong uniform concentration of the chemical prior to the chemotaxis assay, the fraction that migrates toward the stimulus significantly decreases, indicating that *C. elegans* decreases its response to the chemical stimulus after the preexposure treatment (Colbert and Bargmann, 1995).

This decrease was assumed to always be adaptation until Wen et al. (1997) first suggested it was possible to see habituation to a chemical stimulus by using a longer preexposure with a relatively weaker concentration than that used in the sensory adaptation paradigm. They used solubilized Na^+ as the chemical stimulus and found that a smaller number of *C. elegans* migrated toward the Na^+ spot during the chemotaxis assay compared to naïve controls. Importantly, this decrease in approach behavior could be immediately reversed or dishabituated by a short exposure to a high concentration of Na^+ solution, indicating that the change in approach behavior was habituation and not fatigue.

Bernhard and van der Kooy (2000) investigated the difference between chemosensory habituation and adaptation by using various preexposure concentrations. High preexposure concentrations of diacetyl (like those used in the sensory adaptation paradigm) led to a chemotaxis decrement that is irreversible, whereas low concentrations caused a decreased chemotaxis response that was immediately reversible by a dishabituating stimulus. These results suggest that habituation to chemical stimuli can occur in *C. elegans*; however, unless tests for dishabituation are done, the results observed in the sensory adaptation paradigms cannot be considered habituation. Similar to mechanosensory habituation, chemical habituation to diacetyl requires normal glutamate neurotransmission because *C. elegans* mutants lacking the AMPA-type glutamate receptor subunit *glr-1* are not capable of diacetyl habituation (Morrison and van der Kooy, 2001). This is an interesting similarity, but more research needs to be conducted to understand whether the mechanism for habituation is conserved across sensory modalities.

1.32.2.3 Context Conditioning for Habituation

Although habituation and other forms of nonassociative learning are initially dependent only on a single stimulus, later learning concerning the same stimulus can be dependent on environmental conditions during the initial training session (Wagner, 1976, 1978, 1979). One example of this is context conditioning in which there is greater retention of the initial learning during a later training session if some contextual cue was present in the environment during both the initial and later training sessions. This has been observed in a number of organisms, including rats (Evans and Hammond, 1983), crabs (Tomsic et al., 1998), and *Aplysia* (Colwill et al., 1988). *C. elegans* is also capable of such context conditioning using habituation of the response (Rankin, 2000). Worms habituated in the presence of sodium acetate showed greater retention of habituation during a later habituation session only if they were also exposed to sodium acetate during the second session (**Figure 1**(**a**) and (**b**)). It was very important that the worms experience this contextual environment only during the habituation sessions to form the association, as Rankin (2000) showed that if this was not the case, latent inhibition and extinction blocked the enhanced learning (**Figure 1**(**c**)). Latent inhibition occurs when the animal is preexposed to the contextual cue for a period of time before it is used in training. When *C. elegans* was exposed to the sodium acetate for 1 h prior to habituation training, there was no difference between the initial training session and the later test session. Extinction occurs when the contextual cue remains present in the absence of habituation after the initial training. This was done by leaving the worms on sodium acetate between the training session and the test; no difference was observed between sessions in this case either. Finally, reverse context conditioning was observed when worms were reared on sodium acetate plates and then trained and tested on normal growth medium (**Figure 1**(**c**)). Therefore, *C. elegans* is capable of associating a chemical concentration with habituation of the tap withdrawal response, and this context conditioning is sensitive to latent inhibition and extinction.

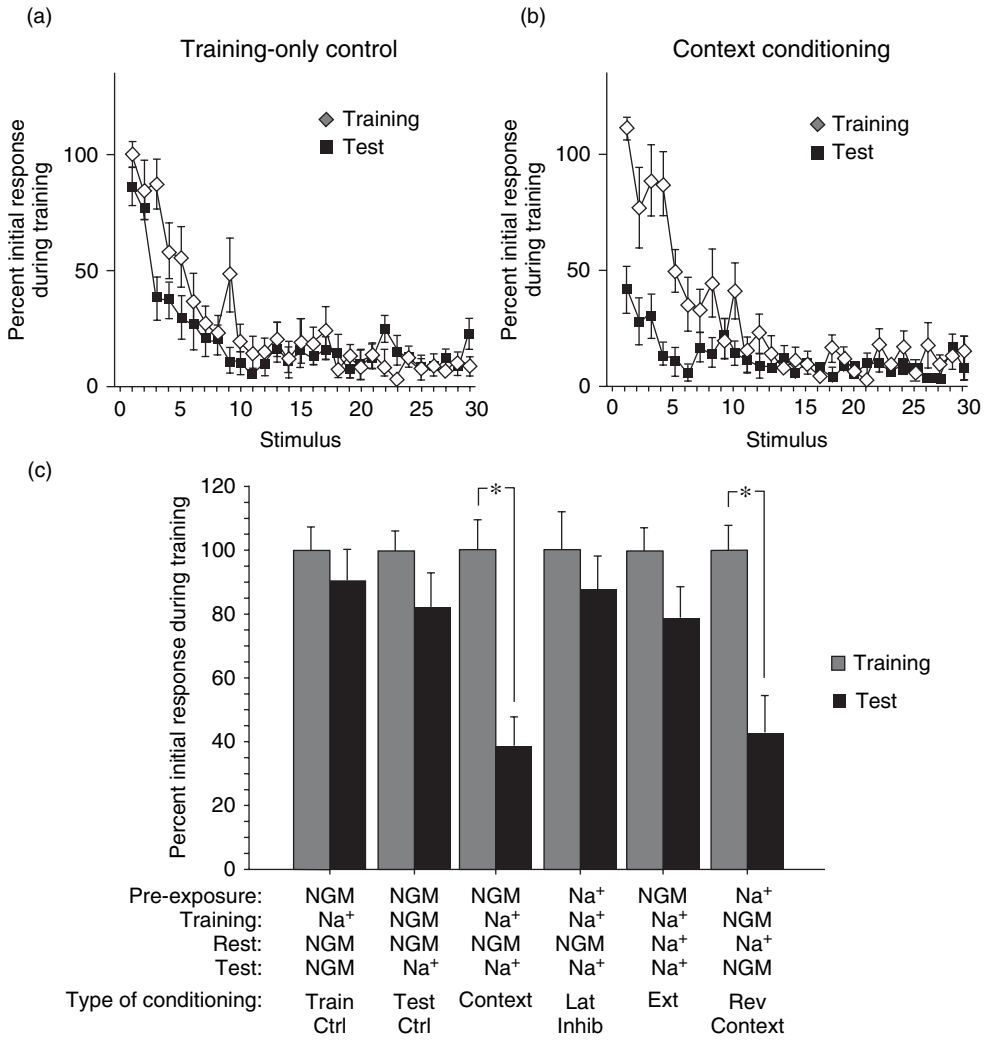

Figure 1 Context conditioning of habituation of the tap withdrawal response. (a and b) Habituation curves during the initial training (light diamonds) and later testing (dark squares) sessions for *C. elegans* that experienced sodium acetate context only during the training session (training-only control; (a)) and *C. elegans* that experienced the sodium acetate context during training and testing (context conditioning; (b)). (c) Initial responses during the initial training (light bars) and later testing (dark bars) habituation sessions for all conditioned groups: training-only control (Train Ctrl; sodium acetate only during training), testing-only control (Test Ctrl; sodium acetate only during testing), context (sodium acetate during training and during testing), latent inhibition (Lat Inhib; sodium acetate for 1 h prior to training, during training and during testing), extinction (Ext; sodium acetate during training, during the 1 h between training and testing, and during testing), reverse context (Rev Context; sodium acetate for the 1 h prior to training and for the 1 h between training and testing). *C. elegans* were exposed to either the sodium acetate (Na^+) context cue or to standard nematode growth medium (NGM) during preexposure, training, testing, and the period in between the training and test sessions (rest). * indicates significant difference between the initial response during training and test sessions. Rankin CH (2000) Context conditioning in habituation in the nematode *C. elegans. Behav. Neurosci.* 114: 496–505. Adapted from APA.

1.32.2.4 State-Dependent Learning

A similar form of learning is known as state-dependent learning because it uses a physiological state within the organism to act as a cue for a nonassociative learning behavior. One method of artificially manipulating internal state in an organism is with the use of neuroactive drugs. Ethanol exposure during the chemotaxis assay did not affect the naïve attraction toward benzaldehyde, nor did it affect benzaldehyde adaptation; however, if *C. elegans* was

preexposed to benzaldehyde in the presence of ethanol, then it would only show adaptation if it was also exposed to ethanol during the chemotaxis assay (Bettinger and McIntire, 2004). This suggests that *C. elegans* can learn to associate a physiological state with a nonassociative training paradigm and that this training will only be expressed when experiencing that same physiological state.

1.32.3 Predicting Food

Associative learning involves the formation of an association between two previously unrelated stimuli. The common stimulus used in most of the associative learning paradigms for *C. elegans* is food. Food is an extremely important resource for any animal; therefore, it is not surprising that most animals studied have the ability to learn to associate specific sensory cues with food. In 1997, Wen et al. were the first to show that even an organism as simple as *C. elegans* can learn to predict the location of food based on previous experience.

1.32.3.1 Appetitive Classical Conditioning using Chemical Cues

Classical conditioning is a form of associative learning that involves presentation of a conditioned stimulus (CS) with an unconditioned stimulus (US) so that an association between the CS and the presence of the US is learned. Wen et al. (1997) used a discriminative classical conditioning paradigm, which involves two CSs, one paired with the US and the other unpaired. They incorporated this paradigm into a chemotaxis assay to investigate associative learning in *C. elegans*. Sodium acetate and lithium chloride concentrations were chosen as the CSs such that *C. elegans* shows the same preference to them; if sodium acetate and lithium chloride are spotted on opposite ends of a chemotaxis plate, 50% of a naïve group will migrate toward sodium, and 50% will migrate toward chloride. When worms were preexposed to sodium paired with food, followed by chloride paired without food, 70–80% of the group migrated toward sodium during the chemotaxis assay; similar results were observed when chloride was paired with food and sodium was unpaired, except the majority of the group moved toward the chloride (Wen et al., 1997).

Using slight variations on this original paradigm, many researchers are now investigating the cellular and molecular mechanisms involved in the association of chemical cues with prediction of food source. For example, genetic screens have been conducted to identify mutants with defects in this behavior (Wen et al., 1997; Saeki et al., 2001); however, the function of the genes identified in these screens (such as *lrn-1* and *lrn-2*) has not yet been identified. Another study found a secretory protein with a low-density lipoprotein receptor (LDLR) motif (*hen-1*) that is important for sensory integration and that played a role in food-related associative learning (Ishihara et al., 2002). It is difficult to understand how these molecules and genes might fit together into a possible mechanism, but as more molecules are identified, the pathway(s) will be more apparent.

1.32.3.2 Thermotaxis

In 1975, Hedgecock and Russell discovered that worms could track changes in temperature and move toward or away from temperatures; they called this behavior thermotaxis. They found that thermotaxis could be altered by experience: *C. elegans* would migrate toward a preferred temperature on a concentration gradient, and the preferred temperature was the temperature at which it is cultivated from hatching until the test assay. Interestingly, animals starved during cultivation would avoid the temperature at which they were cultivated, whereas well-fed worms could change their temperature preference after cultivation in a well-fed environment at a new temperature for several hours. This behavior was investigated using genetic and neural circuit analysis to understand the cellular and molecular mechanisms that mediate it (Hedgecock and Russell, 1975; Mori and Ohshima, 1995), but it was not recognized as a form of learning until several reviews were published in the late 1990s (Bargmann and Mori, 1997; Mori, 1999). In the more recent review, Mori (1999) suggested that for thermotaxis to occur, two processes must take place: first, *C. elegans* must be able to memorize its current cultivation temperature, and then it must be able to associate that temperature with the state of food in its current environment. If there is an abundance of food, then the worms will migrate toward this cultivation temperature in the future. If there is no food, and the worms are starving, this cultivation temperature will be avoided.

After the identification of an intriguing mutant (*aho-2*) that was capable of memorizing a cultivation temperature but was defective at associating that temperature with the food state, thereby always

migrating to its cultivation temperature regardless of whether it was well fed or starved during its previous exposure, Mori and colleagues conducted an elegant behavior dissection of the two processes involved in thermotaxis (Mohri et al., 2005). This was accomplished by cultivating *C. elegans* at a particular temperature (17 °C vs. 25 °C) with a particular food condition (well fed vs. starved). Groups of worms were then switched in one of the conditions, and worms were tested using the thermotaxis assay at various time intervals after the switch (**Figure 2**). This allowed Mohri et al. (2005) to watch as either the new temperature was learned or the new food state was associated as time passed. The results

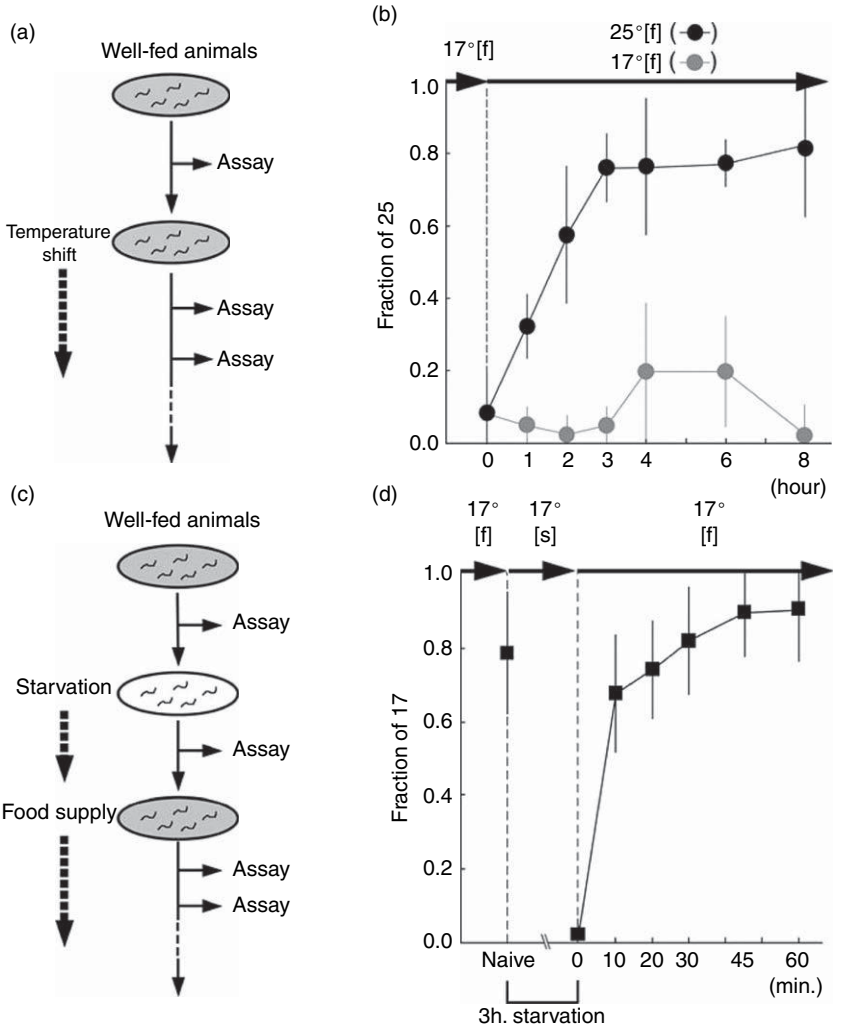

Figure 2 Dissection of temperature memory and food-temperature association of thermotaxis. (a) Procedural diagram for observing the kinetics for learning a new cultivation temperature; *C. elegans* was cultivated at 17 °C on plates with food, assayed, and then switched to a new cultivation temperature of 25 °C and then assayed every hour. (b) The fraction of worms that migrate toward 25 °C during each of the thermotaxis assays for the temperature switch paradigm (upper dark circles). *C. elegans* worms kept at 17 °C were assayed as a control (lower light circles). (c) Procedural diagram for observing the kinetics for association of a new feeding state; *C. elegans* cultivated at 17 °C on a well-fed plate was assayed, transferred to plates with no food for 3 h, assayed again, then transferred back to food and assayed every 10–15 min. (d) The fraction of *C. elegans* that migrates toward 17 °C during each of the thermotaxis assays for the feeding-state switch paradigm. Note that the time scale for (b) is in hours, and (d) is in minutes. Adapted from Mohri A, Kodama E, Kimura KD, Koike M, Mizuno T, and Mori I (2005) Genetic control of temperature preference in the nematode *Caenorhabditis elegans*. *Genetics* 169: 1437–1450.

suggested that it takes *C. elegans* roughly 3 h to learn and memorize a cultivation temperature, whereas it can change the food-state association with that memorized temperature in a matter of minutes (10–25 min, depending on the direction of the switch). This study was instrumental in raising our level of understanding of this behavior, which will be crucial as we attempt to understand the molecular mechanisms that mediate the thermosensory learning and memory of which *C. elegans* is capable.

The neural circuit underlying thermotaxis has been determined (Mori and Oshima, 1995). A number of genes expressed in these neurons have been found to play a role in thermotaxis learning. These include a calcium sensor, *nsc-1* (Gomez et al., 2001); a secretory protein with a LDLR motif, *hen-1* (Ishihara et al., 2002); a LIM homeobox gene, *ceh-1* (Cassata et al., 2000); genes that affect the amount of oxidative stress; and genes that disrupt the insulin/IGF-1/TGF-β endocrine pathway (Murakami and Murakami, 2005; Murakami et al., 2005). A number of mutations have been discovered to disrupt normal thermotaxis as well (such as *aho-2*); however, their respective genes have not yet been identified (Mohri et al., 2005). Unfortunately, to date no relationship between these molecules has been identified, so the mechanisms involved in thermotaxis learning are still unclear.

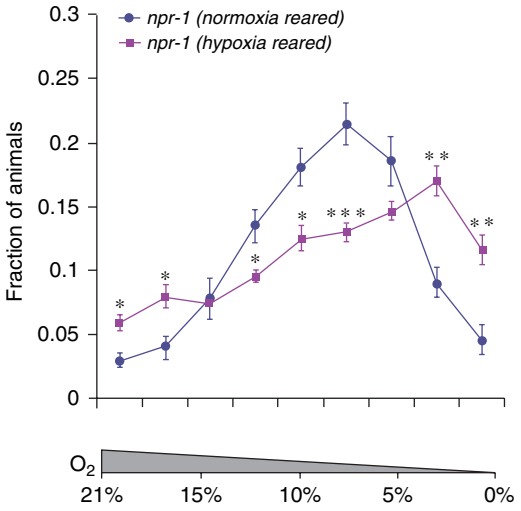

Figure 3 Experience-dependent behavioral plasticity of aerotaxis for *npr-1* mutant *C. elegans*. Fraction of *npr*-1 mutant *C. elegans* (similar variant to Hawaiian strain) that migrate to different oxygen concentrations (0–21%) after being cultivated in normoxia (21%; circles) versus hypoxia (1%; squares) conditions. Asterisks indicate significant differences between fractions at each oxygen concentration. Reprinted from Cheung BH, Cohen M, Rogers C, Albayram O, and de Bono M (2005) Experience-dependent modulation of *C. elegans* behavior by ambient oxygen. *Curr. Biol.* 15: 905–917. Copyright (2005), with permission from Elsevier.

1.32.3.3 Aerotaxis

C. elegans can also sense oxygen concentrations in their environment (Gray et al., 2004). Cheung et al. (2005) invested the effects of oxygen on the behavior of *C. elegans*, as well as the preference for different concentrations of oxygen. Given a concentration gradient, *C. elegans* will migrate toward a preferred oxygen level of 7–12%. This preference is exaggerated by a natural genetic variant found in a strain of *C. elegans* from Hawaii, such that a much larger fraction of the Hawaiian strain accumulates in the 7–12% oxygen content region of the assay plate. These *C. elegans* worms were all cultivated in the laboratory at a standard oxygen concentration of 21%; however, when the worms were cultivated at 1% oxygen concentration, a shift in the oxygen preference was observed during the aerotaxis assay. These hypoxia-reared *C. elegans* migrated to the 0–5% region of the assay plate (**Figure 3**), suggesting that the *C. elegans* learned that a low concentration of oxygen predicts the occurrence of food. Oddly, this learned behavior was only observed in the worms with the recessive Hawaiian genotype, which carry a mutation in a gene called *npr-1* that is homologous to a mammalian neuropeptide Y receptor, and not in the worms with the standard laboratory wild-type (from Bristol, England). This is not surprising, considering that the Bristol strain lacks other oxygen-related behaviors, such as social aggregation during feeding at high levels of oxygen (Cheung et al., 2005). Cheung et al. (2005) identify a set of neurons responsible for the behavior and a set of soluble guanylyl cyclases that are expressed in these neurons that play a role in this learned behavior, suggesting the beginnings of a putative mechanism.

1.32.4 Predicting Harm

Being able to predict the presence of food is not the only thing that is advantageous for survival. The foresight and avoidance of harm or death would also increase the chances of a long and successful reproductive life.

1.32.4.1 Aversive Learning toward Pathogenic Food

One important source of potential harm is food. When *C. elegans* is in its natural environment in the soil, it encounters various types of bacteria, some of which it approaches and some of which it avoids; the types that it is attracted to, it will consume. Unfortunately, a number of these bacteria are pathogenic to *C. elegans*, causing harm and even death. Zhang et al. (2005) investigated whether *C. elegans* could learn to avoid these pathogenic bacteria after a period of preexposure long enough to lead to harmful infection.

Zhang et al. (2005) choose pathogenic bacterial strains, *Pseudomonas aeruginosa* and *Serratia marcescens*, to which *C. elegans* is naively attracted. These bacteria proliferate within the digestive tract of the nematode, causing a harmful infection that can lead to death after long exposures. Zhang et al. (2005) modified the standard chemotaxis assay by using small bacterial lawns grown on opposite sides of the testing plate instead of chemical solutions; one lawn was the test bacteria, and there other was *E. coli* OP50 (a standard food source for *C. elegans* in the laboratory) as a control. Naive *C. elegans* worms (cultivated on *E. coli* OP50) tested in this assay were equally attracted to *P. aeruginosa* and *E. coli* and showed a slight preference for *S. marcescens* compared to *E. coli*. When *C. elegans* was cultivated in an environment that included *E. coli* and *P. aeruginosa* (cultivation in *P. aeruginosa* alone caused severe infections that lead to death), significantly fewer animals migrated toward *P. aeruginosa* during the taxis assay compared to naive worms, suggesting after preexposure to the pathogenic bacteria, the worms now strongly preferred the *E. coli*; the same was true when they were preexposed and tested with *S. marcescens*. This strongly supports the hypothesis that *C. elegans* learns to avoid bacteria that cause infectious harm.

In the preceding experiments *C. elegans* worms were preexposed to the test bacteria for their entire development, which is approximately 4 days. To test the kinetics of this olfactory avoidance learning, Zhang et al. (2005) cultivated *C. elegans* worms on *E. coli* until they were adults and then preexposed animals to the test conditioning environment (test bacteria plus *E. coli*) or a control conditioning environment (*E. coli* alone) for various amounts of time and then tested the groups using the taxis assay. They found that the shortest preexposure time that achieved a similar difference in preference to that observed after preexposure during all of development was 4 h of conditioning. Therefore, adult *C. elegans* worms needed to be exposed to pathogenic bacteria for at least 4 h to learn to avoid it.

This paradigm does not address the question of whether the learning that is occurring is strengthening the aversion toward the pathogenic bacteria, the attraction toward the control bacteria, or both. Zhang et al. (2005) tested this by developing a multiarm maze that contained four types of bacteria, the pathogenic test bacteria, the nonpathogenic control bacteria, novel pathogenic bacteria, and novel nonpathogenic bacteria (**Figure 4**(**a**)). A group of *C. elegans* was placed in a central decision area and then had to choose an arm to move down to get to a bacterial lawn; the percentage of worms that ended up at each bacterial lawn was measured. A greater percentage chose the control bacterial lawn, and a lesser percentage chose the test bacterial lawn in the preexposed group compared to the naive group (**Figure 4**(**b**)). The percentage of worms found in both of the novel bacterial lawns was found to be the same between groups. This suggests that this pathogenesis-induced olfactory aversive learning is caused by both an increase in aversion to the previously experienced pathogenic bacteria and an increase in attraction to the previously experienced nonpathogenic bacteria. Interestingly, Zhang et al. (2005) found that the kinetics of these different aspects were not the same because *C. elegans* worms that were exposed to the pathogenic bacteria for only 4 h showed aversion to the pathogenic test bacterial lawn but not an increased attraction to the nonpathogenic control lawn. This suggests that *C. elegans* can learn the aversive aspect more quickly than it can learn the attractive aspect of this behavior.

Serotonin is an important neurotransmitter for food-related behaviors in *C. elegans* (Croll, 1975; Horvitz et al., 1982; Avery and Horvitz, 1990), so it is not surprising that mutations that affect serotonin neurotransmission disrupt learning of pathogenic bacteria avoidance (Zhang et al., 2005). Using neuron-specific expression of wild-type constructs that rescue these serotonin deficits, the site of plasticity was identified. The researchers believe the ADF serotonergic chemosensory neuron is activated during bacterial infection and releases serotonin onto the interneurons involved in integrating chemosensory information, AIY and AIZ. The serotonin induces some sort of neural plasticity, not yet understood, that leads to the behavioral plasticity observed during the behavior.

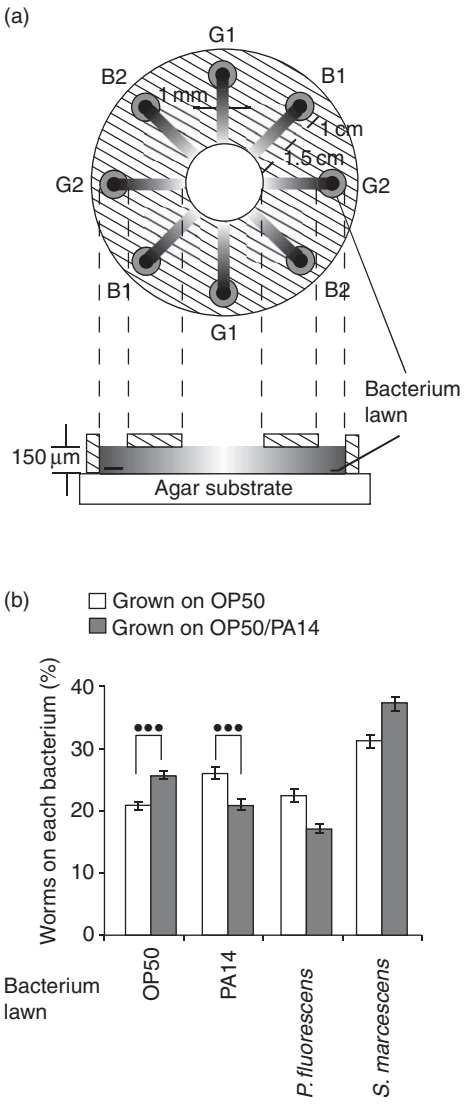

Figure 4 Learned avoidance of pathogenic bacteria. (a) Multiarm maze for testing attractive versus aversive nature of pathogenic bacteria avoidance. *E. coli* OP50, previously experienced nonpathogenic bacteria, was placed at G1; *P. aeruginosa* PA14, previously experienced pathogenic bacteria, was placed at B1; *P. fluorescens*, novel nonpathogenic bacteria, was placed at G2; *S. marcescens*, novel pathogenic bacteria, was placed at B2. *C. elegans* worms were placed in the center of the apparatus and allowed to choose which bacterial lawn to migrate toward. (b) Fraction of *C. elegans* worms, which were preexposed only to *E. coli* OP50 (light bars) versus preexposed to *E. coli* OP50 and *P. aeruginosa* PA14 (dark bars), that migrated to the different bacterial lawn. *** indicates significant differences ($p < 0.05$) between different preexposure conditions. Zhang Y, Lu H, and Bargmann CI (2005) Pathogenic bacteria induce aversive olfactory learning in *Caenorhabditis elegans*. *Nature* 438: 179–184. Adapted by permission from Macmillan Publishers Ltd: Nature, Copyright (2005).

1.32.5 Summary

C. elegans possesses a plethora of behaviors that can change with experience, which covers every aspect of its sensory abilities that has been investigated. Understanding the details of this learning and memory is much easier than understanding the complex behavioral repertoire of higher-order organisms. This detailed understanding is essential for helping to understand the mechanisms that mediate these organisms because a mechanism must be able to explain all aspects of the learning to be complete. *C. elegans* also offers the advantage of accessible manipulation at the molecular level, which allows for investigation of cellular and molecular mechanisms of learning and memory. These results can then be carefully extended from this model system to higher-order organisms in the hopes of better understanding the biological foundations of learning and memory.

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1.33 Computational Models of Hippocampal Functions

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1.33.1 Introduction

In this chapter, a computational approach to the function of the hippocampus in memory is described and compared to other approaches. The theory is quantitative and takes into account the internal and systems-level connections of the hippocampus, the effects on memory of damage to different parts of the hippocampus, and the responses of hippocampal neurons recorded during memory tasks. The theory was developed by Rolls (1987, 1989a,b,c, 1996b, 2007), Treves and Rolls (1992, 1994), and with other colleagues (Rolls et al., 2002; Rolls and Stringer, 2005; Rolls and Kesner, 2006). The theory was preceded by the work of Marr (1971), who developed a mathematical model, which, although not applied to particular networks within the hippocampus and dealing with binary neurons and binary synapses that utilized heavily the properties of the binomial distribution, was important in utilizing computational concepts and in considering how recall could occur in a network with recurrent collateral connections. Analyses of these autoassociation or attractor networks developed rapidly (Gardner-Medwin, 1976; Kohonen, 1977; Hopfield, 1982; Amit, 1989; Treves and Rolls, 1991; Rolls and Treves, 1998). Rolls (1987, 1989b) produced a theory of the hippocampus in which the CA3 neurons operated as an autoassociation memory to store episodic memories including object and place memories, and the dentate granule cells operated as a preprocessing stage for this by performing pattern separation so that the mossy fibers could act to set up different representations for each memory to be stored in the CA3 cells. He suggested that the CA1 cells operate as a recoder for the information recalled from the CA3 cells to a partial memory cue, so that the recalled information would be represented more efficiently to enable recall, via the backprojection synapses, of activity in the neocortical areas similar to that which had been present during the original episode. At about the same time, McNaughton and Morris (1987) suggested that the CA3 network might be an autoassociation network, and that the mossy fiber–to-CA3 connections might implement detonator synapses. The concepts that the diluted mossy fiber connectivity might implement selection of a new random set of CA3 cells for each new memory and that a direct perforant path input to CA3 was needed to initiate retrieval were introduced by Treves and Rolls (1992). Since then, many investigators have contributed to our understanding of hippocampal computation, with some of these approaches described in the section titled 'Comparison with other theories of hippocampal function' and throughout the chapter.

1.33.2 A Theory of Hippocampal Function

1.33.2.1 Systems-Level Functions of the Hippocampus

Any theory of the hippocampus must state at the systems level what is computed by the hippocampus. Some of the relevant evidence comes from the effects of damage to the hippocampus, the responses of neurons in the hippocampus during behavior, and the systems-level connections of the hippocampus, described in more detail elsewhere (Rolls and Kesner, 2006; Rolls, 2007).

1.33.2.1.1 Evidence from the effects of damage to the hippocampus

Damage to the hippocampus or to some of its connections such as the fornix in monkeys produces deficits in learning about the places of objects and about the places where responses should be made (Buckley and Gaffan, 2000). For example, macaques and humans with damage to the hippocampal system or fornix are impaired in object–place memory tasks in which not only the objects seen but where they were seen must be remembered (Gaffan, 1994; Burgess et al., 2002; Crane and Milner, 2005). Posterior parahippocampal lesions in macaques impair even a simple type of object-place learning in which the memory load is just one pair of trial-unique stimuli (Malkova and Mishkin, 2003). Further, neurotoxic lesions that selectively damage the primate hippocampus impair spatial scene memory, tested by the ability to remember where in a scene to touch to obtain reward (Murray et al., 1998). Rats with hippocampal lesions are impaired in using environmental spatial cues to remember particular places (O'Keefe and Nadel, 1978; Jarrard, 1993; Cassaday and Rawlins, 1997; Martin et al., 2000; Kesner et al., 2004). These memory functions are important in event or episodic memory, in which the ability to remember what happened where on typically a single occasion is important.

It will be suggested below that an autoassociation memory implemented by the CA3 neurons enables event or episodic memories to be formed by enabling associations to be formed between spatial and other including object representations.

1.33.2.1.2 The necessity to recall information from the hippocampus

Information stored in the hippocampus will need to be retrieved and affect other parts of the brain in order to be used. The information about episodic events recalled from the hippocampus could be used to help form semantic memories (Rolls, 1989b,d, 1990a; Treves and Rolls, 1994). For example, remembering many particular journeys could help to build a geographic cognitive map in the neocortex. The hippocampus and neocortex would thus be complementary memory systems, with the hippocampus being used for rapid, on-the-fly, unstructured storage of information involving activity potentially arriving from many areas of the neocortex, while the neocortex would gradually build and adjust the semantic representation on the basis of much accumulating information (Rolls, 1989b; Treves and Rolls, 1994; McClelland et al., 1995; Moscovitch et al., 2005). The present theory shows how information could be retrieved within the hippocampus and how this retrieved information could enable the activity in neocortical areas that was present during the original storage of the episodic event to be reinstated, thus implementing recall, by using hippocampo-neocortical backprojections (see **Figure 1**).

1.33.2.1.3 Systems-level neurophysiology of the primate hippocampus

The systems-level neurophysiology of the hippocampus shows what information could be stored or processed by the hippocampus. To understand how the hippocampus works, it is not sufficient to state just that it can store information – one needs to know what information. The systems-level neurophysiology of the primate hippocampus has been reviewed recently by Rolls and Xiang (2006), and a brief summary is provided here because it provides a perspective relevant to understanding the function of the human hippocampus that is somewhat different from that provided by the properties of place cells in rodents, which have been reviewed elsewhere (see McNaughton et al., 1983; O'Keefe, 1984; Muller et al., 1991; Jeffery et al., 2004; Jeffery and Hayman, 2004).

The primate hippocampus contains spatial cells that respond when the monkey looks at a certain part of space, for example, at one quadrant of a video monitor, while the monkey is performing an object–place memory task in which he must remember where on the monitor he has seen particular images (Rolls et al., 1989). Approximately 9% of the hippocampal neurons have such spatial view fields, and about 2.4% combine information about the position in space with information about the object that is in that position in space (Rolls et al., 1989). The representation of space is for the majority of hippocampal

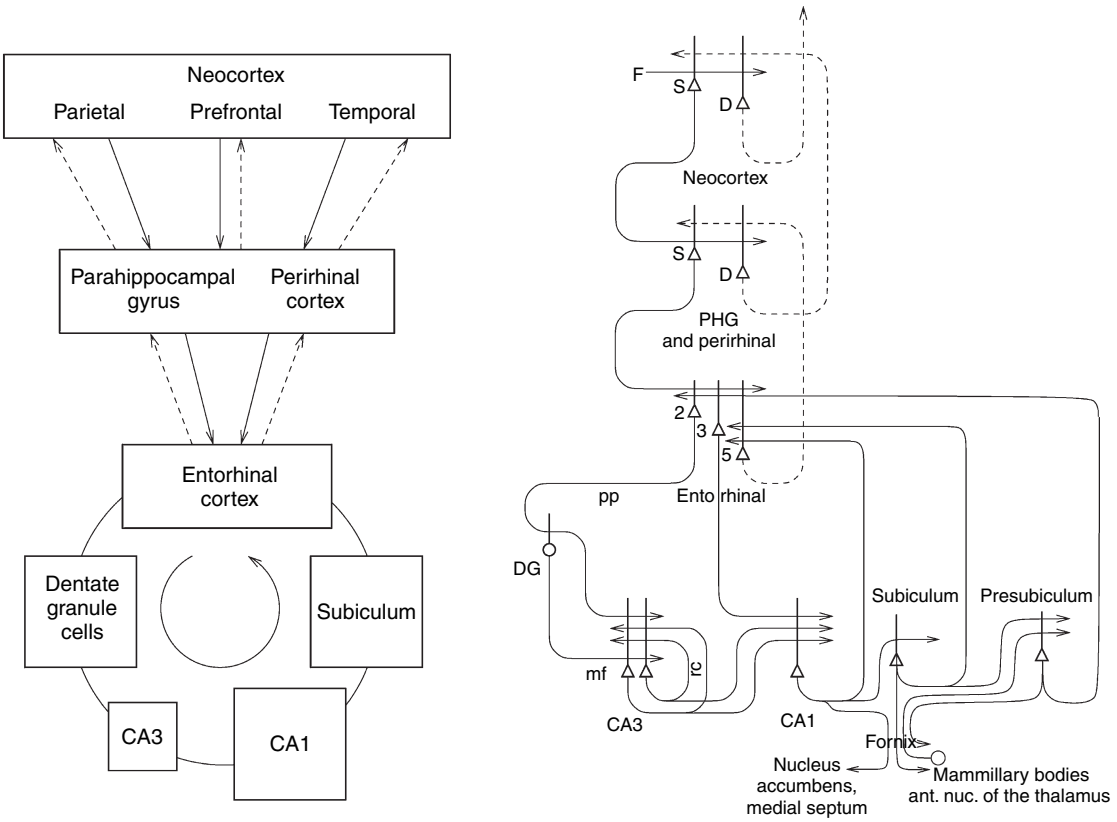

Figure 1 Forward connections (solid lines) from areas of cerebral association neocortex via the parahippocampal gyrus and perirhinal cortex, and entorhinal cortex, to the hippocampus; and backprojections (dashed lines) via the hippocampal CA1 pyramidal cells, subiculum, and parahippocampal gyrus to the neocortex. There is great convergence in the forward connections down to the single network implemented in the CA3 pyramidal cells and great divergence again in the backprojections. Left: block diagram. Right: more detailed representation of some of the principal excitatory neurons in the pathways. Abbreviations: D, deep pyramidal cells; DG, dentate granule cells; F, forward inputs to areas of the association cortex from preceding cortical areas in the hierarchy; mf, mossy fibers. PHG, parahippocampal gyrus and perirhinal cortex; pp, perforant path; rc, recurrent collateral of the CA3 hippocampal pyramidal cells; S, superficial pyramidal cells; 2, pyramidal cells in layer 2 of the entorhinal cortex; 3, pyramidal cells in layer 3 of the entorhinal cortex. The thick lines above the cell bodies represent the dendrites.

neurons in allocentric – not egocentric – coordinates (Feigenbaum and Rolls, 1991). These spatial view cells can be recorded while monkeys move themselves round the test environment by walking (or running) on all fours (Rolls et al., 1997a, 1998; Robertson et al., 1998; Georges-François et al., 1999). These hippocampal spatial view neurons respond significantly differently for different allocentric spatial views, and have information about spatial view in their firing rate, but do not respond differently just on the basis of eye position, head direction, or place. If the view details are obscured by curtains and darkness, then some spatial view neurons (especially those in CA1 and less those in CA3) continue to respond when the monkey looks toward the spatial view field, showing that these neurons can be updated for at least short periods by idiothetic (self-motion) cues including eye position and head direction signals (Rolls et al., 1997b; Robertson et al., 1998).

A fundamental question about the function of the primate including human hippocampus is whether object as well as allocentric spatial information is represented. To investigate this, Rolls et al. (2005) made recordings from single hippocampal formation neurons while macaques performed an object–place memory task that required the monkeys to learn associations between objects, and where they were shown in a room. Some neurons (10%) responded differently to different objects independently of location; other neurons (13%) responded to the spatial view independently of which object was present at

the location; and some neurons (12%) responded to a combination of a particular object and the place where it was shown in the room. These results show that there are separate as well as combined representations of objects and their locations in space in the primate hippocampus. This is a property required in an episodic memory system, for which associations between objects and the places where they are seen are prototypical. The results thus show that a requirement for a human episodic memory system, separate and combined neuronal representations of objects and where they are seen out there in the environment, are present in the primate hippocampus (Rolls et al., 2005). What may be a corresponding finding in rats is that some rat hippocampal neurons respond on the basis of the conjunction of location and odor (Wood et al., 1999).

Primate hippocampal neuronal activity has also been shown to be related to the recall of memories. In a one–trial object place recall task, images of an object in one position on a screen and of a second object in a different position on the screen were shown successively. Then one of the objects was shown at the top of the screen, and the monkey had to recall the position in which it had been shown earlier in the trial and to touch that location (Rolls and Xiang, 2006). In addition to neurons that responded to the objects or places, a new type of neuronal response was found in which 5% of hippocampal neurons had place-related responses when a place was being recalled by an object cue.

The primate anterior hippocampus (which corresponds to the rodent ventral hippocampus) receives inputs from brain regions involved in reward processing such as the amygdala and orbitofrontal cortex (Pitkanen et al., 2002). To investigate how this affective input may be incorporated into primate hippocampal function, Rolls and Xiang (2005) recorded neuronal activity while macaques performed a reward-place association task in which each spatial scene shown on a video monitor had one location, which if touched yielded a preferred fruit juice reward, and a second location that yielded a less preferred juice reward. Each scene had different locations for the different rewards. Of 312 hippocampal neurons analyzed, 18% responded more to the location of the preferred reward in different scenes, and 5% to the location of the less preferred reward (Rolls and Xiang, 2005). When the locations of the preferred rewards in the scenes were reversed, 60% of 44 neurons tested reversed the location to which they responded, showing that the reward–place associations could be altered by new learning in a few trials. The majority (82%) of these 44 hippocampal reward–place neurons tested did not respond to object–reward associations in a visual discrimination object–reward association task. Thus the primate hippocampus contains a representation of the reward associations of places out there being viewed, and this is a way in which affective information can be stored as part of an episodic memory, and how the current mood state may influence the retrieval of episodic memories. There is consistent evidence that rewards available in a spatial environment can influence the responsiveness of rodent place neurons (Hölscher et al., 2003; Tabuchi et al., 2003).

1.33.2.1.4 Systems-level anatomy

The primate hippocampus receives inputs via the entorhinal cortex (area 28) and the highly developed parahippocampal gyrus (areas TF and TH) as well as the perirhinal cortex from the ends of many processing streams of the cerebral association cortex, including the visual and auditory temporal lobe association cortical areas, the prefrontal cortex, and the parietal cortex (Van Hoesen, 1982; Amaral, 1987; Amaral et al., 1992; Suzuki and Amaral, 1994b; Witter et al., 2000b; Lavenex et al., 2004) (see **Figure 1**). The hippocampus is thus by its connections potentially able to associate together object and spatial representations. In addition, the entorhinal cortex receives inputs from the amygdala and the orbitofrontal cortex, which could provide reward-related information to the hippocampus (Suzuki and Amaral, 1994a; Carmichael and Price, 1995; Stefanacci et al., 1996; Pitkanen et al., 2002).

The primary output from the hippocampus to neocortex originates in CA1 and projects to subiculum, entorhinal cortex, and parahippocampal structures (areas TF-TH), as well as prefrontal cortex (Van Hoesen, 1982; Witter, 1993; Delatour and Witter, 2002; van Haeften et al., 2003) (see **Figure 1**), though there are other outputs (Rolls and Kesner, 2006).

1.33.2.2 The Operation of Hippocampal Circuitry as a Memory System

1.33.2.2.1 Hippocampal circuitry

(see Figure 1; Amaral and Witter, 1989; Storm-Mathiesen et al., 1990; Amaral, 1993; Witter et al., 2000b; Naber et al., 2001; Lavenex et al., 2004)

Projections from the entorhinal cortex layer 2 reach the granule cells (of which there are 10^6 in the rat) in the dentate gyrus (DG), via the perforant path (pp) (Witter, 1993). The granule cells project to CA3 cells via the mossy fibers (mf), which provide a sparse but possibly powerful connection to the $3{\cdot}10^5$

CA3 pyramidal cells in the rat. Each CA3 cell receives approximately 50 mf inputs, so that the sparseness of this connectivity is thus 0.005%. By contrast, there are many more – possibly weaker – direct perforant path inputs also from layer 2 of the entorhinal cortex onto each CA3 cell, in the rat on the order of $4{\cdot}10^3$. The largest number of synapses (approximately $1.2{\cdot}10^4$ in the rat) on the dendrites of CA3 pyramidal cells is, however, provided by the (recurrent) axon collaterals of CA3 cells themselves (rc) (see **Figure 2**). It is remarkable that the recurrent collaterals are distributed to other CA3 cells throughout the hippocampus (Amaral and Witter, 1989; Amaral et al., 1990; Ishizuka et al., 1990; Amaral and Witter, 1995), so that effectively the CA3 system provides a single network, with a connectivity of approximately 2% between the different CA3 neurons given that the connections are bilateral. The neurons that comprise CA3, in turn, project to CA1 neurons via the Schaffer collaterals. In addition, projections that terminate in the CA1 region originate in layer 3 of the entorhinal cortex (see **Figure 1**).

1.33.2.2.2 Dentate granule cells

The theory is that the dentate granule cell stage of hippocampal processing that precedes the CA3 stage acts in a number of ways to produce during learning the sparse yet efficient (i.e., nonredundant) representation in CA3 neurons that is required for the autoassociation implemented by CA3 to perform well (Rolls, 1989b; Treves and Rolls, 1992; Rolls and Kesner, 2006; Rolls et al., 2006).

The first way is that the perforant path – the dentate granule cell system with its Hebb-like modifiability is suggested to act as a competitive learning network to remove redundancy from the inputs producing a more orthogonal, sparse, and categorized set of outputs (Rolls, 1987, 1989a,b,d, 1990a,b, Rolls and Treves, 1998; Rolls, 2007). (Competitive networks are described elsewhere: Hertz et al., 1991; Rolls and Treves, 1998; Rolls and Deco, 2002; Rolls, 2008). The nonlinearity in the *N*-methyl-D-aspartate (NMDA) receptors may help the operation of such a competitive net, for it ensures that only the most active neurons left after the competitive feedback inhibition have synapses that become modified and thus learn to respond to that input (Rolls, 1989a). Because of the feedback inhibition, the competitive process may result in a relatively constant number of strongly active dentate neurons relatively independently of the number of active perforant path inputs to the dentate cells. The operation of the dentate granule cell system as a competitive network may also be facilitated by a Hebb rule of the form:

$$\delta w_{ij} = k \,.\, r_i \,(r'_j - w_{ij}), \qquad [1]$$

where k is a constant, r_i is the activation of the dendrite (the postsynaptic term), r'_j is the presynaptic firing rate, w_{ij} is the synaptic weight, and r'_j and w_{ij} are in appropriate units (Rolls, 1989a). Incorporation of a rule such as this which implies heterosynaptic long-term depression (LTD) as well as long-term potentiation (LTP) (see Levy and Desmond, 1985; Levy et al., 1990) makes the sum of the synaptic weights on each neuron remain roughly constant during learning (cf. Oja, 1982; see Rolls, 1989a; Rolls and Treves, 1998; Rolls, 2008; Rolls and Deco, 2002).

This functionality could be used to help build hippocampal place cells in rats from the grid cells present in the medial entorhinal cortex (Hafting et al., 2005). Each grid cell responds to a set of places in a spatial environment, with the places to which a cell responds set out in a regular grid. Different grid cells have different phases (positional offsets) and grid spacings (or frequencies) (Hafting et al., 2005). We (Rolls et al., 2006) have simulated the dentate granule cells as a system that receives as inputs the activity of a population of entorhinal cortex grid cells as the animal traverses a spatial environment and

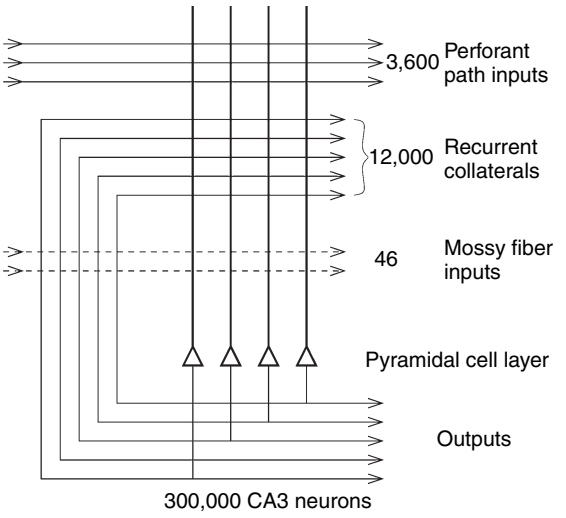

Figure 2 The numbers of connections from three different sources onto each CA3 cell from three different sources in the rat. After Treves A and Rolls ET (1992) Computational constraints suggest the need for two distinct input systems to the hippocampal CA3 network. *Hippocampus* 2: 189–199; Rolls ET and Treves A (1998) *Neural Networks and Brain Function.* Oxford: Oxford University Press.

have shown that the competitive net builds dentate-like place cells from such entorhinal grid cell inputs (see **Figure 3**). This occurs because the firing states of entorhinal cortex cells that are active at the same time when the animal is in one place become associated together by the learning in the competitive net, yet each dentate cell represents primarily one place because the dentate representation is kept sparse, thus helping to implement symmetry breaking (Rolls et al., 2006).

The second way is also a result of the competitive learning hypothesized to be implemented by the dentate granule cells (Rolls, 1987, 1989a,b,d, 1990a,b, 1994). It is proposed that this allows overlapping (or very similar) inputs to the hippocampus to be separated in the following way (see also Rolls, 1996b). Consider three patterns B, W, and BW, where BW is a linear combination of B and W. (To make the example very concrete, we could consider binary patterns where B = 10, W = 01, and BW = 11.) Then the memory system is required to associate B with reward and W with reward, but BW with punishment. Without the hippocampus, rats might have more difficulty in solving such problems, particularly when they are spatial, for

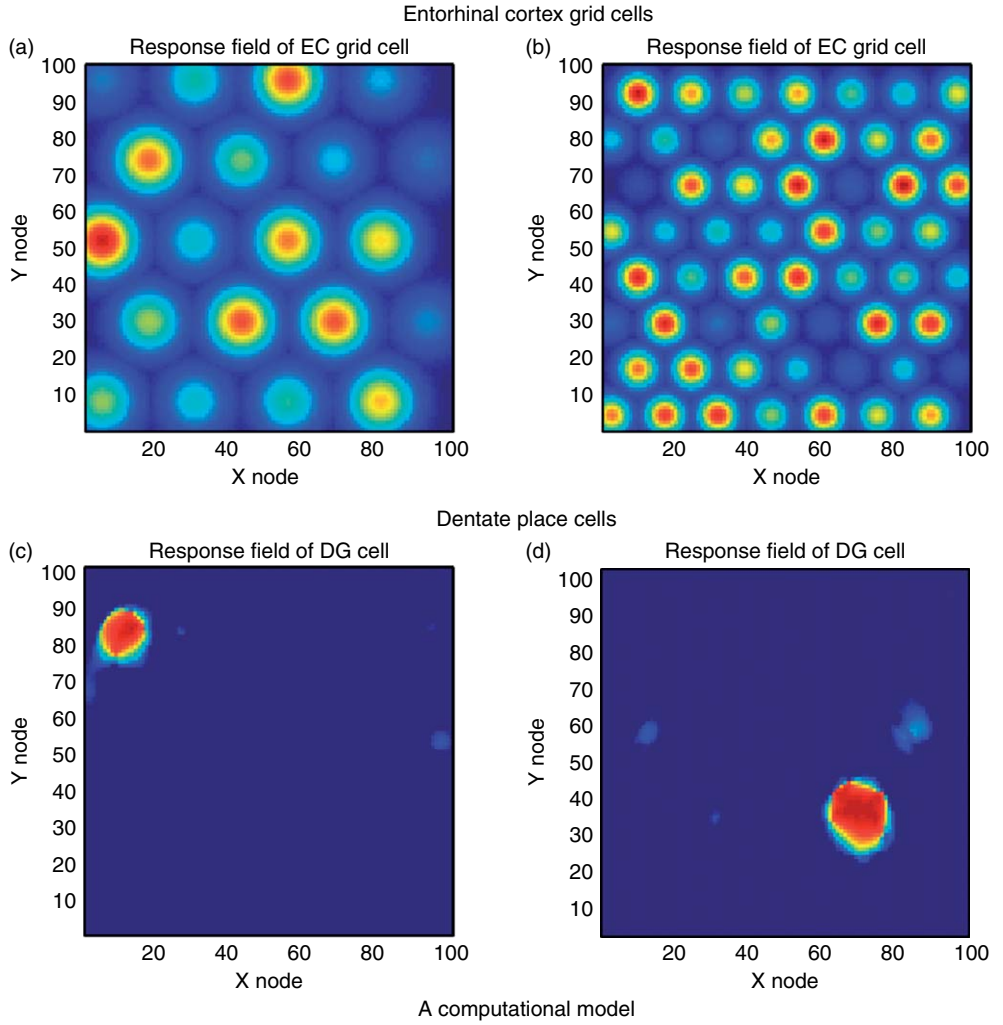

Figure 3 Simulation of competitive learning in the dentate gyrus to produce place cells from the entorhinal cortex grid cell inputs. (a), (b). Firing rate profiles of two entorhinal cortex grid cells with frequencies of four and seven cycles. In the simulation, cells with frequencies of four to seven cycles were used, and with 25 phases or spatial offsets. (A phase is defined as an offset in the X and Y directions, and five offset values were used in each direction.) The standard deviation of the peak heights was set to 0.6. (c), (d): Firing rate profiles of two dentate gyrus cells after competitive network training with the Hebb rule. After Rolls ET, Stringer SM, and Elliot T (2006) Entorhinal cortex grid cells can map to hippocampal place cells by competitive learning. *Netw. Comput. Neural Sys.* 17: 447–465.

the dentate/CA3 system in rodents is characterized by being implicated in spatial memory. However, it is a property of competitive neuronal networks that they can separate such overlapping patterns, as has been shown elsewhere (Rolls, 1989a; Rolls and Treves, 1998; Rolls, 2008); normalization of synaptic weight vectors is required for this property. It is thus an important part of hippocampal neuronal network architecture that there is a competitive network that precedes the CA3 autoassociation system. Without the dentate gyrus, if a conventional autoassociation network were presented with the mixture BW having learned B and W separately, then the autoassociation network would produce a mixed output state and would therefore be incapable of storing separate memories for B, W, and BW. It is suggested, therefore, that competition in the DG is one of the powerful computational features of the hippocampus and could enable it to help solve spatial pattern separation tasks (Rolls and Kesner, 2006).

This computational hypothesis and its predictions have been tested. Rats with DG lesions are impaired at a metric spatial pattern separation task (Gilbert et al., 2001; Goodrich-Hunsaker et al., 2005) (see **Figure 4**). The recoding of grid cells in the entorhinal cortex (Hafting et al., 2005) into small place field cells in the dentate granule cells that has been modeled (Rolls et al., 2006) can also be considered to be a case where overlapping inputs must be recoded so that different spatial components can be treated

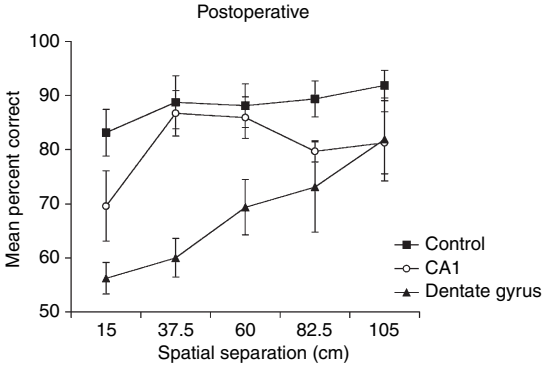

Figure 4 Pattern separation impairment produced by dentate gyrus lesions. Mean percent correct performance as a function of spatial separation of control group, CA1 lesion group, and dentate gyrus lesion group on postoperative trials. A graded impairment was found as a function of the distance between the places only following dentate gyrus lesions. After Gilbert PE, Kesner RP, and Lee I (2001) Dissociating hippocampal subregions: Double dissociation between dentate gyrus and CA1. *Hippocampus* 11: 626–636.

differently. I note that Sutherland and Rudy's configural learning hypothesis was similar but was not tested with spatial pattern separation. Instead, when tested with, for example, tone and light combinations, it was not consistently found that the hippocampus was important (Sutherland and Rudy, 1991; O'Reilly and Rudy, 2001). I suggest that application of the configural concept, but applied to spatial pattern separation, may capture part of what the DG – acting as a competitive network – could perform, particularly when a large number of such overlapping spatial memories must be stored and retrieved.

The third way in which the DG is hypothesized to contribute to the sparse and relatively orthogonal representations in CA3 arises because of the very low contact probability in the mf–CA3 connections and is described in the section titled 'Mossy fiber inputs to the CA3 cells' and by Treves and Rolls (1992).

A fourth way is that, as suggested and explained in the section titled 'Mossy fiber inputs to the CA3 cells,' the dentate granule cell–mf input to the CA3 cells may be powerful, and its use, particularly during learning, would be efficient in forcing a new pattern of firing onto the CA3 cells during learning.

In the ways just described, the dentate granule cells could be particularly important in helping to build and prepare spatial representations for the CA3 network. The actual representation of space in the primate hippocampus includes a representation of spatial view, whereas in the rat hippocampus it is of the place where the rat is. The representation in the rat may be related to the fact that with a much less developed visual system than the primate, the rat's representation of space may be defined more by the olfactory and tactile as well as distant visual cues present and may thus tend to reflect the place where the rat is. However, the spatial representations in the rat and primate could arise from essentially the same computational process as follows (Rolls, 1999; de Araujo et al., 2001). The starting assumption is that in both the rat and the primate, the dentate granule cells (and the CA3 and CA1 pyramidal cells) respond to combinations of the inputs received. In the case of the primate, a combination of visual features in the environment will, because of the fovea providing high spatial resolution over a typical viewing angle of perhaps 10°–20°, result in the formation of a spatial view cell, the effective trigger for which will thus be a combination of visual features within a relatively small part of space. In contrast, in the rat, given the very extensive visual field

subtended by the rodent retina, which may extend over 180°–270°, a combination of visual features formed over such a wide visual angle would effectively define a position in space that is a place (de Araujo et al., 2001).

Although spatial view cells are present in the parahippocampal areas (Rolls et al., 1997a, 1998; Robertson et al., 1998; Georges-François et al., 1999), and neurons with place-like fields (though in some cases as a grid [Hafting et al., 2005]) are found in the medial entorhinal cortex (Moser and Moser, 1998; Brun et al., 2002; Fyhn et al., 2004; Moser, 2004), there are backprojections from the hippocampus to the entorhinal cortex and thus to parahippocampal areas, and these backprojections could enable the hippocampus to influence the spatial representations found in the entorhinal cortex and parahippocampal gyrus. On the other hand, as described above, the grid-like place cells in the medial entorhinal cortex could, if transformed by the competitive net functionality of the dentate cells, result in the place cell activity (without a repeating grid) that is found in dentate and rat hippocampal neurons.

1.33.2.2.3 CA3 as an autoassociation memory

1.33.2.2.3.(i) Arbitrary associations and pattern completion in recall Many of the synapses in the hippocampus show associative modification as shown by long-term potentiation, and this synaptic modification appears to be involved in learning (see Morris, 1989, 2003; Morris et al., 2003; Lynch, 2004). On the basis of the evidence summarized above, Rolls (1987, 1989a,b,d, 1990a,b, 1991) and others (McNaughton and Morris, 1987; Levy, 1989; McNaughton, 1991) have suggested that the CA3 stage acts as an autoassociation memory that enables episodic memories to be formed and stored in the CA3 network, and that subsequently the extensive recurrent collateral connectivity allows for the retrieval of a whole representation to be initiated by the activation of some small part of the same representation (the cue). The crucial synaptic modification for this is in the recurrent collateral synapses. A description of the operation of autoassociative networks is provided by Hertz et al. (1991), Rolls and Treves (1998), Rolls and Deco (2002), and Rolls (2007). The architecture of an autoassociation network is shown in **Figure 2**, and the learning rule is as shown in eqn [1], except that the subtractive term could be the presynaptic firing rate (Rolls and Treves, 1998; Rolls and Deco, 2002).

The hypothesis is that because the CA3 operates effectively as a single network, it can allow arbitrary associations between inputs originating from very different parts of the cerebral cortex to be formed. These might involve associations between information originating in the temporal visual cortex about the presence of an object, as well as information originating in the parietal cortex about where it is. I note that although there is some spatial gradient in the CA3 recurrent connections, so that the connectivity is not fully uniform (Ishizuka et al., 1990), nevertheless the network will still have the properties of a single interconnected autoassociation network allowing associations between arbitrary neurons to be formed, given the presence of many long-range connections that overlap from different CA3 cells.

Crucial issues include how many memories could be stored in this system (to determine whether the autoassociation hypothesis leads to a realistic estimate of the number of memories that the hippocampus could store); whether the whole of a memory could be completed from any part; whether the autoassociation memory can act as a short-term memory, for which the architecture is inherently suited, and whether the system could operate with spatial representations, which are essentially continuous because of the continuous nature of space. These and related issues are considered in the remainder of this section and in more detail elsewhere (Rolls and Kesner, 2006; Rolls, 2008).

1.33.2.2.3.(i).(a) Storage capacity We have performed quantitative analyses of the storage and retrieval processes in the CA3 network (Treves and Rolls, 1991, 1992). We have extended previous formal models of autoassociative memory (see Amit, 1989) by analyzing a network with graded response units, so as to represent more realistically the continuously variable rates at which neurons fire, and with incomplete connectivity (Treves, 1990; Treves and Rolls, 1991). We have found that in general, the maximum number $p_{\max}$ of firing patterns that can be (individually) retrieved is proportional to the number C^{RC} of (associatively) modifiable recurrent collateral synapses per cell, by a factor that increases roughly with the inverse of the sparseness a of the neuronal representation (Each memory is precisely defined in the theory: It is a set of firing rates of the population of neurons – which represent a memory – that can be stored and later retrieved, with retrieval being possible from a fraction of the originally stored set of neuronal firing rates.) The

sparseness of response (or selectivity) of a single cell to a set of stimuli (which in the brain has approximately the same value as the sparseness of the response of the population of neurons to any one stimulus, which can in turn be thought of as the proportion of neurons that is active to any one stimulus if the neurons had binary responses; see Franco et al., 2007) is defined as

$$a = \left(\sum_{i=1,n} r_i/n\right)^2 \Big/ \sum_{i=1,n}\left(r_i^2/n\right), \qquad [2]$$

where r_i is the firing rate to the ith stimulus in the set of n stimuli. The sparseness ranges from $1/n$, when the cell responds to only one stimulus, to a maximal value of 1.0, attained when the cell responds with the same rate to all stimuli. Approximately,

$$p_{\max} \cong \frac{C^{\mathrm{RC}}}{a\ln(1/a)}k, \qquad [3]$$

where k is a factor that depends weakly on the detailed structure of the rate distribution, on the connectivity pattern, and so on, but is roughly on the order of 0.2–0.3 (Treves and Rolls, 1991). The sparseness a in this equation is strictly the population sparseness (Treves and Rolls, 1991; Franco et al., 2007). The population sparseness a^{p} would be measured by measuring the distribution of firing rates of all neurons to a single stimulus at a single time. The single-cell sparseness or selectivity a^{s} would be measured by the distribution of firing rates to a set of stimuli, which would take a long time. These concepts are elucidated by Franco et al. (2007). The sparseness estimates obtained by measuring early gene changes, which are effectively population sparsenesses, would thus be expected to depend greatly on the range of environments or stimuli in which these were measured. If the environment was restricted to one stimulus, this would reflect the population sparseness. If the environment was changing, the measure from early gene changes would be rather undefined, as all the populations of neurons activated in an undefined number of testing situations would be likely to be activated. For example, for $C^{\mathrm{RC}} = 12{,}000$ and $a = 0.02$, $p_{\max}$ is calculated to be approximately 36,000. This analysis emphasizes the utility of having a sparse representation in the hippocampus, for this enables many different memories to be stored. Third, in order for most associative networks to store information efficiently, heterosynaptic LTD (as well as LTP) is required (Fazeli and Collingridge, 1996; Rolls and Deco, 2002; Rolls and Treves, 1990, 1998; Treves and Rolls, 1991). Simulations that are fully consistent with the analytic theory are provided by Simmen et al. (1996) and Rolls et al. (1997b).

We have also indicated how to estimate I, the total amount of information (in bits per synapse) that can be retrieved from the network. I is defined with respect to the information i_{p} (in bits per cell) contained in each stored firing pattern, by subtracting the amount i_{l} lost in retrieval and multiplying by p/C^{RC}:

$$I = \frac{p}{C^{\mathrm{RC}}}(i_p - i_i) \qquad [4]$$

The maximal value $I_{\max}$ of this quantity was found (Treves and Rolls, 1991) to be in several interesting cases around 0.2–0.3 bits per synapse, with only a mild dependency on parameters such as the sparseness of coding a.

We may then estimate (Treves and Rolls, 1992) how much information has to be stored in each pattern for the network to efficiently exploit its information retrieval capacity $I_{\max}$. The estimate is expressed as a requirement on i_{p}:

$$i_p > a\ln(1/a) \qquad [5]$$

As the information content of each stored pattern i_{p} depends on the storage process, we see how the retrieval capacity analysis, coupled with the notion that the system is organized so as to be an efficient memory device in a quantitative sense, leads to a constraint on the storage process.

A number of points that arise are treated elsewhere (Rolls and Kesner, 2006; Rolls, 2007). Here I note that given that the memory capacity of the hippocampal CA3 system is limited, it is necessary to have some form of forgetting in this store, or another mechanism to ensure that its capacity is not exceeded. (Exceeding the capacity can lead to a loss of much of the information retrievable from the network.) Heterosynaptic LTD could help this forgetting, by enabling new memories to overwrite old memories (Rolls, 1996a, 2007). The limited capacity of the CA3 system does also provide one of the arguments that some transfer of information from the hippocampus to neocortical memory stores may be useful (see Treves and Rolls, 1994). Given its limited capacity, the hippocampus might be a useful store for only a limited period, which might be on the order of days, weeks, or months. This period may well depend on the acquisition rate of new episodic

memories. If the animal were in a constant and limited environment, then as new information is not being added to the hippocampus, the representations in the hippocampus would remain stable and persistent. These hypotheses have clear experimental implications, both for recordings from single neurons and for the gradient of retrograde amnesia, both of which might be expected to depend on whether the environment is stable or frequently changing. They show that the conditions under which a gradient of retrograde amnesia might be demonstrable would be when large numbers of new memories are being acquired, not when only a few memories (few in the case of the hippocampus being less than a few hundred) are being learned.

1.33.2.2.3.(i).(b) Recall A fundamental property of the autoassociation model of the CA3 recurrent collateral network is that the recall can be symmetric, that is, the whole of the memory can be retrieved from any part. For example, in an object–place autoassociation memory, an object could be recalled from a place retrieval cue, and vice versa. This is not the case with a pattern association network. If, for example, the CA3 activity represented a place–spatial view, and perforant path inputs with associative synapses to CA3 neurons carried object information (consistent with evidence that the lateral perforant path [LPP] may reflect inputs from the perirhinal cortex connecting via the lateral entorhinal cortex [Hargreaves et al., 2005]), then an object could recall a place, but a place could not recall an object.

A prediction of the theory is thus that the CA3 recurrent collateral associative connections enable arbitrary associations to be formed between whatever is represented in the hippocampus, in that, for example, any place could be associated with any object, and in that the object could be recalled with a spatial recall cue, or the place with an object recall cue.

In one test of this, Day et al. (2003) trained rats in a study phase to learn in one trial an association between two flavors of food and two spatial locations. During a recall test phase they were presented with a flavor that served as a cue for the selection of the correct location. They found that injections of an NMDA blocker (AP5) or alpha-amino-3-hydroxy-5-methyl-isoxazole-4-propionic acid (AMPA) blocker (CNQX) to the dorsal hippocampus prior to the study phase impaired encoding, but injections of AP5 prior to the test phase did not impair the place recall, whereas injections of CNQX did impair the place recall. The interpretation is that somewhere in the hippocampus NMDA receptors are necessary for forming one-trial odor–place associations, and that recall can be performed without further involvement of NMDA receptors.

In a hippocampus subregion test of this, rats in a study phase are shown one object in one location, and then a second object in another location. (There are 50 possible objects and 48 locations.) In the test phase, the rat is shown one object in the start box and then after a 10-s delay must go to the correct location (choosing between two marked locations). CA3 lesions made after training in the task produced chance performance on this one-trial object–place recall task. A control, fixed visual conditional-to-place task (Rolls and Kesner, 2006) with the same delay was not impaired, showing that it is recall after one-trial (or rapid) learning that is impaired. In the context of arbitrary associations between whatever is represented in CA3, the theory also predicts that cued place–object recall tasks and cued place–odor recall tasks should be impaired by CA3 lesions.

Evidence that the CA3 system is not necessarily required during recall in a reference memory spatial task, such as the water maze spatial navigation for a single spatial location task, is that CA3-lesioned rats are not impaired during recall of a previously learned water maze task (Brun et al., 2002; Florian and Roullet, 2004). However, if completion from an incomplete cue is needed, then CA3 NMDA receptors are necessary (presumably to ensure satisfactory CA3–CA3 learning), even in a reference memory task (Nakazawa et al., 2002). Thus, the CA3 system appears to be especially needed in rapid, one-trial object–place recall and when completion from an incomplete cue is required.

In a neurophysiological investigation of one-trial object–place learning followed by recall of the spatial position in which to respond when shown the object, Rolls and Xiang (2005) showed that some primate hippocampal (including CA3) neurons respond to an object cue with the spatial position in which the object had been shown earlier in the trial. Thus, some hippocampal neurons appear to reflect spatial recall given an object recall cue.

1.33.2.2.3.(i).(c) Completion Another fundamental property is that the recall can be complete even from a small fragment. Thus, it is a prediction that when an incomplete retrieval cue is given, CA3 may be especially important in the retrieval process. Tests of this prediction of a role for CA3 in pattern completion have been performed, as follows.

Rats were tested on a cheese board with a black curtain with four extramaze cues surrounding the apparatus. (The cheese board is like a dry-land water maze with 177 holes on a 119-cm-diameter board.) Rats were trained to move a sample phase object covering a food well that could appear in one of five possible spatial locations. During the test phase of the task, following a 30-s delay, the animal needs to find the same food well in order to receive reinforcement with the object now removed. After reaching stable performance in terms of accuracy to find the correct location, rats received lesions in CA3. During postsurgery testing, four extramaze cues were always available during the sample phase. However, during the test phase zero, one, two, or three cues were removed in different combinations. The results indicate that controls performed well on the task regardless of the availability of one, two, three, or all cues, suggesting intact spatial pattern completion. Following the CA3 lesion, however, there was an impairment in accuracy compared to the controls especially when only one or two cues were available, suggesting impairment in spatial pattern completion in CA3-lesioned rats (Gold and Kesner, 2005) (see **Figure 5**). A useful aspect of this task is that the test for the ability to remember a spatial location learned in one presentation can be tested with a varying number of available cues, and many times in which the locations vary, to allow for accurate measurement of pattern completion ability when the information stored on the single presentation must be recalled.

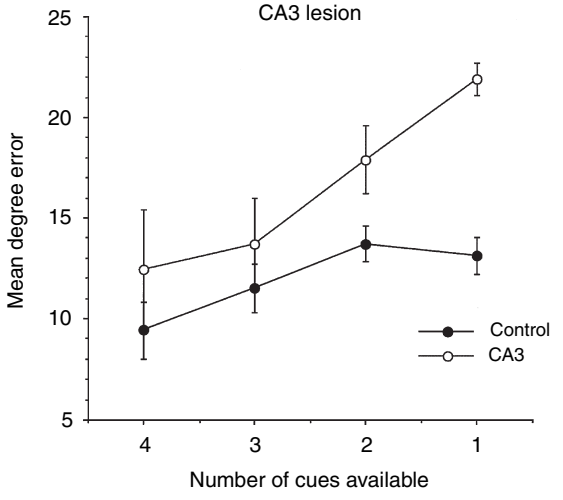

Figure 5 Pattern completion impairment produced by CA3 lesions. The mean (and SEM) degree of error in finding the correct place in the cheeseboard task when rats were tested with 1, 2, 3, or 4 of the cues available. A graded impairment in the CA3 lesion group as a function of the number of cues available was found. The task was learned in the study phase with the four cues present. The performance of the control group is also shown. After Gold AE and Kesner RP (2005) The role of the CA3 subregion of the dorsal hippocampus in spatial pattern completion in the rat. *Hippocampus* 15: 808–814.

In another study, Nakazawa et al. (2002) trained CA3 NMDA receptor-knockout mice in an analogous task, using the water maze. When the animals were required to perform the task in an environment where some of the familiar cues were removed, they were impaired in performing the task. The result suggests that the NMDA receptor–dependent synaptic plasticity mechanisms in CA3 are critical to perform the pattern completion process in the hippocampus.

1.33.2.2.3.(ii) Continuous spatial patterns and CA3 representations The fact that spatial patterns, which imply continuous representations of space, are represented in the hippocampus has led to the application of continuous attractor models to help understand hippocampal function. This has been necessary because space is inherently continuous, because the firing of place and spatial view cells is approximately Gaussian as a function of the distance away from the preferred spatial location, because these cells have spatially overlapping fields, and because the theory is that these cells in CA3 are connected by Hebb-modifiable synapses. This specification would inherently lead the system to operate as a continuous attractor network. Continuous attractor network models have been studied by Amari (1977), Zhang (1996), Taylor (1999), Samsonovich and McNaughton (1997), Battaglia and Treves (1998), Stringer et al. (2002a,b, 2004), Stringer and Rolls (2002), and Rolls and Stringer (2005) (see Rolls, 2007; Rolls and Deco, 2002) and are described next.

A continuous attractor neural network (CANN) can maintain the firing of its neurons to represent any location along a continuous physical dimension such as spatial position and head direction. It uses excitatory recurrent collateral connections between the neurons (as are present in CA3) to reflect the distance between the neurons in the state space of the animal (e.g., place or head direction). These networks can maintain the bubble of neural activity constant for long periods wherever it is started, to represent the current state (head direction, position, etc.) of the animal, and are likely to be involved in many aspects of spatial processing and memory, including spatial vision. Global inhibition is used to keep the number

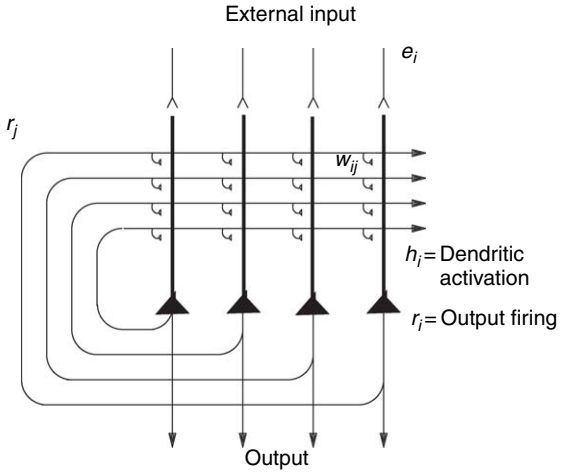

Figure 6 The architecture of a continuous attractor neural network. The architecture is the same as that of a discrete attractor neural network.

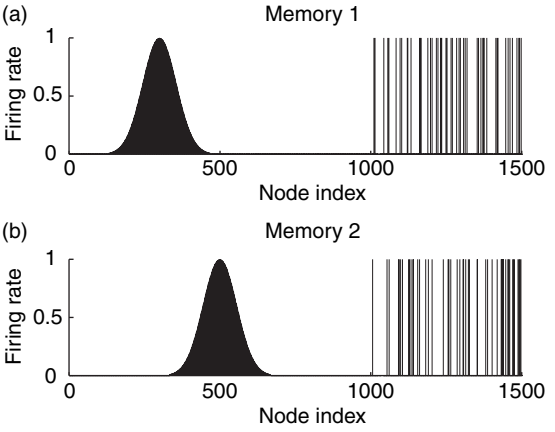

Figure 7 The types of firing patterns stored in continuous attractor networks are illustrated for the patterns present on neurons 1–1000 for Memory 1 (when the firing is that produced when the spatial state represented is that for location 300), and for Memory 2 (when the firing is that produced when the spatial state represented is that for location 500). The continuous nature of the spatial representation results from the fact that each neuron has a Gaussian firing rate that peaks at its optimal location. This particular mixed network also contains discrete representations that consist of discrete subsets of active binary firing rate neurons in the range 1001–1500. The firing of these latter neurons can be thought of as representing the discrete events that occur at the location. Continuous attractor networks by definition contain only continuous representations, but this particular network can store mixed continuous and discrete representations, and is illustrated to show the difference of the firing patterns normally stored in separate continuous attractor and discrete attractor networks. For this particular mixed network, during learning, Memory 1 is stored in the synaptic weights, then Memory 2, etc., and each memory contains a part that is continuously distributed to represent physical space and a part that represents a discrete event or object.

of neurons in a bubble or packet of actively firing neurons relatively constant and to help to ensure that there is only one activity packet. Continuous attractor networks can be thought of as very similar to autoassociation or discrete attractor networks (see Rolls and Deco 2002) and have the same architecture, as illustrated in **Figure 6**. The main difference is that the patterns stored in a CANN are continuous patterns, with each neuron having broadly tuned firing that decreases with, for example, a Gaussian function as the distance from the optimal firing location of the cell is varied, and with different neurons having tuning that overlaps throughout the space. Such tuning is illustrated in **Figure 7**. For comparison, autoassociation networks normally have discrete (separate) patterns (each pattern implemented by the firing of a particular subset of the neurons), with no continuous distribution of the patterns throughout the space (see **Figure 7**). A consequent difference is that the CANN can maintain its firing at any location in the trained continuous space, whereas a discrete attractor or autoassociation network moves its population of active neurons toward one of the previously learned attractor states, and thus implements the recall of a particular previously learned pattern from an incomplete or noisy (distorted) version of one of the previously learned patterns.

The energy landscape of a discrete attractor network (see Rolls and Deco 2002) has separate energy minima, each one of which corresponds to a learned pattern, whereas the energy landscape of a continuous attractor network is flat, so that the activity packet remains stable with continuous firing wherever it is started in the state space. (The state space refers to the set of possible spatial states of the animal in its environment, e.g., the set of possible places in a room.)

So far we have said that the neurons in the continuous attractor network are connected to each other by synaptic weights w_{ij} that are a simple function, for example, Gaussian, of the distance between the states of the agent in the physical world (e.g., head directions, spatial views, etc.) represented by the neurons. In many simulations, the weights are set by formula to have weights with these appropriate Gaussian values. However, Stringer et al. (2002b) showed how the appropriate weights could be set up by learning. They started with the fact that since

the neurons have broad tuning that may be Gaussian in shape, nearby neurons in the state space will have overlapping spatial fields, and will thus be coactive to a degree that depends on the distance between them. The authors postulated that therefore the synaptic weights could be set up by associative learning based on the coactivity of the neurons produced by external stimuli as the animal moved in the state space. For example, head direction cells are forced to fire during learning by visual cues in the environment that produces Gaussian firing as a function of head direction from an optimal head direction for each cell. The learning rule is simply that the weights w_{ij} from head direction cell j with firing rate r_j^{HD} to head direction cell i with firing rate r_i^{HD} are updated according to an associative (Hebb) rule:

$$\delta w_{ij} = k r_i^{\mathrm{HD}} r_j^{\mathrm{HD}}, \quad [6]$$

where δw_{ij} is the change of synaptic weight and k is the learning rate constant. During the learning phase, the firing rate r_i^{HD} of each head direction cell i might be the following the Gaussian function of the displacement of the head from the optimal firing direction of the cell:

$$r_i^{\mathrm{HD}} = e^{-s_{\mathrm{HD}}^2/2\sigma_{\mathrm{HD}}^2}, \quad [7]$$

where s_{HD} is the difference between the actual head direction x (in degrees) of the agent and the optimal head direction x_i for head direction cell i, and σ_{HD} is the standard deviation. Stringer et al. (2002b) showed that after training at all head directions, the synaptic connections develop strengths that are an almost Gaussian function of the distance between the cells in head direction space.

1.33.2.2.3.(iii) Combined continuous and discrete memory representations in the same (e.g., CA3) network, and episodic memory

Space is continuous, and object representations are discrete. If these representations are to be combined in, for example, an object–place memory, then we need to understand the operation of networks that combine these representations. It has now been shown that attractor networks can store both continuous patterns and discrete patterns (as illustrated in **Figure 7**) and can thus be used to store, for example, the location in (continuous, physical) space (e.g., the place out there in a room represented by spatial view cells) where an object (a discrete item) is present (Rolls et al., 2002).

1.33.2.2.3.(iv) The capacity of a continuous attractor network, and multiple charts

If spatial representations are stored in the hippocampus, the important issue arises in terms of understanding memories that include a spatial component or context of how many such spatial representations could be stored in a continuous attractor network. The very interesting result is that because there are in general low correlations between the representations of places in different maps or charts (where each map or chart might be of one room or locale), very many different maps can be simultaneously stored in a continuous attractor network (Battaglia and Treves, 1998a).

1.33.2.2.3.(v) Idiothetic update by path integration

We have considered how spatial representations could be stored in continuous attractor networks and how the activity can be maintained at any location in the state space in a form of short-term memory when the external (e.g., visual) input is removed. However, many networks with spatial representations in the brain can be updated by internal, self-motion (i.e., idiothetic) cues even when there is no external (e.g., visual) input. The way in which path integration could be implemented in recurrent networks such as the CA3 system in the hippocampus or in related systems is described next.

Single-cell recording studies have shown that some neurons represent the current position along a continuous physical dimension or space even when no inputs are available, for example, in darkness. Examples include neurons that represent the positions of the eyes (i.e., eye direction with respect to the head), the place where the animal is looking in space, head direction, and the place where the animal is located. In particular, examples of such classes of cells include head direction cells in rats (Ranck, 1985; Taube et al., 1990; Muller et al., 1996; Taube et al., 1996) and primates (Robertson et al., 1999), which respond maximally when the animal's head is facing in a particular preferred direction; place cells in rats (O'Keefe and Dostrovsky, 1971; McNaughton et al., 1983; O'Keefe, 1984; Muller et al., 1991; Markus et al., 1995) that fire maximally when the animal is in a particular location; and spatial view cells in primates that respond when the monkey is looking toward a particular location in space (Rolls et al., 1997a; Robertson et al., 1998; Georges-François et al., 1999).

One approach to simulating the movement of an activity packet produced by idiothetic cues (which is a form of path integration whereby the current location is calculated from recent movements) is to employ a look-up table that stores (taking head direction cells as an example), for every possible head direction and head rotational velocity input generated by the vestibular system, the corresponding new head direction (Samsonovich and McNaughton, 1997). An analogous approach has been described for entorhinal cortex grid cells (McNaughton et al., 2006). Another approach involves modulating the strengths of the recurrent synaptic weights in the continuous attractor on one but not the other side of a currently represented position, so that the stable position of the packet of activity, which requires symmetric connections in different directions from each node, is lost, and the packet moves in the direction of the temporarily increased weights, although no possible biological implementation was proposed of how the appropriate dynamic synaptic weight changes might be achieved (Zhang, 1996). Another mechanism (for head direction cells) (Skaggs et al., 1995) relies on a set of cells, termed (head) rotation cells, which are coactivated by head direction cells and vestibular cells and drive the activity of the attractor network by anatomically distinct connections for clockwise and counterclockwise rotation cells, in what is effectively a look-up table. However, these proposals did not show how the synaptic weights for this path integration could be achieved by a biologically plausible learning process.

Stringer et al. (2002b) introduced a proposal with more biological plausibility about how the synaptic connections from idiothetic inputs to a continuous attractor network can be learned by a self-organizing learning process. The mechanism associates a short-term memory trace of the firing of the neurons in the attractor network reflecting recent movements in the state space (e.g., of places) with an idiothetic velocity of movement input (see **Figure 8**). This has been applied to head direction cells (Stringer et al., 2002b), rat place cells (Stringer et al., 2002a,b), and primate spatial view cells (Stringer et al., 2004, 2005; Rolls and Stringer, 2005). These attractor networks provide a basis for understanding cognitive maps and how they are updated by learning and by self-motion. The implication is that to the extent that path integration of place or spatial view representations is performed within the hippocampus itself, then the CA3 system is the most likely part of the hippocampus to be involved in this, because it has

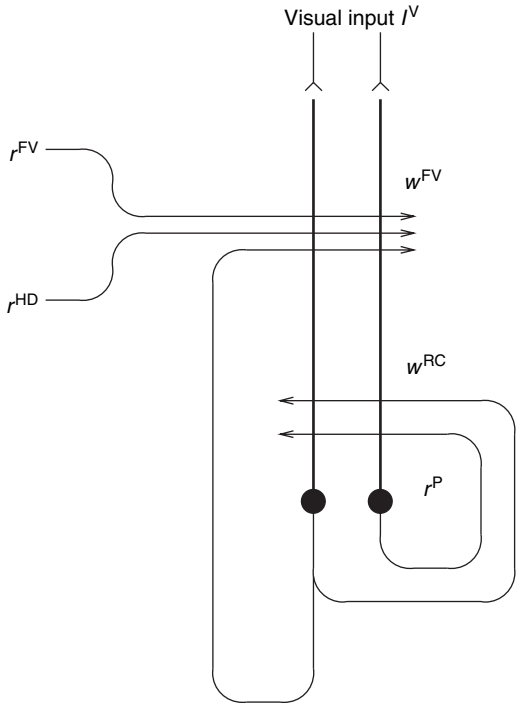

Figure 8 Neural network architecture for two-dimensional continuous attractor models of place cells. There is a recurrent network of place cells with firing rates r^P, which receives external inputs from three sources: (i) the visual system I^V, (ii) a population of head direction cells with firing rates r^{HD}, and (iii) a population of forward velocity cells with firing rates r^{FV}. The recurrent weights between the place cells are denoted by w^{RC}, and the idiothetic weights to the place cells from the forward velocity cells and head direction cells are denoted by w^{FV}.

the appropriate recurrent collateral connections. Consistent with this, Whishaw and colleagues (Maaswinkel et al., 1999; Whishaw et al., 2001; Wallace and Whishaw, 2003) have shown that path integration is impaired by hippocampal lesions. Path integration of head direction is reflected in the firing of neurons in the presubiculum, and mechanisms outside the hippocampus probably implement path integration for head direction.

1.33.2.2.3.(vi) The dynamics of the recurrent network The analysis described earlier of the capacity of a recurrent network such as the CA3 considered steady-state conditions of the firing rates of the neurons. The question arises of how quickly the recurrent network would settle into its final state. With reference to the CA3 network, how long does it take before a pattern of activity, originally evoked in

CA3 by afferent inputs, becomes influenced by the activation of recurrent collaterals? In a more general context, recurrent collaterals between the pyramidal cells are an important feature of the connectivity of the cerebral neocortex. How long would it take these collaterals to contribute fully to the activity of cortical cells? If these settling processes took on the order of hundreds of milliseconds, they would be much too slow to contribute usefully to cortical activity, whether in the hippocampus or the neocortex (Rolls, 1992; Panzeri et al., 2001; Rolls and Deco, 2002; Rolls, 2003).

It has been shown that if the neurons are not treated as McCulloch-Pitts neurons, which are simply updated at each iteration, or cycle of time steps (and assume the active state if the threshold is exceeded), but instead are analyzed and modeled as integrate-and-fire neurons in real continuous time, then the network can effectively relax into its recall state very rapidly in one or two time constants of the synapses (Treves, 1993; Battaglia and Treves, 1998b; Rolls and Treves, 1998; Rolls and Deco, 2002). This corresponds to perhaps 20 ms in the brain. One factor in this rapid dynamics of autoassociative networks with brain-like integrate-and-fire membrane and synaptic properties is that with some spontaneous activity, some of the neurons in the network are close to threshold already before the recall cue is applied, and hence some of the neurons are very quickly pushed by the recall cue into firing, so that information starts to be exchanged very rapidly (within 1–2 ms of brain time) through the modified synapses by the neurons in the network. The progressive exchange of information starting early on within what would otherwise be thought of as an iteration period (of perhaps 20 ms, corresponding to a neuronal firing rate of 50 spikes/s) is the mechanism accounting for rapid recall in an autoassociative neuronal network made biologically realistic in this way. Further analysis of the fast dynamics of these networks if they are implemented in a biologically plausible way with integrate-and-fire neurons, is provided in Section 7.7 of Rolls and Deco (2002), in Appendix A5 of Rolls and Treves (1998), by Treves (1993), and by Panzeri et al. (2001).

1.33.2.2.3.(vii) Mossy fiber inputs to the CA3 cells We hypothesize that the mf inputs force efficient information storage by virtue of their strong and sparse influence on the CA3 cell firing rates (Rolls, 1987, 1989b,d; Treves and Rolls, 1992). (The strong effects likely to be mediated by the mfs were also emphasized by McNaughton and Morris [1987] and McNaughton and Nadel [1990].) We hypothesize that the mf input appears to be particularly appropriate in several ways.

First of all, the fact that mf synapses are large and located very close to the soma makes them relatively powerful in activating the postsynaptic cell. (This should not be taken to imply that a CA3 cell can be fired by a single mf excitatory postsynaptic potential [EPSP].)

Second, the firing activity of dentate granule cells appears to be very sparse (Jung and McNaughton, 1993), and this, together with the small number of connections on each CA3 cell, produces a sparse signal, which can then be transformed into an even sparser firing activity in CA3 by a threshold effect. For example, if only one granule cell in 100 were active in the dentate gyrus, and each CA3 cell received a connection from 50 randomly placed granule cells, then the number of active mf inputs received by CA3 cells would follow a Poisson distribution of average $50/100 = 1/2$, that is, 60% of the cells would not receive any active input, 30% would receive only one, 7.5% two, little more than 1% would receive three, and so on. (It is easy to show from the properties of the Poisson distribution and our definition of sparseness that the sparseness of the mf signal as seen by a CA3 cell would be $x/(1+x)$, with $x = C^{\mathrm{MF}} a_{\mathrm{DG}}$, assuming equal strengths for all mf synapses.) If three mf inputs were required to fire a CA3 cell and these were the only inputs available, we see that the activity in CA3 would be roughly as sparse, in the example, as in the dentate gyrus. C^{MF} is the number of mf connections to a CA3 neuron, and a_{DG} is the sparseness of the representation in the dentate granule cells.

Third, nonassociative plasticity of mfs (see Brown et al., 1989, 1990) might have a useful effect in enhancing the signal-to-noise ratio, in that a consistently firing mf would produce nonlinearly amplified currents in the postsynaptic cell, which would not happen with an occasionally firing fiber (Treves and Rolls, 1992). This plasticity, and also learning in the dentate, would also have the effect that similar fragments of each episode (e.g., the same environmental location) recurring on subsequent occasions would be more likely to activate the same population of CA3 cells, which would have potential advantages in terms of economy of use of the CA3 cells in different memories, and in making some link between different episodic memories with a common feature, such as the same location in space.

Fourth, with only a few, and powerful, active mf inputs to each CA3 cell, setting a given sparseness of the representation provided by CA3 cells would be simplified, for the EPSPs produced by the mfs would be Poisson distributed with large membrane potential differences for each active mf. Setting the average firing rate of the dentate granule cells would effectively set the sparseness of the CA3 representation, without great precision being required in the threshold setting of the CA3 cells (Rolls et al., 1997b). Part of what is achieved by the mf input may be setting the sparseness of the CA3 cells correctly, which, as shown above, is very important in an autoassociative memory store.

Fifth, the nonassociative and sparse connectivity properties of the mf connections to CA3 cells may be appropriate for an episodic memory system that can learn very fast, in one trial. The hypothesis is that the sparse connectivity would help arbitrary relatively uncorrelated sets of CA3 neurons to be activated for even somewhat similar input patterns without the need for any learning of how best to separate the patterns, which in a self-organizing competitive network would take several repetitions (at least) of the set of patterns.

The mf solution may thus be adaptive in a system that must learn in one trial, and for which the CA3 recurrent collateral learning requires uncorrelated sets of CA3 cells to be allocated for each (one-trial) episodic memory. The hypothesis is that the mf sparse connectivity solution performs the appropriate function without the mf system having to learn by repeated presentations of how best to separate a set of training patterns. The perforant path input would, the quantitative analysis shows, not produce a pattern of firing in CA3 that contains sufficient information for learning (Treves and Rolls, 1992).

On the basis of these points, we predict that the mfs may be necessary for new learning in the hippocampus but may not be necessary for recall of existing memories from the hippocampus. Experimental evidence consistent with this prediction about the role of the mfs in learning has been found in rats with disruption of the dentate granule cells (Lassalle et al., 2000).

As acetylcholine turns down the efficacy of the recurrent collateral synapses between CA3 neurons (Hasselmo et al., 1995), then cholinergic activation also might help to allow external inputs rather than the internal recurrent collateral inputs to dominate the firing of the CA3 neurons during learning, as the current theory proposes. If cholinergic activation at the same time facilitated LTP in the recurrent collaterals (as it appears to in the neocortex), then cholinergic activation could have a useful double role in facilitating new learning at times of behavioral activation, when presumably it may be particularly relevant to allocate some of the limited memory capacity to new memories.

1.33.2.2.3.(viii) Perforant path inputs to CA3 cells By calculating the amount of information that would end up being carried by a CA3 firing pattern produced solely by the perforant path input and by the effect of the recurrent connections, we have been able to show (Treves and Rolls, 1992) that an input of the perforant path type, alone, is unable to direct efficient information storage. Such an input is too weak, it turns out, to drive the firing of the cells, as the dynamics of the network are dominated by the randomizing effect of the recurrent collaterals. This is the manifestation, in the CA3 network, of a general problem affecting storage (i.e., learning) in all autoassociative memories. The problem arises when the system is considered to be activated by a set of input axons making synaptic connections that have to compete with the recurrent connections, rather than having the firing rates of the neurons artificially clamped into a prescribed pattern.

An autoassociative memory network needs afferent inputs also in the other mode of operation, that is, when it retrieves a previously stored pattern of activity. We have shown (Treves and Rolls, 1992) that the requirements on the organization of the afferents are in this case very different, implying the necessity of a second, separate input system, which we have identified with the perforant path to CA3. In brief, the argument is based on the notion that the cue available to initiate retrieval might be rather small, that is, the distribution of activity on the afferent axons might carry a small correlation, $q << 1$, with the activity distribution present during learning. In order not to lose this small correlation altogether, but rather transform it into an input current in the CA3 cells that carries a sizable signal – which can then initiate the retrieval of the full pattern by the recurrent collaterals – one needs a large number of associatively modifiable synapses. This is expressed by the formulas that give the specific signal S produced by sets of associatively modifiable synapses, or by nonassociatively modifiable synapses: If C^{AFF} is the number of afferents per cell,

$$S_{\mathrm{ASS}} \sim \frac{\sqrt{C^{\mathrm{AFF}}}}{\sqrt{p}}\, q \quad S_{\mathrm{NONASS}} \sim \frac{1}{\sqrt{C^{\mathrm{AFF}}}}\, q. \qquad [8]$$

Associatively modifiable synapses are therefore needed and are needed in a number C^{AFF} of the same order as the number of concurrently stored patterns p, so that small cues can be effective, whereas nonassociatively modifiable synapses – or even more so, nonmodifiable ones – produce very small signals, which decrease in size the larger the number of synapses. In contrast with the storage process, the average strength of these synapses does not play now a crucial role. This suggests that the perforant path system is the one involved in relaying the cues that initiate retrieval.

1.33.2.2.4 CA1 cells

1.33.2.2.4.(i) Associative retrieval at the CA3 to CA1 (Schaffer collateral) synapses The CA3 cells connect to the CA1 cells by the Schaeffer collateral synapses. The following arguments outline the advantage of this connection being associatively modifiable and apply independently of the relative extent to which the CA3 or the direct entorhinal cortex inputs to CA1 drive the CA1 cells during the learning phase.

The amount of information about each episode retrievable from CA3 has to be balanced against the number of episodes that can be held concurrently in storage. The balance is regulated by the sparseness of the coding. Whatever the amount of information per episode in CA3, one may hypothesize that the organization of the structures that follow CA3 (i.e., CA1, the various subicular fields, and the return projections to neocortex) should be optimized so as to preserve and use this information content in its entirety. This would prevent further loss of information, after the massive but necessary reduction in information content that has taken place along the sensory pathways and before the autoassociation stage in CA3. We have proposed (Treves and Rolls, 1994; Treves, 1995) that the need to preserve the full information content present in the output of an autoassociative memory requires an intermediate recoding stage (CA1) with special characteristics. In fact, a calculation of the information present in the CA1 firing pattern, elicited by a pattern of activity retrieved from CA3, shows that a considerable fraction of the information is lost if the synapses are nonmodifiable, and that this loss can be prevented only if the CA3 to CA1 synapses are associatively modifiable. Their modifiability should match the plasticity of the CA3 recurrent collaterals. The additional information that can be retrieved beyond that retrieved by CA3 because the CA3 to CA1 synapses are associatively modifiable is strongly demonstrated by the hippocampal simulation described by Rolls (1995) and is quantitatively analyzed by Schultz and Rolls (1999).

1.33.2.2.4.(ii) Recoding in CA1 to facilitate retrieval to the neocortex If the total amount of information carried by CA3 cells is redistributed over a larger number of CA1 cells, less information needs to be loaded onto each CA1 cell, rendering the code more robust to information loss in the next stages. For example, if each CA3 cell had to code for two bits of information, for example, by firing at one of four equiprobable activity levels, then each CA1 cell (if there were twice as many as there are CA3 cells) could code for just 1 bit, for example, by firing at one of only two equiprobable levels. Thus, the same information content could be maintained in the overall representation while reducing the sensitivity to noise in the firing level of each cell. In fact, there are more CA1 cells than CA3 cells in rats (2.5×10^5). There are even more CA1 cells (4.6×10^6) in humans (and the ratio of CA1 to CA3 cells is greater). The CA1 cells may thus provide the first part of the expansion for the return projections to the enormous numbers of neocortical cells in primates, after the bottleneck of the single network in CA3, the number of neurons in which may be limited because it has to operate as a single network.

Another argument on the operation of the CA1 cells is also considered to be related to the CA3 autoassociation effect. In this, several arbitrary patterns of firing occur together on the CA3 neurons and become associated together to form an episodic or whole scene memory. It is essential for this CA3 operation that several different sparse representations are present conjunctively in order to form the association. Moreover, when completion operates in the CA3 autoassociation system, all the neurons firing in the original conjunction can be brought into activity by only a part of the original set of conjunctive events. For these reasons, a memory in the CA3 cells consists of several different simultaneously active ensembles of activity. To be explicit, the parts A, B, C, D, and E of a particular episode would each be represented, roughly speaking, by its own population of CA3 cells, and these five populations would be linked together by autoassociation. It is suggested that the CA1 cells, which receive these groups of simultaneously active ensembles, can detect the conjunctions of firing of the different ensembles that represent the episodic memory and

allocate by competitive learning neurons to represent at least larger parts of each episodic memory (Rolls, 1987, 1989a,b,d, 1990a,b). In relation to the simple example above, some CA1 neurons might code for ABC, and others for BDE, rather than having to maintain independent representations in CA1 of A, B, C, D, and E. This implies a more efficient representation, in the sense that when eventually, after many further stages, neocortical neuronal activity is recalled (as discussed later), each neocortical cell need not be accessed by all the axons carrying each component A, B, C, D, and E but, instead, by fewer axons carrying larger fragments, such as ABC and BDE. This process is performed by competitive networks, which self-organize to find categories in the input space, where each category is represented by a set of simultaneously active inputs (Rolls and Treves, 1998; Rolls, 2000; Rolls and Deco, 2002).

1.33.2.2.4.(iii) CA1 inputs from CA3 vs direct entorhinal inputs Another feature of the CA1 network is its double set of afferents, with each of its cells receiving most synapses from the Schaeffer collaterals coming from CA3, but also a proportion (about 1/6; Amaral et al., 1990) from direct perforant path projections from entorhinal cortex. Such projections appear to originate mainly in layer 3 of entorhinal cortex (Witter et al., 1989) from a population of cells only partially overlapping with that (mainly in layer 2) giving rise to the perforant path projections to DG and CA3. This suggests that it is useful to include in CA1 not only what it is possible to recall from CA3 but also the detailed information present in the retrieval cue itself (see Treves and Rolls, 1994).

Another possibility is that the perforant path input provides the strong forcing input to the CA1 neurons during learning and that the output of the CA3 system is associated with this forced CA1 firing during learning (McClelland et al., 1995). During recall, an incomplete cue could then be completed in CA3, and the CA3 output would then produce firing in CA1 that would correspond to that present during the learning. This suggestion is essentially identical to that of Treves and Rolls (1994) about the backprojection system and recall, except that McClelland et al. (1995) suggest that the output of CA3 is associated at the CA3 to CA1 (Schaeffer collateral) synapses with the signal present during training in CA1, whereas in the theory of Treves and Rolls (1994), the output of the hippocampus consists of CA1 firing, which is associated in the entorhinal cortex and earlier cortical stages with the firing present during learning, providing a theory of how the correct recall is implemented at every backprojection stage though the neocortex (see the next section).

1.33.2.2.5 Backprojections to the neocortex – a hypothesis

The need for information to be retrieved from the hippocampus to affect other brain areas was noted in the introduction. The way in which this could be implemented via backprojections to the neocortex is now considered.

It is suggested that the modifiable connections from the CA3 neurons to the CA1 neurons allow the whole episode in CA3 to be produced in CA1. This may be assisted as described above by the direct perforant path input to CA1. This might allow details of the input key for the recall process, as well as the possibly less information-rich memory of the whole episode recalled from the CA3 network, to contribute to the firing of CA1 neurons. The CA1 neurons would then activate, via their termination in the deep layers of the entorhinal cortex, at least the pyramidal cells in the deep layers of the entorhinal cortex (see **Figure 1**). These entorhinal cortex layer 5 neurons would then, by virtue of their backprojections (Lavenex and Amaral, 2000; Witter et al., 2000a) to the parts of cerebral cortex that originally provided the inputs to the hippocampus, terminate in the superficial layers (including layer 1) of those neocortical areas, where synapses would be made onto the distal parts of the dendrites of the (superficial and deep) cortical pyramidal cells (Rolls, 1989a, 1989b, 1989d). The areas of cerebral neocortex in which this recall would be produced could include multimodal cortical areas (e.g., the cortex in the superior temporal sulcus, which receives inputs from temporal, parietal, and occipital cortical areas, and from which it is thought that cortical areas such as 39 and 40, related to language, developed), and also areas of unimodal association cortex (e.g., inferior temporal visual cortex). The backprojections, by recalling previous episodic events, could provide information useful to the neocortex in the building of new representations in the multimodal and unimodal association cortical areas, which by building new long-term representations can be considered as a form of memory consolidation (Rolls, 1989a,b,d, 1990a,b), or in organizing actions.

The hypothesis of the architecture with which this would be achieved is shown in **Figure 1**. The feedforward connections from association areas of the

cerebral neocortex (solid lines in Figure 1) show major convergence as information is passed to CA3, with the CA3 autoassociation network having the smallest number of neurons at any stage of the processing. The backprojections allow for divergence back to neocortical areas. The way in which I suggest that the backprojection synapses are set up to have the appropriate strengths for recall is as follows (Rolls, 1989a,b,d). During the setting up of a new episodic memory, there would be strong feedforward activity progressing toward the hippocampus. During the episode, the CA3 synapses would be modified, and via the CA1 neurons and the subiculum, a pattern of activity would be produced on the backprojecting synapses to the entorhinal cortex. Here the backprojecting synapses from active backprojection axons onto pyramidal cells being activated by the forward inputs to entorhinal cortex would be associatively modified. A similar process would be implemented at preceding stages of neocortex, that is, in the parahippocampal gyrus/perirhinal cortex stage, and in association cortical areas, as shown in Figure 1.

The concept is that during the learning of an episodic memory, cortical pyramidal cells in at least one of the stages would be driven by forward inputs but would simultaneously be receiving backprojected activity (indirectly) from the hippocampus, which would, by pattern association from the backprojecting synapses to the cortical pyramidal cells, become associated with whichever cortical cells were being made to fire by the forward inputs. Then, later on, during recall, a recall cue from perhaps another part of cortex might reach CA3, where the firing during the original episode would be completed. The resulting backprojecting activity would then, as a result of the pattern association learned previously, bring back the firing in any cortical area that was present during the original episode. Thus, retrieval involves reinstating the activity that was present in different cortical areas during the learning of an episode. (The pattern association is also called heteroassociation, to contrast it with autoassociation. The pattern association operates at multiple stages in the backprojection pathway, as made evident in Figure 1.) If the recall cue was an object, this might result in recall of the neocortical firing that represented the place in which that object had been seen previously. As noted elsewhere in this chapter and by McClelland et al. (1995), that recall might be useful to the neocortex to help it build new semantic memories, which might inherently be a slow process and is not part of the theory of recall.

1.33.2.2.6 Backprojections to the neocortex – quantitative aspects

A plausible requirement for a successful hippocampus-directed recall operation is that the signal generated from the hippocampally retrieved pattern of activity, and carried backward toward neocortex, remains undegraded when compared to the noise due, at each stage, to the interference effects caused by the concurrent storage of other patterns of activity on the same backprojecting synaptic systems. That requirement is equivalent to that used in deriving the storage capacity of such a series of heteroassociative memories, and it was shown in Treves and Rolls (1991) that the maximum number of independently generated activity patterns that can be retrieved is given, essentially, by the same formula as eqn (3) above where, however, a is now the sparseness of the representation at any given stage and C is the average number of (back)projections each cell of that stage receives from cells of the previous one. (k′ is a similar, slowly varying factor to that introduced above.) If p is equal to the number of memories held in the hippocampal memory, it is limited by the retrieval capacity of the CA3 network, $p_{\max}$. Putting together the formula for the latter with that shown here, one concludes that, roughly, the requirement implies that the number of afferents of (indirect) hippocampal origin to a given neocortical stage (C^{HBP}), must be $C^{\mathrm{HBP}} = C^{\mathrm{RC}} a_{\mathrm{nc}}/a_{\mathrm{CA3}}$, where C^{RC} is the number of recurrent collaterals to any given cell in CA3, the average sparseness of a representation is a_{nc}, and a_{CA3} is the sparseness of memory representations there in CA3.

This requirement is very strong: Even if representations were to remain as sparse as they are in CA3, which is unlikely, to avoid degrading the signal, C^{HBP} should be as large as C^{RC}, that is, 12,000 in the rat. If then C^{HBP} has to be of the same order as C^{RC}, one is led to a very definite conclusion: A mechanism of the type envisaged here could not possibly rely on a set of monosynaptic CA3-to-neocortex backprojections. This would imply that, to make a sufficient number of synapses on each of the vast number of neocortical cells, each cell in CA3 has to generate a disproportionate number of synapses (i.e., C^{HBP} times the ratio between the number of neocortical and the number of CA3 cells). The required divergence can be kept within reasonable limits only by assuming that the backprojecting system is polysynaptic, provided that the number of cells involved grows gradually at each

stage, from CA3 back to neocortical association areas (Treves and Rolls, 1994) (cf. **Figure 1**).

The theory of recall by the backprojections thus provides a quantitative account of why the cerebral cortex has as many backprojection as forward projection connections. Further aspects of the operation of the backprojecting systems are described elsewhere (Rolls, 2008).

1.33.3 Comparison with Other Theories of Hippocampal Function

The overall theory described here is close in different respects to those of a number of other investigators (Marr, 1971; Brown and Zador, 1990; McNaughton and Nadel, 1990; Eichenbaum et al., 1992; Gaffan, 1992; Squire, 1992; Moscovitch et al., 2005), and of course priority is not claimed on all the propositions put forward here.

Some theories postulate that the hippocampus performs spatial computation. The theory of O'Keefe and Nadel (1978), that the hippocampus implements a cognitive map, placed great emphasis on spatial function. It supposed that the hippocampus at least holds information about allocentric space in a form that enables rats to find their way in an environment even when novel trajectories are necessary, that is, it permits an animal to "go from one place to another independent of particular inputs (cues) or outputs (responses), and to link together conceptually parts of the environment which have never been experienced at the same time" (O'Keefe and Nadel, 1978). O'Keefe (1990) extended this analysis and produced a computational theory of the hippocampus as a cognitive map, in which the hippocampus performs geometric spatial computations. Key aspects of the theory are that the hippocampus stores the centroid and slope of the distribution of landmarks in an environment and stores the relationships between the centroid and the individual landmarks. The hippocampus then receives as inputs information about where the rat currently is and where the rat's target location is and computes geometrically the body turns and movements necessary to reach the target location. In this sense, the hippocampus is taken to be a spatial computer, which produces an output that is very different from its inputs. This is in contrast to the present theory, in which the hippocampus is a memory device that is able to recall what was stored in it, using as input a partial cue. A prototypical example in Rolls' theory is the learning of object–place association memory and the recall of the whole memory from a part, which can be used as a model of event or episodic memory. O'Keefe's theory postulates that the hippocampus actually performs a spatial computation. A later theory (Burgess et al., 1994, 2000) also makes the same postulate, but now the firing of place cells is determined by the distance and approximate bearing to landmarks, and the navigation is performed by increasing the strength of connections from place cells to goal cells and then performing a gradient-ascent style search for the goal using the network.

McNaughton et al. (1991) have also proposed that the hippocampus is involved in spatial computation. They propose a compass solution to the problem of spatial navigation along novel trajectories in known environments, postulating that distances and bearings (i.e., vector quantities) from landmarks are stored, and that computation of a new trajectory involves vector subtraction by the hippocampus. They postulate that a linear associative mapping is performed, using as inputs a cross-feature (combination) representation of (head) angular velocity and (its time integral) head direction, to produce as output the future value of the integral (head direction) after some specified time interval. The system can be reset by learned associations between local views of the environment and head direction, so that when later a local view is seen, it can lead to an output from the network that is a (corrected) head direction. They suggest that some of the key signals in the computational system can be identified with the firing of hippocampal cells (e.g., local view cells) and subicular cells (head direction cells). It should be noted that this theory requires a (linear) associative mapping with an output (head direction) different in form from the inputs (head angular velocity over a time period, or local view). This is pattern association (with the conditioned stimulus local view and the unconditioned stimulus head direction), not autoassociation, and it has been postulated that this pattern association can be performed by the hippocampus (cf. McNaughton and Morris, 1987). This theory is again in contrast to the present theory, in which the hippocampus operates as a memory to store events that occur at the same time and can recall the whole memory from any part of what was stored. (A pattern associator uses a conditioned stimulus to map an input to a pattern of firing in an output set of neurons, which is like that produced in the output neurons by the unconditioned stimulus. A description of pattern associations and autoassociators in a neurobiological context is provided by Rolls (1996a, 2007) and Rolls

and Treves (1998). The present theory is fully consistent with the presence of spatial view cells and whole-body motion cells in the primate hippocampus (Rolls, 1999; Rolls and O'Mara, 1993; Rolls and Xiang, 2006) (or place or local view cells in the rat hippocampus, and head direction cells in the presubiculum), for it is often important to store and later recall where one has been (views of the environment, body turns made, etc.), and indeed such (episodic) memories are required for navigation by dead reckoning in small environments.

The present theory thus holds that the hippocampus is used for the formation of episodic memories using autoassociation. This function is often necessary for successful spatial computation but is not itself spatial computation. Instead, I believe that spatial computation is more likely to be performed in the neocortex (utilizing information if necessary recalled from the hippocampus). Consistent with this view, hippocampal damage impairs the ability to learn new environments but not to perform spatial computations such as finding one's way to a place in a familiar environment, whereas damage to the parietal cortex and parahippocampal cortex can lead to problems such as topographical and other spatial agnosias in humans (see Gruesser and Landis, 1991; Kolb and Whishaw, 2003). This is consistent with spatial computations normally being performed in the neocortex. In monkeys, there is evidence for a role of the parietal cortex in allocentric spatial computation. For example, monkeys with parietal cortex lesions are impaired at performing a landmark task in which the object to be chosen is signified by the proximity to it of a landmark (another object; Ungerleider and Mishkin, 1982).

A theory closely related to the present theory of how the hippocampus operates has been developed by McClelland et al. (1995). It is very similar to the theory we have developed (Rolls, 1987, 1989a,b,d; Treves and Rolls, 1992, 1994; Rolls, 2007) at the systems level, except that it takes a stronger position on the gradient of retrograde amnesia, emphasizes that recall from the hippocampus of episodic information is used to help build semantic representations in the neocortex, and holds that the last set of synapses that are modified rapidly during the learning of each episode are those between the CA3 and the CA1 pyramidal cells, as described above (see **Figure 1**). It also emphasizes the important point that the hippocampal and neocortical memory systems may be quite different, with the hippocampus specialized for the rapid learning of single events or episodes and the neocortex for the slower learning of semantic representations, which may necessarily benefit from the many exemplars needed to shape the semantic representation.

Lisman and colleagues (2005) have considered how the memory of sequences could be implemented in the hippocampus. This theory of sequential recall within the hippocampus is inextricably linked to the internal timing within the hippocampus imposed, he believes, by the theta and gamma oscillations, and this makes it difficult to recall each item in the sequence as it is needed. It is not specified how one would read out the sequence information, given that the items are only 12 ms apart. The Jensen and Lisman (1996) model requires short, time constant NMDA channels and is therefore unlikely to be implemented in the hippocampus. Hasselmo and Eichenbaum (2005) have taken up some of these sequence ideas and incorporated them into their model, which has its origins in the Rolls and Treves model (Rolls, 1989b; Treves and Rolls, 1992, 1994), but proposes, for example, that sequences are stored in entorhinal cortex layer III. The proposal that acetylcholine could be important during encoding by facilitating CA3–CA3 LTP, and should be lower during retrieval (Hasselmo et al., 1995), is an important concept.

Another type of sequence memory uses synaptic adaptation to effectively encode the order of the items in a sequence (Deco and Rolls, 2005). This could be implemented in recurrent networks such as the CA3 or the prefrontal cortex.

In this chapter, we have seen that quantitative approaches to the functions of the hippocampus in memory are being developed by a number of investigators and that these theories are consistent with the quantitative circuitry of the hippocampus as well as with neuronal recordings and the effects of lesions. Moreover, we have seen that the predictions of these theories are now being tested.

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1.34 Neural Computation Theories of Learning

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1.34.1 Introduction

The anatomical discoveries in the nineteenth century and the physiological studies in the twentieth century showed that brains were networks of neurons connected through synapses. This led to the theory that learning could be the consequence of changes in the strengths of the synapses.

The best-known theory of learning based on synaptic plasticity is that proposed by Donald Hebb, who postulated that connection strengths between neurons are modified based on neural activities in the presynaptic and postsynaptic cells:

> When an axon of cell A is near enough to excite cell B and repeatedly or persistently takes part in firing it, some growth process or metabolic change takes place in one or both cells such that A's efficiency, as one of the cells firing B, is increased. (Hebb, 1949)

This postulate was experimentally confirmed in the hippocampus with high-frequency stimulation of a presynaptic neuron that caused long-term potentiation (LTP) in the synapses connecting it to the postsynaptic neuron (Bliss and Lomo, 1973). LTP takes place only if the postsynaptic cell is also active and sufficiently depolarized (Kelso et al., 1986). This is due to the *N*-methyl-D-aspartate (NMDA) type of glutamate receptor, which opens when glutamate is bound to the receptor, and the postsynaptic cell is sufficiently depolarized at the same time (*See* Chapters 1.33, 1.35).

Hebb's postulate has served as the starting point for studying the learning capabilities of artificial neural networks (ANN) and for the theoretical analysis and computational modeling of biological neural systems. The architecture of an ANN determines its behavior and learning capabilities. The architecture of a network is defined by the connections among the artificial neural units and the function that each unit performs on its inputs (*See* Chapter 1.35). Two general classes are feedforward and recurrent architecture.

The simplest feedforward network has one layer of input units and one layer of output units (**Figure 1**, left). All connections are unidirectional and project from the input units to the output units. The perceptron is an example of a simple feedforward network (Rosenblatt, 1958). It can learn to classify patterns from examples. It turned out that the perceptron can only classify patterns that are linearly separable – that is, if the positive patterns can be separated from all negative patterns by a plane in the space of input patterns. More powerful multilayer feedforward networks can discriminate patterns that are not linearly separable. In a multilayer feedforward network, the 'hidden' layers of units between the input and output layers allow more flexibility in learning features. Multilayer feedforward networks have also been applied to solve some other difficult problems (Rumelhart and McClelland, 1986).

In contrast to strictly feedforward network models, recurrent networks also have feedback connections

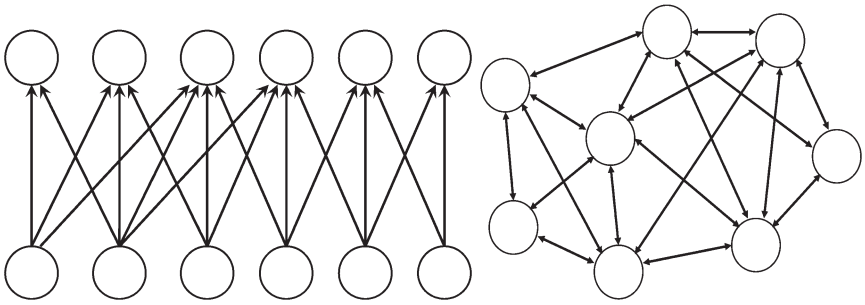

Figure 1 Network architectures. Left: Feedforward network. Right: Recurrent network. Open circles represent neuronal units, and arrowhead lines represent synaptic connections.

among units in the network (**Figure 1**, right). A simple recurrent network can have a uniform architecture such as all-to-all connectivity combined with symmetric weights between units, as in a Hopfield network (Hopfield, 1982), or it can be a network with specific connections designed to model a particular biological system.

Modeling learning processes in networks implies that the strengths of connections and other parameters are adjusted according to a learning rule (*See* Chapter 1.33). Other parameters that may change include the threshold of the unit, time constants, and other dynamical variables. A learning rule is a dynamical equation that governs changes in the parameters of the network. There are three main categories of learning rules: unsupervised, supervised, and reinforcement. Unsupervised learning rules are those that require no feedback from a teaching signal. Supervised learning rules require a teacher, who provides detailed information on the desired values of the output units of the network, and connections are adjusted based on discrepancies between the actual output and the desired one. Reinforcement learning is also error correcting but involves a single scalar signal about the overall performance of the network. Thus, reinforcement learning requires less-detailed information than supervised learning.

A learning algorithm specifies how and under what conditions a learning rule or a combination of learning rules should be applied to adjust the network parameters. For a simple task, it is possible to invent an algorithm that includes only one type of learning rule, but for more complex problems, an algorithm may involve a combination of several different learning rules.

In the following sections, we give an overview of basic learning rules and examples of learning algorithms used in neural network models, and describe specific problems solved by neural networks with adjustable parameters.

1.34.2 Hebbian Learning

Implementations of Hebb's rule can take different forms (Sejnowski and Tesauro, 1988). Simple associative Hebbian learning is based on the coincidence of activities in presynaptic and postsynaptic neurons. The dynamics of Hebbian learning are governed by a differential equation:

$$\frac{\mathrm{d}w_{ij}}{\mathrm{d}t} = \alpha \cdot v_i \cdot u_j$$

where w_{ij} is the weight of a connection from an input unit j with activity u_j to an output unit i with activity v_i, and α is a learning rate.

The Hebbian learning rule has been used to model a wide variety of problems, including feature selectivity and cortical map development.

Cortical neurons respond selectively to particular feature stimuli, such as selectivity for ocular dominance and orientation in the visual cortex. To understand challenges of modeling the development of feature selectivity, consider a network with many input units and one output unit. We would like to explore under what conditions the output unit will respond well to few input units and less to the others. If we apply a stimulus to the input units and allow the connections to develop according to the Hebbian learning rule, then all connections will grow and eventually saturate, and no selectivity will emerge. To develop selectivity, some dependencies among weights are needed, so that changes at one connection will influence the others. There are many different ways to introduce dependencies. One

approach is to introduce weight normalization (Miller and Mackay, 1994). A different approach, based on competition among input patterns, called the BCM (Bienenstock, Cooper, and Munro) rule (Bienenstock et al., 1982), has been used to model the development of orientation selectivity and ocular dominance in neural networks.

Neuronal response selectivity varies across the cortex in regular patterns called cortical maps. Although some aspects of cortical map formation during development are activity independent, neuronal activity can modify the maps. Hebbian learning rules have also been applied to model the effects of cortical activity on map formations. For comprehensive overviews of neural network models that develop orientation selectivity maps and ocular dominance columns, see Swindale (1996) and Ferster and Miller (2000).

Models of cortical map formation can become extremely complex when multiple features, such as retinotopic location, ocular dominance, orientation preference, and others, are considered simultaneously. To deal with such problems, a more abstract class of models was developed by Kohonen (1982). The Kohonen algorithm is usually applied to two-layer networks with feedforward connections from an input layer to an output layer. The input layer is an N-dimensional vector layer. The output layer is normally a one- or two-dimensional array. There are no lateral connections in the output layer, but the algorithm can accomplish what models with lateral connections can achieve at less computational cost. The algorithm does this by a weight updating procedure that involves neighboring units. At every step, it chooses a 'winner' among output units whose weights are closest to the input pattern. Then it updates the weights of the winner and the nearby neighbors of the winner. The number of neighbors that participate in weight updating is controlled through a neighborhood function, which is dynamically changed during learning to ensure convergence. The neighborhood function starts out long range and is reduced as learning proceeds. This allows the network to organize a map rapidly and then refine it more slowly with subsequent learning.

Models based on the Kohonen algorithm perform dimensionality reduction, which facilitates data analysis, taking input vectors from a high-dimensional feature space and projecting them onto a low-dimensional representation.

1.34.3 Unsupervised Hebbian Learning

If the goal of learning is to discover the statistical structure in unlabeled input data, then the learning is said to be unsupervised. A common method for unsupervised learning is principal component analysis (PCA). Suppose the data are a set of N-dimensional input vectors. The task is to find an $M < N$ dimensional representation of N-dimensional input vectors that contains as much information as possible of the input data. This is an example of dimensionality reduction, which can significantly simplify subsequent data analysis.

A simple network that can extract the first principal component (the one with the maximal variance) is a network with N input units and one output unit. At each time step an N-dimensional input vector is applied to the input layer. If we allow the connections to be modified according to the Hebbian learning rule, then in the case of zero mean value of the input vector, the weights will form an N-dimensional vector, along which the variance will be the largest. This is the principal eigenvector or component. A network with N input and M output units, augmented with a generalized Hebbian learning rule, can learn first M components. The projections of the input data onto the components give us M-dimensional representation of the N-dimensional input data.

PCA is appropriate when the data obey Gaussian statistics, but images, audio recordings, and many types of scientific data often do not have Gaussian distributions. As an example of such a problem, consider a room where a number of people are talking simultaneously (cocktail party), and the task is to focus on one of the speakers. The human brain can, to some extent, solve this auditory source separation problem by using knowledge of the speaker, but this becomes a more difficult problem when the signals are arbitrary. The goal of blind source separation (BSS) is to recover source signals given only sensor signals that are linear mixtures of the independent source signals. Independent component analysis (ICA) is a method that solves the BSS problem for non-Gaussian signals. In contrast to correlation-based algorithms such as PCA and factor analysis, ICA finds a nonorthogonal linear coordinate system such that the resulting signals are as statistically independent from each other as possible.

One approach to BSS derives unsupervised learning rules based on information theory. The input is

assumed to be N mixtures of N independent sources, and the goal is to maximize the mutual information between the inputs and the outputs of a two-layer neural network. The resulting stochastic gradient learning rules are highly effective in the blind separation and deconvolution of hundreds of non-Gaussian sources (Bell and Sejnowski, 1995).

ICA is particularly effective at analyzing electroencephalograms (EEG) and functional magnetic resonance imaging (fMRI) data (Jung et al., 2001). Consider, for example, electrical recordings of brain activity at many different locations on the scalp. These EEG potentials are generated by underlying components of brain activity and various muscle and eye movements. This is similar to the cocktail-party problem: We would like to recover the original components of the brain activity, but we can only observe mixtures of the components. ICA can reveal interesting information of the brain activity by giving access to its independent components. ICA also gives useful insights into task-related human brain activity from fMRI recordings when the underlying temporal structure of the sources is unknown.

Another application of ICA is feature extraction (Lee, 1998). A fundamental problem in signal processing is to find suitable representations for images, audio recordings, and other kinds of data. Standard linear transformations used in image and auditory processing, such the Fourier transforms and cosine transforms, may not be optimal, and but it would be useful to find the most efficient linear transformation, based on the statistics of the data, to optimally compress the data.

1.34.4 Supervised Learning

Consider the problem of learning to retrieve an output pattern given an input pattern. To remember the patterns, the Hebbian rule can be applied to adjust weights between input and output units. As mentioned earlier, however, the associative Hebbian learning rule will lead to saturation with multiple repetitions, which reduces the capacity of the network. To resolve this problem, one can augment the Hebbian rule with a weight normalization algorithm as in the case of unsupervised learning algorithms.

Another disadvantage of using the associative Hebbian learning rule is that weight adjustments do not depend on the actual performance of the network. An effective way to adjust weights would be by using information of the actual performance of the network. Supervised learning can do this. Supervised learning requires a teacher, who provides detailed information of the desired outputs of the network and adjusts the connections based on discrepancies between the actual outputs and the desired ones.

The perceptron uses a supervised learning rule to learn to classify input patterns (Rosenblatt, 1958). The perceptron is a two-layer network with one output unit that can classify input patterns into two categories. The Hebbian learning rule can be used to solve the task, but the perceptron with the Hebbian learning rule works well only if the number of input patterns is significantly less than the number of input units. An error-correcting supervised learning algorithm for weight adjustments is more effective for a large number of input patterns:

$$\frac{\mathrm{d}w_{ij}}{\mathrm{d}t} \propto u_j \cdot (R_i - v_i)$$

where w_{ij} is a weight of a connection from the input unit j with activity u_j to an output unit i with activity v_i, R_i is a target value of the output unit, and

$$v_i = \sum_j w_{ij} \cdot u_j$$

The perceptron learning rule uses the performance of the network to decide how much adjustment is needed and in which direction the weights should be changed to decrease the discrepancy between the actual network outputs and the desired ones. If input patterns are linearly separable, then the perceptron learning rule guarantees to find a set of weights that allow pattern classification.

A simple unsupervised Hebbian learning rule adjusts synaptic weights based on correlations between presynaptic and postsynaptic neurons. However, this approach is inefficient when the goal of the network is to perform a specific function, rather than simply represent data. To perform a specific task, the network should receive some information about the task.

An example of how Hebbian plasticity can be incorporated into a supervised learning framework is a two-layer network that was trained to perform a function approximation task (Swinehart and Abbott, 2005). The feedforward connections from input units to output units were modified according to an unsupervised Hebbian rule, and a supervised learning mechanism was used to adjust connections from a supervisor to the network. The supervisor is a network that assesses the performance of the training

network and, based on that information, modifies the gains of the input units using an error-correcting learning rule. The purpose of the supervised modulation was to enhance connections between the input and the output units to facilitate the synaptic plasticity needed to learn the task. Thus, Hebbian plasticity did not have direct access to the supervision, and the supervised modulations did not produce any permanent changes. Nonetheless, this network could learn to approximate different functions. In the initial phase the improvement in the network performance was mostly due to the gain modulation, and the synaptic adjustments were minimal. But later, the synaptic adjustments and the gain modulation were equally involved in shaping the performance. Once the network learned the task with the supervisor, it was possible to turn off the supervision, relying only on further Hebbian plasticity to refine the approximation.

The role of the supervisor in the model was to compute an error by comparing the actual and the desired output of the network and to use this error to direct the modification of network parameters such that the network performance improves. Conventionally, the major targets of this process were the synaptic weights. The novel feature of this supervised learning scheme was that supervision took place at the level of neuronal responsiveness rather than synaptic plasticity.

A simple two-layer perceptron cannot solve higher-order problems, but adding additional layers to the feedforward network provides more representational power. Then new learning algorithms are needed to train multilayer networks. The simple error-correcting learning rule was effective for training two-layer networks. With the rule, the connections from the input layer to the output one are adjusted based on discrepancies between the desired output and the actual output produced by the network. In a multilayer network, however, there are intermediate 'hidden' layers that also need to be trained. The back-propagation learning algorithm was developed to train multilayer networks (Rumelhart and McClelland, 1986). The learning rule relies on passing an error from the output layer back to the input layer. Multilayer networks trained with the back-propagation learning rule have been effective in solving many difficult problems.

An example of a multilayer network that was trained using a back-propagation algorithm is a model of song learning in songbirds (Fiete et al., 2004). Juvenile male songbirds learn their songs from adult male tutors of the same species. Birdsong is a learned complex motor behavior driven by a discrete set of premotor brain nuclei with well-studied anatomy (*See* Chapter 1.17). Syringeal and respiratory motor neurons responsible for song production are driven by precisely executed sequences of neural activity in the premotor nucleus robustus archistriatalis (RA) of songbirds (**Figure 2**). Activity in RA is driven by excitatory feedforward inputs from the forebrain nucleus high vocal center (HVC), whose RA-projecting neural population displays temporally sparse, precise, and stereotyped sequential activity. Individual RA-projecting HVC neurons burst just once in an entire song motif and fire almost no spikes elsewhere in the motif. The temporal sparseness of HVC activity implies that these HVC–RA synapses are used in a special way during song; that is, each synapse is used only once during the motif. The goal of the work was to study the effect of HVC sparseness on the learning speed of the network. They studied multilayer feedforward network with an HVC layer that provides input to a

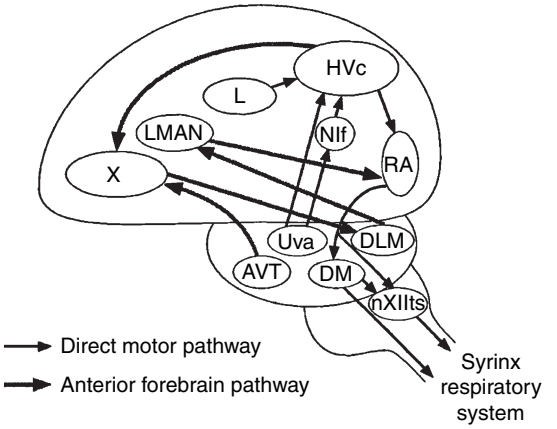

Figure 2 Schematic diagram of the major songbird brain nuclei involved in song control (*See also* Chapter 1.17). The thinner arrows show the direct motor pathway, and the thicker arrows show the anterior forebrain pathway. Abbreviations: Uva, nucleus uvaeformis of the thalamus; NIf, nucleus interface of neostriatum; L, field L (primary auditory area of the forebrain); HVc, higher vocal center; RA, robust nucleus of the archistriatum; DM, dorsomedial part of the nucleus intercollicularis; nXIIts, tracheosyringeal part of the hypoglossal nucleus; AVT, ventral area of Tsai of the midbrain; X, area X of lobus parolfactorius; DLM, medial part of the dorsolateral nucleus of the thalamus; LMAN, lateral magnocellular nucleus of the anterior neostriatum. From Doya K and Sejnowski TJ (2000) A computational model of avian song learning. In: Gazzaninga MS (ed.) *The New Cognitive Neurosciences*, 2nd edn., p. 469. Cambridge, MA: MIT Press; used with permission.

'hidden' RA layer and RA projecting to an output layer of motor units. Song learning is thought to involve plasticity of synapses from HVC to RA because these synapses display extensive synaptic growth and redistribution during the critical period. So in the model, the weights from HVC layer to RA layer were modified. Because there is no evidence of plasticity in the synapses from RA to motor neurons, those connections in the model were kept fixed. For learning, the connections from HVC to RA were adjusted to minimize discrepancy between the desired outputs and the actual outputs produced by the network. They used the back-propagation gradient descent rule and varied the number of bursts in HVC neurons per motif. The network learned the motif for any number of bursts in HVC neurons, but the learning time for two bursts per motif nearly doubled compared to the one burst case and increased rapidly with the number of bursts. Based on these simulations, they concluded that the observed sparse coding in HVC minimized interference and the time needed for learning. It is important to note here that the back-propagation learning algorithm was not used to model the biological learning process itself, but rather to determine if the network architecture can solve the problem and what constraints the representation may have on the speed of learning.

1.34.5 Reinforcement Learning

Learning about stimuli or actions based solely on rewards and punishments is called reinforcement learning. Reinforcement learning is minimally supervised because animals are not told explicitly what actions to take in a particular situation. The reinforcement learning paradigm has attracted considerable interest because of the notion that the learner is able to learn from its own experience at attempting to perform a task without the aid of an intelligent 'teacher.' In contrast, in the more commonly employed paradigm of supervised learning, a detailed 'teacher signal' is required that explicitly tells the learner what the correct output pattern is for every input pattern.

A computational model of birdsong learning based on reinforcement learning has been proposed (Doya and Sejnowski, 2000). A young male songbird learns to sing by imitating the song of a tutor, which is usually the father or other adult males in the colony. If a young bird does not hear a tutor song during a critical period, it will sing short, poorly structured songs. If a bird is deafened during the period when it practices vocalization, it develops highly abnormal songs. Thus, there are two phases in song learning – the sensory learning phase, when a young bird memorizes song templates, and the sensorimotor learning phase, in which the bird establishes the motor programs using auditory feedback. These two phases can be separated by several months in some species, implying that birds have remarkable capability for memorizing complex temporal sequences. Once a song is crystallized, its pattern is very stable. Even deafening the bird has little immediate effect.

The anterior forebrain pathway, which is not involved in song production, is necessary for song learning. In the previously discussed model (Fiete et al., 2004), it was assumed that HVC is a locus of pattern memorization during the first phase of learning, song acquisition, and RA is a motor command area (*See* Chapter 1.17). Therefore, the patterns stored in HVC serve as inputs to RA to produce motor commands. It was also assumed that evaluation of the similarity of the produced song to the memorized tutor song takes place in area X in the anterior forebrain. This assumption is supported by a finding that area X receives dopaminergic input. Depending on how closely the produced song is to the tutor's song, the connections from HVC to RA are modulated via the lateral magnocellular nucleus (LMAN).

The learning algorithm consisted of making small random changes in the HVC to RA synapses and keeping the new weights only if overall performance was improved. The network learned artificial song motifs and was even able to replicate realistic birdsongs within the number of trials that birds take to learn their songs.

Reinforcement learning has thus far had few practical successes in solving large-scale complex real-world problems. In the case of reinforcement learning with delay, the temporal credit assignment aspect of the problem has made learning very slow. However, a method called temporal difference (TD) learning has overcome some of these limitations (Sutton and Barto, 1998). The basic idea of TD learning is to compute the difference between temporally successive predictions. In other words, the goal of learning is to make the learner's current prediction for the current input pattern more closely match the prediction at the next time step. One of the most effective of these TD methods is an algorithm called TD(λ), in which there is an exponentially decaying feedback of the error in time, so that previous estimates for

previous states are also corrected. The time scale of the exponential decay is governed by the λ parameter.

Perhaps the most successful application of TD(λ) is TD-Gammon, which was designed for networks to learn to play backgammon (Tesauro, 1995). Backgammon is an ancient two-player game that is played on an effectively one-dimensional track. The players take turns rolling dice and moving their checkers in opposite directions along the track as allowed by the dice roll. The first player to move all his checkers all the way forward and off his end of the board is the winner.

At the heart of TD-Gammon is a neural network with a standard multilayer architecture. Its output is computed by a feedforward flow of activation from the input nodes, representing the game position, to the output node, which evaluates the strength of the position. Each of the connections in the network is parameterized by a real valued weight. Each of the nodes in the network outputs a real number equal to a weighted linear sum of inputs feeding into it, followed by a nonlinear sigmoid operation. At each time step, the TD(λ) algorithm is applied to the output, which is then back-propagated to change the network's weights.

During training, the neural network selects moves for both sides. At each time step during the course of a game, the neural network scores every possible legal move. The move that is then selected is the move with maximum expected outcome for the side making the move. In other words, the neural network learns by playing against itself. At the start of self-play, the network's weights are random, and hence its initial strategy is random. But after a few hundred thousand games, TD-Gammon played significantly better than any previous backgammon program, equivalent to an advanced level of play. In particular, it is not dependent on a human teacher, which would limit the level of play it can achieve (Tesauro and Sejnowski, 1989). After one million games, TD-Gammon was playing at a championship level.

One of the essential features of reinforcement learning is a trade-off between exploration and exploitation. The learning system should exploit a successful strategy to reach the goal of the task it learns, but it should also explore other strategies to find out if there is a better one. In models, exploration has been implemented by stochasticity. The source of such stochasticity in the brain remains unclear. A model implementing this trade-off between exploration and exploitation has been proposed (Seung, 2003). The model is based on the probabilistic nature of synaptic release by a presynaptic terminal when an action potential arrives at the terminal. The model combines this local synaptic release-failure event and a global reward signal received outside based on the output of the model. The main assumption is that synapses are hedonistic: they increase their probabilities of release or failure depending on which action immediately preceded reward. This concept of the hedonistic synapse is potentially relevant to any brain area in which a global reinforcement signal is received (Klopf, 1982).

This version of reinforcement learning was used to address the matching law phenomenon (Seung, 2003). When animals are presented with repeated choices between competing alternatives, they distribute their choices so that returns from two alternatives are approximately the same. A return is the total reward obtained from an alternative divided by the number of times it was chosen. Before trials, the alternatives are baited with unequal probabilities. The network had to learn a probabilistic strategy in which one alternative is favored over the other one. The network started from equal choices for both alternatives, but over time, it learned a preference that satisfied the matching law.

In the present model, stochastic vesicle release was assumed to be a source of stochasticity in the brain. However, there might be many other possible sources of noise, such as fluctuations in quantal size, irregular action potential firing, and on a slower time scale, the stochastic creation and destruction of synapses. Thus, identifying specific sources of randomness is essential for connecting mathematical models and neurobiology.

1.34.6 Spike-Timing Dependent Plasticity

The traditional coincidence version of the Hebbian learning rule implies simply that the correlation of activities of presynaptic and postsynaptic neurons drives learning. This approach has been implemented in many types of neural network models using average firing rate or average membrane potentials of neurons (*See* Chapter 1.35). Although Hebb's formulation implicitly recognized the idea of causality and relative spike timing (Hebb, 1949; Sejnowski, 1999), this was not appreciated by a generation of modelers because rate coding was generally accepted as the primary form of information processing, and high-frequency

stimulation protocols were used to induce plasticity at synapses. More recently, the relative timing of spikes has been shown to be critical for the direction and magnitude of synaptic plasticity in the cortex as well as the hippocampus (Markram et al., 1997; Bi and Poo, 1998). Potentiation of a synapse takes place if the presynaptic spike precedes the postsynaptic spike, and depression occurs when presynaptic spike follows the postsynaptic spike. This spike-timing dependent plasticity (STDP) is an asymmetric function of relative spike times in the presynaptic and postsynaptic neurons. The time window for the plasticity can be as short as 10 ms and as long as 100 ms, depending on the synapse.

A natural application for STDP is temporal sequence learning (*See* Chapters 1.34, 1.35). If neurons are activated in a sequential manner then, due to the asymmetry of the learning rule, synapses from previously activated neurons to following active neurons will be strengthened. For example, such a spike-timing dependent learning algorithm has been used to train a network to link sequential hippocampal place cells while a rat navigates a maze (Blum and Abbott, 1996). The goal was to predict the direction of a future motion on the basis of a previous experience. Asymmetric synaptic weights develop in the model because of the temporal asymmetry of LTP induction and because place fields are activated sequentially during locomotion. This learning algorithm closely resembles the STDP learning rule. The only essential difference is time scale, which in the model was 200 ms, longer than the STDP windows found in cortical or hippocampal neurons.

This model of a navigational map was based on three observations. First, NMDA-dependent LTP in hippocampal slices occurs only if presynaptic activity precedes postsynaptic activity by less than approximately 200 ms. Presynaptic activity following postsynaptic firing produces either no LTP or long-term depression (LTD). Second, place cells are broadly tuned and make synaptic connections with each other both within the CA3 region and between CA3 and CA1. Third, a spatial location can be determined by appropriately averaging the activity of an ensemble of hippocampal place cells. These three observations imply that when an animal travels through its environment, causing different sets of place cells to fire, information about both temporal and spatial aspects of its motion will be reflected in changes of the strengths of synapses between place cells. Because this LTP affects a subsequent place cell firing, it can shift the spatial location coded by the place cell activity. These shifts suggest that an animal could navigate by heading from its present location toward the position coded by the place cell activity. To illustrate both how a spatial map arises and how it can be used to guide movement, these ideas were applied to navigation in the Morris maze. The network was trained using this spike-timing dependent learning algorithm to form a direction map, which improved with training.

Timing is important in auditory processing, and a number of perceptual tasks, such as sound localization, explicitly use temporal information. Sound localization is important to the survival of many species, in particular to those that hunt in the dark. Interaural time differences (ITD) are often used as a spatial cue. However, the question of how temporal information from both ears can be transmitted to a site of comparison, where neurons are tuned to ITDs, and how those ITD-tuned neurons can be organized in a map remains unclear. A network model based on STDP can successfully account for a fine precision of barn owl sound localization (Kempter et al., 2001). The model converts ITDs into a place code by combining axonal delay lines from both ears and STDP in synapses with distributed delays. The neurons are organized as a single-layer network for each frequency and receive inputs from both ears through axonal arbors. The axons have different time delays. After training, each neuron adjusts its connections to axons with the appropriate time delays in agreement with the neuron's spatial position. In this way, a map with neurons tuned to particular ITDs can be formed.

There is an interesting connection between STDP and TD learning at the computational level (Rao and Sejnowski, 2003). If, consistent with TD learning, synaptic weights between Hodgkin–Huxley type spiking neurons are updated based on the difference in the postsynaptic voltage at time $t + \Delta t$ and at time t, where t is the time when the presynaptic neuron fired a spike, and Δt is a fixed time interval, then the learning rule resembles the conventional STDP learning rule. Networks with this spike-dependent TD learning rule are able to learn and predict temporal sequences, as demonstrated by the development of direction selectivity in a recurrent cortical network. The network consisted of a single chain of recurrently connected excitatory neurons. Each neuron initially received symmetric excitatory and inhibitory inputs of the same magnitude. For training, the neurons in the network were exposed to 100 trials of retinotopic sensory inputs consisting of moving pulses of excitation in the rightward direction.

The effect of learning on the network was in developing a profound asymmetry in the pattern of excitatory connections from preceding and successor neurons. The synaptic conductances of excitatory connections from the left side were strengthened, whereas the ones from the right side were weakened. Because neurons on the left side fired (on average) a few milliseconds before a considered neuron, whereas neurons on the right side fired (on average) a few milliseconds after, as a result, the synaptic strengths of connections from the left side were increased, whereas the synaptic strengths for connections from the right side were decreased. As expected from the learned pattern of connections, the neuron responded vigorously to rightward motion but not to leftward motion.

To investigate the question of how selectivity for different directions of motion may emerge simultaneously, they also simulated a network comprising two parallel chains of neurons, with mutual inhibition between corresponding pairs of neurons along the two chains. As in the previous simulation, a given excitatory neuron received both excitation and inhibition from its predecessors and successors. To break the symmetry between the two chains, they provided a slight bias in the recurrent excitatory connections, so that neurons in one chain fired slightly earlier than neurons in the other chain for a given motion direction. To evaluate the consequences of spike-based TD learning in the two-chain network, the model neurons were exposed alternately to leftward- and rightward-moving stimuli for a total of 100 trials. As in the previous simulation, the excitatory and inhibitory connections to a neuron in one chain showed asymmetry after training, with stronger excitatory connections from the left neurons and stronger inhibitory connections from the right neurons. A corresponding neuron in the other chain exhibited the opposite pattern, and as expected from the learned patterns of connectivity, neurons in one chain were selective to rightward motion, and neurons in the other chain were selective to the leftward motion. This explanation was consistent with the development of directionally selective neurons in the visual cortex of kittens.

1.34.7 Plasticity of Intrinsic Excitability

Several lines of evidence argue for the presence of activity-dependent modification of intrinsic neuronal excitability during development and learning (Daoudal and Debanne, 2003; *See* Chapter 4.40). In the dentate gyrus of the hippocampus, for example, in addition to homosynaptic LTP of excitatory synaptic transmission, the probability of discharge of the postsynaptic neurons to a fixed excitatory synaptic input is enhanced by high-frequency stimulation (HFS, 100 Hz) of the afferent fibers (Bliss et al., 1973). This second component has been called excitatory postsynaptic potential (EPSP)-to-spike potentiation (E-S potentiation) (Frick et al., 2004). Synaptic plasticity (LTP) and nonsynaptic E-S potentiation are complementary. As in LTP, E-S potentiation requires the activation of NMDA receptor (NMDAR) for its induction. These two forms of plasticity may share common induction pathways. In a recent study of deep cerebellar nuclei neurons, tetanization of inputs to these neurons produces a rapid and long-lasting increase in intrinsic excitability that depends on NMDAR activation (Aizenman and Linden, 2000). These studies suggest that plasticity of intrinsic excitability may be important in developmental plasticity and information storage.

Another form of plasticity in intrinsic excitability has been demonstrated in spontaneously firing vestibular nucleus neurons, which may be responsible for learning of the vestibuloocular reflex. Purkinje cells, which are inhibitory, contact a subset of the neuron in the vestibular nucleus, which receive direct vestibular input and project to the oculomotor nuclei. Brief periods of synaptic inhibition or membrane hyperpolarization produced a dramatic increase in both spontaneous firing rate and responses to intracellularly injected current (Gittis and du Lac, 2006). A similar change occurred after silencing the vestibular nerve. Neurons in the vestibular system fire at remarkably high rates in the intact animal, with resting rates on the order of 50–100 spikes/s and responses to head movements ranging up to 300 spikes/s. Loss of peripheral vestibular function silences the vestibular nerve, resulting in a significant loss of spontaneous firing in the neurons of the vestibular nucleus, which then returns to control values within about a week, even in the absence of vestibular nerve recovery. This plasticity of intrinsic excitability could potentially contribute either to adaptive changes in vestibular function during recovery from peripheral damage or to oculomotor learning in intact animals.

A similar phenomenon has been demonstrated in cultured neocortical pyramidal neurons (Desai et al., 1999). Prolonged activity blockade lowers the threshold for spike generation, and neurons fire at a higher

frequency for any given level of current injection. These changes occurred through selective modifications in the magnitude of voltage-dependent currents: sodium currents increase and persistent potassium currents decrease, whereas calcium currents and transient potassium currents are unaltered. Increase of neuronal excitability in response to reduced activity may contribute to the activity-dependent stabilization of firing rates. The stability in neuronal firing rates is maintained through many mechanisms, and regulation of neuronal excitability may be one of them.

Information about the outside world is transformed into spike trains in the nervous system. How do the neurons learn to represent the information, and do they change their behavior based on changing external stimuli? In the discussion of unsupervised learning and the ICA algorithm, it was shown that information theoretical approaches can be effective in solving real-world problems. A similar information theoretical approach can be implemented to search for an optimal representation. A Hodgkin–Huxley type model of a neuron that can adjust its membrane conductances to maximize information transfer has been proposed (Stemmler and Koch, 1999). The slope of the neuronal gain function should line up with the peak of the input to maximize information transfer. The learning rules they implemented in the model performed this matchup by adjusting the membrane conductances. The conductance modulations did not require calculation of mutual information but were based solely on local characteristics of the neuron. They showed that for different input distributions the model could successfully line up the gain function and the input distributions leading to maximization of information transfer. Thus, the ability of activity-dependent selective modification of the gain functions based on the active balance of inward and outward ion channels could serve a number of important functions, including fine-tuning of the output properties of neurons to match the properties of their inputs.

Plasticity of intrinsic excitability can also participate in regulating the conventional synaptic plasticity. For details, see the previously discussed model, which combines Hebbian and supervised learning (Swinehart and Abbott, 2005), in the section titled 'Supervised learning.'

1.34.8 Homeostatic Plasticity

Correlation-based Hebbian plasticity is thought to be crucial for information storage because it produces associative changes in the strength of individual synaptic connections. However, correlation-based learning in neural networks can be unstable. According to the Hebb rule, if a presynaptic neuron participates in firing of a postsynaptic neuron, it leads to strengthening the synapses between the neurons. This makes it more likely that next time the presynaptic neuron fires, it will cause firing in the postsynaptic neuron, which leads to further strengthening of the synapse. Simple associative Hebbian algorithm causes instability in the network by increasing the total activity of the network and losing selectivity among synapses. To keep the network stable and maintain the selectivity of the network, an additional mechanism must stabilize the properties of neuronal networks.

Homeostatic plasticity is a mechanism by which the neurons regulate the network's activity (Turrigiano and Nelson, 2000). There are many different ways neural activities could be regulated to keep them within a functional dynamical range. One mechanism that could maintain relatively constant activity levels is to increase the strength of all excitatory connections into a neuron in response to a prolonged drop in firing rates, and vice versa. This form of homeostatic plasticity is called synaptic scaling.

Regulating synaptic strength is not the only mechanism by which homeostatic activity can be maintained. Previously discussed plasticity of intrinsic excitability also contributes to the homeostatic regulation by controlling the firing rates of the neurons.

All theoretical models implementing associative Hebbian learning rule have to deal with the instability problem. For example, the BCM learning rule deals with unconstrained growth of synaptic weights by dynamically adjusting the threshold between potentiation and depression (Bienenstock et al., 1982). This algorithm is biologically plausible and reflects experimental findings indicating that calcium level is crucial for the direction of plasticity. The dynamical threshold modulation implemented in the BCM rule not only prevents the synapses from unconstrained growth but also maintains the activity level of the units at the appropriate value (*See* Chapters 1.33, 1.35).

In the next section we present some other examples of learning algorithms involving homeostatic plasticity as a critical element of learning.

1.34.9 Complexity of Learning

The learning paradigms discussed earlier were based on a single mechanism for plasticity (e.g., STDP versus homeostatic and synaptic versus intrinsic

neuronal). However, many difficult tasks cannot be solved using a single learning rule, but require combinations of several learning rules working together. Another essential element of modeling learning processes is the time scale of learning. There are multiple time scales for plasticity, from milliseconds to years, and depending on the demands of the task, different mechanisms for plasticity with different time scales may be involved.

Long-term memory is vulnerable to degradation from passive decay of the memory trace and ongoing formation of new memories. Memory based on synapses with two states shows exponential decay, but experimental data shows that forgetting (memory degradation) follows a power law. A cascade model was developed to address this problem (Fusi et al., 2005). In the model, synapses had two states, weak and strong, but in addition to transition between these two states, there were metaplastic transitions within each state. Based on the stage of metaplasticity, the synapses showed the range of behavior from being highly plastic to being resistant to any plasticity at all. The metaplastic transitions effectively introduced multiple time scales into the model.

The cascade model outperformed alternative models and exhibited a power law for the decay of memory as a function of time. The dependence of memory lifetime on the number of synapses in the model is also a power law function. Memory lifetimes diminish when the balance between excitation and inhibition is disturbed, but the effect is much less severe in the cascade model than in noncascade models.

The function of homeostatic plasticity is to maintain the activity of the cortex at a functional level. But are there any other computational or functional advantages of such plasticity? One study has shown that a combination of Hebbian and homeostatic plasticity can lead to temporal sharpening in response to multiple applications of transient sensory stimuli (Moldakarimov et al., 2006). The model included two types of homeostatic mechanisms, fast and slow. Relatively fast plasticity was responsible for maintaining the average activity of the units. To maintain activity in the excitatory neurons at a target homeostatic level, they implemented a learning rule, according to which inhibitory connections have been adjusted. The slow plasticity was used to determine the value of the target average activities. Thus, the model had three time scales for synaptic adjustments: Hebbian, fast homeostatic, and slow homeostatic mechanisms. Repeated presentations of a transient signal taught the network to respond to the signal with a high amplitude and short duration, in agreement with experimental findings. This sharpening enhances the processing of transients and may also be relevant for speech perception.

A standard approach in models of self-organized map (SOM) formation is the application of Hebbian plasticity augmented with a mechanism of weight normalization. A conventional way to normalize weights is based on a sum of weights coming into each neuron: The soma collects information on every weight, sums them, and then decides on the amount of normalization. An alternative approach to weight normalization has been proposed (Sullivan and de Sa, 2006). The normalization algorithm did not need information from every synapse but rather was based on the average activities of the units and homeostatic plasticity. When Hebbian and homeostatic mechanisms were combined, the average activities of the units were better maintained compared to the standard Hebbian models.

Dimensionality reduction facilitates the classification, the visualization, and the storage of high-dimensional data. A simple and widely used method is PCA, which finds the directions of greatest variance in the data set and represents each data point by its coordinates along each of these directions. A new deep network model has been proposed to transform the high-dimensional data into a low-dimensional code (Hinton and Salakhutdinov, 2006). The adaptive multilayer network consisted of two subnetworks, an encoder and decoder. The encoder transformed high-dimensional data into a low-dimensional code. The code layer was then used as the input layer to the decoder network to reconstruct the original input pattern.

The two networks were trained together to minimize the discrepancy between the original data and its reconstruction. The required gradients were obtained using the chain rule to back-propagate error derivatives, first through the decoder network and then through the encoder network. In general, it is difficult to optimize the weights in a multilayer network with many hidden layers. Large initial weights typically lead to poor local minima; with small initial weights, the gradients in the early layers are tiny, making it impossible to train. But if the initial weights are close to a good solution, gradient descent back-propagation works well. A good initial network was obtained with unsupervised learning based on Restricted Boltzmann Machine (RBM) learning algorithm. First, the input layer of the

multilayer network was used as a visible layer of RBM, and the next layer served as a feature layer. After learning one layer of feature detectors, the weights were fixed and used for learning a second layer of feature detectors. This layer-by-layer learning was repeated many times. After pretraining multiple layers of feature detectors, the model was unfolded to produce the encoder and decoder networks that initially used the same weights. The global fine-tuning stage used back-propagation through the whole network to adjust the weights for optimal reconstruction.

They applied the algorithm to multiple tasks including handwritten digits visualization, grayscale images, and documents generalization. In all these tasks, the new algorithm outperformed different approaches based on PCA and other supervised algorithms.

1.34.10 Conclusions

We have discussed learning rules and learning algorithms designed for neural network models and described some problems that can be solved by neural networks with modifiable connections. Neural computation is a broad field that continues to grow; only a few selected studies have been used to illustrate general principles.

Although early modeling efforts focused mainly on traditional synaptic plasticity, such as LTP and LTD, relatively new homeostatic plasticity mechanisms are also being explored. Although synaptic plasticity was once presumed to be the primary neural mechanism of learning, recent models have incorporated changes of intrinsic properties of the neurons as well.

Most experimental studies of learning have studied the mechanisms of synaptic plasticity in reduced preparations. Recently the focus has shifted to relating the changes in the synapses with behavioral learning. For example, inhibitory avoidance learning in rats produced the same changes in hippocampal glutamate receptors as induction of LTP with HFS (Whitlock et al., 2006). Because the learning-induced synaptic potentiation occluded HFS-induced LTP, they concluded that inhibitory avoidance training induced LTP in hippocampus.

Theoretical approaches can integrate local mechanisms with whole system behavior. Even after locating particular sites where changes occur, it is still not clear to what degree those changes are directly related to the learning. Building a computational model that integrates learning mechanisms allows one to evaluate the importance of different sites of plasticity. The observed plasticity for some sites may be secondary, or compensatory to the primary sites of learning (Lisberger and Sejnowski, 1992).

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1.35 Connectionist Memory Models of Hippocampal Function

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1.35.1 Introduction

Connectionist models of memory function distinguish themselves from other models of memory through their explicit analogy to memory function that may be supported by the anatomy of the neural substrate. Unlike semantic memory models, for example, connectionist models do not generally store explicit semantic pieces of information in individual memory locations. Instead, the expression of concepts in connectionist models relies on the simultaneous activity of a number of units that form a unique pattern. The structure that is needed to elicit such patterns of activity is provided by a corresponding unique arrangement of connection strengths between units. The network of units is therefore analogous to anatomical networks of neurons, and the connections in such models are analogous to the pathways, fibers, and synapses that form connections between neurons and groups of neurons.

The degree of functional similarity between connectionist units and connections and their biological counterparts varies greatly between different models. Connectionist models with abstract functions, such as the error back-propagating functions of Rumelhart units, tend to focus on general concepts of learning in networks with distributed storage resembling neural networks. At the other end of the spectrum, computational models that involve a greater degree of biophysical accuracy generally study neural activity that is comparable with electrophysiological data. It is the latter type of connectionist model that is our principal focus here, as those models are directly applicable to the study of neural activity in the hippocampal system.

We will consider connectionist models of the complex and comparatively well-studied memory binding functions of the medial temporal lobes. The anatomical regions included in the medial temporal lobes are the hippocampus (dentate gyrus, Cornu Ammonis fields CA1–CA3, and CA4, which is also called the hilus, and the subiculum) and the surrounding perirhinal, parahippocampal, and entorhinal cortices (*See* Chapter 1.33 for anatomical details).

Three significant forms of memory function are believed to depend on the function of hippocampal and entorhinal networks in particular: (1) context-dependent memory, which is retrieved by association with specific contextual cues; (2) episodic memory, in which specific associations between temporally ordered events are maintained; and (3) spatial memory, which aids spatial navigation through the retrieval of associated spatial features (*See* Chapters 1.14, 1.15, 1.21, 1.23, 1.33, 1.34). In the following text, we will review a number of theories and models that specifically attempt to model functions that have

been proposed to rely on neuronal network activity in the hippocampal and entorhinal regions of the medial temporal lobes.

Connectionist models developed to study different types of learning and memory are evaluated in the context of the computational neurophysiology that underlies function in networks of the entorhinal cortex and hippocampus. Hippocampal function supports specific memory-dependent behaviors in humans and animals. Data are therefore available with which models of the integral system can be tested. The hippocampal system is consequently a rich substrate for studies both at the scale of an individual network and at the scale of dynamic interactions between networks.

Neuropsychological data in human subjects show that lesions of the hippocampus selectively impair specific components of memory function. Hippocampal lesions impair the delayed free recall of information (Scoville and Milner, 1957; Penfield and Milner, 1958; Corkin, 1984). Such lesions have little effect on digit span (Corkin, 1984) or on the recency component of the serial position curve (Baddeley and Warrington, 1970). Neither semantic memory nor consolidated episodic memory appears affected by hippocampal lesions (Zola-Morgan et al., 1983, 1986). As a result of lesion data from nonhuman primates, the impairments seen in patients such as HM have been attributed in part to the removal of perirhinal cortex and parahippocampal gyrus (Suzuki et al., 1993). Lesions that are restricted to the subregions of the hippocampus may, however, cause severe memory impairments, as exhibited by patient RB (Zola-Morgan et al., 1986; Rempel-Clower et al., 1995). For example, in tests of the free recall of ten words from the middle of a 15-word list, patient RB recalls only 10% of the words, whereas controls recall about 40% (Graf et al., 1984). Similar striking differences between controls and patients with hippocampal lesions appear in tests of the free recall of information from a story, which is a common subtest of the Wechsler Memory Scale (Zola-Morgan et al., 1986). The human data support the specific significance of hippocampal subregions for the storage and retrieval of verbal information with context-dependent and episodic associations, as mentioned (*See* Chapters 1.02, 1.14, 1.15).

Memory function in a range of tasks can also be impaired by drug effects and by damage to the cholinergic innervation of regions in the medial temporal lobes. In humans, damage to cortical cholinergic innervation caused by anterior communicating artery aneurysms impairs performance in tests of the free recall of lists of words or information from paragraphs (Heilman and Sypert, 1977; Hodges and Carpenter, 1991), causes enhanced interference in an AB, AC paradigm (van der Linden et al., 1993), and also results in considerable confabulation in memory tasks (DeLuca, 1993). In nonhuman primates, lesions of the fornix, which cuts off most of the cholinergic innervation of the hippocampus, have been shown to impair formation of snapshot memories (Gaffan and Harrison, 1989). In addition to the extensive behavioral data suggesting some role for the hippocampus in human memory function, there is a wealth of data on the anatomy and physiology of this structure, and extensive theoretical work on the function of individual subregions. Here we review simulations of encoding and retrieval of item representations within networks that represent components of the full hippocampal circuit. The connectionist models discussed deal with sequential encoding, delayed free recall, and pattern recognition. In the context of hippocampal learning and memory, we review the published modeling results of many investigators, and in particular the integrative qualities of several system models that include our own work.

1.35.2 Connectionist Modeling of Hippocampal Episodic Memory

The study of memory in neural network models is typically approached in one of two ways: Either a fixed pattern of network connectivity is applied, so that simulations can focus on aspects of the retrieval of information (Hopfield, 1982; Treves and Rolls, 1994), or connectionist models use a protocol of repeated presentations of stimuli, so that they can simulate the training of connection weights by explicitly computing an error during a behavioral task (Schmajuk and DiCarlo, 1992; Gluck and Myers, 1993; Myers and Gluck, 1994). By contrast, recent simulations of hippocampal network activity use Hebbian learning (Jensen and Lisman, 1996; Hasselmo and Wyble, 1997; Koene et al., 2003) and often do not impose learning and recall stages externally (Hasselmo and Wyble, 1997; Koene et al., 2003). Instead, these models explicitly simulate the effect of cholinergic modulation of specific pathways and oscillatory activity at theta rhythm. Electrophysiological evidence suggests that such modulation elicits specific periods of selective

suppression of synaptic transmission and of modulated susceptibility to long-term potentiation (LTP) (Hyman et al., 2003). Conditions favor the retrieval of patterns of activity in associative memory when synaptic transmission is cholinergically suppressed (Huerta and Lisman, 1993; Hasselmo, 1995). Conversely, encoding and self-organization are favored in the absence of synaptic suppression and when LTP is facilitated.

In models of hippocampal memory function that simulate neural activity in terms of values for the firing rate, Hebbian learning rules are justified by experimental evidence of firing rate protocols that elicit LTP (McNaughton et al., 1978; Levy and Steward, 1979; Kelso et al., 1986; Wigstrom et al., 1986; *See* Chapter 1.33). Similarly, experimental evidence by Bi and Poo (1998) supports the hypothesis that spike-timing-dependent potentiation (STDP) of synapses is an effect that implements the Hebbian learning rule. Computational equivalents of STDP are applied in integrate-and-fire neural network models of hippocampal function (Jensen et al., 1996; Koene et al., 2003), also known as spiking neuron models (Gerstner, 1998a,b; Gerstner and Kistler, 2002).

In many cases, a theory of hippocampal function focuses exclusively on the function of a specific subregion of the hippocampus. Such theories commonly do not take into account how interactions between the subregions of the hippocampal system affect behavior in specific tasks. This is not a trivial matter, since hippocampal function is characterized by a dynamic interaction between the activity in its various subregions. It is likely that hippocampus-mediated behavior is not simply a consequence of the gathering of communicated results from isolated regional functions, but is instead elicited and modified through interaction and feedback. A number of reviews have previously summarized subregion-specific theories (Levy, 1989; Eichenbaum and Buckingham, 1990; O'Reilly and McClelland, 1994; Treves and Rolls, 1994).

Our models of learning and memory in the hippocampal system explicitly integrate the simulated functions of multiple regions (Hasselmo and Wyble, 1997; Hasselmo et al., 2002b; Koene et al., 2003; Hasselmo and Eichenbaum, 2005). A similar approach has been taken by other investigators, such as Jensen and Lisman (1996). The structure of these integrated system models of hippocampal function was motivated by experimental data about the anatomy and physiology of the hippocampal formation, and by previous theoretical work on the function of different hippocampal subregions (Marr, 1971; Valentino and Dingledine, 1981; McNaughton and Morris, 1987; Dutar and Nicoll, 1988; Levy, 1989; Eichenbaum and Buckingham, 1990; Levy et al., 1990, 1995; Ratcliff et al., 1990; McNaughton, 1991; Treves and Rolls, 1992, 1994; O'Reilly and McClelland, 1994; Rolls, 1995; Hasselmo et al., 1996, 1998). These models contrast with most earlier simulations in that they do not simulate individual effects and ideas in isolation. Instead, they combine the functions of different regions into a detailed, self-regulated model, generally including network representations of three or more subregions of the hippocampus along with the adjacent entorhinal cortex. Some of these new models address specific human memory tasks, such as free recall and recognition, and the effect of drugs on memory function during such tasks, which was not done in earlier simulations of the full hippocampal network (Rolls et al., 1997). Others address the combination of spatial memory and episodic memory or context-dependent memory tasks involved in rodent spatial navigation behavior. The anatomy of the hippocampal formation and structure of the model are summarized in **Figure 1**.

The hippocampus extends along the ventromedial border of the temporal lobe and receives convergent multimodal input from a wide range of neocortical association areas, most of which project to the hippocampus via neurons of the entorhinal cortex. The hippocampus consists of two interdigitated structures: the dentate gyrus (DG) and cornu ammonis (CA), of which regions CA1 and CA3 feature prominently in many models. Researchers often refer to the classical trisynaptic circuitry of the hippocampus, which consists of a feed-forward flow of information between the different structures (Amaral and Witter, 1989). As shown in **Figure 1**, entorhinal cortex layer II projects via the perforant path to the dentate gyrus. The DG projects via the mossy fibers to region CA3; region CA3 contains extensive excitatory recurrent collaterals (the longitudinal association fibers) and also projects on to region CA1 via the Schaffer collaterals. Region CA1 projects back directly and via the subiculum to entorhinal cortex layer IV. In addition to the trisynaptic circuit, there are also direct projections from entorhinal cortex layers II and III to regions CA3 and CA1 of the hippocampus.

In the recent integrated system models (Hasselmo and Wyble, 1997; Hasselmo and Eichenbaum, 2005),

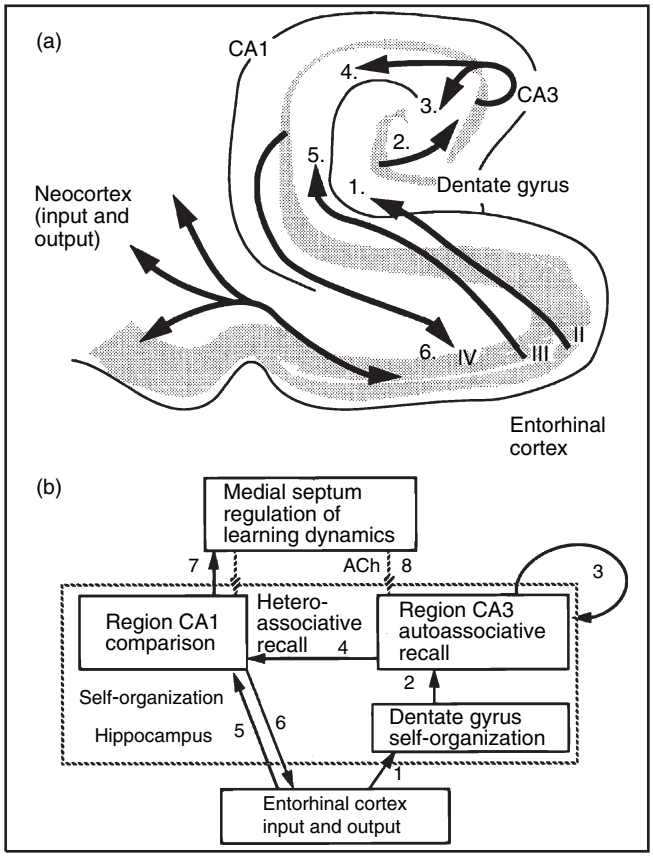

Figure 1 (a) Anatomical connectivity of the hippocampal formation. Connections between the hippocampus and multimodal association cortices pass through the entorhinal cortex. (1) Fibers of the perforant path connect layers II and III of the entorhinal cortex with the dentate gyrus. (2) The dentate gyrus projects to subregion CA3 via the mossy fibers. (3) Longitudinal association fibers connect pyramidal cells within subregion CA3. (4) The Schaffer collaterals connect subregion CA3 with subregion CA1. (5) Perforant path connections also enter subregion CA1 from the entorhinal cortex. (6) Projections back from subregion CA1 enter layer IV of the entorhinal cortex, either directly or via the subiculum. Subregion CA1 can influence activity in the medial septum either directly or via connections with the lateral septum. The medial septum (and the vertical limb of the diagonal band of Broca) provides cholinergic modulation to all hippocampal subregions. (b) Proposed function of individual anatomical subregions in the model by Hasselmo and Wyble (1997). The entorhinal cortex provides input from neocortical structures and transmits output back to neocortical structures. (1) Perforant path synapses undergo rapid self-organization to form new representations of patterns presented sequentially to the entorhinal cortex. (2) Mossy fibers pass the sparse new representation on to subregion CA1 for autoassociative storage. (3) Excitatory feedback in subregion CA3 mediates autoassociative storage and recall of these representations. (4) Schaffer collaterals mediate heteroassociative storage and recall of associations between activity in subregion CA3 and the self-organized representations formed by entorhinal input to subregion CA1. (5) Perforant path inputs to subregion CA1 undergo self-organization, forming new representations of entorhinal cortex input for comparison with recall from subregion CA3. (6) Feedback from subregion CA1 stores associations between activity in CA1 and in entorhinal cortex, allowing representations in subregion CA1 to activate the associated activity patterns in entorhinal cortex layer IV. (7) Output from subregion CA1 regulates cholinergic modulation, allowing a mismatch between recall and input to increase acetylcholine (ACh) and a match between recall and input to decrease ACh. (8) ACh from the medial septum sets appropriate dynamics for learning of new information in the model.

the network representations of individual subregions have the following functions:

1. Entorhinal cortex layer II (ECII). Network activity elicited by input to this model region represents the activity induced in entorhinal cortex in response to experimental stimuli. For example, the presentation of specific words in a behavioral experiment, as well as the shared experimental context, elicits representative patterns of activity in ECII.

2. Dentate gyrus (DG). The network model of this structure forms self-organized representations of each input pattern in ECII. The overlap between stored item and temporal context-specific representations in DG is reduced compared with the corresponding sequence of patterns of activity in ECII. The resulting patterns of output activity in DG target neurons in region CA3.

3. Region CA3. This modeled region encodes and retrieves associations between the shared experimental context (e.g., episode) and the individual items (e.g., words). The CA3 network activity provides the driving force for memory function in the model.

4. Region CA1. This modeled network structure compares the direct input from entorhinal cortex with the output of region CA3. In Hasselmo and Wyble (1997), the output of region CA1 regulates levels of acetylcholine on the basis of how well CA3 retrieval matches direct input. In Hasselmo and Eichenbaum (2005), the convergence of spreading activity in the entorhinal cortex and of episodic retrieval activity in region CA3 generates spiking activity in CA1 that identifies prior temporal context-specific events needed to make a decision at a choice-point in a delayed spatial alternation task.

5. Medial septum. The model network of this region in Hasselmo and Wyble (1997) sets the level of acetylcholine in all the other regions. In our integrated system model of hippocampal function, modulation by acetylcholine influences synaptic modification, synaptic transmission, depolarization, and adaptation.

6. Entorhinal cortex layer IV (ECIV). This modeled region stores associations between the full input patterns and the compressed representations from region CA1, allowing full retrieval of patterns. In prior work by one of the authors (Koene, 2001), this decoding stage emphasized learned connectivity from region CA1 to subiculum and ECIV.

Other connectionist approaches modeling these individual functions are described in the following text.

A number of subregion-specific models have focused on the significance of the perforant path synapses that link the output of entorhinal cortex to the DG (Marr, 1971; McNaughton and Morris, 1987; Eichenbaum and Buckingham, 1990; Treves and Rolls, 1994). It is a common hypothesis that the perforant path synapses encode representations of the patterns of afferent input activity that are manifested by sparse or nonoverlapping, distributed patterns of activity in the DG. Such sparse representations are especially useful to a hippocampal function for the encoding of episodic memory after a single occurrence of a sequence of individual patterns of input activity, in which the individual patterns of simultaneous activity are familiar. This is also called one-shot episodic learning. Recent theory and modeling have begun to address the questions of when and how such sparse representations may be generated and modified in tasks that involve multiple stimulus episodes in which specific patterns of activity may interfere (Koene, 2001; Koene and Hasselmo, 2006). In the section titled 'Dentate gyrus: Generating representations that minimize interference,' we address the issue of 'catastrophic interference' (McCloskey and Cohen, 1989) and the ways in which models of hippocampal memory function deal with that concern.

The Schaffer collateral fibers are another important hippocampal synaptic pathway. Those fibers transfer the output activity of subregion CA3 to subregion CA1. A common hypothesis is that the Schaffer collateral fibers mediate heteroassociative memory function (McNaughton, 1991; Treves, 1995). If this is so, then activity in CA1 may be predicted to a significant degree by the activity in CA3 (Levy, 1989). The involvement of the Schaffer collateral fibers in heteroassociative memory function has been explored in modeling studies with differing hypotheses about their precise role in the process. Possible self-regulation of learning and recall was studied in models specifically of the Schaffer collaterals (Hasselmo and Schnell, 1994), as well as in more inclusive models of the hippocampal network (Hasselmo and Stern, 1996; Hasselmo et al., 1996, 1998). In another set of hippocampal network models, the bulk of heteroassociative memory function was assumed to occur within the CA3 network, and the involvement of the Schaffer collateral fibers was constrained to delivering the activity of heteroassociative memory function to neurons in region CA1 (Jensen and Lisman, 1996; Koene et al., 2003).

The desire to pinpoint the hippocampal structure in which heteroassociative memory function is enabled has frequently focused on the possible function of the third set of significant fibers in the hippocampus, the longitudinal association fibers (or

recurrent fibers) within region CA3. One common hypothesis is that the longitudinal association fibers within region CA3 mediate autoassociative memory function (Marr, 1971; McNaughton and Morris, 1987; Eichenbaum and Buckingham, 1990; Treves and Rolls, 1992; Lisman, 1999). The other common hypothesis is that these association fibers mediate episodic memory by encoding temporal sequences of spiking patterns at the synapses of fibers that connect pyramidal neurons within region CA3 (Levy, 1989; Minai and Levy, 1994; Levy et al., 1995; Jensen and Lisman, 1996; Hasselmo and Eichenbaum, 2005). Models that focused exclusively on the recurrent CA3 network have studied autoassociative learning and retrieval (Hasselmo et al., 1995; Levy et al., 1995). More recently, models of autoassociative function mediated by such a recurrent network have been incorporated into more general (hierarchical) models of the integral hippocampal system (Hasselmo and Stern, 1996; Hasselmo et al., 1996, 2002b; Jensen and Lisman, 1996; Koene et al., 2003; Hasselmo and Eichenbaum, 2005; Koene and Hasselmo, 2007b).

The integral system models of hippocampal and entorhinal memory function tend to include a (sub)-set of modeled subregions, most commonly including networks of entorhinal cortex and hippocampal subregions CA3 and CA1. Each subregion is generally represented by local network circuitry. Those networks receive input and deliver output through connections that represent a subset of the known anatomical pathways in the medial temporal lobes. For example, the Hasselmo and Wyble (1997) model of cholinergically modulated learning and recall includes networks representing ECII, ECIII, DG, hippocampal subregions CA3 and CA1, and ECIV.

This system model is depicted in **Figure 2**. A similarly complex model of episodic recall by Lisman et al. (2005) includes networks that represent hippocampal subregion CA3 and that separately represent the mossy and granule cell layers of the DG. In recent models of temporal context-dependent episodic memory in the hippocampus that were used to simulate behavioral experiments with rodents in spatial tasks, modeling involved the specification of networks representing layer II and III of the entorhinal cortex, the DG, and hippocampal subregions CA3 and CA1, as well as the interactions mediated by fiber pathways between the modeled subregions (Hasselmo and Eichenbaum, 2005; Koene and Hasselmo, 2007b).

1.35.3 Encoding and Retrieval of Items within a Context Cue Presented in Layer II of Entorhinal Cortex

Our understanding of the physiology and of the biophysics of neurons in ECII has improved greatly in recent years (Klink and Alonso, 1997; Fransén et al., 2002), as have the available data about the task-specific responses of neurons from electrophysiology performed in ECII (Hafting et al., 2005). It is now known that both the pyramidal and stellate cell types in ECII can exhibit rhythmic activity during specific tasks and can sustain persistent repeated firing following specific stimulation protocols. Klink and Alonso (1997) showed that an after-depolarizing response exhibited by pyramidal cells in ECII can sustain rhythmic spiking once action potentials are elicited by a short period of depolarization or by synaptic input. These characteristic neural responses in ECII resemble earlier findings about neurons in the prefrontal cortex (Andrade, 1991). The apparent possibility that synaptic input can elicit intrinsic spiking without relying on excitatory fiber loops or synfire chains (Abeles et al., 1993) gave rise to a novel mechanistic hypothesis for short-term spike buffering introduced by Lisman and Idiart (1995). Since then, models of working memory that are based on intrinsic neuron dynamics have been investigated and used as networks specially suited to the rapid acquisition and ordered maintenance of sequences or temporal cues of patterns of spikes (Jensen et al., 1996; Koene and Hasselmo, 2007a). A buffered sequence of spike patterns can be repeatedly delivered to the hippocampus for episodic encoding (Jensen and Lisman, 1996; Koene et al., 2003).

In vivo electrophysiology during spatial tasks has shown that neurons in medial ECII respond in a spatial-location-specific manner that has some resemblance with the place cell activity commonly attributed to hippocampal pyramidal cells (Fyhn et al., 2004), although the place fields associated with location-specific activity in ECII can be large and overlapping with multiple peak response locations. Responses of stellate cells in rat layer II of dorsocaudal medial entorhinal cortex also appear to be rhythmic and directly related to location in a spatial task, but in contrast to the activity of the pyramidal cells, Hafting et al. (2005) found that the place-specific responses of the stellate cells in ECII change in spike frequency with distance from spatial grid points of maximum spike frequency. Active grid

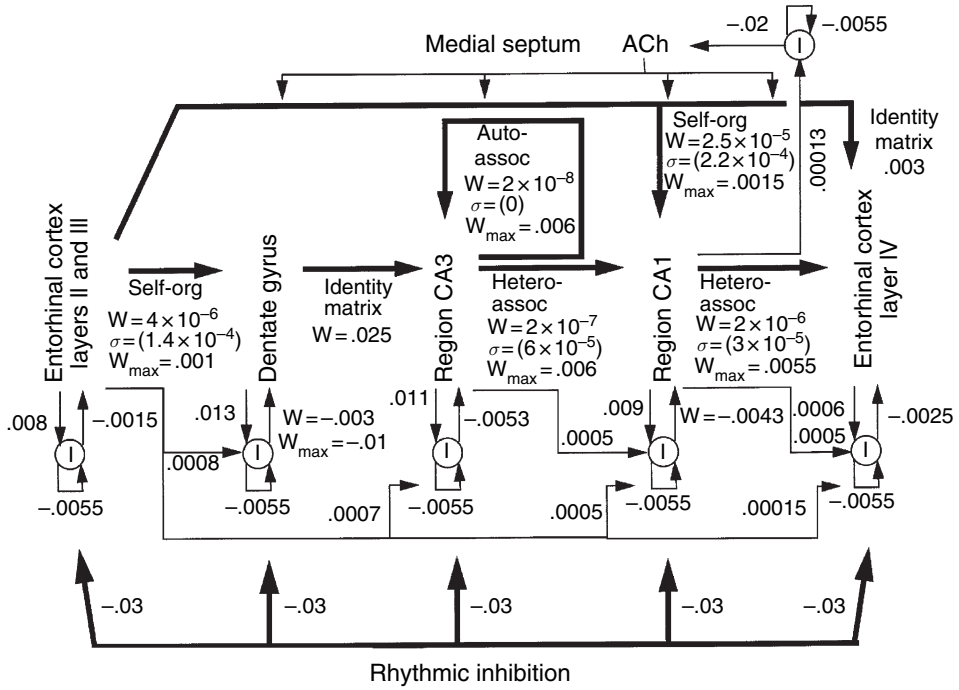

Figure 2 Strength of individual connections within the network simulation of the hippocampal formation in Hasselmo and Wyble (1997). When a single number is specified, this is the homogeneous strength of a set of nonmodifiable connections. When three numbers are specified, these are the mean initial strength, the standard deviation of the initial strength, and the maximum possible strength of a set of modifiable connections. Each modeled layer of entorhinal cortex contained 40 excitatory units, while the other hippocampal subregions each contained ten excitatory context units and 50 excitatory item units. Excitatory connections between regions represent the perforant path projecting from layer III of entorhinal cortex to dentate gyrus, layer III of entorhinal cortex to subregion CA1, the mossy fibers from dentate gyrus to CA3, the recurrent longitudinal association fibers in subregion CA3, the Schaffer collaterals from CA3 to CA1, and projection back from subregion CA1 to layer IV of entorhinal cortex. In each region the pyramidal cells are fully connected with an inhibitory interneuron (marked I) by excitatory connections of the strength shown. The inhibitory interneuron sends back inhibitory connections of the strength shown. In the dentate gyrus and subregion CA1, these inhibitory connections had random initial strength and were modifiable.

points of a particular stellate cell appear throughout an extended spatial field, and the grid points form equilateral triangles. The spatial frequency that is defined by the distance between grid points of an active ECII stellate cell increases when recording from cells in the dorsal medial ECII toward cells in the ventral medial ECII. The possibility that combinations of the rate-coded activity of multiple grid cells can act as a coordinate system that determines a precise spatial location has led to the development of models of path integration that incorporate this stellate activity in ECII as part of the information into the hippocampal system (O'Keefe and Burgess, 2005).

Other connectionist modeling of the memory function of ECII emphasizes encoding of representative patterns for use in hippocampal memory function. In Hasselmo and Wyble (1997), context-dependent encoding of specific word stimuli is assumed to occur in ECII. The corresponding connectionist network model that does this encoding was tested with regard to its performance in subsequent recognition or free recall of the words. In the simulations, both individual words and experimental contexts were represented by binary patterns of activity that were presented to the ECII and ECIII networks of the model. The binary patterns of activity used were strongly overlapping and were chosen randomly. The assumption that underlies the use of separate item and context representation is that a persevering context, provided by input due to (1) environmental features present during trials, (2) features of the presentation medium, and (3) the presence of the experimenter, results in cortical activity that is largely nonoverlapping with item input activity. Each individual word belonged to a specific

list, and the representative patterns of words from the same list were always given in association with the same consistent representation of context. During the presentation of a pattern of activity, which was maintained for 400 time steps of the simulation, the network activity caused by the input propagated to all other subregions of the modeled hippocampal system. In this manner and by manipulation of the model functions, the effects of scopolamine (Ghoneim and Mewaldt, 1975) could be described. A similar representation of stimuli has been used in previous connectionist models of free recall (Metcalfe and Murdock, 1981; Metcalfe Eich, 1982; Gillund and Shiffrin, 1984).

Free recall and recognition test different aspects of memory retrieval. A common means to elicit recognition retrieval in connectionist models is to present partial input patterns. By contrast, free recall tests require that subjects recall items from a list without being given item-specific cues. In the integral hippocampal system model of Hasselmo and Wyble (1997), either type of retrieval could be elicited by providing the corresponding appropriate input to the modeled entorhinal networks. To simulate free recall, only the activity pattern representing a context cue was presented to layers II and III of model entorhinal cortex. The resulting entorhinal activity evoked context-specific activity in the model networks of DG and of hippocampal subregion CA3. In the associative CA3 network, the context cue elicited activity that represents individual items. If one of the item patterns of activity produced in CA3 matched a pattern of activity that was elicited in ECIV in response to the context cue, then successful recall was achieved.

To test the recognition of events in an episode or items from a list in a connectionist model, it is necessary to explicitly avoid presenting a cue that is a partial representation of one learned item. In tests of recognition with human subjects, the subjects are given lists that contain words from a learned list, as well as distractor words. They are instructed to identify words that were encountered during the encoding phase of the experiment. A similar protocol can be simulated in connectionist models. For example, in the model of Hasselmo and Wyble (1997), this was simulated by presenting to layers II and III of the model entorhinal cortex a sequence containing some known and some unknown item patterns. In simulations, the propagation of activity allowed the known item patterns to elicit the retrieval of a corresponding context pattern in the CA3 network. A recognition process of this sort was first proposed by Hollingworth (1913), which led Tulving and Norman (Norman, 1968; Tulving, 1975) to hypothesize that recognition was the inverse function of recall.

Another issue that concerns the presentation of input at entorhinal cortex layers of a hippocampal system model is the relationship between presentation frequency and recognition, the effect of familiarity. This issue was addressed by several connectionist models (Metcalfe and Murdock, 1981; Gillund and Shiffrin, 1984; Hintzman, 1988; Chappell and Humphreys, 1994) and was restated in the integral system model of Hasselmo and Wyble (1997). There and in similar system models, the effect that cue familiarity has on simulated recognition performance may be observed in terms of the temporal delay before a context or item pattern is reactivated by retrieval. This method has been suggested for studies of temporal delays in ECIV.

The potential roles of the entorhinal cortex in models of learning and memory, as the perforant path input to the integrative processing in the hippocampus, are likely to inspire a major part of the modeling and experimental efforts in coming years. In addition to the sustained and specialized rhythmic responses supported by pyramidal and stellate cells in ECII, novel types of intrinsic spiking responses are now known to occur in ECIII and ECV. In layer III, induced sustained spiking may be maintained until deactivated by an equivalent stimulus, regardless of intervening hyperpolarization (Fransén et al., 2006b). In layer V, successive instances of induced depolarization produce a graded increase in the frequency of a sustained spiking response, while following instances of hyperpolarization cause a graded decrease of the sustained spike frequency (Fransén et al., 2006a). Each of these characteristic responses implies a nonsynaptic neural memory function that can play a specialized role in learning and memory.

1.35.4 Dentate Gyrus: Generating Representations That Minimize Interference

Recent models of learning and memory in the hippocampal system propose that the neurons of the DG may self-organize to form novel and sparse (nonoverlapping) ensembles that are associated with a unique temporal context (Hasselmo and Eichenbaum, 2005). In this case, such a temporal context is

identified as a sequence of input activity that originates in the entorhinal cortex, as well as through associations that were established with episodic sequences of patterns of activity in region CA3 of the hippocampus. The unique representations of successive temporal contexts that may be generated in DG could establish associations between those representations within the DG that reflect a specific temporal order. Such associations within the DG may be established through synaptic modification in the fibers that connect the mossy and granule cell layers of DG (Lisman, 1999; Lisman et al., 2005).

Unique coding for temporal context in DG may provide a route of direct access through which input to the DG can cue the retrieval of episodic memory stored by the hippocampus. A process by which unique representations may be generated by coincidental activity has been postulated in earlier studies (Koene, 2001). In connectionist models, a temporal context may be represented as a continually changing sequence of input activity. Presentation of such a sequence to the hippocampus may be aided by a first-in, first-out buffer in ECII, thereby providing a sliding window in which activity is repeatedly presented in order (Koene and Hasselmo, 2007a). The representation of temporal context has been described formally in terms of context drift and its potential significance with respect to the recency and contiguity effects in human recall performance (Howard and Kahana, 2002; Howard et al., in press). Recent unpublished data by J. Manns, obtained by recording in subregion CA1, provides supporting electrophysiological evidence of a mechanism that supports contiguity of context representations.

Connectionist networks, unlike human brains, are prone to a type of forgetting called 'catastrophic interference,' which is not gradual as during normal forgetting observed in experiments with humans (Barnes and Underwood, 1959). When new data interfere catastrophically with existing stored memory in connectionist networks, the earlier memory can be erased rapidly and completely by the interference effects. Attractor neural network models can avoid catastrophic interference by using a protocol of interleaved learning. During interleaved learning, items of a list are presented multiple times in randomized order (McClelland et al., 1995; Hasselmo, 1999).

In humans, interleaved learning may indeed occur during a gradual process of transfer from the hippocampus and memory consolidation in neocortical regions of the brain. For such consolidation to be possible, new information that is generally believed to be processed as part of hippocampal function must first be acquired and encoded in the hippocampal system. During this initial encoding, interleaved learning is not generally feasible, since animals and humans cannot exert absolute control over the environment from which stimuli appear. It will often be impossible or undesirable (as in the case of dangerous events) to repeat stimuli or to randomize their order during repeated presentations (as in the case of causal events). One-shot encoding of episodes is possible without risking catastrophic interference if nonoverlapping representations are involved, as in the case of the unique coding that was hypothesized in DG, as described in preceding paragraphs. A number of spiking neuron models (integrate-and-fire models) of hippocampal function use encoding with sparse patterns in portions of modeled hippocampal network hierarchy to avoid catastrophic interference. In those models, the separated patterns of activity can be maintained in a short-term buffer to repeat ordered presentations that elicit activity in the hippocampus and encode episodic associations (Jensen et al., 1996; Jensen and Lisman, 1996; Koene, 2001; Koene et al., 2003; Hasselmo and Eichenbaum, 2005).

This supposed function of the DG has been described in some connectionist models as a competitive network. There, it is proposed that the divergent connections from entorhinal cortex to DG distribute overlapping input patterns into sparse nonoverlapping representations that reduce interference (McNaughton, 1991; Treves and Rolls, 1994). A detailed analysis of the modification of perforant path synapses by O'Reilly and McClelland (1994) showed that modification can enhance pattern separation, but that study did not explicitly focus on the sequential presentation of different input patterns. A modification of perforant path synapses was explicitly considered when sequences of input were presented in the Hasselmo and Wyble (1997) model. The excitatory perforant path connections from layer II of model entorhinal cortex to the DG network consequently achieved self-organization: random initial connection strengths from ECII to DG resulted in the initial activation of a subset of DG neurons; the connections from active ECII neurons to activated DG neurons were strengthened; the connections from inactive ECII neurons were weakened by synaptic decay in response to the postsynaptic activity; connections from active ECII neurons to inactive DG neurons were weakened due to presynaptically regulated synaptic decay. The effect of these model

mechanisms corresponded to experimental evidence, which has shown that strengthening of perforant path synapses depends on pre- and postsynaptic activity (McNaughton et al., 1978; Levy and Steward, 1979), while weakening of connections has been similarly demonstrated (Levy and Steward, 1979). In Hasselmo and Wyble (1997), the model mechanism was able to establish selective patterns of connectivity between active neurons in the ECII network and neurons in the DG network. The process produced sparsified, less overlapping representations for each of the item patterns that were presented in trial sequences.

The model in Hasselmo and Wyble (1997) did require a novel mechanism to prevent previously established representations from dominating the learning elicited by new pattern input. The novel mechanism involved strengthening the connections from active inhibitory interneurons in DG to active neurons in DG (Hasselmo et al., 1996, 1998). Such inhibitory synaptic enhancement has been demonstrated in other regions of the hippocampus (Haas and Rose, 1984; Morishita and Sastry, 1991; Grunze et al., 1996). The resulting suppression of neurons that participated in previously encountered representations insured that different patterns of activity were elicited in DG by subsequent patterns of entorhinal cortex activity.

The connectionist proposal that DG rapidly establishes new memory patterns to represent changing input leads to neural network models that rely of different rates of learning in DG networks and in networks that represent a hippocampal subregion such as CA3. In Hasselmo and Wyble (1997), this need was met by assuming different thresholds for synaptic modification in the two regions. In more recent models, the comparative ease with which spiking has been shown to occur in cells of the dentate granule layer is explicitly taken into consideration, so that the resulting simulations further suggest that comparatively rapid encoding takes place at synapses in the DG (Lisman et al., 2005; Koene and Hasselmo, 2007b).

1.35.5 CA3: Forming Attractors and Associations between Attractors during Rhythmic Oscillation at Theta Frequency

The hippocampus is essential to the ability of humans to easily acquire new episodic memory. One-shot learning is known to occur, in which known stimuli are presented only once in a specific order. One-shot episodic learning may depend critically on a combination of subregion-specific learning functions. In DG, nonoverlapping and therefore noninterfering representations can be made of activity that originates in and may be buffered in the entorhinal cortex. In subregion CA3, associative memory may be encoded by the LTP of the synapses of excitatory recurrent fibers (the longitudinal fibers of subregion CA3). Jensen and Lisman (1996) have demonstrated successful simulations with a model that combines these elements and achieves one-shot learning of novel episodes that are composed of known item patterns. In that integrate-and-fire network model, item representations are patterns of simultaneous spikes, and individual patterns are separated by competitive recurrent inhibition due to the activation of a network of interneurons after each pattern of item spikes. Lisman and Idiart (1995) theorized that the separation of spike patterns by such inhibition may explain the appearance of gamma frequency in the power spectrum during working memory tasks.

A model of episodic acquisition similar to that of Jensen and Lisman (1996) incorporates novel insights about the modulatory effect of theta rhythm in the hippocampus (Hasselmo et al., 2002a; *See* Chapter 1.37). During each cycle of the simulated theta rhythm in this model, the phase of maximum neuronal membrane depolarization in region CA3 coincides with a suppression of the strength of afferent input transmission. Conditions during that phase favor associative retrieval via the recurrent fibers in subregion CA3. Conversely, conditions at the opposite phase of the theta rhythm encourage the receipt and encoding of novel associations in accordance with afferent input. During that opposite phase of rhythmic modulation, recurrent synaptic transmission is suppressed, and LTP of those synapses is more readily elicited (Hyman et al., 2003).

The theory of differential functional modulation during different phase intervals of the theta rhythm that is observed during hippocampal memory tasks inspired the development of a new category of models of hippocampal episodic memory (Hasselmo et al., 2002b,c; Cannon et al., 2003). In these models, memory encoding and memory retrieval alternate within each cycle of the theta rhythm. Complex integral system models include both the proposed neural network mechanism of buffered (Lisman and Idiart, 1995; Koene and Hasselmo, 2007a) one-shot episodic learning (Jensen et al., 1996; Jensen and Lisman, 1996) and the proposed biophysical model

mechanism of alternating encoding and retrieval conditions. These system models with integrate-and-fire neurons react dynamically to feedback from the environment in response to actions taken and, therefore, perform successfully in tasks during which the need for memory encoding or memory retrieval may not be easily predicted or may be required in a concurrent or ongoing manner (Hasselmo et al., 2002b; Koene et al., 2003). During phase-locked conditions favorable to memory encoding, buffered sequences of spike patterns cause synaptic modification at recurrent excitatory fibers in model subregion CA3 and in model ECIII. During phase-locked conditions favorable to memory retrieval, activity spreads along strengthened connections within the recurrent networks, thereby retrieving representative spike patterns that provide information about known feature associations and heteroassociative information about encountered episodes.

The allocation of network roles in terms of stored association type is a continuing source of debate. For example, in models by Hasselmo et al. (2002b) and by Koene et al. (2003), feature (or place) associations were stored in a network representing subregion CA3, and heteroassociative storage occurred in a network representing ECIII. In later models by Hasselmo and Eichenbaum (2005) and by Koene and Hasselmo (2007b), the roles of the two recurrent networks were reversed. In either case, it was assumed that a convergence of streams of spreading activity in the two recurrent networks elicited spike activity at pyramidal neurons in hippocampal subregion CA1. The spiking of neurons in subregion CA1 can inform actions to be taken to successfully perform a specific task. The models of rhythmically alternating encoding and retrieval, and of activity that is gated by converging streams of spreading activity during retrieval, were applied together by Koene et al. (2003) to successfully simulate the performance of rat spatial navigation and by McGaughy et al. (2005) to simulate conditioned responses in a delayed nonmatch to sample task.

A number of connectionist models have proposed that hippocampal subregion CA3 provides autoassociative memory function, based on the extensive and strong excitatory recurrent connectivity in that neural network (McNaughton and Morris, 1987; Levy, 1989; Eichenbaum and Buckingham, 1990; Treves and Rolls, 1992; Hasselmo et al., 1995; Hasselmo and Stern, 1996). In contrast to the biophysical and structural complexity of the models described in preceding paragraphs, neural network models with excitatory recurrent connections that focus on the principles of attractor networks have been used extensively to model memory function (Little and Shaw, 1975; Anderson et al., 1977; Hopfield, 1982; Anderson, 1983; Ruppin and Yeshurun, 1991; Chappell and Humphreys, 1994; Hasselmo et al., 1995; *See* Chapters 1.33, 1.34). Those networks have the characteristics of fixed-point attractor models, due to recurrent connections with specific patterns of connection strengths. When network activity settles into final stable states, those stable states represent stored memories. Each final stable state may be reached by a different state of initial activity.

From a computational perspective, fixed-point attractors present an appealing model of human memory function, since the final memory states can exhibit robustness to variation in the input cues. Therefore, attractor dynamics are particularly useful for models that are used to simulate free recall. In tests of free recall, the input cues are relatively weak, and associations with an experimental context are shared by a number of items in the same list. Given initial activity that reflects these item and context input characteristics, free recall performance is simulated when attractor dynamics lead from the initial state to a final stable state that is a known representation in the trial context. It is worth noting that attractor dynamics in a more biophysically detailed model of hippocampal subregion CA3 may mediate the retrieval of autoassociative memory, even if network activity does not settle into persisting fixed-point attractors. The dynamics of activity in subregion CA3 may nonetheless be dominated by the approach to an attractor, during the interval defined by a single oscillation at gamma frequency, or during complex spike activity.

As perpetuated in recent system models (Hasselmo and Eichenbaum, 2005; Koene and Hasselmo, 2007b), the hypothesized role in Hasselmo and Wyble (1997) for the memory function of subregion CA3 was that of an attractor network that specialized in the formation of robust episodic representations of items and context. These associations were learned by modifying excitatory recurrent connection strengths, so that attractor states were encoded. The encoding of weaker associative links between context and item attractors was also assumed. Free recall and recognition were primarily achieved through the attractor dynamics. During encoding, the activity of a sufficiently active unit in model DG propagates through an identity matrix of connections that represent mossy fibers to activate individual CA3 units. In the presence

of cholinergic suppression, the pattern of activity in the model is determined primarily by this afferent input, instead of being subject to strong recurrent excitation. Consequently, the connections between neurons that were activated by input from the DG are selectively strengthened, which results in increased activity in model subregion CA3 and a greater output of activity to subregion CA1.

Earlier studies have explored the stability of the CA3 network when modeled with a number of different patterns of connectivity (Minai and Levy, 1994). In comparatively abstract neural network models, the possibility of an exponential explosion of excitatory activity is prevented by assuming that total activity is normalized (Treves and Rolls, 1992). In continuous-firing-rate models, a similar limitation of network activity is achieved through the use of feedback inhibition (Hasselmo et al., 1995; Hasselmo and Wyble, 1997). The same method may be applied in spiking neuron models.

In the Hasselmo and Wyble (1997) integral system model, increased activity in subregion CA1 causes a decrease in simulated cholinergic modulation throughout the model network, which in turn causes greater recurrent excitation in subregion CA3. Therefore, it is possible to enter a stable fixed-point attractor pattern that represents a stored context or item, if a pattern of cue activity is presented. An emerging item or context attractor state can (a) drive ensuing activity into an associated item or context attractor state, and (b) drive output activity in region CA1 and ECIV. Attractor states are terminated in the model by neural adaptation and by the rhythmic inhibition through interneuron activity, which is a model mechanism of hippocampal theta rhythm (Bland and Colom, 1993).

With the model mechanisms in Hasselmo and Wyble (1997), free recall is simulated when a cue elicits a context attractor state in model subregion CA3. The activity spreads into a number of weakly associated item attractor states in CA3. As the activity of item attractor states grows, they compete by inhibiting each other. At most one winning attractor state persists, which represents the free recall of an item. That attractor state is eventually terminated by cyclic inhibition, so that the context attractor can evoke the attractor state of another associated item. Inhibitory oscillations have been shown to provide a potent means of competitive neural network learning (Norman, 2006).

Recognition is simulated when input of an individual learned item pattern of activity in model ECII elicits the corresponding item attractor state in subregion CA3. When a familiar item attractor state is reached, that attractor elicits associated context attractor activity. Such context attractor activity does not appear for novel items.

1.35.6 CA1: Comparing and Gating of Input from Region CA3 and Entorhinal Cortex

There are two common functional hypotheses concerning the arrangement of synapses in the important Schaffer collateral fiber path from hippocampal subregion CA3 to subregion CA1. The first supposes that the Schaffer collaterals undergo a process of self-organization (Treves and Rolls, 1994). The second supposes that the Schaffer collaterals provide a heteroassociative memory function (McNaughton, 1991). The two are not mutually exclusive, and the relative amount of these two functions depends upon how strongly the Schaffer collaterals influence activity in subregion CA1.

If the Schaffer collaterals dominate postsynaptic activity in region CA1 during learning, then they will predominantly undergo self-organization. This must be assumed if no mechanism for the modulation of synaptic transmission is incorporated in models. However, in models that include the cholinergic suppression of synaptic transmission at the Schaffer collaterals, the perforant path input can more strongly influence CA1 activity during learning, thereby allowing heteroassociative memory function. The heteroassociative memory function may be necessary if it is assumed that one of the functions of subregion CA1 is to provide a comparison between the recall activity generated by subregion CA3 and episodes of direct input transferred from entorhinal cortex, as has been proposed by some researchers (Levy, 1989; Eichenbaum and Buckingham, 1990; Lisman, 1999).

Experimental results have shown that the strength of input from entorhinal cortex to subregion CA1 is insufficient to achieve significant activation in subregion CA1, unless supported by converging input from hippocampal subregion CA3. The Schaffer collateral input from CA3 therefore gates the activation of neurons in subregion CA1. In this regard, recent integral system models of the hippocampal system, such as the model of Hasselmo and Eichenbaum (2005) and the model of Koene and Hasselmo (2007b), implement an explicit mechanism of

convergence and gating of activity in CA1 by Schaffer collateral input.

The model of Hasselmo and Wyble (1997) proposes that, during sequential self-organization in DG, similar self-organization also takes place in subregion CA1, resulting in a similar modification of excitatory and inhibitory connections. The possibility of long-term changes of inhibitory potentials is supported by considerable data obtained by recording in subregion CA1 (Grunze et al., 1996). During encoding of a novel pattern, random initial connection strengths of the activated Schaffer collateral fibers elicits a distributed pattern of activity in subregion CA1. This postsynaptic activity can interact with the presynaptic activity of input from perforant path fibers to form a new self-organized representation. Hasselmo and Wyble supposed that during recall, even though the perforant path input initially has a greater influence on the activity of subregion CA1, those patterns of Schaffer collateral activity that represent familiar stimuli are a sufficient match to perforant path input, so that the output of CA1 elicits a reduction of the cholinergic modulation. One consequence of this is the removal of cholinergic suppression of synaptic transmission through the Schaffer collateral fibers. Then, the input to CA1 through Schaffer collaterals comes to dominate activity there. The mode suggests that it is through such a mechanism that the output activity of hippocampal subregion CA3 can drive the neural activity in subregion CA1 to produce a pattern of activity that is associated with memory retrieved in CA3.

1.35.7 Medial Septum: Feedback Regulation of Cholinergic Modulation and Selective Emphasis of Encoding or Retrieval

In the regulatory system of cholinergic modulation that was supposed in the models discussed in preceding paragraphs, the propagation of hippocampal activity through the medial septum has been explicitly associated with the feedback regulation and consequent selective emphasis of memory encoding or memory retrieval processes. In recent simulation studies, the proposed selection mechanism has been further refined to include ongoing alternation of preferential encoding and retrieval in separate phase intervals of each cycle of theta rhythm. Rhythmic modulation of hippocampal networks at theta frequency establishes opposing periods of relative depolarization and relative hyperpolarization of the neural membrane.

In Hasselmo et al. (2002a), this network-wide modulation of membrane potentials is combined with the aforementioned phase-locked selective suppression of recurrent or afferent fiber transmission during distinct intervals of the theta rhythm. Also included was an explicit mechanism of the theta-phase specific effectiveness of LTP in the hippocampus (Hyman et al., 2003). The sum of the theta-modulated transmission and plasticity effects may enable alternation between conditions that favor encoding and conditions that favor retrieval during each theta cycle. This model of ongoing encoding and retrieval has been applied successfully to simulated spatial navigation tasks (Hasselmo et al., 2002b; Koene et al., 2003) and to delayed nonmatch to sample tasks (McGaughy et al., 2005), as well as to a biophysical implementation of reinforcement learning in prefrontal cortex (Koene and Hasselmo, 2005).

According to Hasselmo and Wyble (1997), the level of output activity elicited in subregion CA1 is a direct determinant of activity in the medial septum. That in turn determines the degree of cholinergic modulation that is applied to all modeled subregions through connections from the medial septum. The cholinergic modulation was deemed responsible for all cellular effects of acetylcholine, namely: (1) selective suppression of synaptic transmission at connections from neurons in CA3 through recurrent connections to other neurons in CA3, and at connections from neurons in CA3 to neurons in CA1, as well as at connections from neurons in CA1 to neurons in the entorhinal cortex; (2) network-wide depolarization of all neurons; (3) suppression of adaptation in the response of excitatory neurons; and (4) enhancement of synaptic plasticity (Hyman et al., 2003). In the model implementation, default levels of cholinergic modulation were high, and changes were effected by the active inhibition of cholinergic input. This inhibition was hypothesized to occur due to the activation of interneurons in the medial septum by output activity from subregion CA1.

In summary therefore, when both the output activity from model subregion CA3 to CA1 and the activity delivered to CA1 via the perforant path generate a match and elicit consequent activity in the CA1 network, then the output activity from CA1 feeds back into the hippocampal system in the form of a decrease of cholinergic modulation via the medial septum, which was proposed to effectively

switch the modeled hippocampal networks from encoding to retrieval dynamics. This proposed switching system has a slower time course than the rhythmic alternation of modulation that was proposed in Hasselmo et al. (2002a) and subsequent published models.

1.35.8 The Translation of Representations in Layer IV of Entorhinal Cortex and Feedback to the Neocortex

ECIV receives direct input from ECII. For this reason, layer IV may be considered an ideal network in which activity represents the output of the hippocampal system during simulations of tests of free recall and of recognition (Hasselmo and Wyble, 1997). Simulated activity elicited in ECII is transmitted directly to ECIV, propagation that is done through an identity matrix of connections in the model. During the initial learning of a novel pattern, it is that input which dominates activity in layer IV of model entorhinal cortex. After all, feedback from subregion CA1 is suppressed by cholinergic modulation during this time. Associations between the patterns of activity in ECIV and the activity of episodic representations in subregion CA1 are encoded.

During simulated retrieval, partial input cues given to the ECII network result in inactive or incomplete item pattern activity in ECIV. Those patterns of activity can be completed only once output activity spreads from subregion CA1 to ECIV, which can reconstitute previously encoded item or context information. Transmission through the feedback connections from subregion CA1 to ECIV is not suppressed during retrieval. Consequently, recognition tests could be simulated and evaluated with the model by measuring whether activity in the ECIV network generated a known context pattern when individual familiar or novel item patterns were presented (Norman and O'Reilly, 2003).

1.35.9 The Hippocampal Model of Temporal Context-Dependent Episodic Memory

Insights gained in work with earlier integral system models, such as in the models of Hasselmo and Wyble (1997), Jensen and Lisman (1996), and Koene et al. (2003), and through the application of those models to simulated tasks that depend on the specific functions of each layer of the entorhinal cortex and subregions in the hippocampus, led those researchers to formulate novel hierarchical models. Recent models in Hasselmo and Eichenbaum (2005) and in Lisman et al. (2005) incorporate proposed functions of context-dependent episodic memory that enable complex behavioral performance in tasks such as delayed spatial alternation. Each of these models takes into consideration associative encoding on the recurrent fibers of two networks. In the case of the Hasselmo and Eichenbaum (2005) model, these networks are subregion CA3 and ECIII. In the case of the Lisman et al. (2005) model, they are subregion CA3 and the recurrent network that is formed by the reciprocal synaptic fibers between the mossy and granule cell layers of the DG. Associative encoding on the recurrent fibers in DG was also taken into account for the unique sparse encoding in DG of the models of Hasselmo and Eichenbaum (2005) and Koene and Hasselmo (2007b), although the implementation of the network was more abstract in those models.

The Hasselmo and Eichenbaum (2005) model has been applied to simulated tasks in which performance depends on the ability to concurrently encode and retrieve temporal context-dependent episodic memory. In these simulations, input activity that is elicited by stimuli in a simulated environment causes activity in a layer II network of model entorhinal cortex. In an integrate-and-fire implementation of the model hypotheses (Koene and Hasselmo, 2007b), the ECII network acts as a working memory of short sequences of sequences of spike patterns that are sustained by persistent firing characteristics of the pyramidal neurons in ECII (Koene and Hasselmo, 2007a). Several studies have addressed the possible mechanisms by which executive mechanisms and attention may gate input to working memory (Grossberg and Stone, 1986; O'Reilly and Frank, 2005). The output of the ECII network provides repeated episodes of spiking activity to the model ECIII, as well as to model subregion CA3 and model DG.

Hasselmo and Eichenbaum have proposed that the synapses of recurrent fibers in ECIII encode associations between items distinguished by specific features, such as associations between the place cells of adjacent place fields in spatial navigation tasks. In this model, the associative network properties of the DG are used to generate unique temporal context representations that are associated with a buffered

episodic cue of activity in the ECII network. The third associative network, model subregion CA3, was assigned a heteroassociative role, storing episodic memories of the sequences of activity propagated from the output of ECII. During encoding, the modification of synapses at fibers from DG to CA3 creates associations between the unique temporal context-specific representations in DG and the episodic memory traces stored in subregion CA3. In this manner, activating a representation stored in the DG provides a direct way to elicit the retrieval of a specific instance of episodic memory that was stored in the hippocampus.

A significant characteristic of episodic encoding in the CA3 network of the models of Hasselmo and Eichenbaum (2005) and Koene and Hasselmo (2007b) is that episodic order is reversed in the encoded associations at recurrent fibers. Thus, during episodic retrieval in the model CA3 network, the sequence of retrieved activity can trace events in reverse, resembling reversal of activity shown during *in vivo* electrophysiology in linear track experiments (Foster and Wilson, 2006) and during spatial alternation behavior in multiple T-mazes (Johnson and Redish, 2006).

Activity in the model subregion CA1 was elicited by the convergence of spreading activity elicited during retrieval in the ECIII and CA3 recurrent networks. Simulation results with the model showed that it could successfully simulate performance in delayed spatial alternation tasks. The activity of neurons in the model subregion CA1 could be decoded to represent specific spatial locations and, in the stem of a T-maze, exhibited the selective activity and spiking behavior of experimentally observed 'splitter cells' (Hasselmo and Eichenbaum, 2005; Koene and Hasselmo, 2007b). In a spatial alternation task, splitter cells that respond to motion in the same head direction and in the same location of the stem of a T-maze fire selectively, depending on whether an animal must turn left or right at the top of the stem in order to perform a specific run of the alternation task successfully.

With the development of several hierarchical models that include the interacting functions of multiple subregions of the hippocampal system, it has become possible to simulate full-network activity and thereby to study the interactions between the subregions. Early full-network simulations focused either on behavioral phenomena (Schmajuk and DiCarlo, 1992; Gluck and Myers, 1993) or on hippocampal physiology (Rolls, 1995; Hasselmo and Stern, 1996), but the recent models explicitly simulate a combination of system behavior and biophysical detail. That combination of modeling levels has already led to new insights about mechanisms necessitated by the interaction and improved physiological realism of individual subregion networks. Full-network simulations in Hasselmo and Wyble (1997) indicated the need for inclusion of additional hippocampal subregions, so that nonoverlapping representations could be generated (DG), and so that encoding and retrieval conditions could be regulated within the simulations (a comparison function in CA1, plus medial septum). Entorhinal regions were included to decode self-organized representations in the hippocampus and allow the study of the interaction of input and output with neocortical regions. A set of simulations by Jensen et al. (1996) and by Jensen and Lisman (1996) showed how a succession of hippocampal network stages could generate incrementally more complex associative information, from short buffered sequences to auto-associative item representations and sequence representations of episodic memory, and emphasized how the characteristic physiology of *N*-methyl-D-aspartate (NMDA) receptors in the subregions involved could selectively mediate the different associative functions. Simulations by Koene et al. (2003) clearly demonstrate the involvement of synchronization by theta rhythm throughout the hippocampal system during active task behavior, while simulations by Hasselmo and Eichenbaum (2005) demonstrate the functional significance of spreading retrieval activity in regions CA3 and ECIII that converge in region CA1.

In Hasselmo and Wyble (1997), simulations with the neural network model were able to replicate list length and list strength effects seen in the human data.

The list strength effect appears during free recall, where list strength is the ratio of recall performance between strong items (items presented several times) and weak items (items presented once). There was no effect of list strength on performance during recognition. In the model, items interfere only when simultaneously active, such as when competing during free recall. As more items compete, simulated recall becomes more difficult, with a greater threshold for retrieval in subregion CA3.

This difference in the experimental human data, between free recall and recognition performance, was not replicated by earlier composite models, in which each item contributes to the variance of the entire

system. In such models, the variance deteriorates recognition accuracy just as it deteriorates recall accuracy (Ratcliff et al., 1990). The list strength effect has been achieved differently by a model of Chappell and Humphreys (1994), in which the activity of units is controlled by intrinsic dynamics. The list length effect, which is seen during recognition, and the list strength effect are achieved by the same process in composite models. In contrast, the list length effect appears in the Hasselmo and Wyble (1997) model when the chance that a distractor item matches one of the list of items learned by DG is great enough to cause an erroneous recognition. This effect is due to increasing intrusions with longer lists, rather than due to increasing variance assumed in the composite models.

Simulations in full-network, hierarchical models of hippocampal function may also be compared to data on the effect of hippocampal lesions. Removal of a model's ability to rapidly encode episodic memory after a single presentation can simulate anterograde amnesia and partial retrograde amnesia, as experienced by patients such as HM (Scoville and Milner, 1957). Temporally graded, partial retrograde amnesia (McClelland et al., 1995) may result if episodic memories encoded with the hippocampal system are involved in a gradual formation of representations in the neocortex (Buzsáki, 1989; Treves and Rolls, 1994; Hasselmo et al., 1998). A transfer of data back from the hippocampus to deep entorhinal cortex during periods of decreased cholinergic modulation is supported by physiological data (Chrobak and Buzsáki, 1994). Compared to the more abstract and fundamental models of human memory (Gluck and Myers, 1993; Myers and Gluck, 1994), full-network simulations clarify the link between the model and specific anatomical or physiological features of the hippocampal system.

The internal representation of item activity in recent models of hippocampal function, though established through a biologically plausible rationale, has similarities with earlier and nonconnectionist models of human memory. Entorhinal activity resembles coding in composite trace models, where the individual features that compose items elicit subsets of an item representation (Metcalfe and Murdock, 1981; Murdock, 1982). By contrast, after sparse nonoverlapping encoding in DG, representations have more in common with those used in separate trace models (Gillund and Shiffrin, 1984; Hintzman, 1988). For example, the storage of representations in CA3 in (Hasselmo and Wyble, 1997) resembles that used in the search of associative memory model (Gillund and Shiffrin, 1984), where a matrix of weights between 0 and 1 stores the relative values of associations between different items, but using attractor dynamics for retrieval. To cue retrieval, a number of hippocampal models use context (Hasselmo and Wyble, 1997; Hasselmo and Eichenbaum, 2005; Koene and Hasselmo, 2007b). Such cuing resembles the retrieval mechanism used in convolution-correlation models Metcalfe and Murdock (1981).

During retrieval, a procedure of cleanup, error correction, or pattern completion is used by many models of human memory, from the more abstract approaches (Hintzman, 1988) to those in which dynamic interactions allow activity to spread through different regions (Hasselmo and Wyble, 1997; Hasselmo and Eichenbaum, 2005). In the Lisman (1999) model, the combination of perforant path and Schaffer collateral input to CA1 allows error or novelty detection, while dynamic pattern completion during the retrieval of spiking patterns in episodic recall is enabled by recurrent activation through the reciprocal loop from CA3 to the granule and mossy cell layers of DG and back to CA3. In the Hasselmo and Wyble (1997) model, competition between different patterns in model subregion CA3 must result in a clear winner before the output of CA3 is propagated to subregion CA1 and to the model ECIV, since the model specifies that activity in CA1 can be elicited only by complete retrieved patterns.

In the model of temporal context-dependent episodic memory of Hasselmo and Eichenbaum (2005) and its variant based on integrate-and-fire neurons (Koene and Hasselmo, 2007b), spiking in CA1 can be elicited only when there is a convergence between spreading activity representing the retrieval of feature associations in entorhinal cortex and spreading activity in CA3 that represents retrieved episodic memory in the temporal context. In their implementation of cleanup, those latest models incorporate competition between items retrieved by auto- and heteroassociative attractor processes, as well as the gating of perforant path input by Schaffer collateral input, which had been demonstrated in prior full-network models (Lisman, 1999; Koene et al., 2003). In addition to this, the integrate-and-fire neuron implementation of the model (Koene and Hasselmo, 2007b) of temporal context-dependent episodic memory does not depend on a specific complete pattern size, but instead depends on activity that occurs at specific phases of the cycles of theta rhythm.

Some models of free recall (Metcalfe and Murdock, 1981; Gillund and Shiffrin, 1984) prevent the reactivation of previous responses through a sort of repetitive cued recall, in which previously recalled items provide cues that move recall more efficiently from item to item. In contrast, the cues presented for free recall in the Hasselmo and Wyble (1997) model remain constant during recall. Instead, that model uses adaptation based on previous activation to effectively remove a pattern, once activated, from the pool of possible responses. The adaptation properties of the model have been used to simulate functional magnetic resonance imaging data that showed a decrease in hippocampal activation during the viewing of a single repeated stimulus when compared to the activation during the viewing of different novel stimuli (Stern et al., 1996; Stern and Hasselmo, 1997).

Simulations with neural network models that include mechanisms of interactions between networks with specific characteristics, such as those described in the preceding text, can be used to make specific and testable predictions. For example, in the case of the Hasselmo and Wyble (1997) neural network model, by blocking simulated cholinergic effects, it was possible to make clear and specific predictions of the effects of scopolamine on human memory function. The selectivity of the effects predicted corresponded to effects demonstrated in psychopharmacological experiments (Ghoneim and Mewaldt, 1975, 1977; Peterson, 1977; Mewaldt and Ghoneim, 1979). Specifically, encoding of new words was impaired, but retrieval of words in a free recall test was not, and the recognition of words was not impaired. The connectionist model therefore pointed out a direct link between physiological effects at the level of neurons and synapses and the behavioral effects of drug administration in a human experiment. This example demonstrates how the use of connectionist simulations to link cellular physiology with behavior may be used to constrain models of memory function in accordance with experimental data in neurophysiology and molecular biology.

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1.36 Theory of Reward Systems

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1.36.1 Introduction

Except, perhaps, for the unabashed moralist, knowledge alone does not determine choice of action, i.e., knowing that "action A leads to X and action B leads to Y" does not 'entail' choosing A or B. What enables choice, given this information, is some nonarbitrary means of establishing the relative merits of achieving X or Y. We argue that it is the reward system that provides this means. From this perspective, although the reward system is an extension of the general motivational processes of animals, its function is limited to actions over which animals can exert control and that are instrumental to achieving some goal or other, i.e., to goal-directed instrumental actions. Of course, although most aspects of an animal's behavioral repertoire can be described in goal-directed terms, many of these activities are not goal-directed at all and are reflexive responses elicited by stimuli or relations between stimuli. Establishing criteria for discerning goal-directed and non-goal-directed actions is a necessary step, therefore, in limiting our discussion of the reward system. In this chapter, we consider first the criteria for defining an action as goal-directed and then use that definition to describe the nature and function of the reward system in establishing primary rewarding events, like foods and fluids, both with respect to encoding reward value and to retrieving that value in order to choose between competing courses of action.

This research has established that the value of reward is determined by the quality of the emotional response associated with an event, the latter dependent on current motivational state, i.e., value essentially maps onto the relationship between the specific sensory features of an event and the particular, pleasant or unpleasant, emotional feedback generated when that event is contacted. This issue is taken up in more detail in the section titled 'Reward processes,' where we examine one of the main predictions of this account, that, in the context of secondary rewards, any event associated with a pleasant emotional reaction will support the performance of goal-directed actions. These are sensory events that acquire reward value through association with primary rewards (commonly mislabeled conditioned reinforcers). The procedures used to establish secondary rewards are identical to those commonly used to establish Pavlovian conditioned responses to a stimulus, raising the possibility that the functioning of the reward system can be reduced to the motivational processes that support Pavlovian conditioning. In the section titled 'Secondary reward' we examine this possibility and conclude, based on the extensive evidence standing against this claim, that Pavlovian conditioned responses (CRs) and

goal-directed actions are controlled by fundamentally distinct incentive processes.

1.36.2 Reward Processes

1.36.2.1 Goal-Directed Actions and Behavioral Control

The critical distinction between reflexive and goal-directed actions is that the latter are controlled by a causal relationship to their consequences, whereas the former are not. There are many illustrations of this distinction but perhaps the most apposite is Sheffield's (1965) analysis based on the salivary response of dogs. Salivation was the conditioned and unconditioned reflex studied by Pavlov (1927). Nevertheless, from a goal-directed perspective, it is possible that dogs control this response in order to facilitate digestion or to improve the taste of food. Sheffield arranged a standard pairing between conditioned and unconditioned stimuli, in this case presentation of a tone followed by food delivery, but with a twist: If the dog salivated during the tone the food was not delivered on that trial. This arrangement maintains a Pavlovian relationship between the tone and food but abolishes any instrumental contingency between salivation and food. He reasoned that, if the salivation was goal-directed then this omission contingency should ensure that they stop salivating; indeed having never had the opportunity to learn that salivating improved the rewarding impact of the food by enhancing its flavor or improving its ingestion, they should never acquire salivation to the tone in the first place. Sheffield found that it was clearly the Pavlovian relationship controlling performance; during the course of over 800 tone-food pairings the dogs acquired and maintained salivation to the tone even though this resulted in them losing most of the food they could otherwise have obtained.

Salivation may be the exception of course, but in numerous studies over the last 40 years it has been established in a range of species that Pavlovian conditioned responses do not adjust to this kind of contingency, i.e., one in which performance of the conditioned response leads to the omission of the unconditioned stimulus. Rats acquire conditioned approach responses during a conditioned stimulus (CS) when doing so omits the food (Holland, 1979), pigeons peck at keys (Williams and Williams, 1969), chicks chase food away (Hershberger, 1986), and so on. In all of these studies, the evidence confirms that the performance of the Pavlovian CR does not depend on the relationship between the CR and the US. (*See* Chapter 1.03).

In contrast, experiments assessing the performance of actions acquired during instrumental conditioning have found evidence that these responses do indeed depend on the contingency between action and outcome. Take, for example, instrumental lever pressing. Rats will acquire lever pressing for food quickly and without explicit shaping. Putting this response on an omission contingency, in which responding leads to the omission of an otherwise freely delivered food, rapidly reduces the performance of that response, more rapidly than simply delivering the outcome in an unpaired manner (Davis and Bitterman, 1971; Dickinson et al., 1998; *See* Chapter 1.06). Furthermore, numerous studies have demonstrated the exquisite sensitivity of the performance of instrumental lever pressing to changes in the net probability of outcome delivery given the action (i.e., the difference between probability of an outcome given a response and the probability of the outcome given no response). These changes can be highly selective; degrading one action–outcome contingency by delivering the outcome associated with that action noncontingently often has no effect on the performance of other actions (Colwill and Rescorla, 1986; Dickinson and Mulatero, 1989; Balleine and Dickinson, 1998a).

1.36.2.2 The Effect of Changes in Reward Value

Generally, therefore, goal-directed actions are those that, unlike Pavlovian CRs, are sensitive to the causal relation between the performance of the action and its specific outcome. It is important to note, however, that lever press responses can be controlled by two kinds of association. The first is the relationship between action and outcome described earlier. After extensive instrumental training, however, performance of an action can become habitual, elicited by various situational cues connected with the action through a process of sensorimotor association (Adams, 1981; Dickinson, 1985, 1994). Although the formation of these associations diminishes sensitivity to omission (Dickinson et al., 1998), it does not necessarily abolish it and, although this test distinguishes actions from Pavlovian conditioned reflexes, it does not provide an adequate assessment in itself to distinguish goal-directed actions from habits. Fortunately, there is a clear distinction between the functions of the instrumental outcome in the two

forms of learning. Whereas the outcome serves as the second term of the action–outcome association that supports the acquisition and performance of goal-directed actions, it serves merely to strengthen or to reinforce the stimulus–response (S–R) associations that form habits. As such, the outcome forms no part of the associative structure that supports habitual performance. Based on this analysis, therefore, and combined with an assessment of sensitivity to changes in the instrumental contingency, the standard test of whether an action is goal-directed or not involves an assessment of the sensitivity of performance to a posttraining change in the reward value of the outcome. From an S–R perspective, when conducted posttraining, i.e., after a substantial S–R association has been established, a change in outcome value should be expected to have little if any effect on the subsequent tendency to perform the action. If an action is goal-directed, however, the change in value should potently alter performance.

Consider the case in which a hungry rat is trained to press a lever for a particular type of food pellet. According to a goal-directed account, it is the reward value of the food pellets that motivates performance. Consequently, if having trained the rat to perform this action, the reward value of the food pellets is reduced in some way, we should expect this devaluation to affect performance, i.e., the rat should be less inclined to press the lever after the devaluation. Given this scenario, the question at issue is whether the devaluation affects performance via the animal's knowledge of the contingency between lever pressing and the food pellets. In the first appropriately controlled study along these lines, Adams and Dickinson (1981) assessed this by training rats with two types of food pellets, sugar and grain, with only one type being delivered by lever pressing. The other type of pellet was presented independently of any instrumental action. Thus, any particular rat might have to work for sugar pellets by lever pressing, while receiving free deliveries of grain pellets every so often. The issue was whether the animals would reduce lever pressing more after the devaluation of the response-contingent pellets, the sugar pellets in our example, than after devaluation of the free pellets, the grain ones. Such an outcome could only occur if the effect of the devaluation was mediated by the instrumental contingency between lever pressing and the sugar pellets.

In this study, the pellets were devalued using conditioned taste aversion procedures; it is well established that a food aversion can be conditioned by inducing gastric illness, for example by the injection of lithium chloride (LiCl), shortly after the animal has consumed the food (Bernstein, 1999). In the Adams and Dickinson study, having trained the rats to lever press, half had a taste aversion conditioned to the sugar and half to the grain pellets. During aversion conditioning, the levers were withdrawn and the animals were given a series of sessions in each of which they were allowed to eat one type of pellet. The animals in the devaluation group received a LiCl injection after sessions in which they received the pellets that had been contingent on lever pressing during training but not following sessions with the free pellets. The control group, by contrast, had the aversion conditioned to the free pellets rather than the response-contingent ones. Although such food aversions can be established with a single pairing of consumption with illness when the food is novel, the treatment had to be repeated a number of times to suppress consumption in the present study. This is because the pellets were already familiar to the rats, having been presented during instrumental training.

After inducing these aversions, Adams and Dickinson were now in a position to ask whether devaluing the pellets that acted as the reward for lever pressing during training had a greater impact on performance than devaluing the freely delivered pellets. This result would be expected if the motivational properties of rewards are mediated by their instrumental relation to the action. In fact, this is just what Adams and Dickinson found: when subsequently given access to the lever again, the devaluation group pressed significantly less than the control group. Note that this test was conducted in extinction, during which neither type of pellet was presented, for if the pellets had been presented during testing, the reluctance of the devaluation group to press the lever could be explained simply in terms of the direct suppressive effect of presenting this aversive consequence. By testing in extinction, however, different performance in the two groups must have reflected integration of knowledge of the consequences of lever pressing acquired during training with the current reward value of the pellets. This suggestion was further confirmed by Colwill and Rescorla (1986) using a choice test. They trained hungry rats to perform two instrumental actions, lever pressing and chain pulling, with one action earning access to food pellets and the other earning access to a sucrose solution. The rats were then given several trials in which they were allowed to consume one of the outcomes with the levers and chains

withdrawn and were then made ill by an injection of LiCl. All animals were then given a choice extinction test on the levers and chains again conducted in extinction, i.e., in the absence of either of the outcomes. Although S–R accounts should predict no effect of this treatment, Colwill and Rescorla found that animals performed less of the action whose training outcome was subsequently paired with LiCl than the other action, indicating that the rats had indeed encoded the consequences of their actions.

The importance of these demonstrations of the outcome devaluation effect lies in the fact that, together, they provide strong evidence that animals encode the specific features of the consequences or outcome of their instrumental actions. Furthermore, these studies show that instrumental performance is not only determined by the encoding of the action–outcome relation but also by the current reward value of the outcome. In recent years, considerable attention has been paid to the processes that contribute to the encoding of reward value, and the advances that have been made have come largely from asking how outcome devaluation works to change instrumental performance: How does taste aversion work to modify the rats' evaluation of the outcome and so change the course of its instrumental performance?

1.36.2.3 Incentive Learning and the Encoding of Reward Value

Perhaps the simplest account of the way taste aversion learning works to devalue the instrumental outcome can be derived from accounts of aversive conditioning generally according to which pairing the instrumental outcome with illness changes the evaluation of the outcome through the formation of a predictive association between the food or fluid and the aversive state induced by illness. The result of an effective pairing of the outcome with illness is, therefore, that the animal learns that the outcome now signals that aversive consequence. From this perspective, the outcome devaluation effect is the product of a practical inference process through which a previously encoded action–outcome relation is combined with learning that the outcome signals an aversive consequence to reduce subsequent performance of the action.

In contrast, Garcia (1989) introduced a more complex account according to which the change in the evaluation of the outcome induced by taste aversion learning is not due to changing what the outcome predicts but due to changes in how it tastes. Garcia related the change in taste to negative feedback from a system sensitive to illness that he identified as inducing a disgust or distaste reaction. It is important to see that this view implies that taste aversion learning involves not one learning process but two: (1) an effective pairing of the outcome with illness initially enables a connection between the sensory properties of the outcome and processes sensitive to illness; (2) this association is activated when the outcome is subsequently contacted to generate a distaste reaction and allow the animal to associate the outcome representation with disgust or distaste. This account predicts that, to induce outcome devaluation, it is not sufficient merely to pair the outcome with an injection of LiCl. Rather, a change in value is not induced until the second process is engaged when the outcome is again contacted.

The procedures employed to induce instrumental outcome devaluation, such as that described by Adams and Dickinson (1981), do not differentiate between these two accounts of taste aversion learning because the conditioning of an aversion to the outcome is usually conducted using multiple pairings of the outcome with illness. Clearly the pairings themselves would be sufficient to establish a signaling relation between the outcome and an aversive consequence. But the fact that the animals were allowed to contact the outcome on subsequent pairings could have provided the opportunity for the animals to associate the outcome representation with distaste. If a substantial aversion to the outcome could be conditioned with a single pairing of the outcome with illness, however, then these accounts of outcome devaluation make divergent predictions: On the signaling account, a devaluation effect should emerge, providing that an effective pairing between the taste and illness was produced; on Garcia's (1989) account it should not emerge until the rats have been reexposed to the devalued outcome. In a test of these divergent predictions, Balleine and Dickinson (1991) trained thirsty rats to lever press for water. After acquisition, the outcome was switched to sugar solution for a single session, after which the rats were given an injection of LiCl either immediately or after a delay (the latter treatment, as an unpaired control, should have induced relatively little aversion to the sucrose on either account). The critical question was whether, in the absence of further contact with the sucrose, the rats in the immediately poisoned group

would display reduced performance on the lever relative to the delayed group.

To assess the influence of reexposure to the sucrose, half of each of the immediate and delayed groups were allowed merely to taste the sucrose, whereas the remainder were given water before two tests were conducted on the levers. The first test was conducted in extinction to assess the effects of devaluation and reexposure on the tendency to press. A second, punishment test was then conducted in which responding on the lever again delivered the sucrose, which allowed us to assess the strength of the aversion to sucrose. If a substantial aversion to the sucrose was conditioned in the immediately poisoned groups, then not only should a reliable punishment effect have emerged in the second test, but, on the signaling account, responding should also have been reduced in the extinction test in all of the immediately poisoned rats. In contrast, in Garcia's account, responding in the extinction test should be reduced in those immediately poisoned rats given reexposure to the sucrose. In fact, in this and in several other experiments along similar lines, Balleine and Dickinson (1991) and Balleine (1992) found consistent evidence for Garcia's account; although a single pairing between sucrose and illness invariably produced a reliable punishment effect in immediately poisoned rats, a devaluation effect only emerged in the critical extinction test if reexposure to the sucrose was given prior to the test.

These results suggest that outcome devaluation depends upon the interaction of two learning processes. The first process involves the conditioning of an association between the outcome and processes that are activated by the induction of illness by LiCl. The failure of this learning process to directly affect instrumental performance suggests that it is not, alone, sufficient to induce outcome devaluation. Rather, it appears to be necessary for feedback from this first learning process to become explicitly associated with the specific sensory features of the outcome itself for a change in the reward value of the instrumental outcome to occur and for performance to change. Indeed, considerable evidence now suggests that this second learning process critically determines the encoding of the rewarding properties of the instrumental outcome, a process referred to as incentive learning (Dickinson and Balleine, 1994, 1995).

The reason for emphasizing the role of incentive learning in instrumental outcome-devaluation effects is that it also appears to be the process by which other primary motivational states, such as hunger and thirst, encode the reward value of other goals such as foods and fluids. It is well established that the motivational state of rats is a major determinant of their instrumental performance; not surprisingly, hungry animals work more vigorously for a food reward than sated ones. But what current evidence suggests is that this is because a food-deprived state induces an animal to assign a higher incentive value to nutritive outcomes when they are contacted in that state and that this high rating of the incentive value of the outcome is then reflected in a more vigorous rate of performance. Although this suggestion stands contrary to general drive theories of motivation that suppose that increments in motivation elicit their effects on performance by increases in general activation (Hull, 1943), there are good empirical grounds for arguing that motivational states do not directly control performance (Dickinson and Balleine, 1994, 2002; Balleine, 2001). Balleine (1992) trained groups of undeprived rats to lever press for a food reward. After training, half of the rats were shifted to a food deprivation schedule, whereas the remainder were maintained undeprived before both groups were given an extinction test on the levers. Balleine found that performance of the groups on test did not differ even though the shift in motivational state was clearly effective. In a subsequent test where the animals could again earn the food pellets, the food-deprived rats pressed at a substantially higher rate than the undeprived rats. Although motivational state clearly did not exert any direct control over performance, as was found in taste aversion conditioning, the motivational state could control performance if the rats were given the opportunity for incentive learning by allowing them consummatory contact with the instrumental outcome in the test motivational state prior to the test. To demonstrate this, Balleine (1992) trained two further groups of rats to lever press when undeprived. Both groups were given prior exposure to the instrumental outcome when food-deprived before the test in which one group was tested undeprived and the other food-deprived. Now a clear difference in performance was found in that the rats tested when food-deprived and allowed to consume the instrumental outcome when food-deprived prior to test pressed at a higher rate than the other three groups that in turn did not differ. Balleine (1992) was able to confirm that this incentive learning effect depended upon the instrumental contingency. He trained undeprived rats to perform two actions, lever pressing and chain pulling,

with one action earning access to food pellets and the other to a maltodextrin solution. All rats were then given a choice extinction test on the levers and chains. Prior to the test, however, the animals were given six sessions in which they were allowed to consume one instrumental outcome when food deprived and, on alternate days, the other outcome in the training, i.e., undeprived, state. On test, Balleine found that animals performed more of the action that, in training, had delivered the outcome reexposed in the food-deprived state prior to the test than the other action.

It should be noted that this role for incentive learning in instrumental performance following a shift in motivational state is not confined to posttraining increases in food deprivation. The same pattern of results was also found for the opposite shift, i.e., where rats were trained to lever press for food pellets when food-deprived and then tested when undeprived. In this case, rats only reduced their performance when food deprivation was reduced if they were allowed to consume the instrumental outcome when undeprived prior to the test (Balleine, 1992; Balleine and Dickinson, 1994). Finally, the generality of this role of incentive learning in instrumental performance has been confirmed for a number of different motivational systems and in a number of devaluation paradigms. For example, in addition to taste aversion learning, incentive learning has been found to mediate (1) specific satiety-induced outcome devaluation effects (Balleine and Dickinson, 1998b); (2) shifts from water deprivation to satiety (Lopez et al., 1992); (3) changes in outcome value mediated by drug states (Balleine et al., 1994, 1995a); and changes in the value of (4) thermoregulatory rewards (Hendersen and Graham, 1979) and (5) sexual rewards (Everitt and Stacey, 1987; Woodson and Balleine, 2002) (see Dickinson and Balleine, 1994, 2002; Balleine, 2001, for reviews). In all of these cases, it is clear that animals have to learn about changes in the incentive value of an instrumental outcome through consummatory contact with that outcome before this change will affect performance.

1.36.2.4 Incentive Learning as an Emotional Process

Traditional neobehaviorist learning theories argued that CRs, what were called fractional anticipatory goal responses, could exert a motivating effect on instrumental performance (Hull, 1943, 1952; Spence, 1956). Largely due to the subsequent work of Konorski (1967) and Mowrer (1960), however, it is now widely accepted that these effects reflect the conditioning of an affective state that can exert a direct modulatory influence over consummatory responses and, through a change in the emotional responses elicited during ingestion, on instrumental performance (Rescorla and Solomon, 1967; Dickinson, 1989). Recent research investigating the microstructure of orofacial taste reactivity responses in rats to various tastes has provided evidence, not only of specific ingestion and rejection responses to sweet and bitter tastes, but also that the ingestive taste reactivity responses are increased in hungry rats to tastes previously paired with nutrients (Myers and Sclafani, 2001). Likewise, rejection-related taste reactivity responses are increased to tastes previously paired with illness (Berridge et al., 1981). With respect to incentive learning, this approach suggests that, during this form of consummatory exposure, activation of the outcome representation activates its associated motivational system, which, through activation of attendant affective processes, generates feedback in the form of an emotional response. This process is illustrated in **Figure 1**. On this account, incentive learning depends on two processes: a feedback process: (**Figure 1 (a), (b)**) and a feedforward process (**Figure 1 (c)**). Presenting the instrumental outcome in some motivational state or other provides the opportunity for the formation of an association between the outcome representation and the motivation system (**Figure 1(a)**) that acts to open a feedback loop (**Figure 1(b)**). When the outcome is subsequently contacted, activation of the outcome representation acts to produce specific motivational activity that results directly in activity in affective structures productive of an emotional response. Incentive learning

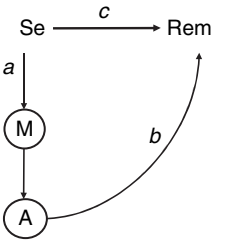

Figure 1 The structure of incentive learning. (a) Sensory features of the instrumental outcome (Se) are associated with a motivational process (M). (b) Through connections with affective structures (A) this connection provides feedback in the form of an emotional response (Rem). (c) Incentive learning reflects the association between Se and Rem based on their contiguous activity.

(Figure 1(c)), then, is the formation of a feedforward association between the outcome representation and an emotional response.

Taste aversion-induced outcome devaluation effects provide a good example of this process. In this case, this perspective argues that a taste is first associated with activation of a disgust system induced by LiCl. After this pairing, reexposure to the taste can drive the disgust system to activate the aversive affective system to generate an aversive emotional response. It is the contiguous pairing of the taste and the emotional response that, from this perspective, drives the reduction in reward value induced by reexposure. Notice that, if pairing a taste with illness conditions an association between the taste and disgust, then blocking the activity of the disgust system at the time of conditioning using an antiemetic, i.e., a drug that prevents or relieves illness or nausea, should be predicted to attenuate the formation of that association with the effect that, in the test sessions, rats should prefer a taste poisoned under the antiemetic to some other poisoned taste. But furthermore, if the expression of a previously conditioned aversion, and the consequent change in reward value, depends upon the ability of the taste representation to access the disgust system via an established connection, blocking the activity of the disgust system with an antiemetic during reexposure should be predicted to block the incentive learning effect; see Figure 2.

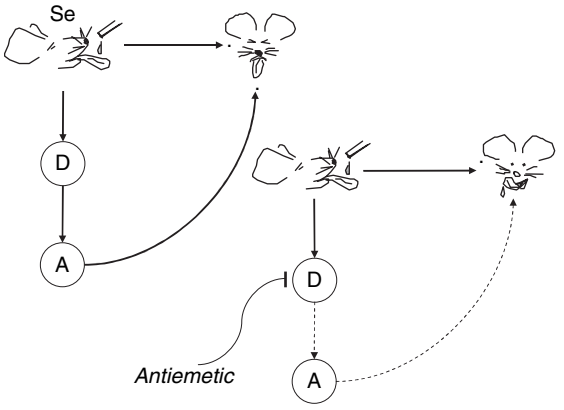

Figure 2 Incentive learning and taste aversion-induced outcome devaluation. The left panel shows the effect of reexposure to a poisoned outcome; the association between the taste (Se) and disgust (D) induced by pairing the taste with illness provokes feedback in the form of a disgust response, allowing the rat to learn about the change in value of the outcome. The right panel shows the predicted effect of an antiemetic on incentive learning. By blocking activity in the disgust system, the antiemetic should reduce the unpleasant emotional feedback and hence the change in value produced during reexposure to the taste. A, affective structures.

In accord with this suggestion, Limebeer and Parker (2000) reported that the antiemetic ondansetron blocked the expression of the aversive taste reactivity responses induced by a taste previously paired with illness. Furthermore, we have assessed this prediction by assessing the influence of ondansetron on reexposure to a poisoned taste on instrumental choice performance (Balleine et al., 1995b). In this experiment, thirsty rats were trained in a single session to perform two actions, lever pressing and chain pulling, with one action delivering a sucrose solution and the other a saline solution on a concurrent schedule. Immediately after this training session, all of the rats were given an injection of LiCl. Over the next 2 days the rats were given brief periods of reexposure to both the sucrose and the saline solutions. Prior to one reexposure session, rats were injected with ondansetron in an attempt to block the emotional effects of reexposure, whereas prior to the other session they were injected with vehicle. The next day, the rats were given a choice extinction test on the lever and chain. If reexposure devalues the instrumental outcome via the ability of the outcome representation to access the disgust system, blocking the activity of that system with ondansetron should attenuate the effects of reexposure such that, on test, the action that, in training, delivered the outcome subsequently reexposed under ondansetron should be performed more than the other action. This is, in fact, exactly what was found (Balleine et al, 1995a). The attenuation of incentive learning by ondansetron provides, therefore, strong confirmation of the suggestion that incentive learning depends critically upon negative feedback generated by an association between the outcome representation and a disgust system.

1.36.2.5 Retrieving Reward Value

Given the role of incentive learning in the encoding of reward, it is interesting to consider how the value conferred by this process is retrieved to determine choice performance. Because the choice tests are often conducted many days after incentive learning, in extinction the rat is forced to rely on their memory of specific action–outcome associations and the current relative value of the instrumental outcomes. So how is value encoded for retrieval during this test?

A currently influential theory, the somatic marker hypothesis (Damasio, 1994), proposes that value is retrieved through the operation of the same processes through which it was encoded. According to this view, decisions based on the value of specific goals are determined by reexperiencing the emotional effects associated with contact with that goal. With regard to outcome devaluation effects, for example, the theory could not be more explicit:

> When a bad outcome connected with a given response option comes to mind, however fleetingly, you experience an unpleasant gut feeling . . . that forces attention on the negative outcome to which the given action may lead, and functions as an automated alarm signal which says: Beware of danger ahead if you choose the option that leads to this outcome. The signal may lead you to reject, *immediately*, the negative course of action and thus make you choose between other alternatives (Damasio, 1994: 173).

An alternative theory proposes that reward values, once determined through incentive learning, are encoded abstractly (e.g., X is good or Y is bad and so on) and, as such, from this perspective they are not dependent on the original emotional effects induced by contact with the goal during the encoding of incentive value for their retrieval (see Balleine and Dickinson, 1998a; Balleine, 2005, for further discussion).

We have conducted several distinct series of experiments to test these two hypotheses and, in all of these, the data suggest that after incentive learning, incentive values are encoded abstractly and do not involve the original emotional processes that established those values during their retrieval (Balleine and Dickinson, 1994; Balleine et al., 1994, 1995a,b). One test of these two accounts was derived from consideration of the role of associations between the outcome representation and the disgust system in outcome devaluation described in the previous section. If the impact of outcome devaluation on performance is carried by emotional feedback induced by activation of the disgust system by the outcome representation, then, according to the somatic marker hypothesis, reducing the ability of the outcome representation to activate the disgust system during retrieval of incentive value on test by administering ondansetron prior to the test should be predicted to attenuate the effects of outcome devaluation on performance. This experiment replicated the procedures used in the experiment described earlier (Balleine et al., 1995b) except that, prior to the choice extinction test, half of the animals were injected with ondansetron, whereas the remainder were injected with vehicle. Based on the previous study, it was anticipated that the group given the injection of vehicle prior to the test would perform more of the action that, in training, had delivered the outcome reexposed under ondansetron. More importantly, if activation of the disgust system critically mediates the retrieval of incentive value during the test, as the somatic marker hypothesis suggests, then any difference found in the vehicle group should be attenuated in the group injected with ondansetron on test.

The results of this experiment were very clear; contrary to predictions of the somatic marker hypothesis, the injection of ondansetron on test had no impact whatsoever on performance in the choice extinction test. Whether injected with vehicle or ondansetron prior to the test, the action that, in training, delivered the outcome reexposed under ondansetron was performed more than the other action and to a similar degree. This finding suggests that, although activity in the disgust system determines the effects of incentive learning, the disgust system does not play a role once incentive learning has occurred, i.e., the retrieval of incentive value is not based on the same process through which it was encoded. In line with the proposal that reward value is encoded abstractly or symbolically and in contradiction to predictions from the somatic marker hypothesis position, in this and other similar studies we have found that the processes that determine the encoding of reward value are not required during the retrieval of that value during free choice tests in order for animals to select a course of action.

1.36.3 Secondary Reward

The suggestion that the reward process supporting instrumental conditioning is derived from an association between the sensory features of an event and an emotional response, together with the evidence for the abstract encoding of reward value, provides an immediate explanation as to how events not directly associated with primary motivational systems can serve as the goals of instrumental actions; from this perspective, any stimulus associated with an emotional response should be able to serve as a goal and so support the performance of goal-directed actions. In the past, the seemingly arbitrary nature of goals has been explained in terms of a process called

conditioned reinforcement (Skinner, 1938). Within that literature, this process was proposed as the means by which arbitrary things, like colored pieces of paper, could serve as reinforcers supporting the development of new response tendencies through the acquisition of various stimulus-response associations. It is our view that the term conditioned reinforcement is a misnomer; it implies that the actions that they support are no more than habits. Of course, most human actions are acquired and maintained by goals that are associated with primary rewards and so have only an indirect connection to primary motivational systems and as such are more likely to be goal-directed actions than habits. We propose that the process that determines the acquisition of these goals be referred to, therefore, as secondary reward (SdR). Nevertheless, it is clear that the goal-directed status of these actions is something that stands in need of direct assessment.

There is, in addition, a further implication of this account. Although one should anticipate that secondary rewards will be the more potent, what this account of incentive learning portends is that, if the emotional response associated with an event determines whether it can serve as a goal, essentially any event can serve as the goal of an action providing it induces a positive change in emotional tone. In this section we describe research indicating that both stimuli associated with already established rewards and salient sensory events can serve as goals, allowing animals to acquire new responses based on the relationship of actions to these, sometimes weakly but nevertheless apparently rewarding, consequences.

1.36.3.1 Sensory Versus Secondary Reward

As mentioned, the older literature dealing with the phenomenon of conditioned reinforcement proposed that when neutral stimuli were associated with reinforcing ones, they could become conditioned reinforcers. A problem widely neglected within this literature, however, is the fact that apparently neutral stimuli turn out to be very difficult to come by. Indeed, the vast majority of experimentally utilized stimuli are demonstrably not neutral with respect to their ability to support instrumental responding even prior to any pairing with primary reward (Kish, 1966). The capacity of environmental stimuli, or more correctly, of change in the state of environmental stimuli to support instrumental behavior can, however, be well enough handled by the current claim that reward value is controlled by the emotional response associated with that event providing it is accepted that change in environmental stimuli provides a sufficiently positive change in that response. From this perspective, therefore, events that are sufficiently mild to induce a positive change provide a source of sensory reward (SeR), whether it is derived from generalization or perhaps by another source of motivation, such as a form of preparatory state produced by general affective arousal (Konorski, 1967) or perhaps, as has occasionally been proposed in the past, by a primary motivational process such as curiosity (Berlyne, 1960).

In order to use secondary reward as a tool to establish the way apparently arbitrary events can become the goals of instrumental action, it is important first to compare the influence of secondary and sensory rewards on the performance of actions. The question is, which secondary reward procedure should one employ to do so? The central position of this notion in Hull's conception of learning (Hull, 1943) and Skinner's utilization of it to explain the origin of human actions without apparent reinforcement (Skinner, 1938) drove considerable research during the middle part of the last century intended to establish or to disprove its applicability to the conditioning process. The most commonly used procedure to analyze SdR has been in chain schedules of instrumental reinforcement, where both instrumental training with the SdR and the pairing of the event with reward presumed to support that conditioned reinforcer occurred within a common sequence of behavior. Zimmerman (1969), for instance, gave rats the opportunity to press one lever in order to obtain the presentation of a stimulus light on a fixed interval. Once that stimulus was presented, a response on a second lever would result in the delivery of food. The stimulus light, via its forward pairings with the food, should have accrued associative strength over the course of performance. Because, however, responding on the first component of the chain also activated the second manipulandum in the chain, it is difficult to assert that the animal was responding for the stimulus rather than the opportunity to respond on that second manipulandum. Chain schedules, therefore, typically require some further intervention in order to partition the sources of support for instrumental responding. In this case, Zimmerman took advantage of the fact that the pattern of responding on fixed interval and variable interval schedules differs to assess whether the light was controlling performance on the first lever as a secondary reward.

To do this, he put the rewarding impacts of the light and food into competition with each other on the first lever. In a test phase, the light was presented as a result of responding on the first lever on a variable interval schedule, whereas the food was presented on the fixed interval schedule that had previously delivered the light and the second lever was shifted to an extinction schedule. Zimmerman found that the pattern of responding on the first lever shifted from that typical of a fixed interval schedule to that typical of a variable interval schedule, a finding consistent with the development of conditioned reinforcing properties by the stimulus light.

Although commonly employed, the difficulty of ruling out alternative interpretations of the source of instrumental performance on chain schedules leaves something to be desired. Since the second response, as in Zimmerman's study (1969), often becomes superfluous in the critical phase testing for the presence of SdR, it follows that it may not be necessary at all. Extinction studies of SdR reify this possibility, by utilizing a training phase where an instrumental action is paired with a stimulus that is immediately followed by the delivery of a reward. Because of the presence of the reward during training, the second, third, and higher components of the instrumental chain used to provide further conditioned stimuli and eventual primary reinforcement in chain studies of SdR are eliminated from the outset. A test phase is again required to detect the role of the SdR in the maintenance of that instrumental behavior. If the SdR plays no role in the maintenance of instrumental responding, then with or without its presence at an extinction test phase, animals should extinguish at the same rate. Instead, researchers usually find that animals extinguish much more slowly when the instrumental response leads to the delivery of the putative conditioned reinforcer than when it leads to no stimulus consequences (Bugelski, 1938).

Although these studies appear to confirm the basic effect, the most direct way to demonstrate and compare the secondary or sensory reward value of some event or other is to assess its ability to serve as the goal during the acquisition of a new action. If stimuli acquire the ability to reward instrumental actions in the course of pairing them with a primary reward, then it follows that one should be able to demonstrate the acquisition of instrumental actions that have as their sole outcome the delivery of a stimulus with a history of this pairing. This logic has been frequently employed in the detection of SdR, and procedures employing it have generally been referred to as acquisition of a new response, or simply, acquisition tests of SdR. Especially attractive is the absence of confounding effects of primary reward during training that could interfere with SdR interpretations of instrumental behaviors (Wike, 1966). Numerous experiments along these lines have been conducted by giving prior stimulus–outcome associations followed by training on a lever that delivers that stimulus. Work by Trevor Robbins and colleagues has demonstrated particularly clear acquisition of lever-press performance when that lever delivered a stimulus that was previously associated with food relative to an inactive lever that the rats could press but that had no scheduled consequences (Taylor and Robbins, 1984; Robbins et al., 1989).

We have conducted a similar experiment to those of Robbins using two different versions of their procedure, firstly to replicate their basic result but also to examine the effects of using a different control condition in which one lever delivered a stimulus that had previously been paired with food and the other lever delivered a familiar stimulus but that had not been paired with any rewarding consequence; a sensory reinforcement control (SeR). The results of this study are presented in **Figure 3**. As is clear from this figure, a good conditioned reinforcement effect was observed in both conditions: responding on the lever delivering the SdR was greater than on the inactive lever (left panel) and greater on the lever delivering the SdR than on the lever delivering the SeR. It is also clear, however, that the net size of the SdR effect is really much smaller than one might be led to believe from the difference between the active and inactive levers.

1.36.3.2 Do Secondary Rewards Reward, Reinstate, or Reinforce?

Describing events associated with primary reward as SdRs suggests that the responses that animals learn to gain access to SdRs are goal-directed. This is, however, a matter of dispute. It has often been argued in the past that, rather than developing reward value, the stimulus acquires the ability to drive instrumental responding in an S–R fashion, i.e., rather than acting as a goal in and of itself, it acts to reinforce the connection between situational cues and the response. That the conditioned reinforcing stimulus itself might not be the object of an instrumental action, but rather an elicitor of that action, is an explanation that has seen some theoretical and experimental exploration. Bugelski (1956), for instance, reinterpreted his

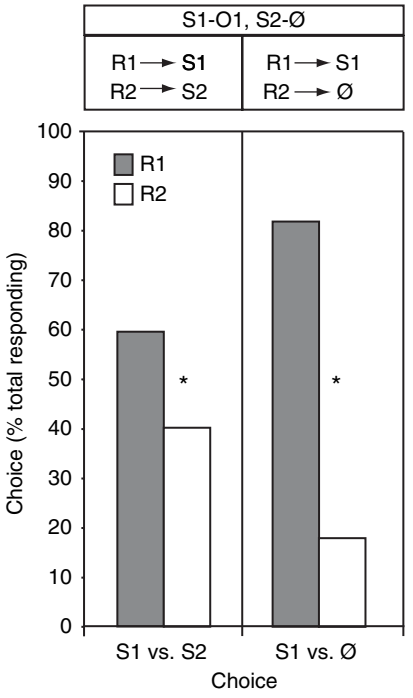

Figure 3 An assessment of secondary reward conducted in a choice test on two levers. Rats were first given pairings between one stimulus (S1) and a rewarding outcome (O1), whereas another stimulus was presented unpaired (S2). Some rats were then allowed to press two levers. In one group, one lever delivered S1 and the other S2 (left panel) in the other group, one lever delivered S1 and the other nothing (Ø). It is clear that both tasks revealed a secondary rewarding effect of S1 on performance. However, the effect is somewhat exaggerated by the choice between S1 and Ø. When sensory reward is taken into consideration, as it is in the choice between S1 and S2, the net secondary reward effect is significantly smaller.

earlier extinction test-conditioned reinforcement data (Bugelski, 1938) using this framework and found that it provided a satisfactory account of the results. During acquisition training, the SdR not only follows the instrumental response as a consequence, but on all trials except the first bears a forward predictive relationship with later occurrences of that response. It is at least possible that during acquisition the instrumental action is reinforced solely by the primary reinforcer, whereas the SdR becomes associated with the response itself. In extinction, it is argued, the conditioned reinforcer then acts, following the first response, to delay extinction through this conditioned ability to evoke or reinstate subsequent instrumental responses.

Wyckoff (1959) attempted to produce a quantitative model of SdR effects emphasizing the eliciting function, or cue properties, of the conditioned reinforcer. This model was based on the results of an experiment reported by Wyckoff et al. (1958) in which rats were given conditioning trials where a buzzer was followed by the delivery of water. Following this training, experimental rats were given the opportunity to press a lever in order to secure the delivery of the buzzer without water, and control rats were placed on an omission schedule, where the buzzer was delivered if they refrained from pressing the lever. Performance between the two groups was not reliably different, which led Wyckoff et al. to conclude that the buzzer functioned primarily not to reward lever pressing, in which case the experimental group should have pressed significantly more than the control animals, but to elicit lever pressing. This result, however, has not been replicated, suggesting that some feature of the experimental design, or a simple lack of power, prevented Wyckoff et al. from observing cue-independent conditioned reinforcing effects. Indeed, Ward (1960) conducted a formally very similar experiment, substituting food reward for water and the random delivery of the cue in the control group for the omission schedule, and demonstrated a reliably greater level of responding in experimental animals than in control animals.

An important source of evidence against the response elicitation account of SdR effects comes from an experimental series performed by Crowder and his colleagues. They employed a yoked control procedure in several different paradigms to demonstrate the existence of secondary reward above and beyond the effects of stimulus-based response elicitation. In all experiments, the experimental animals performed an instrumental action that was followed by the delivery of SdR. At the same time as that delivery, the yoked controls received noncontingent presentation of that same stimulus. If the stimulus elicited or reinstated further responding, it should have done so equally in both groups. Instead, in the extinction test paradigm (Crowder et al., 1959a), the acquisition of a new response paradigm (Crowder et al., 1959b), and in reacquisition (Crowder et al., 1959c) and retention SdR paradigms (Crowder et al., 1959d), they found superior performance in the experimental subjects whose actions were correlated with the delivery of the SdR. Although these results are not completely immune to criticism derived from the analysis of systematic sources of error in the

yoked group (Church, 1964), they indicate the relatively small degree of support that elicitation or reinstatement accounts provide for instrumental responding in SdR paradigms.

One published study has attempted to assess whether lever pressing for the SdR is goal-directed by devaluing the primary reward previously associated with the SdR (Parkinson et al., 2005). In this study rats were given pairings of a light stimulus paired with sugar after which the sugar was paired with illness. Although this reduced responding to the sucrose, it did not affect the ability of the light to serve as a secondary reward for lever pressing; lever pressing was acquired and maintained to a comparable degree whether the sucrose had been devalued or not. The authors concluded that, as the lever pressing appeared to be independent of the value of the primary reward, performance acquired through SdR should be considered habitual. But what was not confirmed in this study, however, was whether the devaluation of the sucrose was successful in modifying the reward value of the light. Indeed, as the SdR value depends on the association of the light with the emotional response elicited by the sucrose, rather than by the sucrose itself (see **Figure 4**), it seems unlikely that SdR could be undermined in this way. Rather, what this account predicts is that devaluation of the SdR could only be induced by counterconditioning, i.e., pairing the light previously paired with sucrose with a noxious consequence, such as foot shock. Would lever pressing still have been maintained after this treatment? To date no studies along these lines have been conducted, although there is plenty of evidence from studies of conditioned punishment to conclude that at least the sensory

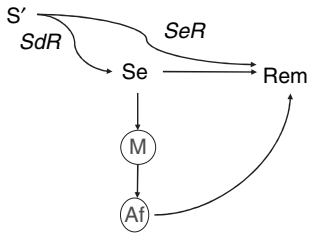

Figure 4 Sensory reward (SeR) is derived from the emotional effects (Rem) of stimulus change that can be produced by the presentation of even quite neutral stimuli (such as S′). Secondary reward (SdR) is derived from the pairing of S′ with an excitatory sensory event (Se in this diagram) previously established as a primary reward and through its association with an emotional response generated by its connection with both motivational (M) and affective (Af) processes (see **Figure 1**).

rewarding component of stimuli is abolished by this means of devaluation (Killcross et al., 1997).

Other studies from our laboratory have, however, confirmed that actions acquired for a secondary reward are essentially goal-directed. As discussed with respect to primary reward, one of the criteria for defining an action as goal-directed is that it is sensitive to the causal relationship between the action and reward. In this experiment, rats were first given pairings between two distinct visual cues with one cue paired with sucrose and the other with food pellets. After this training, the rats were trained to press two levers, each associated with a different visual cue. In these sessions, one of the visual cues was also presented noncontingently; as such the noncontingent cue was the same as that presented contingent on pressing one lever but different from that presented for pressing the other lever. As such the specific R-SdR contingency was maintained on one lever but was degraded on the other. The results of this study are presented in **Figure 5**. As is clear from this figure, the rats were sensitive to the specific lever press–SdR contingency, reducing performance on the action delivering the same SdR as that delivered noncontingently relative to the other action. This result is not consistent with either the reinforcing or reinstating functions of SdRs (Winterbauer, 2006).

An important aspect of the establishment of a secondary reward is its pairing with primary reward. The procedures that establish SdRs are, in fact, identical to those used to establish Pavlovian CRs to a stimulus. The possibility that stimuli require something more than Pavlovian conditioning to become conditioned reinforcers has been entertained; Skinner (1938) proposed, for example, in a thesis later considered in detail in the work of Keller and Schoenfeld (1950), that only stimuli that act to set the occasion for responding to other Pavlovian stimuli could serve as SdRs. Again, as Wike (1966) suggested, although occasion setters may make better SdRs, there seems to be no requirement that all conditioned reinforcers be occasion setters. Work in our laboratory has largely confirmed this view, in that we show perfectly reasonable SdR effects without special modifications to the Pavlovian conditioning phase (Winterbauer, 2006). But this raises an important issue: if SdR can be established using Pavlovian procedures, are the processes underlying reward and those underlying Pavlovian incentive motivation all one and the same? Or is this no more than a superficial, procedural similarity?

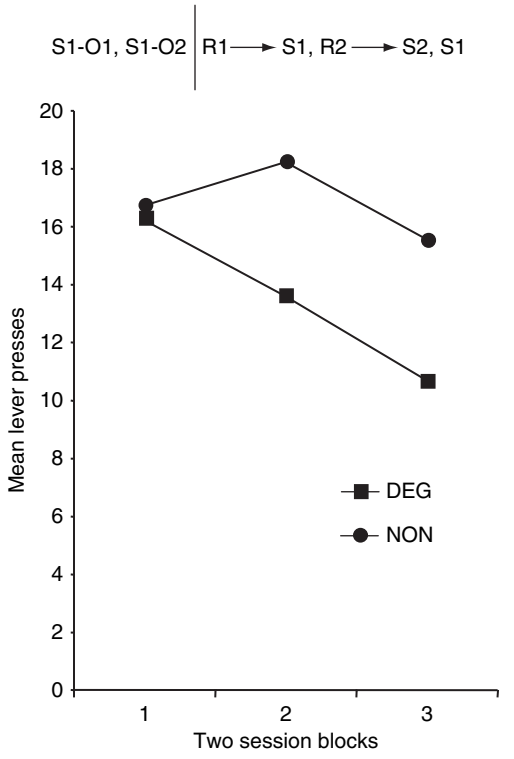

Figure 5 Contingency degradation in instrumental conditioning using secondary rewards. After establishing two stimuli (S1 and S2) as secondary rewards by pairing them with primary rewards, rats were trained to perform two lever-press responses (R1 and R2) with one earning S1 and the other S2. Noncontiguous presentations of one secondary reward (e.g., S1) degraded the response–outcome contingency (DEG) and caused a significant reduction in responding on the lever delivering S1 (DEG) relative to that delivering S2 (NON).

1.36.4 Reward and the Anticipation of Reward

The preceding sections have reviewed the considerable evidence suggesting that the influence of changes in reward value on goal-directed instrumental actions is an important determinant of action selection and of instrumental performance generally. Other factors can clearly influence performance, however. One of the most obvious, and perhaps best-documented, influences on action selection is that produced by stimuli 'associated' with reward. Advertising has a clear influence on action selection; if it did not the advertising industry would be a vacuous waste of time and of advertisers' money. Of course, advertisers are hoping that the stimuli that they associate with a particular product will provide the basis for quite specific changes in choice performance and, of course, by and large they do. It is important to recognize, however, that, despite a superficial similarity in some of the procedures used to establish the reward value of particular events, notably SdRs, there is substantial evidence suggesting that the influence of cues associated with reward on goal-directed instrumental actions is not mediated by the reward system. In this section, we describe this evidence as it has emerged from analyses of the relationship between Pavlovian and instrumental conditioning, particularly those proposing that the motivational processes engaged by reward and by the anticipation of reward are the same or, at the very least, interact with one another.

1.36.4.1 Pavlovian-Instrumental Interactions

In fact, some of the earliest evidence that the representation of the instrumental outcome takes part in action selection was found by studying how Pavlovian and instrumental learning processes interact. For instance, Trapold (1970) trained rats on a biconditional discrimination in which, on any given trial, subjects were allowed to choose between two actions (left and right lever press). Trials were initiated by the presentation of one of two discriminative stimuli (tone and clicker), signaling which of the actions would be rewarded (e.g., S1 → R1 and S2 → R2). The novel feature of this experiment, however, was that these cues also signaled the identity of the outcome that could be earned on that trial. Whereas the control groups earned either food pellets (food control) or sucrose solution (sucrose control) on both actions (e.g., S1 → R1 → O1 and S2 → R1 → O1), the experimental group was rewarded with one outcome (O1; e.g., pellets) for performing one action and a different outcome (O2; e.g., sucrose) for performing the other action (e.g., S1 → R1 → O1 and S2 → R2 → O2). Consequently, the experimental group differed from the control groups in that, for the former, each discriminative stimulus signaled not only a different response but also a different outcome. Interestingly, Trapold (1970) found that the experimental group acquired more rapidly than either control group despite the fact that the S–R arrangements needed to solve the discrimination were the same across conditions.

This phenomenon, known as the differential outcomes effect, provides clear evidence that reward expectations can be used to guide action selection.

Moreover, the representation mediating this effect appears to consist of richly detailed information about the sensory properties of the reward. In Trapold's (1970) study, the sucrose solution and grain-based pellets used to differentially reward the two actions were both nutritive outcomes and so should have held a similar incentive value for hungry rats. Since this motivational variable does not appear to have been used to discriminate between actions, rats probably relied instead on the sensory features (e.g., texture, odor, taste) of the anticipated outcome. There is even evidence that this effect can be obtained using outcomes that differ in one motivationally irrelevant sensory feature. Fedorchak and Bolles (1986) trained thirsty rats on a biconditional lever press discrimination task in which each correct response was rewarded with water. For two groups, the delivery of water was occasionally paired with a flashing light; whereas the light exclusively followed just one of the two S–R arrangements in the differential outcomes group, it followed both responses with an equal probability in the nondifferential control group. For a third group, the light was never paired with water. Once again, the group that received differential outcomes acquired more rapidly than the other two groups, demonstrating that the expectancy of a sensory event extraneous to outcome itself could be used to guide action selection.

How does differential outcomes training provide an advantage in discriminating between two actions? Clearly, it must have something to do with the Pavlovian contingencies embedded in the task (see **Figure 6**, top panel). It has long been argued that Pavlovian learning plays an important role in the control of instrumental performance (Rescorla and Solomon, 1967). Although we will discuss alternative accounts shortly, let us first consider the model Trapold and Overmier (1972) devised to explain the differential outcomes effect and similar findings (see **Figure 6**, middle panel). Their model was built within the general framework of traditional S–R theory (Hull, 1943), and so instrumental learning was assumed to involve the gradual recruitment of S–R associations through a conventional reinforcement process. However, Trapold and Overmier (1972) proposed that reward deliveries engage a second, Pavlovian learning process capable of supporting the acquisition of stimulus–reward associations. It was argued that through such learning, stimuli acquired the capacity to elicit a reward expectancy comprising the sensory features of that event. The final step in their argument was in allowing this reward

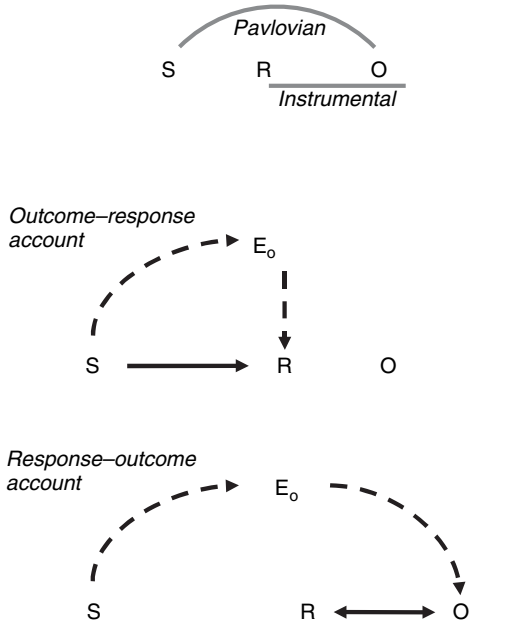

Figure 6 Schematic diagrams illustrating the associative structure proposed to underlie instrumental conditioning on various accounts. As shown in the top panel, the introduction of an instrumental (R–O) contingency is typically accompanied by an imbedded Pavlovian (S–O) contingency, arising from incidental pairings between contextual cues and reward. Two-process accounts of action selection have proposed that Pavlovian learning results in the generation of an outcome expectancy (E_o), which may guide performance by entering into association with the instrumental response (middle panel), or by retrieving any response that had earned the expected outcome (bottom panel).

expectancy to enter into S–R associations like any other sensorial event in the training environment, i.e., the expectation of reward was assumed to acquire discriminative control over performance. According to this analysis, the experimental group in Trapold's (1970) study was provided with an additional source of stimulus support for action selection; the correct choice was signaled by both an auditory cue and an expectation of the reward that could be earned on that trial.

The differential outcomes effect provides strong evidence that the Pavlovian learning can influence instrumental performance through a highly specific representation of the mediating outcome. Further evidence for this claim comes from studies of the so-called Pavlovian-instrumental transfer effect. For instance, Kruse et al. (1983) first trained rats using a biconditional procedure quite similar to that used in differential outcomes studies (e.g., Trapold, 1970;

Fedorchak and Bolles, 1986), such that each stimulus (clicker and tone) signaled both the response (left or right lever) that would be rewarded and the identity of its outcome (pellets or sucrose solution). During a separate Pavlovian training phase, the group of interest to our current discussion received pairings between a stimulus (pause in the background white noise) and one of the two outcomes (either pellets or sucrose). In a subsequent test phase, Kruse et al. (1983) found that presentations of this stimulus facilitated instrumental performance in an outcome-dependent manner; rats preferentially increased their performance of the action that had shared a common outcome with that cue, relative to the other action. Importantly, this test was conducted in extinction, indicating that this effect relied entirely on information acquired during earlier training phases.

Following Kruse et al. (1983), there have been numerous demonstrations of outcome-specific transfer (Colwill and Rescorla, 1988), even using actions that had been acquired through free operant training (Colwill and Motzkin, 1994; Delamater, 1995; Holland, 2004). The latter finding is important because it reveals that Pavlovian learning can influence action selection even under conditions in which anticipating reward provides no obvious advantage in obtaining reward. According to Trapold and Overmier (1972), the transfer effect emerges because the Pavlovian outcome expectancy selectively retrieves the response it signaled during training through the activation of an outcome–response association (see **Figure 6**, top panel). This account applies equally well to the free operant situation. Note that in any instrumental conditioning study there exists an embedded Pavlovian relationship between contextual cues and the reward delivery. In this case, cues that best predict reward should come to elicit an expectancy of reward capable of entering into association with the response.

Of course, the two-process account of Trapold and Overmier (1972) does not provide the only explanation for the influence of Pavlovian reward expectancies over instrumental performance. For instance, several two-process theories have been proposed that assume instrumental learning involves encoding some approximation of the action–outcome contingency arranged by the experimenter (Bolles, 1972; Asratyan, 1974). According to this view, Pavlovian outcome expectancies guide action selection by retrieving the action that had actually earned that outcome during training (see **Figure 6**, bottom panel).

1.36.4.2 The Two-Process Account of Reward Value

How is Pavlovian-instrumental transfer relevant to our interpretation of instrumental performance as an instance of goal-directed action? Recall that in order to be considered goal-directed, a behavior must be performed because of its expected consequences; performance should depend on the subject's capacity to (1) anticipate the outcome of the action (i.e., action–outcome learning) and (2) evaluate the incentive properties of that outcome (i.e., incentive learning). Two-process theories, however, tend to attribute incentive effects, such as the sensitivity of instrumental performance to outcome devaluation, to the Pavlovian process (Rescorla and Solomon, 1967). These accounts typically assume that Pavlovian learning provides the motivational support for instrumental performance. Even Trapold and Overmier (1972), who took an expressly associative approach, entertained the possibility that incentive manipulations have their effect by disrupting the capacity of the Pavlovian outcome expectancy to mediate response selection (e.g., through generalization decrement). Others have taken a more explicitly motivational position. Bolles (1972), for instance, proposed that Pavlovian and instrumental processes interact based on their shared outcome expectancies, but that this interaction is gated by the incentive value of mediating outcome. The two-process approach, therefore, provides a compelling explanation for the influence of reward value over performance. According to this account, instrumental responding is depressed following outcome devaluation, not because of a reduction in the reward value of the outcome and knowledge of the underlying response–outcome contingency, but because this treatment diminishes the Pavlovian support for performance.

The claim that Pavlovian learning plays a part in action selection is beyond doubt. The critical question, however, is whether these processes are responsible for the influence of reward value over performance. If so, it would be necessary to abandon the goal-directed interpretation of instrumental performance altogether. Note that since the two-process account uses the Pavlovian–instrumental interaction responsible for transfer to explain the sensitivity of performance to outcome devaluation, it predicts that these two apparently distinct forms of action selection should share a common associative structure. One way to evaluate this prediction, therefore, is to assess whether the associations guiding

transfer and outcome devaluation are acquired at roughly the same rate. For instance, it has been repeatedly shown that, while sensitivity to outcome devaluation emerges with rather limited training (Holland, 2004; Yin et al., 2005), depending on training parameters used (e.g., number of action–outcome contingencies), this effect is either maintained (Colwill and Rescorla, 1985a) or attenuated (Adams, 1981; Holland, 2004) with training that is more extensive. Alternatively, recent evidence suggests that Pavlovian-instrumental transfer increases in magnitude with more extensive instrumental training (Holland, 2004).

It should also be possible to evaluate the two-process account by analyzing the content of the associations that mediate transfer and outcome devaluation. However, it is important to remember that individual two-process theories do not agree on what that content should be. Trapold and Overmier (1972), for instance, argued that the response becomes associated with an expectancy of reward generated by prevailing stimuli, resulting in an outcome–response association. As we have mentioned, others (e.g., Asratyan, 1974; Bolles, 1972) have proposed that the response becomes associated with the outcome it actually produces during training, in the form of a response–outcome association. Two-process theories, therefore, can be distinguished by determining whether the association responsible for action selection reflects the actual response–outcome contingency, or whether it is, instead, the product of the incidental stimulus–outcome contingency present during training. However, investigating the relative contribution of these two contingencies to instrumental learning is no trivial task. In any typical instrumental conditioning study, the outcome earned by the response is also predicted by the prevailing situational cues (i.e., the anticipated and earned outcomes are the same). Thus, one approach to the problem is to create a training situation in which this in not the case.

Several studies have used this basic strategy to assess the associative structure underlying transfer and outcome devaluation. For instance, Colwill (1994) reported evidence of outcome selective transfer with responses that had been concurrently trained on distinct action–outcome contingencies. Similarly, Colwill and Rescorla (1985b) reported that rats display an outcome-specific devaluation effect after concurrent training of this kind. Since rats given concurrent training are allowed to alternate freely between responses, the context should be associated equally with both outcomes, thereby preventing the development of specific outcome–response associations. The specificity of transfer and outcome devaluation despite this treatment, therefore, seems to suggest that both effects can be supported by response–outcome learning.

Rescorla and Colwill (1989) and Rescorla (1992) have attempted more directly to compare the relative contribution of outcome–response and response–outcome associations to these effects. For instance, Rescorla and Colwill (1989) investigated this issue by first pretraining rats on a common nose-poke response with four distinct stimuli; two stimuli (S1 and S3) signaled a pellet reward and two others (S2 and S4) signaled a sucrose solution. Next, they were given discrimination training on two responses (R1 and R2), such that one response, say R1, earned pellets and the other response, R2, earned sucrose. However, each response was also signaled by a stimulus that had previously been paired with the alternative outcome (i.e., $S2 \rightarrow R1 \rightarrow O1$ and $S1 \rightarrow R2 \rightarrow O2$). According to Trapold and Overmier's (1972) two-process account, this should have resulted in the formation of, for example, a sucrose–R1 association, even though R1 had actually been followed by pellets. During the transfer test, rats were allowed to perform each response in extinction while S3 and S4 were occasionally presented. In contrast to the predictions of the outcome–response view, it was found that stimulus presentations selectively facilitated performance based on the identity of the outcome that 'followed' a response during training (e.g., S3 increased R1 relative to R2). Furthermore, in a separate experiment, Rescorla and Colwill (1989) used the same strategy to investigate the structure underlying outcome devaluation performance. They found that, as with transfer, the sensitivity of instrumental performance to reward value was dominated by response–outcome learning; performance was suppressed by devaluing the outcome that the action had actually earned during training, not the outcome that was signaled by the discriminative stimulus (e.g., devaluing O1 decreased R1 relative to R2).

There is, however, reason to question whether these experiments provide a fair test of the outcome–response account. This basic approach, of course, depends entirely on the experimenter's capacity to create a situation in which the expectation of reward differs from the reward that is obtained by responding. In Rescorla and Colwill's (1989) study, for instance, each discriminative stimulus was pretrained so that it would signal a different outcome

from the one that would be earned on that trial. Since this phase of the experiment was conducted over 4 days, however, it is possible that rats were able to learn the new stimulus–outcome relationships (e.g., S1 → O2), nullifying the effects of pretraining. Rescorla (1992) addressed this issue in an experiment otherwise quite similar to the first (Rescorla and Colwill, 1989), except that, during the discrimination phase, each stimulus continued to be paired with the outcome that it predicted during initial pretraining, while at the same time signaling that responding could earn the opposite outcome. These additional Pavlovian trials were added to encourage the persistence of the initial stimulus–outcome learning, thereby providing greater opportunity for any potential outcome–response associations to form during the instrumental discrimination training. Using this new procedure, Rescorla (1992) once again found no evidence that outcome–response associations play a part in outcome devaluation performance. However, the results of transfer testing were less straightforward. He observed that stimulus presentations tended to increase the performance of both responses, although this effect was larger for the response that had 'earned' the outcome signaled by the transfer stimulus than it was for the response that had been trained in 'anticipation' of that outcome. Thus, while these findings suggest that both outcome devaluation and transfer are dominated by response–outcome learning, they also indicate that outcome–response associations may play some, albeit limited, role in the latter.

This conclusion does not help the two-process account of reward value. According to this account, the processes underlying transfer and outcome devaluation should be identical. Perhaps more importantly, however, these studies illustrate the difficulty in attempting to dissociate the contributions of Pavlovian and instrumental learning to performance. Indeed, even in these studies it is possible that the subjects were able to confound the experimenter's intentions and acquire appropriate stimulus–outcome associations during instrumental training based on the relationship between the features of the individual response manipulanda and the outcome earned by those responses. For instance, rats trained to press a lever for pellets and pull a chain for sucrose solution may come to associate the lever itself with pellets and the chain with sucrose. Such learning would ensure that the rat anticipated the reward that they would actually obtain for performing the response, even in the presence of a context that signaled both rewards (e.g., Colwill and Rescorla, 1985b; Colwill, 1994) or a Pavlovian cue that signaled a different reward (e.g., Rescorla and Colwill, 1989; Rescorla, 1992).

This problem can be avoided, however, by training distinct action–outcome contingencies on a common response manipulandum. For instance, Dickinson et al. (1996) trained rats to push a vertically positioned pole to the left and right for different outcomes; for half the rats, left pushes earned food pellets and right pushes earned a maltodextrin solution, whereas the other half was trained with the opposite arrangement. Rats were then sated on one of the two outcomes in order to selectively reduce its reward value. Immediately after this treatment, they were given an extinction test in which the pole was available and could be pushed freely in either direction without consequence. Dickinson et al. (1996) found that, despite having both actions trained on a common manipulandum, the rats were able to use response–outcome training relationships to guide their action selection according to outcome value; rats were less likely to push the pole in the direction that had earned the now devalued outcome, relative to the other direction. This finding is incompatible with the two-process account, which predicts that outcome-selective devaluation should never emerge in the absence of differential stimulus–outcome contingencies. Instead, it provides strong support for the view that instrumental performance is goal-directed and that its sensitivity to reward value depends on response–outcome learning.

One final method for evaluation of the two-process account of reward value involves assessing the interaction between transfer and outcome devaluation. If these phenomena rely on the same underlying structure, then the capacity of a Pavlovian cue to facilitate performance should depend on the value of the mediating outcome representation. Colwill and Rescorla (1990) directly investigated the role of incentive value in outcome selective transfer. Rats were initially given biconditional discrimination training using differential outcomes, such that one stimulus (S1) signaled that pellets could be earned on one response (R1) and the other stimulus (S2) signaled that sucrose could be earned on a different response (R2). Subsequently, they were given free operant training on two new responses (R3 and R4), such that each earned a unique outcome (either pellets or sucrose). One outcome was then devalued through

conditioned taste aversion and then a transfer test was conducted in extinction, with both R3 and R4 available. Although rats were, in general, less likely to perform the response that had earned the devalued outcome than the other response, both responses were selectively facilitated by presentations of the stimulus with which they shared a common outcome. Moreover, the magnitude of this transfer effect, measured in the difference from baseline performance, was comparable across responses. This basic finding, that devaluing an outcome fails to diminish its capacity to mediate Pavlovian-instrumental transfer, has since been replicated in a number of studies (Rescorla, 1994; Holland, 2004).

Altogether, there appears to be scant support for the two-process account of reward value. The associative processes supporting outcome devaluation and transfer appear to be acquired at different rates and encode somewhat different content. Furthermore, instrumental responses remain sensitive to outcome devaluation under conditions that cannot support differential stimulus–outcome learning. Finally, the Pavlovian-instrumental interaction responsible for transfer does not appear to depend on the reward value of the retrieved outcome. Instead, these findings strengthen the goal-directed view of instrumental action and, while demonstrating that reward anticipation influences action selection, it is also clear that this effect is not mediated by the reward system.

1.36.5 Summary and Conclusions

We have argued that the reward system is a specialization that developed in the service of goal-directed action allowing animals to encode the relative values of specific environmental events. These values provide the basis for choice, allowing animals to decide on a course of action based not only on knowledge or information as to the consequences of an action but on the basis of the value of those consequences.

Encoding the reward value of a particular event involves the formation of an association between the specific sensory representation of that event and an emotional response. In the case of primary rewards, the emotional response is directly determined by the activity of specific motivational and affective processes engaged during consummatory contact with the outcome. Thus, by virtue of their biologically active properties (e.g., nutrient, fluidic, pheromonal), rewarding events (food, fluid, sex objects, and so on) are readily able to activate these underlying systems that modify emotional responses as one of the consequences of that activation. Basing the evaluation of primary rewards on emotional responses is adaptive if those responses are determined by the operation of these basic motivational and affective systems, which is essential if the animal's choice between alternative courses of action is to remain, by and large, adaptive too. In the case of secondary rewards, the emotional response is, of course, determined by the primary reward with which it is paired. By basing the transfer of value from primary to secondary rewards on an emotional response, the selection of actions, even when they are directed toward achieving apparently quite arbitrary goals, can be understood as being constrained by primary motivational processes through their influence on emotional responses.

Finally, we addressed the distinction between the role the reward system plays in assigning reward value and the processes controlling the anticipation of reward. These are quite distinct aspects of behavioral control; although cues that signal forthcoming rewards can provide information that can be used by the goal-directed system, they do not depend, ultimately, on the reward system to play that role. As such, the influence of reward-related cues on action selection does not replace or explain away the functions of the reward system in this regard. Rather, the distinct processes mediating the effects of reward and of the anticipation of reward provides the basis for understanding the role that cognitive processes generally play in goal-directed action. Because it constrains the event relations to which an animal is exposed, there has been a long tradition of using Pavlovian conditioning to model the cognitive control of behavior. The fact that, ultimately, this system is concerned with the production of reflexive responses would, however, appear to render this approach perhaps a little too abstract. It makes more sense to study the role of cognition in a behavioral system within which information can act to influence performance. Based on the evidence reviewed here that animals are able to exert control over their instrumental actions, choose between actions based on the relative value of their consequences, and use predictive information to influence action selection, we suggest that instrumental conditioning provides the more precise model of this capacity.

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1.37 Synchronous Oscillations and Memory Formation

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1.37.1 Introduction

It is commonly held that the neuronal mechanisms supporting the formation of memories consist of use-dependent long-term modifications of synaptic transmission. In order to explain the formation of new associations, Donald Hebb had postulated that connections among neurons should strengthen if the coupled neurons are repeatedly active in temporal contiguity (Hebb, 1949). This prediction has been confirmed by the seminal discovery of long-term potentiation (LTP) in the hippocampus by Bliss and Lomo (1973). They had found that tetanic stimulation of excitatory pathways led to a long-lasting enhancement of the efficacy of the synapses between the activated fibers and the respective postsynaptic target cells. Later it had been shown that this increase in synaptic efficacy occurred only if the postsynaptic cells were actually responding with action potentials to the tetanic stimuli, thus fulfilling the criterion of contingent pre- and postsynaptic activation. If postsynaptic cells were prevented from responding, modifications either did not occur or had opposite polarity, i.e., they consisted of a reduction of synaptic efficacy. This phenomenon has become known as long-term depression (LTD). It is now well established that both modifications depend on a surge of calcium in the subsynaptic space of the postsynaptic dendrites and that the polarity of the modifications depends on the rate of rise and the amplitude of this calcium increase (Bröcher et al., 1992b; Hansel et al., 1996, 1997). Fast and strong increases lead to LTP, while slow and smaller increases trigger LTD. Moreover, the source of the calcium increase is of importance. Calcium entering through *N*-methyl-D-aspartate (NMDA) receptor-associated channels favors the induction of LTP, whereas calcium entering through voltage-dependent calcium channels is more likely to trigger LTD. However, both modifications can be obtained merely by raising intracellular calcium concentrations through the liberation of caged calcium in a concentration-dependent manner (Neveu and Zucker, 1996). A vast number of studies have subsequently been performed in order to elucidate the molecular cascades mediating these use-dependent changes in synaptic transmission which involve both changes in transmitter release and postsynaptic susceptibility. This has led to a deep understanding of the extremely complex regulatory processes that translate neuronal activity into lasting changes of synaptic transmission (*See* Chapters 1.33, 1.34, 1.35).

It had long been held that the polarity of use-dependent synaptic gain changes depended merely on the extent to which pre- and postsynaptic activity was correlated in time as initially proposed by Hebb. The evidence that activation of NMDA receptors was one of the decisive variables in determining the polarity of synaptic gain changes agreed with this notion because these channels open and become permeable for calcium ions only if the excitatory transmitter glutamate is bound to the receptor and if the postsynaptic cell is sufficiently depolarized to remove the magnesium block that makes the channel impermeable at hyperpolarized membrane potential levels (Artola and Singer, 1987; Kleinschmidt et al., 1987; Bear et al., 1990). Thus, LTP would occur in all cases where presynaptic afferents are sufficiently active while the postsynaptic cells are sufficiently depolarized (Artola et al., 1990). Since the level of depolarization of the postsynaptic neuron not only depends on the level of activity of the pathways under consideration, but also on all the other excitatory and inhibitory inputs, this mechanism can also elegantly account for the cooperativity of synaptic modifications. Even weak inputs can increase their gain if at the same time strong inputs are active to assure sufficient depolarization of the postsynaptic membrane to remove the magnesium

block. Likewise, concomitant activation of inhibitory inputs can prevent NMDA receptor activation at levels of presynaptic activity that would normally induce LTP. In this case, the outcome is usually LTD (Artola et al., 1990).

In conclusion, this mechanism is ideally suited to strengthening excitatory interactions among pairs of cells that are frequently activated in temporal contiguity, and because of cooperativity, it strengthens excitatory inputs converging on a common target cell if these inputs are frequently active in temporal contiguity. Conversely, modifications of opposite polarity occur if the respective activation patterns are anti-correlated. In this conceptual framework, the crucial variable that determines the occurrence and the polarity of synaptic gain changes is the discharge rate of the pre- and postsynaptic elements and the temporal coherence of fluctuations in discharge rate. The precise timing of individual spikes appeared irrelevant in this context.

1.37.2 Spike Timing Synaptic Plasticity

This notion has changed considerably over the last few years because of three initially independent lines of research that converged in the conclusion that the precise timing of spikes matters both in signal processing and synaptic plasticity (Gray and Singer, 1989; Gray et al., 1989; Markram et al., 1997). In experiments based on paired recordings from coupled neurons in slices, it was deduced that the polarity of synaptic gain changes depended crucially on the temporal relation between excitatory postsynaptic potentials (EPSPs) and postsynaptic action potentials. It was discovered that somatic action potentials can propagate backward into the dendrites because of activation of voltage-dependent dendritic sodium channels and electrotonic propagation and that contingency of this back-propagating action potential with a simultaneously generated EPSP could be sufficient to remove the magnesium block of the NMDA channels and in certain cases even allow for the generation of a dendritic calcium spike, thus allowing for a sufficient increase in postsynaptic calcium to induce LTP (Stuart and Häusser, 2001). By varying the timing between the EPSP and the back-propagating spike, in this case elicited by somatic current injection, it was revealed that the timing between individual EPSPs and back-propagating spikes was critical both for the induction of long-term modifications and the determination of their polarity. No changes occurred when the interval between the EPSP and the back-propagating spike was longer than about 50 ms. When the EPSP preceded the back-propagating action potential, the probability of obtaining LTP increased with decreasing delays and then there was a sharp transition toward LTD as soon as the EPSP occurred after the back-propagating spike. This discovery of spike timing-dependent plasticity (STDP) had two important implications: First, it showed that the precise timing of spikes matters in determining the occurrence and polarity of synaptic gain changes. Second, the mechanism subserving synaptic modifications not only evaluates simple covariations between pre- and postsynaptic firing rates, but also evaluates causal relations. It increases the gain of excitatory connections whose activity can be causally related to the activation of the postsynaptic neuron and it weakens connections whose activity could not have contributed to the postsynaptic response (Markram et al., 1997; but see Stiefel et al., 2005).

At about the same time, evidence was obtained suggesting that the precise timing of individual spikes is relevant in signal processing. In vivo recordings from higher visual areas, the auditory and the somatic sensory cortex, revealed that the discharge latencies of individual neurons could signal the temporal structure of stimuli with an extreme precision in the millisecond range, suggesting that precise timing of discharges can be preserved despite numerous intervening synaptic transmission steps (Buracas et al., 1998; Reinagel and Reid, 2002). Simulation studies, partly based on the concept of synfire chains proposed by Moshe Abeles (1991), confirmed that conventional integrate-and-fire neurons are capable of transmitting temporal information with the required precision (Mainen and Sejnowski, 1995; Diesmann et al., 1999).

Parallel to these discoveries, evidence has been obtained in the visual system for the existence of mechanisms capable of adjusting the timing of individual spikes, again with millisecond precision independently of external stimuli (Gray and Singer, 1989; Gray et al., 1989; Engel et al., 2001; Fries et al., 2001a). Using multielectrode recording techniques, it was shown that neurons in the visual cortex engage in oscillatory firing patterns in the range of the gamma frequency band and that these periodic discharges are synchronized with a precision in the millisecond range. This synchronization was observed not only between neurons located within the same cortical area, but also between cells in different cortical areas

and even among cells in corresponding areas of the two hemispheres (Roelfsema et al., 1997; Singer, 1999). While synchronization probability reflected to some extent the layout of corticocortical association connections (Löwel and Singer, 1992), it soon turned out that this was the result of a highly dynamic self-organizing process that enables rapid reorganization of synchronized cell assemblies as a function of stimulus configuration, central state, and attentional mechanisms (for review, see Singer, 1999; Engel et al., 2001). Initially, this precise synchronization of discharges of spatially distributed neurons was seen mainly in the context of low-level visual processes such as feature binding and figure ground segregation. Later it became clear that it is a ubiquitous phenomenon in most structures of the nervous system (Castelo-Branco et al., 1998; Brecht et al., 1999) and with all likelihood serves a large number of different functions that all have to do with the coordination and selective routing of activity in the context of distributed processing (Womelsdorf et al., 2006). Synchronized discharges have a stronger impact on target neurons than temporally dispersed inputs and this effect means that synchrony can be used as a complementary mechanism to rate increases in order to raise the saliency of neuronal signals (Biederlack et al., 2006). The difference is that synchrony can enhance the saliency of discharge patterns on a spike-by-spike basis with very high temporal resolution.

Extensive experimental and theoretical studies have since demonstrated that the oscillatory patterning of neuronal activity is crucial for the adjustment of precise spike timing (König et al., 1995; Volgushev et al., 1998). Because of reciprocal coupling via chemical and electrical synapses, networks of inhibitory interneurons engage in oscillatory activity, the frequency of which is often characteristic for particular networks and conveys periodic inhibition to excitatory neurons, in the case of the neocortex and the hippocampus pyramidal cells (Whittington et al., 2001). The effect is that the timing of spikes generated by pyramidal cells does not solely depend on the timing of the arriving EPSPs. During the hyperpolarizing phase of the oscillation cycle, arriving EPSPs have only a small chance to drive action potentials in the postsynaptic neuron because of the initial shunting and then hyperpolarizing effect of the barrages of IPSPs. This limits spiking essentially to the depolarizing phase in the peak of the oscillation cycle, causing synchronization of discharges in cells oscillating in phase. Moreover, the precise timing of spikes generated during the depolarizing cycle does depend on the strength of the excitatory input (Fries et al., 2007). Strong excitatory input will elicit spikes earlier during the depolarizing cycle than a weak input. Thus, the phase relation between the time of occurrence of a spike and the peak of the oscillation cycle does reflect the amplitude of the excitatory drive that generated the spike. Through this mechanism, known as phase precession in the hippocampus for example, rate-coded amplitude values can be converted into a temporal code that is expressed in the timing of spikes relative to the oscillation cycle. As has been shown in numerous theoretical studies, such temporal codes are advantageous for fast processing because information on spike times can be transmitted and read out much more rapidly than information encoded in discharge rates because it does not require temporal integration, neither for transmission nor for readout (for review, see Fries et al., 2007).

There is thus a mechanism that can evaluate the precise temporal relations among individual spikes for the gating of synaptic plasticity, and there is a mechanism to adjust the precise timing of spikes during signal processing and to convert rate-coded information into timing relations. Both the classical Hebbian mechanism as well as STDP, which most certainly do coexist, evaluate temporal relations among activity patterns in order to convert correlations into synaptic gain changes. For these modifications to be meaningful, the signatures of relatedness used for engram formation must be exactly the same as those used for signal processing. Otherwise, correlations occurring spuriously during signal processing would lead to changes in synaptic coupling that are functionally meaningless. Because STDP does depend so critically on the precise timing of spikes and is susceptible to changes in timing relations on the order of the millisecond, relations between the distributed firing of neurons need to be defined and evaluated with similar precision during signal processing. However, this requires that neurons can act as coincidence detectors and distinguish between precisely synchronized and temporally dispersed input activity.

A third line of independent evidence also favors the notion that precise spike timing may play an important role in neuronal processing and memory formation. Data are accumulating which indicate that cortical circuits, synaptic properties, and the characteristics of neurons are optimized for the transmission and detection of coincident activity. A prominent feature of cortical connectivity is sparseness and as proposed by Abeles and confirmed later in extensive

simulation studies (Mainen and Sejnowski, 1995; König et al., 1996; Diesmann et al., 1999), such networks strongly favor transmission of synchronized activity over transmission of temporally dispersed activity. Likewise, the frequency adaptation of transmitter release and the adaptation of postsynaptic receptors attenuate transmission of frequency-coded information. These adaptive mechanisms favor transmission of singular synchronized events and tend to filter out discharge sequences occurring at high frequencies. Sensitivity to single but coincident EPSPs is further enhanced by cooperative mechanisms in the postsynaptic dendrites. The existence of voltage-dependent sodium and calcium channels in the dendrites and their ability to convert high-amplitude EPSPs such as result from coincident input into regenerative spikes greatly enhances the coincidence sensitivity of cortical neurons (Stuart and Häusser, 2001; Ariav et al., 2003). Finally, there is evidence that spike thresholds lower as the depolarizing slope preceding the spike becomes steeper. This also favors responses to coincident inputs as compared to temporally dispersed inputs. Last but not least, the membrane time constants of cortical neurons are shorter than previously assumed, especially when the neurons are in the up state: this is due to the reduced membrane resistance caused by the simultaneous bombardment by EPSPs and IPSPs in the up state.

Thus, several lines of evidence indicate that cortical networks that are thought to support memory formation by mechanisms of use-dependent synaptic plasticity are capable of exploiting precise temporal relations among the discharges of interconnected neurons for signal processing, the encoding of information, and the induction of corresponding gain changes.

However, if precise timing of discharges is achieved through an oscillatory modulation of neuronal excitability, especially when it comes to the coordination of timing relations over larger distances, and if synaptic gain changes depend essentially on STDP, a problem arises. If coupled neuron groups engage in oscillatory activity in the same frequency range but oscillate 180 degrees out of phase, one can anticipate situations in which EPSPs always arrive in the trough of the oscillation cycle and hence at equal distance to the preceding and the following peak of enhanced excitability where spikes tend to occur. Thus, EPSPs are preceded and followed by spikes at the same interval. In this case, different outcomes are predicted by the classical Hebbian correlation rule and STDP. If the pre- and postsynaptic neurons discharge at high frequencies, as would be the case, for example, if they are engaged in gamma oscillations, the classical Hebbian rule would predict LTP because of the contingency of high-frequency pre- and postsynaptic activation. The STDP rule cannot make a clear prediction because EPSPs are preceding and following spikes at about the same interval, which should lead to a cancellation of the antagonistic effects. This problem has recently been addressed in an in vitro study on slices of the visual cortex (Wespatat et al., 2004). The results indicated that inputs oscillating at the same frequency as the target cell (20 or 40 Hz) underwent LTP when they were in phase with the depolarizing peak of the oscillation cycle, whereby it was irrelevant whether the EPSPs arrived shortly before or after the action potentials, whereas the same input underwent LTD when it oscillated 180 degrees out of phase so that the EPSPs arrived during the hyperpolarizing troughs of the oscillation cycle of the postsynaptic neuron.

1.37.3 Evidence for Relations between Oscillatory Activity, Synaptic Plasticity, and Learning

Given that learning mechanisms evaluate temporal correlations among the activity patterns of coupled neurons and that the temporal patterning of neuronal activity is often structured by an oscillatory modulation, one expects to find close relations between memory processes and oscillatory activity. Although research on this issue is still at its very beginning, such evidence is indeed available.

Indirect evidence for a relation between synaptic plasticity and oscillatory patterning comes from studies relating oscillatory activity to central states and attentional mechanisms. While synaptic modifications can be induced with artificial stimulation conditions irrespective of the state of neuronal networks and even in fully deafferented slice preparations, there is consensus that under natural conditions learning-related modifications of synaptic gain occur only when the brain is in an activated state and when attentional mechanisms are functional. The reason is that synaptic modifications require the presence of an appropriate mix of neuromodulators such as acetylcholine, dopamine, and noradrenaline and that these modulators are available in the required concentrations only when the brain is in an awake and activated state. Another likely reason is that the induction of synaptic modifications requires a minimum of cooperativity, for example a sufficient amount of synchronized input activity, and that such cooperativity is only achieved in the

activated brain. These activated brain states favor the emergence of oscillatory activity in various frequency bands and the synchronization of the oscillations across structures that need to cooperate for memory processes. In this context, the most important frequency bands appear to be the theta, the beta, and the gamma. Gamma oscillations in the neocortex and the associated precise synchronization of neuronal discharges are greatly facilitated by the presence of acetylcholine and its action on muscarinic receptors (Munk et al., 1996; Herculano-Houzel et al., 1999). Acetylcholine also facilitates use-dependent synaptic modifications and it is likely that there is a relation between the two phenomena (Bröcher et al., 1992a; Wespatat et al., 2004). Likewise, attention facilitates gamma oscillations (Fries et al., 2001b) and favors learning (Bröcher et al., 1992; Wespatat et al., 2004). Preliminary evidence suggests that there may be a causal relation between the occurrence of synchronized oscillatory activity in the gamma-frequency band and the induction of synaptic plasticity. It is possible to modify the receptive fields of neurons in the visual cortex by appropriate visual stimulation even in anesthetized preparations if the brain is concomitantly activated by electrical stimulation of the mesencephalic reticular formation (unpublished observation). This stimulation increases the release of plasticity-enhancing neuromodulators and at the same time favors the occurrence of gamma oscillations in response to the applied stimuli. Post hoc analysis of the neuronal responses to the change-inducing light stimuli revealed that only those trials caused lasting changes in receptive field properties that were associated with a strong oscillatory modulation and synchronization of neuronal responses in the gamma band. A similar relation, albeit in the theta-frequency range, has been found in the hippocampus (Huerta and Lisman, 1995). Here, the so-called beta-burst stimulation that entrains the hippocampus in the characteristic theta rhythm of the structure turned out to be particularly effective for the induction of long-lasting synaptic modifications. Moreover, when the hippocampal circuits were engaged in spontaneous theta oscillations, the effectiveness of the stimuli applied for the induction of synaptic gain changes and the polarity of the resulting synaptic modifications depended critically on the phase relation between the ongoing theta activity and the change-inducing stimuli (Huerta and Lisman, 1995). A similar finding has been obtained in the somatosensory barrel cortex, where synchronous oscillatory activity occurs in conjunction with the whisking movements. Again, the efficacy of the change-inducing electrical stimuli depended critically on the timing of the stimuli relative to the whisking cycle. Finally, it was shown in experiments in which hippocampus theta was recorded while animals were exposed to classical conditioning paradigms that memory traces could be established only if the conditioning stimuli were given during a particular phase of the theta cycle. While these experiments only showed a relation between the timing of inducing stimuli relative to oscillatory activity, more direct evidence for an instrumental role of long-range synchronization of oscillations in memory processes has been obtained with multielectrode recordings from structures relevant for memory formation (Fell et al., 2001; Tallon-Baudry et al., 2001, 2004). In human subjects implanted with depth electrodes for the localization of epileptic foci, it was found that successful formation of episodic memories was accompanied by transient increases in gamma- and theta-oscillatory synchrony between the hippocampus and neighboring entorhinal cortex, structures known to be involved in memory formation. In trials in which memory formation was not successful, these changes in synchronization were not observed (Fell et al., 2001, 2003). Likewise, simultaneous recordings from limbic structures (amygdala and hippocampus) have shown that fear conditioning is associated with transient synchronization of oscillatory activity between the two structures (Seidenbecher et al., 2003; Narayanan et al., 2007). Electroencephalographic and magnetoencephalographic studies in healthy human subjects revealed that classical Pavlovian conditioning leads to a lasting enhancement of the synchronization of gamma oscillations between cortical areas encoding the conditioned and nonconditioned stimuli, respectively (Miltner et al., 1999).

The recall of memories also appears to be associated with enhanced oscillatory patterning of neuronal responses in the theta, beta, and gamma bands (Raghavachari et al., 2001; Tallon-Baudry et al., 2001; Rizzuto et al., 2003; Sederberg et al., 2003; Herrmann et al., 2004; Guderian and Duzel, 2005). Auditory stimuli that matched a previously stored template led to significantly more gamma band synchrony than those that did not match (Debener et al., 2003). Visual stimuli for which subjects have a long-term memory representation caused significantly greater gamma oscillations in the occipital cortex than similar stimuli that were not stored in long-term memory (Herrmann et al., 2004). A close relation between encoding and retrieval of memory contents and enhanced gamma oscillations has also been found in experiments on working memory (Tallon-Baudry et al., 1997; Strüber et al., 2000;

Tallon-Baudry et al., 2001, 2004). Here, the increase in oscillatory activity was particularly pronounced over parietal, central, and frontal regions of the brain. Finally, there appears to be a relation between the access to declarative memory and long-range synchronization of gamma oscillations (Melloni et al., 2007). In humans performing a delayed matching-to-sample task on stimuli that were only perceived in a subset of trials and gained access to consciousness and declarative recall, it was possible to demonstrate that only those stimuli that were consciously perceived and encoded in declarative memory evoked large-scale phase synchronization of gamma oscillations across distributed cortical networks. In addition, only those trials were associated with sustained, enhanced theta activity throughout the hold period. The latter finding agrees well with the growing evidence that large-scale synchronization in the theta frequency band is closely related to the encoding and recall of stimulus material in declarative memory. Successful recognition of known faces is associated with increased activity in a distributed network that includes prefrontal mediotemporal and visual areas in occipital cortex (Guderian and Duzel, 2005). Finally, invasive recordings in patients suggest that theta band oscillations are implicated in spatial navigation, working memory, and episodic memory (Raghavachari et al., 2001; Caplan et al., 2001, 2003; Rizzuto et al., 2003; Sederberg et al., 2007).

Last but not least, there is recent evidence from studies in human subjects that consolidation of memories during sleep is closely related to an oscillatory patterning of neuronal activity (Marshall et al., 2006).

1.37.4 Conclusion

So far, most of the evidence suggesting a relation between synchronized oscillatory activity in various frequency bands and memory formation is correlative in nature and does not allow one to conclude that synchronized oscillatory activity is a necessary prerequisite for the induction of memory-related synaptic gain changes. However, the evidence reviewed concerning the importance of precise temporal relations in memory processes and the pivotal role of oscillations for the establishment of precise temporal relations between neuronal activities provides strong support for the hypothesis that oscillations and the associated synchronization of spike discharges play a crucial role in the coordination of distributed neuronal processing on the one hand and engram formation on the other. This conclusion is further supported by the growing evidence that cognitive deficits, including impairments in short- and long-term memory such as occur in schizophrenia, Alzheimer's disease, and epilepsy, are associated with abnormal patterns of neuronal oscillations and reduced synchronization (for review, see Uhlhaas and Singer, 2006).

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1.38 The Neuronal Workspace Model: Conscious Processing and Learning

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1.38.1 Introduction

Researchers in the field of computational biology have mostly focused their attention in the recent years on the sequences of eukaryotic genomes, on their annotation, and on the understanding of how these linear sources of information give rise to the three-dimensional organization of the body and, in

particular, of the brain. Ultimately, from the DNA sequences already stored *in silico* of the human genome, one should be able to compute the main features of the species-specific functional organization of our brain. Yet, the brain strikingly differs from the other organs of the body in several respects. First, the complexity of its cellular and supracellular organization is orders of magnitudes higher, and second, it is able to learn and store information from the outside world. Finally, its structure is under constant reorganization as a function of its internal physiological states of activity, either endogenously generated or evoked by signals from the outside world. These are a few of the many reasons why computational neuroscience has recently developed as a rather autonomous and fast-moving discipline. Its principal project is to understand the multiple modes of signal processing by the brain ultimately resulting in behavior and/or tacit mental events. It is also to build up formal models, expressed in terms of neuronal networks, that link the molecular, neuronal, physiological, and behavioral/mental data in a coherent, noncontradictory though minimal form (Changeux and Dehaene, 1989). Ultimately implemented as 'formal organisms' (Changeux et al., 1973), these neurocomputational models should altogether account for the available data and produce experimentally testable predictions at all those levels. Being minimal, they are not anticipated to give an exhaustive description of reality, but nevertheless to validate or invalidate theories and, if necessary, give rise to new ones, thus contributing to our understanding of how the human brain works.

If multiple attempts have been successfully done to model the relationships between the states of activity of neuronal networks and overt behaviors in simple systems (Grillner and Graybiel, 2006), a most fascinating intellectual challenge of today's neuroscience remains to understand the explicit processing of the mental events which invest our brain, in other words to establish a comprehensive theory of consciousness on the basis of the presently available scientific knowledge. As stated by Delacour (1997),

> Multiple explanations have already been suggested like such-and-such a sophisticated algorithm, the oscillation of the extracellular electrical field in the cortex, the probabilistic character of synaptic transmission, or some still mysterious property of 'quantum gravity.' These theories represent little advance over the pineal gland theory of René Descartes. (Delacour, 1997: 127)

The case of consciousness is indeed exceptional. First of all, a fundamental ambiguity exists in the use of the term which, depending on context and author, can equally refer to waking state, personal experience, mental processing, or the multifaceted concept of self. Second, its comprehensive description crosses multiple disciplines ranging from basic neuroscience to molecular biology, psychology, and philosophy which, in the present situation of the academic community, show considerable difficulties to interact and cooperate. Last, any plausible theory on consciousness has to refer to a phenomenal subjective experience reported through introspection that a long tradition, from positivism to behaviorism and, most of all, the current practices of bench work research in neuroscience or pharmacology, has banned from any serious form of scientific enquiry.

Nevertheless, in the past decades, the situation has significantly changed because of a 'renaissance' (Zeman, 2005) of empirical research on consciousness and the proposal of constructive and plausible mechanistic theories, which aim at accounting for the subjective experience of a unified or global 'space or scene,' where some kind of *synthesis* between past, present, and future takes place, where multimodal perceptions, emotions, and feelings (present), and evoked memories of prior experiences (past), together with anticipations of actions (future), become subjectively integrated in a continuously changing and dynamic flow of consciousness, "altogether one and multiple at any of its moments" (Fessard, 1954; Bogen, 1995; Edelman and Tononi, 2000; Crick and Koch, 2003; Dehaene and Changeux, 2004). Among these theories, Zeman (2005) distinguishes the following.

1.38.1.1 Information Processing Theories

Following the lead of William James in associating consciousness with selective attention and memory, Baars (1989) has proposed a psychological model which postulates that the content of consciousness is broadcasted to the whole brain through a 'global workspace' which recruits the operation of multiple unconscious and automatic processors. Yet, Baars proposed as the essential neural basis of his global workspace the ascending reticular formation, the nonspecific nuclei of the thalamus, and only casually mentioned that "it is possible that corticocortical connections should also be included."

Shallice (1988) has also suggested an integrative role of consciousness through a 'supervisory attentive system' which would control the activities of lower level psychological systems mediated by some kind of 'contention scheduling' system and has placed emphasis on parieto-prefrontal networks in relation with the supervisory system (see also Frith et al., 1999).

1.38.1.2 Neurobiological Theories

In their pioneering efforts to specify the neural correlates of consciousness, Crick and Koch (Crick, 1994; Crick and Koch, 1995, 2004) have successively emphasized the importance of gamma-band oscillations around 40 Hz as a correlate of conscious processing; then, successively, the role of connections to and from prefrontal cortex in conscious perception (though more recently they have defended the opposite view that prefrontal cortex works as an 'unconscious homunculus'); and last, the possible role of the claustrum in the integration of conscious percepts (Crick and Koch, 2005).

Edelman and Tononi (2000) and Tononi and Edelman (1998) have emphasized the role of information integration and of reentrant connections in establishing a shifting assembly or 'dynamic core' linking distributed cortical and thalamic neurons. Its representation content, at the same time diversified and unitary, could not be localized to single parts of the brain and would vary significantly among individuals, but yet would correspond to the content of phenomenal consciousness. Lumer et al. (1997a,b) have developed a formalism in their simulations focused on early visual processing, with reentrant connections but without establishing a link with the notion of consciousness and specifically with the dynamic core hypothesis.

The hypothesis of a conscious 'neuronal workspace' (Dehaene et al., 1998; Dehaene and Changeux, 2000; Dehaene and Naccache, 2001; Dehaene et al., 2003b) emphasizes the role of distributed neurons with long-distance connections, particularly dense in prefrontal, cingulate, and parietal regions, interconnecting multiple specialized processors and broadcasting signals at the brain scale in a spontaneous and sudden manner, forming a conscious 'global neuronal workspace.' This model is extensively presented and discussed in this chapter.

1.38.1.3 Social Theories

The philosopher Strawson (1974) has argued that the concept of one's own mind presupposes the concept of other minds. This, together with the notion that language is critical for human consciousness, has led to the notion that conscious experience would be more a social construction than a physiological or psychological phenomenon (Rose, 1999). Without contesting the importance of social relationships and in particular of language (see Edelman, 1989) in human consciousness, one may wonder, however, whether this is the adequate level of explanation for a comprehensive theory of consciousness, which should ultimately capture such basic phenomena as sleep and anesthesia, masking, or attentional blink (see following).

In this chapter we successively analyze: (1) the theoretical premises of the neuronal workspace hypothesis; (2) the formal representation of the neuronal workspace model; (3) simulations with the workspace model of states of consciousness and access to consciousness in cognitive tasks; and (4) the neuronal workspace model and the evolution of consciousness.

1.38.2 The Neuronal Workspace Hypothesis: Premises and Theoretical Statements

The views presented in this chapter developed from three sets of complementary data: anatomical, computational, and psychophysical.

1.38.2.1 Anatomical Data

Early observations by Cajal (1892) (see DeFelipe and Jones, 1988) underlined the 'special morphology' of the pyramidal cells from the cerebral cortex and suggested that they might be "the substratum of the highest nervous activities," calling them '*the psychic cells*.' Cajal mentioned their very numerous and complex dendritic cells and also noted that "the pyramidal cells from cortical layers II and III possess long axons with multiple collaterals." Cajal further distinguished in the white matter: projection fibers which enter the cerebral peduncle, callosal fibers which associate the two hemispheres, and fibers of association that "bring into relation . . . different territories and different lobes of the same hemisphere" (Cajal, 1892). He also noted that these fibers of association increase in number in humans and large

mammals, where they form "the main mass of the white matter." Recent investigations have confirmed the view that the corticocortical and callosal fibers primarily (though not exclusively) arise from layer II-III pyramids (Jones, 1984) (**Figure 1(d)**).

Furthermore, von Economo (1929), a follower of Brodman, distinguished in his "Cytoarchitectonics of the human cerebral cortex" five "fundamental types of cortical structure" (**Figures 1(a) and 1(b)**) and among them: the "frontal type 2 (which) . . . possesses large, well-formed, and well-arranged pyramidal cells in layers III and V" while in the "parietal type 3 (these cells) are smaller, more slender, and numerous . . . ." Von Economo also noted that the type 2 is "spread over the anterior two-thirds of the frontal lobe, over the superior parietal lobule . . ." as well as over the cingulate cortex, among other cortical areas and concludes that "type 2 and 3 isocortex . . . are the chief station for the commemorative and higher psychic functions."

Interestingly, recent quantitative analysis of the dendritic field morphology of layer III pyramidal neurons in the occipitofrontal cortical 'stream' revealed a continuous increase of complexity up to the prefrontal cortex within a given species (Elston and Rosa, 1997, 1998; DeFelipe and Farinas, 1992) (**Figure 1(c)**) and from lower species (owl monkey, marmoset) to humans (Elston, 2003). A correlative increase of the relative surface of the prefrontal cortex accompanies this increased complexity (see Changeux, 2004). Moreover, mapping of long-range connections in the monkey cerebral cortex revealed long-range connections linking, among others, the prefrontal cortex (area 46), the superior temporal sulcus, parietal area 7a, and the hippocampus together with the contralateral anterior and posterior cingulum, area 19, and the parahippocampal gyrus (Goldman-Rakic, 1988). These circuits were suggested to contribute to working memory (Goldman-Rakic, 1994) and

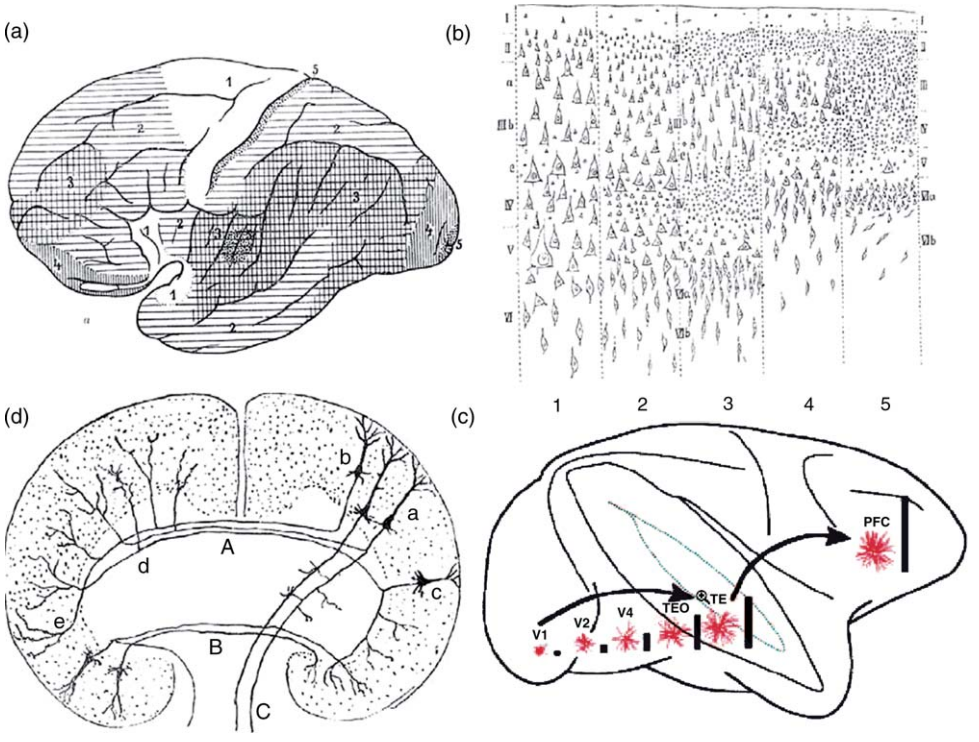

Figure 1 The anatomical basis of the neuronal workspace hypothesis. The pyramidal neurons with long corticocortical axons from layers 2–3 of the cerebral cortex (d) display increased complexity of the basal dendrites (and thus increased connectivity) from primary visual areas (V1) to prefrontal cortex (PFC) (here in the monkey (c)) and from primitive vertebrates to humans. These neurons are particularly abundant in what von Economo referred to as type 2 cortex (b), which primarily occupies the frontal, parietotemporal, and cingulate areas (a). Parts (a) and (b) from von Economo C (1929) *The Cytoarchitectonics of the Human Cerebral Cortex.* London: Oxford University Press; used with permission. (c) from Elston GN (2003) Cortex, cognition and the cell: New insights into the pyramidal neuron and prefrontal function. *Cereb. Cortex* 13: 1124–1138; used with permission from Oxford University Press. (d) from Cajal S (1892) El nuevo concepto de la histologia de los centros nerviosos. *Rev. Ciencias Med.*18: 457–476; used with permission.

proposed to contribute to the anatomical basis of the neuronal workspace model (Dehaene et al., 1998).

1.38.2.2 Computational Data

The present neurocomputational approach to conscious versus nonconscious processing originates from the design of neural network models that aimed at specifying the contribution of prefrontal cortex to increasingly higher cognitive tasks (Dehaene and Changeux, 1989, 1991, 1997; Dehaene et al., 1998). Successively, these models considered the issues of how a network could retain an active memory across the long delay of a delayed-response task (Dehaene and Changeux, 1989), how it could encode abstract rules that might be selected from external or internal rewards (Dehaene and Changeux, 1991), and finally how networks based on those principles could pass complex planning tasks such as the Tower of London test or the Stroop test (Dehaene and Changeux, 1997; Dehaene et al., 1998). The 'conscious *neuronal* workspace' model (Dehaene et al., 1998; Dehaene and Naccache, 2001; Dehaene et al., 2003b; Dehaene and Changeux, 2005) is the last development of these models which emphasize the role of distributed neurons with long-distance connections, particularly dense in prefrontal, cingulate, and parietal regions, which are capable of interconnecting multiple specialized processors and can broadcast signals at the brain scale in a spontaneous and sudden manner.

1.38.2.3 Cognitive Psychology, Neuropsychology, and Human Neuroimaging

A long psychophysical and neuropsychological tradition, dating back to Hughlings Jackson and perpetuated among others by Baddeley, Shallice, Mesulam, or Posner, has emphasized the hierarchical organization of the brain and separates lower automatized systems from increasingly higher and more autonomous supervisory executive systems. It has also been influenced by Fodor's distinction between the vertical 'modular faculties' and a distinct 'isotropic central and horizontal system' capable of sharing information across modules. Empirically, finally, it has taken advantage of a variety of experimental techniques, starting with behavioral analysis, neuropsychological observation in brain-lesioned patients, and most recently, human neuroimaging with functional magnetic resonance imaging (fMRI) and causal interference with transcranial magnetic stimulation (TMS).

It is beyond the scope of this chapter to discuss the variety of experimental contributions that are relevant to the ongoing science of consciousness (see, e.g., Laureys, 2005), but they can be briefly sketched as belonging to two main lines of research. The first line, starting with the pioneering research of Weiskrantz on blindsight and Marcel on subliminal priming, has investigated the extent of nonconscious processing in humans. In combination with fMRI, this research has uncovered that not only subcortical, but in fact a variety of specialized cortical systems were capable of activating in the absence of any conscious report of stimulus presence (for review, see Naccache and Dehaene, 2001; Naccache et al., 2005; Dehaene et al., 2006). The second line, exemplified by Posner's or Shallice's work, has studied the properties of a central executive or executive attention system, whose activity seems to index conscious top-down attention and control (Norman and Shallice, 1986; Amati and Shallice, 2007). Through the use of Baars's contrastive method, which consists of opposing two minimally different conditions, one of which is conscious and the other is not, it has been observed that executive control is deployed only following consciously perceived trials (e.g., Kunde, 2004) and is consistently associated with a sharp increase in dorsal and midline prefrontal as well as anterior cingulate and, in many cases, inferior or posterior parietal activation (see Gusnard and Raichle, 2001; Dosenbach et al., 2006).

1.38.2.4 The Model

Inspired by these observations, our theoretical work attempted to capture them within a minimal theoretical model. In the following, the headlines of the theoretical premises will be presented following the initial presentation of Dehaene et al. (1998), yet updated in a few of its formulations in the subsequent papers of Dehaene et al. (2003a) and Dehaene and Changeux (2005).

1.38.2.4.1 Two computational spaces

The neuronal workspace hypothesis distinguishes, in a first approach, two main computational spaces within the brain (**Figure 2**), each characterized by a distinct pattern of connectivity:

a. A processing network, composed of a set of parallel, distributed, and functionally specialized

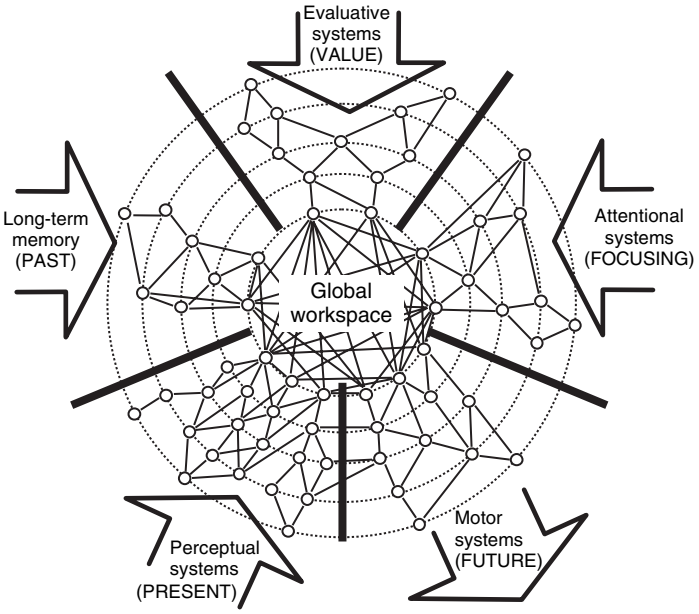

Figure 2 Schematic representation of the neuronal workspace hypothesis as initially proposed by Dehaene et al. (1998). The model distinguishes two computational spaces: (1) specialized processors, which are modular, encapsulated, and automatic, labeled here as perceptual systems, long-term memory (including autobiographic memory and self), and attentional and evaluative systems and (2) the global workspace, with long-range axon neurons broadcasting signals to multiple areas yielding subjective experience and reportability. From Dehaene S, Kerszberg M, and Changeux JP (1998) A neuronal model of a global workspace in effortful cognitive tasks. *Proc. Natl. Acad. Sci. USA* 95: 14529–14534; used with permission from the National Academy of Sciences.

processors (Baars, 1989) or modular subsystems (Shallice, 1988) subsumed by topologically distinct cortical domains with highly specific local or medium-range connections that encapsulate information relevant to its function. This specialized network processes information in a bottom-up manner (see Miyashita and Hayashi, 2000).

b. A global workspace, consisting of a distributed set of cortical neurons characterized by their ability to receive from and send back to homologous neurons in other cortical areas, horizontal projections through long-range excitatory axons (which may as well impinge on excitatory or inhibitory neurons). Such long-range corticocortical tangential connections include callosal connections and mostly originate from the pyramidal cells of layers 2 and 3. We therefore propose that the extent to which a given brain area contributes to the global workspace would be simply related to the fraction of its pyramidal neurons contributing to layers 2 and 3, which is particularly elevated in von Economo's type 2 (dorsolateral prefrontal) and type 3 (inferior parietal) cortical structures (von Economo, 1929). The pyramidal neurons from layers 2 and 3 establish, in addition, vertical and reciprocal connections with layer 5 neurons and thus corresponding thalamic nuclei. These connections contribute to both the stability and the dynamics of workspace activity, via, for instance, self-sustained circuits, but also mediate the direct access to and from the processing networks (Brecht et al., 2003). The global network neurons typically process information in a top-down manner.

1.38.2.4.2 Content of the global workspace

In the original formulation of the neuronal workspace hypothesis (Dehaene et al., 1998), five major categories of processors were distinguished which could be dynamically mobilized and multiply reconfigured (**Figure 2**).

a. *Perceptual circuits* give the workspace access to the present state of the external world. Empirically, perceptual circuits may include the primary and secondary sensory areas together with the object-oriented ventral and lateral areas of the temporal lobes in both visual (Mishkin and Ungerleider, 1982; Goodale et al., 1991; Fang and He, 2005) and auditory (Rauschecker and Tian, 2000) modalities as well as

the temporal and inferior parietal areas involved in language comprehension (including Wernicke's area) (Mesulam, 1998). Accordingly, the content of any external stimulus, attended object, or linguistic input can access the global workspace. Such access may take place stepwise through hierarchical stages of processing through primary and secondary sensory areas such as V1 and FEF (Lamme and Roelfsema, 2000) and then higher association areas of temporal, frontal, and cingulate cortex. In Dehaene et al. (2003a) and Dehaene and Changeux (2005) formulations, each area was further assumed to establish with the neighboring area bottom-up feedforward connections and top-down feedback projections, the top-down connections being slower, more numerous, and more diffuse (Felleman and van Essen, 1991; Salin and Bullier, 1995). Moreover, bottom-up connections were thought to impinge on glutamate α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA) receptors, whereas the top-down ones would primarily mobilize glutamate *N*-methyl-D-aspartate (NMDA) receptors (see Lumer et al., 1997).

b. *Motor programming circuits* allow the content of the workspace to be used to guide future motor behaviors and actions. A hierarchy of nested circuits implements motor intentions, from the highest level of abstract plans to individual actions, themselves composed of moves and gestures (Jeannerod and Jacob, 2005).

Empirically in humans, these circuits include premotor cortex, posterior parietal cortex, supplementary motor area, basal ganglia (notably the caudate nucleus), and cerebellum, as well as the high-level speech production circuits of the left inferior frontal lobe, including Broca's area. Connections of the workspace to motor and language circuits at the higher levels of this hierarchy endow any active representation in the workspace with the property of reportability (Weiskrantz, 1997; Dehaene et al., 2006), namely, the fact that it can be described or commented on using words or gestures.

c. *Long-term memory circuits* provide the workspace with an access to stored past percepts and events.

These long-term memory stores are likely distributed throughout the cortex according to their original content and modality; hippocampal and parahippocampal areas through reciprocal links with workspace neurons may play a special role in mediating the storage in and retrieval from these long-term stores.

d. *Evaluation circuits* (Dehaene and Changeux, 1989, 1991; Friston et al., 1994; Schultz et al., 1997) allow representations in the workspace to be selected according to a positive or negative value.

Empirically, the main anatomical systems in this respect include mesocortical noradrenergic, dopaminergic, serotoninergic, and cholinergic pathways together with the orbitofrontal cortex, anterior cingulate, hypothalamus, amygdala, and ventral striatum (see Everitt and Robbins, 2005). Autoevaluation systems develop from reciprocal projections allowing evaluation circuits to be internally activated by the current workspace content (Dehaene and Changeux, 1991) and, conversely, to selectively maintain or change workspace activity according to whether its value is predicted to be positive or negative (Dehaene and Changeux, 1991, 1995, 1997). These evaluation systems are the targets of drugs as instrumental reinforcers eventually resulting in drug self-administration or drug taking and mobilizing in particular medial prefrontal cortex and nucleus accumbens core (Everitt and Robbins, 2005; Christakou et al., 2004).

e. *Attention circuits* allow the workspace to mobilize its own circuits independently from the external world. Changes in workspace contents need not necessarily lead to changes in overt behavior, but may result in covert attention switches to selectively amplify or attenuate the signals from a subset of processor neurons.

Although all descending projections from workspace neurons to peripheral modular processors are important in this selective amplification process, a particular role is played by areas of the parietal lobe in visuospatial attention (Posner, 1994; Posner and Dehaene, 1994).

1.38.2.4.3 Global modulation of workspace activation

The state of activation of workspace neurons is assumed to be under the control of global vigilance signals from the ascending reticular activating system. Empirically they may include cholinergic nuclei in the upper brainstem and basal forebrain, noradrenergic nuclei (e.g., from the locus coeruleus), a histaminergic projection from the posterior hypothalamus, and dopaminergic and serotoninergic pathways arising from the brainstem (McCarley, 1999) together with recently identified orexin neurons from lateral hypothalamus (de Lecea et al., 1998; Harris et al., 2005). Much if not all of the influence exerted by these pathways is mediated by the thalamus and characterized by an increase in the excitability of the corticothalamic neurons.

Slow-wave sleep, on the other hand, coincides with a reduction of activity in the cholinergic, noradrenergic, and histaminergic nuclei, the anterior hypothalamus and basal forebrain being candidates for a critical role in sleep induction (Zeman, 2001). These signals are powerful enough to control major transitions between the awake state (workspace active) and slow-wave sleep (workspace inactive) states. Others provide graded inputs that modulate the amplitude of workspace activation, which is enhanced whenever novel, unpredicted, or emotionally relevant signals occur and, conversely, drops when the organism is involved in routine activity.

In the waking state the corticothalamic neurons are tonically depolarized by a blocking of hyperpolarizing potassium conductance (Steriade, 1999) (e.g., by acetylcholine acting on muscarinic receptors and norepinephrine on α_1-adrenergic receptors) switching them out of the slow-bursting mode and into fast gamma-band oscillations (Steriade et al., 1993; Llinas and Steriade, 2006). Similar effects can be obtained by electrical stimulation of the brainstem or by direct application of acetylcholine (McCormick and Bal, 1997). Moreover, mutations in the $\alpha 4$ and $\beta 2$-subunit genes of the nicotinic acetylcholine receptor cause autosomal dominant frontal lobe epilepsy (Steinlein, 2004), and deletion of the $\beta 2$-subunit is accompanied by a decrease of micro-arousals, which take place during slow-wave sleep (Léna et al., 2004).

As a consequence, the corticothalamic neurons show increased excitability: their signal-to-noise ratio is increased, and the response to sensory stimuli is facilitated. Their spontaneous activity is high and characterized by stochastic independence of time intervals between successive action potentials (Llinas and Paré, 1991; Steriade et al., 1993; Llinas and Steriade, 2006). By contrast, during slow-wave sleep, where consciousness is absent, signal-to-noise ratios to sensory responses are decreased, and most neurons tend to discharge in bursts synchronized over large populations, thus introducing distortions or blocking information transmission (Livingstone and Hubel, 1981).

In the resting awake state, the brain is the seat of an important baseline (Gusnard and Raichle, 2001; Raichle and Gusnard, 2005) or ongoing metabolic activity; a very large fraction of it (about 80%) being correlated with glutamate cycling and, hence, active synaptic signaling processes (Shulman and Rothman, 1998; Hyder et al., 2002; Raichle and Gusnard, 2002; Shulman et al., 2004). During slow-wave sleep, anesthesia, or coma, global cerebral glucose metabolism falls by about 20% (Heiss et al., 1985; Buchsbaum et al., 1989; Shulman et al., 2004), particularly in frontal and parietal cortices (Laureys, 2005; Laureys et al., 2006). Interestingly, optical imaging of visual cortex in anesthetized animals revealed structural states of activity which have a similar global organization as activity patterns evoked by external stimuli (Tsodyks et al., 1999; Kenet et al., 2003).

1.38.2.4.4 Spatiotemporal dynamics of workspace activity

From a theoretical point of view, the global workspace is considered the seat of a particular kind of brain-scale activity state characterized by the spontaneous activation, in a sudden, coherent, and exclusive manner, referred to as ignition (Dehaene and Changeux, 2005), of a subset of workspace neurons, the rest of workspace neurons being inhibited. The transition to this state of highly correlated activity is fast and characterized by an amplification of local neural activation and the subsequent activation of multiple distant areas. The entire workspace is globally interconnected in such a way that only one such workspace representation can be active at any given time (see Sigman and Dehaene, 2005, 2006). This all-or-none invasive property distinguishes it from peripheral processors in which, due to local patterns of connections, several representations with different formats may coexist.

A representation which has invaded the workspace may remain active in an autonomous manner and resist changes in peripheral activity (see Dehaene and Changeux, 1989, 1991). If it is negatively evaluated, or if attention fails, it may, however, be spontaneously and randomly replaced by another discrete combination of workspace neurons. Functionally, this neural property implements an active generator of diversity which constantly projects and tests hypotheses (or prerepresentations) on the outside world (Dehaene and Changeux, 1989, 1991, 1997). The dynamics of workspace neuron activity is thus characterized be a constant flow of individual coherent episodes of variable duration and their selection.

Although a variety of processor areas project to the interconnected set of neurons composing the global workspace, at any given time only a subset of inputs effectively accesses it. We postulate that this gating is implemented by descending modulatory projections from workspace neurons to more peripheral processor neurons. These projections may selectively amplify or extinguish the ascending inputs from processing neurons, thus mobilizing, at a given time, a specific set of

processors in the workspace while suppressing the contribution of others. In other words, the pattern of mobilized processor neurons defines the actual subjective content of conscious perception.

1.38.3 Formal Representation of the Neuronal Workspace Model

Since the initial formulation of Dehaene et al. (1998), the general architecture and dynamics of the neural network representing the global workspace and the relevant processors have been further specified in Dehaene et al. (2003a) and Dehaene and Changeux (2005). It is well understood that in any instance these computer simulations are partial and incomplete. Yet they are expected to point to the importance of particular components or features of these minimal architectures, thus leading to critical experimental tests. In our work, we found it useful to develop two quite distinct types of computer simulations. Some of them, referred to here as 'Type 1' models, were intended to describe an entire task-related cognitive architecture and thus focused more on global connectivity than on fine physiological details (e.g., Dehaene et al., 1998). Others, referred to as 'Type 2' models, were intended to capture some fine-grained physiological characteristics of neuronal firing trains and event-related potentials during conscious and subliminal perception. These simulations therefore necessarily incorporated considerably more physiological details of receptor types and cortical layers, but were not extended in a brain-scale architecture solving a precise task (e.g., Dehaene et al., 2003a; Dehaene and Changeux, 2005). Here, we describe their principles in turn.

1.38.3.1 Detailed Physiological Simulations of Access to the Conscious Workspace

In those Type 2 simulations, we intended to describe only part of the workspace (Dehaene and Changeux, 2005), but to do so with physiological details. The goal was to simulate the bottom-up/top-down interactions occurring between four hierarchically organized areas, the lowest of which was in contact with the external world while the highest was assumed to contact other workspace areas (not simulated).

1.38.3.1.1 Single-neuron model

The model (**Figure 3(a)**), adapted from Lumer et al. (1997a,b), was simulated at the level of single-compartment integrate-and-fire neurons whose membrane potential evolved according to semirealistic differential equations taking into account realistic temporal delays and AMPA, NMDA, and gamma-aminobutyric acid (GABA) currents. Neurons also received a diffuse neuromodulator input summarizing the known depolarizing effects of ascending activating systems, such as those from cholinergic, noradrenergic, and serotoninergic nuclei in the brainstem, basal forebrain, and hypothalamus (Steriade et al., 1993; Llinas and Steriade, 2006). This parameter was used to control the level of wakefulness (see following discussion).

1.38.3.1.2 Columnar structure

The neurons were organized into simulated thalamocortical columns comprising 80 excitatory and 40 inhibitory neurons and organized in a three-layered structure, schematizing supragranular, infragranular, and layer IV cortical neurons and a corresponding thalamic sector (**Figure 3(b)**). A fairly realistic scheme of connections was implemented, whereby thalamic excitatory neurons projected to layer IV (AMPA, 3 ms delay) and, with lesser strength, to infragranular neurons (AMPA, 3 ms). Layer IV excitatory neurons projected to supragranular neurons (AMPA, 2 ms). Supragranular excitatory neurons projected to infragranular neurons (AMPA, 2 ms). Finally, infragranular excitatory neurons projected to layer 4 (AMPA, 7 ms), to supragranular neurons (AMPA, 7 ms), and to the thalamus (AMPA, 8 ms). Those principles and parameter values capture the major properties of translaminar connections (Lumer et al., 1997a,b; Douglas and Martin, 2004), though they do not attempt to capture the possible functional roles of the different layers (see, e.g., Raizada and Grossberg, 2003).

1.38.3.1.3 Long-range connections

For corticocortical projections, supragranular excitatory neurons of each area projected to layer IV of the next area (AMPA, 3 ms). In agreement with physiological observations (Felleman and Van Essen, 1991; Salin and Bullier, 1995), top-down connections were slower, more numerous, and more diffuse. They connected the supra- and infragranular excitatory neurons of a given column to the supra- and infragranular layers of all areas of a lower hierarchical level. Strong top-down connections linked columns coding for the same stimulus, whereas weaker top-down connections projected to all columns of a lower area. Both were NMDA mediated, and transmission delays increased with cortical distance (delay $= 5 + 3\delta$ ms,

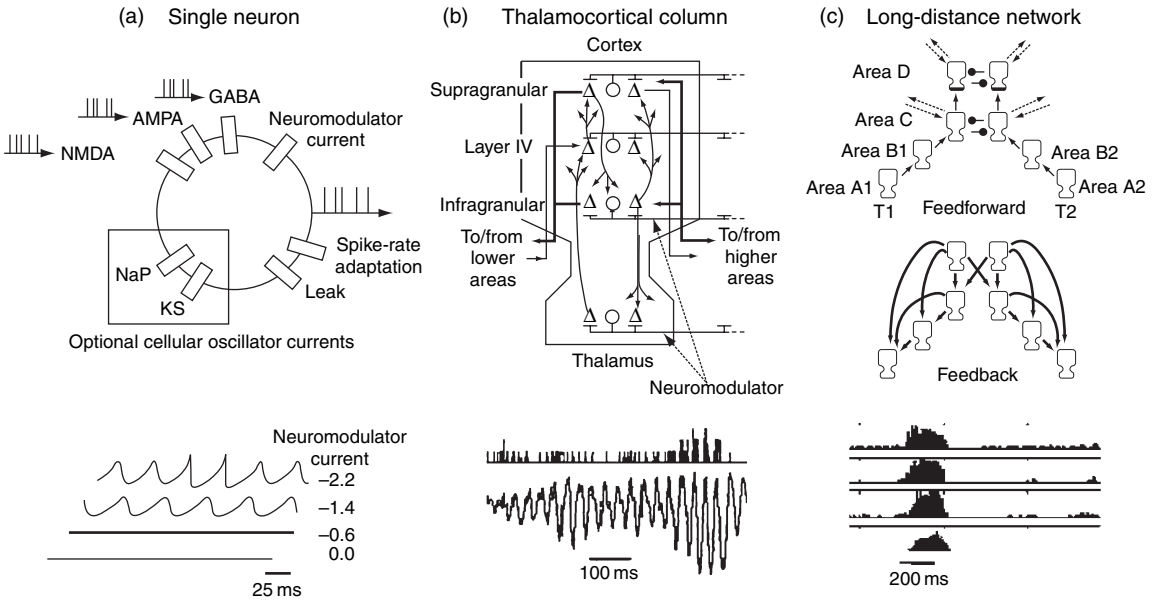

Figure 3 Detailed implementation, at the cellular and molecular levels, of the neuronal workspace model in the case of access to consciousness as in Dehaene et al. (2003b) and Dehaene and Changeux (2005). Single neurons may generate sustained oscillations of membrane potential at high enough level neuromodulator current (a) but only the thalamocortical column (b), and global network levels do generate complex waxing and waning EEG-like oscillations (b) and metastable global states of sustained firing (or 'ignition') (c). From Dehaene S, Sergent C, and Changeux JP (2003b) A neuronal network model linking subjective reports and objective physiological data during conscious perception. *Proc. Natl. Acad. Sci. USA* 100: 8520–8525, with permission from the National Academy of Sciences; and Dehaene S and Changeux JP (2005) Ongoing spontaneous activity controls access to consciousness: A neuronal model for inattentional blindness. *PLoS Biol.* 3: 910–927, with permission from the Public Library of Science.

with $\delta = 1$ for consecutive areas, 2 for areas two levels apart in the hierarchy, etc.).

1.38.3.1.4 Spontaneous activity

Although we studied stimulus-evoked activity (see later discussion), a most important goal of these stimulations was to also capture spontaneous corticothalamic activity and its modulation with states of vigilance. We studied two possible sources for spontaneous activity, both of which were meant as theoretical idealizations on a continuum of possibilities. The first case, hereafter the 'cellular oscillator model,' was a purely deterministic model in which neurons follow simple differential equations incorporating persistent sodium and slowly inactivating potassium currents whose interplay generates intrinsic gamma-band oscillations of membrane potential (Wang, 1993), comparable to those recorded experimentally (Llinas et al., 1998). We also described another simulation, hereafter called the 'random spikes' model, in which stochastic spontaneous activity arose from fast random fluctuations in membrane potential, capturing the joint effects of synaptic and postsynaptic noise on spike initiation. Both cellular sources turned out to have a similar effect on global spontaneous corticothalamic states (for details, see Dehaene and Changeux, 2005).

1.38.3.2 Minimal Models of Cognitive Architectures for Effortful Tasks

Another line of models, Type 1 simulations, attempted to capture behavioral and neuroimaging observations on higher-level 'executive' tasks that depend on prefrontal cortex (Dehaene et al., 1998), such as the Delayed-Response, Stroop, Wisconsin, or Tower of London tests (Dehaene and Changeux, 1989, 1991, 1997). We have not found it possible to design a pertinent model of such tasks while working at the level of detailed spiking neurons and columns. Thus, those models were of a more abstract nature and incorporated neural 'units' formally similar to single neurons or clusters whose average firing rate was simulated by McCulloch-Pitts units. No attempt was made to capture intracolumnar or thalamic dynamics, but the network incorporated a series of assemblies assumed to represent relevant cortical activity at several hierarchical levels.

1.38.3.2.1 Learning by reward

An interesting advantage of these coarser cognitive models is that they allowed for the simulation of longer periods of time and, therefore, of selective learning by reward, which empirically can be accompanied by drastic changes in vigilance and conscious access to task-relevant features. The network received a reward signal (R) provided after each network response ($R = +1$, correct; $R = -1$, incorrect). This reward led to two types of internal changes: classical synaptic weight changes of the Hebbian type, and a more original hypothesis of direct workspace activity modulation.

1.38.3.2.2 Synaptic weight changes

In the initial formulation of the theory (Dehaene et al., 1998) and for simplicity, only the synaptic weights between two excitatory units were assumed to be modifiable according to a reward-modulated Hebbian rule $\Delta w^{post,pre} = \varepsilon\ R\ S^{pre}\ (2\ S^{post} - 1)$, where R is the reward signal, pre is the presynaptic unit, and post is the postsynaptic unit (Dehaene and Changeux, 1989).

1.38.3.2.3 Workspace activity changes

Starting with our earliest modeling approaches (Dehaene and Changeux, 1989), we have assumed that reward entry can have either a stabilizing or destabilizing effect on prefrontal neuron activity. In Dehaene et al. (1998), we assumed that workspace neuron activity is under the influence of both vigilance and reward signals. The vigilance signal V is treated as having a modulatory influence on all workspace neurons. It is updated after each response: if $R > 0$, then $\Delta V = -0.1\ V$, otherwise $\Delta V = 0.5\ (1 - V)$. This has the effect of a slowly decreasing vigilance with sharp increases on error trials. The reward signal R influences the stability of workspace activity through a short-term depression or potentiation of synaptic weights (Dehaene and Changeux, 1989, 1991, 1997). A plausible molecular implementation of this rule has been proposed in terms of allosteric receptors (Dehaene and Changeux, 1989, 1991). It postulates that the time coincidence of a diffuse reward signal and of a postsynaptic marker of recent neuronal activity transiently shifts the allosteric equilibrium either toward, or from, a desensitized refractory conformation (Heidmann and Changeux, 1982, see also Changeux and Edelstein, 2005). Through this chemical Hebb rule, negative reward destabilizes the self-sustaining excitatory connections between currently active workspace neurons, thus initiating a change in workspace activity.

1.38.4 States of Vigilance as Spontaneous Thalamocortical Rhythms and Their Brain Imaging

The simulations of the vigilance states by Dehaene and Changeux (2005) type 2 modeling incorporate only minimal physiological mechanisms such as changes in a single current $I_{neuromodul}$, the depolarizing influence of ascending neuromodulation systems onto thalamic and cortical neurons (**Figure 3(a)**). They nevertheless suffice to generate a dynamical phase transition whose properties bear interesting similarity with actual empirical observations. We observed a robust threshold value of ascending neuromodulatory signaling beyond which structured neuronal activity emerged in the form of spontaneous thalamocortical oscillations in the gamma band (20–100 Hz, with a peak of the power spectrum around 40 Hz) (see also Bush and Sejnowski, 1996; Fuentes et al., 1996). The simulated waxing and waning synchronous bursts of oscillations bear similarity with empirical observations of transient periods of thalamocortical resonance, detected as bouts of gamma-band oscillations using electrophysiological recordings, for instance in the cat thalamus and cortex (Steriade et al., 1993; Steriade et al., 1996), or in humans using electro- and magneto-encephalography (Llinas and Ribary, 1993) (**Figure 3(b)**). They are proposed to represent the state of consciousness referred to as vigilance.

An original feature of the simulations is to characterize precisely the change in state in terms of a dynamical phase transition, referred to as a Hopf bifurcation. The Hopf bifurcation is continuous in the amplitude of spontaneous activity, which increases steadily from zero as vigilance increases. Thus it implements a true continuum of consciousness states, from high vigilance to drowsiness, and the various states of sleep anesthesia, or coma (Gajraj et al., 1999; Bonhomme et al., 2000; Sleigh et al., 2001). However, the Hopf bifurcation is also discontinuous in frequency space as the ascending neuromodulation increases. This may capture the observation that, during awakening or returning from anesthesia, there is a definite threshold for regaining of consciousness, which coincides with the threshold for emergence of high-frequency spontaneous thalamocortical oscillations.

Anatomically, the model predicts that in the awake state, spontaneous activity is present in all areas, but exhibits a higher degree of organization in higher cortical association areas, whose neurons are tightly interconnected by long distance into a global neuronal workspace and mobilize other low-level areas in a top-down manner. Thus, the model predicts that brain territories particularly rich in 'workspace neurons' with long-distance connections (i.e., prefrontal, parietal, superior temporal, and cingulate cortices) show the most intense and consistent spontaneous activity in the awake state. This prediction fits with the observation that the 'baseline' activity of the awake human brain at rest points to a network linking dorsal and ventral medial prefrontal, lateral parietotemporal, and posterior cingulate cortices (Gusnard and Raichle, 2001; Mazoyer et al., 2001; Raichle et al., 2001) which constantly fluctuates in synchrony with changes in electroencephalographic spectral content (Laufs et al., 2003) and shows the greatest drop in metabolism during anesthesia, sleep, coma, or the vegetative state (Maquet and Phillips, 1998; Fiset et al., 1999; Laureys et al., 2000; Paus, 2000; Balkin et al., 2002; Heinke and Schwarzbauer, 2002; Shulman et al., 2003). In striking agreement with the workspace model, volatile anesthetics have been recently shown to disrupt frontoparietal recurrent information transfer at gamma frequencies in the rat (Imas et al., 2005).

1.38.5 Interactions between External Stimuli and Ongoing Spontaneous Activity: Facilitation versus Competition

A most original aspect of Dehaene and Changeux's (2005) type 2 simulations on ongoing spontaneous activity concerns its interactions with external stimuli. These interactions can be facilitatory (higher spontaneous activity facilitates the detection of weak stimuli) or inhibitory (very high spontaneous activity preventing access to other external stimuli).

First, spontaneous activity may affect activation caused by external stimuli. The model predicts that ascending neuromodulatory current and the external input current combine in a smooth and largely additive fashion. The threshold for conscious access (ignition) is not fixed, but decreases as vigilance increases. At one extreme, very low levels of vigilance completely prevent the possibility of ignition, even by long and intense stimuli. Such stimuli only lead to a short pulse of activation through the thalamus and the early sensory areas of the model. Thus, we expect that early sensory signal can be processed, while higher cortical ones are attenuated, during altered states of consciousness. This prediction is consistent with empirical observations of auditory processing during sleep (Portas et al., 2000) or the vegetative state (Laureys et al., 2000), where stimuli activate the thalamus and auditory cortex, but fail to generate the distributed state of correlated prefrontal, parietal, and cingulate activity observed in awake normal subjects. Similar observations have been made with tactile or pain stimuli, suggesting that the lack of prefrontal–parietal–cingulate ignition is quite characteristic of those states (Laureys et al., 2002; Laureys et al., 2004).

Pharmacological agents such as nicotine, which can mimic and potentiate ascending cholinergic systems, might also have an influence on the perceptual threshold in visual masking or other psychophysical tests, which should be measurable both psychophysically and with brain imaging measures of ignition (e.g., prefrontal–cingulate activity in fMRI, P300 in event-related potentials). In schizophrenic patients, subliminal processing is intact, but the threshold for conscious perception of masked visual stimuli is increased, possibly relating to an impairment of top-down prefrontal–cingulate connectivity (Dehaene et al., 2003b; Del Cul et al., 2006). In such patients, we predict that nicotine might partially bring the conscious access threshold back toward its normal value.

Second, the model predicts that very high levels of spontaneous activity can prevent ignition by external stimuli (**Figure 4**). As mentioned, optical imaging of visual cortex in anesthetized animals has revealed structured states of spontaneous ongoing activity, which have the same global organization as activity patterns evoked by external stimulation (Tsodyks et al., 1999; Kenet et al., 2003). Moreover, high levels of spontaneous activity inhibit the sensory responses evoked by external stimuli, for instance, by whisker deflection in somatosensory cortex (Petersen et al., 2003). Such interactions with ongoing activity can provide an explanation for the large variability in spike trains evoked by repeated identical sensory stimuli (Arieli et al., 1996; Petersen et al., 2003).

The complete blocking of some incoming stimuli that occurs in the simulations of the model offers a plausible explanation for the psychological phenomenon of inattentional blindness (Newby and Rock, 1998). In this phenomenon, human observers engaged into an intense mental activity (such as

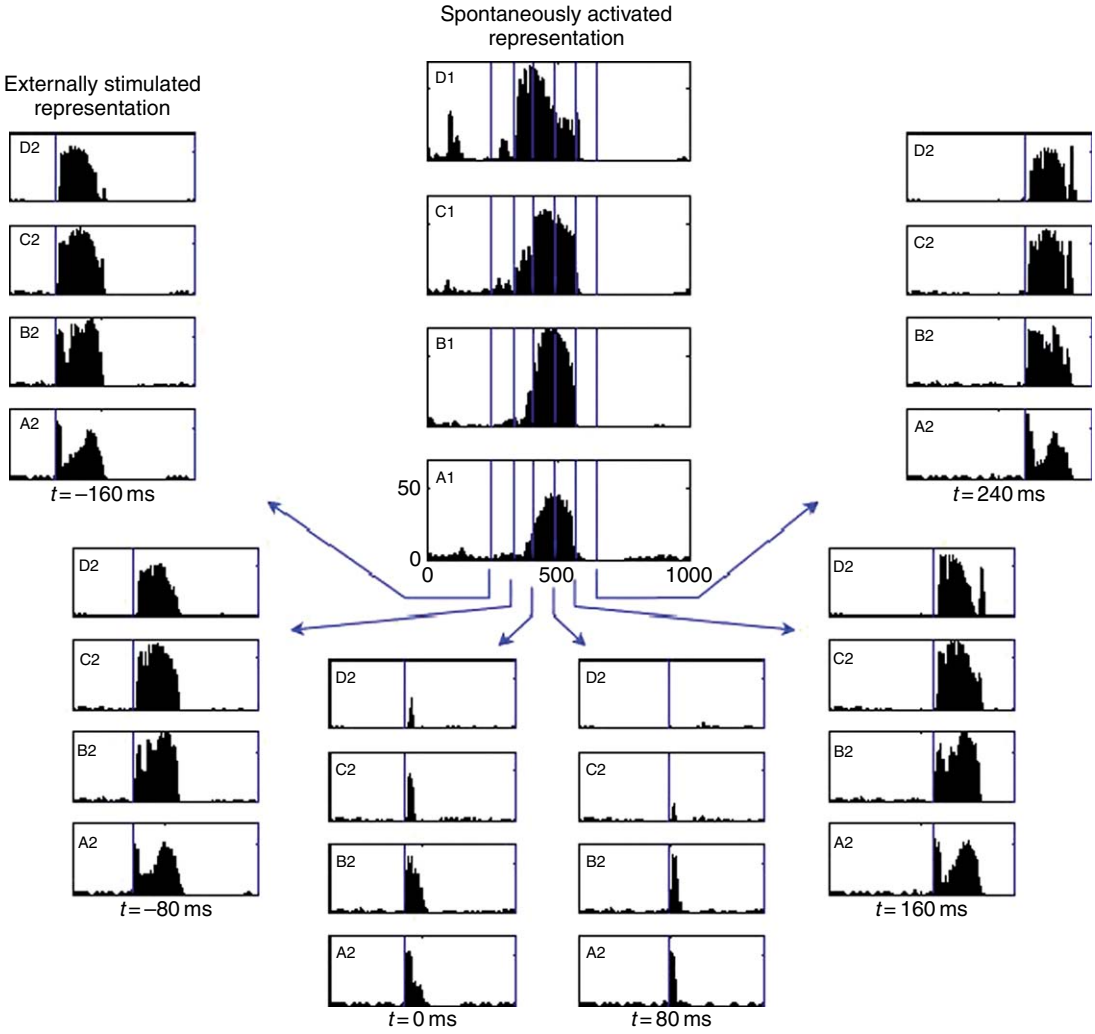

Figure 4 Competition between spontaneous workspace activity and external sensory simulation—a plausible model of the inattentional blindness state (Dehaene and Changeux (2005)). In the state of inattentional blindness, the subject fails to consciously detect external stimuli during periods of spontaneous thought. This can be reproduced by the simulations. In the boxes on the left of the figure, *before* the period of ongoing spontaneous activity, the external stimulus propagates in a bottom-up manner from the lowest (A) to the highest (B) areas of the sensory system yielding activation of the workspace (see **Figure 3(c)**). In the right boxes the same occurs *after* the period of spontaneous activity. On the other hand, when the external stimulus coincides with the period of spontaneous activity (center), its access to the workspace is inhibited. From Dehaene S and Changeux JP (2005) Ongoing spontaneous activity controls access to consciousness: A neuronal model for inattentional blindness. *PLoS Biol.* 3: 910–927; used with permission from the Public Library of Science.

detecting or counting stimuli of a certain type) become totally oblivious to other irrelevant stimuli, even when they occur within the fovea for a long duration (Simons and Chabris, 1999; Chun and Marois, 2002). Although inattentional blindness is typically studied in the laboratory by placing subjects in a predefined task, the simulations suggest that spontaneous trains of thought, unrelated to external stimuli and instructions, may also exert a temporary blocking. The model predicts that this state should be characterized by (1) an intense prefrontal–parietal–cingulate activation by the distracting thought or object prior to the presentation of the target stimulus; and (2) a proportional reduction of the target-induced activation to a brief bottom-up activation in specialized processors. fMRI studies provide direct support for those predictions (Rees et al., 1999; Weismann et al., 2005). Future research should extend those paradigms using time-resolved neuroimaging methods such as event-related potentials to

test the prediction that early bottom-up activation is preserved, but top-down recurrent reverberations are suppressed in an all-or-none manner.

1.38.6 Competition between Sensory Stimuli for Access to Consciousness: Looking for Objective Records of Subjective Perception

The model was also studied under conditions simulating a classical perceptual phenomenon referred to as the attentional blink where two sensory stimuli compete for access to reportable conscious perception (Raymond et al., 1992). In a typical experiment, participants are asked to process two successive targets, T1 and T2. When T2 is presented between 100 and 500 s after T1, the ability to report it drops, as if the participants' attention had 'blinked.' In other words, rather paradoxically, perception of a first visual stimulus may prevent the subjective perception of a second one. The paradigm is sufficiently simple and explicit to study why some patterns of brain activity have access to subjective experience and thus to establish, in a causal, mechanistic manner, a link between subjective reports and objective physiological recordings.

The minimal network proposed (Dehaene et al., 2003a; **Figure 3**) is composed of four hierarchical stages of processing where stimuli T1 and T2 evoke neuronal assemblies. At the lower level, A and B correspond to primary and secondary visual areas and C and D to higher association areas, including temporal and frontal cortex. At the lower level, the assemblies do not inhibit each other (see Arnell and Jolicoeur, 1997), but further on, T1 and T2 reach higher association areas C and D, where they compete for global access via reciprocal inhibitory interactions.

With the network placed in a regime of spontaneous thalamocortical oscillations, corresponding to a state of wakefulness (see earlier discussion), one distinguishes two principal modes of signal processing by the network. First, let us consider the simple case where T1, in the absence of competing stimuli, is consciously perceived. T1 evokes a short burst of phasic physiological activity that propagates across the A to D corticothalamic hierarchy. Reaching the highest cortical levels, the sensory input generates top-down amplification signals which, about 80 ms later, cause sustained firing in areas A and B. In a larger-scale simulation, such a long-lasting dynamic state would generate brain-scale propagation of stimulus information into the entire workspace network. It is proposed that this global broadcasting constitutes the physiological basis of conscious reportability.

The network also simulates the conditions of the 'blink' when T1 and T2 are presented in close succession. Experimentally it is known that when T2 is either presented simultaneously with T1, or long after, it is, in both cases, subjectively perceived. The network simulation indeed shows that in both conditions, sustained firing supported by joint bottom-up activation and top-down amplification takes place. Yet, under conditions of close temporal succession, a T2 stimulus presented during T1-elicited global firing elicits bottom-up activation restricted to levels A and B, but fails to propagate to higher cortical levels. As a result, the second phase of top-down amplification does not occur. The T2 stimulus is blinked from conscious perception (**Figure 5**).

The simulation predicts that a temporary drop in firing rate of pyramidal cells coding for T2 in areas C or D is associated with a loss in performance typical of the attentional blink. The model also shows a global drop of power emitted in the gamma band and of cross-correlations between distant T2-coding neurons. Thus, several indexes of firing and synchrony all point to a drop in global activity during the blink, particularly evident in the higher areas C and D.

An original property of the model is the distinction of two modes of signal processing – a nearest-neighbor bottom-up propagation of sensory stimulation across the hierarchy of areas and a long-distance top-down network that sends amplification signals back to all levels below it. In particular, it predicts a dynamic all-or-none bifurcation between the two modes associated with different subjective perception of the stimulus. Indeed, objective physiological data indicate that during this blink, T2 fails to evoke a P300 potential, but still elicits event-related potentials associated with visual and semantic processing (P1, N1, and N400) (Vogel et al., 1998).

The prediction of an all-or-none loss of conscious perception and of T2-induced higher-level brain-scale activation during the attentional blink was tested experimentally (Sergent and Dehaene, 2004; Sergent et al., 2005). We used a modified attentional blink paradigm in which human subjects merely had to report to what extent they had seen a word (T2) within a rapid letter stream that contained another target letter string (T1). To obtain a continuous measure of subjective perception, subjects were asked to move a

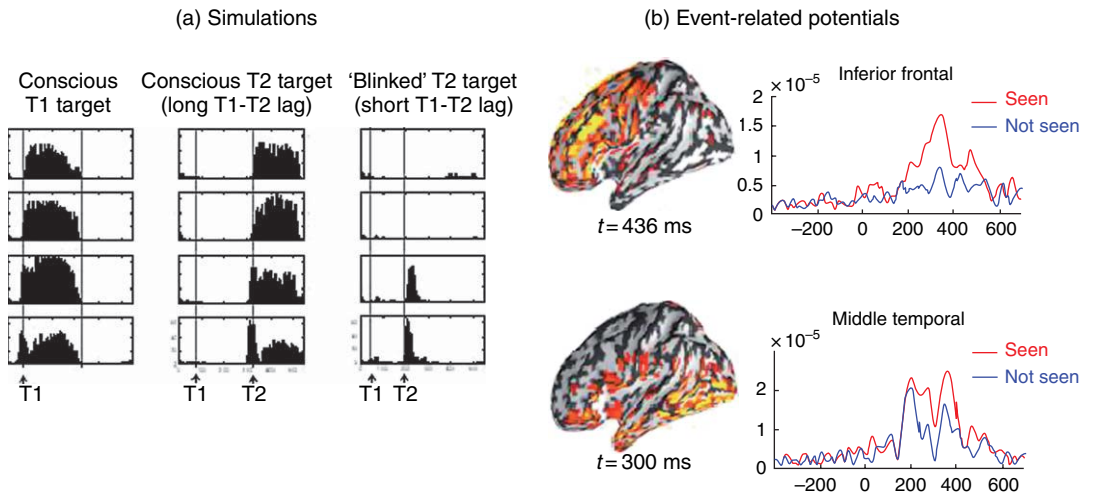

Figure 5 Comparison of the predictions of the neuronal workspace model (from Dehaene et al., 2003b) with the temporal dynamics of cortical electrical activity evoked by seen and unseen stimuli (from Sergent et al., 2005) during the attentional blink task. (a) Simulation of three trials of the attentional blink task. In each column with eight boxes (reproducing the superposed areas of **Figure 3(c)**) is shown the evolution of the computed firing rate in excitatory neurons. *Left column:* the sensory stimulus T1 accesses the workspace; *central column*: the lag between T1 and the second stimulus T2 is such that the sustained activity elicited by T1 has decayed in such a way that T2 accesses the conscious workspace; *right column*: the short lag between T2 and T1 is such that the sustained activity in T1 interferes with the access of T2 to the workspace. (b) Event-related potentials recorded during the attentional blink task at the level of the inferior frontal cortex where differences are noted between subjectively seen and not seen stimuli. Such difference is not observed in the initial bottom-up activation of the temporal cortex (200 ms) but converges later on during a global reverberation phase (300–400 ms). From Dehaene S, Sergent C, and Changeux JP (2003b) A neuronal network model linking subjective reports and objective physiological data during conscious perception. *Proc. Natl. Acad. Sci. USA* 100: 8520–8525, with permission from the National Academy of Sciences; and Sergent C, Baillet S, and Dehaene S (2005) Timing of the brain events underlying access to consciousness during the attentional blink. *Nat. Neurosci.* 8: 1391–1400, with permission from Nature Publishing Group.

cursor on a continuous scale, from 'not seen' on the left to 'maximal visibility' on the right. The results indicate that the reported subjective perception during the blink is indeed all-or-none (Sergent and Dehaene, 2004; Sergent et al., 2005) and relates to the loss of activation in a distributed, synchronous network prominently involving inferior and lateral prefrontal cortices as well as anterior cingulate (Marois et al., 2004; Gross et al., 2004; Sergent et al., 2005; Hommel et al., 2006).

The model in its simple formulation is coherent with previous proposals of a role of top-down recurrent (Lamme and Roelfsema, 2000), reentrant (Edelman, 1993), or resonant (Llinas et al., 1998) connections in the integrative processing of consciously perceived signals. It is also supported by recent experimental data. Because the blink is attributed to competition for workspace access, the proportion of T2 targets that are blinked, as a function of time, roughly traces the inverse shape of the neural activity evoked by T1 in higher-level areas C and D. In actual experiments, similarly, there is an inverse relation between the P300 waveform evoked by T1 and the size of the blink (Sergent et al., 2005). Furthermore, the fMRI activation elicited by T1 in parietal, frontal, and cingulate areas predicts the size of the blink (Marois et al., 2000). Last, the timing of the brain events during the attentional blink using letter strings was recently resolved by event-related potentials. The data show that early potentials (P1 and N1) were equally evoked by seen and not seen words, indicating that these early brain events do not fit with conscious perception. However, a rapid divergence was observed around 270 ms, with late brain events solely evoked by seen words (Sergent et al., 2005) (**Figure 5**). The data are thus fully consistent with the proposal of the model (Dehaene et al., 2003a) that top-down amplification signals and sustained firing into the workspace network constitutes the physiological basis of conscious reportability.

Although there have been no single-neuron recordings during the attentional blink, the simulated profiles of single-neuron activity to seen and blinked T2 targets can be compared to electrophysiological recordings obtained in other paradigms of conscious and unconscious processing. In perceptual areas A and B of the

model, neurons fire phasically in tight synchrony with the stimulus, then show a broader period of late amplification only in seen trials, not in blinked trials. This parallels experimental recordings in areas V1 and IT under conditions of inattention, reduced contrast, masking, or anesthesia, where late amplification occurs only for reportable stimuli (Lamme and Roelfsema, 2000; Super et al., 2001; Lamme et al., 2002). The presently available physiological data are thus consistent with the proposal of the neuronal workspace model that conscious access of reportable signals is a sudden self-amplifying bifurcation leading to a global brain-scale pattern of activity in the workspace network.

1.38.7 Preconscious States of Activity

Despite considerable progress in the empirical research on the brain imaging of conscious perception, debates have arisen about the coherence of these data. For instance, some researchers emphasize a correlation of conscious visual perception with early occipital events (Zéki, 2003), others with late parietofrontal activity (Sergent et al., 2005). Also, following Weiskrantz (1997), we insisted on the notion that subjective reports are the basic criterion that can establish whether a percept is conscious or not. Yet, the philosopher Ned Block (2005) has suggested that in reality, we may experience conscious experiences that are richer in content than what we can report. For instance, when an array of letters is flashed, viewers claim to see the whole array, although they can later report only one subsequently cued row or column. The initial processing of the array might already be considered as 'phenomenally' conscious though not 'seen' in a fully conscious manner (Lamme, 2003; Block, 2005).

We have expressed our disagreement with the phenomenal/access distinction, whose empirical testability is debatable, and have argued instead that within nonconscious processing, one must introduce a transient preconscious state of activity in which information is potentially accessible, yet not accessed (Dehaene et al., 2006). This led to the formal distinction (Dehaene et al., 2006) (**Figure 6**) within nonconscious information processing of:

(1) ***Subliminal processing*** of input signals that may occur when bottom-up activation is insufficient to trigger a large-scale reverberating state. The described simulations of a minimal thalamocortical network (Dehaene and Changeux, 2005) show that in a global network of neurons with long-range axons exhibiting nonlinear self-amplifying properties, a well-defined dynamic threshold exists beyond which activity quickly grows until a full-scale ignition is seen, while a slightly weaker activation quickly dies out.

(2) ***Preconscious processing***, a term coined to design a neural process that potentially carries enough activation for conscious access, but is temporarily buffered in a nonconscious store. Such a buffering might result

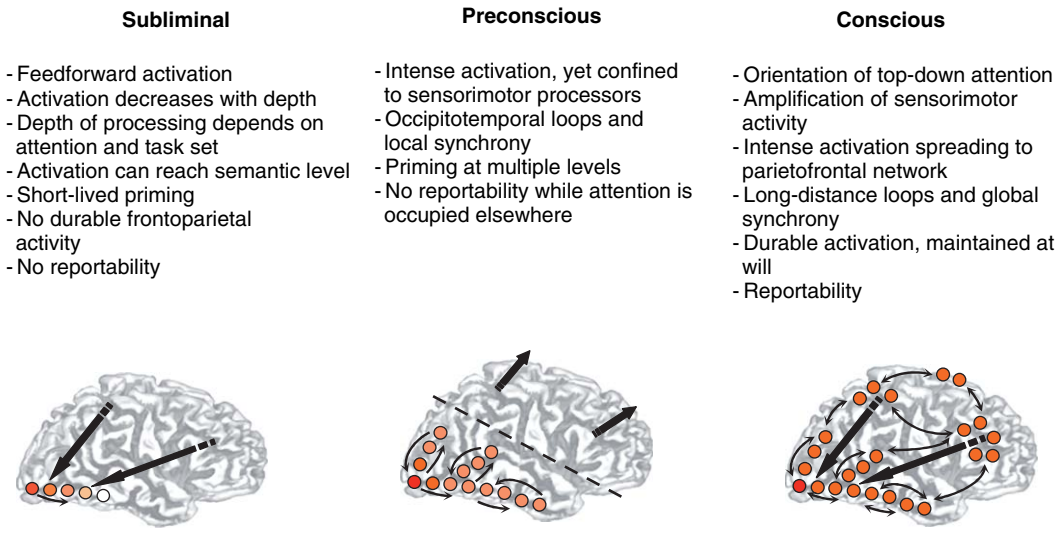

Figure 6 Schematic representation of the subliminal, preconscious, and conscious states of processing of visual stimuli. From Dehaene S, Changeux JP, Naccache L, Sackur J, and Sergent C. (2006) Conscious, preconscious, and subliminal processing: A testable taxonomy. *Trends Cogn. Sci.* 10: 204–211; used with permission from Elsevier.

from a lack of top-down attentional amplification, for example, owing to transient occupancy of the central workspace system (see preceding discussion and Dehaene and Changeux, 2005). The formal analysis of the attentional blink and inattentional blindness paradigms, indeed, has shown that even strong visual stimuli may remain temporarily preconscious. They are potentially accessible (they could quickly gain access to conscious report if they were attended), but they are not consciously accessed at the moment. At the neurocomputational level, preconscious processing is proposed to involve resonant loops within medium-range connections which maintain the representation of the stimulus temporarily active in a sensory buffer for a few hundred milliseconds.

In a fair attempt to establish objective recordings of subjectively reported conscious perception, these various conditions have to be experimentally and theoretically examined. In particular, other independent methods for decoding conscious states based, for instance, on trained pattern classifiers of fMRI or alternative physiological signals might be used to 'objectively' track signal processing in the course of conscious perception (Haynes and Rees, 2005, 2006), thus offering closer tests of the theoretical models.

1.38.8 Performance of an Effortful Deduction Task: The Stroop Task

Our modeling approach was developed along two distinct lines: *type 2* models were intended to capture some fine-grained physiological characteristics of neuronal firing trains and event-related potentials during conscious and subliminal perception. These simulations therefore necessarily incorporated considerably more physiological details of receptor types and cortical layers, but were not extended in a brain-scale architecture solving a precise task (e.g., Dehaene et al., 2003a; Dehaene and Changeux, 2005). On the other hand, the *type 1* models were intended to describe an entire task-related cognitive architecture and thus focused more on global connectivity than on fine physiological details (e.g., Dehaene et al., 1998). The Stroop task (McLeod, 1991) was selected as a simple experimental paradigm where a subject has to make a decision about the meaning of a written word under conditions where interference may occur. For instance, the subject is asked to give the color of the ink with which a color word is printed, and the meaning of the word may differ from the actual color of the printed word. Under this last condition, the subject has to make a conscious effort to give the correct response. The task was originally simulated with the standard neuronal workspace model (Dehaene et al., 1998), in which four input units were dedicated to encoding four color words, four other input units encoded the color of the ink used to print the word, and four internal units corresponded to the four naming responses. Routine color naming and word naming are implemented by direct one-to-one connections between these units and the corresponding output naming units. Workspace activation is not needed for any of these tasks. However, the effortful task (color naming with word interference) consists of providing conflicting word and color inputs and rewarding the network for turning on the naming unit appropriate to the ink color. When the naive network is switched to the effortful condition, an initial series of errors takes place as the network steadily applies the routine naming response. Yet, the delivery of negative reward leads to an increase in vigilance and to the sudden activation of variable patterns among workspace units resulting in a search phase for the next $\sim$30 trials. Workspace activation varies in a partly random manner as various response rules are explored, but the workspace activation patterns that lead to activating the incorrect response unit are negatively rewarded and tend to be eliminated in subsequent trials (**Figure 7**). Eventually, the network settles into a stable activation pattern, with a fringe of variability that slowly disappears in subsequent trials. This stable pattern, which leads to correct performance, is characterized by the differential amplification of the relevant word units relative to color units and by strong excitatory connections among active workspace units maintaining the pattern active in the intertrial interval.

Following the search phase, the network goes through a phase of effortful task execution in which workspace activation remains indispensable to correct performance. During this phase, workspace activity remains high, even on occasional trials in which the word and ink color information do not conflict. When performance is correct for a series of consecutive trials, vigilance tends to drop. However, any lapse in workspace activation is immediately sanctioned by an error. Each error is immediately followed by an intense reactivation of the workspace. Progressively, though, the task becomes routinized as the Hebbian rule applied to processor units tends to increase the color-to-name connections and to decrease the

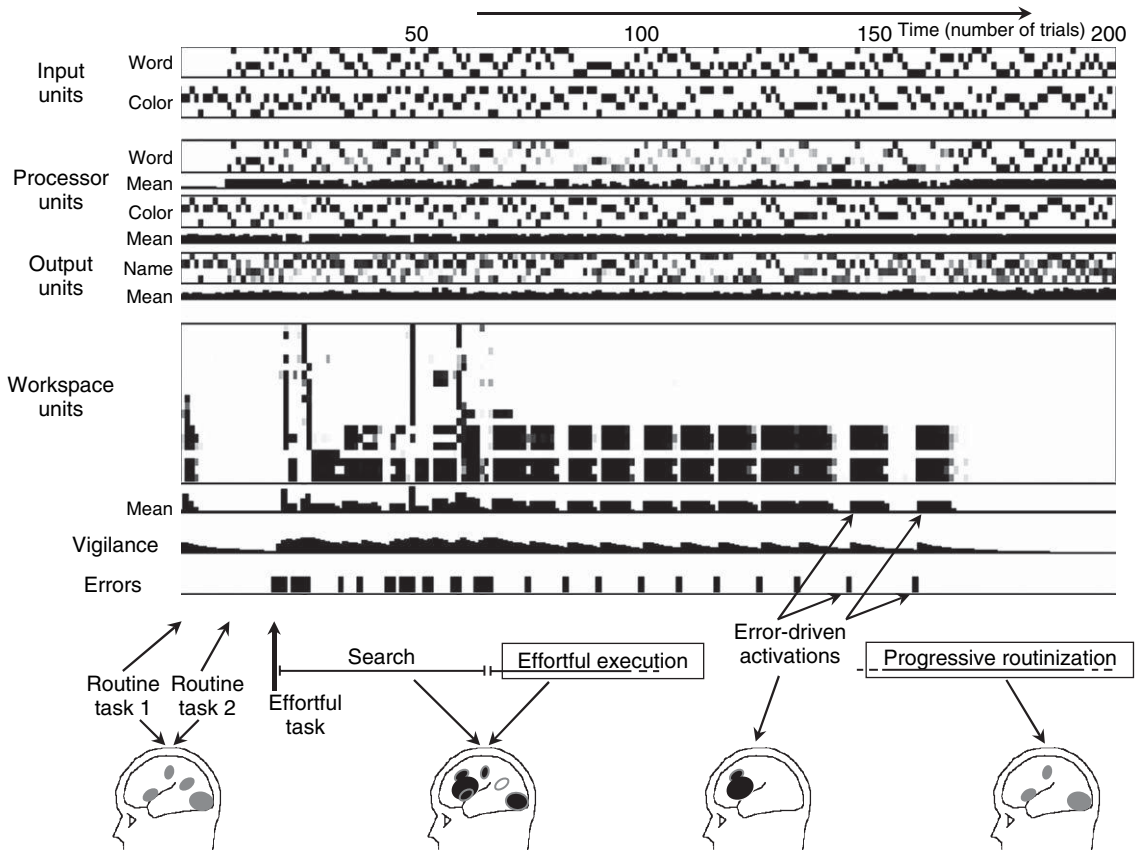

Figure 7 Simulation of the temporal dynamics of the Stroop task based on the original simulation of the neuronal workspace model of Dehaeneet al. (1998). The Stroop task was introduced without warning after routine trial no. 20. Note the selective activation of workspace units with a simultaneous amplification of color processors and a suppression of word processors. Workspace unit activation is seen in the initial phase of searching the appropriate response rule during the effortful execution of the task and following each erroneous response. Lower line: putative brain imaging correlates of workspace activation and routine are shown. From Dehaene S, Kerszberg M, and Changeux JP (1998) A neuronal model of a global workspace in effortful cognitive tasks. *Proc. Natl. Acad. Sci. USA* 95: 14529–14534; used with permission from the National Academy of Sciences.

word-to-name connections. Routinization is characterized by increasingly longer periods of correct performance in the absence of workspace activation.

The key empirical prediction of our hypothesis in the domain of brain imaging is the existence of a strong correlation between cortical areas that are found active in conscious effortful tasks and areas that possess a strong long-distance corticocortical connectivity (**Figure 7**). The global activation of neurons dispersed in multiple cortical areas is expected to be visualized as a temporary increase in the long-distance coherence of brain activity in electro- and magnetoencephalography or in studies of functional connectivity with fMRI.

The model also predicts that areas rich in workspace neurons will appear as suddenly activated when a novel, nonroutine task is introduced while being absent during routine tasks and will vary semi-randomly during the initial learning of a novel task. The level of activation should be high and stable during execution of a known but not yet routinized effortful task and should decrease during routinization, but should resume sharply following an error.

Brain-imaging experiments indicate that the workspace network which includes dorsolateral prefrontal cortex (dlPFC) and anterior cingulate (AC) is active in effortful cognitive tasks, including the Stroop test, with a graded level of activation as a function of task difficulty (Pardo et al., 1990; Cohen et al., 1997; Paus et al., 1998). With automatization, activation decreases in dlPFC and AC, but it immediately recovers if a novel, nonroutine situation occurs (Raichle, 1994). AC activates in tight synchrony with subjects' errors (Dehaene and

Cohen, 1994; Carter et al., 1998). Data consistent with the model have been recently obtained with a rather elaborate reward-based logical deduction task referred to as 'master-brain' task (Landmann et al., 2006). In the course of this trial-and-error learning process, the subjects have to infer the identity of an unknown four-key code on the basis of successive feedback signals but also through internal autoevaluation deductions. This search period is followed by a routine period during which subjects merely repeatedly execute the identified sequence. fMRI measurements reveal a sudden activation during search of the expected workspace circuits which include bilateral frontoparietal (particularly the lateral orbitofrontal and dorsolateral prefrontal cortex) and anterior cingulated cortex, striatum, and midbrain together with the cerebellum (**Figure 8**). This activation collapses during ensuing periods of routine sequence repetition. Furthermore, examination of brain activation during logical and chance discovery showed an early collapse when the correct logical sequence could be deduced without waiting to receive an actual external reward. In agreement with the simulations of the neuronal workspace model in the case of the Stroop task (Dehaene et al., 1998), the data reveal large-scale changes in interconnected distant cortical areas, which may take place tacitly according to an autoevaluation process. They add an important aspect, the differential activation of the striatum: The activation of the putamen and a part of the right caudate body indexes the sign of the reward prediction error (referred to as a ventral 'critic'), and the head of the right caudate indexes the available information that can be extracted from it (referred to as a dorsal 'actor') (see Sutton and Barto, 1981; Schultz et al., 1997; O'Doherty, 2004). This relationship between the workspace network and the underlying subcortical reward networks,

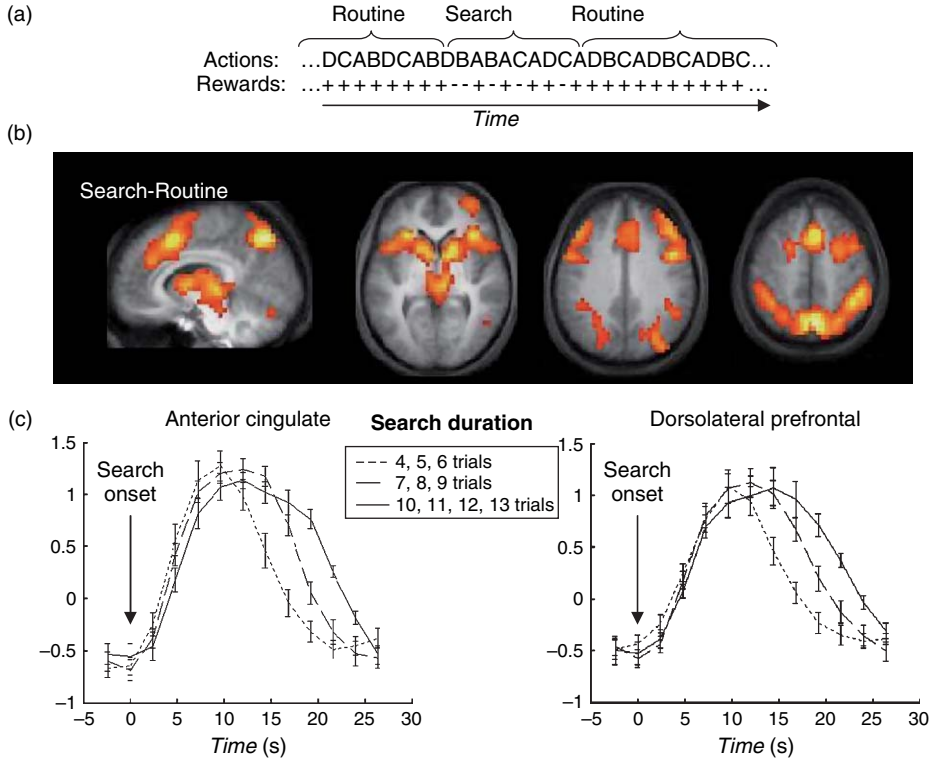

Figure 8 Brain imaging fMRI data of the dynamics of prefrontal and cingulate activity during a reward-based logical deduction task (Landmann et al., 2006). (a) Subjects were engaged in a motor trial-and-error learning task, in which they had to infer the identity of a hidden four-key press by trial-and-error (here ADBC). The design of the task allowed subjects to base their inferences not only on the feedback they received but also on internal deductions and evaluations. (b) fMRI imaging revealed, in agreement with the neuronal workspace model (**Figure 7**), a large bilateral activation of parietal, prefrontal, cingulate, and striatal networks during the search period that collapsed during ensuing routine execution. From Landmann C, Dehaene S, Pappata S, et al. (2006) Dynamics of prefrontal and cingulate activity during a reward-based logical deduction task. *Cereb. Cortex* 17: 749–759; used with permission from Oxford University Press.

which was not taken into consideration in the detailed model of access to consciousness, becomes accessible on both theoretical and experimental grounds.

1.38.9 The Evolution of Consciousness

1.38.9.1 Animal and Human Consciousness

Since the provocative statements of Thomas Huxley that "we are conscious automata," that "brutes" share consciousness with humans (1874) and that "all states of consciousness in us, as in them, are immediately caused by molecular changes of brain substance," nonhuman species and especially laboratory animals have served as experimental models for the scientific investigation of behavior but also of animal consciousness (Thorndike, 1898; Yerkes, 1916; Barresi and Moore, 1996; Jasper, 1998; Koch, 2004; Changeux, 2006). In the framework of the present discussions about neurocomputational models of consciousness, a first challenging issue is thus to what extent the neuronal workspace model may be usefully exploited to define and evaluate consciousness in animals. A second related question is whether or not functionally homologous (rather than analogous) neural structures might be at work in these nonhuman species. Answers to both questions are of importance, in particular if one wishes to use laboratory animals like the mouse as experimental models to investigate the neural bases of consciousness (see Changeux, 2006).

As early as 1921, the Italian neurologist Luigi Bianchi stated that "among the phenomenal factors of the activities of living organisms" arises a "bond of coherence" which "progresses with the development and complexity of living organisms and of their nervous system" that he referred to as consciousness. He further specified that "the dawn of higher consciousness coincides with the apparition of the frontal lobes in the evolution of the brain" and also noted their "inhibitory power" and their capacity for "intellectual syntheses." Bianchi in many respects anticipated several of the presently suggested ideas and theories about consciousness (Changeux, 1983; Shallice, 1988; Edelman, 1989; Crick, 1994; Crick and Koch, 2003; Dehaene and Changeux, 2004; Changeux and Edelstein, 2005; Naccache, 2005; and Naccache et al., 2005, among many others). Following these views, one may then argue that lower species like mice, or even birds, which have a reduced or even absent prefrontal cortex – a critical component of the neuronal workspace circuits – have little if any consciousness. Would experimental and theoretical investigations with the laboratory mouse be simply irrelevant to a scientific investigation of consciousness (see Block, 2005)?

It is of interest to reevaluate these issues in the framework of the neuronal workspace model with nonhumans as well as with humans in the course of both ontogenetic and phylogenetic evolutions. A first point to note is that looking at the developing human infants, Preyer already stated in 1894, "There is not the least reason for assuming in advance that every human being comes into the world endowed with complete consciousness of self" and "there are several grades of consciousness." Developmental studies with humans from the newborn to the adult (Zelazo, 1996; Lagercrantz et al., 2002; Zelazo, 2004; Johnson, 2005; Lagercrantz, 2005; Bartocci et al., 2006) reveal without ambiguity that, beyond its diverse definitions, consciousness cannot be viewed as an irreducible and unique global entity. A similar conclusion emerges from ethological and experimental studies with evolutionarily distant species, such as mice, monkeys, and apes (see Boakes, 1984; Trivers, 1985; Barresi and Moore, 1996; Changeux, 2002). Comparative analysis of these systems suggests a breakdown of consciousness into multiple nested hierarchical levels. Here, we tentatively propose a first classification into four levels, all the while realizing that it remains necessarily arbitrary and simplistic (see Changeux, 2006). Note that these represent landmark points on a continuum rather than sharp distinctions, because our goal is to emphasize a continuity of phylogenetic and ontogenetic stages to full-blown adult human consciousness. The proposed levels comprise:

a. A lower level of minimal consciousness for simple organisms, like rats or mice, which undergo cycles of sleep and wakefulness, possess the capacity to display spontaneous motor activity and to create representations, for instance from visual and auditory experience, to store them into long-term memory and use them for approach and avoidance behavior; these organisms exhibit what is referred to as exploratory behavior (see Thinus-Blanc et al., 1996; Granon et al., 2003); they are amenable, as humans, to trace conditioning (which in humans requires awareness) and to delay conditioning (which does not) (Han et al., 2003). These organisms do not make reference to an *explicit* sense of self and display

minimal social interactions.

In humans, the 25- to 30-week preterm fetus processes tactile and painful stimuli in the sensory cortex (see brain imaging studies by Bartocci et al., 2006) and might thus perceive pain when awake; he/she might have reached a stage of brain maturation analogous (though not identical) to that of a newborn rat/mouse (see Lagercrantz, 2005).

b. Basic consciousness, present, for instance, in vervet monkeys (possibly also in some birds), manifests itself by functional use of objects, protodeclarative pointing, and searching for hidden objects; organisms at this level may display elaborate social interactions, imitations, social referencing, and joint attention; they possess the capacity to hold several mental representations in memory simultaneously and are able to evaluate relations of others to self.

In humans, newborn infants exhibit, in addition to sensory awareness, the ability to process memorized mental representations (e.g., of a pacifier), to express emotions, and to show signs of shared feelings or empathy (Singer, 2006). Even newborns differentiate between self and nonself touch (Rochat, 2003) and imitate the tongue protrusion of an adult (Meltzoff, 1990). At a few months of age, responses to novelty are present and include a late negativity wave which has been tentatively assigned to prefrontal cortex (Dehaene-Lambertz and Dehaene, 1994; Reynolds and Richards, 2005). Prefrontal cortex is active in response to speech, but only in awake infants, not when asleep (Dehaene-Lambertz et al., 2002). Thus access of sensory information to consciousness may already be present, though capacities for internal manipulations in working memory are reduced if not absent.

c. Explicit self-consciousness develops in infants at the end of the second year, together with working and episodic memory and language; it is characterized by self-recognition in mirror tests and by the use of single arbitrary rules with knowledge of one's own behavioral potential and self–other distinction; to some extent chimpanzees might reach this level (see Boakes, 1984).

d. Reflective consciousness with theory of mind and full conscious experience, first-person ontology, and explicit report, is unique to humans and develops after 3–5 years in children.

Examination of this first preliminary classification leads to a simple and unambiguous conclusion. First, adult human consciousness develops progressively, starting from rather rudimentary dispositions in the newborn. Second, mice and rats do not go far beyond the level of minimal consciousness.

1.38.9.2 Minimal Consciousness in Mice and Rats

Careful examination of mouse and rat behavior (see Brown and Bowman, 2002; Granon et al., 2003; Han et al., 2003) in the context of the neuronal workspace and as possible models of human psychiatric disorders (see Granon and Changeux, 2006) leads to the following conclusions.

a. Multiple states of consciousness (such as wakefulness, sleep, coma, general anesthesia, epileptic seizures) and the regulation of their reversible transitions occur in the mouse, as in all mammals, including humans. The circadian sleep–waking cycle mobilizes rather universal mechanisms (Llinas and Steriade, 2006) and is controlled throughout vertebrate species by brainstem reticular formation and intralaminar nuclei of the thalamus (Bogen, 1995; Jones, 1998) with complex patterned releases of neuromodulatory substances. For instance, in the framework of the current studies on nicotinic acetylcholine receptors (nAChR) (Changeux and Edelstein 2005), gene inactivation studies demonstrate the positive contribution of the $\beta 2$ subunit of the nAChR to the phasic expression of arousal promoting mechanisms by endogenous acetylcholine (Cohen et al., 2002; Léna et al., 2004), and similar phenotypes as those noticed in the knockout mice are observed after chronic in utero exposure of the fetus to nicotine (Cohen et al., 2005). These mice thus offer a plausible animal model of sudden infant death syndrome, whose prevalence is known to increase in smoking pregnant women (Cohen et al., 2005).

The use of the neuronal workspace formalism (see Dehaene and Changeux, 2005) might, in this respect, be of some help. One may, for example, view the various graded states of consciousness (from deep anesthesia and coma to full awareness) as directly related to the spontaneous activity of recurrent thalamocortical loops and reticular thalamic nuclei (see Llinas and Paré, 1991; Steriade et al., 1993) as described in the model. Conclusively, the description of the neural mechanisms involved in the transition to wakefulness appears relevant to the case of the mouse as well as its application to models of human pathologies.

b. Delayed-response tasks, exploratory behavior, and flexible goal-directed behaviors. Since the early 1990s (Kolb, 1990), a rich body of behavioral and pharmacological observations has revealed that rats display working memory, attentional processes, and flexible goal-directed behavior which rely on the contribution of the prefrontal cortex (Kolb, 1990). For example, rats may perform an effortful, counterinstinctive, delayed-response task referred to as delayed matching-to-sample that lesions of the prefrontal cortex selectively impair, at variance with a spontaneous delayed nonmatching-to-sample task (Granon et al., 1994). Moreover, in these pioneering studies, it was already shown that cholinergic pathways (Nordberg and Winblad, 1986; Levin, 1992) and specifically their nicotinic component selectively control these cognitive processes (injection of neuronal bungarotoxin into the prelimbic area of the prefrontal cortex selectively impairs the task) (Granon et al., 1995).

In the mouse, exploratory activity is a spontaneous behavior (Thinus-Blanc et al., 1996; Poucet and Herrmann, 2001) that serves to gather and store spatial information which allows allocentric coding of space, itself necessary for flexible navigational processes. Quantitative analyses (Faure et al., 2003) with mice lacking the high-affinity nAChR further reveal that the balance between navigation and exploration shifts in favor of navigation to the detriment of more precise exploration of the environment. Additional comparative studies including several objects presentations in an open-field arena and elementary social behavior (between a test resident mouse and a social intruder) revealed that mice deleted for the nAChR $\beta 2$ subunit are more rigid and exhibited less behavioral flexibility than wild-type mice (Granon et al., 2003; Maskos et al., 2005). The prefrontal cortex is particularly reduced in size in the mouse, yet its lesions (Granon, unpublished) cause evident deficit in the aforementioned conflict-resolution situations and, moreover, create a behavioral phenotype which displays several features in common with the loss of $\beta 2$-nAChR.

These studies unambiguously demonstrate: (1) the specialization of neural circuits engaged in such executive functions which most often (though not always) mobilize the prefrontal cortex; (2) the gating of these functions by nAChRs activated through endogenously released acetylcholine; and last, (3) the intimate relationships between reward and cognition evidenced by the joint recovery of exploratory behavior and reward functions by the targeted reexpression of the $\beta 2$ subunit in the ventral tegmental area dopaminergic nucleus (Maskos et al., 2005).

In conclusion, mice under these experimental conditions do far more than to simply react to sensory information. They engage in complex extended behaviors geared toward far-removed goals and sensitive to rewards. Using the word of Denton (2005), they would display some kind of curiosity being able to orchestrate locomotor behaviors according to what might tentatively be named – at our own risks – conscious intentions. Moreover most, if not all, of all these behaviors need the integrity of the prefrontal cortex. Even if in the case of rats and mice the exact homologies with primates and humans prefrontal cortex areas are still debated (Brown and Bowman, 2005), one may – still hypothetically – propose that the flexible goal-directed behaviors examined with the mouse fall into the category of conscious processes described by the neuronal workspace model.

The model was primarily designed to account for access to consciousness and reportability (see Dehaene et al., 1998). Reporting responses have been demonstrated with some animal species, for instance, using a commentary key (Weiskrantz, 1991; Cowey and Stoerig, 1995) in the case of macaque blind-sight. Yet, a still unanswered question is whether or not reportability can be demonstrated in a species like the mouse. In any case, the invention of a reliable assay with this species is urgently needed. In conclusion, the neuronal workspace model obviously deals with important features of *minimal consciousness*. It accounts, for instance, for the active maintenance of abstract rules through top-down amplification and the flexible control of tasks that require a novel interconnection of existing processors as it typically occurs in the Stroop task (see earlier discussion) as well as with the aforementioned mice behaviors. It deals, *a fortiori*, with the active maintenance of information during a delay period (see Han et al., 2003; Koch, 2004). Even though the relevant simulations have not been carried out, the neuronal workspace architecture should adequately fit exploratory behavior and offer an appropriate mechanism for the ultimate stage of spatial processing introduced by Poucet's model (1993) where, in a workspace homologue, unified location-independent representations with one unique reference direction are being built.

1.38.9.3 Social Relationships and Consciousness

The hierarchical scale of levels of consciousness mentioned at the beginning of this section underlines the importance of social interactions to the extent that empathy was viewed as a characteristic feature of newborn consciousness. Social organisms, including humans, represent intentional relations of themselves and other agents, yet at different levels. They unambiguously distinguish their own intentional relations (or first-person information) from the qualitatively different information available about other agents' intentional relations (or third-person information). In this respect, one should remember that the analysis of the exploratory behavior leads to the distinction between allocentric and egocentric motor behavior in the mouse (Rondi-Reig et al., 2006), pointing to the still highly speculative occurrence of a self. In the mouse, such self is primarily oriented toward the outer physical world, though empathy to pain has been recently reported in the mouse (Langford et al., 2006). No evidence was found at this stage with the mouse for comparability between the actions of self and others, as is found in higher species, and no sign of imitating goal-directed activity or of understanding the viewpoints of others was observed (Barresi and Moore, 1996). In other words, the presently available evidence does not support the occurrence of authentic social relationships in the adult mouse.

The human infant at birth is already at a stage more advanced than the adult mouse; in this respect, as mentioned earlier, he or she may already distinguish between his own and others' movements, in particular by touch, and the newborn displays rudiments of imitations (Meltzoff and Gopnik, 1993; Barresi and Moore, 1996; Lagercrantz, 2005). Moreover, human neonates display emotional contagion by responding more with crying when hearing another newborn crying than when hearing white noise or their own cry (see Decety and Jackson, 2004). Thus, the mouse cannot be a good animal model to investigate intentional relations and social understanding whose highest level is reached exclusively in humans. On the other hand, it may serve as *baseline* to define the elementary neural circuits mobilized by these social relationships in higher mammals and humans.

Extension of the neuronal workspace model to these issues may help in the definition of the minimal components of neural networks able to simulate what may be referred to as social consciousness. One has first to realize that several successive hierarchical levels have to nest the basic states of consciousness of the newborn to reach the full reflective consciousness of the human adult. They include imitation, social referencing, and joint attention, but also what is referred to as the standard theory of mind (see Premack and Woodruff, 1978; Baron-Cohen et al., 2000; Frith and Frith, 2003; Gusnard, 2005). This disposition to represent other people's intentions and beliefs, commonly referred to as propositional attitudes, mobilizes circuits distinct from empathy (Singer, 2006). It develops relatively late in the child (4–5 years), long after empathy. Moreover, both empathy and mentalizing are the objects of a severe maturation before the child reaches the stage of reflective consciousness; it includes in particular the general use of symbols (linguistic or not). Interestingly, this evolution through childhood and adolescence is accompanied by a nonlinear loss of gray matter in the cerebral cortex linked to the selective stabilization (pruning) of synapses (Giedd et al., 1999a,b; Singer, 2006), which takes place during postnatal development (see Changeux, 1983, 2004).

Considerable work has to be developed to establish a useful match between an extended neuronal workspace model and reflective consciousness.

1.38.9.4 The Neuronal Workspace and Human Pathologies

On the clinical side, the neuronal workspace model offers simple interpretations of a variety of human pathologies which cannot be reviewed here. For instance, the neuronal workspace model may account for characteristic deficits caused by frontal lobe lesions in performing delayed-response tasks like the Wisconsin card sorting task (Dehaene and Changeux, 1991) or the Stroop task (see the first simulations of the model; Dehaene et al., 1998) and/or in working memory or declarative memory tasks (Squire, 1987–1988; Ungerleider, 1995; Naccache, 2005; Zeman, 2005).

Frontal lobe pathology is also associated with senescence and dementia (Parkin and Walter, 1992). Particularly relevant to the theory is the case of frontal lobe dementia. This degenerative disease is characterized by apathy, unconcern, disinhibition, distractibility, loss of social awareness, and loss of emotional empathy (Brun, 1987; Neary et al., 1988; Baker et al., 2006). Interestingly, it can be caused by either a mutation in the microtubule-associated protein tau at Chr17q21 (characterized by cytoplasmic neurofibrillary inclusions) or a null mutation in the gene of a growth factor, progranulin, at the Chr17q21

31 locus (characterized by ubiquitin–immunoreactive neuronal inclusions) (Baker et al., 2006; Mackenzie et al., 2006). It manifests itself by a selective loss of layers 2 and 3 pyramidal cells of the prefrontal cortex, the long axon neurons which were postulated as the basic anatomical components of the workspace circuits (Dehaene et al., 1998). In other words, this disease offers a striking example of a genetic dissection of the neuronal workspace in the adult human brain.

Impairments at the level of workspace neurons might also shed some light on the cognitive deficits underlying psychiatric diseases such as schizophrenia. Indeed, cognitive deficits in schizophrenia often affect a broad variety of cognitive tests, and thus may fit better within the present perspective than within the classical neuropsychological perspective, whereby an individual patient's deficits are explained by a local impairment within a modular architecture of specialized subsystems. Many neuroimaging studies suggest decreased frontal and anterior cingulate activation in schizophrenia, as well as decreased long-distance connectivity (Andreasen et al., 1997; Friston, 1998). Furthermore, a dissociation between preserved subliminal processing and impaired conscious access has been reported: the threshold for masking is systematically elevated in schizophrenia, and preserved visual, semantic, and even motor priming suggest that this deficit is due to a central integration impairment, not a basic sensory impairment (Dehaene et al., 2003; Del Cul et al., 2006). Interestingly, similar deficits of access to consciousness are also seen in patients with early multiple sclerosis (MS) and diffuse white matter damage (Reuter et al., 2007). The parallels between MS and schizophrenia, and the conceptualization of at least part of their cognitive deficits as affecting a global workspace for flexible conscious processing, offer interesting avenues for future research.

Another consequence of the workspace theory is that it leads to a plausible interpretation of drug (e.g., nicotine) addiction. Addiction may indeed be viewed as an escape from the voluntary control of drug taking behavior, for instance, as a consequence of the disconnection of a reciprocal-loop linking the neuronal workspace circuits, including prefrontal cortex, dopaminergic neurons, and striatum, thus uncovering the compulsive nonconscious aspect of drug addiction (for discussion see Gutkin et al., 2006).

Moreover, as noted earlier, the nicotinic receptor knockout mice which are compulsively navigating without pausing for exploration may offer an animal model for human attention-deficit/hyperactivity disorder behavior, for which hyperactivity symptoms are known to improve with nicotine treatment (Shytle et al., 2002; Granon and Changeux, 2006).

Last, the differential role of the ventral tegmental area in the recovery of some aspects of cognitive functions in the mouse by local reexpression of nicotinic receptors (Maskos et al., 2005) points to an analogy with the human disease called auto-activation deficit described by Laplane and Dubois (2001). Human patients display a characteristic inertia—they stay at the same place all day long without signs of spontaneous activity but may perform complex activities when stimulated. They show an empty mind for hours yet without cognitive impairment but with stereotyped activities and flattened affects. Their deficit is caused by striatopallidal lesions accompanied by frontal hypometabolism, suggesting, as in the case of the mouse model, a close link between reward and, here, the content of consciousness.

These are a few examples of human pathologies in which the Neuronal Workspace model offers simple and productive interpretations.

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2.01 Introduction and Overview

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2.01.1 The Cognitive Psychology of Memory: Introduction

The main problem in the scientific study of memory is that it proceeds on many different fronts. Neurochemical and neurobiological approaches propel some researchers; systems neuroscientists examine changes in larger pathways in the nervous system; animal behaviorists examine learning and memory as reflected in behavior of (mostly) infrahuman animals, such as birds finding caches of seed; cognitive psychologists study human memory through behavioral means using measures such as recall and recognition; computer scientists endorse computational approaches to memory that sometimes pay little attention to behavioral or neuroscience constraints; and, of course, the study of memory has been the topic of discourse by philosophers for over 2000 years. This four-volume series covers a huge selection of topics that are central to the scientific study of memory. In a different edited volume, Roediger et al. (2007) considered 16 critical concepts in the science of memory from the various viewpoints described above.

2.01.2 Cognitive Approaches to Memory

Practitioners of what is today called cognitive psychology have a long tradition of the experimental study of various aspects of memory. Experimental psychology is often dated from the founding of Wilhelm Wundt's laboratory in Leipzig in 1879. Coincidentally, that same year marks the year that Hermann Ebbinghaus (1850–1909) began his painstaking research that led to his great book, *Über das Gedächtnis (On Memory)* in 1885 (Ebbinghaus, 1885). Ebbinghaus conducted meticulous experiments that asked many fundamental questions about learning and memory, and virtually all his results have stood the test of time in that they have been widely replicated. His work dates the start of the cognitive/behavioral study of learning and memory in humans, although of course centuries of speculation and theorizing (particularly by the British empiricist philosophers) preceded and informed his first experimental efforts. Bower (2000) provides a brief historical overview of this approach to studying learning and memory.

Cognitive psychologists approach the problem of memory through careful experimentation to examine theories that vary in their levels of specification. Some theories (say, transfer-appropriate processing) are broad and seek to capture a wide range of performance across many situations, whereas other approaches (such as mathematical models of performance in specific tasks) are more formal and often attempt to capture memory performance only in tightly structured paradigms.

Traditionally, up until perhaps 30 years ago, cognitive psychologists paid little attention to neuroscience discoveries, and likewise, neuroscientists paid little attention to the experimental work of the cognitive psychologists. Although this division of labor is honored to some degree in the separate volumes of this work, the interests of scientists are clearly broader today. Unlike the case 30 years ago, cognitive psychologists today follow advances in neuroscience with great interest, and many of the concepts and tasks used by neuroscientists were originally developed by psychologists (either those studying animal learning and memory or those applying cognitive methods to these topics in humans). Although this volume is largely devoted to the cognitive/behavioral study of memory, many chapters lean heavily on neuroscience findings. The authors were given leeway to cover their particular topic from

the vantage they deemed most appropriate, bringing in the types of evidence they considered most relevant. Some chapters rely heavily on neuroscience evidence, whereas others refer to purely behavioral experimentation. I see this as perfectly appropriate for the various topics covered in this volume.

2.01.3 Organization of the Volume

One time-honored procedure in the study of cognitive processes is sorting (e.g., Mandler, 1967). An experimenter can give a subject a set of concepts and ask him or her to sort them into groups. The hope is to discover something about how the subject's mind organizes experiences into concepts or categories. The titles of chapters for this volume were originally listed alphabetically, but then the editors of each volume were asked to organize them in some meaningful way, which corresponds reasonably well to a sorting task. I took several trials to reach criterion on this task and can still quibble with myself on various decisions. Luckily, the editors were not asked to create sections of the volume and to label our categories. Here I provide some rationale for the ordering of the chapters and, at the same time, outline the contents of the volume.

The volume begins with a chapter on attention and memory by Neil Mulligan (*See* Chapter 2.02). After all, events in the world that are not attended will not be encoded well and cannot be remembered later, so this seemed a logical starting point. Nelson Cowan's chapter on sensory memory (*See* Chapter 2.03) follows this one. Sensory memory (iconic storage, echoic storage, and similar processes in other modalities) lies at the borderline between perceiving and remembering. No one has ever proposed a good solution to the question of where perceiving ends and remembering begins, and ideas about sensory storage bridge this gap. Susan Gathercole's chapter on working memory comes next (*See* Chapter 2.04). The topic of how people hold information in mind while manipulating it in reasoning and solving problems represents a huge topic in cognitive psychology over the past 40 years. The next chapter is on serial learning by Alice Healy and William Bonk (*See* Chapter 2.05). Most research on serial learning uses paradigms requiring short-term recall (such as digit span and similar procedures), so placing it after the working memory chapter seems reasonable. However, the chapter also covers long-term processes in serial organization.

Robert Greene provides a chapter on the fundamental topic of repetition and spacing effects (*See* Chapter 2.06). Perhaps the first principle of learning and memory is that repeated experiences are (almost) always better remembered than single experiences; further, having two experiences distributed in time (up to some limit that differs for various tasks and retention intervals) leads to greater performance. Another fundamental principle, dating at least to George Miller's (1956) pioneering work on recoding in memory, is that events are not remembered as they are presented in the outside world (events do not somehow leap into the brain as veridical copies of experience), but, rather, events are coded (or recoded) as they are filtered through an individual's personal experiences (or apperceptive mass, to bring back a useful term from early in psychology). Events are remembered as they are coded and not as they necessarily 'are' in the environment. Reed Hunt's chapter on coding processes brings out this important point and shows how recoding can improve retention in some cases but in other cases can lead to errors (*See* Chapter 2.07). Mental imagery is one type of code that has received great attention in the literature, and Cesare Cornoldi, Rossana DeBeni, and Irene Mammarella review this literature in the next chapter (*See* Chapter 2.08).

An event that differs dramatically from many other events that are themselves similar is usually well remembered, which constitutes a distinctiveness effect. For example, a picture of a horse embedded in the middle of a 99-word list of other concrete nouns is much better remembered than if the word 'horse' is presented in a uniform list of 100 words (with 'horse' embedded in the analogous position in the list). This outcome occurs even if the mode of recall is verbal (i.e., people must recall the word 'horse' both when it is presented as a picture and as a word). Distinctiveness effects are ubiquitous in memory research, and Stephen Schmidt provides a review of what is known about this topic in his chapter 'A Theoretical and Empirical Review of the Concept of Distinctiveness in Memory Research' (*See* Chapter 2.09). The next chapter, 'Mnemonic Devices: Underlying Processes and Practical Applications,' by James Worthen and Reed Hunt, brings together the chapters on recoding, imagery, and distinctiveness by reviewing techniques for memory improvement that have been developed over the years (*See* Chapter 2.10). Some of these techniques date back to the ancient Greeks, but modern research has helped to uncover the reasons for their effectiveness. Many of these techniques depend on

imagery, and some (such as the method of loci) rely on humans' ability to remember routes and spatial layouts well, especially ones experienced repeatedly. Timothy McNamara, Julia Sluzenski, and Bjorn Rump review the interesting topic of human spatial memory and navigation (*See* Chapter 2.11).

The next chapters in the volume have to do with memory losses and errors. Forgetting refers to the loss of information over time, and James Nairne and Josefa Pandeirada review the topic in their chapter by that name (*See* Chapter 2.12). A complementary topic is on inhibitory processes, a chapter by Karl-Heinz Bauml (*See* Chapter 2.13). Inhibitory processes are concerned with another set of phenomena that have to do with forgetting. The basic idea is that forgetting may result from an active process of memories being inhibited and therefore forgotten, at least temporarily. Forgetting is often considered an error of omission – information does not come to mind when we try to retrieve it – but errors of commission are of great interest, too. False memories arise when we retrieve information differently from the way it was experienced or, in the most dramatic cases, when we retrieve confident memories of events that never happened at all. Elizabeth Marsh, Andrea Eslick, and Lisa Fazio review this topic in their chapter titled 'False Memories' (*See* Chapter 2.14). Eric Eich, Elke Geraerts, Jonathan Schooler, and Joseph Forgas provide a chapter on mood and emotion in memory, titled 'Memory in and About Affect' (*See* Chapter 2.15). When people are in different moods when they experience events and then try to retrieve them later, they often remember more poorly than if the moods are the same between encoding and retrieval (the phenomenon of mood-congruent memory). However, when people experience greater emotional states during encoding (e.g., strong fear), they often remember events well.

The next few chapters have to do with retrieval of information from memory, as well as associated states of consciousness and processes during this process. Suparna Rajaram and Sarah Barber provide an overview of retrieval processes in memory (*See* Chapter 2.16). John Gardiner writes on the distinction between remembering and knowing, which are responses representing two states of conscious awareness during retrieval (*See* Chapter 2.17). Asher Koriat, Morris Goldsmith, and Vered Halamish discuss control processes in voluntary remembering, dealing with issues such as the criterion people use when deciding that recovered information should be reported as a memory and the factors affecting memory reports (*See* Chapter 2.18). Stephen Lindsay writes on the related topic of source monitoring, or the issue of how people recollect the source of information they report as a memory – did I read the fact in the newspaper, did a friend tell me, or was it learned from television (*See* Chapter 2.19)? Janet Metcalfe and John Dunlosky write on the issue of metamemory, or what people know about their own memories and the strategic processes used in regulating encoding and retrieval of information (*See* Chapter 2.20). Alan S. Brown has provided two chapters on puzzling phenomena of memory retrieval, the experience of déjà vu (when a person has the strange sensation that an event or scene has been experienced previously), and the tip-of-the-tongue phenomenon (the annoying experience when a desired bit of information can almost, but not quite, be retrieved) (*See* Chapters 2.21, 2.22).

Colleen Parks and Andrew Yonelinas provide the chapter 'Theories of Recognition Memory' (*See* Chapter 2.23), with particular emphasis on whether a single-factor or two-factor theory best accounts for the data. William Hockley writes about the related topic of memory search in various types of memory tests, including short-term recognition (S. Sternberg's (1966) item recognition test), long-term recognition, free recall, and other tasks (*See* Chapter 2.24). Both the chapter on recognition and the chapter on memory search involve considerations of mathematical modeling, and the next chapter by Jeroen Raaijmakers explicitly considers mathematical models of human memory (*See* Chapter 2.25). His chapter is followed by a related one by Michael Kahana, Marc Howard, and Sean Polyn on associative retrieval processes in episodic memory (*See* Chapter 2.26). Karl Szpunar and Kathleen McDermott provide an overview on the concept of episodic memory as it has developed since Tulving's seminal chapter in 1972 (Tulving, 1972) (*See* Chapter 2.27). They discuss how neural processes involved in episodic memory may also subserve a person's envisioning the future as well as recollecting the past.

The next series of chapters involves memory of a different kind from episodic memory. David Balota and Jennifer Coane's chapter on semantic memory concerns representation of well-learned information such as words and their meanings (*See* Chapter 2.28). Brian Ross, Eric Taylor, Erica Middleton, and Timothy Nokes survey the related field of how humans learn concepts and categories in 'Concept and Category Learning in Humans' (*See* Chapter 2.29). Gideon Deák and Anna Holt describe research on the critical issue of language learning and report how theories have advanced over the years (*See*

Chapter 2.30). Peter Frensch and Hilde Haider discuss research on the venerable topic of transfer and expertise (*See* Chapter 2.31), a topic that really runs throughout the book in many ways.

Pierre Perruchet reviews the evidence concerning implicit learning, which uses transfer designs as a major tool for understanding (*See* Chapter 2.32). Dale Stevens, Gagan Wig, and Daniel Schacter then review recent evidence on the related topic of implicit memory and priming (*See* Chapter 2.33). Timothy Lee and Richard Schmidt provide an overview on the topic of motor learning and memory, which is related to implicit learning in some ways (*See* Chapter 2.34). Much recent work has shown that procedural and motor skills (as well as some other forms of learning) consolidate while people sleep. Jessica Payne, Jeffrey Ellenbogen, Matthew Walker, and Robert Stickgold review this exciting frontier in memory research in 'The Role of Sleep in Memory Consolidation' (*See* Chapter 2.35).

The next group of chapters is concerned with development of memory across the lifespan, as well as individual differences among people in memory ability. Carolyn Rovee-Collier and Kimberly Cuevas review evidence about infant memory (*See* Chapter 2.36), and then Peter Ornstein, Catherine Haden, and Priscilla SanSouci consider the development of skilled remembering in children (*See* Chapter 2.37). Elena Grigorenko discusses developmental disorders of learning (*See* Chapter 2.38), and Michelle Dawson, Laurent Mottron, and Morton Gernsbacher describe learning in autism (*See* Chapter 2.39). Michael Kane and Tina Miyake write about individual differences in episodic memory among adults (*See* Chapter 2.40), and Moshe Naveh-Benjamin and Susan Old discuss aging and memory (*See* Chapter 2.41). Finally, Anders Ericsson describes research on superior memory of mnemonists and experts in various domains (*See* Chapter 2.42).

The next few chapters of the book focus on more applied aspects of learning and memory research. Mark McDaniel and Aimee Callendar discuss work on cognition, memory, and education, focusing on applying principles from learning and memory research to educational practice (*See* Chapter 2.43). Jeffrey Neuschatz and Brian Cutler discuss the important issue of eyewitness identification (*See* Chapter 2.44). Since the advent of DNA evidence, over 200 people convicted of crimes – often on the basis of eyewitness evidence - have been released from prison, exonerated by DNA evidence. This state of affairs has caused a searching examination of the typical methods used by police to conduct eyewitness identifications. Gilles Einstein, Mark McDaniel, Richard Marsh, and Robert West discuss another popular topic in recent research – how people remember to do things in the future, such as taking an antibiotic pill four times a day when fighting an infection. Their chapter, 'Prospective Memory: Processes, Lifespan Changes, and Neuroscience,' discusses this interesting line of research (*See* Chapter 2.45).

The last three chapters of the volume examine memory from a broader perspective. Most chapters previously described are based on laboratory tasks concerned with learning and memory. Martin Conway and Helen Williams write on the nature of autobiographical memory, which is concerned with how people recollect the events of their lives (*See* Chapter 2.46). Michael Ross, Craig Blatz, and Emily Schryer discuss social memory processes, which includes the issue of how people influence one another as they remember (as well as other topics) (*See* Chapter 2.47). Finally, James Wertsch discusses the emerging topic of collective memory (*See* Chapter 2.48), which is a representation of the past that is shared by members of a group. The group might be people in a nation recollecting an important historical event, such as how people in the United States remember the Revolutionary War. Different groups may see the past in different ways, as Wertsch brings out in his chapter. The empirical study of collective memory is an emerging topic but one that is sure to be more important in the future.

2.01.4 Conclusion

The 47 substantive chapters in this volume represent a marvelous, state-of-the art digest by leading scholars as they summarize what is known about many of the critical topics in the cognitive psychology of learning and memory. The entries range from topics that have a long history (e.g., transfer) to those that have emerged only recently (prospective memory, collective memory). Editing the volume has caused me to learn much, and I believe every reader of this volume will share this experience.

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2.02 Attention and Memory

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> Very great is the dependence of retention and reproduction upon the intensity of the *attention* and *interest* which were attached to the mental states the first time they were present. [italics in original] (Ebbinghaus, 1885: 3)

> Whatever future conclusions we may reach as to this, we cannot deny that *an object once attended to will remain in the memory,* whilst one inattentively allowed to pass will leave no traces behind. [italics in original] (James, 1890: 427)

2.02.1 Introduction

This chapter provides a brief overview of the relationship between attention and memory. The above quotations, although specifically written about the relationship between attention and long-term memory, provide a traditional viewpoint articulating the intimate connection between these constructs. Indeed, as Norman (1969) notes, a researcher would have little difficulty finding similar speculations in the earliest surviving writings in philosophy of mind. Ancient practical manuals on memory and rhetoric begin with the fundamental assumption that successful memory starts with attention (Yates, 1966). Modern researchers in cognitive psychology are likewise interested in attention and memory, but typically for theoretical reasons rather than the practical demands of the rhetorician's 'art of memory.' We shall see that, despite disputes about the details, these quotations remain quite apt regarding many aspects of the relationship between attention and memory.

At the outset, it should be noted that there is a tremendous amount of research under the general heading of 'attention and memory,' enough to require a book-length review even in the early days of the 'cognitive revolution' (Norman, 1969), and even more so in later eras (Cowan, 1995). Consequently, this short review is necessarily selective and incomplete. My goal is simply to describe some of the most important trends and results and to point the interested reader in the direction of additional resources. To begin, we briefly discuss the varieties of memory and the varieties of attention before proceeding to discuss the relationship between the two.

2.02.1.1 Varieties of Memory

Psychologists have long found it useful to differentiate among forms or aspects of memory. The most basic distinction is based on duration, the difference between immediate, fleeting retention of recently presented material and longer-lasting, more permanent aspects of memory. This distinction was captured early on in James's distinction between primary and secondary memory and was similarly

delineated in the modal model of Atkinson and Shiffrin (1968), with its distinction between the short-term and long-term stores. Contemporary psychology continues to distinguish between shorter-term and longer-term memory, as well as among different forms of long-term memory, based on neuropsychological, neuroscientific, and behavioral evidence (e.g., Schacter et al., 2000).

Working memory is the current term for short-term or primary memory, although this conception is more complicated than the unitary short-term store envisaged in the modal model. Working memory is the system responsible for the short-term storage and manipulation of mental representations and contains three primary components, the phonological loop, the visuospatial sketchpad, and the central executive (Baddeley, 1986). The phonological loop is the mechanism responsible for the storage and rehearsal of phonological/verbal information. In a similar way, the visuospatial sketchpad provides temporary maintenance of visual and spatial patterns. The central executive coordinates the operation of these two subsidiary components and mediates the manipulation and transformation of information in these subsystems. The central executive is often associated with limited-capacity attentional resources (Engle, 2002), as discussed later. Finally, a recent version of the working memory model (Baddeley, 2002) proposes an additional component, the episodic buffer, responsible for binding together information represented in different forms or codes (**Figure 1**).

As short-term memory has been fractionated into multiple components, recent theorizing also distinguishes among multiple forms of long-term memory. These distinctions are based on several dimensions, key among them differences in phenomenology and differences in informational (or representational) content. First, it is common to differentiate among varieties of long-term memory based on phenomenology at retrieval, that is, whether the retrieval produces conscious recollection or not. This is critical to the distinction between explicit memory, which refers to conscious, intentional recollection of the past, and implicit memory, which refers to unconscious or unintentional influences of the past. Numerous differences (or dissociations) between explicit and implicit memory lend credence to the importance of this distinction (e.g., *See* Chapter 2.33; Mulligan, 2003b). The distinction between recollection and familiarity made in dual-process models of memory similarly relies on differences in the phenomenology of retrieval (Yonelinas, 2002).

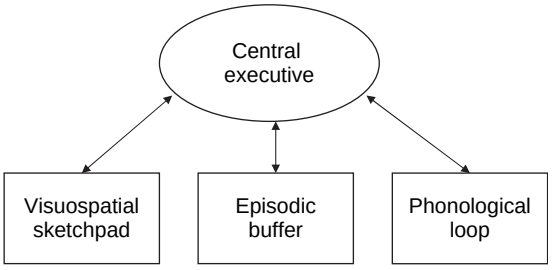

Figure 1 The working memory model. Adapted from Baddeley AD (2002) Is working memory still working? *Eur. Psychol.* 7: 85–97, with permission.

Distinctions based on informational content are important to the multiple systems view of long-term memory (e.g., Schacter et al., 2000), a view that overlaps with distinctions based on phenomenology. First, and most important for our present purposes, is episodic memory, long-term memory for personally experienced events. This form of memory records autobiographical experiences that occurred at a specific time in a specific place. As noted by Tulving (2002), this form of memory permits 'mental time travel,' allowing a person to reexperience an event from the first-person perspective. Episodic memory overlaps with the concept of explicit memory because it supports the conscious and intentional recollection of the past. Semantic memory refers to the organized body of general knowledge about the world. This form of memory includes concepts, categories, vocabulary, and so on. This form of memory is distinguished from episodic memory by its depersonalized nature. Retrieving information from semantic memory is tantamount to retrieving facts rather than reexperiencing prior episodes.

The perceptual representation system (PRS) is

> a collection of domain-specific modules that operate on perceptual information about the form and structure of words and objects. (Schacter et al., 2000: 635–636)

This system represents long-term knowledge of perceptual objects in the modality in which they are processed. Finally, procedural memory is the system that represents skilled behaviors, including perceptual and motor skills, as well as more abstract cognitive skills. This system is marked by slow, incremental strengthening of representations through repeated practice. Procedural memory encompasses the learning of motor skills, such as riding a bike or

swinging a golf club, as well as cognitive skills, such as learning to read.

Conscious recollection (explicit memory) is associated with the episodic system. Implicit memory is more various, forms of which appear to be mediated by each of the other nonepisodic systems. For example, perceptual priming (one manifestation of implicit memory) is attributed to the PRS, whereas conceptual priming is attributed to the operation of the semantic system. Finally, the expression of procedural skills is also associated with implicit memory.

2.02.1.2 Varieties of Attention

Just as memory is multifaceted, so too is attention. To begin, let us examine James' classic definition of attention:

> It is the taking possession by the mind, in clear and vivid form, of one out of what seems several simultaneously possible objects or trains of thought. Focalization, concentration of consciousness are of its essence. It implies withdrawal from some things in order to deal effectively with others. (James, 1890: 403)

From such a description, researchers have typically teased out several aspects or dimensions of attention. First, attention is used for selection among the multitude of stimuli that impinge on our senses at any given moment. Perception would lack coherence if we attempted extensive analysis of every stimulus in the perceptual field. Second, attention implies a limited ability to process information. This is often characterized as a limited pool of attentional resources. Third, attention pertains to control in several ways: control of the flow of information, control of ongoing behavioral responses, and control of prepotent responses (e.g., inhibition).

The dominant distinction in recent research is between peripheral (or modality-specific) aspects of attention (such as visual attention) and central (or modality-independent) aspects of attention. For example, Johnston et al. (1995) propose a distinction between input and central attention, which distinguishes two limited-capacity mechanisms: one responsible for selective aspects of attention, and the other involved in higher-level mental functions (decision making, response selection, etc.). The distinction between selective and central attention corresponds quite closely to similar distinctions made in the literature on attention systems (Wickens, 1984; Duncan et al., 1997; Duncan, 1999), such as the distinction between perceptual and decisional attention (Ashby et al., 1998; Maddox, 2002), the distinction between perceptual and executive attention (Pashler, 1998; Engle, 2002), and the distinction raised in the neuroscience literature between the posterior and the anterior attention systems (Posner and Peterson, 1990; see also Freiwald and Kanwisher, 2004; Humphreys and Samson, 2004). Finally, the concept of central attention is often associated with the central executive component of Baddeley's (1986) model of working memory (e.g., Engle, 2002).

Studies of selective attention and central attention have typically used different experimental paradigms. In the typical experiment on selective attention, multiple stimuli are presented in the same modality, and the participant's attention is either directed at a critical stimulus or directed away from the critical stimulus by requiring the participant to respond to other (distracter) stimuli. Studies of central aspects of attention typically use divided-attention (or dual-task) paradigms. In these experiments, the participant is presented with two streams of stimuli in different modalities (e.g., visual and audition) and required to divide attention across the two sets. This is usually done by requiring the participant to respond to both streams of stimuli and by instructing the participant that both types of responses are important (i.e., encouraging the participant to divide attention equally over both tasks).

2.02.2 Attention, Memory, and the Beginnings of the 'Cognitive Revolution'

2.02.2.1 Cherry's Dichotic Listening Studies

Many of the landmark studies of the studies of the modern era of cognitive psychology focused on attention and memory. Specifically, studies of dichotic listening sought to analyze the role of selective attention in perception and memory. The initial study was that of Cherry (1953), who pioneered the experimental paradigm that would have such profound effect on later empirical and theoretical work. Cherry and the other early researchers were taken by the 'cocktail party problem,' which serves as a natural example of selective attention. At a cocktail party, there may be numerous conversations occurring all around the listener, any one of which could, in principle, be comprehended if attention were so directed.

A person in a conversation must attend to one speaker (and filter out all of the competing messages) to successfully take part in the conversation. How does this occur, and what is the fate of the unattended message(s)? To study this problem, Cherry developed an experimental analogue that would set the agenda for much of the early research on attention and memory. In this experimental technique, participants attended to one of two simultaneously presented messages. The messages were presented over different tracks of a stereo headset so that one message was presented to one ear, and a different message was presented to the other ear. The attended message was human speech, and the ignored message might consist of other human speech (in English or in another language), reversed speech, music, a steady tone, and so on. To ensure that subjects were attending to the correct message, the message was repeated by the subject as it was heard – a task called shadowing. The message that was not shadowed can be characterized as ignored or irrelevant rather than unattended. Although the goal of the dichotic listening task is to render the irrelevant message unattended, this may not always happen. It is possible that subjects might shift their attention on occasion to the nonshadowed message, undermining the goal of the experimental technique. This possibility makes a more neutral term (ignored or irrelevant) a better designation for the nonshadowed input.

Cherry found that subjects were successful in the shadowing task when the two messages differed in terms of their physical properties. The typical physical difference between the messages was spatial location, with one message presented to one ear and the competing message presented to the other. The messages could also be successfully segregated (and one successfully shadowed) when both messages were presented on the same track, provided the messages differed on another physical dimension, such as a male versus a female voice. However, if the two messages were physically similar (e.g., same location, or input ear, and same or similar voices), then the shadowing task became extremely difficult even if the messages differed in other ways, such as the topic or meaning of the messages. Cherry concluded that selection could only operate on the physical characteristics of the message and not on their content.

Most important for the present purposes was the memorial fate of the ignored message. Consistent with his conclusions about selection, Cherry (1953) found that only the physical properties of the ignored message were later remembered. After the shadowing task was over, subjects were asked what they remembered of the ignored message. Typically, subjects could remember whether it was human speech as opposed to a tone or music. Furthermore, they could recall whether it was a male or female voice. However, the subjects showed very little memory for the content of the unattended speech. That is, they showed little memory for words or phrases in the unattended message. Moray (1959) showed that, even when the same word was presented repeatedly – as many as 35 times – in the ignored ear, there was no memory for the stimulus. Furthermore, subjects were not entirely certain that the language was English. Reversed speech and speech in other languages were rarely recognized as non-English. Cherry's broad conclusion was that the unattended material is analyzed at the level of gross perceptual characteristics, but that selective attention is required for the analysis of and long-term memory of detailed aspects of the message such as the language spoken, the identity of individual words, and semantic content.

2.02.2.2 The Filter Model and the Debate between Early and Late Selection Theories

From research on dichotic listening, Broadbent (1958) developed an early, highly influential information processing account of attention, perception, and memory (**Figure 2**). In this model, Broadbent depicted cognition as a series of discrete, serial information-processing stages. Processing begins with sensory systems, which can process large amounts of raw sensory information in parallel. Other research (e.g., split-span studies) indicated that sensory information may be preserved for a short time prior to selection. Thus, Broadbent argued that the initial sensory processing of the perceptual characteristics of inputs was deposited into a short-term memory buffer. This is the point at which attention operates in Broadbent's model, acting as a selective filter. The filter blocks out unwanted inputs based on selection criteria that reflect the goals of the cognitive system. For example, given that the subject's goal is to succeed at the shadowing task, selection is based on the physical location of the to-be-attended message. The selection criteria operate on one of the perceptual characteristics of inputs in the memory buffer. The attended material gains access to a limited-capacity perceptual system (the P system in **Figure 2**), which allows analysis for content, conscious awareness, and ultimately, encoding into long-term memory.

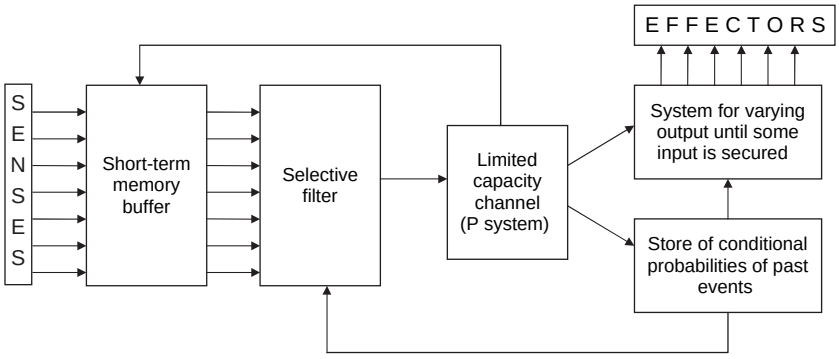

Figure 2 The filter model. Adapted from Broadbent DE (1958) *Perception and Communication.* London: Pergamon Press, with permission.

It should be noted that Broadbent's model has many similarities to the Atkinson and Shiffrin (1968) modal model. The memory buffer in Broadbent's model corresponds to the sensory register of Atkinson and Shiffrin, in that both store raw sensory information prior to selective attention and refined perceptual analysis. The limited-capacity perceptual system of Broadbent is analogous to the short-term store of Atkinson and Shiffrin in terms of its limited capacity, its equation with the contents of awareness, and its role as a conduit to long-term storage.

Broadbent's model proposed that the selective filter operates on the basis of physical characteristics of the message and prior to the analysis of meaning. Consequently, this model is referred to as an early-selection model of attention. Subsequent research on the filter theory raised questions about early selection and gave rise to important competitor models. For example, Moray (1959) found that participants often noticed their own name when it was presented in the ignored channel. According to the filter model, detailed content such as the identity of a word or name should be unavailable from an unattended message. A converging result came from Treisman's (1960) study, in which one story was presented to the attended ear (and shadowed by the subject) and a second story was presented to the ignored ear. Partway through the study, the first story switched tracks and replaced the story in the ignored ear (the first story itself being replaced in the attended track with a new, third, story). According to the filter theory, if the subject is not attending to the irrelevant ear, then they should not process any of its content, and thus should have no awareness that the first story continued in the irrelevant channel. However, subjects typically continued to shadow the first story even after it switched ears. Neither of these results comports with Broadbent's original filter model.

Treisman (1964) handled these new findings by modifying Broadbent's theory. Treisman argued that selective attention does not operate as an all-or-none filter but, rather, operates like a gain control, attenuating unattended inputs. Such attenuated stimuli still might be recognized (i.e., their content fully analyzed) if the stimulus is very important (such as one's own name) or has been primed by attended semantic context. This attenuation view preserves the early placement of the selective filter (now an attenuator) as operating prior to semantic analysis and the processes required for encoding into long-term memory.

An alternate approach was adopted by late selection models (e.g., Deutsch and Deutsch, 1963; Norman, 1968). These models proposed that stimuli routinely undergo substantial analysis (up to identification processing), whether attended or not. The selective mechanism in these models operates after perceptual and content analysis but before response selection. Under this view, semantic analysis helps determine which stimulus is most relevant for current goals and should guide behavioral response.

A number of subsequent results were taken as supportive of late selection. For example, Lackner and Garret (1972) found that the interpretation of ambiguous sentences in the attended ear was biased by words presented in the ignored channel. The results of Corteen and associates (Corteen and Wood, 1972; Corteen and Dunn, 1974) were similarly interpreted as supporting late selection and memory access without attention. Participants in these studies initially underwent a learning phase in which a set of words was paired with electric shock. In a subsequent phase of the experiment, participants showed a heightened galvanic skin response (GSR) to the

shock-paired words even when these words were presented to the ignored channel in a dichotic listening task.

Results such as these imply semantic analysis of unattended information (and late selection) but have been controversial because of the possibility of covert shifts of attention. It is possible in dichotic listening tasks (and in other selective attention tasks) that a subject's attention might wander to the nominally unattended ear. The critical question is whether results such as the above represent semantic analysis of unattended information or momentary shifts of attention to the ignored ear. As framed by Lachter et al. (2004), the issue is whether there is leakage (penetration of the selective filter by semantic content) or slippage (covert, perhaps unintentional, shifts of attention). In a review of the literature, Holender (1986) concluded that results that appear to support late selection in auditory selective attention are actually the result of such attentional slippage. A more recent review comes to the same conclusion for studies of both auditory and visual selective attention (Lachter et al., 2004). Furthermore, Lachter et al. (2004: 884–885) argue that the potential for slippage was underestimated in early research because estimates of the time necessary for attentional shifts were quite high (estimated to be 500 ms or longer in Broadbent (1958)). More modern estimates are as low as 150 ms for voluntary (endogenous) shifts of attention and 50 ms for involuntary (exogenous) shifts. If attention can be so rapidly shifted from one stimulus (or channel) to another, it raises the possibility that rapid shifts of attention might go unnoticed by the experimenter.

This issue has been raised regarding a number of studies. Following up on the results of Corteen and Dunn (1974), Dawson and Schell (1982) presented shock-associated words in the ignored channel of a dichotic listening task. Trials were separated based on evidence for attentional shifts to the ignored channel (based on on-line performance such as shadowing errors). The heightened GSR effect was much greater on trials exhibiting evidence of attention shifts than on trials exhibiting no such evidence. In a similar vein, Wood et al. (1997) varied the difficulty of the primary shadowing task (by increasing the rate of speech to be shadowed) under the assumption that, as the shadowing task becomes more demanding, covert attentional shifts to the ignored channel would be less likely. Evidence for semantic processing and long-term memory of the ignored channel was only found for easier shadowing tasks; no such evidence was found for the more demanding shadowing task, consistent with the idea that covert shifts of attention may give the appearance of semantic processing and long-term memory for unattended materials (for thorough review of these issues, see Holender, 1986; Lachter et al., 2004).

Finally, working memory resources, to be discussed in more detail next, may play a critical role in selective attention. For example, Conway et al. (2001) investigated the cocktail-party effect of Moray (1959), with participants of high and low working memory capacity. Prior research indicates that low working memory capacity is associated with distractibility. Conway et al. (2001) reasoned that more distractible subjects are more likely to allow their attention to shift to the irrelevant channel. This study found that the low-capacity subjects were much more likely to notice their name in the irrelevant channel. This result is consistent with the previous research suggesting that attention shifts may be responsible for the appearance of semantic processing of the ignored channel. In addition, this result indicates a close connection between working memory and selective attention. Similarly, deFockert et al. (2001) found that increasing a working memory load made subjects more susceptible to distraction in a visual selective-attention task. Several researchers have now suggested that selective attention processes are controlled by working memory resources (e.g., Engle, 2002; Lavie et al., 2004). That is, working memory capacity may dictate the extent to which we can successfully focus on one input in the face of distraction (as in dichotic listening). From a historical perspective, this is an interesting inversion. Psychology has traditionally described attention as controlling access to memory structures, but this view implies that a memory structure (working memory) controls the function of an attentional process (selective attention).

2.02.3 Working Memory and Attention

The concept of immediate or short-term memory has long been defined in terms associated with attention. For example, in the modal model of Atkinson and Shiffrin (1968), the presence of information in the short-term store rendered it available to consciousness and allowed the information to guide and control ongoing behavior. Attention is likewise associated with conscious awareness and control of behavior. The short-term store is defined as having a sharply limited capacity, as is attention when

discussed in terms of processing resources. The same points could be made about the limited-capacity perceptual system in Broadbent's filter model. Of course, short-term memory is currently conceptualized in terms of the working-memory model (Baddeley, 1986), which contains specialized subsystems. However, the subsystems are similarly characterized as limited-capacity processing resources, although in the case of the phonological loop and visuospatial sketchpad, the resources are applied for specialized purposes.

In the modal model, the digit span task was the standard measure of the capacity of short-term memory, which along with numerous other assessments of capacity, yielded the famous estimate of 7 ± 2 (Miller, 1956). In the contemporary working memory model, digit span (along with other simple span tasks) is interpreted as a measure of the phonological loop. Analogous measures assess the capacity for storing visual and spatial information in the visuospatial sketchpad. For example, in one visual span task (Logie, 1996), a subject is presented with a matrix of blocks for a brief time. Some of the blocks are filled in, and others are unfilled. After a brief delay, the matrix is represented with one block changing from filled to unfilled (or vice versa). The participant's task is to identify the changed block. This task is relatively easy if the matrix consists of few blocks but becomes increasingly difficult as the size of the matrix increases. The matrix size at which the subject can no longer reliably identify the changed block is taken as the upper bound of the storage capacity of the visuospatial sketchpad.

The phonological loop and sketchpad are subject to dual-task interference, another of the ways in which the subsystems of working memory can be characterized as capacity-limited resources. In the case of these subsystems, the limitations are most clearly exhibited for distracter tasks requiring the same working memory resource. For example, if subjects simultaneously carry out a digit span task and a verbal distracter task (both tasks that draw on the phonological loop), the measure of capacity (i.e., digit span) is dramatically reduced relative to baseline. Alternatively, if digit span is carried out along with a distracter task requiring visual or spatial imagery (which should draw on the sketchpad and not the phonological loop), minimal reduction of digit span occurs. Visual span exhibits the opposite pattern of interference. A secondary task requiring visual or spatial imagery greatly reduces visual span, whereas a verbal secondary task produces much less of an effect. This pattern of selective interference provides part of the rationale for positing the two distinct storage systems (Logie, 1996). Furthermore, this demonstrates that the two subsystems are limited-capacity resources for specialized purposes or information types.

Although the phonological loop and visuospatial sketchpad can be related to the construct of attention via limited capacity, it is the central executive component of working memory that is most typically associated with attention. In contrast to the specialized subsystems, the central executive is a general, amodal processing resource that monitors and controls the actions of the working memory subsystems. Increasingly, the central executive is seen as playing the more general role of an attentional controller, prompting Baddeley (1993) to wonder whether working memory, particularly the central executive component, might be better labeled 'working attention.'

To assess the relationship between the central executive (and the entirety of working memory) and attentional control, it is useful to have a general measure of working memory capacity. These assessments do not solely measure the central executive, because the executive is assumed to regulate and control the other processes in the system rather than provide active storage of information itself. Measures of working memory are called complex span tasks because they seek to assess the joint processing and storage capacities of the whole system, in contrast with simple span tasks (like digit span) that predominantly measure passive storage of a single subsystem (such as the phonological loop). One example is the reading span task (Daneman and Carpenter, 1980) in which subjects read a series of sentences and simultaneously try to maintain the last word from each sentence. At the end of a set of sentences, the participant recalls the final words. The number of words recalled serves as the measure of working-memory span. This task requires both processing (for comprehension) and storage. A similar task is the operation span task (Engle, 2002), every trial of which consists of an equation and a word (e.g., "$4/2 - 3 = 6$? (yes or no) DOG"). The participant's task is to verify whether the equation is correct and remember the word for subsequent recall. At the end of a small set of trials (e.g., 2–7), the words are recalled (again serving as the measure of working memory span).

Working memory capacity, as measured in these tasks, correlates with a number of higher cognitive skills, such as language comprehension, reading ability, reasoning, and general intelligence (see Engle

and Kane (2004), for a review). Importantly for the present purposes, working memory capacity also relates to the control of attention. As noted earlier, individuals with higher working memory spans are less distractible in dichotic listening tasks and exhibit greater control over inhibitory processes (e.g., Conway et al., 2001). Experimental manipulations of working memory capacity produce similar effects in selection and inhibition paradigms (deFockert et al., 2001; Engle and Kane, 2004; Lavie et al., 2004).

2.02.4 Attention and Episodic Memory

The preceding section focused on the relationship between attention and working memory. Returning to the quotations that began this chapter, we now turn to research on attention and long-term memory, beginning with episodic memory. The vast majority of research on long-term memory has focused on episodic (or explicit) memory, conscious recollection of the past on direct tests of memory such as recognition, free recall, and cued recall. Furthermore, most of this research on memory and attention examines the role of attention during encoding. The bulk of this research provides little challenge to the intuitive sense that attention is critical for long-term memory; that is, memory for a stimulus is negatively affected by manipulations of selective or divided attention. Rather, research in this domain typically examines whether some aspects of stimulus encoding might be less reliant on attention than others and whether any elements of episodic encoding can be thought of as being free of attentional influence. In addition, there has been recent interest in determining whether retrieval from episodic memory requires attention to the same degree as encoding.

2.02.4.1 Attention and Encoding

The deleterious effects of reduced attention during encoding have been amply documented from the earliest days of psychological research (Smith, 1895). This holds for manipulations of selective attention as well as dual-task manipulations meant to divide central attentional resources. Beginning with selective attention, the research on dichotic listening makes clear that memory for the content of material presented in the unattended channel is greatly diminished on explicit memory tests such as recognition and recall (Broadbent, 1971). Furthermore, it is often the case that there is no measurable episodic memory at all for ignored material. For example, Eich (1984) found that recognition memory was at chance for words presented in the ignored channel of a dichotic listening study. Of course, it is also clear from this early research that at least some rudimentary perceptual information about the unattended message persists. Even in studies with tight controls for covert attentional switching, subjects recall whether the ignored channel was human speech, a male or female voice, a tone, and so on, implying that some encoding of perceptual information occurs without selection (Lachter et al., 2004). On the other hand, it should also be noted that, even if perceptual information about ignored stimuli is encoded to some degree, the encoding of this information is enhanced by focused attention (Cowan, 1995).

Research on visual selective attention reveals similar results. For example, Wolford and Morrison (1980) developed a visual analogue to dichotic listening in which the study trials consisted of a word flanked by two digits (e.g., 3 DOG 5). In one condition, participants attended to the digits, judging if they were of the same parity (both odd or both even) or of different parity. Although the word was focally presented, it was not the object of attention. In another condition, participants attended to the word. Several blocks of trials were presented to fully accustom the participants to the encoding task. Later, participants were given a recognition test for the words. Not surprisingly, recognition memory was greater when participants attended to the words as opposed to the digits. In addition, when tested on words from the final study blocks (after practicing the parity judgment task over many trials), participants in the parity-judgment condition exhibited no recognition memory for the words. This is quite similar to dichotic listening results that imply that selective attention is critical for later long-term memory and may be absent in an unattended condition (Merikle and Reingold, 1991).

It is clear that selective attention to a stimulus enhances (and may be necessary for) long-term episodic memory for the stimulus. Substantial research has also been conducted to examine the role of central attentional resources in memory encoding, typically by using a dual-task paradigm (i.e., a divided-attention manipulation), in which encoding is carried out under full or divided attention conditions. For example, in the full attention condition, the participant might read a series of study words and

attempt to memorize them for a later test. In the divided-attention condition, the participant attempts to read and memorize the study words while simultaneously carrying out a secondary task. The secondary tasks take on a number of forms but are all designed to compete for central attentional resources. For example, in the three-odd task, the participant hears a series of digits and signals whenever he/she detects a sequence of three odd numbers in a row. Alternatively, one might divide attention with a short-term memory load, in which the participant keeps in mind a set of digits or letters while reading and trying to memorize a set of study materials (the participant recalls the memory load at the end of the study trial to ensure that the material was kept in mind).

It is abundantly clear that dividing attention with tasks such as these degrades later memory on explicit tests, such as recognition or free and cued recall (e.g., Craik et al., 1996; Mulligan, 1998). It should be noted, however, that even quite demanding secondary tasks are unlikely to eliminate explicit memory (Mulligan, 1997, 1998). In addition, the effects of divided attention on encoding are graded: As the secondary task becomes more and more difficult, later memory for the study materials is reduced accordingly (e.g., Mulligan, 1997).

Much of the research in this area focuses on whether there are certain aspects of episodic memory that are more or less dependent on central attention. For example, Castel and Craik (2003) examined whether memory for item versus associative information has differential reliance on attention. Item memory refers to the recognition or recall of individual stimuli as having been present in some particular episode (e.g., the study phase of a memory experiment). Associative memory refers to memory for a newly formed association between stimuli. This goes beyond memory for which stimuli were present in a given context but requires remembering which stimuli were associated with one another. Castel and Craik argued that the formation of new associations requires binding processes that are carried out in prefrontal cortex and are highly dependent on central attention (e.g., Moscovitch, 2000). Consequently, these authors predicted that dividing attention during encoding should disrupt associative memory more than item memory. To test this prediction, participants were presented with pairs of study words under either full or divided attention (attention was divided with the three-odd task). Item memory was assessed by a recognition test for the second word of each pair. That is, participants merely had to remember whether the test word was presented during the study phase. Associative memory was assessed with a paired recognition test, using several types of test pairs. Some of the test pairs were identical to pairs on the study list (intact pairs), some test pairs consisted of two old words from different study pairs (rearranged pairs), and other test pairs contained one or two new words. On this test, participants try to recognize intact pairs from the study list, a discrimination requiring memory for a particular association formed during the encoding phase. Divided attention reduced accuracy in both of the item and associative tests, but as predicted, the deficit was substantially greater for associative recognition. Similar results are found on tests of context and order memory, tests that likewise require episodic binding of previously unrelated information (e.g., Troyer et al., 1999; Troyer and Craik, 2000).

Dual-process models of episodic memory, which propose two independent memory processes, recollection and familiarity (Jacoby, 1991; Yonelinas, 2002), are similar to the distinction between item and associative information. Recollection refers to consciously remembering both the specific test item and the context in which it occurred, whereas familiarity is an undifferentiated feeling that a stimulus was previously encountered. Recollection is assumed to entail a recall-like search process that is consciously controlled and intentional. Familiarity, however, is assumed to be unconscious and unintentional, reflecting processing fluency. Recollection is reliant on binding processes during encoding that associate items and their spatiotemporal context. This suggests that recollection should be quite sensitive to divided attention during encoding. Alternatively, familiarity is assumed to have much less reliance on associative processing, and consequently less reliance on attention. Several lines of research support these notions. First, different types of episodic tests show differential sensitivity to divided attention at encoding, with free recall being the most affected and recognition memory the least (e.g., Craik et al., 1996). Given that free recall is assumed to be heavily reliant on recollective search processes and recognition is more heavily influenced by familiarity, it makes sense that recall is more affected by divided attention than recognition. Second, methods used to tease apart recollection and familiarity within recognition memory (such as the process-dissociation and remember/know procedures) generally indicate that dividing attention during encoding has robust effects on later recollection but little effect on familiarity (see Yonelinas (2002) for a review).

2.02.4.2 Attention and Retrieval

Because the traditional view states that attention is critical for the *creation* of memory traces (e.g., James, 1890; Cherry, 1953; Broadbent, 1958; Norman, 1969), it is not surprising that the vast majority of the research on attention and episodic memory has focused on encoding. Interest in the role of attention in memory retrieval is a more recent development. In an early study on the topic, Baddeley et al. (1984) examined the effects of divided attention on both episodic encoding and retrieval using free-recall, cued-recall, and recognition memory tests. As one would expect, dividing attention during encoding decreased performance on all the memory tests. However, when attention was divided during retrieval there was little decrease in memory accuracy, leading Baddeley et al. to conclude that retrieval processes are relatively automatic.

Craik et al. (1996) likewise varied attention during encoding and during retrieval but came to somewhat different conclusions. Craik et al. used a secondary task that permitted two complementary assessments of the role of attention in memory retrieval. First, as in Baddeley et al. (1984), accuracy in the memory task was compared under the full and divided attention conditions. Second, performance on the secondary task in the divided-attention condition was compared with performance in this task when performed alone (in a baseline condition, in which the secondary task was the sole task). The first comparison indicates whether the secondary task has an effect on memory retrieval. The second comparison indicates whether memory retrieval has an effect on the secondary task. Such secondary task costs are a traditional way to measure whether a process (like memory retrieval) requires attention. The results of the first comparison were consistent with Baddeley et al. (1984): dividing attention during retrieval produced little decline in memory accuracy (of course, divided attention during encoding produced the typical reduction in memory performance, **Figure 3**). However, the second comparison indicated that performance in the secondary task was disrupted when paired with memory retrieval. This contradicts the proposal that retrieval processes are automatic. Rather, Craik et al. argued, retrieval processes make use of attentional resources (as evidenced by the secondary task costs) but proceed in an obligatory (or 'protected') manner (Naveh-Benjamin et al., 2000). Under this view, retrieval takes precedence over other ongoing activities, protecting retrieval accuracy from the deleterious effects of a secondary task.

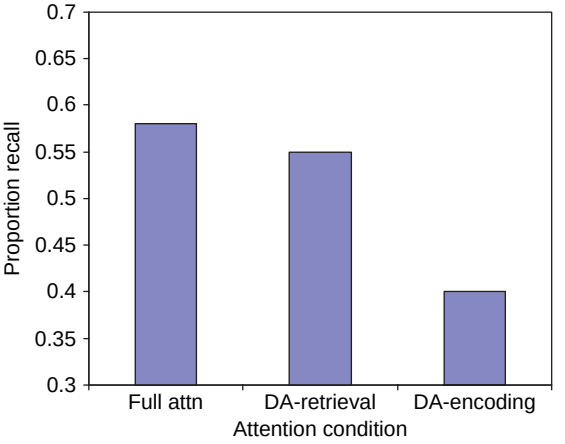

Figure 3 Craik et al. (1996) found that dividing attention during encoding produced a substantial decrease in recall, whereas dividing attention during retrieval produced minimal effect. Full attn = full attention at both encoding and retrieval; DA-encoding = divided attention during encoding, full attention during retrieval; DA-retrieval = full attention during encoding, divided attention during retrieval.

Although Baddeley et al. (1984), Craik et al. (1996), and several other studies (see Craik (2001) for a review) found no effect of divided attention at retrieval on memory accuracy, such effects sometime emerge. Some studies report significant effects on free and cued recall without finding a comparable effect on recognition accuracy (e.g., Park et al., 1989; Anderson et al., 1998). In other studies, a significant decrement to recognition accuracy is reported (Fernandes and Moscovitch, 2000; Hicks and Marsh, 2000; Lozito and Mulligan, 2006). It is important to ascertain why such effects might be found.

Fernandes and Moscovitch (2000, 2003) have argued that memory retrieval is more likely to be disrupted when the secondary task and the memory task use the same type of materials. For example, Fernandes and Moscovitch (2000) used either a number-based or word-based secondary task as participants carried out a recall or recognition test for studied words. The number-based task did not disrupt test accuracy (consistent with Craik et al., 1996), whereas the word-based task significantly reduced memory accuracy. Although the results indicate a divided attention deficit in recognition, Fernandes and Moscovitch argued that decrements obtained were not caused by competition for central attentional resources but were, rather, a result of competition for word-specific representational

systems. Fernandes and Moscovitch labeled this a material-specific interference effect.

Hicks and Marsh (2000), in contrast, posited that competition for central resources can also lead to a divided attention effect under certain conditions. They based this claim on the dual-process model described above, arguing that search-based recollection processes should be disrupted by a secondary task, whereas familiarity processes should be less susceptible. Consequently, encoding conditions that produce higher levels of recollection should exhibit divided-attention effects. Hicks and Marsh had participants either read or generate words during encoding under the standard assumption that generation enhances later recollection. During the recognition test, memory accuracy was reduced by divided attention in the generate condition but not in the read condition. Lozito and Mulligan (in press) also found significant divided-attention effects on both conceptual and perceptual recognition tests, converging on a similar conclusion: Memory retrieval that is recollective in nature is susceptible to divided-attention effects during retrieval. In light of the results of Fernandes and Moscovitch (2000), it is critical to note that the secondary tasks of Hicks and Marsh and of Lozito and Mulligan used numbers, whereas the memory test used words, implying that the divided-attention effect is general (i.e., implicating central attention), rather than material specific.

2.02.5 Attention and Other Forms of Long-Term Memory

2.02.5.1 Attention and Implicit Memory

At the outset of this chapter we differentiated between explicit (episodic, recollective, intentional, conscious) memory and implicit (unconscious, unintentional, nonepisodic) memory. Explicit memory is typically measured with traditional memory tests, such as recognition and free and cued recall, in which participants are directed to think back about a prior event and report on it. Implicit memory tests simply require participants to perform a task (e.g., completing word fragments, generating category examples) without reference to any prior experience. Memory for prior events is inferred from the increased ability to complete, generate, identify, or otherwise process recently presented stimuli. The enhanced processing is called priming.

The principles that govern implicit and explicit memory appear to differ in a number of ways, as evidenced by striking population and functional dissociations (*See* Chapter 2.33; Roediger and McDermott, 1993; Mulligan, 2003b). Coupled with neuroimaging evidence (e.g., Schacter and Badgaiyan, 2001), these dissociations indicate separable components of memory underlying implicit and explicit memory phenomena. Given the centrality of attention in theories of memory encoding and the rather uniform effects of divided attention on episodic memory, it is important to evaluate the role of attention in implicit memory.

With regard to manipulations of selective attention, the results are quite clear: Directing attention away from a stimulus diminishes the amount of priming detected (e.g., Eich, 1984; Bentin et al., 1998; Crabb and Dark, 1999; Mulligan and Hornstein, 2000). This implies that selective attention is necessary for robust levels of priming. A separate question is whether implicit memory can arise in the absence of attention. This question is a matter of some debate. A study by Eich (1984) indicated this possibility. In this dichotic listening experiment, subjects shadowed a prose passage presented to one ear while word pairs were presented in the other ear. Each word pair consisted of a homophone and a word biasing its less common meaning (e.g., taxi-FARE). Subjects showed no explicit recognition for these words. In an implicit test condition, subjects were given a spelling test for aurally presented words. This test contained the homophones presented during the shadowing task as well as a control set of homophones. The subjects were more likely to choose the uncommon spelling for homophones from the ignored channel than for the control homophones, demonstrating above-chance priming for the word pairs presented in the irrelevant channel. This has been taken as evidence of implicit memory for unattended stimuli. It should be noted that, when the word pairs were presented to the shadowed ear, even greater levels of priming resulted. So Eich's results demonstrate reduced priming for the ignored versus attended channel, coupled with above-chance priming for the material presented in the ignored ear. Merikle and Reingold (1991) showed a similar pattern of results for visual selective attention.

Although the results of Eich (1984) were impressive and indicate that implicit memory has less reliance on selective attention than does explicit memory, there is reason to wonder whether these results represent implicit memory for unattended material. Wood et al. (1997) suggested that the presentation rate of the material in Eich's (1984) study was too slow to preclude rapid, covert switches of

attention. As noted in the earlier discussion of dichotic listening, there is always the concern of attentional slippage. Wood et al. investigated this issue by varying the speed with which the attended materials was presented. At the slower rate used by Eich, Wood et al. found the same results: chance-level recognition coupled with above-chance priming on the spelling task. However, at the faster rate (a rate at which subjects could still successfully shadow), priming in the spelling task was not above chance. Wood et al. argued that a faster rate in the attended channel renders the shadowing task more attention demanding and diminishes the likelihood of covert (and undetected) switches of attention to the putatively unattended channel. Consequently, even though the unattended materials were presented at the same slow rate as in the Eich study, no priming was found. This argues against the notion that implicit memory is found for unattended stimuli. Similarly, Berry et al. (2006) recently failed to replicate Merikle and Reingold's results with visual selective attention, strengthening the concern about implicit memory in the absence of attention.

A related line of research has used dual-task paradigms to examine the role of central attention resources in implicit memory. As with selection attention studies, initial studies implied important differences in the role of attention in implicit and explicit memory, with later studies modifying this conclusion. Consider an experiment by Parkin and Russo (1990), in which participants named pictures of everyday objects during the study phase. In the full-attention condition, this was the sole task. In the divided-attention condition, participants named the pictures and carried out a tone-monitoring task, in which a series of tones were categorized as high, medium, or low. Participants were later given an explicit test, in which they recalled the names of the pictures, or an implicit test, in which they identified fragmented pictures. Priming in this task is indicated by identification of studied (or old) pictures at lower levels of clarification than the new pictures. As would be expected, recall was greatly diminished by divided attention. However, the amount of priming on the picture-fragment task was essentially the same for the full- and divided-attention conditions. Several other studies produced similar results (see Jacoby et al., 1989; Parkin et al., 1990; Russo and Parkin, 1993; Mulligan and Hartman, 1996; Schmitter-Edgecombe, 1996a,b), giving rise to the claim that implicit memory has little reliance on attention and largely reflects automatic encoding processes (e.g., Jacoby et al., 1989, 1993; Parkin et al., 1990; Parkin and Russo, 1990; Bentin et al., 1995; Isingrini et al., 1995; Aloisi et al., 2004).

The notion that implicit memory reflects automatic encoding processes has not withstood subsequent research, however (see Mulligan and Brown (2003) for a review). First, this initial research focused on perceptually based priming tasks (such as perceptual identification and word and picture fragment completions), in which degraded or partial perceptual cues guide memory retrieval. Implicit memory may also be assessed with conceptually based tests in which memory retrieval is guided by conceptual cues, such as category names or associates. Mulligan and Hartman (1996; Mulligan, 1997, 1998) demonstrated that conceptual priming is quite sensitive to division of attention during encoding. Second, even perceptually based implicit tests have proven susceptible to divided-attention manipulation under some conditions. For example, Mulligan (2003a) found that a divided-attention task requiring frequent response selection affected perceptual priming, whereas a divided-attention task requiring less frequent response selection left priming unaffected.

To summarize the results, it is now clear that implicit memory is generally affected by a variety of selective- and divided-attention manipulations, indicating that implicit memory relies on both selective attention and central attentional resources. In addition, the effects of these attention manipulations are generally larger on explicit than implicit memory. At present, the results indicate that implicit and explicit memory differ quantitatively rather than qualitatively in terms of their reliance on attention. Both forms of memory rely on attention (in both the selective and central-resources senses), but explicit memory appears to rely on attention more heavily (see Mulligan, 2003a, for speculation as to why).

2.02.5.2 Attention and Procedural Learning

Procedural learning encompasses the acquisition of perceptual, motor, and cognitive skills and is another form of nonconscious memory. Skill acquisition is characterized by gradual improvements in performance over many sessions of practice. To isolate processes of procedural learning and make it amenable to experimental analysis, researchers have typically turned to a small set of laboratory tasks. The most prominent example is the serial reaction time (SRT) test. In this test, participants identify the location of a target as it moves among a fixed set of

(usually four to seven) locations by pressing the key that corresponds to each new location. Embedded in the sequence of locations is a repeating pattern. Reaction times gradually decrease over hundreds or thousands of trials. Procedural learning is assessed in a transfer block in which the pattern is no longer present. An increase in reaction times indicates that something about the repeating pattern had been learned. Another commonly used task is the artificial grammar (AG) learning task developed by Reber (1967). In this task, participants are presented with strings of elements (e.g., letters) that were produced by a complex set of rules – the underlying grammar – that dictates the order in which the elements may occur. After exposure to the learning set, a new set of strings is presented, some of which are consistent with the grammar (i.e., these are strings generated by the grammar), and others that violate the grammar. The participant's task is to categorize the test strings as grammatical or nongrammatical. Above-chance accuracy on this test in the absence of verbalizable knowledge of the grammatical rules is usually taken as evidence of implicit learning of (at least some aspects of) the underlying grammar (see Dienes and Berry, 1997, for a review).

The bulk of the research on attention and procedural learning has examined the role of central attentional resources, using dual-task paradigms. In an early study, Nissen and Bullemer (1987) reported that dividing attention during learning eliminated procedural learning in the SRT task. The dual task used in that study was a tone counting task, in which participants heard high and low tones and kept a running count of the number of low tones. However, there is evidence that the tone-counting task may disrupt procedural learning by interfering with the timing or organization of responses in the SRT task rather than through its demands on central attention (e.g., Stadler, 1995). An arguably less problematic secondary task, the symbol counting task, produces similar effects, disrupting procedural learning relative to a full-attention condition (e.g., Shanks and Channon, 2002; Shanks et al., 2005). Research using the AG task produces similar results. Procedural learning in this task is reduced by a concurrent decision task (Dienes et al., 1991). The learning of complex motor skills has a similar reliance on attention (Wulf and Prinz, 2001). Thus, the acquisition of procedural skills appears to rely on central attentional mechanisms. However, the expression of highly learned skills may not. For example, Helman and Berry (2003) found that dividing attention during the final test in an AG task produced no effect on the expression of procedural information that was initially acquired in a full attention condition. Eversheim and Bock (2001) present a single study of procedural learning, which reveals the typical pattern of results across early acquisition trials and later expression of a practiced skill. These authors used a tracking task in which the visual feedback had been reversed. Over the early trials of the tracking task, performing a secondary task greatly diminished the rate of improvement. However, after extensive practice with the task, a dual-task condition produced little decrement in performance. That is, the acquisition of this new perceptual-motor skill was harmed by distraction, whereas the expression of the attained skill was little disrupted by divided attention.

Although the majority of the relevant studies examined central-attentional resources, a few have examined the relationship between selective attention and procedural learning. Perhaps not surprisingly, most have concluded that procedural learning requires selective attention to the stimuli of the learning task (e.g., Jiang and Chun, 2001; Turk-Browne et al., 2005; see also Wulf and Prinz, 2001).

2.02.6 Concluding Comments

This chapter began with quotations from James and Ebbinghaus articulating the traditional view that attention is critically important for memory. Modern researchers differentiate among multiple forms of both memory and attention, which has allowed a more fine-grained analysis of this relationship. We have seen the ways in which attention is intertwined with the concept of working memory, so much so that aspects of working memory (i.e., the central executive) are sometimes equated with central attention. It is also apparent that attention is critical for acquisition in long-term memory. This is especially clear with regard to episodic memory, although reliance on attention might be greater for certain aspects of episodic encoding (e.g., associative information) than for others (e.g., item information). Furthermore, recent research highlights the importance of attention during encoding for nonepisodic forms of memory, such as implicit and procedural memory phenomena. It is only in the case of long-term memory retrieval that the role of attention is debated. Retrieval from episodic memory appears to be attention demanding, albeit less so than memory encoding. In contrast, retrieval in procedural memory, especially of

well-learned skills, is much less dependent on attention. Finally, there is little research on the role of attention in implicit memory, an area in need of systematic research.

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2.03 Sensory Memory

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2.03.1 Introduction

Sensory memory refers to the short-lived memory for sensory details of events. This can include how things looked, sounded, felt, smelled, and tasted. To some extent, this type of information must persist in long-term memory. It allows us to recognize a familiar voice over the telephone or to recognize the taste of a favorite food. However, the human information processing system seems to be designed in such a way that the richness of sensory memories is quickly lost, leaving behind more categorized memories. For example, when one hears or reads a sentence, the gist is strongly saved but the exact manner in which the material was presented is more quickly lost; verbatim wording is readily accessible only for the most recent phrase, and it is incomplete even for that phrase (Sachs, 1967; Jarvella, 1970). Physical features of stimuli do leave long-term memory traces that influence later behavior (Kolers, 1974; Cowan, 1984; Weldon et al., 1995), but these may lack the fine-grained subtlety of short-lived sensory memories. Sensory memory is typically distinguished from mental imagery, which can include sensory-like qualities but is typically less detailed. For example, one may form an image of a U.S. penny (one-cent coin), but it is distinguished from a sensory memory in its vagueness, such as not really knowing which way Lincoln's head faces.

It has long been recognized that at least some sorts of sensory memory operate in a manner that is different from other forms of memory. In classic research on attention, subjects were asked to repeat a message presented to one ear and ignore a conflicting message presented to the other ear concurrently. Although they could recall little of the message presented to the ear to be ignored, when the task ended the last few words of that message often could be recalled (Broadbent, 1958). This suggested that the information in the unattended message was held temporarily in a form that soon would be lost if it were not attended. A natural way to understand that phenomenon is that the temporary storage consists of sensory information and a longer-lasting record can be saved only if that sensory information is attended before it fades away, which allows the formation of memory for the words that were spoken.

Sperling (1960) carried out a classic study that dramatically illustrated the difference between

sensory and abstract forms of memory for a recent event. Subjects saw a briefly presented array with three rows of characters and then received a tone indicating whether the top, middle, or bottom row should be recalled (a partial-report cue). If the tone was presented soon enough, almost all characters in the designated row could be recalled, provided that there were four or fewer characters in the row. As the delay between the array and the tone cue was increased, performance diminished, quickly at first and then more slowly, curving down to a steady level (asymptote) within 1 s. By that point, the cue came too late to be of use, and subjects could remember only as many characters as one would expect if the limit were about four characters retained from the entire display. This pattern of results could be accounted for on the basis of two types of memory: a sensory memory that held the entire display like a snapshot for a very short time, even though the number of characters was far too large to be attended at once, and a more abstract memory that could hold only about four items. The partial-report cue could influence which part of the sensory memory was transferred to an abstract form and then reported, but the cue had to be presented before the sensory memory faded.

One can see from this finding that an understanding of sensory memory is critical for an understanding of performance on memory tests. Even if one is trying to assess the availability of abstract forms of memory, the contribution of sensory memory must be either eliminated or taken into account. Beyond that, sensory memory is an important aspect of our conscious experience of the outside world, as we will see.

One thing that makes sensory memory controversial is that the boundaries between it and other types of memory are debatable. By some accounts, there is a distinction between sensory storage that comes before perception and postperceptual storage (Massaro and Loftus, 1996). It does appear that there are two phases of temporary memory for stimulus qualities: a brief phase that seems like a continuation of the stimulus for about a quarter second, and a second phase that seems like a vivid recollection for some seconds (Massaro, 1975a; Cowan, 1984, 1988, 1995). However, one could argue that the process of perception begins quite rapidly in the brain, and that the first phase of memory for sensation is already part of perception. When this brief memory begins to fade away, it is not the same thing as a picture becoming hazy or indistinct, as if viewed through a fog. Instead, it is as if different features of each object can be lost separately. In Sperling's (1960) type of procedure, for example, the errors that crop up as the partial-report cue is delayed (or omitted) appear to be primarily location errors, in which a character is reported at the wrong location rather than forgotten completely (Townsend, 1973; Mewhort et al., 1981; Irwin and Yeomans, 1986). Consistent with this, a great deal of neurological research suggests that different features of an object, such as its identity versus its location, are perceived using different neural subsystems (in vision: Haxby et al., 1991; in hearing: Alain et al., 2001; in touch: Pons et al., 1992), and that perception of an object therefore requires a subsequent integration of these features. Sensory memory could include a constellation of features that have not necessarily been integrated. The full integration of these features into objects could require attention (Treisman and Gelade, 1980), which could result in a nonsensory type of memory.

It also may be that the brief sort of sensory memory is not truly a memory as such but, rather, a side effect of perceptual processing. The difference is that the neural effect of a stimulus giving rise to sensation begins before the stimulus ends and, for very brief stimuli, may not even reach a peak until some time after the stimulus has ended. The duration of this sensory memory appears longer for less intense stimuli. Such findings suggest that the sensory persistence of the stimulus may reflect conscious access to neural processes involved in perception (cf. Weichselgartner and Sperling, 1985; Dixon and Di Lollo, 1994; Massaro and Loftus, 1996; Loftus and Irwin, 1998).

Given such debates, it is important to back up and examine the distinctions regarding sensory memory that often are taken as its defining characteristics.

2.03.2 Defining Characteristics of Sensory Memory

2.03.2.1 Memory for Stimuli As Opposed to Ideas

Consider how the perception of a newborn infant must differ from that of an adult: it differs in many ways, but one important difference is as follows. The infant has a range of sensory experiences, but there is not yet any way to attach significance or meaning to most of those experiences. An adult does have meanings, or ideas, to attach to experiences. For example, if an American adult

sees a red octagon, it may remind him or her of a traffic stop sign. Yet it seems reasonable to believe that there is perception and memory of the shape and color that can be separated from its meaning. That may not be exactly true, inasmuch as it is difficult for people to remember or perhaps even to perceive objects without some influence of their knowledge that apples are red, grass is green, and so on (Bartleson, 1960; Ratner and McCarthy, 1990). After identification of a stimulus and its meaning has taken place, there may be neural feedback to the parts of the brain that perceive sensations. Nevertheless, to a first approximation, we can think of sensory memory as the memory for the knowledge-free, sensation-based characteristics of stimuli that resemble what a newborn would perceive. If it turns out to be impossible to think of sensory memory in this way, the postulation of a distinction between sensory and nonsensory forms of memory may have to be weakened.

2.03.2.2 Memory for Information More Fine-Grained Than a Familiar Category

A critical step in perception is categorization: gaining the knowledge that a particular stimulus is an example of items in a certain category, whether or not one knows a verbal label for that category. For example, one can perceive two successive piano notes as coming from different tone categories even if one does not know or remember the names of those categories. Sensory memory is typically viewed as coming before that step of categorization. As a result, it can include fine details that distinguish items within a category, such as differences in tone frequency too small to change the identity of the note, except to make it slightly out of tune (Keller et al., 1995), differences between two slightly different pronunciations of the same vowel (Pisoni, 1973), or differences between two slightly different shades of the same color (Massaro, 1975a). In each case, if slightly different stimuli are presented in close succession (ideally within about 250 ms of each other), the sensory memory of the first stimulus can be compared to the second stimulus with good acuity. This characteristic could be important, as when one is trying to learn exactly how to imitate a word spoken in an unfamiliar language or, in infants, learning language in the first place.

2.03.2.3 Memory Even for Unattended Stimuli

As mentioned in the introduction, the concept of sensory memory arose to explain how people can briefly retain more information than they can process. The concept of a short-lived sensory memory for unattended information was present in the selective listening procedure (Broadbent, 1958) and the partial-report procedure using visual arrays (Sperling, 1960). Darwin et al. (1972) brought these two lines of research together by constructing an auditory analogue of Sperling's procedure. In this analogue, an array of three simultaneous spoken characters presented to left, center, and right spatial locations was followed by a second such array and then a test for the information presented at one location. Similar findings were obtained using arrays of tones (Treisman and Rostron, 1972; Rostron, 1974).

In the auditory studies, it was not possible to present 12 or more simultaneous stimuli as Sperling (1960) did in the visual modality. Consequently, it is possible that subjects were able to carry out a perceptual analysis of the sounds. Nevertheless, other evidence suggests that the perceptual analysis is incomplete and that the items need not have received full attention to have been remembered in the procedure of Darwin et al. (1972). Cowan et al. (1990) carried out a procedure in which nine syllables (*bee, bih, beh, dee, dih, deh, gee, gih, geh*) were presented at random intervals through headphones while the subject ignored them and silently read a novel. When a light signal occasionally appeared, the subject was to stop reading and identify the last spoken syllable, which occurred 1, 5, or 10 s ago. Generally, performance was good at a 1-s retention interval and decreased dramatically across the longer intervals. However, in an experiment in which subjects had to monitor the speech stream while reading (pushing a button if *dih* was presented), there was no forgetting across retention intervals, even though the monitoring task was performed correctly on only 60% of the trials. In another experiment, the reading was whispered by the subject and recorded so that diversions in attention away from the reading could be observed (as 1-s pauses in reading). It was found that consonant perception was much better on trials in which there were diversions of attention while the target syllable was presented. The sensory form of memory may only be adequate for vowel perception, and memory for consonants may be nonsensory in nature and may require attention to be formed. Vowels are more or less steady-state sounds, whereas

stop consonants involve rapid acoustic changes that may be too complex to be maintained in a long form of sensory memory.

In sum, it seems apt to say that sensory memory is a special type of memory that may not require attention to be formed. It cannot hold all aspects of the environment; perhaps it comprises a snapshot of information or slice of time and is unable to hold much information that changes over time. Nevertheless, it is sufficient to save information about far more than one can attentively process at once and thereby serves as a sort of multichannel bulletin board helping the perceiver to shift attention from one sensory channel to another as the incoming information warrants.

2.03.3 Why Study Sensory Memory?

Cognitive psychologists and other students of human behavior have not been consistently enthralled by the concept of sensory memory. The majority of them are interested in learning how meaningful information is processed, and for that enterprise, the fate of sensory information seems to be of secondary interest. Taking this paucity of interest further, Haber (1983) asserted that sensory memory is a byproduct of processing that is not really of any use in ecologically relevant circumstances, with rare exceptions such as when one wants to read on a dark, rainy night by the light produced by flashes of lightning. However, there are several key reasons why sensory memory is of interest. Some important phenomena in the modern world make use of visual sensory memory; without its smearing of the effects of sensation, we would not perceive motion pictures as moving but, rather, as a rapid succession of still frames. It seems likely, as well, that there are analogous phenomena in the natural world. As one spots a deer running across the forest, the continual disappearance of parts of the animal behind trees and reappearance of those parts is reconstructed by one's perceptual system to form a continuous event, the deer running. Sensory memory may be critical in allowing this smooth percept to be formed. In audition, the case is straightforward, inasmuch as sounds inherently change over time. Some mental device must capture segments of the sounds in order to interpret them. For example, as we already have noted, language learning may depend on sensory memory for how words are pronounced. Some less obvious reasons to be interested in sensory memory are as follows.

2.03.3.1 Understanding Qualia and Consciousness

Philosophers speak of qualia, the essential mental states corresponding to experiences. These may be considered the building blocks of conscious experience. The equivalent notion within psychology is the study of subjective experience that can be traced back to the introspectionist method of Wilhelm Wundt, who founded the first laboratory of experimental psychology in 1879. Experimental methods related to introspection make use of similarities in the verbal descriptions of an event across individuals. Descriptions of fleeting events get at the smallest temporal unit of consciousness, or psychological moment, during which all events appear simultaneous even if they are not (e.g., Stroud, 1955; Lichtenstein, 1961; Allport, 1968; Eriksen and Collins, 1968; Creel et al., 1970; Robinson and Pollack, 1971). In the typical experiment to examine this concept, multiple visual displays (such as two sets of dots) are presented rapidly, and when they are presented in close enough temporal proximity, they are perceived as a single image that includes all of the presented items (e.g., all of the dots together). The presentation time within which there is perceived simultaneity depends on stimulus factors but is typically in the range of 100 to 200 ms. One account of the psychological moment states that the sensory memory of the first presentation still must be sufficiently active when the second presentation arrives, so that it becomes impossible to tell the difference between sensory memory and sensation, allowing them to be fused into an apparently simultaneous percept. On that basis one can explain, for example, why a loud noise and a closely following quieter noise can be perceptually fused into a single noise if the gap between them is no more than 100 ms or so (Plomp, 1964). The second noise must be mistaken for part of the decaying sensory trace of the first noise, and the chances of that happening increase as the gap between them gets shorter and the second noise gets quieter.

There were two competing hypotheses of the psychological moment, and only one of them is consistent with a sensory memory account. According to a continuous-moment hypothesis, the psychological moment is a sliding window of time. This is compatible with the notion that the sensory memory of each stimulus can be combined with immediately successive stimuli. In contrast, according to a discrete-moment hypothesis, there are successive windows defined by internal neural events (e.g., oscillation in

the firing of neurons so as to collect incoming sensory signals). Two brief events occurring, say, 80 ms apart would fall in either the same psychological moment or in different moments, depending on when they happened to occur relative to the boundary between successive moments.

Allport (1968) carried out an experiment to decide between these hypotheses that seems especially elegant and decisive. On every trial, 12 horizontal lines were presented in rapid succession, over and over, progressing from a line high on the oscilloscope screen to lines lower and lower on the screen. Only one line was presented at a given time, but because of perceived simultaneity, multiple lines were visible at once. The rate of succession was adjusted for each subject until exactly 11 of the 12 lines were visible at once. At this rate, one could observe what was termed shadow movement as the remaining line that could not be seen changed over time. Now, according to a continuous-moment hypothesis, the shadow should move from top to bottom. While the 12th, lowest line is presented, the sensory afterimage of the 1st, highest line is the oldest and fades. While this line 1 is again presented, the sensory afterimage of line 2 becomes the oldest and fades; while line 2 is again presented, the sensory afterimage of line 3 becomes the oldest and fades; and so on. In contrast, according to a discrete moment hypothesis, the shadow should move from bottom to top. If lines 1–11 fit within one discrete perceptual moment, line 12 is not visible. Then line 12, along with the next presentation of lines 1–10, will all fit into the next moment, and line 11 will not be visible; then lines 11–12 along with the next presentation of lines 1–9 will all fit into the next moment, and line 10 will not be visible; and so on. The results consistently showed downward shadow movement, supporting a continuous psychological moment and a sensory memory explanation.

2.03.3.2 Understanding Group Differences in Information Processing

If the psychological moment is determined by sensory memory, then group differences in sensory memory have important implications for how the groups perceive the world. It determines which events will be grouped together within the duration of sensory memory and which will be separated, beyond that duration (e.g., Dixon and DiLollo, 1994; Loftus and Irwin, 1998).

A study by Cowan et al. (1982) suggested that 8- to 9-week-old infants have a longer first phase of sensory memory than adults do. The procedure that was used was one of auditory backward recognition masking (Massaro, 1975b). In that type of procedure in adults, two brief sounds are presented in rapid succession, and the subject is to identify the first sound in a multiple-choice test. Performance improves as the time between the onsets of the first and second sounds (the stimulus onset asynchrony or SOA) increases to about 250 ms. Even if the choices are so close that performance is substantially below 100%, performance levels off to an asymptotic level at about that SOA. The explanation (in concert with other, convergent procedures that we discuss later) is that information must be extracted over time from sensory memory into a more abstract form of memory until the second sound masks or overwrites the sensory memory of the first sound, interrupting the process of extracting information. By an SOA of 250 ms, the auditory sensory memory fades, so delays of the second, masking stimulus beyond that point would not help. For infant study, an *ah-ah* vowel pair was presented repeatedly, but with different SOAs among the pairs. Sometimes, an *eh-ah* pair was presented instead, but the access to this different pair was restricted to pairs with a particular SOA. The dependent measure of sound discrimination was how long and how vigorously infants were willing to suck on a pacifier that yielded not food but access to pairs that changed from *ah-ah* to *eh-ah,* rather than being stuck with a monotonous repetition of *ah-ah.* (For other infants, the assignment of the two vowels was reversed.) Higher sucking rates for this condition than for a condition in which there was no acoustic benefit of sucking yielded evidence of sound discrimination when the changes occurred within pairs with a 400-ms SOA, but not when the changes occurred within pairs with the 250-ms SOA that is sufficient for optimal performance in adult studies.

Various methods also have been used to show that the duration of sensory memory may differ from the norm in children with mental retardation (Campbell, and Meyer, 1981) or reading disability (Sipe and Engle, 1986) and in patients who have had unilateral temporal lobectomy (Efron et al., 1985). It is not known whether sensory memory abnormality contributes to cognitive disabilities in these groups.

2.03.3.3 Eliminating Contamination from Nonsensory Aspects of Cognition

Even if one is interested in abstract forms of memory, it is necessary to examine sensory memory in order

to control its contribution in various test procedures. A good example is the procedure of Luck and Vogel (1997) to examine working memory. An array of simple, schematic objects is presented and then followed by a second array identical to the first or differing in the identity of one of the objects. In such procedures, people can remember about four items (cf. Sperling, 1960). If the interest is on that working memory limit, then one needs to be sure that sensory memory has faded away before the test. One could use a partial-report cue to determine when it has faded, as Sperling did. Another method is to mask the array with another, interfering array after various intervals and to determine how much information already has been transferred from sensory memory to working memory. Woodman and Vogel (2005) did that and suggested that sensory memory for items in an array was transferred to working memory at a rate of about 50 ms per item in the array.

A similar point could be made with respect to understanding attention. Imagine an experiment in which different word lists are presented simultaneously to the left and right ears. Suppose one has evidence suggesting that an individual can attend to both channels of speech at once. Such evidence may be misleading. If the speech is presented too slowly, there is the possibility of instead (1) attending to the word presented to one ear, and then (2) switching attention to the other ear in time to perceive the sensory memory of the word presented to that ear. If this is the case, speeding up the presentation may eliminate evidence that both channels are being perceived. For an example of this see Wood et al. (1997). In attention research, as in working memory research, the effect of sensory memory must be taken into account.

2.03.4 Techniques to Examine Sensory Memory

Of necessity, we already have discussed a number of techniques used to examine sensory memory. Now it should be helpful to take a brief inventory of these methods. In taking this inventory it is important to keep in mind that different methods disagree. Still, it may be proposed that different outcomes theoretically might result from a common sensory memory. For example, a subjective impression of the duration of a stimulus might result from the duration for which the sensory neural response exceeds a certain intensity, whereas a measure of information about the stimulus might result from an integration of the neural response intensity over time (cf. Cowan, 1987; Loftus and Irwin, 1998).

2.03.4.1 Sensory Persistence Procedures

In the most straightforward types of investigation, stimuli extended over time are perceived as being simultaneous, as in the investigations of the psychological moment described above. Johann Andreas Segner, a German physicist and mathematician living in the 1700s, attached a glowing coal to a cartwheel, rotating the wheel at various speeds. He found that a complete circle was perceived if the wheel was rotated at a rate of at least 100 ms per rotation. This implies that the sensory memory of the glowing coal fell below some minimal level of brightness by about 100 ms.

Efron (1970a,b,c) carried out quite a nice set of experiments to refine the sensory persistence procedure. An indicator (e.g., a click) stimulus was presented along with a target stimulus (e.g., a light flash), and the study estimated when the indicator sounded as if it occurred at the same time as the offset of the target (or, in a control condition, the onset of the target). The result was that the onset of the target was only very slightly overestimated, whereas the offset was overestimated by up to about 200 ms. The nature of the overestimation depended heavily on the duration of the target, such that the target appeared to have a minimal perceived duration of about 200 ms. Very similar results were obtained when the target was visual and when it was auditory. The duration of a perception appears to be the duration of the perceptual response, and if the target stimulus is brief, it reflects the duration of the target plus its perceptual afterimage or sensory memory.

2.03.4.2 Partial-Report Procedures

We already have described the procedures like that of Sperling (1960) and Darwin et al. (1972), in which an array of items is followed by a partial-report cue that allows part of the sensory memory representation to be transferred to a more abstract, categorized, reportable state, working memory. One enigma worthy of consideration is that, whereas persistence procedures have produced similar results for vision and audition, partial-report procedures seem to produce much shorter periods of cue utility for vision (under 1 s) than for audition (about 4 s). We return to this enigma when discussing theories of forgetting from sensory memory.

2.03.4.3 Selective-Attention Procedures

We have touched upon procedures in which an auditory stimulus is ignored at the time of its presentation and only subsequently receives the benefit of attention applied to the sensory memory (Broadbent, 1958; Cowan et al., 1990; there are various other examples such as Treisman, 1964; Norman, 1969; Glucksberg and Cowen, 1970).

It is possible also to examine memory for unattended material in the visual modality. Various techniques can be used to get the subject to contract or expand the focus of visual attention (Eriksen and St. James, 1986; LaBerge and Brown, 1989), and one can then examine memory for information inside or outside of the attentional focus. One difficulty is that there is poorer perceptual analysis of information in the periphery of the visual field, regardless of whether that part of the field is the focus of attention. Usually, the center of the visual field of gaze is also the focus of attention, so attention and visual acuity are confounded. (That is not the case in audition, for which there is no change in the effectors accompanying attention.) Luckily, it is possible to direct a subject's attention to an area outside of the center of gaze (e.g., Brefczynski and DeYoe, 1999). For this reason, it is theoretically possible to determine the effects of attention on visual perception and examine memory for centrally presented but unattended stimuli. It does seem clear that visual attention affects perception, but more research is needed to reveal the details of unattended sensory images in the center of gaze.

2.03.4.4 Backward-Masking Procedures

We already have touched upon the technique of backward masking, in which a brief target stimulus is followed by a mask and impedes recognition of the target. Notably, little masking occurs if the mask precedes the target (Massaro, 1973). This confirms that the critical aspect of backward masking is overwriting of the target's sensory memory by the mask, not the mere proximity of target and mask. It is also noteworthy that the period of backward masking obtained in the auditory modality is quite similar to the visual modality (Turvey, 1973). That appears to be true also in persistence procedures, but not in partial-report or selective-attention procedures, a point to which we return shortly.

One benefit of the masking procedure is that it can be used to show that the neural locus of sensory memory is not entirely peripheral. Turvey (1973) showed that substantial backward masking occurs even when the target was presented to one ear and the mask was presented to the other ear. (For convergent evidence of a central locus of the visual afterimage using a persistence technique, see Haber and Standing, 1969, 1970.) Kallman and Morris (1984) showed something similar in audition, though the opposite conclusion is often cited (Hawkins and Presson, 1977). Cowan (1995, Section 2.5) cited physiological studies supporting the notion that auditory sensory memory has a central locus.

2.03.5 Theories of Forgetting From Sensory Memory

2.03.5.1 Modality-Specific Rates of Decay

It is interesting to see how a scientific field responds to inconsistency. Although the methods described above have been present for quite some time, most theoretical mentions of sensory memory in textbooks seem to go along with the conclusion that auditory memory outlasts visual memory. That could account for the findings of partial-report and selective-attention procedures, but not the findings of persistence and backward-masking procedures. An alternative view is described in the sections that follow.

2.03.5.2 Two Phases of Sensory Memory with Different Rates of Decay

The study of Sperling (1960) was truly seminal in the field. When Darwin et al. (1972) found that sensory memory appeared to be useful for a much longer period in audition (about 4 s) than Sperling found in vision (less than 1 s), it led to the belief that auditory memory lasts longer than visual memory. However, Massaro (1976) offered a different interpretation. Whereas Sperling's experiments used a large number of simultaneous characters (e.g., 12), the smaller number of simultaneous characters used by Darwin et al. (1972) allowed perceptual analysis. According to this view, the sensory memory observed by Sperling was preperceptual, whereas that was not true of the memory observed in the auditory studies. Consistent with Massaro's view, Cowan (1984, 1988, 1995) summarized evidence from various procedures that there are two phases of sensory memory in both the visual and auditory modalities (as well as in other modalities): a short, literal phase lasting about 250 ms and a longer, second phase lasting several seconds. The second phase was said to comprise temporarily

activated sensory features in long-term memory and was said to be both functionally similar to temporarily activated semantic features in long-term memory and much more processed than the first phase.

A couple of studies, one in vision and one in audition, provide striking evidence that there are two types of sensory memory. Phillips (1974) presented two spatial patterns of black and white squares that differed in at most the fill of one square. At short interpattern delays, performance was excellent but was harmed if there was a displacement of the screen location from the first pattern to the second. It was as if the subject actually could see the superimposition of the patterns. In contrast, at longer delays, performance was poorer, and displacement of the screen location did not matter. This suggests that the longer representation was more abstract than the shorter representation.

Kallman and Massaro (1979) carried out a backward-masking procedure in which a standard tone had to be compared to a subsequent comparison tone. Either the standard or the comparison tone was followed by a masking tone. At issue in this experiment was the effect of the similarity between the mask and the tone it masked. When the mask followed the standard tone, it could result in either interference with extraction of information from the sensory trace, which could be termed Masking Type 1, or overwriting of information about the tone even after it has been extracted from the sensory trace, which could be termed Masking Type 2. As the interval between the standard tone and the mask increased, Masking Type 1 presumably disappeared, whereas Masking Type 2 remained. On other trials, it was the comparison tone that was masked, and therefore, only Masking Type 1 was possible; a judgment could be made as soon as information was extracted from that comparison tone. Given that similarity effects were obtained only for Masking Type 2, it was possible to distinguish two phases of auditory memory with different properties. These were termed preperceptual auditory storage and synthesized auditory memory, respectively. These terms correspond to the short and long auditory stores of Cowan (1984).

2.03.5.3 No-Decay Theories

Last, it must be mentioned that some investigators have proposed that there are no decaying memories, including no decaying sensory memory. These investigators view the decline in performance with increasing retention intervals as a matter of a loss of temporal distinctiveness of the items at the end of the list (Neath and Crowder, 1990; Crowder, 1993; Nairne, 2002). That type of theory, combined with the notion that there is better temporal distinctiveness in the auditory modality (e.g., Glenberg and Swanson, 1986), could help to explain why there is an advantage for items at the end of a verbal list presented in the auditory as opposed to the visual modality (cf. Penney, 1989; Marks and Crowder, 1997; Beaman and Morton, 2000; Cowan et al., 2004). However, it is not an easy matter to distinguish between decay and distinctiveness accounts.

Cowan et al. (1997) considered that there might be a distinctiveness explanation for performance in two-tone comparison procedures, in which performance decreases as a function of the time between the standard and comparison tones. As that time increases, it may become larger than the time between trials, so that the tones are not neatly grouped in episodic memory into the trials to which they belong. To overcome this problem Cowan et al. manipulated the time between trials as well as the time between the standard and comparison tones within a trial. Examining trials with the ratio between these two times held constant, and performance decreased only slightly as the time between the standard and comparison tone increased, until it exceeded 6 s. Between 6 and 12 s, the drop was a bit more severe. However, Cowan et al. (2001) reexamined the evidence, taking into account distinctiveness caused by intervals before the penultimate trial, and found no strong evidence of decay (although the data were preliminary in this regard).

A remaining possibility is that, in these procedures, sensory memory information that is attended can be rehearsed (Keller et al., 1995). A study that argues against the effects of time on nonsensory short-term memory (Lewandowsky et al., 2004) takes into account verbal rehearsal, but not the possibility of an attention-based, possibly nonverbal type of rehearsal (cf. Barrouillet et al., 2004). It remains to be seen whether sensory information that is unattended at the time that it is presented, and thus cannot be rehearsed, is lost over time in a way that can be explained by temporal distinctiveness or in a way that cannot be so explained.

2.03.6 Comments on the Future of Research on Sensory Memory

Sensory memory is one of the oldest topics in experimental psychology. Currently, there is only a small to moderate amount of ongoing research on the topic,

but that does not imply that the great problems in the field have been solved, or that the field has become trivial or uninteresting. Sensory memory is an intrinsically fascinating set of neural mechanisms that must be strongly associated with basic conscious experience. As brain researchers investigate how humans know what is real and what is only imaginary, their research no doubt will lead them back to the persistent mysteries within the topic of sensory memory.

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2.04 Working Memory

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2.04.1 Introduction

A common feature of our everyday mental life is the need to hold information in mind for brief periods of time. We frequently have to remember a new telephone or vehicle registration number, to write down the spelling of an unusual name that has been dictated to us, or to follow spoken instructions to find our destination in an unfamiliar environment. At other times, we need to engage in mental activities that require both temporary storage and demanding cognitive processing. Mental arithmetic provides a good example of this: successfully multiplying two numbers such as 43 and 27 in our heads involves storing not only the numbers but the products of the intermediate calculations, accessing and applying the stored rules of multiplication and addition, and integrating the various pieces of information to arrive at the correct solution. Our conscious experience of the calculation attempt is of a kind of mental juggling, in which we try to keep all elements of the task – the numbers we are trying to remember as well as the calculations – going at the same time. Often, the juggling attempt will fail, either because the capacity of working memory is exceeded, or because we become distracted and our attention is diverted away from the task in hand.

Working memory – which is the term widely used by psychologists to refer to the set of cognitive processes involved in the temporary storage and manipulation of information – supports all of these activities and many more. A useful informal way of conceptualizing working memory is as a mental jotting pad that we can use to record useful material for brief periods of time, as the need arises in the course of our everyday cognitive activities. Although it a valuable and highly flexible resource, working memory has several limitations: its storage capacity is limited, and it is a fragile system whose contents are easily disrupted. Once lost from working memory, material cannot be recovered.

The basic features of working memory are described in this chapter. Leading theoretical accounts of the cognitive processes involved in working memory are described, and key findings and experimental phenomena are outlined. As it is now also known that

working memory is important not only for the temporary retention of information, but also for the acquisition of more permanent knowledge, theories of how different aspects of working memory mediate learning are also considered in this chapter.

2.04.2 The Working Memory Model

One influential theoretical account of working memory has framed much of the research and thinking in this field for several decades. In 1974, Baddeley and Hitch advanced a model of working memory that has been substantially refined and extended over the intervening period. The influence of the working memory model extends far beyond the detailed structure of its cognitive processes, which are considered in the following sections. The radical claim made by Baddeley and Hitch was that working memory is a flexible multicomponent system that satisfies a wide range of everyday cognitive needs for temporary mental storage – in other words, it does important work for the user. The distinction between short-term memory and working memory is a key element in the philosophy of this approach. The term working memory refers to the whole set of cognitive processes that comprise the model, which as we will see includes higher-level attentional and executive processes as well as storage systems specialized for particular information domains. Activities that tap a broad range of the functions of working memory, including both storage and higher-level control functions, are often described as working memory tasks. The term short-term memory, on the other hand, is largely reserved for memory tasks that principally require the temporary storage of information only. In this respect, short-term memory tasks tap only a subset of working memory processes. Detailed examples of each of these classes of memory task are provided in later sections.

A further key element of the Baddeley and Hitch (1974) approach is its use of dual-task methodology to investigate the modular structure of the working memory system. These researchers have developed a set of laboratory techniques for occupying particular components of the working memory system, which can then be used to investigate the extent to which particular activities engage one or another component. By the logic of dual-task methodology, any two activities that are unimpaired when conducted in combination do not tap common limited capacity systems. In contrast, performance decrements when two tasks are combined indicate that they share a reliance on the same component. This empirical approach has proved invaluable in fractionating working memory into its constituent parts, leading to the most recent version of the working memory model, advanced by Baddeley in 2000 (Baddeley, 2000) (**Figure 1**).

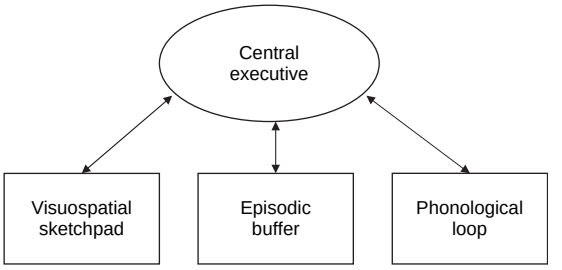

Figure 1 The Baddeley (2000) working memory model.

This model consists of four components. Two of these components, the phonological loop and the visuospatial sketchpad, are slave systems that are specialized for the temporary storage of material in particular domains (verbal and visuospatial, respectively). The central executive is a higher-level regulatory system, and the episodic buffer integrates and binds representations from different parts of the system. The nature of each of these components and associated empirical evidence are described in the following sections. Note also that components of working memory are directly linked with longer-term memory systems in various informational domains. The nature of the interface between working memory and the acquisition of knowledge is considered in later sections of the chapter.

2.04.2.1 The Phonological Loop

Originally termed the articulatory loop by Baddeley and Hitch (1974), the phonological loop is a slave system dedicated to the temporary storage of material in terms of its constituent sounds, or phonemes. The two-component model of the phonological loop advanced by Baddeley in 1986 is shown in **Figure 2**. Representations in the phonological short-term store are subject to rapid time-based decay. Auditory speech information gains obligatory access to the phonological store.

Subvocal rehearsal reactivates serially the contents of the short-term store, in a process that corresponds closely to overt articulation (speaking), but which does

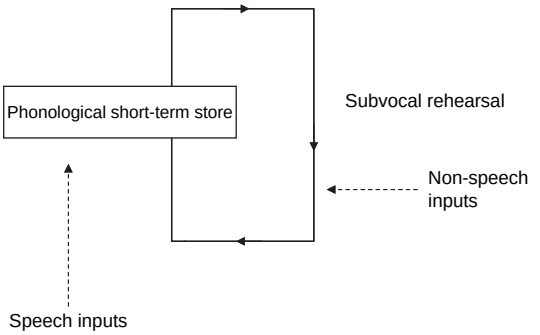

Figure 2 The phonological loop model, based on Baddeley (1986).

not necessarily involve the movement of the speech apparatus or the generation of speech sounds. Representations in the phonological store that are rehearsed before they have time to decay can be maintained in the phonological loop indefinitely, provided that rehearsal continues. Rehearsal consists of the high-level activation of speech-motor planning processes (Bishop and Robson, 1989; Caplan et al., 1992) and is a time-limited process in which lengthier items take longer to activate than short items. Material that is not presented in the form of spoken language but which is nonetheless associated with verbal labels, such as printed words or pictures of familiar objects, can enter the phonological store via rehearsal, which generates the corresponding phonological representations from stored lexical knowledge.

2.04.2.1.1 Empirical phenomena

This fractionated structure to the phonological loop is consistent with a wide range of experimental phenomena from the serial recall paradigm, in which lists of items are presented serially for immediate recall in the original input sequence. Evidence that verbal material is held in a phonological code is provided by the fact that irrespective of whether the memory lists are presented in auditory form or in the form of print, recall is poorer for sequences in which the items share a high degree of phonological similarity (e.g., C, G, B, V, T) than for those which have little overlap in phonological structure (e.g., X, H, K, W, Q). This effect of phonological similarity, first reported by Conrad (1964) and replicated many times subsequently, indicates that serial recall is mediated by phonological representations. Degradation of these representations, possibly due to decay, will thus cause confusion between representations of items with highly similar phonological structures.

The obligatory access of auditory speech information to the phonological loop is demonstrated by the irrelevant speech effect. Serial recall of visually presented verbal items is impaired if spoken items are presented during list presentation, even though participants are told to ignore these stimuli. Moreover, the recall advantage to phonologically distinct over phonologically similar sequences is eliminated under such conditions of irrelevant speech (Colle and Welsh, 1976; Suprenant et al., 1999). This finding indicates that irrelevant speech operates on the same process that gives rise to the phonological similarity effect, so that the unwanted stimuli generate representations in the phonological store that disrupt those of the list items to be recalled.

Evidence for the existence of a distinct subvocal rehearsal process that operates on the contents of the phonological store is provided by other empirical phenomena. An important finding first reported by Baddeley et al. (1975) is that serial recall accuracy is impaired when memory lists contain lengthy items (e.g., aluminum, hippopotamus, tuberculosis) than otherwise matched short items (e.g., zinc, stoat, mumps). Detailed analyses have established that a linear function related recall accuracy to the rate at which participants can articulate the memory sequence: items that are be spoken more rapidly are recalled more accurately, to a commensurate degree. This phenomenon, known as the word length effect, is present for visually and auditorily presented verbal material and is suggested to reflect the serial rehearsal process, which requires more time to re-activate lengthy than short items. As a consequence, representations in the phonological store of lengthy items are more likely to have decayed between successive rehearsals, leading to decay and loss of information.

Support for this interpretation is provided by findings that the word length effect disappears if participants engage in articulatory suppression by saying something irrelevant such as “hiya, hiya, hiya” during presentation of the memory list, for both visually and auditorily presented lists (Baddeley et al., 1975, 1984). These results can be simply explained. Having to engage in irrelevant articulation during a memory task prevents effective rehearsal of the memory items themselves – it simply is not possible to say one thing and to rehearse subvocally something else. As rehearsal is prevented in this condition, there can be no further impairment of recall with lengthy as opposed to short memory items, as this effect is also tied to the rehearsal process.

It should be noted that because visually presented material requires rehearsal to access the phonological store, preventing rehearsal via articulatory suppression should also eliminate the phonological similarity effect with visual presentation, as the material will not reach the store for the similarity-based interference to occur. This prediction has been supported by findings from many studies (Murray, 1968; Peterson and Johnson, 1971).

The claim that the word length effect arises only from subvocal rehearsal has not gone uncontested. Lengthier items are slower not only to rehearse but also to recall, and there is convincing evidence that the increased delay in recalling longer items is one cause of lower performance, probably due to the increased opportunity for time-based decay of the phonological representations. Cowan et al. (1992) employed mixed lists composed of both short and long words to investigate the effects of recall delay. They found a linear relation between the amount of time elapsing from the beginning of the recall attempt and the accuracy of recall, with recall declining as the delay increased (see also, Cowan et al., 1994). One possibility is that the word length effect is multiply determined, and that the slower rate of rehearsal for long than short items is just one of several mechanisms causing lower levels of recall accuracy for lists composed of lengthy stimuli.

Debate concerning the detailed processes underpinning experimental phenomena such as the effects of word length and irrelevant speech (e.g., Neath et al., 2003; Jones et al., 2006) continues, and will in time result in a fuller understanding of the precise mechanisms of serial recall. More generally, though, the broad distinction between the short-term store and rehearsal subcomponents of the phonological loop has received substantial support from several different empirical traditions. It is entirely consistent with evidence of developmental fractionation of the subcomponents of the phonological loop during the childhood years (see Gathercole and Hitch, 1993; Palmer, 2000, for reviews). The phonological store appears to be in place by the preschool period: by roughly 4 years of age, children show adult-like sensitivity to the phonological similarity of the lists items for auditorily presented material (Hitch and Halliday, 1983; Hulme and Tordoff, 1989). The subvocal rehearsal strategy, in contrast, emerges at a later time, typically after 7 years of age. Flavell et al. (1967) observed many years ago that very young children do not show the overt signs of rehearsal, such as lip movements and overt repetition, that characterize older children. Children below 7 years of age are also not disrupted by recalling memory sequences composed of lengthy rather than short items (Hitch and Halliday, 1983), although word length effects do emerge in children as young as 5 years of age if they are trained in the use of rehearsal strategies (Johnson et al., 1987). Also, there is also no consistent association between the articulatory rate and memory span in 5-year-old children, although strong links are found in adults (Gathercole et al., 1994a). Together, these findings indicate that although the phonological store is present at a very early point in children, the use of subvocal rehearsal as a means of maintaining the rapidly decaying representations in the store emerges only during the middle childhood years.

The phonological store and rehearsal process also appear to be served by distinct neuroanatomical regions of the left hemisphere of the brain. Evidence from patients with acquired brain damage resulting in impairments of verbal short-term memory indicates that short-term phonological storage is associated with the inferior parietal lobule of the left hemisphere, whereas rehearsal is mediated by Broca's area, in the left premotor frontal region (see Vallar and Papagno, 2003; Muller and Knight, 2006, for reviews). Findings from neuroimaging studies using methods such as positron emission tomography and functional magnetic resonance imaging to identify the areas of the brain activated by verbal short-term memory tasks in typical adult participants have further reinforced the neuroanatomical distinction between the phonological store and rehearsal (see Henson, 2005, for review).

2.04.2.1.2 A computational model of the phonological loop

Despite its simplicity, the Baddeley (1986) model of the phonological loop is capable of explaining much of the evidence outlined in the preceding section and several other experimental phenomena. It does, however, have one notable shortcoming as a model of serial recall. Although this paradigm requires the accurate retention of both the items in the memory list and their precise sequence, the model focuses exclusively on the representation of item information and therefore fails to account for how the serial order of list items is retained in the phonological loop. As a consequence, it cannot accommodate many detailed aspects of serial recall behaviour. One important characteristic of serial recall is the serial position function, the asymmetric bow-shaped curve that arises from high levels of accuracy of recalling initial

list items (the primacy effect), relatively poor recall of mid-list items, and a moderate increase in accuracy for items at the end of the sequence (the recency effect). Another key finding is that the most common category of errors in serial recall is order errors, in which items from the original position migrate to nearby but incorrect positions in the output sequence (Bjork and Healy, 1974; Henson et al., 1996). The Baddeley (1986) model of the phonological loop provides no explanation of either of these features of verbal short-term memory.

Burgess and Hitch (1992) addressed this problem by developing and implementing a connectionist network model that incorporated a mechanism for retaining the serial order of items in addition to temporary phonological representations and an analog of rehearsal that corresponds to the phonological loop. The structure of the model is shown in **Figure 3**. It consists of four separate layers of nodes that represent input phonemes, words, output phonemes, and a context signal. Serial order is encoded by associating the activated item representation with a slowly evolving context signal containing a subset of active nodes that change progressively during presentation of the list, and can be conceptualized as a moving window representing time such that successive context states are more similar to one another than temporally distant states. Presentation of an item causes temporary activation of input phoneme nodes, word nodes, and output phoneme nodes via existing interconnections. When one item node succeeds in becoming the most active, a temporary association is formed between the winning item node and currently active context nodes. The item node is then suppressed, allowing the same process to be repeated for the next item in the sequence. In this model, rehearsal consists of feedback from the output phonemes (activated following selection of the winning item node) to the input phonemes.

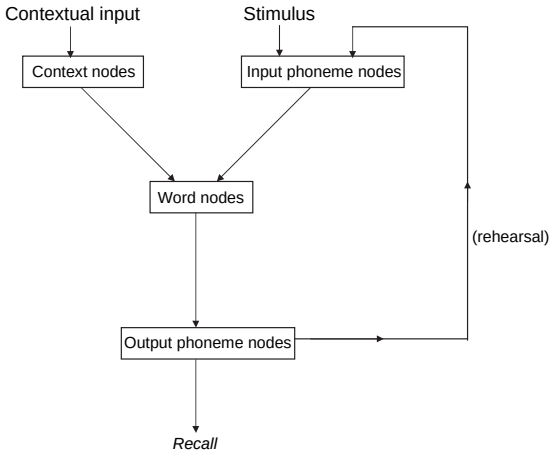

Figure 3 Simplified architecture of the Burgess and Hitch (1992) network model of the phonological loop.

At recall, the original context signal is repeated and evolves over successive items in the same way as at input. For each signal, item nodes receive activation based on their initial pairing with context in the original sequence, and the winning item is selected and activates consistent output phonemes. Noise is added to this final selection process to induce errors. Where serial order errors do occur – that is, incorrect item nodes are selected – they tend to migrate to target-adjacent positions as a consequence of the high degree of overlap in the context nodes active in successive context states.

In addition to generating the classic experimental phenomena associated with the phonological loop such as the phonological similarity, word length, and articulatory suppression effects, this model simulates many of the features of serial order behavior that the phonological loop model on which it was based could not address. Consider first the serial position function. Primacy effects arise largely from the greater number of rehearsals received by early list items, and recall of both initial and final items is enhanced by the reduced degree of order uncertainty at these terminal list positions. A preponderance of order errors is also readily generated because context signals, like item representations, degrade with time. Thus on some occasions, the retrieved item representation will have been associated with an adjacent context signal, yielding recall of a list item at an incorrect output position. When items do migrate in simulations of the model, they show the bell-shaped migration function in which the distances traveled in the sequence are usually small rather than large, which has also been established in the behavioral data.

2.04.2.1.3 The phonological loop and language

Although the cognitive processes underpinning the phonological loop are well understood, one puzzle for many years was exactly why this system exists. It may have turned out to be useful for remembering telephone numbers, but why do we have the system in the first place? A number of possibilities were considered. One plausible hypothesis was that the phonological loop acts as a buffer for planned speech.

The presence of a phonological output buffer that stores the retrieved phonological specifications of intended lexical items and enables the smooth and rapid production of speech has long been recognized as a logical necessity by speech production theorists (e.g., Bock, 1982; Romani, 1992). There is, however, little evidence that the phonological store fulfills this function (see Gathercole and Baddeley, 1993 for review). It has consistently been found that adult neuropsychological patients with very severe deficits of verbal short-term memory leading to memory spans of only one or two items can nonetheless produce spontaneous speech normally: utterance length rates of hesitations and self-corrections are comparable to those of control adults (Shallice and Butterworth, 1977).

A second hypothesis was that the phonological loop provides an input buffer to incoming language that is consulted in the course of normal comprehension processes (Clark and Clark, 1977). Once again, findings from adult short-term memory patients provided the opportunity to test this hypothesis. The prediction was clear: if short-term memory plays a significant role in comprehension, the very low memory span of short-term memory patients should lead to substantial impairments in processing the meaning of language. Findings from many research groups and many different patients provided little support for this prediction. Despite severe deficits in phonological loop functioning, short-term memory patients typically had few difficulties in processing sentences for meaning, except under conditions in which lengthy, unusual, and ambiguous syntactic structures were used, or the sentences were essentially memory lists (see Vallar and Shallice, 1990; Caplan and Waters, 1990; Gathercole and Baddeley, 1993; for reviews). It therefore appears that although under most circumstances the language processor operates online without recourse to stored representations in the phonological loop, these representations may be consulted in an off-line mode to enable backtracking and possible re-analysis of spoken language under some conditions (McCarthy and Warrington, 1987).

There is, however, one area of language functioning in which the phonological loop appears to play a central role, and that is in learning the sound structure of new words. Evidence from many sources converges on this view. Studies of typically developing children have consistently found close and selective associations between measures of verbal short-term memory and knowledge of both native and foreign language vocabulary (e.g., Gathercole and Baddeley, 1989; Service, 1992; Cheung, 1996; Masoura and Gathercole, 1999). The accuracy of nonword repetition – in which a child hears a spoken nonword such as woogalamic and attempts to repeat it immediately – is particularly highly correlated with vocabulary knowledge, although so too are more conventional measures of verbal short-term memory such as digit span (Gathercole et al., 1994b). A similar link is found between verbal memory skills and the rate of learning nonwords in paired-associate learning paradigms, in which participants learn to associate unfamiliar phonological forms with either novel objects (Gathercole and Baddeley, 1990a, used toy monsters with names such as Pimas), unrelated words (such as fairy-kipser), or semantic attributes (e.g., bleximus is a noisy, dancing fish). Both of the latter examples are from a study reported by Gathercole et al. (1997), in which the phonological memory skills of the participating 5-year-old children were in contrast found to be independent of the ability to learn word–word pairs.

Further evidence that the phonological loop is involved in the long-term learning of phonological structures in particular has been provided by the study of individuals with developmental or acquired deficits in language learning. Specific language impairment (SLI) is a condition in which children fail to develop language at a normal rate despite normal intellectual function. Word learning represents a particular problem for affected children. It has consistently been found that children with SLI have substantial impairments of nonword repetition and of other measures of verbal short-term memory (e.g., Gathercole and Baddeley, 1990b; Bishop et al., 1996; Archibald and Gathercole, 2006). A corresponding neuropsychological patient, PV, had a severe deficit of the phonological loop, and was found to be completely unable to learn word–nonword pairings such as rose–svieti, but performed within the typical range on a word–word learning task (Baddeley et al., 1988). Experimental studies of paired-associate learning with normal adult participants have shown that word–nonword learning is disrupted by variables known to interfere with phonological loop functioning, such as phonological similarity and articulatory suppression (Papagno et al., 1991; Papagno and Vallar, 1992). In contrast, learning of word–word pairs is not influenced by these variables.

On this basis, it has been proposed that the primary function of the phonological loop is to support

learning of the sound structures of new words in the course of vocabulary acquisition (Baddeley et al., 1998b). It is suggested that initial encounters with the phonological forms of novel words are represented in the phonological short-term store, and that these representations form the basis for the gradual process of abstracting a stable specification of the sound structure across repeated presentations (Brown and Hulme, 1996). Conditions that compromise the quality of the temporary phonological representation in the phonological loop will reduce the efficiency of the process of abstraction and result in slow rates of learning. In a recent review of this theory and associated evidence, Gathercole (2006) has suggested use of the phonological loop to learn new words is a primitive learning mechanism that dominates at the early stages of learning a language and remains available as a strategy throughout life. However, once a substantial lexicon is established in a language, word learners increasing rely on lexically mediated learning of new words, thereby building on the phonological structures that they have already acquired.

2.04.2.1.4 Summary

The phonological loop model advanced by Baddeley (1986), consisting of a short-term store and a subvocal rehearsal process, is the most influential current account of verbal short-term memory. Convergent evidence for the model is provided from a range of research traditions including experimental cognitive psychology, developmental psychology, neuropsychology, and neuroimaging. A similar diverse range of findings indicate that the phonological loop plays a key role in vocabulary acquisition (Baddeley et al., 1998; Gathercole, 2006).

The successful implementation of the model in the form of a connectionist network by Burgess and Hitch (1992) is an important development that has stimulated competing computational models of serial recall with distinct architectures. The network model has also been further developed to simulate learning of novel sequences by the phonological loop (Burgess and Hitch, 1999). The availability of detailed models of short-term memory and the reciprocal stimulation of empirical findings and computational simulations is a sign of advanced theoretical development that is in large part due to the guiding influence of the phonological loop concept on this field over many years.

2.04.2.2 The Visuospatial Sketchpad

2.04.2.2.1 Theory and empirical phenomena

The second slave system of the working memory model is the visuospatial sketchpad, specialized in the storage and manipulation of information that can be represented in terms of either visual or spatial characteristics. Short-term memory for visuospatial material is associated with increased activity in the right hemisphere regions of the inferior prefrontal cortex, anterior occipital cortex, and posterior parietal cortex, and acquired damage to these regions of the brain leads to selective deficits in remembering these domains of material (see Gathercole, 1999, for review).

Several tasks have been designed to tap the visuospatial sketchpad. These include recognizing the pattern of filled squares in a two-dimensional grid (Phillips and Christie, 1977; Wilson et al., 1987), remembering the order in which a set of blocks are tapped (often known as the Corsi blocks task), using a grid to generate a mental image corresponding to a set of spatial instructions (Brooks, 1967), and recalling the path drawn through a maze (Pickering et al., 2001).

Like its sister slave system the phonological loop, the sketchpad has now been fractionated into two distinct but interrelated components: A visual store or cache that preserves the visual features of perceived or internally generated objects and a spatial or sequential component that may serve a recycling function analogous to subvocal rehearsal (Logie, 1995). The strongest evidence for the separation of the sketchpad into these two components is provided by studies of neuropsychological patients with acquired brain lesions resulting in selective impairments of visual storage but preserved spatial short-term memory (Hanley et al., 1991) and converse deficits in spatial but not visual short-term memory (Della Sala et al., 1999; Della Sala and Logie, 2003).

Dual task studies have played an important role in illuminating the functional organization of the visuospatial sketchpad. One popular method for tapping the capacity for the generation and temporary storage of spatial material is the Brooks (1967) task, in which the participant is presented with a 4×4 empty grid in which one particular cell was designated as the starting square. The experimenter then gives a series of verbal instructions which participants are encouraged to remember by mentally filling in the grid, as shown in **Figure 4**. Following the

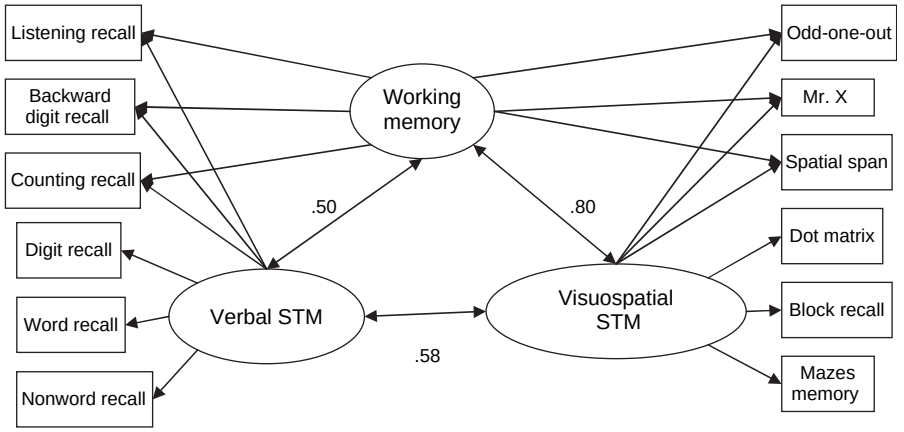

Figure 4 Measurement model of working memory, based on data from children aged 4–11 years. STM, short-term memory. From Alloway TP, Gathercole SE, and Pickering SJ (2006) Verbal and visuo-spatial short-term and working memory in children: Are they separable? *Child Dev.* 77: 1698–1716; used with permission from Blackwell Publishing.

instructions, participants recall the sequence by filling in the grid with the numbers. This condition facilitates the use of spatial imagery and hence the visuospatial sketchpad. In a control condition which does not encourage the use of such spatial imagery, the spatial terms up, down, left, right were replaced by the nonspatial adjectives good, bad, slow, and fast to yield nonsensical sentences. Presumably, recall in this condition was supported by the phonological loop rather than the sketchpad.

Evidence that participants use mental imagery to mediate performance in the spatial but not the nonsense condition is provided by the superior levels of recall accuracy in the former condition (Brooks, 1967). In a systematic study of the effects of concurrent activities on memory performance in the two cases, Baddeley and Lieberman (1980) reported further evidence that distinct components of working memory are employed in the two conditions. Performing a concurrent task – tracking an overhead swinging pendulum – disrupted recall in the spatial but not the nonsense condition. Thus, spatial recall appears to be selectively impaired by the encoding of unrelated spatial content, consistent with the employment of a spatial code to mediate recall performance.

Subsequent investigations indicate that eye movements may play a key role in the maintenance of spatial images in the sketchpad. Postle et al. (2006) reported a series of experiments showing that voluntary eye movements impair memory for spatial locations but not for nonspatial features of visual objects, providing further support for a distinction between visual and spatial components of the sketchpad. Other studies have shown that the engagement of other movement systems such as hands (finger tapping), legs (foot tapping), and arms exerts similar disruptive influences on memory for spatial sequences such as Corsi block recall (e.g., Smyth and Scholey, 1988). It therefore appears that the maintenance of spatial representations in the sketchpad is supported by a central motor plan that is not specific to any particular effector system, by which is recruited the planning and execution of movements in the full range of motor systems.

There is some evidence that the visual storage component of the spatial sketchpad is selectively disrupted by the concurrent perception of irrelevant visual features. In a series of studies reported by Quinn and McDonnell (1996), memory for detailed visual characteristics was selectively impaired by visual noise that corresponded to a randomly flickering display of pixels similar to an untuned television screen that participants were required to view but asked to disregard. This finding is important, as it is directly analogous to the irrelevant speech effect in verbal serial recall (Colle and Welsh, 1976), which has been interpreted as reflecting obligatory access to the short-term store component of the phonological loop for auditory speech material.

One interpretational problem raised by many studies of visuospatial short-term memory is the extent to which these and other similar tasks reflect a genuinely distinct component of working memory, or alternatively draw on the more general resources of the central executive. The central executive, which is described in more detail in the following section, is a

limited-capacity domain-general system capable of supporting a wide range of cognitive activities. Several different lines of evidence indicate that the central executive plays a major role in many visuospatial short-term memory tasks. Performance on visual storage tasks has been found to be strongly disrupted by concurrent activities that lack an overt visuospatial component but which are known to tax the central executive, such as mental arithmetic (Phillips and Christie, 1977; Wilson et al., 1987). Also, studies of individual differences have consistently shown that measures of visuospatial short-term memory are much more closely associated with performance on central executive tasks than are phonological loop measures, in both typically developing children (Gathercole et al., 2004a, 2006a; Alloway et al., 2006) and in a clinical study of adults with bipolar mood disorder (Thompson et al., 2006).

2.04.2.2.2 Summary

Although current understanding of the detailed cognitive processes involved in visuospatial short-term memory is less well advanced than that of the phonological loop, two basic facts have now been established. First, the sketchpad functions independently of the phonological loop – it is associated with activity in the right rather than the left hemisphere of the brain and is selectively disrupted by concurrent activities that do not influence the phonological loop. Second, the processes involved in manipulating and storing visual features and spatial patterns appear to be distinct from one another, again showing neuropsychological and experimental dissociations. It is rather less clear to what extent the visuospatial sketchpad represents a distinct component of working memory that is dissociable from the central executive.

2.04.2.3 The Central Executive

At the heart of the working memory model is the central executive, responsible for the control of the working memory system and its integration with other parts of the cognitive system. The central executive is limited in capacity, and is closely linked with the control of attention and also with the regulation of the flow of information within working memory, and the retrieval of material from more permanent long-term memory systems into working memory. Neuroimaging studies indicate that the frontal lobes of both hemispheres of the brain, and particularly of the prefrontal cortex, are activated by activities known to tax the central executive (See Collette and Van der Linden, 2002; Owen et al., 2005, for reviews).

2.04.2.3.1 The supervisory attentional system

In 1986, Baddeley suggested that central executive may correspond in part at least to the model of the supervisory attentional system (SAS) advanced by Shallice (1982) to explore the control of attention in action. The SAS has two principal components. The contention scheduling system consists of a set of schemas, which are organized structures of behavioral routines that can be activated by either internally or externally generated cues. When a schema reaches a particular level of activation, it is triggered and the appropriate action or set of actions is initiated. Thus, we have schemas that govern all our skilled behaviors: walking and talking, breathing and jumping, opening doors and using a telephone. Schemas can be hierarchically organized. Skilled car drivers, for example, will have a driving schema that is composed of linked subschemas such as steering and braking schemas. Many of our actions are governed by the automatic activation of these schemas in response to environmental cues. So, once we are behind the wheel of a moving car, the sight of a red brake light in the car in front will probably be sufficient to trigger the automatic activation of the braking subschema. Activation levels of all incompatible schemas (such as the accelerating schema, in this case) are inhibited when a schema is triggered.

The second component, the SAS, controls behavior via a very different process. The SAS can directly activate or inhibit schemas, thereby overriding their routine triggering by the contention scheduling system. The intervention of the SAS corresponds to volitional control and prevents us from being endless slaves to environmental cues – it allows us to choose to change the course of our actions at will. However, because the SAS is a limited capacity system, there are finite limits on the amount of attentional control we can apply to our actions.

Baddeley's (1986) suggestion was that the central executive corresponds to the limited-capacity SAS. He also proposed that two types of behavioral disturbance associated with damage to the frontal lobes arise from malfunctioning of the central executive, and coined the term dysexecutive syndrome to describe this disorder. These neuropsychological patients are typically characterized by one of two

possible types of behavior. Perseveration is a form of behavioral rigidity in which the individual continually repeats the same action or response. An example would be greeting a newcomer by saying "Hello" and then continuing to make the same response many times to the same individual, increasingly inappropriately. Distractibility consists of unfocused behavior in which the individual fails to engage in meaningful responses but may, for example, continuously walk around a room manipulating objects. Baddeley suggested that such individuals have an impairment in central executive resources that reduces their capacity for volitional control of behavior via the SAS, which is instead dominated by the contention scheduling system. Perseveration results when a schema becomes highly activated and cannot be effectively inhibited by the SAS to allow the triggering of other appropriate behaviors, and distractibility results from the background triggering of behavior by environmental cues with no overriding focus by the SAS.

This conceptualization of the central executive has proved useful in guiding the development of laboratory tasks that engage the central executive. One such task is random generation (Baddeley, 1986). In a typical task, the participant is required to generate in a random manner exemplars from a familiar category, such as digits or letters, paced by a metronome. The importance of generating random sequences rather than stereotyped ones such as 1, 2, 3 or a, b, c is emphasized. In 1998, Baddeley et al. (1998a) conducted a series of experiments to investigate the hypothesis that the central executive is needed to intervene to override the activation of stereotyped response sequences in this task. There were several key findings consistent with this view. First, the degree of randomness of the sequences generated by the participants diminished (i.e., the responses became more stereotyped) when the generation rate was increased. This result indicates that the randomness of the responses was constrained by a limited capacity process. Second, the degree of randomness of the generated sequences was not impaired when the task was combined with other activities requiring stereotyped responses such as counting, but was substantially disrupted by nonstereotyped concurrent activities such as maintaining a digit load or generating exemplars of semantic categories. Applying the logic of dual-task methodology, it appears that both tasks tap a common limited-capacity mechanism, the central executive.

2.04.2.3.2 Complex memory span

The central executive also plays a key role in complex memory span tasks, which require both processing and storage. The first reported complex span task, reading span, was developed by Daneman and Carpenter in 1980. In this task, participants must read aloud each of a sequence of printed sentences, and at the end of the sequence they must recall the final word of each sentence in the same order as the sentences were presented. The number of sentences read on each trial is then increased until the point at which the participant can no longer reliably recall the sequence of final words. Findings from this task were impressive – complex memory span scores were highly correlated with the performance of the participating college students on their scholastic aptitude tests completed on entry to college. Importantly, the correlations with scholastic aptitude were considerably higher than those found with storage-only measures of verbal short-term memory.

A range of other complex span paradigms have been subsequently developed, all sharing the common feature of requiring both memory storage while participants are engaged in significant concurrent processing activity. A listening span version of the reading span test in which the sentences were heard rather than read by participants was employed by Daneman and Carpenter (1983), and was found to be correspondingly associated with academic abilities. Complex span tasks suitable for use by young children have also been developed. One popular task is counting span, in which the child has to count the number of elements in a series of visual displays, and at the end of the sequence to recall the totals of each array, in the order of presentation (Case et al., 1982). The odd-one-out task (Russell et al., 1996; Alloway et al., 2006) is a complex memory span task that requires visuospatial rather than verbal storage and processing (see also, Shah and Miyake, 1996). Participants view a series of displays each containing three unfamiliar objects, two identical and one different. The task is to point to the location of the odd one out, and then at the end of the sequence to recall the sequence of spatial locations of the different items. In other complex span tasks, the material to be stored is distinct from the contents of the processing activity. An example of one such task is operation span (Turner and Engle, 1989), in which participants attempt to recall digits whose presentation is interpolated with a sequence of simple additions that must be completed.

Despite the large degree of variation in both the processing and storage demands of the different

complex memory span tasks, a highly consistent pattern of findings has emerged. Performance on such tasks is strongly related to higher-level cognitive activities such as reasoning and reading comprehension (e.g., Kyllonen and Christal, 1990; Engle et al., 1992), and also to key areas of academic achievement during childhood such as reading and mathematics (e.g., Swanson et al., 1996; Hitch et al., 2001; Jarvis and Gathercole, 2003; Gathercole et al., 2004b, 2006a; Geary et al., 2004; Swanson and Beebe-Frankenberger, 2004). In the majority of these studies, associations with learning were much higher for complex memory span measures than measures such as digit span of verbal short-term memory. Corresponding closer links with measures of intellectual functioning in adulthood such as reading comprehension, scholastic aptitude, and fluid intelligence have also been consistently found in adult populations (for reviews, see Daneman and Merikle, 1996; Engle et al., 1999b).

In order to understand why complex span measures of working memory performance are so strongly associated with learning abilities and other measures of high-level cognition, it is necessary first to consider what cognitive processes these measures tap. It has been suggested that the processing portions of these tasks are supported by the domain-general resources of the central executive, whereas the storage requirements are met by the respective domain-specific slave system (Baddeley and Logie, 1999). By this view, both the central executive and the phonological loop contribute to performance on verbal complex span tasks such as reading span, listening span, and counting span, whereas performance on visuospatial complex span tasks is mediated by the central executive and the visuospatial sketchpad.

There is now substantial evidence to support this proposal. A common processing efficiency factor has been found to underlie both verbal and visuospatial complex memory tasks (Bayliss et al., 2003). Two recent studies have investigated the latent factor structure underlying individuals' performance on both simple (storage-only) and complex span measures in both the verbal and visuospatial domains, in children (Alloway et al., 2006) and in adults (Kane et al., 2004). In both cases, the best-fitting model is a structure consisting of distinct verbal and visuospatial short-term storage components (corresponding to the phonological loop and visuospatial sketchpad, respectively), plus a domain-general factor corresponding to the central executive. A summary of the factor structure of the model from Alloway et al. (2006) is shown in **Figure 4**. It can be seen that the complex span tasks load both on the domain-general factor and the respective domain-specific storage system. These data provide an impressive degree of support for the basic structure of the working memory model.

So why is it the case that slow rates of academic learning therefore characterize children who perform poorly on complex memory measures of working memory (e.g., Pickering and Gathercole, 2004; Gathercole et al., 2006a)? We have suggested that the reason is that working memory acts as a bottleneck for learning (Gathercole, 2004; Gathercole et al., 2006b). The acquisition of knowledge and skill in complex domains such as reading and mathematics requires the gradual accumulation of knowledge over multiple learning episodes, many of which will take place in the structured learning environment of the classroom. Learning is thus an incremental process that builds upon the knowledge structures and understanding that have already been acquired: any factor that disturbs this acquisition process will have deleterious consequences for the rate of learning, as the necessary foundations for progress will not be in place. It is proposed that working memory capacity is one of the factors that constrains learning success in potential learning episodes. Many classroom activities require the child to keep information in mind while engaging in another cognitive activity that might be very demanding for that individual. Mental arithmetic is an example of such a demanding working memory activity for adults. In children, whose working memory capacity is considerably smaller and who do not have the same bedrock of stored knowledge and expertise to support cognitive processing, working memory challenges of a comparable magnitude are present in much simpler activities, such as writing sentences, adding up totals of objects displayed on cards, or detecting rhyming words in a poem read by the teacher. Children with poor working memory capacities will face severe difficulties in meeting the demands of these situations and, as a result of their working memory overload, will fail in part or all of the learning activity. Such situations represent missed learning opportunities and if they occur frequently, will result in a slow rate of learning.

2.04.2.4 The Episodic Buffer

The episodic buffer is the most recent addition to the working memory model, and was first outlined in a seminal paper by Baddeley in 2000 (Baddeley, 2000).

In this article, Baddeley argued the need for a separate buffer capable of representing and integrating inputs from all subcomponents of working memory and from long-term memory systems in a multidimensional code.

One justification for the episodic buffer is that it solves the binding problem, which refers to the fact that although the separate elements of multimodal experiences such as seeing an object moving and hearing a sound are experienced via separate channels leading to representations in modality-specific codes, our perception is of the event as a coherent unitary whole. At some point, the representations must therefore converge and be chunked together and experienced consciously as a single object or event; Baddeley's suggestion was that the episodic buffer may fulfill this function.

Other evidence also points to a close interface between the subcomponents of working memory and other parts of the cognitive system. It has long been known that meaningful sentences are much better remembered than jumbled sequences of words, with memory spans as high as 16 words compared with the six or seven limit for unrelated words (Baddeley et al., 1987). This indicates that representations in the phonological loop are integrated at some point with conceptual representations arising from the language processing system. Importantly, patients with acquired impairments of verbal short-term memory show reduced memory span for sentences as well as for word lists, but still show the relative advantage of meaningful over the meaningless material. Patient PV, for example, had a sentence span of five and a word span of one (Vallar and Baddeley, 1984). As PV's long-term memory was entirely normal, the reduction in her sentence span must arise from the point of interaction between verbal short-term memory (or the phonological loop). Baddeley (2000) proposed that the episodic buffer may provide the appropriate medium for linking the phonological loop representations with those from long-term memory, and that the central executive may control the allocation of information from different sources into the buffer.

The characteristics of the episodic buffer have been explored in a subsequent experimental programme by Baddeley and collaborators. One line of investigation has looked into whether the episodic buffer plays a role in the binding of different visual features of objects into chunks by comparing memory for arrays of colors or shapes with memory for bound combinations of these features (Allen et al., 2006). In a series of experiments, recognition memory for visually presented objects was tested by presenting an array of objects followed by a probe; the participants' task was to judge whether the probe was present in the original display or not. Across conditions, recognition memory was tested either for shape by presenting a display of different unfilled shapes, for color with a display of squares of different colors, or for both color and shape by presenting objects composed of unique shape/color combinations. In line with previous findings from this paradigm (Wheeler and Treisman, 2002), recognition performance was found to be as accurate in the feature combination as the single feature conditions. Thus, feature binding appears to be a relatively efficient process.

Allen et al. (2006) investigated whether this binding process depends on central executive resources, as might be predicted from the working memory model shown in **Figure 1**, in which information is fed into the episodic buffer from the central executive. To test this possibility, participants also performed demanding concurrent tasks that would be expected to require executive resources – counting backwards and retaining a near-span digit load – while viewing the object arrays. The results were clear: although recognition memory was generally less accurate under dual task conditions, memory for bound features was not selectively disrupted. The only condition that did lead to a greater impairment of recognition for feature combinations than single features was one that involved sequential rather than simultaneous presentation of objects.

On the basis of these findings, Allen et al. concluded that binding the features of simple visual features takes place in the visuospatial sketchpad and does not require executive support. However, it was suggested that storage of such automatically bound information is fragile and may fall apart when further feature combinations need to be encoded and stored in visuospatial memory.

The possible role of the attentional resources of the central executive in integrating linguistic information with representations in the phonological loop in the episodic buffer was investigated by Jefferies et al. (2004). The main focus of this study was the substantial advantage found in the immediate recall of prose compared with unrelated words, which Baddeley (2000) had suggested may be mediated by the integration of linguistic and phonological information in the episodic buffer. Jefferies et al.

conducted a series of experiments in which the relative difficulty of different kinds of lists was equated for individual participants. Thus, an example of an unrelated word list that corresponds to 50% above span for an average participant with a word span of six was the nine items essay, marmalade, is, lots, clowns, wine, spaces, often, a. In the sentence condition of a corresponding level of difficulty, an average participant with a sentence span of 13 would receive the following sequence of unrelated sentences for immediate recall: Railway stations are noisy places. Guns can cause serious injuries. Water is boiled in kettles. Pink roses are pretty flowers. In a further story condition, the sentences were thematically related, as in the following example: A teenage girl loved buying clothes. She went shopping with her mom. They traveled into town by bus.

The possible engagement of attentional processes associated with the central executive was investigated by comparing the impact of a continuous reaction time (CRT) task completed during the presentation of the memory sequence on performance in the different conditions. Following Craik et al. (1996), the CRT task involved pressing one of four keys corresponding to the spatial location of a visual target that appeared on a computer screen; as soon as the key was pressed, the next stimulus was presented. This task is known to place significant demands on controlled attentional processing. If the central executive does play a crucial role in loading phonological and linguistic information into the episodic buffer where it can be integrated into a multidimensional code underpinning sentence span, a selective decrement in the recall of sentences relative to unrelated words would be expected in the concurrent CRT conditions.

Jefferies et al. (2004) found that recall of unrelated words was more or less unaffected by the concurrent task, as was the recall of thematically organized material in the story condition. These findings indicate that the use of the phonological loop places few demands on attentional resources, and also that the activation of preexisting representations relating to the semantic and syntactic content of the stories occurs relatively automatically. In contrast, CRT did markedly impair performance in the condition involving the recall of unrelated sentences. It therefore appears that substantial attentional support from the central executive is required for the retention of unrelated chunks of linguistic information, possibly within the episodic buffer.

Although the study of the episodic buffer is still in its infancy, the concept is being refined in light of new evidence and is proving useful in guiding research on memory for relatively complex forms of material. The simple idea that the central executive is required to feed information through to the episodic buffer for the purposes of feature binding has not received strong support from the research completed so far: there is little evidence for central executive involvement in either the binding of simple visual features (Allen et al., 2006) or in the recall of coherent prose, although attentional support does appear to be crucial for the temporary retention of chunks of unrelated linguistic information (Jefferies et al., 2004). Ongoing and future research designed to delineate the precise conditions under which the central executive and episodic buffer interact seems certain to provide further fruitful insights into the role played by working memory in the storage and manipulation of complex and structured information.

2.04.2.5 Other Models of Working Memory

The multicomponent model of working memory initially advanced by Baddeley and Hitch (1974) is the most enduring and influential theoretical framework in the field. Its success rests with the breadth of scope of the model – incorporating verbal and visuospatial short-term memory, as well as attentional processes – and also with the capacity of the model to evolve in light of incoming evidence. Although the original tripartite structure of the 1974 model has been largely preserved, each component has been elaborated and differentiated over the intervening years, largely but not exclusively by using the dual task methodology to identify distinct subcomponents of the system. The model has also proved successful in accommodating evidence from a wide range of empirical traditions including cognitive development, neuropsychology, and neuroscience in addition to experimental psychology. It is, however, by no means the only model of working memory, and there are currently several other conceptualizations that are proving to be highly effective in guiding research and thinking in the area. Some of the significant alternative theoretical accounts of working are outlined in the following.

2.04.2.5.1 Attentional based models

One influential theoretical account of working memory of this type is Cowan's (1995, 2001) embedded process model, summarized in **Figure 5**. According to this model, long-term memory can be partitioned

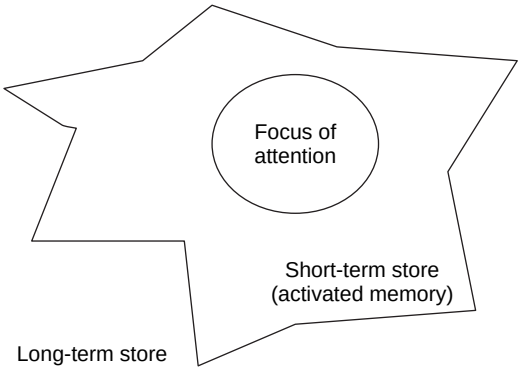

Figure 5 Cowan's (1995) embedded process model of working memory.

in three ways: the larger portion that has relatively low activation at any particular point in time, a subset that is currently activated as a consequence of ongoing cognitive activities and perceptual experience, and a smaller subset of the activated portion that is the focus of attention and conscious awareness. The focus of attention is controlled primarily by the voluntary processes of the executive system that are limited in capacity in chunks. Recent work indicates that typically between three and five chunks of information can be maintained in the focus of attention (Cowan, 2001; see also Chen and Cowan, 2005; Cowan et al., 2005). In contrast, long-term memory activation is time-limited and decays rapidly without further stimulation.

Cowan et al. (2005) have put forward an interpretation of complex memory span performance and its links with scholastic aptitude measures that is markedly divergent from the explanation based on the working memory model considered in the section titled 'The central executive.' By this account, the crucial feature of complex span tasks is that the processing activity prevents the usual deployment of control strategies such as rehearsal and grouping, and thus exposes more directly the scope of the focus of attention, as indexed by the number of chunks that can be maintained simultaneously. Learning ability will be constrained by having a relatively poor scope of attention, laid bare by complex memory span tasks.

An attentional-based account of working memory function has been also advanced by Engle and associates (e.g., Engle et al., 1999b). In some respects, Engle's model shares a similar architecture with the Baddeley and Hitch (1974) framework, combining domain-specific storage of verbal and visuospatial material with controlled attention. The detailed functioning of the components is, however, quite different. Short-term memory consists of traces that have exceeded an activation threshold and represent pointers to specific regions of long-term memory. They therefore do not represent temporary representations in a specialized temporary store, as in the phonological loop. Controlled attention is a domain-general resource that can achieve activation through controlled retrieval, maintain activation, and block interference through the inhibition of distractors.

Unsworth and Engle (2006) have recently put forward a new explanation of why complex memory span tasks correlate more highly with measures of higher-order cognitive function than simple memory span, based upon the distinction between primary and secondary memory. According to this account, memory items that have been recently encountered are held in primary memory, and may also be transferred into the more durable secondary memory system (Waugh and Norman, 1965). The processing activity in complex span tasks displaces items from primary memory, so that recall performance is supported principally by residual activation in secondary memory. Unsworth and Engle suggest that it is the ability to retrieve items from secondary memory that is crucial to more cognitive activities such as reasoning. Note that this interpretation is somewhat similar to that advanced by Cowan et al. (2005); in both cases, the claim is that learning is served most directly by the quality of activation of long-term memory, and not by the capacity of the controlled attention process that generates conscious experience.

2.04.2.5.2 The resource-sharing model

A contrasting theoretical perspective on working memory was provided by Daneman and Carpenter (1980, 1983; Just and Carpenter, 1992). These researchers conceived working memory as an undifferentiated resource that could be flexibly deployed either to support temporary storage or processing activity. By this account, individuals with relatively low span scores on complex memory span tasks were relatively unskilled at the processing element of the activity (reading, in the case of reading span), thereby reducing the amount of resource available for storage of the memory items. This idea that working memory is a single flexible system fueled by a limited capacity resource that can be flexibly allocated to support processing and storage was applied by Case et al. (1982) to explain developmental increases in working memory performance across the childhood years.

They proposed that the total working memory resource remains constant as the child matures, but that the efficiency of processing increases, releasing additional resource to support temporary storage. Consistent with this view, Case et al. found in a study of 6- to 12-year-old children that counting spans were highly predictable from individual counting speeds. Furthermore, counting spans were reduced to the level typical of 6-year-old children when adults' counting efficiency was reduced by requiring the use of nonsense words rather than digits to count sequences. It was concluded that the decreased memory spans resulted from the greater processing demands imposed by the unfamiliar counting task, leading to a processing/storage trade-off that diminished storage capacity.

2.04.2.5.3 Time-based theories

The resource-sharing model of working memory has been challenged substantially in recent years. Towse and Hitch (1995) proposed that participants do not process and store material at the same time in complex span tasks as assumed by the resource-sharing approach, but instead strategically switch between the processing and storage elements of the task. Evidence consistent with this task-switching model has been provided in a series of studies that have either varied counting complexity while holding retention interval constant (Towse and Hitch, 1995) or manipulated retention requirements in counting, operation, and reading span tasks, while holding constant the overall processing difficulty (Towse et al., 1998). In each case, the period over which information was stored was a better predictor of complex memory span than the difficulty of the processing activity. This has led to the claim that complex memory span is constrained by a time-based loss of activation of memory items (Hitch et al., 2001).

The consensus view at present is that no single factor constrains complex memory span (Miyake and Shah, 1999; Bayliss et al., 2003; Ransdell and Hecht, 2003). A more complex model recently advanced by Barrouillet and colleagues (Barrouillet and Camos, 2001; Barrouillet et al., 2004) combines concepts of both temporal decay and processing demands in a single metric of cognitive cost that is strongly related to performance on complex span tasks. In this model, the cognitive cost of a processing task is measured as the proportion of time that it requires limited-capacity attentional resources, for example, to support memory retrievals. When attention is diverted from item storage to processing in this way, memory representations cannot be refreshed and therefore decay with time. The heaviest cognitive costs and therefore the lowest levels of complex span performance are therefore expected under conditions in which there is the greatest ratio of number of retrievals to time. Experimental findings reported by Barrouillet et al. (2004) are entirely consistent with this prediction. Using a complex memory span paradigm in which they separately manipulated the rate of presentation of the memory items and the number of intervening items to be processed, complex memory span was found to be a direct linear function of the cognitive cost of the processing activity, computed as a ratio of the number of processing items divided the period over which they were presented. Thus, processing intervals that had relatively high loads (in other words, a relatively large number of items per unit time) were associated with lower span scores than processing intervals with low cognitive loads (low numbers of items per unit time).

2.04.2.5.4 Summary

In this section, a number of alternative theoretical accounts of working memory have been considered. It can be argued that some of these conceptualizations provide valuable specifications of the nature of central executive processes and are not necessarily incompatible with the Baddeley and Hitch (1974; Baddeley, 2000) model. Certainly, the emphasis on time-based loss of information by Towse and Hitch and the ideas of Barrouillet and colleagues concerning cognitive load could readily be accommodated in an elaborated model of the central executive and its interface with the phonological loop. The majority of these alternative approaches also emphasize the role of attention in working memory, a concept given prominence also by Baddeley (1986). However, other claims that working memory is an activated subset of long-term memory and does not exist as a temporary storage medium distinct from preexisting knowledge are less easy to reconcile.

2.04.3 Overview

The ability to hold information in mind for brief periods of time, termed working memory by cognitive psychologists, is an essential feature of our everyday mental life. The purpose of this chapter is to provide a contemporary overview of current theoretical understanding of the cognitive processes

of working memory. According to the influential model advanced originally by Baddeley and Hitch (1974) and revised and elaborated over the subsequent years (Baddeley, 1986, 2000; Burgess and Hitch, 1992, 1999), working memory consists of an attentional controller, the central executive, supplemented by slave systems specialized in the storage of verbal and nonverbal information (the phonological loop and visuospatial sketchpad, respectively). An additional component is the episodic buffer, capable of integrating information from different parts of working memory and other parts of the cognitive system. Each component of the model is limited in capacity.

This relatively simple model of working memory has proved capable of accommodating a wide range of empirical findings. Its fractionated structure has been informed by findings from experimental studies using dual task methods, by developmental dissociations in studies of children, and by evidence of distinct underlying brain from the fields of neuropsychology and neuroimaging. In the area of the phonological loop in particular, understanding of the underlying cognitive processes is sufficiently well advanced to allow the development of a computational model capable of simulating many detailed aspects of verbal short-term memory behavior.

Two components of the working memory model – the central executive and phonological loop – appear to play key roles not only in the temporary retention of information, but also in supporting longer-term learning, particularly during the childhood years. The phonological loop is important for learning the sound patterns of new words in the course of acquisition of vocabulary in native and foreign languages, whereas the central executive mediates academic learning in areas including reading and mathematics. Detailed theoretical accounts of the possible causal roles of working memory in these elements of learning are considered.

There are also several alternative theoretical accounts of working memory that are currently proving useful in guiding further research and understanding in this field. Some of these theories conceive of working memory as the subset of representations in long-term memory that have been activated either automatically via our interactions with the environment or effortfully, by being the focus of a consciously controlled attentional resource. Whereas the role played by attention is acknowledged in almost all current models of working memory, the distinction between models that assume specialized temporary storage mechanisms and those that see working memory as a property of preexisting knowledge representations is a fundamental one, yet to be resolved by empirical evidence. A further common feature of many theories is that time-based forgetting is a crucial feature of working memory.

Research in the field of working memory continues, stimulated by the availability of detailed theoretical accounts that guide empirical investigations of both typical and atypical working memory functioning. There is also increasing recognition that our current understanding of working memory can be put to more practical use, particularly in the fields of education and remediation (e.g., Gathercole and Alloway, in press). In this respect, working memory represents a strong example of how laboratory investigations of basic cognitive processes have the potential to enhance less esoteric elements of our everyday cognitive experience.

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2.05 Serial Learning

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In many activities in everyday life, we are required to learn the serial order of a set of elements. For example, whenever we acquire a new word, we must learn a novel order of sounds. As Lashley (1951) pointed out in his classic paper, the problem of learning serial order is that elementary movements occur in many different orders in different actions. How, then, can an individual who knows the elementary movements in an action learn to produce them in the correct sequence? For example, how can a pianist who knows the notes occurring in a given song learn to produce the notes in the correct order? That is the central problem involved in serial learning.

2.05.1 Concepts

To address this problem, we need to define several important concepts and make some crucial distinctions: Performing a serial task requires subjects to display knowledge of both the elements in the task and their arrangement. The task elements are items, and their arrangement is their order. In discussing serial learning, order is usually based on the temporal sequence in which the items occur, but in some cases order is based instead on the spatial locations of the items. Thus, letters are the items in words, and the letters must appear in a fixed order for a given word to be identified. The words 'tap' and 'pat' have the same items, but their different orders create different meanings. We can describe the order of the items either in terms of their ordinal positions without reference to their relational sequence (in 'tap,' 't' is first, 'a' is second, and 'p' is third) or in terms of their relational sequence without reference to their ordinal positions (in 'tap,' 't' precedes 'a' and 'p' follows 'a').

In cognitive psychology, learning typically refers to the process of acquiring information over time, whereas memory usually refers to the retention (or forgetting) of information. Thus, in the study of serial learning, we are most concerned with the acquisition of order information, but we also need to understand the underlying memory processes that provide the foundation for such learning over time. In practice, assessments of learning typically involve multiple study and test episodes, but assessments of memory usually involve a single study episode followed by a single test. Thus, memory research can be viewed as providing a snapshot of the first stage of the learning process.

2.05.2 Tasks

The original procedure used to investigate serial learning was established by Ebbinghaus (1885/1913).

In this repeated study–test procedure, a list of items is studied and then tested by requiring recall of the items in the order in which they were shown. This procedure is repeated until the subject reaches a criterion of recalling the list without error. Later researchers replaced this procedure with the method of anticipation, in which subjects are shown one item for a fixed amount of time and are then required to anticipate the next item in the sequence. Subsequently, the next item is shown, which provides feedback to subjects as to the correctness of their last response. This procedure continues throughout the presentation of a list, and list presentation is repeated until the subject reaches a criterion, perhaps one time through the list without error. The investigator tabulates how many presentations of the list are required to reach the criterion.

In recent investigations, the focus has shifted from the learning of order information to immediate memory for order information. Consequently, the most popular procedure is that of serial recall. In this case, subjects are given a series of items to study and are then required to recall the entire list in sequential order. Serial recall can be contrasted with free recall, in which subjects are free to report the items in any order they want and do not need to indicate the sequence information in any way. In serial recall tasks, subjects learn multiple different lists rather than the same list repeatedly. Often the investigator includes a delay between the presentations of successive items (interitem interval) or between the presentation of the last item on the list and the recall test (retention interval). Sometimes extraneous distracting items are interpolated during either the interitem interval or the retention interval to prevent subjects from rehearsing (practicing) the items during those intervals.

In the recall procedures, to respond correctly, subjects must remember the items. Another method was developed to isolate memory for order even further by eliminating the need for the subjects to remember the items. Specifically, the items are given to the subjects either in advance or during the trial, and the subjects simply have to reconstruct the order in which the items occurred. For example, for the list ABCDEF the subjects might be told that the items were BFACED, and they would have to rearrange the items into the correct sequence by placing A into the first slot, B into the second slot, and so on. In reconstructing the order, the subjects thus place each item into its appropriate position, perhaps in a horizontal array of slots, but the slots do not necessarily have to be filled in order from left to right. If a left-to-right response is required, the task is serial reconstruction of order, whereas if no constraints on response order are specified, the task is free reconstruction of order, using the same distinction described earlier for serial and free recall.

A new repeated study–test procedure has been developed to investigate both memory for and learning of serial lists, with successive snapshots of the learning process taken until a criterion is reached (Bonk, 2006). This procedure can be viewed as a combination of three common tasks already described: serial learning, serial recall, and serial reconstruction of order. Under this procedure, subjects view a display showing a set of items including both targets and distractors. The targets are then highlighted one at a time to indicate the required sequence. Subjects observe this presentation and then reconstruct the sequence by choosing one item at a time. The items can vary in type, but in the initial study were clip art pictures. The sequences can vary in length, and in the initial study they were from 6 to 15 items long. To respond correctly, subjects must remember both the identity of the target items and the order in which they occurred. A given sequence of target items is shown and recalled multiple times until the subject reaches the criterion of two perfectly recalled sequences in a row.

2.05.3 Results

The most widely cited experimental result in the study of serial order is the serial position function, first described by Nipher (1878; see also Stigler, 1978) for serial recall. To obtain this function, every position in the list is scored separately, and the total number of correct responses at a given position is computed either across repetitions of the list (in a learning paradigm such as the method of anticipation) or across different lists (in a memory paradigm such as serial recall). The function typically takes on a bow shape (like a bow in archery), wherein items at the start and end of the list are remembered better than intermediate items. The advantage for the initial items is the primacy effect, and the advantage for the final items is the recency effect. In serial learning, the primacy advantage is typically much larger and includes more items than the recency advantage, which sometimes includes only a single item. Asymmetrical bow-shaped functions for the initial test of a given list in the new repeated study–test procedure developed by Bonk (2006) are shown in **Figure 1** for each of 10 list lengths. Asymmetrical

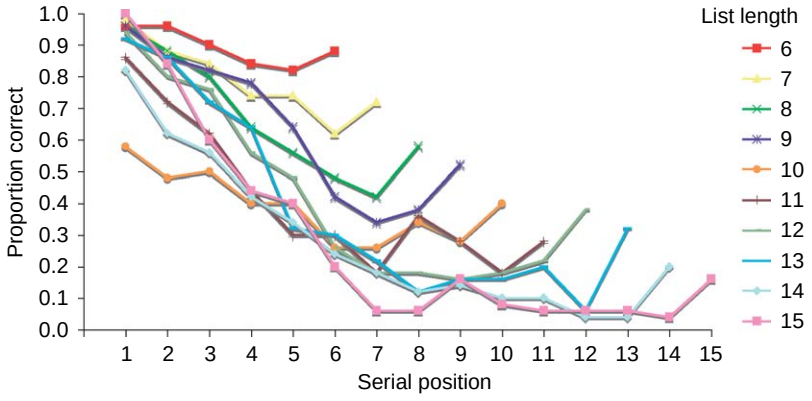

Figure 1 Mean proportion of correct responses as a function of list length and serial position for initial tests in serial learning experiment by Bonk (2006).

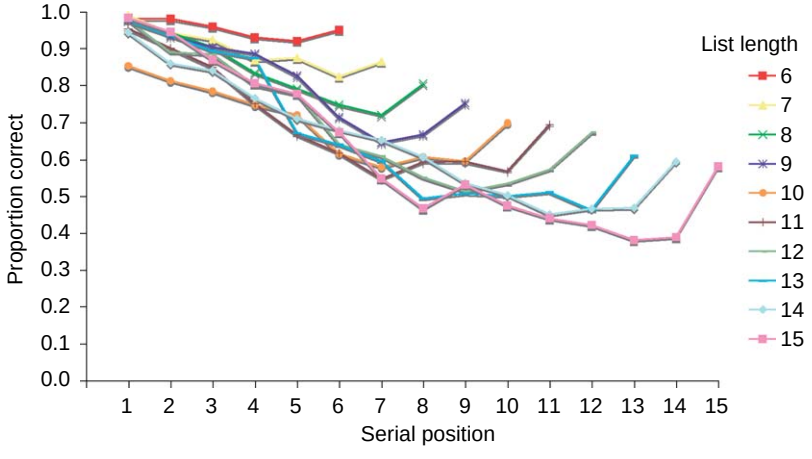

Figure 2 Mean proportion of correct responses as a function of list length and serial position for all attempts through the first perfect recall of a given list by a given subject in serial learning experiment by Bonk (2006).

bow-shaped functions are shown in **Figure 2** for all tests of a given list through the first perfect recall, again for the same 10 list lengths. **Figure 1**, thus, shows curves reflecting serial recall, whereas **Figure 2** shows curves reflecting serial learning. The curves are different, but both show an asymmetrical bow shape. Although the level of performance in serial recall or serial learning may depend on many factors, such as the rate at which the items are presented or the familiarity of the items, the serial position curve typically takes on the same shape when it is normalized. Normalization requires computing the proportion of all correct responses that occur at each serial position of a given list by a given subject. For example, if a subject on a six-item list took three attempts to reach the criterion and during those attempts made a total of 15 correct responses, with 3 of them on the first serial position, the normalized proportion correct for that position would be $3/15 = 0.20$. The fact that the shape of the normalized serial position function is constant across serial learning conditions was first demonstrated by McCrary and Hunter (1953). The normalized functions for the serial learning results of Bonk (2006) are shown in **Figure 3**, again for all 10 list lengths.

Other widely studied results involve the errors made by subjects in tasks requiring serial order. A frequent type of error is one in which the correct item is given but is not placed into its correct position. In a serial recall task, this is a transposition error. Typically, transposition errors occur in pairs because the positions of two adjacent items are confused. For example, if subjects are given the list ABCDEF and they recall ACBDEF, they have transposed a pair of

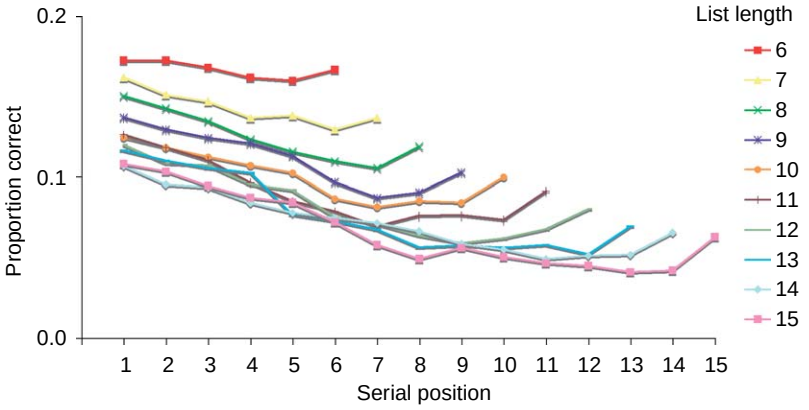

Figure 3 Mean normalized proportion of correct responses as a function of list length and serial position for all attempts through the first perfect recall of a given list by a given subject in serial learning experiment by Bonk (2006).

letters, B and C. Such a paired transposition would result in errors at two of the six list positions, positions 2 and 3. A nontransposition error is any other type of error in this task, such as when an item that did not occur in the list is substituted for a correct letter. For example, if subjects respond with YBCDEZ to the sample list, they have made nontransposition errors at two of the six list positions, positions 1 and 6.

Even with shorter lists, a bow-shaped serial position function is found for serial recall. In a procedure known as the distractor paradigm, items are briefly presented, followed by a retention interval filled with an interpolated task, often consisting of items of a different type, all of which must be read aloud by the subjects. For example, a list of four letters might be presented followed by a variable number of digits, with subjects reading aloud both the letters and digits before they recall the letters in the order shown. This procedure allows the investigators to examine the amount of information remaining in memory after various delays when rehearsal of the information is prevented. Using this procedure and differentiating between transposition and nontransposition errors, Bjork and Healy (1974) found that symmetrical bow-shaped serial position functions were found for total errors at each of three different retention intervals (3, 8, or 18 interpolated digits). These functions, when decomposed into transposition and nontransposition errors, showed a bow shape only for the transposition errors; the functions for nontransposition errors were much flatter, as shown in **Figure 4**.

Transposition errors can be further described in terms of a positional uncertainty gradient, which is a function of the distance between the input positions of the correct item and the item that substitutes for it in the subject's recall response. When the distance is short, the probability of an error is typically larger than when the distance is long. Such error gradients are shown in **Figure 5** for two different conditions in which order information was isolated by telling the subjects in advance which items would occur and using the same set of items on every trial of the experiment (Healy, 1975). The list items occurred one at a time in different spatial locations arranged in a row, with the spatial and temporal positions independently manipulated. In the temporal condition, the items occurred in fixed spatial locations, so only the temporal sequence of the items needed to be learned and remembered. In the spatial condition, the items occurred in a fixed temporal sequence, so only the spatial locations of the items needed to be learned and remembered. The items in this experiment were four consonant letters in each condition. As in the experiment by Bjork and Healy (1974), there were three different retention intervals, with 3, 8, or 18 interpolated digits. These functions show three striking differences in the retention of temporal and spatial order information. First, the decline in accuracy across retention intervals is sharp in the temporal condition but modest in the spatial condition. Second, the serial position function (evident by examining correct responses) is bow-shaped in the temporal condition but not in the spatial condition. Third, the error gradients are steeper in the temporal condition than in the spatial condition.

One specific type of nontransposition error that often occurs is a confusion error, in which a given item is replaced with another item that is confusable

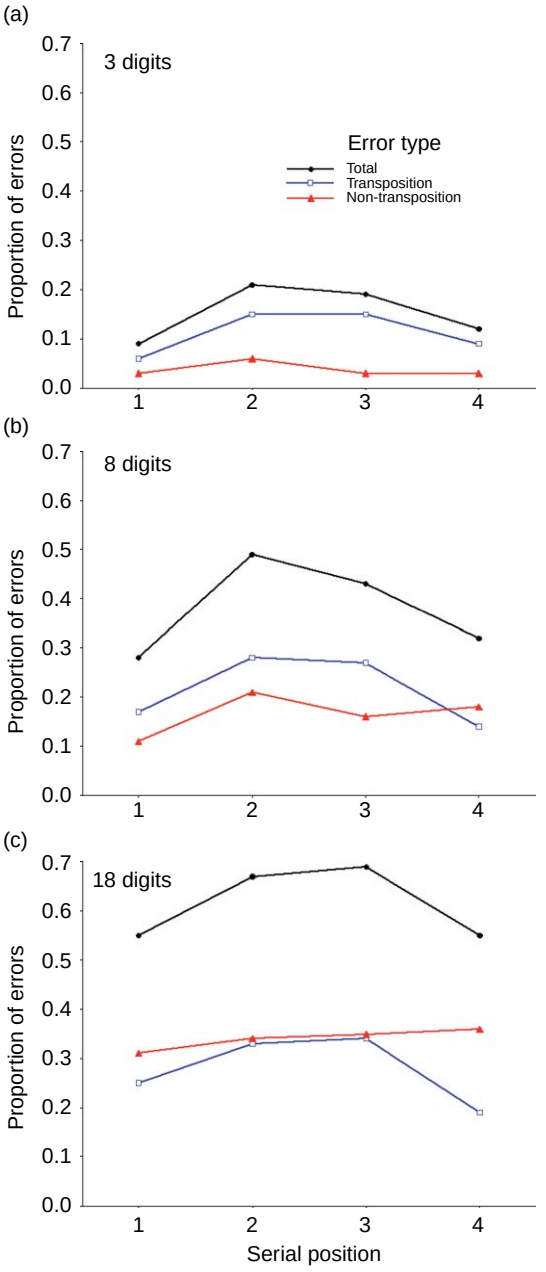

Figure 4 Mean proportion of errors as a function of retention interval (i.e., for 3, 8, and 18 interpolated digits), and serial position for all errors (total) and separately for transposition and nontransposition errors in serial recall experiment by Bjork and Healy (1974).

with it. In our example, a confusion error would occur in the second position if subjects respond with AGCDEF. The confusion error is presumably a result of similarity between the original item and the one replacing it, such as the similarity in sound between the letters B and G in the example. Such an error could be classified as a phonological confusion error. Other types of confusion errors are also possible. For example, if the items were words, the confusions could be based on similarity of meaning rather than sound, in which case they would be semantic confusion errors (e.g., replacing 'cot' with 'bed').

Often a nontransposition error is not based on item similarity but, rather, on positional similarity. Specifically, subjects show a tendency to replace an item in a given list with an item from the same position in an earlier list (e.g., Conrad, 1960; Estes, 1991). For example, if subjects see the list ABCDEF followed by the list GHIJKL and they recall the second list as GHIJEL, they have replaced the item in the fifth position of the second list with the item in the same position of the previous list. This type of error is a serial order intrusion.

Both the serial position functions and the different types of errors give us clues that help us understand the cognitive processes underlying memory for and learning of serial order information.

2.05.4 Theories

2.05.4.1 Classic Theories

The classic theories are largely theories of serial learning because the most popular experimental paradigm used at the time they were developed was the method of anticipation, and this paradigm provided the data that were to be explained by the models.

2.05.4.1.1 Associative chaining

An early description of serial learning was based on an associative chaining model wherein one item in a sequence was linked to (associated with) the next item in a chain (see, e.g., Crowder, 1968). This model was a natural outgrowth of the serial learning task involving the method of anticipation in which each item in the list is explicitly given as a cue for the next item. In our example of the list ABCDEF, the letter A would be linked to the letter B, B to C, and so on. However, even for that task, the simple associative chaining model may not be appropriate, as is evident intuitively from the observation that missing one item in a serial list does not lead to failure to report all subsequent list items. For example, a chaining model may predict that, in memorizing a complete poem, if any word is forgotten then it would be impossible to recall subsequent words in the poem. This particular problem is overcome if there are associative links of

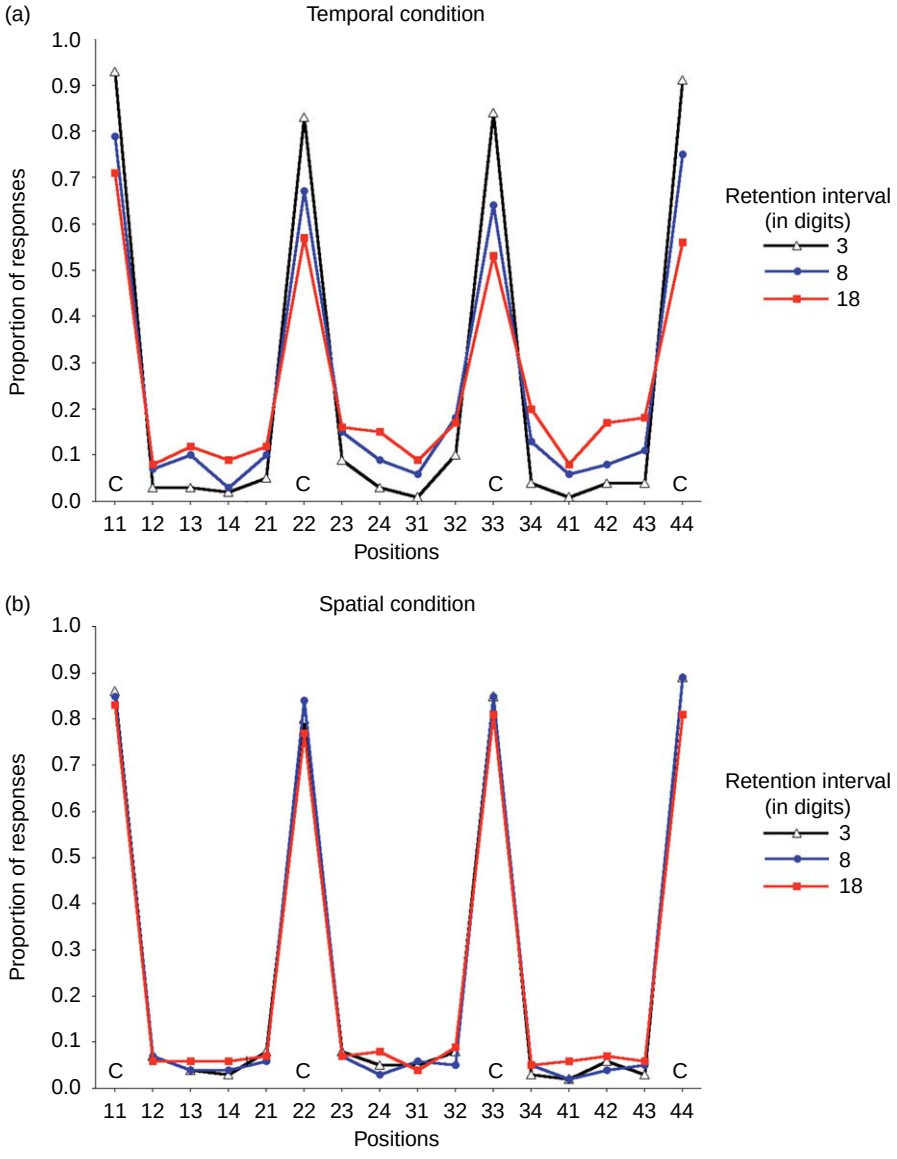

Figure 5 Mean positional uncertainty gradients for temporal and spatial conditions of experiment by Healy (1975). The point plotted for position ij represents the proportion of instances in which the response occurring at position i was the item appearing in input position j of the trial. The label C indicates correct responses.

varying strength among all items in the list, not just neighboring items, with the associations for adjacent items stronger than the more remote associations linking items that are not adjacent in the list. Thus, if a word in a poem is forgotten, subsequent words could still be recalled on the basis of remote associations from earlier words in the poem that could serve as cues. However, even such compound chaining could not overcome other types of evidence against this class of models. For example, in one experiment using the method of anticipation, subjects learned a serial list of adjectives to a criterion of one perfect trial. Then they were given a task to learn a set of paired associates, with experimental pairs formed from adjacent adjectives in the previous list and control pairs formed from unrelated adjectives. Subjects learned the experimental pairs no faster than they learned the control pairs in the paired associate task (Young, 1962), which seems inconsistent with the assumption that in the serial learning task subjects formed strong associations between adjacent items

(but see Crowder, 1968, for counterevidence supporting the existence of such associations).

2.05.4.1.2 Positional coding

Another early description of serial learning is also based on associations between stimuli and responses; it involves a simple positional coding model. In this case, the associations are not from one item to the next but, rather, between a given item and its ordinal position (see, e.g., Young et al., 1967). In our example, the letter A would be associated with ordinal position 1, B would be associated with ordinal position 2, and so on. One version of this theory is a box model (Conrad, 1965), according to which each successive item in a list is entered into a box, with the boxes preordered in memory. Item information in the boxes gets degraded with the passage of time, and at recall subjects output items for each box in turn using whatever information is still available. Transposition errors occur in this model not because of a reordering of the boxes but, rather, because information about an item in a given box is degraded so that the remaining partial information may be consistent with another list item, leading to report of that other item rather than the correct one for that position. This simple model was also refuted by experiments testing it. For example, in a study like the earlier one testing the chaining model, subjects learned, using the method of anticipation, an ordered list of adjectives to a criterion of one perfect trial. Then they were given a task to learn a set of paired associates, in this case with the ordinal position numbers as stimuli and the serial list adjectives as responses. Subjects did not perform as well on the paired associate task, at least on the intermediate items, as they should have if they had in effect learned those associations previously during the serial learning task (Young et al., 1967).

2.05.4.1.3 Positional distinctiveness

A simple but powerful model was proposed by Murdock (1960) to account for the serial position function in serial learning solely in terms of the distinctiveness of the positions. By this model, a given position's distinctiveness is determined merely by comparing its ordinal position value to the values of all of the other list positions. For example, in a five-item list, the difference between the ordinal position value for the first position and the value for the other positions is the sum of $|1-2|$, $|1-3|$, $|1-4|$, and $|1-5|$, which is $1+2+3+4=10$. In contrast, a similar calculation for the third position yields $|3-1|$, $|3-2|$, $|3-4|$, and $|3-5|$, which is $2+1+1+2=6$. Thus, as is also clear intuitively, the first position is more different from the other positions than is the third position. The actual calculation of distinctiveness is a bit more complex because log values are used instead of the ordinal numbers themselves. The use of log values allows the model to account for the finding that primacy effects are typically stronger than recency effects. According to this model, the serial position function should be the same shape for all lists of a given length, even if the lists vary in terms of their presentation time or the familiarity of the items that comprise them. Indeed, as mentioned earlier, normalized serial position functions have the same shape across all experimental conditions (McCrary and Hunter, 1953).

2.05.4.2 Contemporary Theories

Unlike the classic theories, contemporary theories are largely theories of immediate serial memory because the most popular experimental methodology became the immediate serial recall paradigm, and this paradigm provided much of the data that were to be explained by the theories. Thus, the emphasis has shifted from the learning of serial order information to immediate memory for serial order information. The new data by Bonk (2006), which examine serial recall on successive learning trials, provides an empirical integration of serial memory and serial learning results, but little theoretical integration has yet been proposed.

2.05.4.2.1 Perturbation model

An elegant model was proposed by Estes (1972) to account for serial recall performance in the distractor paradigm. Like the classic models, the perturbation model is based on simple associations. However, the associations in this case are between an individual list item and a control element, which represents the given context or environment in which the list was learned. At the core of the model is the concept of a reverberating loop that links the control element to a given list item, with a recurrent reactivation of the list item each time the control element is accessed. Because all the items in a list are associated to the same control element, the difference in reactivation times reflects their input order. The timing of the reactivations, thus, provides the basis for knowledge of the order of the items in a list. This knowledge is assumed to be perfectly stored in memory immediately after the list is presented. Loss of such

information, resulting in failure to recall the items in the correct order, may then occur for one of two reasons. First, the subject may lose access to the control element, perhaps because the experimental context has shifted as a function of time or because of some interpolated, interfering activity. Second, there may be perturbations, or disturbances, in the timing of the recurrent reactivations, presumably resulting from random neural activity. If the timing perturbations are large enough, two adjacent list items may be interchanged so that the later item is reactivated before the earlier item, thus leading to transposition errors in recall. The perturbation process can account for the symmetrical bow-shaped serial position functions found in immediate serial recall of short lists because the likelihood of interchanges resulting from timing perturbations is greater for intermediate list items (which have neighboring items on both sides) than for end items (which have a neighboring item on only one side). This same mechanism easily accounts for the positional uncertainty gradients observed for temporal (but not spatial) order recall, wherein the likelihood of a transposition error decreases as the distance in time increases between the input positions of the correct item and the one replacing it.

After its original formulation, the perturbation model was refined to account for the fact that order information can be viewed as hierarchical (Lee and Estes, 1981). If lists are divided into subsets, perhaps by adding pauses between groups of items, then subjects need to know on which list a given item occurred and in which subset of the list it occurred, as well as its relative position in the subset. According to the refined version of the perturbation model, each item is coded for its placement in this three-tier hierarchy. The hierarchy of codes is repeatedly reactivated, and the perturbation process applies independently at each level, so at each reactivation there is a probability that the relative position of adjacent lists, subsets, or items will be disturbed. This hierarchical perturbation process produces serial order intrusion errors, when an item in a given list or list subset is replaced by an item from the same position in an earlier list or list subset.

2.05.4.2.2 Start-end model

The start-end model (Henson, 1998) was proposed to account for the serial position functions, the positional uncertainty gradients, and the distributions of different types of errors in the serial recall task. At the heart of this model is the observation that the start and end of a list are most salient and therefore serve as anchors, or markers, to code for each item's position in the list (see Feigenbaum and Simon, 1962, for an earlier use of the notion of list end items serving as anchors). Each item gets a two-value code based on the strength of both the start and end markers at that point in the list. The start marker is assumed to be strongest at the beginning of the list and to get progressively weaker for subsequent list items. In contrast, the end marker is assumed to be weakest at the beginning of the list and to get progressively stronger for subsequent list items. Although the end is not evident at the start of the list, subjects anticipate the end (at least when they know the list length), and that expectation allows for the use of the end marker. The model reproduces the general finding that primacy effects are larger than recency effects by giving greater strength to the start marker than to the end marker.

This model makes use of a distinction between types and tokens as a way of representing items. A given item, such as a word, may occur in multiple lists or on multiple occasions in a given list. Each time the word occurs, the item is the same type, but the different instances of the word constitute different tokens. In the start-end model it is assumed that each item token codes both identity and positional information. The identity information specifies the content of that item (e.g., which word has occurred). The positional information is derived from the strength of the start and end markers for that item token. According to the model, the item tokens are unordered in memory; instead, they are ordered at the time of recall. Specifically, at recall the position of a given item is cued by its start and end marker strength values; the identity of the item that matches the cued strength values most closely is recovered and then recalled at that position. Another assumption made by the model is that once an item is recalled, its type is suppressed so that subjects will be less likely to recall a given item type more than once in a trial. This aspect of the model allows it to account for the Ranschburg effect (e.g., Jahnke, 1969), whereby subjects are likely to fail to recall second occurrences of a given item.

2.05.4.2.3 Primacy model

The primacy model (Page and Norris, 1998) is related to both the perturbation and start-end models but was formulated to account for a different set of results. The results in this case are those that formed the basis of Baddeley and Hitch's (1974) model of the phonological loop, which is a qualitative

description of working memory that describes rehearsal processes but does not provide any specific mechanisms for serial recall. Thus, the primacy model can be viewed as a computational version of the phonological loop model (see also Burgess and Hitch, 1999, for an alternative quantitative version of this model). The primacy model does not specifically code position information, but such information is derived at the time of recall from the relative activation strengths of list items. These activation strengths vary as a function of the time when the list items occurred, forming a primacy gradient, with the strength greatest for the first item and declining for successive items in the list. These activation strengths can be thought of as reflecting the degree to which the context defining the start of the list is associated with each successive list item. By this view, the start-of-the-list context resembles both the control element of the perturbation model and the start marker of the start-end model. However, unlike the start-end model, there is no corresponding end marker in the primacy model.

To model the recall process, the primacy model implements the assumption that in a repeating cycle, the item with the greatest activation is selected for recall, and after it is recalled, it is suppressed. Subsequently, the item with the next highest activation is recalled and then suppressed, and so on. During the recall process, the activations for all list items decay exponentially with time. Errors result from the fact that there is noise in the process of selecting the item with the strongest activation (which can be viewed as noise in the perception of the activation strengths), even though there is no noise in the activation strengths themselves. Primacy effects fall out of the model naturally because of the primacy gradient, but recency effects occur because end items can only participate in a paired transposition error in one direction (i.e., with one neighbor), whereas intermediate items can participate in paired transposition errors in both directions (i.e., with neighbors on both sides). Paired transposition errors occur in this model whenever the perceived activation strength of a given list item is either less than the perceived activation strength of a subsequent list item or greater than the perceived activation strength of a preceding list item. Such paired transposition errors also rely on a property of the model called fill in, which is the assumption that when an item is missed in recall because of a transposition it is likely to be recalled in the next position. This model is, thus, consistent with the observation from Bjork and Healy (1974) that transposition errors show a bowed serial position function but nontransposition errors do not. Nontransposition errors typically increase as a function of serial position. To account for this finding, the primacy model assumes that once the item with the strongest perceived activation is selected, the activation is compared to a threshold value. If the activation is above threshold, the item will be recalled, whereas if it is below threshold, it will be omitted and the subject will resort instead to guessing an item, with this threshold comparison subject to noise. Thus, the primacy model can account for nontransposition errors as well as transposition errors.

2.05.4.2.4 OSCAR

A novel approach to explaining serial recall was taken by Brown et al. (2000) in their oscillator-based computational model OSCAR. Oscillators are timing mechanisms that generate continuously changing rhythmic output. Oscillators occur at different frequencies, with high-frequency oscillators repeating more often than low-frequency oscillators. An analogy can be made to the hands in a clock face. The second hand completes its cycle more rapidly than the minute hand, which in turn completes its cycle more rapidly than the hour hand. OSCAR accounts for the learning of order by making use of oscillator timing mechanisms presumed to occur naturally in the mind. In OSCAR, during list presentation, associations are formed between a vector (an ordered series of numbers) representing a list item and a vector representing successive states of the learning context. The learning context is the current state of the dynamically changing internal set of timing oscillators. Thus, OSCAR, like other models, makes use of associations between items and a representation of the learning context. However, in OSCAR, unlike other models, the learning context changes continuously during list presentation. Just as Lee and Estes (1981) postulated a hierarchy of codes in the perturbation model, the oscillators in OSCAR vibrate at different rates, reflecting different levels of a three-tier hierarchy, including item position within a subset, subset position within a list, and list position within a session. Unlike the perturbation model, however, order errors arise in OSCAR solely during the retrieval stage. Specifically, at the time of retrieval, a sequence is recalled by reinstating the states of the set of oscillators that comprise the learning context. Each successive learning context vector is used as a probe recovering the list item vector that is

associated with it. Retrieval errors occur based on the quality of the learning context vector and the extent to which that vector is specific to a particular item. Items occurring close together in time have similar learning context vector values; thus, noise in the retrieval process leads to positional uncertainty gradients like those found for the temporal condition in the study by Healy (1975). This model, unlike some of the others, can thereby explain observed differences between recall of temporal and recall of spatial order information.

2.05.4.2.5 TODAM

Unlike the other contemporary theories reviewed here, which are restricted to memory for serial order, a model by Lewandowsky and Murdock (1989) is designed to account for serial learning as well as serial recall. This theory of distributed associative memory (TODAM) also differs from the other contemporary models in being based on associative chaining. Although, as mentioned earlier, problems had been found for the classic associative chaining model, these were largely overcome in TODAM. A third difference between TODAM and the other models reviewed here is that TODAM provides a more general account of memory, not being restricted to serial order (see Anderson et al., 1998, for another general model incorporating serial recall). A fourth difference is that the memory representations in TODAM are not localized but are, rather, distributed.

Specifically, in TODAM the representations of all list items are stored together in a common memory vector. The numbers making up the memory vector in TODAM represent values of individual features. Successive items are associated using a mathematical operation convolution that blends the constituent item vectors. The resulting convolution is also added to the common memory vector. If all information is contained in a single memory vector, how can the model recover the individual list items when needed? The retrieval mechanism used for this purpose is correlation, which is the inverse of convolution (i.e., it essentially undoes that operation). Thus, a memory probe representing a particular stimulus item can be correlated with the common memory vector to yield another vector that approximates the response item with which it had been associated. Once the approximation to the response item is generated via the correlation process, it must be deblurred (interpreted) before it can be recalled. If the deblurring process yields an overt recall response, the new vector resulting from that response can then be used as a stimulus probe to recover the next item in the list. The deblurring process might not result in an actual overt response. Nevertheless, the recall process can move forward to the next item in the list because the vector approximation can be used as a stimulus for a subsequent response. This implementation allows TODAM to overcome one of the key problems mentioned earlier plaguing the classic chaining model, namely, that missing one item in a serial list does not lead to failure to report all subsequent items. A subsequent version of TODAM (Murdock, 1995) also uses associations between higher-order chunks of items to avoid problems with simple associative chaining models.

To model serial learning occurring across repeated presentations of the same list, in a closed-loop variant of TODAM, the new information added to the memory vector for an item is reduced by the amount of information already present in the vector. This aspect of the model captures the idea that gradually less is learned about each item during successive repetitions of a list.

2.05.5 Theoretical Issues and Conclusions

A variety of theoretical mechanisms have been proposed to account for serial memory and learning, but there is little consensus as to which is the best. The various models differ along numerous important dimensions, such as the relation between item and order information and whether or not position or sequence information is explicitly coded. Some models do not discriminate between temporal and spatial order, whereas others apply only to temporal order. Crucially, most models do not attempt to provide a theoretical integration of serial memory and serial learning results. Thus, despite the theoretical insights and innovations in the five decades since Lashley (1951) first discussed the importance of this problem, we have not yet achieved a full and widely accepted understanding of the processes underlying serial order behavior, which provides the foundation for many activities in everyday life.

Acknowledgments

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2.06 Repetition and Spacing Effects

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Coyne (2006) dedicated himself to becoming as good a golfer as he could possibly be. He spent a year in this quest, hitting more than 100 000 golf balls and playing 5418 holes. He found that practice at this skill indeed led to improvement but was frustrated to find out that not all practice was equally effective. The beginning of his regimen led to the fastest improvement, an observation that led Coyne to compare his situation to that of a dieter finding that the first few pounds lost were the easiest. Coyne's observations are typical, as the importance, as well as the limitations, of repetition in the mastery of information or skill can be documented in countless domains (Ericsson, 2005).

That repetition is key to learning but that not all repetitions are equally effective are central observations underlying all serious thought on the topic. The central importance of repetition was recognized by many ancient and medieval thinkers. Hermann Ebbinghaus (1885/1964), who initiated the modern era of memory research, carried out a series of experiments that showed that retention improved as a function of the number of times that information had been studied. Ebbinghaus's theoretical approach assumed the centrality of repetition in the acquisition and strengthening of learning.

The fact that learning and memory are sensitive to repetition is not in question. However, the basis of repetition effects is still far from clear. Boundary conditions where repetition has no effect are particularly interesting in determining why repetition affects retention. A related topic of interest in the literature deals with the temporal distribution of repetitions. Generally, repetitions are more effective if they are spaced apart than if they are massed together (the spacing effect).

Anderson and Schooler (1991) have noted that both repetition and spacing effects are rational; that is, if one were designing an ideal memory system, one might construct it so that it would exhibit both of these phenomena. They pointed out that human memory evolved to manage a huge body of information containing millions of facts and experiences. Expecting perfect retrieval of all these facts is unlikely in the face of our limited physical abilities; it may also be unwelcome because we may quickly find ourselves overwhelmed by the massive amount of information we have stored. A rational memory system would be one where the retrievability of a memory is strongly related to the probability of that particular memory being needed. Repetition would surely be one clue indicating that a particular fact was important. If we had encountered or used a fact numerous times in the past, it would be more likely that we will need it again in the future than if we had only used that fact once. Similarly, when a particular fact or experience had been encountered or used at widely spaced intervals, it is probably more likely to

be used again in the future than a fact used equally often but only in a massed cluster. Anderson and Schooler based their reasoning on theories of information retrieval, which underlie many of the computerized search systems found in libraries or on the Internet. Still, acknowledging that repetition effects and spacing effects are good phenomena for well-designed memory systems to show does not address the issue of the mechanisms that lead to these effects in human memory.

2.06.1 Continuity, Discontinuity, and Repetition

Coyne (2006) found that his golf practice (when not interrupted) led to a steady, albeit sometimes agonizingly slow, improvement in his performance. The seemingly continuous, but always slowing, effect of repeated practice is a ubiquitous finding in the field of learning and memory. The total amount learned increases as a function of repetition, but the rate of learning seems to change systematically across trials. The traditional learning curve depicted as a function of practice demonstrates negative acceleration, with the greatest learning occurring on the first trial. The amount learned on each subsequent trial seems to decline continuously. Eventually, the change in performance as a function of further practice is too small to be measurable. The phenomenon of *registration without learning* (Hintzman et al., 1992; Hintzman and Curran, 1995) is a demonstration of this, with participants showing no evidence of learning more information about the details of a stimulus, even while continuing to register its repeated presentations. Cleary et al. (2001) showed a similar pattern in learning about associations: When presented with word pairs that are repeated many times, participants may note the occurrence of individual items without showing improved associative memory. Because people seem to pay much less attention to later occurrences of repeated stimuli, first impressions become particularly important. Participants may not demonstrate evidence of having noticed small changes that are made to a stimulus after its first presentation (DiGirolamo and Hintzman, 1997). Miller et al. (2004) provided additional evidence regarding which features of a stimulus are most likely to be overlooked; specifically, accidental properties that are not inherent aspects of a stimulus are commonly ignored in later repetitions.

Authors such as Guthrie (1935) and Estes (1955) have long noted that this seemingly continuous improvement in performance does not necessarily mean that the learning process itself must be gradual or continuous. Many learning situations can be broken down into smaller components, and one cannot rule out the possibility that these components may be learned suddenly, possibly as a result of insight. If these components are learned at different times, the seemingly gradual nature of learning may instead reflect the accumulation of mastered components, each of which had been learned in a sudden all-or-none fashion. Distinguishing between a truly gradual learning process and the accumulation of numerous small insights is difficult, and it became common to assume that learning may be either gradual or sudden, depending on the situation and the nature of the participant. Harlow (1949) argued that learning to novel situations may be slow and continuous, but that sudden flashes of insight may occur when learning takes place in a familiar situation.

Rock (1957) was the first to move beyond the general concern that learning curves obscure sudden transitions in performance to an empirical methodology. He developed what became known as the drop-out procedure. He used a paired-associate methodology, in which participants had to learn a list of letter–number pairs. Study trials alternated with test trials, on which letters were presented and participants had to recall the corresponding numbers. After each test, Rock removed pairs that had not been answered correctly on that trial and replaced them with new pairs. Performance in this condition was compared to learning in a control condition, where participants were given the same unchanging list on multiple trials. The major finding was that there was no difference between the drop-out condition and the control condition; both groups mastered the list at the same rate. The fact that replacing old (but not yet mastered) pairs with new pairs did not impair performance led Rock to conclude that pairs were either completely learned or completely unlearned. Rock reasoned that if participants had been acquiring partial information about unrecalled pairs, performance should be impaired if such pairs were replaced by new ones. Because no impairment was found, he concluded that participants had not learned anything about a pair until the trial on which it could be recalled. That is, he argued for all-or-none learning: Rock believed that the fact that performance on a paired-associate list gradually improves across repeated study–test cycles obscures the fact that each pair is either entirely learned or

unlearned, with the number of pairs moving from the unlearned to the learned state increasing across trials.

Rock (1957) acknowledged the fact that there was a potential flaw in his procedure. Some pairs are harder than other pairs. Presumably, the pairs that are not remembered on a particular trial would tend to be the most difficult pairs for that participant. In the drop-out condition, these particularly difficult pairs are being replaced on each trial by new pairs. Because the new pairs are randomly chosen to be added to the list, taken together they would be average in difficulty. Therefore, the drop-out group will end up with an easier list than the control group, which is stuck with the list that had been presented on the first trial. If this methodological flaw is serious, Rock would be mistaken in concluding that this methodology supports an all-or-none interpretation of learning. In later research, Steinfeld and Rock (1968) went to great lengths to argue that this artifact did not invalidate the drop-out procedure, but it is impossible to determine exactly how serious the problem is. Similar methodologies (e.g., that of Estes, 1960) run into analogous difficulties. In a review of this literature that was written just as research on this topic was winding down, Crowder (1976) noted this line of experimentation proved to be inconclusive but was valuable in shaking "the almost axiomatic belief of Ebbinghaus that repetition strengthens a unitary memory trace" (p. 273).

2.06.2 Basis of Repetition Effects

2.06.2.1 Theoretical Approaches

The philosopher Ward (1893) anticipated many of the later developments in theories of repetition effects. He distinguished between functional and atomistic accounts of repetition effects. Functional accounts would assume that repetition affects memory by altering a single location or representation. Every stimulus or experience has a particular trace in memory that is altered when it is encountered again. These alterations have the effect of making information about that stimulus easier to locate. Perhaps the most straightforward way of devising a functional account is to assume that memory traces differ along a single dimension, such as trace strength, so that this sort of approach is sometimes called strength theory. This approach is based on the assumption that repetition influences strength and that stronger traces are easier to remember than weaker traces. Ebbinghaus (1885/1964) seems to have implicitly adopted such a strength position, though his focus was on the strength of associations. Underwood (1969a) developed a somewhat more complex theory in which repetition-based strength was just one attribute that could vary among memory traces. On the other hand, atomistic theories maintain that two occurrences of an item lead to independent and distinct memory traces. Each of these traces would contain information about when it had been formed. Atomistic theories are more likely to be referred to now as multiple-trace theories. The German scientist Richard Semon, whose life and work in the early twentieth century has been described by Schacter (1982), was the first prominent multiple-trace theorist, although the model of memory proposed by the scientist Robert Hooke in 1682 can be interpreted in multiple-trace terms (Hintzman, 2003).

A fundamental difference between functional (strength) and atomistic (multiple-trace) accounts is in whether information about individual encounters with a repeated stimulus is maintained in memory. Functional accounts assume that repetition leads to the strengthening or alteration of a single location so that details about particular occurrences are lost. On the other hand, atomistic approaches claim that every occurrence leads to the formation of a separate memory trace, thereby maintaining the specificity of individual encounters. One class of experimental test of these approaches has focused on this fundamental difference by asking participants to make judgments about details of individual presentations of repeated events (see Hintzman, 2000, for a review of this approach). A pure strength theory would assume that participants would only be able to make a judgment about a stimulus by drawing an inference based on its strength; for example, a stronger stimulus can be assumed to have occurred more recently or more frequently. In contrast, multiple-trace accounts typically assume that each occurrence of a stimulus leads to the formation of a trace that records the context in which it was formed; therefore, participants would have access to detailed information about individual presentations of a repeated stimulus.

2.06.2.2 Judgments of Recency

To see how one might use memory judgments to discriminate between strength and multiple-trace approaches, a simplified account of a recency-judgment task (first developed for this purpose by

Morton, 1968) may be helpful. A participant may be given a list of digits and have to remember which of two test items had been presented later on the list. Imagine that a sequence like 7 5 2 9 1 6 5 8 4 3 is presented, and a participant is asked whether 4 or 5 had been presented later on the list. Note that 4 is the correct answer, but that the alternative 5 had been presented twice. According to strength theory, participants may tend to make the wrong choice because they may mistakenly attribute the heightened strength of the digit 5 to a recent presentation, as opposed to multiple presentations. In contrast, a multiple-trace account would claim that participants perform this task by retrieving occurrences of each digit and attempting to determine recency by examining details of each trace. Flexser and Bower (1974) carried out a systematic investigation of this task and concluded that multiple-trace approaches offered a better explanation of recency judgments. Hacker (1980) reached a similar conclusion by measuring response times in recency judgment.

2.06.2.3 Judgments of Frequency

There are several different ways in which one could test memory for frequency. One method would be a test of background frequency, which refers to the number of times that an event has occurred in one's lifetime. For example, Attneave (1953) studied the ability to make background-frequency judgments of letters of the English alphabet. These background-frequency judgments correlated .88 with the true frequency of occurrence in the English language, demonstrating that people are indeed sensitive to how often letters occur. Howes (1954) showed that people are sensitive to the background frequency of occurrence of words.

For the purpose of distinguishing between strength and multiple-trace accounts of repetition effects, tests of situational-frequency judgment are more useful. The prototypical way of studying situational-frequency memory is to present a list of words, which may be repeated varying numbers of times. At the time of test, list words are shown, and people have to indicate how often each was presented on the list. This sort of information is considered *situational* because participants do not have to remember how often items occurred throughout their whole lives but rather how often they occurred in a particular situation (i.e., on the laboratory list). People under typical testing circumstances are often very accurate, even when not expecting a test (Greene, 1984), leading some investigators to conclude that this sort of frequency information is encoded automatically (Hasher and Zacks, 1979, 1984). Although this claim of automatic encoding of frequency information is unlikely in the face of evidence that strategic factors may have a major impact on this task (Greene, 1984, 1986), all theoretical accounts of repetition effects must address why some information about frequency of occurrence is seemingly stored without intention. Strength and multiple-trace theories offer different explanations for how participants are able to estimate situational frequency. A strength theory would assume that participants access the memory trace corresponding to a test item and then assess the strength of that trace. The judgment of frequency is then based on the strength of that trace. For example, a very strong trace would be evidence that the corresponding item had occurred frequently on the list. On the other hand, a multiple-trace theory would assume that participants try to retrieve as many traces as possible of the test item. Those items that had occurred in the context of the list would then be counted, and a judgment of frequency would be based on that count (possibly after some adjustment to fit the expected ranges).

Results from the frequency-judgment literature tend to favor multiple-trace approaches. Although strength theory would have no problem in accounting for judgments of background frequency, it is not clear how this approach could explain people's ability to offer situational-frequency estimates that are independent of an item's overall background frequency. For example, strength theory would predict that background frequency should always contaminate situational frequency so that people should give higher situational judgments to items that occur more often in everyday life. In reality, situational judgments are largely independent of background frequency, and the slight effect obtained goes in the wrong direction: When situational frequency is held constant, participants give higher situational-frequency estimates to words of low background frequency than to words of high background frequency (Rao, 1983). Accuracy of frequency judgments is better for low-frequency words than for high-frequency words (Greene and Thapar, 1994).

Hintzman and Block (1971) carried out the classic demonstration of participants' ability to come up with specific situational frequencies. In this study, people were shown two lists of words, separated by

a 5-minute interval. Each word occurred zero, two, or five times on each of the lists. These list frequencies were factorially crossed, so that the frequency of occurrence on one list would be completely uninformative as to the frequency of occurrence on the other list. After seeing the lists, participants were asked to estimate situational frequency of occurrence of each test word for each of the lists. The most important finding is that participants were very accurate on this task, so that their estimates of list frequency were chiefly determined by the true frequencies on the list being judged. Frequency of occurrence on the other list had only a small influence. The finding that people are able to make separate situational-frequency estimates for the same item in two lists is awkward for strength theory to explain: If these estimates were based solely on a unidimensional construct like strength, participants would not be able to make up these separate and largely independent estimates. On the other hand, multiple-trace theory would have no difficulty with this pattern, because these estimates would be based on a count of individual traces, each carrying information about the context in which it was created.

Greene (1990a, Experiment 5) carried out an even more extreme comparison of strength and multiple-trace approaches. Participants were given a list of words without being told what sort of test to expect. After the list had been presented, participants were shown a word accompanied by two other words. Participants had to choose which of the two accompanying words had been presented more frequently immediately before the single word. (For example, participants may have seen the word GOAT on the list three times. It may have occurred immediately after the word DIME two times and immediately after WALL once. DIME and WALL occurred elsewhere on the list, so that their total frequencies were equal. On the test, participants could be presented with GOAT, accompanied by DIME and WALL, and asked to pick the word that had preceded GOAT more often on the list. To be correct, they would have to pick DIME.) Participants had to answer this type of question for 36 words. Although this was a difficult test, all participants were able to perform above chance. Clearly, repetitions do more than merely strengthen a trace. Rather, every time a stimulus is presented, it leaves some sort of trace that records the context in which it had occurred.

2.06.2.4 Limitations on Multiple-Trace Accounts

Data from frequency-memory experiments provide strong evidence against the notion that repetition merely strengthens memories. It seems necessary to assume that repetition of stimuli leads to the storage of information that includes precise information about the time or context in which each presentation had occurred. However, this does not necessarily mean that repetition only leads to the formation of multiple traces. After all, it is possible that repetition has multiple effects. For example, it could lead both to multiple traces and to a strengthening of some representation in memory. There is some evidence that multiple-trace theories are not adequate for a complete account of the effects of repetition on memory.

2.06.2.4.1 Effects of repetition on nonrepeated items

A simple and pure multiple-trace account would claim that repeated presentations of an item should have the same effect on memory as presenting multiple once-presented items. For example, presenting 10 words three times each should lead to a functional list length of 30 items.

Let us consider a single once-presented item that may be presented in one of three conditions. In one condition, it is presented along with 10 once-presented words. In a second condition, it is presented along with 10 words, each presented three times, so that there would be a total of 31 presentations on the list. In a third condition, the item is presented along with 30 words presented once, so that there would again be a total of 31 presentations. We then administer a recognition test. We are interested only in recognition of the once-presented item. Recognition will certainly be influenced by the length of the list, but the critical issue would be if length should be defined in terms of total presentations (so that the second and third conditions are equivalent and should be more difficult than the first) or in terms of number of novel words presented (so that the first two conditions are equivalent, with the third being more difficult than the others). If one believes that repetitions act like presentations of new items by creating independent traces, then the second and third conditions should be equivalent. In reality, recognition is not influenced by total number of presentations but by the number of novel presentations so that the first and second conditions are both

approximately equal and easier than the third (Ratcliff et al., 1990). The empirical picture is somewhat less clear-cut in free recall, but even here the effect of repeated presentations does not come close to matching the effect of added novel items (Tulving and Hastie, 1972; Ratcliff et al., 1990). Additional evidence that repetitions do not affect other items in the same way as a comparable number of distinct items was reported by Tussing and Greene (2001). This type of finding suggests that a simple application of the multiple-trace approach to repetition effects is inadequate.

2.06.2.4.2 Superadditive effects of repetition on memory

Waugh (1963) observed that one can use simple laws of probability to derive expectations for memory of repeated items based on performance on once-presented items. Let us assume that two presentations of a repeated item lead to two completely independent memory traces. Recall or recognition of that item would be successful if the participant was able to retrieve one or both of these traces. If we define P as the probability of remembering a once-presented word, then the probability of remembering at least one occurrence of a repeated word should be $(2P - P^2)$ if the occurrences act like purely independent traces. This value can be considered an independence baseline, an indication of how high memory for repeated items should be if retention of separate occurrences was completely independent. Although not all studies find that recall of repeated stimuli significantly surpasses the independence baseline (e.g., Glanzer, 1969), there are numerous examples in the literature in which this has occurred (e.g., Johnston and Uhl, 1976; Goldman and Pellegrino, 1977; Overton and Adolphson, 1979). For reasons that are still unclear, the nature of the memory test seems critical, with performance on repeated items being more likely to exceed the independence baseline on recall tests than on recognition tests (Begg and Green, 1988).

At first glance, any reports of memory for repeated items exceeding the independence baseline might appear to be inconsistent with multiple-trace theories of repetition effects. After all, if multiple presentations of an item lead to the creation of separate traces, shouldn't retrieval of these traces operate just like retrieval of once-presented items? However, this argument is too simplistic. It overlooks the possibility that the memorability of an item might be affected by the fact that there were other occurrences of that item. For example, when a word is presented for a second time on a list, this repetition may remind the participant of the earlier occurrence, leading to further rehearsal and increased retrievability of the first presentation. (There is independent evidence that repeated presentations may lead to such a reminding process; see Hintzman and Block, 1973; Tzeng and Cotton, 1980; Winograd and Soloway, 1985). Thus, one cannot reject the multiple-trace approach simply because recall of repeated words may exceed the independence baseline. Rather, the multiple-trace approach suggests that the probability of recall should be predictable based on the probability of retrieval of the separate occurrences. To test the multiple-trace approach, some way must be found to allow for the experimenter to determine whether the participant is able to recall the first, second, or both occurrences of a repeated item.

Watkins and Kerkar (1985) devised such a study. The general procedure in their experiments was to ask participants to learn a list of once- and twice-presented words. Every presentation was tagged by an arbitrarily selected distinguishing attribute. Participants would be required to recall the words. Then they would be asked to remember the attributes. The goal was to determine whether memory for the items could be predicted on the basis of memory for the detailed attributes associated with particular presentations. Their first experiment can serve as an example of their approach. This experiment required recall of lists, each of which was composed of five words presented once and five words presented twice. The attribute manipulated here was the color of the presentations, with 10 different colors being used in printing the words. After the list was presented, participants were asked to recall the words. They recalled 0.18 of the once-presented words and 0.46 of the repeated words. (Note that recall of the repeated words greatly exceeded the independence baseline.) Then, Watkins and Kerkar attempted to determine whether recall of repeated items could be attributed to retrieval of particular occurrences. To determine how well particular occurrences could be retrieved, Watkins and Kerkar asked participants to remember the color that each word had been presented in, including noting two colors for repeated words. Participants were much less accurate in recalling the colors of repeated words than those of once-presented words. Thus, although people are able to recall repeated words better than would be expected based on recall of once-presented words, they actually remember the

details of each occurrence of repeated words less well than they did for once-presented words. Watkins and Kerkar argued that there is a collective recollection of occurrences, which they called generic memory, that can benefit memory for repeated items separately from retrieval of the particular instances. Watkins and LeCompte (1991) presented further evidence that memory for repeated information can exceed recall of specific occurrences.

Hintzman (2004) had another demonstration that multiple-trace accounts cannot offer a complete account of repetition effects. He took what is commonly seen as the strongest evidence for these accounts, namely, participants' ability to estimate the frequency of occurrence of repeated items. He showed that these judgments of frequency were, in fact, more strongly related to presentation frequency than is performance on a recognition-memory test. Essentially, he dissociated memory for items from memory for how often they occur, although multiple-trace accounts attribute both to a common process (namely, the retrieval of separate occurrences). This finding is inconsistent with a pure strength-theory of repetition effects as well, because such a theory would also attribute the effects of repetition on recognition and frequency judgment to a single process, namely, the strengthening of a single trace for each stimulus. Fisher and Nelson (2006) carried out a conceptual replication of this finding by showing that the size of the repetition effect is numerically greater on frequency judgments than on recognition memory. These results suggest that any one-process account of repetition effects will be insufficient.

Overall, the literature suggests that repetition leads to the creation of separate memory traces, each of which contains information about the context in which it occurred. However, findings such as those of Watkins and Kerkar (1985) and Hintzman (2004) indicate that repetition has additional effects on memory beyond the creation of traces. These additional effects are at present still poorly understood. It is possible that a combination of strength and multiple-trace approaches will be necessary; formal models, such as that of Hintzman (1988), where repetitions create multiple traces that may be combined in various ways at the time of test, may eventually shed light on this. At present, however, we know that simple approaches are inadequate without yet being able to offer a successful complex explanation of repetition effects.

2.06.3 Spacing Effects in Memory

Ebbinghaus (1885/1964) noted that "with any considerable number of repetitions a suitable distribution of them over a space of time is decidedly more advantageous than the massing of them at a single time" (p. 89). This advantage of spaced practice over massed practice became one of the laws of memory formulated by Jost (1897). Spacing of repetitions became a widely used manipulation in studies of learning and memory (Bruce and Bahrick, 1992). Reviews of early research on this topic were carried out by Ruch (1928) and McGeoch and Irion (1952). However, given the wide variety of procedures used, many conflicting results were found, and researchers such as Underwood (1961) despaired of being able to demonstrate consistent and unambiguous benefits of repetition spacing. Melton (1967) rectified this by popularizing a straightforward way of demonstrating the beneficial effects of spacing. With this method, a list of words is presented one at a time to participants. Some of the words are presented two or more times, and the number of intervening items between occurrences of repeated items is carefully controlled. Some repeated words (massed words) are presented twice in a row, whereas other repeated words (spaced words) have one or more intervening words between occurrences. When participants are given a test on their memory for the words, a clear benefit for spaced repetitions over massed repetitions can be demonstrated. Recent reviews have established the advantage for spaced repetitions beyond serious question (Janiszewski et al., 2003; Cepeda et al., 2006). Although this conclusion had to be based on controlled laboratory experimentation, people evidently reach a similar conclusion based on their everyday experiences, as they may choose to devote spaced rehearsals to challenging material (Benjamin and Bird, 2006).

Several distinctions among commonly used terms may be useful. The advantage in memory for a repeated item over a once-presented item is a repetition effect. The advantage in memory for spaced items (repeated items that had their occurrences separated by intervening stimuli) over massed items (repeated items presented consecutively) is a spacing effect (sometimes called a distributed-practice effect). When one looks only at spaced items, any advantage in memory as the number of intervening stimuli is increased beyond one would be called a lag effect. Whether increases in spacing

beyond one or two intervening items hold much benefit in remembering is less clear, although evidence increasingly suggests that this is the case (Kahana and Howard, 2005; Cepeda et al., 2006).

Spacing effects have received a large amount of attention from both theorists and experimenters. One reason why they are seen as particularly important is their wide generality. They may be found in a large number of subject populations, including nonhuman animals (e.g., Davis, 1970; Sunsay et al., 2004), human infants (Cornell, 1980), children (Toppino, 1991, 1993), and the elderly (Balota et al., 1989; Benjamin and Craik, 2001). Also, spacing effects have been found in educational settings for typical course materials, suggesting that the distribution of practice may be a useful way to improve retention without requiring additional time (Dempster, 1988). Bahrick (2005) has argued for the importance of viewing spacing effects from an educational perspective, and it is certainly true that spacing can influence long-term retention of material typically learned in school (e.g., Reder and Anderson, 1982; Rea and Modigliani, 1985; Bahrick and Phelps, 1987; Dempster, 1987; Rawson and Kintsch, 2005; Balch, 2006; Kerfoot et al., 2007).

A wide variety of theoretical explanations have been offered for spacing effects over the years. Although spacing effects may be found in many domains and using many tests, theorists have focused on the literature on human memory for word lists. At least with respect to this memory literature, two classes of explanation have been particularly influential, one emphasizing the importance of encoding and the other emphasizing the importance of retrieval.

2.06.3.1 Deficient-Processing Accounts

Some theorists (e.g., Hintzman, 1976; Zechmeister and Shaughnessy, 1980; Cuddy and Jacoby, 1982) have claimed that repetition spacing influences the processing of the second occurrence of repeated items. Typically, the claim is that massing repetitions leads to deficient processing of the second occurrence, relative to spaced repetitions. Hintzman et al. (1973) showed that repetition spacing seemed to influence memory for the details of the second occurrence, but not of the first occurrence, for repeated items.

This deficient processing of the second occurrence of repeated items may in part be due to involuntary processes akin to habituation; that is, we may not be able to keep ourselves from paying less attention to a stimulus that we had just encountered than one whose previous occurrences were more distant in time. However, it may well be that voluntary, strategic processes play a major role here. Zechmeister and Shaughnessy (1980) argued that, as participants are encoding a list of items, they are constantly deciding how to distribute their rehearsals between the current stimulus and the previous ones. The amount of rehearsal that a participant devotes to an item may be influenced by whether he or she feels that it is already well learned. There would be no point in devoting further rehearsal to an item that has already been mastered. Zechmeister and Shaughnessy found that participants overestimate the degree to which they will remember massed stimuli. They argued that this can lead to participants choosing to devote fewer rehearsals to such stimuli than to spaced items. Bahrick and Hall (2005) proposed a variant of this approach specifically aimed at retention over very long intervals.

There are many sources of evidence that converge on the claim that participants do not adequately encode the second presentation of massed items. When participants are asked to rehearse words aloud, they give fewer overt rehearsals to the second presentations of massed items than to the second presentations of spaced items (Rundus, 1971; Ciccone and Brelsford, 1974). When participants are allowed to pace themselves through a list presented on slides, they devote less exposure time to massed items than to spaced items (Shaughnessy et al., 1972; Zimmerman, 1975). Dilation of the pupil in the eye (a measure that is related to cognitive effort) is greater when participants are seeing spaced repetitions than when they are seeing massed repetitions (Magliero, 1983).

One straightforward test of this voluntary encoding account is to see whether spacing effects are eliminated when participants are not expecting a test on their memory for the material. That is, if participants are not deliberately rehearsing any items for a later test, then they would have no reason to treat spaced and massed items differently. The evidence here is strikingly mixed. Spacing effects are sometimes eliminated when lists are learned incidentally, but the details of the study and testing procedure are critical (e.g., Jensen and Freund, 1981; Greene, 1989, 1990b; Challis, 1993; Greene and Stillwell, 1995; Russo et al., 1998).

A second test for voluntary encoding-deficit accounts would involve an examination of the importance of experimental design. Repetition spacing

could either be manipulated within lists (where participants are given a list containing both massed and spaced repetitions) or between lists (where participants receive a pure list, consisting either only of massed repetitions or spaced repetitions). The vast majority of studies in this area has used the within-list design. The magnitude of spacing effects in this design may be exaggerated because participants may rehearse spaced items during the presentation of massed items. If spacing effects result from an encoding deficit for massed items, you may see a reduction (and possibly a complete elimination) of spacing effects on between-list designs. Unfortunately, the literature on this point is very inconsistent, yielding all sorts of conflicting findings, and it is possible that the details of the design, study instructions, and memory test may be critical (Underwood, 1969b, 1970; Waugh, 1970; Greene, 1990b; Hall, 1992; Toppino and Schneider, 1999; Delaney and Knowles, 2005; Kahana and Howard, 2005).

Attempts to reduce spacing effects entirely to a voluntary deficient-encoding process, while inspiring some supportive results, have failed to present a consistent empirical picture. This has convinced some theorists that at least one other process is needed to explain spacing effects completely (Greene, 1989, 1990b; Braun and Rubin, 1998; Russo et al., 1998). In addition, it has been difficult to see how deficient-processing accounts developed to explain how participants remember intentionally learned lists of words can be easily expanded to cover the range of situations, materials, and subjects that can demonstrate advantages for spaced repetitions.

2.06.3.2 Encoding-Variability Accounts

Encoding-variability accounts of spacing effects make the assumption that the distribution of repetitions affects the likelihood that at least one of the occurrences will be successfully retrieved. Typically, it would be assumed that greater spacings would increase the probability that each presentation of a repeated stimulus would be encoded in a very different way, thereby making it more likely that a participant would be able to retrieve at least one of the occurrences.

One analogy that I have found useful in explaining this approach would be to liken this to the probability of being able to find a particularly important piece of paper. If you want to be sure that you will always be able to find the paper when you need it, you may try to have multiple copies of it. However, it would make no sense to place all of those copies in the same place. Rather, you should scatter the copies around at many different places. Although this would make it more difficult to locate all of the copies, it is assumed that you only need to locate one copy. Similarly, if every repetition of an item leads to a separate memory trace and if one only needs to retrieve one of the traces to remember the item, it would clearly be better if the separate traces are somehow distributed throughout memory.

This notion that repetition spacing influences encoding variability, which facilitates the probability of remembering at least one occurrence of an item, has been expressed in several forms. Landauer (1975) adopted this concept literally. He proposed a model in which memory traces are stored at random locations in a memory system. Traces for the occurrences of spaced items would tend to be stored farther apart than traces for the locations of massed items. If one assumes that only part of the memory space is searched during retrieval, then there would be an advantage for the spaced items.

An alternative way of envisioning encoding variability is with respect to the information with which items may become associated. For example, Glenberg (1979) suggested that interitem associations are critical on many memory tasks, particularly free recall. When two occurrences of a repeated item are presented in massed fashion, the two traces tend to become associated with the same items. In contrast, when the two occurrences are spaced apart, the resultant traces are associated with different items. Glenberg argued that the probability of retrieving at least one occurrence of a repeated item increases with the number of different associations that had been formed. Raaijmakers (2003) showed how this approach can be incorporated into a broader mathematical model of memory.

Gartman and Johnson (1972) pointed out that words can be interpreted in slightly different ways. This is clearest in the case of homographs like IRON or TOAST, where the same pronunciation and spelling pattern would be associated with seemingly unrelated meanings. Even when a word is perceived as having only one meaning, there may be slightly different connotations that could come to mind; depending on the context, the word PIANO could be encoded primarily as a musical instrument or as a heavy object. Gartman and Johnson suggested that repetition spacing influences the probability that each occurrence of a repeated word would receive a

somewhat different interpretation and that this variability in meaning would increase the probability that at least one occurrence could be retrieved. Indeed, Gartman and Johnson reported that recall of homographic words does not show a spacing effect, presumably because different meanings may be invoked even at short spacings.

The notion that some variant of encoding variability underlies the spacing effect has been popular among theorists even in the absence of direct evidence that encoding variability benefits memory at all. Some interpretations of this approach would claim that encoding variability should influence memory for once-presented items; that is, the probability of retrieving at least one of two unrelated, once-presented words should increase as a function of the spacing between them. However, this prediction has been falsified, as spacing seems to have no effect on recall of once-presented words (Ross and Landauer, 1978). Also, direct attempts at controlling the presentation context of repeated items have found that encoding variability typically leads to a decrease in memory performance (Bellezza and Young, 1989; Greene and Stillwell, 1995; Verkoeijen et al., 2004).

Even in the absence of direct empirical support, the concept of encoding variability as at least one component in a theory of spacing effects continues to be popular. One reason is that some variants of this approach can offer a straightforward explanation for an otherwise puzzling finding, namely, the fact that memory for massed items may be superior to memory for spaced items if the test is administered very briefly after presentation. Glenberg and Lehman (1980) presented a particularly compelling empirical picture and proposed a proportionality rule that states that "when the retention interval is short relative to the spacing of the repetitions, performance is negatively correlated with repetition spacing; when the retention interval is long relative to the spacing intervals, performance is positively correlated with spacings of the repetitions" (p. 528). Glenberg and Lehman demonstrated this proportionality rule in free recall, with similar findings being obtained in other memory tasks (Peterson et al., 1963; Glenberg, 1976; see Cepeda et al., 2006, for a quantitative review of this pattern). This advantage for massed items after short retention intervals suggests that it is better to have two occurrences presented in contexts very similar to the testing context rather than to have only one. Although this finding of a massed-item advantage after brief retention intervals is only indirect support for encoding-variability approaches, it has been difficult to develop alternative explanations for this finding.

2.06.3.3 Multiprocess Accounts

Theorists have increasingly abandoned the attempt to reduce spacing effects to a single factor and have instead turned to multiprocess explanations, where spacing influences several aspects of memory (e.g., Glenberg, 1979; Greene, 1989, 1990b; Braun and Rubin, 1998; Russo et al., 1998). For example, Greene (1989, 1990b) has argued that deficient processing of the second occurrence of massed items largely explains spacing effects on tests where cues are provided to participants; such cued-memory tests would include recognition or frequency judgment. On the other hand, free recall is an uncued test because no retrieval cues are explicitly given to participants; some variants of the encoding-variability approach offer a better explanation of spacing effects on this test. This sort of explanation reflects the fact that one can find manipulations that have different effects on the spacing effect found in free recall or cued tests (e.g., Glenberg and Smith, 1981; Greene, 1989; Kahana and Greene, 1993). Still, the details of multiprocess approaches have yet to be worked out satisfactorily, as none has been able to offer a comprehensive account of the literature.

The popularity of multiprocess accounts largely reflects the fact that single-process explanations to date have necessarily left large portions of the literature unexplained. One limitation even for multiprocess accounts is that they have largely been applied only to results from memory experiments using adult human participants. In principle a factor like encoding variability can be applied to nonhuman animals (and indeed the concept owes much to the stimulus-sampling theory developed by Estes, 1955, to explain findings in the animal-learning literature). However, there has been little effort paid to seeing whether one can take theories of the spacing effect developed in the field of human memory and apply them in a fruitful way to other domains, such as animal conditioning or human skill acquisition. Until theorists in this area feel compelled to account for a wider range of empirical data, it will be impossible to claim that we have an adequate explanation of spacing effects.

2.06.4 When Repetition Does Not Improve Learning

Because repetition so clearly is an important factor in learning, it is understandable that we have focused on cases where there is a positive relationship. However, sometimes repetition is ineffective in promoting learning. The classic demonstration of this is the poor memory Americans have for the characteristics of the penny. Although Americans have seen this coin countless numbers of times, Nickerson and Adams (1979) showed that they can have quite poor recollection for its details. They may not be able to recall what words are on the penny or where the date is located. After all, people presumably use the color (brown) of the penny to distinguish it from other coins, so they do not need to attend to its other features. If people are given 15 s to study an unfamiliar coin (the mercury dime, which was in use from 1916 to 1945), they remember its details better than they remember those of the penny (Marmie and Healy, 2004). This illustrates the point that repetition in the absence of attention is strikingly ineffective in promoting learning.

The ineffectiveness of repetition in the absence of attention is also illustrated by the fate of items memorized through maintenance, or rote, rehearsal (e.g., Glenberg et al., 1977; Rundus, 1977). In these experiments, participants have to repeat items aloud over and over. When they are given an unexpected memory test on the rehearsed words, there is at best a very weak relationship between the number of overt rehearsals devoted to an item and later memory (Greene, 1987). Simply repeating an item over and over has little benefit for memory in the absence of attention or more elaborative processing of the material.

Repetition may impair learning if memory is tested for only one occurrence. If an item has been presented in several contexts, it may become difficult to retrieve the occurrence that is being tested. An early demonstration of this (although initially interpreted in a somewhat different way) was the negative part-whole transfer effect reported by Tulving (1966). In this procedure, a control group and an experimental group first learn a list of 18 words and then learn a list of 36 words. In the control group, the two lists are unrelated. In the experimental group, the earlier list of 18 words was then included in the list of 36 words. If repetition inevitably leads to improved memory, then the experimental group should have an advantage over the control group. However, Tulving found an effect in the opposite direction, with the control group outperforming the experimental group. A critical issue here is that there is increased opportunity for confusion between the lists when they overlap. Because participants in the experimental group do not necessarily realize that the 18-word list is entirely contained in the 36-word list, they may have difficulty when they try to restrict their recall to the second list (Sternberg and Bower, 1974). In a similar vein, preexposing some items on a list may impair recognition memory for them, at least in part because participants have difficulty knowing whether the familiarity of the items is due to the preexposure or to presentation on the list (Greene, 1999). Repetition may impair memory when the critical task requires participants to disregard some occurrences of a repeated stimulus.

A striking case where repetition may lessen memory is in serial (ordered) recall of short lists. When participants have to recall short lists of digits or letters, memory is impaired if one item is repeated on the list. This phenomenon, known as the Ranschburg effect, was introduced into the modern psychological literature by Crowder and Melton (1965). Crowder (1968) carried out a systematic manipulation of all possible locations of repeated items. He found that, when the two occurrences of a repeated item occupy immediately adjacent serial positions, recall of the series is enhanced. However, when the two occurrences are spaced apart, recall is impaired, with the greatest decrement occurring when there are two intervening items. The impairment is very localized, with only recall of the second occurrence being negatively affected. The Ranschburg effect is also rather delicate, leading Murdock (1974) to label it the "Ranschburg (non) Effect" (p. 297). Later research has shed light on the boundary conditions of this phenomenon, as changes in the nature of either the instructions given or the nature of the test can eliminate the effect (Greene, 1991). This effect seems to occur because recall of the first occurrence of the repeated item may inhibit output of the second occurrence (Greene, 2001).

2.06.5 Conclusion

Much of the literature on repetition and spacing effects has been carried out in the empirically minded spirit of functionalist psychology, so it is perhaps appropriate that strong conclusions can be

drawn about empirical patterns but only tentative ones about theoretical implications. First, it is clear that the development of learning as a function of repetition is established beyond question in the research literature. Second, performance improves as a smooth, negatively accelerated function of frequency of study, though this does not necessarily imply that all aspects of learning take place gradually and continuously. Third, as a result of repeated practice, we form memories that contain the details of each occurrence and that we can access individually. Fourth, the effects of repetition cannot be reduced merely to retention of these separate episodes, as we seem to form generic memories that capture what these individual presentations have in common. Fifth, the effects of repeated study are enhanced if the study episodes are spaced apart in time. Sixth, these spacing effects are most likely due to a combination of factors, such as deficient processing of massed repetitions and superior retrieval for spaced repetitions. Seventh, repetitions may not always enhance memory, particularly when little attention is paid to a stimulus or when accurate remembering requires access to one particular occurrence of an event.

Although the theoretical implications of repetition and spacing effects remain to be worked out, their practical importance is beyond question (Dempster, 1988; Bahrick, 2005). Admittedly, much of the literature on these topics has followed standard laboratory methods employing word lists and college-student participants, thereby exhibiting the strenuous task reductionism typical of memory research (Crowder, 1985). Still, a meta-analysis carried out by Cepeda et al. (2006) suggested that repetition and spacing effects may influence learning for a wide variety of materials and over long retention intervals. As a wider range of procedures and perspectives are directed at these issues, we may hope to achieve greater theoretical progress in understanding these central manipulations for learning and memory.

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2.07 Coding Processes

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The questions of what people know and how they come to know it have been a topic of scholarly inquiry forever. At the level of individual knowledge, all that any of us know is represented in memory, and memory representations are just that, representations. Whatever the event may have been, the processes of perception and comprehension yielded a psychological experience, and it is that experience of the event that becomes the memory. In this sense, the memory is a code that represents the original event, but that code is all the knowledge we as individuals have of the event. With these premises, the importance of studying coding processes in memory is obvious. Answers to the questions of what the nature of the code is and how the code is formed are answers to the venerable questions concerning the nature of knowledge.

Widespread interest in issues of coding only appeared with the cognitive renaissance of the 1950s and 1960s. The concept of coding is rarely found in psychology texts prior to 1950, but by 1972, coding was said to be truly central to modern theories of memory (Bower, 1972). In this chapter, we examine the origins

of the concept, which reveal the theoretical function served by coding and the reason for its centrality in modern theory. An overview of the methods used to study coding is provided, as well as some description of factors known to affect the coding process. Finally, two broad metaphors used to describe the memory code will be discussed, along with several specific ideas that represent each of the metaphors. The reader is warned of a major caveat about the discussion, namely, that the effects of variables associated with the memory test will not be described, nor will the theoretical coaction of coding and retrieval be part of the discussion. One must keep in mind that many of the effects described in this chapter are relative to the conditions of retrieval (*See* Chapter 2.16).

2.07.1 The Coding Process

The process of coding is an integral component of many natural and artificial phenomena. Generally, coding can be defined as the transformation of messages, signals, or states from one representational form to another. With this definition, the presence of coding in activities from espionage to metabolism becomes fairly obvious. One might reasonably wonder, however, why phenomena require the apparently superfluous process of changing forms of representation. That is, if the representations denote the same thing, why insert a process to change the form of the representation? Leaving aside esoteric forms of coding for purposes of deception and secrecy, the principal need for coding processes seems to be that the end user cannot work with the original form of the representation, and coding is necessary to transform the original representation into a useable form. Relevant examples here range from the various transformations of energy after it contacts sensory receptors and before it is "used" in the brain to the necessity of transforming a visual experience to linguistic code to inform someone of the experience.

2.07.1.1 Coding from the Computer Model

The meteoric rise in the use of coding as a psychological concept is largely because of the developments in information science and computer technology between 1948 and 1960. Shannon (1948) published a mathematical theory of an abstract communication system, an important component of which was the bits of information transmitted by the system. The theory was written in a general mathematical form such that information could take any conceivable form and be transmitted over any kind of channel. Some psychologists soon realized that the idea could be applied to human information transmission (e.g., Miller, 1956).

Dovetailing with the abstract notion of information transmission was the more concrete model of the computer as an information processor. By 1958, Newell et al. advocated that the mind be described as an information processing system modeled on the workings of computing machinery. Early in the history of computer science, the computer was conceptualized as a general-purpose symbol manipulator, and the insight that the mind could be construed as a symbol-manipulating system (e.g., Newell and Simon, 1972) provided the foundation for the use of a computer model in theoretical psychology. With this foundation in place, the analogy of specific computer functions such as buffers, stores, and retrieval to human information processing, especially memory, was recognized quickly. Among these specific functions was the process of coding.

For the computer, the fundamental process of coding is the transformation of the external input into the representation defined by the machine language. The analogy here to energy transformation in human sensation and perception is patent. For example, the effective energy contacting the visual receptors is electromagnetic energy, but the human brain cannot use this form of energy. The first coding in vision is the transformation from electromagnetic energy to chemical energy at the level of the rods and cones. In turn, the chemical energy is transformed to electrical energy for transmission through the optic nerve for use in the cortex. Less concrete but equally compelling analogies were drawn to cognitive descriptions of learning and memory. By the 1950s, verbal learning researchers knew that some nonsense syllables were more nonsensical than others. Syllables such as FDR, KLM, and CBS obviously were treated differently than JQN, XFV, PGW. The assumption that the nominal stimulus was the functional stimulus had given way to the admission of a proximal stimulus. With that concession, behavioral theory now needed a psychological process of coding to account for the transformation from nominal to proximal stimulus.

2.07.1.2 The Function of a Code in Psychological Theory

Codes serve as representations for some other object or event. Codes carry information, perhaps not in the

precise form specified by Shannon's (1948) theory, but information in the sense of symbolically representing something else. For our purposes, codes are the psychological manifestation of prior experience. The experience can be in the immediate past, in which case we call the code a perception. As the past grows more distant, we refer to the code as a memory. All psychologists agree that behavior and thought are influenced by prior experience, and because the code is the representation of that experience, understanding codes and the processes that produce them looms large for cognitive theorists.

Codes serve another function for psychological theory that is rarely discussed. That is, codes dispel the mystery of action at a distance. To say that current thought and behavior are caused by prior experience begs the question of how something in the past can cause something to happen in the present. The stock answer to this question is that experience changes the individual, but the customized answer includes the form of this change. Different theories propose different kinds of codes, but in all cases, the stored code solves the problem posed by causal action at a temporal distance. The original event does not cause current thought and behavior but, rather, the coded version of that event, which is accessible at the time of the behavior or thought. Not everyone agrees that this use of the code to bridge the temporal gap is a good thing. Watkins (2002), for example, noted that memory has been reified by assuming that a residual of the original experience is maintained over time and that this characterization is unrealistic. Nonetheless, the search for the contents of the memory code has been quite active.

2.07.2 Breaking the Code

On the assumption that the memory code is the proximate cause of the past's influence on current thought and behavior, the question of what is encoded from a given experience has become a popular research agenda in learning and memory. As with many other concepts in science, the memory code cannot be observed directly. Consequently, a variety of methodologies have been developed to infer the nature of the representation of a particular experience. Each method comes with assumptions that allow the inference to follow, and thus it is important to explicitly acknowledge these assumptions. The following discussion is intended to update an earlier review by Tulving and Bower (1974).

2.07.2.1 Transfer Paradigms

The transfer paradigm is a venerable method for studying the effect of prior experience and inferring the nature of the code for that experience. The use of transfer rests on the assumption that the effect of prior experience is proportional to the similarity of the prior experience and the current task. Perhaps the first use of the transfer paradigm to measure encoding and storage was Ebbinghaus's (1964) savings method. The savings score is a ratio of the number of trials required for original criterion learning to the number of trials to reach the criterion on a subsequent attempt. This ratio is assumed to index the stored memory from initial learning in that memory, for the original experience obviates the need for new learning on the second experience. Thus, the goal of the savings method is to determine the amount of the original experience that is available at a later time.

In contrast, contemporary use of the transfer paradigm has focused on the qualitative characteristics of encoding, as attested to by the wide acceptance of the principle of transfer-appropriate processing (Morris et al., 1977). The reasoning is straightforward. If performance on the criterion test varies as a function of the similarity between the test and the prior experience, one infers that the code includes values from that dimension of similarity. As an example, consider an experiment by Jacoby (1983). Subjects studied words for memory either by reading the words or by generating the words from a fragment. Half of the subjects were given a test of recognition memory, and half were given a test of perceptual identification. Perceptual identification requires that words be read under conditions of severe visual degradation. Prior experience with the words facilitates perceptual identification accuracy, but as Jacoby (1983) showed, only if that experience is reading. Generation of the study words produced no positive transfer to perceptual identification, although generation yielded much higher recognition memory than reading. Jacoby's demonstration of differential transfer from reading and generating at study illustrates the use of transfer paradigm to infer the content of the code.

Negative transfer also can be used to infer the contents of the code. An early example is the release

from proactive inhibition (PI) paradigm (Wickens et al., 1963; Wickens, 1970). The basic paradigm involved presentation of short lists of words for immediate recall. The lists were similar on some dimension; for example, all words could be exemplars of the same category. As can be seen in **Figure 1**, performance declined quickly over the first two to three study-test trials. The decline was identified as PI, the cause of which is competition among the codes. The basis of the competition is assumed to be similarity of the codes. Thus, the presence of proactive interference provides a basis for inferring that the code contains information corresponding to the dimension of similarity. The inference is validated by eliminating the similarity on the final list in the series and observing better performance than on the previous trial, the so-called release from PI, which is depicted in **Figure 1** in the shift condition.

Straightforward inferences about the code from the release from PI paradigm are complicated by data reported by Gardiner et al. (1972). They presented different instances from the same category over three study-test trials and observed PI buildup over the trials, as would be expected. On the fourth trial, subjects continued to see instances from the same category but under different conditions. The standard control condition received no special instructions and continued to show PI on the last trial. In another condition, subjects were informed prior to studying the list that the instances were all exemplars of a subcategory (e.g., water birds). Memory improved with these instructions, as would be expected if the dimension of encoding shifts from that of the first three trials. The critical condition is the third condition, in which the special instructions concerning the material were given after study and prior to recall. These subjects also evidenced release from PI, results consistent with an alternative view of the cause of PI buildup and release. That is, PI reflects the increasing ineffectiveness of a cue as more items are subsumed by that cue, all in accord with the principle of cue overload (Watkins and Watkins, 1975). Release from PI then is caused by the availability of an appropriate new cue. Note that in this interpretation, negative transfer in the release from PI paradigm is not informative about coding processes.

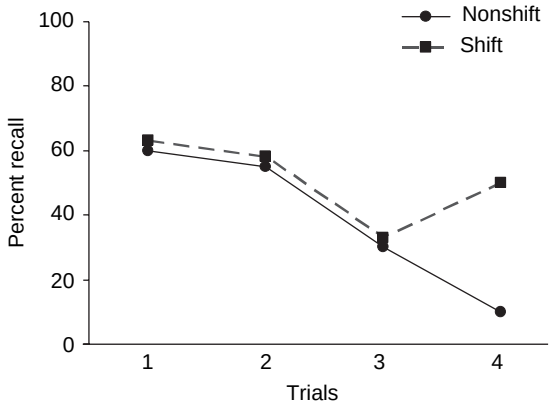

Figure 1 Memory for material from the same category over the first three trials illustrates proactive interference. The shift condition sees material from a different category on the fourth trial, resulting in release from proactive interference. Adapted from Wickens DD, Born DG, and Allen CK (1963) Proactive inhibition and item similarity in short-term memory. *Journal of Verbal Learning and Verbal Behavior* 2: 440–445.

2.07.2.2 Retrieval Cuing

The relative effectiveness of retrieval cues frequently is used to make inferences about the nature of a code. The reasoning is the same as that underlying the use of the transfer paradigm, except that here the appeal is to the principle of encoding specificity (Thomson and Tulving, 1970; Tulving and Thomson, 1973). Encoding specificity states that as a necessary condition for successful memory, the cue must have been present at encoding. If a cue does lead to correct memory, one can assume, based on encoding specificity, that information shared by the cue and its target was encoded originally.

As an example, Nelson et al. (1974) used this logic to investigate semantic and phonetic coding of words. Word lists were studied for memory and then cued for recall by either rhymes or synonyms of the studied words. Half of the subjects who received rhyme cues had studied the words in the presence of the rhymes, and half had studied the words alone. Likewise, half of the subjects receiving synonym cues had studied the words in the presence of the synonyms, and half had studied the words alone. The results showed that recall to a rhyme cue was equally good for words studied in the presence of the rhyme and for words studied alone. Recall in the presence of the synonym cues was much better for the group that had seen the synonym cues at study than for the group that studied the items alone. From these results, Nelson et al. concluded that phonetic information about a word is encoded even when the word

is not modified by a study context. Semantic information corresponding to the synonym is not encoded unless the study context biases that coding process. The results and interpretation make perfect sense, in that words have few alternative sound patterns, whereas the number of nuanced meanings of a word can be large.

The cuing methodology has been used extensively to study the interface between comprehension and memory. For example, Anderson et al. (1976) asked people to study sentences that contained a general noun (e.g., "The woman was outstanding in the theatre"). Later they were given a cued recall test and instructed to recall the last word of the studied sentences. The cue could be the general subject noun (e.g., 'woman') or a specific term that previous norming showed represented the comprehended instantiation of the general noun (e.g., 'actress'). Recall was better for the last word of the sentences when the cue was the particular instantiation rather than the general term that had appeared in the sentence. Using the logic of encoding specificity, Anderson et al. concluded that general terms are comprehended and encoded as particular instantiations rather than as abstract core meaning. This example nicely illustrates the relationship between questions about the memory code and the broad issue of the nature of knowledge. The conclusion from the Anderson et al. study suggests that the meaning of abstract nouns is represented by specific instances of those nouns rather than some abstract meaning that goes beyond the instances.

Using retrieval cues to infer the coded experience is limited to the logic of encoding specificity, namely, the presence of a cue at encoding is a necessary condition of cue effectiveness, not a sufficient condition. The implication of this limitation is that one cannot infer the nature of coding from the absence of a particular cue effect. For example, the declining performance over trials in a buildup of PI paradigm cannot be used to infer a corresponding decline in the encoding of the dimension of similarity across the trials, indeed, quite the opposite inference usually is made. In short, memory performance is affected by factors other than the presence of an appropriate cue. What can be done with some confidence is to infer that particular information was encoded when cue information facilitates memory. Nairne (2002) offers an interesting discussion of the limitations of cue effectiveness as a basis for inferences concerning the encoded trace.

2.07.2.3 Materials Effects

The existence of different kinds of codes also has been inferred from differential effects of material on memory. Perhaps the seminal modern instance of this approach involves comparing memory for a list of pictures versus memory for a list of words that are the names of the pictures. Other things being equal, the pictures will be better remembered than the words (e.g., Paivio, 1971). Paivio and others have interpreted these data as consistent with the idea that at least two classes of codes exist in memory, verbal and imaginal (Paivio, 1995). Pictures can be coded in both forms, whereas words are most likely to be coded in the verbal form, and the multiple forms of code for the pictures confer an advantage in memory performance. Alternatively, Nelson et al. (1976) suggested that the picture superiority effect is not a result of qualitative differences in the code but, rather, to a quantitative difference in the distinctiveness of the sensory code for pictures. Nelson et al. (1977), however, did argue for a difference in the necessity of semantic coding for words and pictures in that pictures require semantic coding prior to phonetic coding, whereas the semantic coding of words is not necessary for phonetic coding. Again, the nature of coding was inferred from the differences in performances for the classes of materials.

Variations in the memory code accompanying manipulations of materials have been used to explain other memory effects, including differences in memory as a function of clinical diagnoses. For example, clinically depressed patients tend to remember negatively valenced words better than positive words, whereas nondepressed people do not show this effect (e.g., Bradley and Mathews, 1983; McDowell, 1984). This effect is eliminated in tests that do not request intentional memory (e.g., Denny and Hunt, 1992; Watkins et al., 1992). Because prior research has shown that meaningfully elaborated codes are better remembered than less elaborated codes on intentional memory tests, these data have led to the conclusion that the encoded representation of negative experiences is more elaborate than the representation for positive experiences in depressed patients. The difference in memory for different types of materials as a function of clinical diagnosis is explained by inferring quantitative differences in the codes. Depressed patients have more elaborate codes representing negative events than do nondepressed people.

A potential problem associated with inferring the type of code from materials effects concerns the decision axis for postulating a class of codes. One would not postulate a qualitatively different class of codes for every dimension along which people can discriminate; otherwise, the kinds of codes would prove practically infinite. What was needed in 1974 (Tulving and Bower) is still not entirely obvious today, and that is a clear set of rules specifying when a differential effect of materials is evidence for different kinds of code. Moreover, care must be exercised to avoid the assumption that labeling material effects as different memory codes has explained anything. Discussing this issue, Tulving and Bower (1974) said, "it is of interest to note that it has not yet been made clear by anyone how the task of explaining memory phenomena is materially aided by the hypothesized existence of different memory stores" (p. 273).

2.07.2.4 Decision Time

Another method used to infer the nature of encoded material is the time taken to respond to queries about the prior experience. The assumption underlying this technique is that response time will be faster if the code contains the information requested by the query. The more inferences from the code required to answer the query, the longer the time will be. A good example of this approach comes from Posner's (1969) research. Subjects were shown two letters in succession, the first of which is the target letter, and the second is the probe. A decision is made as quickly as possible as to whether the probe matches the first target. Various matching rules can be used to instruct the subjects. Suppose the rule is that the two letters have the same name, and the target letter is a capitalized 'A.' The probe can either be a matching capitalized 'A' or one of two nonmatching probes, a lowercase 'a' or a different letter. When 'A' is followed by 'A,' the positive decision is made more quickly than when 'A' is followed by 'a.' The difference in decision latency decreases as the interval between the two letters increases. Assuming that the target letter is held in memory until the match is completed, Posner and his colleagues interpreted this pattern to indicate that the initial representation is visual, thus producing faster matches for visually identical patterns. As the encoding process continues, the visual code is supplemented by a phonetic code, leading to faster responding to the 'A-a' pair at longer interletter intervals.

Reaction time has been used extensively to infer imaginal coding in decision-making tasks (e.g., Brooks, 1967; Cooper and Shepard, 1973; Farah, 1985). As an example, Cave and Kosslyn (1989) showed stimuli consisting of two superimposed rectangles, one drawn in light lines and the other in dark lines. One of the rectangles was drawn vertically, and the other was superimposed diagonally over the first rectangle. The same two objects were used on each trial, although the relative length of the lines in the objects differed from trial to trial. The task was to decide whether the sides of the object drawn in light lines were equal to those of the object drawn in light lines on the previous trial. The principal manipulation was the subject's expectations of the size of the object to be judged and of which of the two rectangles would be drawn in light lines. The manipulation was performed by instructing the subjects that most of the time the same rectangle would be judged on the next trial and that the object would be of the same size as on the preceding trial. These instructions conformed to 75% of the trials. Thus, on 25% of the trials, the size, the object, or both were different from the preceding trial. The time to correctly decide about the targeted rectangle was affected by both expectations. Responses to unexpected objects as well as to unexpected sizes were slower. Considering just the expected object trials, response time increased linearly with the unexpected change in object size. These data indicate that the stimulus from the preceding trial affects performance on the current trial and that this effect systematically varies with the relative size of the stimuli. A reasonable interpretation is that the encoded representation from the previous trial affects performance on the current trial and that representation contains specific size information. That is, the representation is a visual image.

Although studies such as those of Posner and Cave and Kosslyn illustrate that reasonable inferences can be drawn about the nature of the code from decision latencies, one must be aware of the effect of speed–accuracy trade-offs when using latency data. Latencies in most tasks will vary as a function of the emphasis on speed or accuracy, and that trade-off can change the results of an experiment dramatically. Such changes could result in different conclusions about the nature of the code, when in fact the difference is essentially a strategy shift. Moreover, the use of decision latency tends to be limited to material on which accuracy of performance will be near perfect. Thus, the method is not appropriate for new learning or large amounts of to-be-remembered material.

2.07.2.5 False Memories

Memory is not always veridical to the past. People do remember things that did not occur, or at least did not occur as they are remembered. This fact has long been known and has been used as a tool to infer the qualitative nature of the original code. The assumption underlying this inference is that healthy memory is not capricious; rather, errors of commission in memory reflect the content of the coded representation of the probed experience. For example, early studies of false responding in recognition memory showed that synonyms and antonyms of studied words were seductive lures (Anisfeld and Knapp, 1968; Fillenbaum, 1969). The high false alarm rates for these distracters were taken to indicate that the coded representation of the study items was dominated by meaning. The same conclusion was drawn from studies that showed false recognition for sentences that expressed the same idea as studied sentences but were otherwise syntactically different from the studied sentences (e.g., Bransford and Franks, 1971). The coded representation of sentential content seemed to be the abstracted meaning of the sentence.

An important line of research that uses false memory to infer the nature of the code was initiated by studies of inferential processing in comprehension. The idea is that inferences are an integral aspect of normal comprehension and that the information implied in the inference would be part of the coded memory. For example, Johnson et al. (1973) reported a study in which subjects were asked to read several short descriptive stories consisting of two or three sentences. One story was: "John was fixing the birdhouse. He was pounding the nail when his father came out to watch him and help him do the work." The control condition saw the same story except 'pounding the nail' was replaced with 'looking for the nail.' In addition to the actual sentences presented in the story, the recognition test included inference sentences such as: "John was using the hammer to fix the birdhouse when his father came out to watch him and help him do the work." The test instructions were to recognize the sentences that were exactly the same as those presented at study. The group that received the study sentences that invited the tested inferences recognized approximately the same percentage of inference test items and studied sentences. Subjects given the control study sentences made few false alarms to the inference test items. These data are important indications that the coded representation of the prior experience includes the information from the inference, which apparently is indistinguishable from the presented material.

In a similar vein, Deese (1959) reported that people will intrude associatively related words in recall after studying a list of words that are all associated with unpresented words. For example, the study words might include 'sharp,' 'thread,' 'sew,' and 'pin,' but not the word 'needle.' On later recall tests, the probability of recall for the nonpresented associate often is equivalent to the recall of study items. Roediger and McDermott (1995) resuscitated this paradigm, and extensive research has been conducted using the paradigm to study false memories (see Roediger and McDermott, 2000a,b, for a review). A favored interpretation of the intrusions and false alarms that occur in this paradigm is that the critical item comes to mind during study and thus is encoded in the study episode. (*See* Chapter 2.14 for a thorough discussion of false memory.)

Past research has interpreted false memory to be the result of encoding either a general dimension or specific content such as an inference. This interpretation seems reasonable and, additionally, renders false memory less mysterious and capricious in that false memory very often is the product of the normal processes of comprehension of targeted material. One issue concerning inferences from false memory is whether the false memories result from encoding processes or occur at retrieval. For example, false memory of inferences from the Deese/Roediger/McDermott paradigm may be the result of the critical item coming to mind in the presence of targets in recall or recognition. That is, the inference occurs during the test rather than at study. Although this possibility cannot be ruled out entirely, two findings mitigate against an exclusive retrieval interpretation. One is that Roediger et al. (2001) report that false recall is negatively related to correct recall. If the false item were coming to mind as the result of recalling its associates, one would expect a positive relationship between these factors. The second finding is that warnings about false recall are more effective if issued prior to study rather than following study (McDermott and Roediger, 1998; Gallo et al., 2001; Neuschatz et al., 2003; but see McCabe and Smith, 2002, for contrary data).

2.07.2.6 Orienting Tasks

Orienting tasks, usually judgments that the subject makes concerning the to-be-remembered material,

can have a powerful influence on recall and recognition (e.g., Hyde and Jenkins, 1969). With Craik and Lockhart's (1972) levels of processing came a wave of experiments manipulating orienting tasks, all of which assumed that these tasks exert their effect at least in part through specific encoding of a dimension of the material. The logic here is straightforward. If the memory trace is a by-product of perception and comprehension, and the focus of perception and comprehension can be controlled by the orienting instructions, then the qualitative content of the trace can be identified with the dimension specified by the orienting task. For example, Jacoby and Goolkasian (1973) gave subjects lists of word pairs, each of which was related either categorically or acoustically. The orienting task was to rate the degree of the relationship within the pairs. The subjects rating categorical relations recalled more of the items than the subjects who rated the acoustic relationship. The difference in memory is attributed to the nature of the trace (i.e., representations of categorical meaning lead to better memory than sound patterns).

Orienting tasks often are used in conjunction with other methods to infer the nature of the code. For example, Chan et al. (2005) combined the use of orienting tasks and the Deese false memory paradigm to examine the effect of associative versus phonological encoding on false memory. Study lists were either semantically or phonologically related words, and in both cases all the study words were related to a word that was not presented (e.g., 'bed,' 'rest,' 'awake,' or 'sweep,' 'steep,' and 'sleet' are all related to 'sleep,' which itself was not presented). The orienting instructions were to concentrate on the relationship among the words' meanings or among the sound patterns of the words. These instructions were orthogonal to the type of relationship among the words in the lists. The results, which are shown in **Figure 2**, showed an impressive crossover interaction between type of list and orienting task on false memory for the nonpresented items. Considerably more false memory occurred when the orienting task was congruent with the dimension of similarity in the study list. A reasonable interpretation of these data is that the orienting task controls the dimension of encoding and that the critical associate will only come to mind if the study items are coded on a dimension shared by the critical item. That is, if I see *sweep, steep, and sleet*, I will only think about *sleep* if I am attending to the sound of the words.

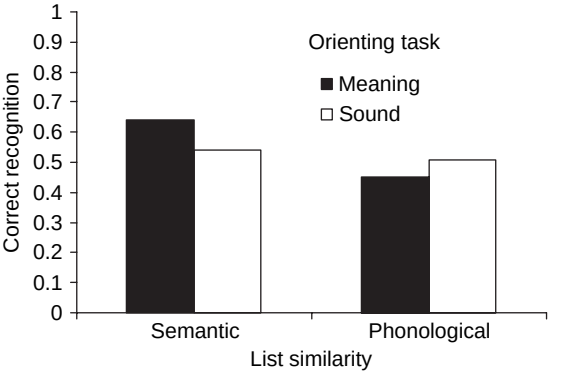

Figure 2 False recognition of critical words as a function of the type of list similarity and orienting task. Adapted from Chan JC, McDermott KB, Watson JM, and Gallo DA. (2005) The importance of material-processing interactions in inducing false memory. *Mem. Cognit.* 33: 389–395.

Despite enormous amounts of research using the technique, we know that orienting tasks alone are not sufficient to identify the content of the code. Leaving aside the possibility that subjects may intentionally focus on dimensions other than that associated with the orienting task, incontrovertible evidence shows that orienting tasks do not control completely the dimension of encoding. For example, Nelson et al. (1979) found that the meaning of a word was encoded in a context that emphasized phonetic features. Hunt et al. (1979) report that visual features of words are encoded during semantic orienting tasks. The encoding of sensory attributes in the course of making a semantic judgment is quite reasonable given that the semantic processing requires sensory input, but this fact complicates the inference one can make about the code following an orienting task.

2.07.2.7 Neural Indices of the Code

A relatively new technique for identifying the code is the use of noninvasive, neural-dependent measures that record brain activity during encoding. The logic of identifying psychological functions from observations of neural dependent measures was formulated in the early 1800s by pioneers such as Gall and Spurzheim, but the development of powerful imaging techniques has made the idea more appealing than ever. A succinct statement of the neuroscientific approach to identifying the memory code begins with the assumption that experiencing an event results in activation of neural pathways that are dedicated to the processing of those types of events.

This activation leaves a trace in the form of altered neural functioning such as increased connectivity. This neural trace will be a critical participant in later memory of the event when activation occurs under appropriate conditions. Using this logic, the trace is identified as the site of the brain activity. To translate the neural code to a psychological code, one appeals to the assumed localization of the psychological functioning (e.g., visual/occipital, auditory/temporal, verbal/temporal-frontal, spatial/parietal, emotional/limbic).

An informative example of the logic underlying the neural identification codes is the study of imagery and memory. A dispute erupted in the 1970s over the nature of the code in memory for an imagined event. The dispute had two aspects. Is the code a modality-specific representation or some more abstract, post-perceptual code (e.g., Shepard, 1978)? And is the mental image a spatial representation or a propositional representation (e.g., Kosslyn, 1980)? Although these appear to be straightforward empirical issues, they proved resistant to adjudication by standard methods of experimental psychology (see, e.g., the following sequence of papers, Kosslyn et al., 1978; Pylyshyn, 1981; Intons-Peterson, 1983).

In the face of this stalemate, some argued that neural techniques are the panacea, "neural measures have the potential to be more decisive on these issues because they provide more direct evidence on the internal processing stages intervening between the stimulus and response in imagery experiments" (Farah, 1995, p. 964). In her reviews of research using various techniques to monitor regional brain activity during mental imagery, Farah (1995, 2006) showed that virtually every study implicates occipital activity in mental imagery, demonstrating that imagery and perception share cortical representations. Moreover, some of these shared cortical representations include spatially mapped areas of the occipital lobe. Thus, if one is willing to assume that the cortical representation is the psychological experience, the neural techniques have resolved successfully what appeared to be an intractable debate about the code underlying mental imagery.

Another interesting example of the use of brain measures to address a question of coding is described by Tulving (1989). The question was, Are there different kinds of codes for knowledge and memory? Tulving reported PET scans of brain activity when the subject was thinking of a recent Sunday afternoon picnic and when thinking of news accounts of French elections. In the best of the Ebbinghaus tradition, Tulving himself was the subject. The results of the scan showed activity in different brain regions when remembering the Sunday picnic than when reflecting on knowledge of the French elections, evidence interpreted to be consistent with the notion that memory and knowledge are represented by different kinds of codes.

Another important line of research using neural techniques was initiated by Wagner et al. (1998). The goal was to identify the locus of brain activity during encoding that is associated with remembered items but not forgotten items. fMRI scans were performed as the to-be-remembered words were presented, and then the scans were backsorted following a recognition memory test to determine what differentiated recognized items from nonrecognized items. Comparing high-confidence hits to misses, greater activity was seen in multiple prefrontal regions and left parahippocampal and fusiform gyri for the hits. Effectively, the conclusion is that relatively high activation in these areas established the code for successful memory. Paller and Wagner (2002, p. 93) have labeled this technique the "subsequent memory paradigm," which yields the contrast between neural activity for successfully remembered and forgotten items. Subsequent use of the paradigm has replicated the original Wagner et al. (1998) results and moved on to issues such as the correlates of coding underlying the subjective experiences of remembering versus knowing (see Paller and Wagner, 2002; Kahn et al., 2004, for reviews).

Research on coding processes that use neural-dependent measures is an exciting development that has produced new information about the brain correlates of learning and memory. However, as with all the other techniques described here, certain cautions are in order if one's goal is to specify as precisely as possible the memory code. Especially important is an issue raised by Henson (2005, 2006) and Poldrack (2006), which is analogous to deductive versus inductive inferences from behavioral data. In the case of behavioral studies of coding processes, a deductive inference would be one in which memory is predicted from a theoretical view of the nature of the code, whereas an inductive inference would be one in which the nature of the code is induced from the behavior. The latter, of course, is the much frowned upon *post hoc* explanation. In the case of the neural measures, the deductive inference is one in which brain activity is predicted from some theoretical idea. Tulving's (1989) study described earlier is an example of the deductive inference based on

neural measures. The hypothesis was that two different kinds of memory codes exist, and the study measured brain activity in the situations that hypothetically activate one or the other of the codes. Notice that the precise regions of brain activity are not critical for the conclusion drawn from the data.

In contrast, the inductive inference, the postulation of a memory code from observation of brain activity, is based entirely on the region of the brain that is activated. Farah's (1995) conclusion that mental imagery is modality specific is based on the assumption that visual perception is mediated by the occipital cortex. Activation of that structure is used to infer visual experience. Not only is the specific brain region important when making these inductive inferences from brain to psychological function but the mapping of structure to function also must be one-to-one (Henson, 2005, 2006; Poldrack, 2006). If any reason existed to assume that the occipital cortex were involved in propositional coding as well as visual perception, one could not infer anything about a particular code from its activation. Thus, if the goal is to specify a particular encoding operation from neural data, one must be able to specify not only what brain area is associated with that operation but also that the brain area is only associated with that particular type of code.

2.07.2.8 Summary of Methods

Memory scientists have been extraordinarily clever at developing techniques to study the nature of the representation in memory. The work is difficult because no direct observation of the memory code is possible, but rather, the code must be inferred from observations of behavior and/or brain activity. The use of indirect inferences to establish the nature of the representation is not at all unique to the question of coding, or even to psychology. It is the same hypotheticodeductive strategy that has led to the postulation of planets and subatomic particles. In contrast, the rules that govern an inference from observations to a specific hypothetical code must be made explicit in each case and examined for their validity.

The challenge confronting the attempt to precisely specify the code in any given circumstance was argued by Anderson (1978). Anderson's point was that inferences about the code are based on data, which in turn were collected under the auspices of theory-driven experiments. That is, a theory predicts some outcome given some setting condition. The problem arises when we realize that a theory postulating a particular code (e.g., a visual image) also assumes some set of processes. Any data explained by this theory also may be explained by an alternative theory that postulates an alternative form of representation (e.g., a propositional code) combined with alternative processes. Anderson concluded that for this reason, arguments about contents of the code cannot be adjudicated by behavioral research. Anderson's only suggested solution was the possibility that neural-dependent measures would become available, and as evidenced by Farah's opinion quoted earlier, some scientists believe that the solution has been achieved in the intervening years. That belief, however, rests on the assumption that the neural measure is a more direct observation of the code.

2.07.3 Factors Affecting the Coding Process

Acquisition and retention of information are determined in part by the circumstances surrounding the initial experience. It is these factors that are classified as the variables affecting encoding. Understanding the effects of these variables is complicated by the fact that criterion performance is affected not only by the coding process but also by the circumstances surrounding the test. Among the major advances in the study of learning and memory is the widespread appreciation for the relativity of the effect of both study conditions and test conditions, each of which constrains the other. Thus, the effect of encoding variables on later performance is relative to the nature of the test. Consequently, in contrast to earlier conceptualizations of learning and memory, we no longer make absolute statements about the general effect of acquisition variables. Nonetheless, the study of factors constituting the encoding environment continues to be a focal area of memory research. In this section, some of that research and its allied phenomena are described.

2.07.3.1 Intent to Remember

Intuitively, intent to remember emerges as a dominant factor affecting later memory, but research on encoding processes has shown that intuition unequivocally to be wrong (e.g., Postman, 1964; Hyde and Jenkins, 1969; Craik and Lockhart, 1972; Challis

et al., 1996). As we shall see, what does matter is the type of processing performed on the material, but trying to remember does not ensure that optimal processing will be engaged. **Figure 3** depicts the results reported by Hyde and Jenkins (1969). Subjects were asked to determine the words' pleasantness, check all of the 'e's in the word, or count the vowels in each word. For three groups of subjects, the orienting tasks were given as incidental memory instructions, and for another three groups the orienting tasks were accompanied by instructions to try to remember the words. As can be seen in **Figure 3**, adding intentional instructions improved performance in the nonsemantic orienting groups but had no effect on the performance following the pleasantness rating task. It is not the intent to remember but, rather, the nature of the processing that is important.

Indeed, an enduring contribution of levels of processing (Craik and Lockhart, 1972) is the acceptance of memory as a by-product of the processes of perception and comprehension of the original experience rather than as the intentional object of processing. After all, how many times during the course of the day does one try to remember, and yet healthy adults can remember most everything that happened yesterday. Furthermore, only occasionally do we know what, if anything, about current experience will be required from memory, rendering intent to remember any part of the experience a gamble against future demands. In light of these considerations, the lack of direct effects of intentional memory is understandable.

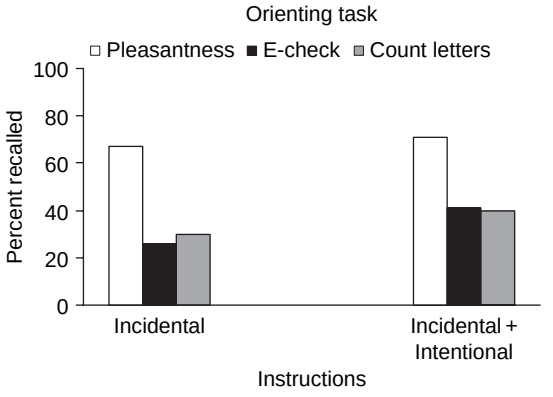

Figure 3 Memory as a function of type of orienting task and intention to remember at study. Adapted from Hyde TS and Jenkins JJ (1969) Differential effects of incidental tasks on the organization of recall of a list of highly associated words. *J. Exp. Psychol.* 82: 472–481.

2.07.3.2 Attention

Coding processes to memory are influenced substantially by both quantitative and qualitative aspects of attention. Qualitative aspects of attentional processing are inferred from the effects of selective attention on memory, and those effects are encompassed by levels of processing. In addition to the effects of selective attention, evidence indicates powerful effects of the amount of attention devoted to encoding. Dividing attention between processing a to-be-remembered event and another activity at encoding comes at a cost to both.

Seminal projects by Baddelely et al. (1984) and Craik et al. (1996) both found that memory was affected negatively by dividing attention at the time of study and also that performance on the secondary task used to divide attention was negatively affected. This research clearly shows that coding processes require attentional capacity. More recent reports (e.g., Fernandes and Moscovitch, 2000; Naveh-Benjamin et al., 2005) have substantiated the earlier conclusion, rendering as apparent the fact that optimal memory for a prior experience requires allocation of conscious processing to that experience. This conclusion only applies to memory tests in which the individual intends to remember, an important example of the caution urged about conclusions concerning encoding without consideration of the retrieval context.

2.07.3.3 Types of Processing

Beginning with Hyde and Jenkins (1969), it became increasingly apparent that memory could be influenced powerfully by asking subjects to perform tasks that focused attention on various aspects or dimensions of the to-be-remembered material. Hyde and Jenkins concluded that the effect of these tasks can be attributed to the "nature of the stored trace" (1969, p. 480). As research intensified, the characteristic of the trace that determined performance took center stage. Hyde and Jenkins had contrasted tasks that required subjects to rate the pleasantness of words, to count the number of 'e's in the words, or to estimate the number of letters in the words. The pleasantness rating task invariably produced better recall, and in contrasting the three tasks, Hyde and Jenkins speculated that the difference lay in the fact that the pleasantness rating task required the words to be treated as meaningful units. This idea would be refined and elaborated by Craik and Lockhart (1972) in one of the most influential papers in the coding literature.

2.07.3.3.1 Levels of processing

Beginning with Estes's (1959) stimulus sampling theory, the notion that objects and events can be conceptualized as multidimensional has been routinely adopted in memory research. Encoding processes function to analyze experience along its various dimensions and select values on those dimensions to represent an experience in memory. The code can be described as the set of these values or features, or alternatively, at a more macro level, the code can be identified with a broad dimension (e.g., phonetic code). Research such as that of Hyde and Jenkins (1969) suggested that encoding semantic features yielded better memory for the event than encoding orthographic features, and Craik and Lockhart (1972) systematized findings such as these with their idea of levels of processing. The idea itself will be discussed later, but the empirical work surrounding the idea uncovered a powerful factor affecting the coding process.

The idea of levels of processing was simple, and the experimental paradigm that it fostered was easily implemented and produced huge effects, factors responsible for dozens of published papers demonstrating the basic effect in the wake of Craik and Lockhart's paper (Watkins, 2002). The effect originally reported by Hyde and Jenkins is that semantic encoding produces superior memory, but the research expanded the characterization to a broad dichotomy between semantic and nonsemantic encoding. The superiority of semantic encoding was demonstrated not only for memory for lists of words, but also for higher-order language constructions (e.g., Perfetti, 1979), and even faces (e.g., Bower and Karlin, 1974). **Figure 4** represents the results of Bower and Karlin's study, in which subjects studied faces by judging their honesty, likableness, or gender. Subsequent recognition memory for the faces was much more accurate following judgments of honesty and likableness. In the wake of the immense volume of literature provoked by levels of processing, one marvels that the basic effect of superior memory following semantic processing remains unexplained (Roediger and Gallo, 2002).

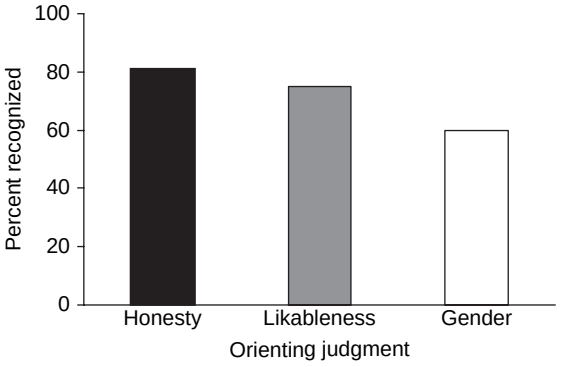

Figure 4 Recognition of faces as a function of judging honesty, likeableness, or gender when studying the faces. Adapted from Bower GH and Karlin MB (1974) Depth of processing of pictures, faces, and recognition memory. *J. Exp. Psychol.* 103: 121–156.

2.07.3.3.2 Self-generation

Slamecka and Graf (1978) convincingly showed that memory for self-generated material is better than memory for externally provided material. This generation effect is operationally distinct from levels of processing in that the generation paradigm requires subjects to generate a word in the presence of highly constraining cues. For example, subjects may be told to generate antonyms of cue words that also fit the letter fragment (e.g., 'hot–c__d'). Other subjects either hear or see lists of the same word pairs and are asked to remember the second member of each pair. Other things being equal, the generated items are much better remembered than the externally provided items.

Similar to levels of processing, generation effects have no consensually agreed upon explanation, and little empirical work currently is devoted to this problem. Nonetheless, generation is a powerful encoding factor. Just how powerful is nicely illustrated in a study by Slamecka and Fevreiski (1983). They arranged a generation list that would yield tip-of-the-tongue states. Subjects were asked to generate words in response to dictionary definitions, and sometimes subjects could not generate the word but would report feeling that they knew that word. The control condition read the definitions followed by the word. **Figure 5** depicts the remarkable outcome, which was that the words that were not generated but were on the tip of the tongue were recalled better that the same word when it had been read.

The generation effect is limited to meaningful material; no generation advantage in memory occurs with meaningless material (Graf, 1980; McElroy and Slamecka, 1982), suggesting a possible connection between the psychological processes mediating generation and levels of processing effects. The generation effect also was eliminated in a study by Donaldson and Bass (1980), in which the subjects in the nongenerate condition were required to judge the quality of the relationship between the cue word and the target. The effect of this manipulation was to

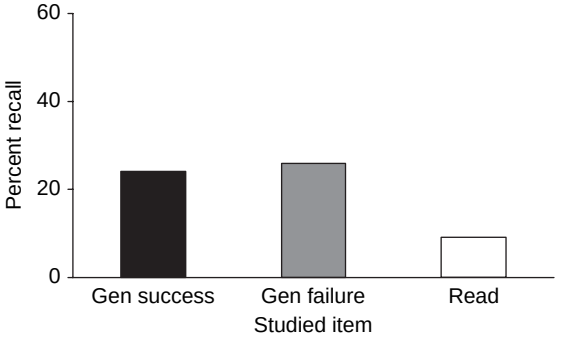

Figure 5 Correct recall as a function of successfully generating, trying but failing to generate, or reading the word at study. Adapted from Slamecka NJ and Fevreiski J (1983) The generation effect when generation fails. *Journal of Verbal Learning and Verbal Behavior* 22: 153–163.

elevate performance in the nongenerate group to the level of the generate group, suggesting that the effect is a result of the attentional focus required by generation.

Begg and his colleagues have made a similar argument based on the premise that the generation effect was the result of impoverished processing of nongenerated items. Begg et al. (1991) demonstrated that generated and read items are equally well remembered if the quality of the processing of the items is equated. For example, if both the generation and read condition are asked to construct images of the words, no difference appears in memory as a function of generation. If both conditions are asked to pronounce the words, the generation condition now shows an advantage in memory. The interpretation of these data entails an important applied message about memory coding; namely, generation requires discriminative encoding processes that transfer to later memory demands, whereas perceptual processing of incoming information may or may not attract beneficial discriminative encoding.

2.07.3.3.3 Organizational processing

Levels of processing and generation research largely have studied memory for unrelated words and have produced descriptions of item-specific coding processes. However, coding of relationships among items has long been known to be important to memory (e.g., Katona, 1940). The modern era of research on organizational coding was launched by Miller's (1956) proposal that discrete elements could be coded into higher-order chunks for storage in short-term memory. The function of such coding was to increase storage capacity by increasing the amount of information in the stored unit. Tulving (1962) later emphasized enhanced retrieval efficiency as the functionally significant impact of organization.

The most straightforward evidence for the importance of organizational processing is found in studies of memory for materials that contain known relationships. For example, word lists consisting of exemplars of known categories are better remembered than random lists, and categorized lists are better remembered when the category exemplars are presented contiguously than when they are presented in random order (Bousfield, 1953; Mathews, 1954). That an active coding process intervenes between list presentation and recall can be inferred from the fact that recall of randomly presented categorized lists tends to come out organized by category. Further evidence of active organization comes from Tulving's (1962) demonstration of subjective organization. Here multiple study-test trials occur for an unrelated list of words, which is presented in a different order on each study trial. Over the trials, a stable output ordering tends to develop – an ordering that is different from the various input orders and is usually idiosyncratic across subjects. The importance of organizational coding to memory has been argued from the almost perfect correlations Tulving found between his measure of organization and free recall.

The dependence of memory on organizational processing also is evident from the phenomena of whole-to-part and part-to-whole negative transfer. Tulving (1966) demonstrated that when subjects learn an initial list to criterion and then are given a second list to learn, half of which comprises the first list items, learning of the second list is impaired relative to a condition that learns an unrelated first list. Similar negative transfer was reported by Tulving and Osler (1967) when the second list consisted of half of the items from the first list. Tulving (1968) suggested that these data indicate the importance of organizational encoding to learning, in that the organization of the first list items interferes with learning (active organization) of the second list. This interpretation subsequently was supported by data showing that positive transfer could be arranged from list 1 to list 2 if list 1 organization is appropriate to list 2 learning (Bower and Lesgold, 1969; Ornstein, 1970).

2.07.3.3.4 Distinctive processing

Laboratory studies promoting the importance of distinctiveness to retention have been reported for over 100 years (e.g., Calkins, 1894), research that parallels the intuition about the memorability of distinctive

events. Despite, or perhaps because of, the intuitive appeal of distinctiveness as a factor at encoding, some confusion surrounds the meaning of the concept of distinctiveness (Hunt, 2006). The most common usage has distinctiveness as a property of events, as in the polka-dot Volkswagen in the funeral procession. In this view, distinctiveness is a property of events, and as such, distinctiveness cannot be explained by appeal to distinctiveness for obvious reasons of circularity. Rather, the standard approach has been to appeal to processes at encoding that entailed a subjective experience, usually of surprise or salience (Green, 1956), which in turn garnered attention in the form of additional processing of the distinctive event (Jenkins and Postman, 1948). In this view, distinctiveness has a quantitative effect on the encoding process.

Interestingly, the standard account of distinctiveness cannot explain the data from von Restorff's (1933) classic paper, which is peculiar because the standard account has been developed largely from data on the isolation effect. The isolation effect refers to enhanced memory for a target item that differs from the other items in the context, which themselves are similar on some dimension. The isolation effect refers to superior memory for the isolated item compared with memory for the same item, in the same serial position of a nonisolated control list. The items of the control list can be either all similar on some dimension or all different. The isolation list conforms to intuitions about what constitutes a distinctive event in that the target item violates the prevailing context. von Restorff was not the first person to use an isolation paradigm, but she was the first person to place the target item early in the list. Her reasoning was that at the early serial position, no context for the list has been established, and the isolate will not be perceived as salient. Nonetheless, the early isolate was better remembered than the corresponding control item. von Restorff's data pose a problem for the standard interpretation of distinctiveness as extraordinary processing attracted by the salience of isolate.

An alternative approach to distinctiveness began as an effort to integrate levels of processing and organization. Humphreys (1976) made the case that optimal encoding of an experience would include both the relationship among the elements comprising the experience as well as information about the elements themselves. Einstein and Hunt (1980) and Hunt and Einstein (1981) were able to demonstrate that the combined effects of organizational encoding and item-specific encoding led to better memory than either alone. Hunt and McDaniel (1993) suggested that the combination of relational and item-specific processing constitutes distinctive processing, using the argument that relational processing refers to processing of dimensions common to all items of an event, and item-specific processing refers to processing of properties of individual items not shared by other items in the event. The combination of relational and item-specific processing then precisely specifies a particular prior item, a description that captures the important discriminative function of distinctive processing. In this view, distinctive processing at encoding is defined as the processing of difference (item-specific properties) in the context of similarity (relational information). Nairne (2006) also has developed ideas about distinctiveness and memory that treat distinctiveness as a psychological phenomenon rather than as a property of events. Nairne's approach defines distinctiveness as the extent to which a particular cue complex specifies a particular event. As with the combination of item-specific and relational processing, Nairne's theory attributes the benefit of distinctive processing to the development of diagnostic information used in retrieval, rather than to quantitative differences in coding processes as specified by the standard treatment of distinctiveness. (For a more extensive discussion of distinctiveness, *See* Chapter 2.09.)

2.07.3.4 Prior Knowledge

Experience within a domain enhances memory for new events within the experienced domain. The more you know about something, the more likely it is that you will remember new information about something (Kimball and Holyoak, 2000). Moreover, this effect is presumed to be largely the result of encoding processes. One line of research leading to this conclusion is the study of experts' memory. Chase and Simon (1973) compared the memory of master chess players with that of novices for various arrangements of pieces on a chessboard. In some cases, the pieces occupied the positions of actual games in play, and in other cases the pieces were randomly arranged on the board. After briefly viewing the board, the subjects were asked to reproduce what they had seen. The experts remembered more than the novices when the boards were based on actual games, but when the pieces were randomly presented, memory was no longer affected by differences in prior knowledge. The superior memory of the experts was explained as the use of prior

knowledge to organize the encoding of a new experience. The experts' advantage disappears outside the domain of expertise, such as with the random arrangement of chess pieces. The results described here for chess apply to other domains, such as bridge, music, medicine, and computer programming (see Ericsson and Lehman, 1996, for a review).

Without disputing the contribution of organizational encoding, evidence has been offered to show that prior knowledge also increases the likelihood of distinctive encoding. Van Overschelde et al. (2005) asked people to remember a list of names of college and professional football (American-style) teams. The list consisted of 10 teams' names. In one case, nine of the teams were professional, and one was a college team, whereas in the other case, all 10 teams were college teams. The lists thus comprised an isolation paradigm. Typically, the isolated item is better remembered than the corresponding item in the all-similar list, an effect attributed to distinctive processing of the target item in the isolation list. Van Overschelde et al. selected people for their experiment who were either knowledgeable about football or not. The result was that the experts recalled the critical item better when it appeared in the isolation list than in the control list, but no isolation effect occurred for the nonexperts. This result suggests that experts encode not only the similarity among items within their domain of expertise but also the differences among these items. That is, prior knowledge seems to influence not only organizational encoding but also distinctive processing.

2.07.4 Characterizations of the Code

A final science of memory will know the neural code for prior experience and have a set of mapping rules to relate that code to the psychological states of memory. Until we reach that final stage, psychological concepts of memory codes will guide our thinking about phenomena of learning and memory. The specific nature of stored information has been characterized in virtually countless ways (e.g., traces, engrams, nodes, images, processes, features, vectors of features, production rules, or logogens). A particular characterization of codes often is driven by the phenomenon under study. For example, research into the effects of learning and memory in problem solving and skill learning often uses a production rule as the code (e.g., Anderson, 1983). What is learned and stored in memory is characterized as an if–then statement in the form of condition–action sequences. This characterization is descriptive of skilled performance in the domain of problem solving in that successful problem solving entails producing a particular action in a particular circumstance. Proposals for particular codes also result from the processing requirements of a theory in which the code is embedded. For example, Neisser's (1967) pioneering theory of pattern recognition proposed that the first step of the recognition process was the analysis of the sensory pattern into units that are stored in long-term memory. These units essentially serve as the data on which processes operate, much as real numbers serve as the database for arithmetic operations. The units used by Neisser were features – a concept that continues to have broad appeal.

Among the myriad descriptions of the memory code, one can detect two general issues that distinguish classes of codes. One of these issues is whether the code should be characterized as structure or process. The structural metaphor perhaps is the more common and more intuitive conceptualization. Here the memory code is a residual, often called the trace, of the prior experience, which is stored in a memory system. The alternative metaphor is that of skill. Here the memory code is represented as a mental process. The skill metaphor is very different from the structural metaphor in that processes are not stored; just as skills are nowhere when you are performing them, memory is nowhere when you are not remembering. These different metaphors lead to interesting differences in memory research, including that on coding, and we examine briefly some of the principle representatives of the two metaphors.

The second general issue concerns the existence of abstract codes; that is, codes for prior experience that are not bound by a particular prior context. The existence of abstract codes has been championed by philosophers as the product of rational thought since at least the time of Plato, and no one seriously studying cognitive processes questioned the reality of abstract codes until quite recently. To some extent, the positions on the abstraction issue are correlated with the first general issue, with the structural metaphor being more compatible with the notion of abstract representations; however, the correlation is not perfect.

2.07.4.1 The Structural Metaphor

Structural analysis of the mind evolved from two important but very different models. The first is

exemplified by Titchener's (1898) admirably clear statement of structuralism, in which of the goal of psychology was modeled on morphology in biology. According to Titchener, the job of the experimental psychologist was to perform "vivisection of the mind which shall yield structural results" (p. 450). The parts of the mind would be revealed by this analysis in much the same way that parts of the body are revealed by morphological analysis. It is precisely this kind of thinking that leads to research attempting to determine the constituents of the memory code that was described earlier.

The second model influencing modern structuralism is the computer. Application of the computer model to human cognition allowed a distinction between structural components and control processes that modeled the distinction between programming commands that are fixed and commands that are contingent on the content. Atkinson and Shiffrin's (1968) theory proposed that certain psychological processes are voluntary (control processes) and that certain structural components of information processing are fixed. The fixed components were memory systems, which are defined in part by the kind of code stored. Thus, coding processes are intimately bound to particular memory systems within a structural analysis. Different structural analyses yield different kinds of codes, as we see in the following discussion.

2.07.4.1.1 Stage theory of information processing

Atkinson and Shiffrin's theory exemplifies the stage analysis of the mind that characterized the halcyon days of information processing. Learning was a matter of transporting information from sensory reception to storage in long-term memory. The trip occurred in three stages. Each stage was a memory storage system, and each system required a different code.

The first stage of processing was the sensory memory store, which theoretically held codes in the form of raw sensory information. Groundbreaking work by Sperling (1960) provided evidence for the existence of a very short-lived memory that contained no meaning, characteristics that fit perfectly with a sensory code. Sperling's work on the visual store was complemented by Darwin et al.'s (1972) report of data suggesting the existence of an auditory store that holds acoustic sensory codes.

The second stage of processing culminated in storage in short-term memory. An important part of the processing was the recoding of the sensory information to its corresponding phonetic form. Evidence for a phonetic code in short-term memory was derived from studies showing interference in memory for short lists of words as a function of phonetic similarity (e.g., Baddeley, 1966). The argument that the code for short-term memory is the sound pattern of the material fit neatly with the importance assigned to rehearsal. That is, rehearsal typically is assumed to be a verbal process, and verbal processes require speech codes.

The final stage of processing was the transfer of information from short-term to long-term memory. Rehearsal was assumed to be the important mechanism of transfer, by which the phonetic code was recoded into its corresponding semantic representation. Again, the evidence for a semantic code in long-term memory was derived from studies showing that semantic similarity interfered with performance on tests of long-term memory (e.g., Baddeley, 1966).

Thus, the stage theory assumed that information processing is characterized in part by coding processes that recoded the information from a previous stage to a form appropriate for storage in the higher stage. In the strongest statement of the theory, the form of the code was assumed to be a structural component, which means that the code has to be in the specified form if the information is stored in the particular system. That is, nothing but a phonetic trace can be stored in short-term memory. Consequently, it is not surprising that research showing that the code in short-term memory could be visual (e.g., Posner, 1969), or even semantic (e.g., Shulman, 1974), raised concern about the stage model. One reaction to the theoretically incongruent data was to revise the model of short-term memory in light of the new evidence on the nature of the code.

2.07.4.1.2 Working memory

The concept of working memory emerged as a revised description of short-term memory (Baddeley and Hitch, 1974) in the wake of the stage model's failure. Working memory is different from the stage model in several dimensions, the most important of which for us is the question of coding. Rather than assume a single store containing a phonetic code, working memory's structure includes three separate storage structures to accommodate three different codes. The structure of working memory includes a phonological loop that stores a phonetic code, a visuospatial sketchpad that stores a visual code, and an

episodic buffer that stores semantic codes (Baddeley, 2000).

Evidence for the independence of the storage systems and their structurally bound codes was inferred from studies of interference. For example, the existence of a phonetic code has been inferred from the interference produced by the phonetic similarity effect (Baddeley, 1966), but this effect can be eliminated under certain circumstances. If the word lists are presented visually and the subject is required to repeat the word 'the' as rapidly as possible during list presentation, phonetically similar lists are remembered just as well as control lists. If, however, presentation of the lists is auditory, the phonetic similarity effect does occur; that is, the phonetically similar list is more poorly remembered than the control list. These data and their interpretation are drawn from Baddeley et al. (1984), who argued that auditory input gains obligatory access to the phonological loop but that visual input requires recoding to a phonetic form. This recoding is performed by the articulatory control process, but that process also is responsible for the production of speech. Thus, rapidly repeating the word 'the' during visual presentation of the list prevents phonetic recoding and storage in the phonological loop. As a consequence, the visual presentation accompanied by interference does not yield a phonetic similarity effect because the words are never coded phonetically.

The existence of a visual-spatial code has been adduced from analogous experiments that involve visual presentation of material accompanied by visual secondary tasks (e.g., Baddeley et al., 1973). In addition, neuroimaging research has offered support for the independent existence of a short-term visuospatial system of the sort proposed by working memory (Smith and Jonides, 1999). The episodic buffer has received little research attention, and at this time no evidence is available concerning the hypothesized code for that system.

The theory of working memory continues to be developed and has provoked a good deal of research, much of it related to the nature of codes in short-term memory. The theory has nothing to say about codes stored in long-term memory, and for the structural view of long-term memory, we turn to the memory systems approach.

2.07.4.1.3 Memory systems

The paragon of modern structuralism in cognitive psychology is the idea generally known as the memory systems approach. Essentially the approach advocates the existence of multiple memory systems in both short- and long-term memory and sets a research agenda of discovering the systems and delineating subsystems. A variety of classification schemes have been proposed, and the one described here is that of Schacter and Tulving (1994; Schacter et al., 2000). Schacter and Tulving outlined a list of defining features for a system that includes the operating rules, the neuroanatomical location of the system, and the type of information stored in the system. The later characteristic is the one of interest to us.

In 1972, Tulving proposed a distinction between what he called semantic memory and episodic memory. Semantic memory stores context-free information that corresponds to knowledge. For example, semantic memory contains information such as 'St. Louis is in Missouri, tomatoes are fruit, Dick Cheney shoots at birds.' These representations are abstract in the sense that the information coded in semantic memory is not constrained by time or space. In contrast, episodic memory stores information bound by its spatial and/or temporal context. For example, episodic memory contains information such as 'I went to the pharmacy yesterday, my wife and I saw Spamalot last Friday, I was told yesterday that John does not like tequila.' Note that the information in episodic memory has a personal as well as a temporal and/or spatial reference. The information stored in episodic memory corresponds to what we normally take to be memory rather than knowledge.

Since Tulving's original proposal, additional memory systems with their associated codes have been discovered. One is the procedural system that contains representations of cognitive and motor skills. These codes are similar to the codes in semantic memory in that they are abstract but differ from semantic memory in the kind of content. The code for the procedural memory literally is the representation of how to do something, like tie your shoes. The procedural code is not readily recoded verbally (try describing how to tie a shoe without using your hands), which is very different from the code in semantic memory. Another recently proposed system is the perceptual representation system. This system contains codes representing the visual and the auditory form of words. For example, the visual form 'cat' is stored in the perceptual representation system as well as a separate code representing the sound of that visual pattern. As with the semantic and procedural codes, the codes in the perceptual representation system do not contain contextually defining information. Unlike those two systems, the perceptual

representation is just that, viz., the modality-specific representation of a word or object. Finally, working memory also is included as one of the components of the system.

2.07.4.2 Summary of Structural Approaches

Memory codes are fundamental to the structural view of the mind in that a defining feature of any theoretical structure is the kind of code it contains. That is, the type code is one of the inherent characteristics of a system, just as skin is an inherent characteristic of mammals. The combination of system and characteristic code then is used as the principal explanation for performance. For example, consider the following situation: Subjects are asked remember a small amount of material but are prevented from rehearsing that material after its presentation and then tested within 30 s of the presentation. Memory for the material will be surprisingly poor (e.g., Peterson and Peterson, 1959). One explanation is that the test drew on short-term memory, which contains a limited duration trace, an aspect of the code contained in short-term memory. As with all metaphors used in science, the structural metaphor not only serves to explain performance but also molds the form of research. Under the structural umbrella, a prominent and respectable research activity is that of identifying and classifying the nature of codes. Thus, much of the research provoked by any of the previously mentioned structural views will be devoted to a description of the memory code. In this function, the structural notion of a memory code has been invaluable for cognitive neuroscience. As mentioned previously, most of the cognitive neuroscience of memory is devoted to identifying brain sites associated with memory phenomena – sites that then are taken to be the brain codes for the prior experience.

2.07.4.3 Process Metaphor

A very different characterization of memory in general and coding in particular arises if mental functioning is assumed to be analogous to a process or skill. The idea is that memory performance is determined by the mental processes operating at the time of an experience rather than by where the memory trace is stored. Craik and Lockhart's (1972) framework, levels of processing, was the seminal impetus for the processing metaphor, and in 1993 it was declared the most successful theory of learning and memory in the previous 25 years (Roediger, 1993). According to its authors, levels of processing "suggested that the memory trace could be thought of simply as the record of those analyses that had been carried out primarily for the purposes of perception and comprehension and that deeper, more semantic analyses yielded records that were more durable" (Lockhart and Craik, 1990, p. 88). Thus, levels of processing assume that the coding process focuses on either the meaning of an event or on nonsemantic properties, such as visual or phonetic features of the event. Attention to semantic features is considered deeper processing, and research has shown time and again that all other things being equal, semantic processing leads to better retention.

The gradual discovery of boundary conditions to semantic processing superiority has led to revisions to the original idea, wherein the central role of depth has been replaced by concepts such as elaborative processing (Craik and Tulving, 1975), distinctive processing (Jacoby and Craik, 1979), and sensory-semantic processing (Nelson, 1979), but the effect of Craik and Lockhart's thinking is manifested in the assumptions these revisions all share with the original view. Chief among these is that coding processes yield a memory trace consisting of qualitative features representing the event. Certain types of traces are more beneficial for retention than others; which type varies with the theorist, but in all cases memory is determined by the qualitative nature of the code. The qualitative nature of the trace is determined by the encoding processes, not by where the trace is stored, as is assumed by most structural theories.

In close temporal and spatial contiguity to Craik and Lockhart's work, a more radical version of the process metaphor began its development with Kolers' (1973) work.

In accord with levels of processing, memory systems played no role in the explanation of performance, but in addition, memory traces of the sort used by structural theories and by levels of processing were shed. The important role of memory traces as conceptual bridges between the past and present was assigned to the psychology processes brought to bear on the current event: The analytic operation of coding the experience becomes what is remembered. The implications of this shift for the concept of coding are far-reaching, as is evident in the following quotation from Kolers (1979):

> On the present view, every encounter with a stimulus elicits a different analysis from every other. . . . In other words, recognition is achieved by virtue of the correlation between the operations carried out on the two encounters with the stimulus event. The more similar the operations, the readier the recognition. But as nothing ever repeats itself exactly, recognition is based on the transfer of skills across occasions and partial correlation. *If the operations that are activated are themselves the record of the stimulus, then as the operations change, the representation of the stimulus also changes; there is no permanent trace of an object, nor even a fixed trace, but skill-developed and occasion-dependent representations.* (p. 383, italics added)

The position expressed here eventually would be known as proceduralism (Kolers and Roediger, 1984). The basic tenet of coding in proceduralism is that the code is the set of psychological processes engaged for perception and comprehension of an event. Levels of processing, in contrast, assumed that these processes produced a trace or code, which was stored in memory. Proceduralism adheres to a much stricter use of the skill metaphor, whereby there is no stored trace or code. After all, where is your typing skill when you are not typing or your adding skills when you are not adding? Rather than assuming a stored trace, the connection between the past and present in proceduralism is represented by the similarity of the psychological processes engaged by the present event to some operations engaged in the past. The more similarity between the two sets of processes, the greater will be the transfer. The metric of similarity includes the modality through which the events are experienced on the reasonable premise that the psychological processes of vision and audition, for example, are different. Thus, one can see that the code for a given event is quite particular and tightly bound by the processing context.

As counterintuitive as the idea is for our intuitions about memory, Kolers (e.g., 1974) offered programmatic evidence for the approach that was sufficiently persuasive to produce important progeny in cognitive psychology. Most notable of these in memory are Roediger's ideas about data-driven and conceptually driven processing and Jacoby's process dissociation theory, both of which take proceduralism's assumptions about coding as foundational.

2.07.4.3.1 Data-driven and conceptually driven processing

The distinction between data-driven and conceptually driven processing emerged as an explanation for dissociations in performance on different types of memory tests. Roediger et al. (1989) argued that these dissociations reflect differences in the processing demands of differences between study/test conditions. The basic assumption is that a particular type of prior processing may be more effective for one type of test than for another. An important example for the development of the data-driven/conceptually driven distinction is the previously discussed research of Jacoby (1983). As a brief reminder, Jacoby asked people to study words under different conditions. In one condition, the words were read without any context. In another condition, the words were generated by the subjects in the context of semantic clues. On a later recognition test, the people who generated the items at study performed better than those who read them – the standard generation effect. However, if the test was to identify visually degraded words, previous reading of the word led to better identification than did previous generation.

Roediger et al. (1989) used Jacoby's (1983) work as the basis for distinguishing the coding of meaning (conceptually driven processing) and the coding of perceptual features (data-driven processing). The dichotomy between semantic and sensory-based codes is not new to our discussion, having been an important component of the stage model, the memory systems, and levels of processing. What is different is that Roediger et al. couched the distinction in processing language. The code for meaning is the psychological processes engaged to analyze the event, and the effect of this prior processing will be revealed only in future circumstances demanding similar analysis of the event. The code created by generating the word 'cat' will not facilitate future demands to read the word 'cat'. In that sense, one can appreciate Kolers' previous quotation to the effect that as the operations change, the representation of the event changes.

The distinction between data-driven and conceptually driven coding has been a powerful stimulant for research and has served an impressive role in classification and organization of memory tests (e.g., Blaxton, 1989; Rajaram and Roediger, 1993). Situations have arisen, however, that resist clean dichotomizing into data-driven and conceptually driven processing. A very simple example is the standard recognition memory test, in which one must process the test item perceptually before a decision concerning its status can be reached. On the face of it, this situation seems to require both

data-driven and conceptually driven processing. The authors of the idea recognized this implication early on: "Tests may involve both types of processes. Indeed, a more useful assumption is to describe two continua, one for each type of processing, to acknowledge that these two modes of processing can be varied orthogonally" (Srinivas and Roediger, 1990, p. 390). The interesting point for our discussion of coding is the realization that characterizations of the trace for an event as exclusively perceptual or conceptual, the very assumption that caused trouble for the stage model, are too simplistic. Advocates of the data-driven/conceptually driven view avoid that mistake by assuming that the code will include both types of information in most all cases.

2.07.4.3.2 Process dissociation theory

Another descendant of Kolerian proceduralism is the process dissociation framework (Jacoby, 1991). Like the memory systems and data-driven/conceptually driven ideas, Jacoby's theory was motivated largely by the challenge of understanding test dissociations. Unlike the other approaches, process dissociation explicitly disavows any effort to identify processes, codes, or systems on the basis of the type of task. Both the memory systems and the data-driven/conceptually driven schemes use the task confronting the subject to identify the type of code that will be required by that task. As research began to discover violations of the prescribed system- or process-task relationship, both the systems and the data-driven/conceptually driven approaches moved to a middle ground: the code for any given event is likely to be mixed. The same data suggested to Jacoby (1991) that these approaches cannot succeed in explaining memory phenomena. The reason is that the explanatory (predictive) power of either approach rests heavily on the ability to identify the theory-specified code representing an event. The primary means for doing so is to assume a code-task purity (i.e., a particular kind of task will recruit a particular kind of code). At best, the inability to identify task-pure codes robs these approaches of some of their precision.

Jacoby's alternative is to specify the nature of the processes as *a priori* rather than identify the operative processes on the basis of task performance. The details of the theory accomplishing this specification are beyond the purview of this chapter, but the assumption about the nature of the code is pure Kolerian proceduralism. Psychological processes are brought to bear on tasks with which we are confronted. These processes vary not only with task demands but also with both external and internal contexts, intent being an important component of the later. In this view, precisely the same process (a.k.a., code) is unlikely to be repeated, a position identical to that expressed in the italicized portion of the quotation from Kolers listed earlier. Consequently, every memory has a unique code in that no two situations engage identical psychological processes.

2.07.4.4 Summary of Process Metaphor

One can conceptualize cognitive activities including learning and memory as analogous to motor skills. Just as particular motor tasks require particular motor processes, so do particular cognitive tasks engage particular processes. In both cases, performance on a task is determined by prior processing as it is related to the task. That is, the effect of prior processing can be either positive or negative. For example, my racquetball game improves with racquetball practice, but my squash game deteriorates as I practice racquetball. The same is true of cognitive tasks. Although it may be that the structural metaphor can encompass all of the memory phenomena marshaled by the process camp, the differences in the metaphors are important influences on research. Three implications of the process metaphor are quite different from anything derived from structural thinking.

Perhaps the least intuitive of these implications is that the influence of the past is not carried by a permanently existing code stored in the system. If learning and memory are thought of as processes, then like typing, or for that matter digestion, memory is nowhere when you are not remembering. Within the process metaphor, the function of the memory code is assigned to transient psychological processes, and research focused on the overlap of processes from one task to another is essentially the process approach to studying the code. However, the vast amount of research sponsored by the structural metaphor aimed at describing memory systems would never occur under the auspices of a process metaphor.

A second and only slightly less counterintuitive implication of proceduralism is that abstract codes of the kind traditionally associated with knowledge do not exist. Take, for example, the following information: George Washington was the first president of the United States. An abstract code for this

information would be stripped of any contextually specific aspects of prior experience with the information, such as when, where, or through what modality the experience occurred. If, however, we adopt a proceduralist's definition (i.e., the code representing an experience is the set of operations that yielded that experience), no representation would be free of context-specific content. The operations producing the experience include those sensory–perceptual processes associated with the modality of processing, and in most cases, it is reasonable to assume that other aspects of the context would influence the encoding operations. The implied lack of an abstract code poses a challenge for proceduralist's accounts of learning and use of concepts, where a concept traditionally is assumed to be distilled from, but not identical to, any particular prior experience. The difference between the abstractionist's and the proceduralist's positions is the venerable difference between rational and empirical knowledge, and it is exciting to see empirical work emerging on this important epistemological issue (e.g., Whittlesea et al., 1994; Heit and Barsalou, 1996; Hannah and Brooks, 2006).

A third implication of the process metaphor extends beyond the issue of coding and is of general importance to conceptualization of cognition. In two different senses, proceduralism is integrative, whereas structuralism leads to modularity. The first sense of integration is that proceduralism need not distinguish various cognitive processes (e.g., perception, memory, reasoning) except on operational grounds for clarity of communication. The aforementioned principles of proceduralism apply regardless of the operational classification of the process, rendering distinctions between such concepts as memory and reasoning unnecessary. The second dimension along which proceduralism is integrative is mind–body dualism. Crowder made this important point by noting that many theories explicitly distinguish between perception and cognition, which he suggests is dualism in a different guise because:

> perceptual skills are considered more legitimately bodily processes than the "mental" cognitive functions such as generation, reflection, and creativity. For example, Tulving and Schacter relegate priming to the activity of the perceptual representation system, as distinct from the episodic memory system. (Crowder, 1993, p. 143)

Taking skill as a metaphor for all cognitive processing, perceptual as well as conceptual, removes any boundary between body functions and mental functions.

2.07.5 Summary of Coding Processes

An enormous amount of research has been directed toward understanding coding processes and their resultant memory representations. The reason for the effort is that the memory code is viewed as the concept that carries the effect of prior experience into the present. In that capacity, the memory code will determine the similarity metric among prior events and between prior and current events. This similarity will determine the types of events that will interfere with each other as well as the effectiveness of certain kinds of cues in the retrieval of particular memories.

All the methods that have been used to infer the memory code are of necessity indirect, based on observations of behavior or brain activity, but the descriptions of the codes inferred from these methods point to three main ways in which the coding process operates. Codes can be a select portion of a complex event, in which case the representation is the portion of the event selected for attention. Codes can be a transformation in the form of the input such as the verbal coding of a picture or the organization of discrete units into a whole. Elaboration is a third form of coding, which yields a representation that contains more than was in the literal physical energy of the original experience. The code resulting from elaborative processing reflects the influence of prior knowledge on perception and comprehension of an event.

Placed in proper context, research on memory coding is an indication of the value of psychology in advancing our knowledge about fundamental questions of mental functioning. This chapter began with the assertion that the problem of knowledge, what can people know and how do they come to know it, is the impetus for coding research. The research has yielded a range of descriptions of particular types of codes that are the fodder for systematic theoretical classification. With that theoretical classification lays the promise of resolution to the perennial issues of the nature of knowledge, issues such as that between abstract, universal knowledge versus particular, contextually bound knowledge.

The resolution has not been achieved, but it is exciting to know that research in psychology has such ambitious aims.

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2.08 Mental Imagery

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2.08.1 Introduction to Imagery and Definitions of Mental Imagery

In our everyday life, during interactions with people and with the environment, a crucial role is fulfilled by the ability to maintain and to recall images (i.e., mental representations including perceptual information). Most of the time, mental images are incidentally activated, such as, for example, when we think back to people's faces or certain episodes of our life. Other times, images are easily retrieved to answer particular questions; for example, people can recall and visualize in their mind how many windows there are in their house and the color of the curtains.

Mental images have always fascinated philosophers; in fact, Greek philosophers such as Plato and Aristotle discussed mental images, the latter considering imagery crucial in both cognition and thinking. Aristotle's theory could be considered the antecedent of the modern analogical imagery view, which maintains a close relationship between perception and imagery (e.g., Shepard and Metzler, 1971; Kosslyn, 1980, 1994). In fact, according to Aristotle, a mental image is an inner representation of real objects, like a copy of real-life scenes. Moreover, medieval thinkers like Augustine and Aquinas gave a central position to imagery in their interpretation of psychological processing from sensation to imagination and memory. Similarly, the British empiricists, for example, Berkeley, Hobbes, and Hume, considered mental images as traces of sensory information. As for more recent times, it is only from the 1970s that psychology reconsidered imagery as a central topic of study (i.e., ever since the advent of cognitive psychology). In fact, behaviorists had failed to consider imagery as a serious topic for experimental investigation because it was not directly observable and thus could not be investigated with a completely objective methodology. The cognitive sciences represented a new era for research on imagery processes. Interest toward mental events was once again a central topic in psychology, and different experimental paradigms were applied to the investigation of higher cognitive processes, such as imagery. Individual reports and subjective introspective experiences were considered objects of interest for experimental psychology, and imagery was included as a legitimate topic of investigation.

In the psychological literature, various definitions and theories regarding mental images were put forward, and most of them were influenced by the interdisciplinary interests surrounding this process. According to Holt (1964), a mental image refers to all the subjective awareness experiences within a

sensory modality, which are not only perceptual. Intons-Peterson and McDaniel (1991) affirmed that mental images might be produced by the interaction between visual representation and a subject's knowledge, suggesting that they are knowledge-based products. Carroll (1993, p. 277) defined imagery as "the ability in forming internal mental representations of visual patterns and in using such representations in solving spatial problems" and proposed a relationship between spatial abilities, visual perception, and imagery. Similarly, Richardson (1999) postulated that mental images are complex mental products, inner representations where information on the actual perceptual appearance of objects can be described and transformed.

Mental imagery is a private and a subjective experience because we cannot observe whether other people have a mental image nor directly know its properties. This implies that its scientific investigation depends on verbal reports and on the phenomenal experience of participants (Richardson, 1980). However, psychological investigation has tried to operationalize or give construct validity to the concept of mental image. For example, according to Paivio (1971), a mental image is defined on the basis of three kinds of operations, that is, (a) by variations in stimulus properties (e.g., high vs. low imagery value), (b) by processing instructions (e.g., instructions to imagine vs. instruction to verbalize), and (c) by individual differences in imagery ability. According to Kosslyn (1980, 1994), a mental image is not simply a phenomenal experience, but a form of internal representation in which information about the visual appearance of a physical object can be manipulated: Visual mental images correspond to short-term memory displays, which are generated from more abstract representations in long-term memory (Kosslyn, 1980; Denis and Kosslyn, 1999). However, the methodological difficulties in the study of imagery have affected a recursive debate between imagery theorists (who support an analogical or pictorial position) and propositionalists (who maintain the existence of an amodal representation, e.g., Pylyshyn, 1973).

2.08.1.1 Debate on the Nature of Representations

The contrast between empirical and rational theories on the acquisition and on the representation of knowledge is recurrent in the history of thinking (e.g., Aristotle against Plato, British Empiricists against Descartes in philosophy and propositionalists against imagery theories in psychology). To summarize, the propositionalists affirm that mental images are epiphenomena, having a symbolic-like format, with no sensorial properties and an explicit explanation of the relations between elements (Pylyshyn, 1973, 1981). According to propositionalists, the representations that underlie the experience of mental imagery are similar to those used in language. The second position, upheld by imagery theorists like Kosslyn (1980, 1994), holds that mental imagery representations are able to depict, not describe, objects; they are analogical representations of objects in our mind and correspond to a quasi-perceptual experience, with a specific modality format.

The initial debate focused on behavioral results such as those obtained by Kosslyn et al. (1978). In the original study, participants were required to memorize a map of an island, where a series of landmarks was drawn, then to imagine the map and to pay attention to one place (e.g., the beach). When the experimenter gave the name of a second place (e.g., the tower) participants had to imagine moving from one place to the other and to press a button as soon as they had reached the second place in their mental image of the map. The results of the study revealed that the further away the second place was from the initial place (the beach), the longer it took for participants to give the response. The conclusion of the authors was that mental images have spatial properties.

The critiques made by Pylyshyn (2002) were as follows: It is not clear whether the results revealed a property of the cognitive architecture or a property of what people know or believe about imagery functioning. Pylyshyn (1981) repeated the experiment by showing participants a map with lights going on and off at the target locations; participants were required to imagine when a light was on and to press a button when they could see it at a second place. Results did not reveal correlations between the distance on the imagined map and reaction times. Similarly, Cornoldi et al. (1996a) found that the distance effect is in relationship to the theories people have about imagery functioning. However, differently from Pylyshyn (2002), they concluded that naïve theories do not simply affect responses but also affect imagery retrieval processes (for a complete debate on these paradigms, see Denis and Kosslyn, 1999).

The debate moved into a new phase when neuroimaging began to be used to study brain activation during mental imagery (see for reviews, Cabeza and Nyberg, 2000; Kosslyn et al., 2001). During mental imagery, some functional magnetic resonance

imaging (fMRI) studies showed an activation of topographically mapped brain areas depicting shapes (see Thompson and Kosslyn, 2000). Furthermore, if these areas are damaged, visual imagery is impaired (Kosslyn et al., 1999). A crucial result to reach the conclusion that imagery involves quasi-perceptual experiences would be if the same visual areas were activated when an object is perceived and when it is imagined. Despite a great number of studies carried out to investigate the involvement of primary visual cortex in imagery, the issue is still open, because results are controversial. In a series of positron emission tomography (PET) experiments, Kosslyn and colleagues found an increased blood flow in Brodmann Area 17 (primary visual cortex) during imagery activity (Kosslyn et al., 1993, 1997); however, other studies with fMRI methods suggest that primary visual cortex is not activated during mental imagery (e.g., D'Esposito, 1997; for a review, see Cabeza and Nyberg, 2000).

In a recent research, Kosslyn and coworkers (Slotnick et al., 2005) tried to disambiguate the long-standing debate on the nature of mental imagery representations and found evidence supporting the depictive view of visual mental imagery. In fact, the authors' contrasting imagery and attention retinotopic maps showed that visual mental imagery can evoke topographically organized activity in striate and extra-striate cortex, in accordance with the stringent criterion required for supporting a depictive theory, as mentioned by Pylyshyn (2002).

Alongside the two opposite positions (propositionalists vs. imagery theorists), intermediate models have been proposed. For example, mental images have been considered as being generated from long-term memory information, thus representing an intermediate format between amodal abstract representations and perceptions. The information in long-term memory would be represented in a more abstract format, whereas conscious mental images would acquire a more sensorial format (Marschark and Cornoldi, 1990). In fact, it is possible that short-term visual memories maintain perceptual detail, but during the integration of information in long-term memory, they lose part of their sensorial properties (Cornoldi et al., 1998).

2.08.1.2 Perceptual and Conceptual Representations: Visual Traces and Generated Images

Cornoldi et al. (1998) proposed a distinction between a visual trace, sharing a large number of characteristics with perception, and a generated image, more dependent on conceptual processes but still distinguishable from an amodal representation. In fact, not only the psychological literature but also subjective experience supports a differentiation between a visual memory based on a recent perceptual experience and a generated mental image. In synthesis, a visual trace is directly received from perception while it happens. For example, when we perceive a shape, we try to maintain it in our mind. In contrast, a generated image is derived from long-term memory information as, for example, when we try to activate an image of our previous car. If the first requires a low degree of attentional control to be kept activated, the latter requires a higher degree of control; moreover, the analogy with perception would be almost complete for a visual trace and only partial for a generated image, and a visual trace would be characterized by sensorial and phenomenic properties and a generated image by perceptual–conceptual properties. It must be noted that a visual trace is also different from a perceptual experience and that, even after short time intervals, a visual experience can be affected by long-term memory reconstructive processes and thus approximate the features of a generated image. The well-known experience of the inability to remember well-known patterns (e.g., the shape and colors of a common banknote, see the example of a 20-euro piece in **Figure 1**) is common to mental images but also concerns recent visual traces.

Figure 1 People are not able to accurately remember the visual appearance of objects, even if they are frequently exposed to them.

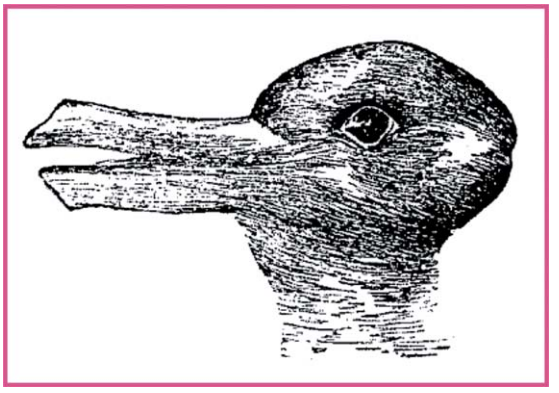

Figure 2 The reversal of a mental image is particularly difficult but can be facilitated by the prevention of verbalization.

The distinction between a visual trace and a generated image can be also used to explain the phenomenon of verbal overshadowing, or the difficulties people meet in the reversal of mental images (Cornoldi et al., 1996b). According to Brandimonte and colleagues (Brandimonte et al., 1992a; Brandimonte and Gerbino, 1993), verbal recoding of visual stimuli occurs almost automatically when pictures are easily nameable; the verbal recoding uses long-term memory knowledge and influences the image of an ambiguous visual stimulus, determining the verbal overshadowing effect. However, if verbalization is prevented, the visual trace still maintains its perceptual properties. If you look at the picture presented in **Figure 2** and then close your eyes, you will explore the experience of a well-established image that cannot be easily reverted. If, for example, a person has in mind the image of a rabbit, that person will have difficulty visualizing a duck, and vice versa.

2.08.2 Different Kinds of Mental Images

Given the fact that mental images are extensively present in human life and respond to different functions, it is not surprising that they appear heterogeneous and may be differentiated. The literature on imagery has proposed a series of taxonomies among different kinds of imagery representations. Richardson (1969), for example, proposed a classification of mental images considering their different properties along the following dimensions: conscious control, phenomenological quality, intentional content, spatial location, and mode. For example, memory images were defined as conscious control phenomena with a quasi-perceptual quality, content related to experience without spatial location and with a potentially amodal format. It is noteworthy that at a higher cognitive level, a distinction can be made between mental images derived from memory and images derived from the imagination. The first refers to all images that we could evoke from memory, such as the image of the Eiffel Tower in Paris. Memory images could differ in vividness, clarity, details, colors, or multisensorial properties. Furthermore, they can be distinguished from fantasy images, created by combining elements stored in memory in new ways, as, for example, in the image of a flying horse. In fact, the creation of integrated images obtained by combining together single images represents a typical mental imagery situation, derived from the classical mnemonic tradition showing that interactive images facilitate the retrieval of an element if the other element is available. **Figure 3** presents an example of interactive image created with the support of fantasy. If the image of a rainbow and the image of a guitar must be integrated in a single interactive image, one could, for example, imagine a natural-looking guitar and a rainbow illuminating it.

The distinction between images from memory and those from the imagination is not rigid because images from memory are not exact reproductions of the equivalent perceptual experiences, whereas fantasy images are created on the basis of features derived from visual experiences. A related distinction refers to common and bizarre images. The first type represents objects, as we know them in the real world; in contrast, the latter are impossible and strange

Figure 3 People trying to include in a single interactive image the images of a guitar and a rainbow could use fantasy to create an effective original representation.

representations of an object. Examples of bizarre images could be a dog smoking a cigar and a man chewing a bone, whereas the corresponding more common image would be a man smoking a cigar and a dog chewing a bone. This distinction has memory implications and has been the basis of mnemonic art. Cornoldi et al. (1988) found that bizarre representations improved memory recall if subjects could evoke the kind of image they wanted. Further studies (Einstein et al., 1989) reported that recall can be influenced by the distinctiveness of materials. They found that in a list of words where all the stimuli were imagined in the bizarre modality, recall was no better than when using common images. When the bizarre and common mental images were generated alternatively in the same list of words, however, the bizarre images produced a better memory performance, indicating that bizarreness *per se* does not produce superiority in memory but needs to be accompanied by another factor, such as distinctiveness.

Other classifications are more focused on the spatial properties of layouts. For example, Kosslyn (1987) distinguished between exact metric coordinates and memory for the relative relation between objects (i.e., coordinate vs. categorical representations; see also Lansdale, 1998). Studies regarding the specialized involvement of different brain structures show that the right hemisphere is important in processing metric spatial information, whereas the left hemisphere participates in processing relative spatial relations (Kosslyn et al., 1989). Other classifications of spatial images refer to the type of processing (sequential vs. simultaneous), to the reference center (egocentric vs. allocentric), and to personal space (peripersonal vs. extrapersonal).

2.08.2.1 General, Specific, Contextual, and Episodic-Autobiographical Images

Can mental images also represent general concepts? The existence of general and prototypical images is an ancient topic of debate. We know that the ability to create a universal or prototypical representation from the analysis of particular cases is a fundamental process in knowledge and conceptual development, but we do not know whether this representation can assume an imaginal format. Radical empiricists (Locke, Hume, Berkeley) denied the existence of general images by arguing that mental images are always based on the representations of specific objects that have been experienced, whereas a general term evokes an abstract idea. From this position two different interpretations developed: one affirming that general images representing the essential properties characterizing the general term (such as a prototypical representation) can be generated, and the other suggesting that general terms are either abstract or refer to an exemplar representation. The distinction between general, specific, contextual, and episodic-autobiographic mental images embraces the first option. The distinction has received support in recent years by cognitive (Cornoldi et al., 1989; De Beni and Pazzaglia, 1995; Helstrup et al., 1997) and neuro-anatomical data (Gardini et al., 2005). An example of a general image may consist of a skeletal representation of the main features of a bird including both coordinate and categorical spatial information. A specific image of a bird should be a particular exemplar of the category (e.g., a canary); moreover, a contextual image refers to an image in which the object or the exemplar is inserted within a context, for example, the canary in the cage. Finally, episodic-autobiographical images correspond with images of single life-episodes connected with an object and having a specific self-reference, for example, my canary escaping from its cage during a sunny day of May. Episodic-autobiographical images involve the retrieval of available episodic traces resulting from autobiographical events. The retrieval of autobiographical memories, even in the absence of specific instructions, seems strictly associated with the activation of mental images (Brewer, 1988).

Cornoldi et al. (1989) investigated the relationship between the generation of different kinds of mental images and memory recall performance. By comparing memory performance for general, specific, and autobiographical mental images, they found that the recall of general and specific mental images did not differ, but autobiographical mental images produced a better recall performance with respect to general and specific ones. De Beni and Pazzaglia (1995) distinguished between self-referred mental images within the personal autobiographical context, in which an individual imagines himself or herself together with the object without a precise episodic reference, and episodic-autobiographical images, representing a specific episode of the subject's life in relation to the object. Episodic-autobiographical mental images increased memory performance with respect to the contextual images but required longer generation times compared with other image categories. This finding could be explained by the fact that the generation of this kind of image requires the

previous generation of a general image. It could also be explained by the fact that the search of a particular episode related to the object must take place, or it might be a result of the richness of details of this kind of image.

According to Cornoldi and coworkers (Cornoldi et al., 1989; De Beni and Pazzaglia, 1995) (see **Figure 4**), the mental image generation would start with the retrieval of a global shape information of the object (which can be used to generate a general image) and be subsequently enriched with details, for example, those of a particular exemplar belonging to the category of an object, or contextual information when an object is imagined in a particular context or with reference to characteristics of a familiar type of an object, thus creating the conditions for generating, respectively, a specific and a contextual image. In contrast, the generation of episodic-autobiographical mental images would undergo a different generation process directly involving the retrieval of the image from the episodic-autobiographical memory store.

In an fMRI study, Gardini et al. (2005) provided anatomical support for the results repeatedly observed in cognitive studies. The researchers, in fact, investigated the neural correlates of general and specific mental images from concrete nouns. Results showed different brain activities for both types of images; in particular, general images activated right frontal areas to a greater extent than specific images, whereas specific ones mainly activated the left-superior frontal region and the right thalamus, demonstrating that general images involve brain areas associated with the generation of global images differently from the specific images, requiring additional support from areas in charge of retrieving visual details.

2.08.3 Models of Mental Imagery and Memory

Most of the authors studying imagery agree with the idea that not only do the generation and the recall of mental images involve memory systems, but also their maintenance and transformation require temporary systems or subsystems of memory devoted to the treatment of visual and spatial information.

In the following paragraphs, we focus on three different approaches to the relationship between mental imagery and memory. We describe the models of Paivio and Kosslyn, and we discuss the potential role played by the visuospatial working memory system in maintaining and elaborating visual and spatial representations.

2.08.3.1 Paivio's Dual-Code Theory

To explain the effectiveness of imageability in predicting memory performance, Paivio (1971) proposed two different categories of processes that people can use when they encode information: images and verbal processes. He proposed studying mental images by observing the influence of imagery on memory performance, starting with a series of studies on nouns

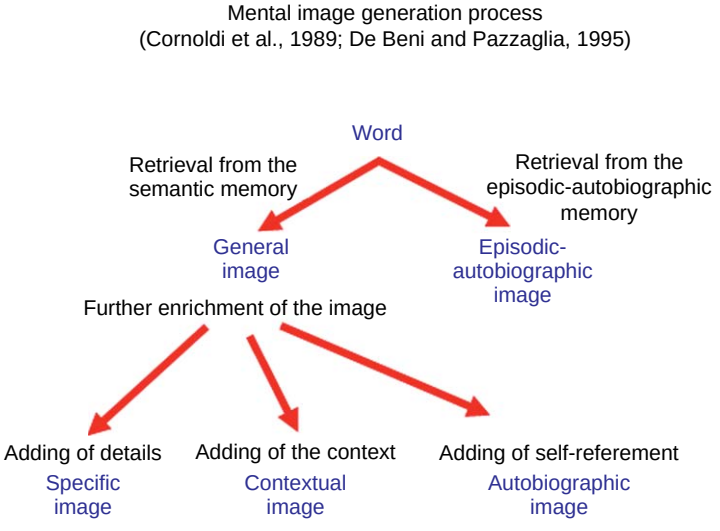

Figure 4 The generation process of mental images (Cornoldi et al., 1989; De Beni and Pazzaglia, 1995).

and paired-associates recall. Paivio et al. (1968) examined the implications of different indexes of verbal stimuli such as meaningfulness (i.e., the number of verbal associations that a word would elicit in a given period of time), concreteness, the extent to which words refer to a tangible object, imagery value (which refers to the ease or difficulty with which words prime a mental picture), and familiarity for memory performance. Results obtained by Paivio et al. (1968) demonstrated that imagery value and concreteness were strongly related to recall, and meaningfulness and familiarity did not produce an effect on recall when the imagery value was controlled for. A particular result, concerning paired-associate recall, was that concrete-abstract pairs were recalled better than abstract-concrete pairs. The result was interpreted on the basis of the assumption that a prior image can represent an appropriate imaginal tag for subsequent information.

Paivio (1971) proposed a dual-coding theory of mental processing (see a schematic representation of the theory in **Figure 5**). The theory assumes that cognitive behavior is mediated by two independent systems specialized for encoding, transforming, storing, and retrieving information. The verbal system is responsible for the encoding and processing of verbal material and the nonverbal for encoding nonverbal input, such as images. The kind of encoding that a stimulus undergoes depends on three types of variables, that is, the nature of the material (high vs. low imagery value), the instructions given to the participants, and their imagery abilities. Paivio's model (1971, 1978) identifies three levels at which information might be processed. The first is the representational level, where the sensory trace activates the appropriate symbolic representation in long-term memory: The logogens are the basic units in long-term memory for verbal stimuli, whereas the imagens represent the basic units for imagery material. The second is the referential level, where symbolic representations in one system activate corresponding representations in the other system. These interconnections are assumed to be involved in naming objects or in creating the image of an object. In particular, referential connections between the two processes are activated when one kind of information (verbal or imagined) activates the other system. In this case there is double encoding. According to the theory, nouns with high imagery value are subjected to double encoding. "The increased availability of both codes increases the probability of item recall because the response can be retrieved from either code" (Paivio, 1971, p. 208). Finally, the associative level involves associative connections among both verbal representations and images.

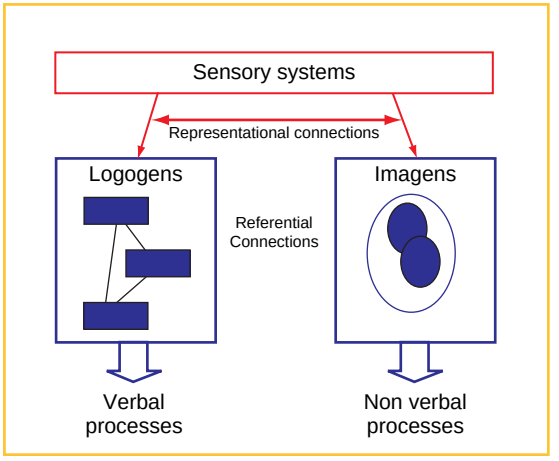

Figure 5 A graphic representation of Paivio's (1971) Dual-Codes Theory.

2.08.3.2 Kosslyn's Visual Buffer

Kosslyn (1980, 1994) proposed a computational model of image generation and provided a description of the functional structures supporting this process. His model is valid for both imagery and high-level visual perception, considered to share structures, functions, and properties. Imagery is a multicomponential process, involving a series of different processes, like image generation, maintenance, inspection, and transformation. Mental generation involves the activation of an image using long-term memory information, inspection of the different parts of an image, and arrangement of the details of an image (Kosslyn et al., 1992, 1995). Mental images do not correspond to simple visual memories but are the result of a multicomponential process that assembles the different parts together and generates a new representation.

Kosslyn's model (1980) was created based on an analogy with computer graphics. Computer graphic files store information in a compressed and nonpictorial form; when they are displayed, they are translated into a mathematical map (bitmap), which specifies the color of each pixel (tiny dot) on the screen. Kosslyn suggested the involvement of two kinds of deep representations: image files containing information regarding the perceptual characteristics

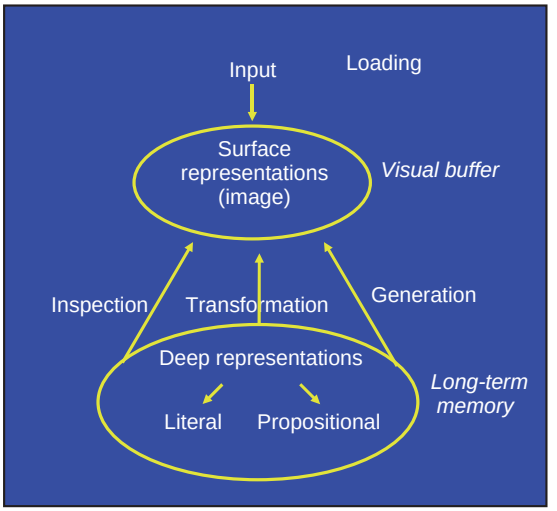

Figure 6 Schematic representation of the interaction between long-term memory and visual buffer (Kosslyn, 1980).

of the skeletal image of objects, and propositional files containing abstract descriptions of objects and their parts expressed in a propositional format (see a schematic representation of the model in **Figure 6**).

Mental imagery activity can be distinguished in a series of processes. For example, the PUT process arranges parts into an image, the FIND guides the top-down search in associative memory, and the PICTURE is responsible for the activation of stored visual representations. However, a different set of processes is hypothesized to be involved in the inspection and transformation of images: REGENERATE refreshes and sharpens the existing images, LOOK FOR searches for a named part within the existing images, SCAN repositions the image by means of a linear transformation, ZOOM increases the resolution of the image, and so on. A critical component of Kosslyn's model is the Visual Buffer, which is considered to be a short-term memory system with spatial properties (x, y, z coordinates, adjacent cells, etc.) located in the occipital lobe. In fact, the images are generated in the visual buffer either on the basis of information loaded by perception or on the basis of information stored in long-term visual memory (see **Figure 7**). In 1994, Kosslyn revised the model in his book *Image and Brain*, addressing the importance of seven basic components:

- The visual buffer, which holds spatially organized patterns of activation;
- The attention window, which focuses on the information in the visual buffer selected for further processing;

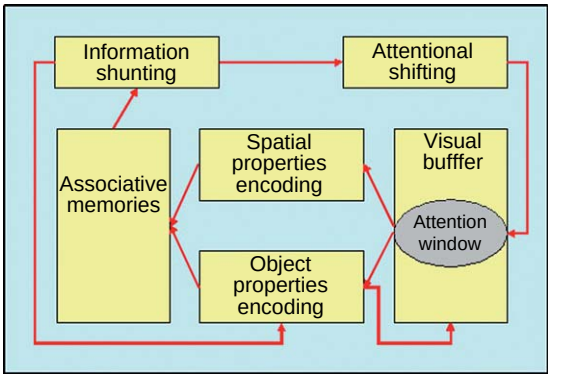

Figure 7 Processes interacting with the visual buffer (Kosslyn, 1994).

- The object properties encoding system, analyzing the physical properties of an object;
- The spatial properties encoding system, which analyses the spatial location of an object;
- The associative memory, containing information about the physical and conceptual properties of the objects;
- The information shunting, which uses stored information to collect further information about an object with top-down processes; and
- The attention shifting mechanism, which shifts the focus of attention from a specific location to a different one.

2.08.3.3 The Visuospatial Working Memory Approach

A partially different approach to imagery was adopted by other researchers, based on the idea that there is a memory system involved during the generation, maintenance, and manipulation of visual information and mental images. The visuospatial working memory (VSWM) approach (Baddeley, 1986; Logie, 1995) to imagery shares many characteristics with the visual buffer system postulated by Kosslyn (1994). However, experimental studies and research paradigms within this framework have followed different directions. The first studies with implications for the link between visual imagery and VSWM were carried out by Brooks in the late 1960s. In particular, in a 1968 study, participants were required to visualize a block capital letter and, following its contour, to answer 'yes' if the corner they mentally followed was on the bottom or the top of the figure and 'no' if it was inside the figure. Moreover, participants could

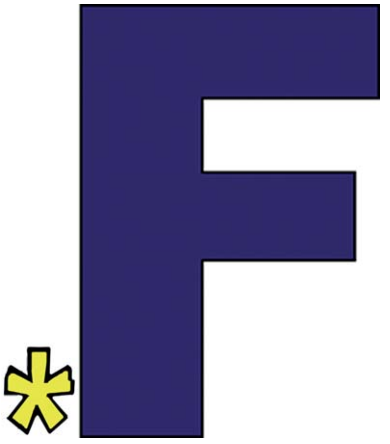

Figure 8 An example of a mental imagery scanning task used by Brooks (1968): People are invited to mentally move along the contour of a capital letter and decide about the properties of its corners.

respond either vocally or by pointing to the letters Y and N on a sheet (see an example in **Figure 8**).

Starting from the bottom left-hand corner (indicated by the yellow asterisk) and going in the direction of the arrow, the correct responses would be: yes-yes-yes-no-no- and so on. Brooks (1968) observed that participants performed worse when responding by pointing to the letters on a sheet than when giving a verbal response, whereas the opposite happened when they had to scan a sentence rather than an image. Brooks concluded that the visualization of a block capital letter involves different cognitive resources than those used to point to printed words on a sheet. These results were consistent with the view that there is a specialized cognitive system able to process visual inputs and to generate and retain images.

The original working memory model proposed by Baddeley and Hitch (1974; Baddeley, 1986) is a nonunitary system comprising three separate components. The central executive has attentional functions and coordinates the activities of the two slave systems, the phonological loop, which maintains and processes verbal information, and the visuospatial sketchpad, in which visual and spatial information are maintained and processed by two different, but complementary, visual and spatial subcomponents (Della Sala et al., 1999). Salway and Logie (1995) attempted to examine the role of working memory and of VSWM, in particular in imagery tasks, by using several kinds of interferences on a single main imagery task. Specifically, they analyzed the effect of random generation (usually interfering with the central executive component), articulatory suppression (interfering with the phonological loop), and spatial tapping (damaging VSWM) on the performance of the Brooks matrix and a verbal task (1968). Participants were asked, in one condition, to imagine placing consecutive numbers in consecutive squares of a visualized matrix; in the second condition, they had to retain a sequence of consecutive words, for example, 'good, bad, slow,' and so on, placing them in a set of nonsense sentences that were to be retained without the use of any form of visual image. Results showed that articulatory suppression damaged the second (verbal) condition, that spatial tapping disrupted the first (spatial) condition, and that random generation interfered with both tasks in an important way. In 1995, Logie suggested a revised model of the VSWM involving a passive visual store (i.e., the visual cache) and an active rehearsal mechanism (i.e., the inner scribe). The visual cache provides a temporary store for visual information (color and shape), whereas the inner scribe handles information about movement sequences and provides a mechanism by which visual information can be rehearsed in working memory.

Within the classic multicomponent model of working memory, there is a debate as to whether mental imagery, having a specific visuospatial format, involves a working memory component, which maintains the specific properties of the information (i.e., the visuospatial sketchpad) or whether it also requires central processes, such as those involved in the central executive system. In the modified view of VSWM (e.g., Logie, 1995), in fact, the generation of an image appears to be the prerogative of the central executive, whereas the retention of its visual and spatial properties may be the responsibility of a temporary store, such as the VSWM system.

On this basis, we could also wonder whether there is a potential overlap between the concepts of VSWM and Kosslyn's visual buffer. From Logie's point of view, if the central executive is responsible for imagery generation and manipulation, it would also host the visual buffer controlling the operations involved in imagery maintenance. Kosslyn's processes acting on the contents of the visual buffer such as GENERATE, ZOOM, SCAN, and so on, could be seen as procedures activated from long-term memory and also operated by the central executive. However, in this case, specific modal processes, in Kosslyn's model clearly attributed to a specialized system, would be included in the amodal activity of a Central System. Moreover, the visual buffer

would contain the visual properties of the image, the information about its location, and any semantic information associated with the image, but these operations would better match the operations of the visuospatial component of working memory.

Within this context, Bruyer and Scailquin (1998), using the dual-task paradigm, analyzed which working memory component is involved in the generation, maintenance, and transformation of mental images, demonstrating that both spatial tapping and random generation interfere with the generation of mental images, but only spatial tapping damages the maintenance of mental images, whereas random generation produces major interference on the transformation (i.e., rotation) of mental images. The difficulties in associating mental imagery with a specific working memory component could be overcome within a continuity model, which assumes that control can be involved at different degrees. In this framework, the VSWM processes may be distinguished according to the degree of controlled activity (see **Figure 9**); in particular, at a lower level, a simple recall of previously acquired information is required, whereas at the higher level, an elaboration of information to produce an output different from the originally presented stimulus is involved (Cornoldi and Vecchi, 2000, 2003). According to this view, a plausible specification of the imagery tasks along the continuum could involve maintenance, inspection, generation, selection, combination, and transformation, respectively ordered on the basis of the active control required (see Cornoldi and Vecchi, 2003).

Another crucial issue in VSWM concerns the nature of the components and of the type of representation maintained in memory. In fact, although there is converging evidence supporting the multicomponential nature of VSWM, there is no agreement on the number and identity of its components. A great number of neuroanatomical data, starting from the work of Ungerleider and Mishkin (1982), has supported the distinction between a spatial and a visual component, for example, by focusing on a where system or on a dorsal stream, processing spatial information, and on a what system or on a ventral stream, processing the features of perceived objects. Another fractionation in VSWM processing was suggested by Pickering et al. (2001), who distinguished between a static format of the generated representations (e.g., a matrix in which locations are presented simultaneously) and a dynamic format (e.g., a matrix in which locations are presented one at a time). A similar distinction was made also by Pazzaglia and Cornoldi (1999) between spatial-sequential and spatial-simultaneous processes: A spatial-sequential task requires recalling spatial positions presented in a sequential format, whereas in a spatial-simultaneous task, participants have to recall positions presented simultaneously. Pazzaglia and Cornoldi (1999) distinguished between these two spatial components and a visual one in which participants have to memorize objects with different shapes, colors, and textures.

2.08.4 Paradigms in the Study of Mental Imagery and Memory

Mental images are mental representations, which, just like thoughts, are not directly observable. As Richardson (1969) stated in the early days of the

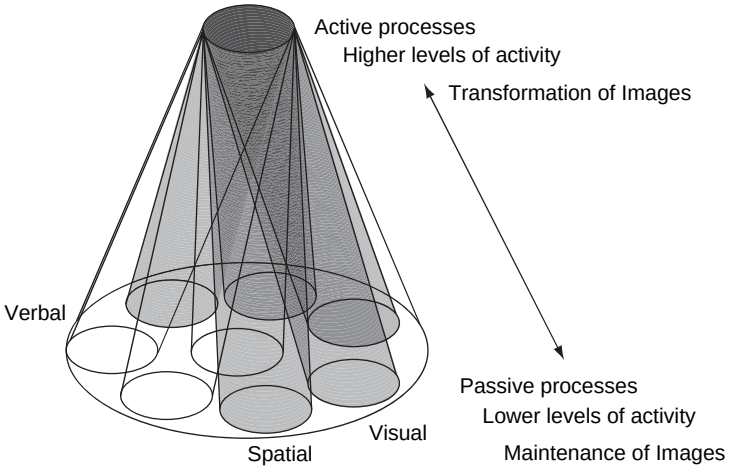

Figure 9 The continuity model of working memory: Mental imagery involves different portions of working memory differentiated according to the type of content and the degree of control (Cornoldi and Vecchi, 2003).

renaissance of research in this area, the problem regarding mental image investigation is to find a valid method to measure imagery. In fact, from the early days of cognitive psychology, this problem has been at the center of psychological questions concerning imagery. In the present state of research, different methodologies have been developed and applied to the imagery domain, offering the possibility of further investigating this phenomenon.

2.08.4.1 Cognitive Paradigms of Mental Imagery Processes

Within cognitive psychology, imagery has been investigated mainly in two different ways. The first examines mental imagery as a dependent measurable variable. It studies qualitative and subjective aspects of imagery and the extent to which mental images are similar to the physical objects that are being imagined. The latter concerns the use of mental images as independent variables (manipulated by researchers) in which observable aspects are reflected in behaviors, and especially in the performance obtained by participants (Richardson, 1999). In this paragraph we focus on the direct study of imagery processes, whereas in subsequent paragraphs, we examine the implications of two main manipulations of variables related to imagery (i.e. materials and abilities).

Studies directly focused on mental imagery representations and processes have used a large range of different paradigms. A few of them have been based on the examination of the subjective imagery experience, as, for example, when people are invited to rate the vividness, or other properties, of their mental images. For example Cornoldi et al. (1992) studied which characteristics influence a vividness judgment; Baddeley and Andrade (2000) examined which manipulation depresses mental imagery, inferred by decreases in the experience of vividness.

A problem with the use of subjective experience ratings is that they are affected by the criterion people use to decide whether or not their image is of a high quality. Since Galton's (1883) first started using vividness ratings to find people with different imagery abilities, the problem has been to decide to what extent vividness ratings describe differences in representations and to what extent they describe differences in criteria. For this reason, the majority of studies focusing on the properties of mental images have also used more objective criteria, such as time, effects of the experimental manipulations, verbal descriptions of the results of imagery processes, drawings, and external ratings (for a review, see Pearson et al., 2001). For example, in the mental pathway task, people are invited to imagine a matrix with a series of cells (e.g., a simple 5×5 board), imagine following a pathway on the board in correspondence with the verbal instructions given (go left, down, etc.), and point, on request, to the position reached at the end of the instructions on a corresponding blank matrix. This task has been particularly successful in studying the strengths and deficits of mental imagery processing in totally congenitally blind individuals (e.g., Cornoldi et al., 1991).

The chronometric study of mental imagery has produced the main experimental paradigms in the field. The most popular paradigm is surely represented by the so-called mental rotation tasks, derived from the traditional psychometric literature on spatial abilities testing, requiring a decision of whether two figures are differently oriented examples of a single figure, or if one of them is actually different (typically its mirror image) from the other. In a series of very influential studies, Shepard and coauthors (e.g., Shepard and Metzler, 1971) reconsidered the method and manipulated the angular variation between the two pictures (see examples in **Figure 10**). They found that the time necessary for giving a response was a function of the rotation angle, showing how the decision was not based on a consideration of the properties of the object (feature x is on the right of feature y, feature z is above feature y, etc.), which would not have been affected by the rotation angle but was based on a process of mentally rotating one figure to see whether it perfectly matched the other one.

There are many other examples of chronometric studies of mental imagery. For example, in the preceding paragraphs, we provided examples of image generation tasks, such as images generated from a verbal label, and mental scanning tasks (e.g., scanning an island or a capital letter). In fact, in the popular taxonomy of mental imagery processes, generation, scanning (and inspection), and transformation represent three classic cases. A fourth case is

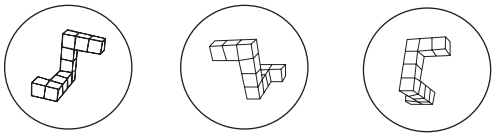

Figure 10 Examples of pictures used for a mental rotation task: People must decide as quickly as possible if the two figures are identical or mirror images.

imagery maintenance, which can be studied either with the classic paradigms of visual memory or with reference to subjective experience (people are invited to press a button when they realize their image is vanishing).

In the field of imagery transformation, the quality of the image can be directly derived from the verbal response of the individual. For example, in a mental subtraction task, people must generate an image from the visual exposure of a drawing and then a subsequent image from the visual exposure of a part of it; their task is to subtract the second image from the first and report the verbal label of the resulting image (see **Figure 11**). Brandimonte et al. (1992b) found that the performance in this task is facilitated by the block of verbalization.

Other studies focused on different types of mental synthesis, where participants are required not only to maintain and combine different images but also to produce a different image unrelated with the single primitive images. This particular case, given the novelty and the potential originality of the final result, has also been considered an example of creative imagery. Finke and his colleagues (Finke et al., 1989) asked subjects to carry out a series of transformations on imagined figures mediated by verbally presented instructions. The transformations were designed so that the final pattern would resemble a familiar object, as in the following example of the task: Imagine a B, now rotate the B 90 degrees to the left, then add a triangle below the rotated B, and finally report what the resulting pattern looks like (see **Figure 12**).

A different and more recent example of an imagery transformation task was suggested by Vecchi and Richardson (2000), who devised the jigsaw puzzle test, considered by the authors as an active VSWM task, because participants must hold in mind the arrangement of the puzzle pieces and actively mentally manipulate their combination. In fact, the task requires that the puzzle be solved by moving the pieces only mentally, without actually touching or moving the pieces in question (see an example in **Figure 13**). Drawings represent common, inanimate objects derived by Snodgrass and Vanderwart (1980), with a high value of familiarity and of image agreement. Each puzzle is numbered, and participants give their responses by writing down the corresponding number of each piece on a response sheet.

The study of imagery processes can also be based on a fractionation of complex processes into more simple ones and on the individuation of measures tapping the most simple components, as illustrated by a series of studies by Postma and coauthors. In an analysis of memory for locations, Postma and De Haan (1996) separated the object location memory into three processes: the first process requires encoding metric information and the coordinates of a particular object located in the environment; the second process, called object-location binding, requires linking the object's identity to its position; and finally, the last process integrates the first two mechanisms and combines metric information with object identity and location (Kessels et al., 2002a,b).

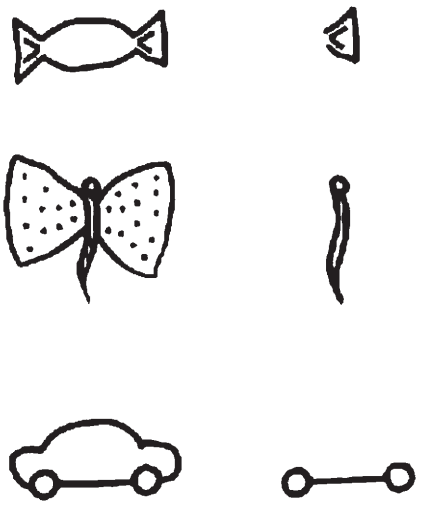

Figure 11 Examples of stimuli used for mental subtraction tasks: People, after exposure to the visual stimuli, must imagine the subtraction of the second part from the first and decide which is the resulting pattern (in this case a fish, a papillon, and a cloud).

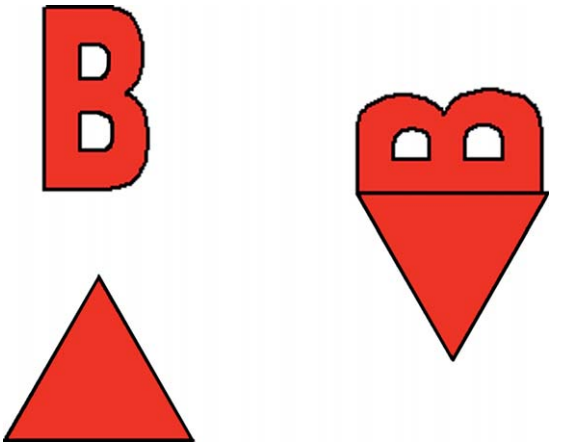

Figure 12 Example of a creative synthesis task.

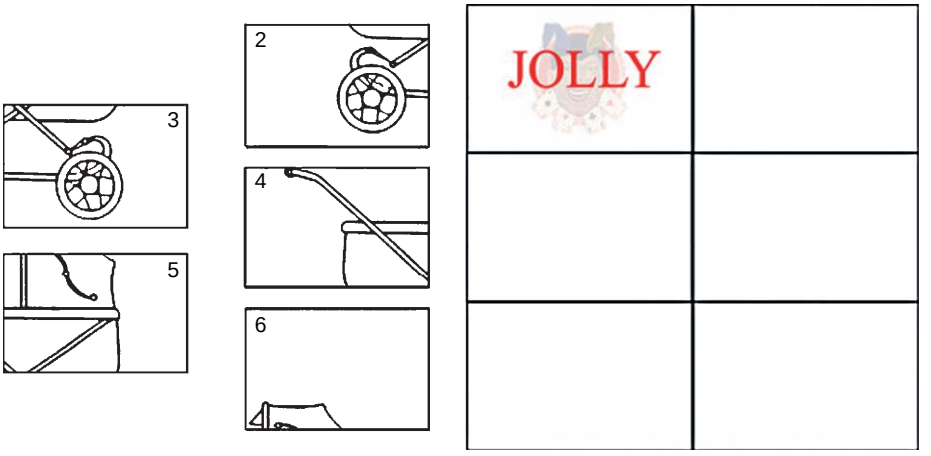

Figure 13 Example of an item derived from the jigsaw puzzle test (adapted from Vecchi and Richardson, 2000).

2.08.4.2 Neural Implications

Neuropsychological paradigms have also been useful in the study of visuospatial imagery. They offer data that have increased, in many respects, our knowledge of mental imagery processes and representations. In particular, the study of single cases has revealed important dissociations and relationships. For example, Bisiach and Luzzatti (1978) reported evidence that a unilateral neglect disorder, which usually causes patients to ignore the left half of the visual field, can also result in deficits in mental imagery. They offered the example of Milan's famous Piazza del Duomo: A patient with an unilateral neglect in front of the cathedral will typically ignore the left part of the square, but – in the case of Bisiach and Luzzatti's patient – the same experience happened in the area of a mental imagery representation. In fact, invited to imagine being in front of the cathedral, the patient was not able to describe its left part. This difficulty was not related to a preceding perceptual difficulty, nor to the particular characteristics of the square, because – when invited to imagine the square giving his back to the cathedral – his imagery block involved the other side of the square (i.e., the side accurately described when facing the cathedral).

Deficits can also be purely confined to visual imagery (Beschin et al., 1997). Farah et al. (1988) reported the case of a patient who had perceptual abilities and object recognition preserved. He was also able to copy drawings correctly and to draw objects from memory, but he could not correctly answer sentences requiring imagery for verification, for example, "An elephant is larger than a mouse." Goldenberg (1989, 1992) demonstrated that patients with left temporo-occipital lesions were unable to take advantage of imagery instructions in verbal learning tasks.

The study of severe neuropsychological patients has been integrated with the study of groups of individuals hypothesized, for different reasons, to have specific difficulties in visuospatial working memory or in mental imagery tasks (for a review, see Cornoldi and Vecchi, 2003). Examples of these groups are blind individuals (e.g., Cornoldi et al., 1991; Cornoldi and De Beni, 1988), children with nonverbal/visuospatial learning disabilities (Cornoldi et al., 1999; Cornoldi and Guglielmo, 2001; Mammarella and Cornoldi, 2005), elderly people (Vecchi and Cornoldi, 1999; Richardson and Vecchi, 2002), and individuals with specific genetic syndromes (Lanfranchi et al., 2004).

With the development of more sophisticated brain-mapping techniques, the study of imagery has taken further steps forward. In fact, from the pioneering work of Roland and Friberg (1985), who studied the variations of cerebral blood flow on the basis of the visualization of familiar routes, a great number of imagery studies have been carried out with neuroimaging techniques. Some of the questions research has tried to answer are: How do differences in brain activation inform us about the nature of different kinds of imagery? What kinds of brain areas are activated during generation, maintenance, and transformation of mental images? Does mental imagery share common cortical structures with perception, memory, or motor control?

Neuroimaging methods can be classified into two main groups: electromagnetic techniques, such as MEG (magneto-encephalography) and ERPs (event-related potentials), which have excellent temporal

resolution but poor spatial resolution, and techniques, such as PET and fMRI, which have good spatial resolution and coarse temporal resolution. It is noteworthy that although neuroimaging techniques can identify regions associated with a cognitive task, they cannot determine which of these regions are crucial for performing the task. For this reason, neuroimaging findings may be complemented with data provided by experimental and neuropsychological methods.

A central issue in the field of imagery is whether those visual areas involved when an object is perceived are also involved when an object is imagined. For example, Kosslyn's theory (1994) predicts activation during mental imagery activity in early visual processing regions (i.e., striate and extrastriate cortex). A series of experiments by Kosslyn and colleagues (Kosslyn et al., 1993, 2001; Kosslyn and Thompson, 2003) provided support for similarities between visual perception and visual imagery; other studies, however, have suggested that primary visual cortex is not activated during visual imagery (Roland and Gulyas, 1994; D'Esposito et al., 1997; see Cabeza and Nyberg, 2000 for a review). Roland and Friberg (1985) asked subjects to visualize the successive view along a route in a familiar environment. The task induced blood flow increase in the superior prefrontal cortex and, in particular, in the superior occipital cortex, the postero-inferior temporal cortex, and the postero-superior parietal cortex. These associative regions are also activated during processing of visual information. However, the authors did not find an activation of the primary visual cortex. According to Kosslyn et al. (1995), the right hemisphere is responsible for generating mental images from memory, whereas the left hemisphere is advantaged in the generation of visual mental images (see also Trojano and Grossi, 1994).

Charlot and coauthors (1992) selected high- and low-imagery participants and involved them in a task requiring the conjugation of abstract verbs (verbal task) and in a mental exploration of a memorized spatial configuration (imagery task). Their results revealed different patterns of activation for high (left-sensory motor cortex in the verbal task and left temporo-occipital cortex in the imagery task) and low imagers, who showed less differentiated increase of their cerebral activity. In Mellet et al.'s (1995) study, participants were required to inspect and memorize a map of an island with six landmarks. The cerebral blood flow was recorded as the participants performed either perceptual (the subjects were shown the map and asked to scan from landmark to landmark) or imaginal scanning of the map (performing a mental scan without looking at the map). The results revealed a common network of cerebral areas, in particular, bilateral superior external occipital regions, reflecting the processes involved in the generation and maintenance of visual images, and the precuneus (left internal parietal region), reflecting the scanning process. In this study, the parietal regions were involved in an imagery process with a spatial component. This latter result is consistent with the already mentioned hypothesis (Ungerleider and Mishkin, 1982) of a distinction between a dorsal stream (spatial system), running from the occipital to the parietal cortex, involved in the perception of spatial locations, and a ventral stream (visual system), running from occipital to the infero-temporal cortex, involved in the recognition of objects. The results obtained by Mellet and colleagues (1995; see also Mellet et al., 1996) revealed that the spatial system is engaged in both mental scanning of visual images and mental scanning of mental images.

2.08.4.3 Imagery Value

In the study of the indexes of verbal materials best predicting memory performance, imagery value has usually resulted as the best predictor (Paivio, 1971), whereas the effects of item frequency/familiarity and associative value/meaningfulness resulted in weaker results and were partly explained by imagery value. This result was interpreted by Paivio (1971) as proof of the dual-code theory; in fact, a high-imagery-value item (e.g., the word 'train') evokes both a verbal and an imaginal encoding, and the double encoding enhances enhance the probability to get back the item. On the contrary a low-imagery-value item (e.g., the word 'range') only evokes a verbal encoding and thus has a poorer trace and a reduced probability to be recovered. Paivio (1971) also assumed that pictorial material has, by definition, a high imagery value and thus, if verbalizable, has the highest probability of having a double encoding; in other words, the picture superiority effect (better recall of the verbal label of a presented picture than of the corresponding presented word) should be caused by the same reasons that explain the superior recall of high-imagery words with respect to the recall of low-imagery words. However, this conclusion raises a series of perplexities (for a review, see Cornoldi and Paivio, 1982; Marschark and Cornoldi, 1990) because high-imagery verbal material differs from low-imagery verbal material in many respects only partly related to the use of mental imagery.

Different paradigms have been used to analyze the specific effects of mental imagery on the recall of high-imagery-value materials. For example, in a dual-task paradigm, participants are presented with a main task, which in this case can involve the retention of the high-imagery-value material. Simultaneously, they are also required to perform a secondary task, most of the time tapping one of the working memory components. (Some studies that have employed the dual-task paradigm have been presented in the preceding paragraphs, e.g., Salway and Logie, 1995; Bruyer and Scailquin, 1998). In a series of studies (e.g., Colpo et al., 1977), we simultaneously presented words, either with high or low imagery value, and pictures, and we found that the memory for high-imagery-value words was selectively impaired, suggesting that high-imagery words and pictures relied on the same type of resources.

A different paradigm concerns the manipulation of instructions. In general, specific instructions encouraging the use of mental imagery enhance memory with respect to instructions encouraging the use of a non-imagery strategy (e.g., repetition); furthermore, this effect seems to interact with the imagery value of the material, although the latter effect is not always clear (for a review, see Richardson, 1999).

2.08.4.4 Individual Differences in Imagery Abilities

The study of mental imagery has often used an individual differences approach, as already anticipated in the section concerning neuropsychological evidence. Also, within normal populations (i.e., in the absence of severe deficits), imagery ability differences can be observed. Actually, some of the tasks used for measuring spatial abilities require the maintenance and the manipulation of mental images, as in the case of tasks requiring mental rotation or the mental transformation of parts (mental folding, mental assembly, etc.). The traditional preference for imagery transformation tasks over other spatial tasks in the psychometric assessment of spatial abilities seems to result from the fact that the imagery transformation tasks involve a high degree of control and are thus central and related to the central structures of intelligence; however, at the same time, they maintain specific spatial features and are not only theoretically but also empirically distinguishable from other high-control tasks tapping verbal functions (Cornoldi and Vecchi, 2003).

Individual differences found with the use of objective measures are related to independent measures obtained with neurological (e.g., Charlot et al., 1992) and cognitive procedures. An example of this relationship is represented by the ability of solving ambiguous figures (Cornoldi et al., 1996). For example, Mast and Kosslyn (2002) found that the ability of rotating images was highly associated with reports of image reversals subsequent to an 180-degree rotation of a figure, whereas other imagery ability measures were not.

In the mental imagery field, the repertoire of differential measures has been enriched by many other measures, including subjective ones. Despite their methodological limitations, the self-report measures have been largely used and have produced a series of different tools. For example, the VVIQ test (Marks, 1973) requires that a person imagines, with either open or closed eyes, a scenario (e.g., a sunset) and reports how vivid his/her image is. The imagery ability is directly inferred by the sum of the values given to the different activated images. There have been concerns regarding the value of this subjective measure, voiced from different sides, but there is evidence that in some cases it may be a useful method (for a review, see McKelvie, 1995). For example, a VVIQ score may be predictive of the performance in a task requiring the memorization of visual objects. However, there is evidence that in some cases other types of subjective reports may have better predictive power. In particular, Graham and Morris (2003) suggested that a lack of a relationship between subjective measures and performance could be because they tap different components of mental imagery. To investigate this, the researchers administered two spatial tasks and two different self-report questionnaires to a group of subjects. One self-report, the VVIQ, mainly involved subjective visual experiences derived from long-term memory, whereas the other included items of the same kind used in the spatial tasks. They found that only the latter items predicted spatial performance, whereas there was no relationship between VVIQ and objective measures.

2.08.5 Educational and Other Applied Implications

The role of mental imagery in human cognition, for example, in reasoning, comprehension, and creativity, has been illustrated in several studies (Paivio, 1971; Richardson, 1999). This evidence suggests that training to effectively use visualization processes could enhance cognitive performance in many areas.

A well-known example of how mental imagery can be used in an educational context is represented by imagery mnemonics. This point was already emphasized by ancient Greeks and Romans, who stressed the importance of using mnemonics to improve learning. However, in the history of culture and education, the attitude toward mnemonics alternated between moments of enthusiasm, resulting from the enhancement of memory, and criticisms, resulting from the artificial connection between memory content and memory cues. For example, Cicero and Giordano Bruno were in favor of mnemonics, but Erasmus and Montaigne were not (Yates, 1966). Despite these different views, there is general agreement that mnemonics enhance memory and that they rely largely on the use of mental imagery (for a review, see Higbee, 1988). One key principle of imagery mnemonics is the creation of interactive images, which facilitates the retrieval of an element when the element imagined in interaction is available.

Mnemonics can be distinguished into two categories according to whether they rely on a specific rule or they require the prior creation of a cues file, and both categories of mnemonics very often rely on the use of mental imagery. Mnemonics based on a rule are of many different types. In the chaining mnemonics, people form a series of interactive images by combining in pairs the sequence of items. For example, given the sequence 'radio, cat, window, salad, etc.,' one could form the interactive image of a radio turned on by a cat, then a subsequent image of a cat jumping onto the windowsill, a window placed above a bowl of salad, and so on.

Another rule-based mnemonic that has received a lot of educational uses is represented by the keyword technique, mainly used for the study of foreign languages. The technique requires people to give a concrete imaginable meaning to the foreign word by associating it with a word in their own language with a similar sound. They then form an interactive image including the images of the new word and the word corresponding to the real meaning of the foreign word. The example given in **Figure 14** shows how a person could remember the Russian word 'likor' for 'battleship'. His first task would be to find an imaginable word similar in sound to the Russian word 'likor,' for example, Lincoln or liquor. The second task would be to create an interactive image including, for example, Lincoln and a battleship. As the two elements are very different in size, the person could use a zooming process to give a name to the small individual represented on the deck of the battleship.

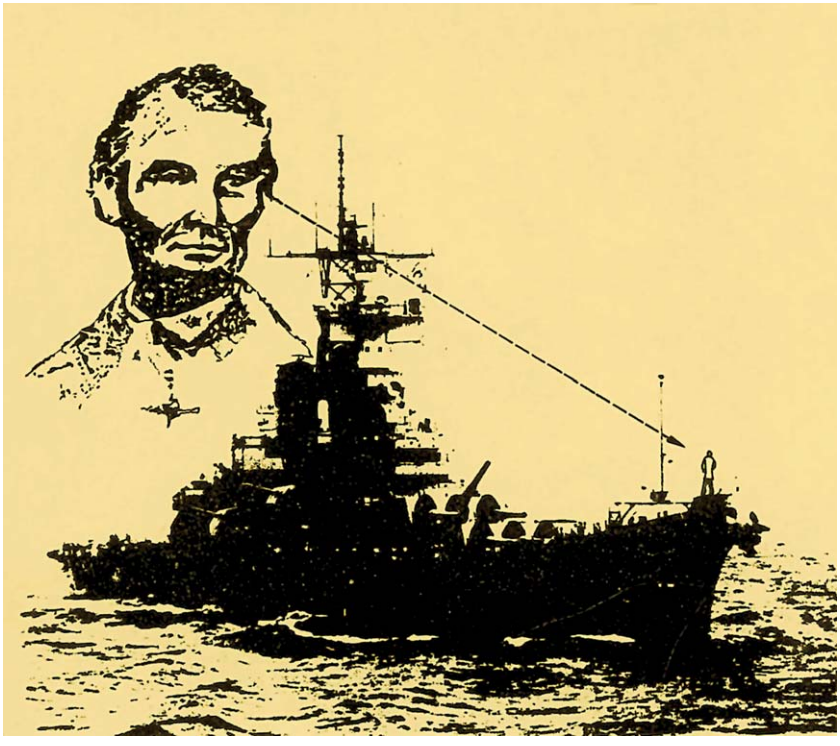

Figure 14 Example of the use of the key-word technique to learn a foreign word ('likor' for 'battleship').

Despite the fact that imagery mnemonics can be considered artificial and distinguished from mnemonics based on semantic associations, in many cases the two components can act together. The example given in **Figure 15** represents this possible synergy. In fact, a child trying to memorize the patterns illustrated in the matrix could simply rely on the semantic associations between items (e.g., a dish and a bowl can stay together), or the more effective rule of categorical clustering (a hen, a duck, a chicken, and a rabbit are all animals). However, the child could also take advantage of the memory of the visual form of the represented objects and the possibility of imagining them in interaction (a farm scenario with the animals, a bowl above a dish, etc.).

Mnemonics based on a cues file requires people to have already created a file containing a series of organized images that will be imagined in interaction with the new to-be-remembered material. At retrieval, the well-known cues file will be used for retrieving the new information memorized in interaction. Expert memorizers have always given preference to this category of mnemonics because it offers the possibility of storing huge amounts of material. There are reports of experts who created files of thousand of cues, either organized around spatial layouts or (more often) organized sequentially in correspondence with the number sequence. The most well known mnemonic based on a cues file is represented by the loci mnemonics, where people use landmarks arranged along a well-known pathway as cues. The example given in **Figure 16** is adapted from the illustration given by Lindsay and Norman (1977) in their psychology handbook, where the pathway was in fact the route followed by Donald Norman to reach the Department of Psychology of the University of California at San Diego, starting from his home. The first landmarks selected by Norman were in sequence: (a) his own home, (b) the bay, (c) the train railway, and so on. Having to memorize a sequence of words, Norman could associate them with the sequence of landmarks (e.g., a radio left close to the gate of his home, a cat looking at a boat in the bay, a broken window on a train). If required to remember the sequence of words in perfect order, people using the loci mnemonics have no

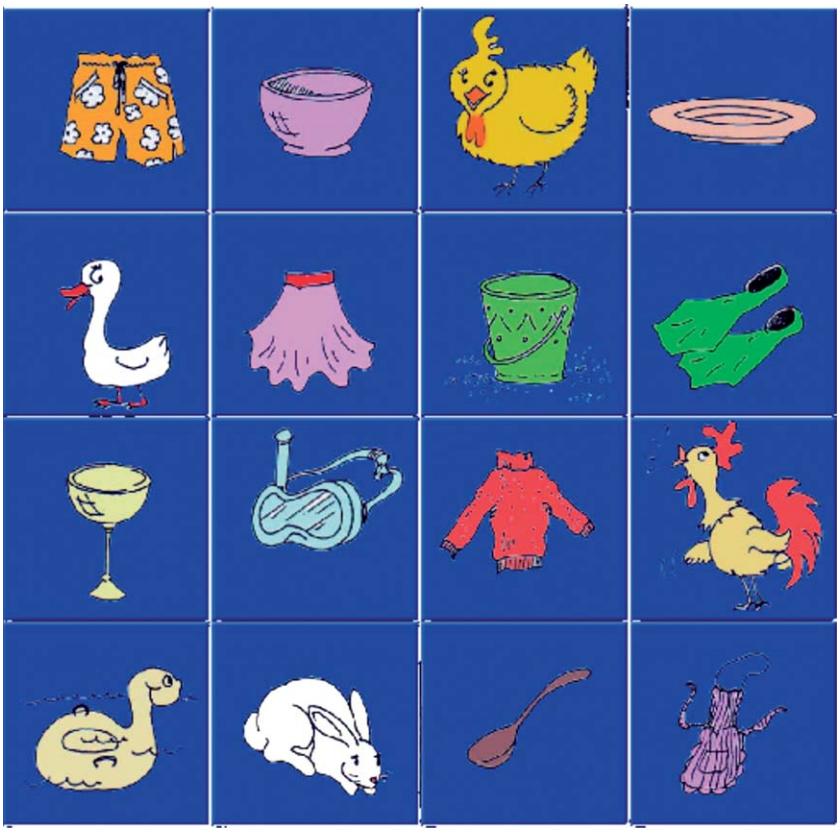

Figure 15 Example of memory material that can take advantage of both semantic and mental imagery strategies.

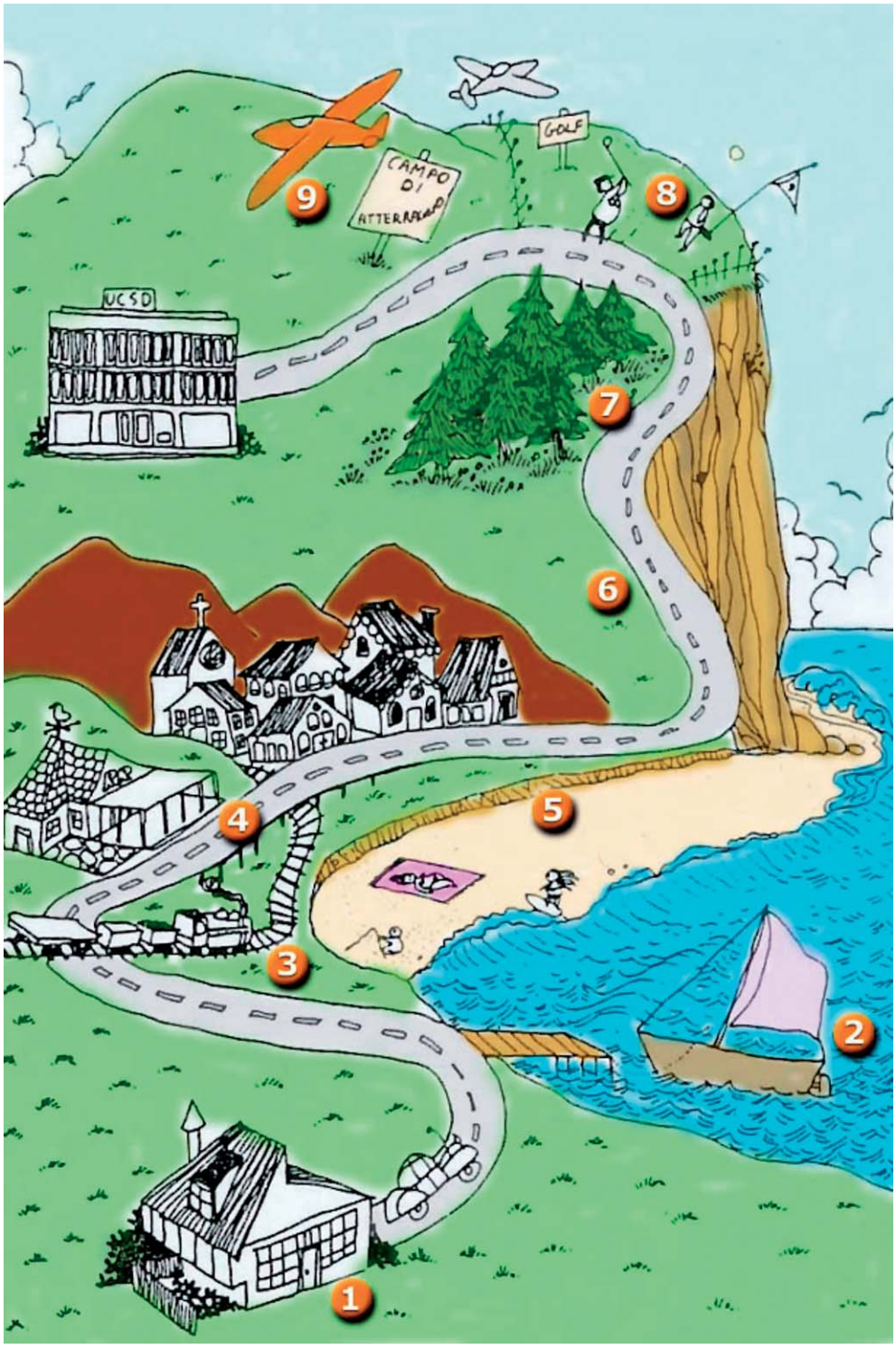

Figure 16 An example of figure employed for using the loci mnemonic. Adapted from Lindsay PH and Norman DA (1977) *Human information processing,* 2nd edn. New York: Academic Press.

problems in retrieving their well-known route with its landmarks and thus retrieve the elements imagined in interaction with their landmarks.

A critique advanced against the loci and other imagery mnemonics is that they can be successfully used for memorizing isolated arbitrary series of items but do not offer an advantage in memorizing meaningful texts. However, there is evidence that loci mnemonics can also enhance memory for texts, although its advantage is more evident when a text is orally presented than when it is written, because of the competition of visual processes involved in imagining and reading (Cornoldi and De Beni, 1991).

2.08.6 Concluding Comments

Mental imagery represents a very relevant part of mental life. Because of its pervasiveness, internal status, and complexity, its study raises a series of methodological problems and requires differentiations and specifications. In this chapter we described mental imagery with reference to different approaches and theories. In particular, we illustrated the debate between prepositional and imagery theorists and the efforts devoted to distinguishing between different imagery processes and representations. The classical problem concerning the extent of the analogy between visual perception and visual mental imagery may find a response in the consideration of the differences from images directly derived from experience and images generated from long-term memory information. Furthermore, the consequences resulting from the use of different types of long-term memory information can be examined within the approach distinguishing between general, specific, and other types of mental images. However, many issues in the field, for example, the dynamic nature of mental images, their role in different life activities (like creative processes, thinking, meditation, and so on), and the study of individual differences, appear still in need of further research developments.

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2.09 Distinctiveness and Memory: A Theoretical and Empirical Review

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2.09.1 Introduction

My memories appear as a varied landscape, with fields and trees and distant mountains. I can distinguish each blade of grass at my feet, I see the individual veins on the leaves on a nearby tree and the brown corrugated grain of the tree's bark. Farther away, the blades of grass are lost in a field of green, and the trees on the hillside are marbled clumps of color. However, I can clearly see a lone tree in the middle of a distant field. On the horizon I can see the blue haze of the mountains, occasionally interrupted with an outcrop of white rock. I know the mountains must be covered with trees, but I cannot separate one from another. The clarity of my memories, like the clarity of my perceptions, appears to be determined by both distance and contrast. In this manner, contrast, or distinctiveness, is a fundamental concept in any theoretical treatment of memory.

Theory and research concerning distinctiveness and memory have long and fascinating histories that have been recounted in detail elsewhere (see Wallace, 1965; Schmidt, 1991; Hunt, 1995). These histories unfailingly begin with Hedwig von Restorff, a postdoctoral assistant in Wolfgang Köhlers's laboratory. von Restorff was one of the first to argue that the Gestalt perceptual principles applied to memory traces (Geissler, 2001). Principles of organization and the perception of figure and ground lend themselves nicely to the clarity of memory for that metaphorical lone tree in the field. von Restorff (1933) argued that "the rules according to which the

reproducibility of items is a function of the structure of the list are consistent with the laws governing whether individual parts in the field of vision remain independent or integrated into a perceptible whole." (Von Restorff, 1933: p. 323) The perception of a single "figure in a whole" (or tree in a forest) is impaired because it becomes a part of the whole. The perception of a tree in a field does not suffer from such interference (Von Restorff, 1933: p. 327). Studies of the von Restorff effect and distinctiveness effects in general can be seen as attempts to understand the good memory for the metaphorical lone tree.

In the following pages, I provide a summary of theoretical and empirical treatments of the concept of distinctiveness in memory research. I begin with a few of my own organizational principles, followed by three theoretical perspectives on distinctiveness in memory. Then I provide a rather long catalog of empirical phenomena that roughly fit under the distinctiveness umbrella. I conclude with a cautionary note concerning the usefulness of distinctiveness as a theoretical construct.

2.09.2 Organizing Principles

Imagine that I am looking at a giant oak tree at the height of fall colors. My visual field is completely filled with the image of the tree, and every leaf on the tree, save one, has turned bright red. The green leaf stands out in this perceptual field. There is nothing unusual about a green leaf; it gains its distinctive character by virtue of its context. In contrast, I do not recall ever seeing a purple polka-dot leaf on a tree. Perhaps a purple polka-dot leaf on a tree would be distinctive in any context. Note that individual leaves on a tree full of purple polka-dot leaves would not stand out from one another. However, the tree as a whole would stand out in a typically green forest. Thus, when one tries to define the distinctiveness of an experience, one must consider the domain, or to push the metaphor, the field of view.

Toward this end, researchers often distinguish between context-dependent, or primary, distinctiveness and absolute or secondary distinctiveness (Schmidt, 1991). Primary distinctiveness (from W. James's primary memory) is defined relative to the immediate context or, for example, a list of words in a memory experiment. For example, a tall tree in the middle of a field of grass stands out in its immediate context, and a single word printed in red stands out in the context of a list of words all printed in black. Secondary distinctiveness is defined with regard to the previous history of the observer and to experiences stored in long-term or James's secondary memory. Examples of secondary distinctiveness might include seeing a purple polka-dot tree or reading the sentence: The banker floated across the puddle on a newspaper.

This discussion of primary and secondary distinctiveness naturally leads to a discussion of experimental design or, more specifically, list structure. The study of primary distinctiveness requires a within-subjects or mixed-list design. That is, the participants must experience material that stands out within the experimental context. In contrast, secondary distinctiveness can be studied in both mixed-list and between-subjects design. For example, suppose one were interested in the effect of word frequency on memory. In a between-subjects design, one group of participants could view a list of frequent words, while a second group would view a list of infrequent (i.e., distinctive) words. In a within-subjects design, participants would view a mixed list of frequent and infrequent words. Interestingly, the word frequency effect in recall may only appear in mixed-list designs (DeLosh and McDaniel, 1996).

Researchers have also wrestled with the problem of the locus of the effect of distinctiveness in memory research. von Restorff addressed this issue when she stated, "Either similar forces are at work in the 'trace field' as are in perception, *or* [her emphasis] our results are merely the direct results of Gestalt effects in perception" (von Restorff, 1933: p. 323). In modern terms, we might ask whether the effects of distinctiveness result from processing differences during encoding or differences in the stored representations or arise during the retrieval stage. von Restorff concluded that the effects operate on the memory traces, combining representational and retrieval views of distinctiveness. Other researchers have concluded that all three stages contribute to the effects of distinctiveness (see Schmidt, 1991, and Hunt, 1995).

McDaniel and Geraci (2006) argued that the effects of primary distinctiveness typically occurred at the retrieval stage, whereas secondary distinctiveness effects resulted from both encoding and retrieval processes. With manipulations of primary distinctiveness, memory for the nondistinctive surrounding items on a list is not impaired, particularly on measures of recognition memory (Schmidt, 1985). This suggests that the processing of the distinctive item does not distract from the processing of the common items on the list. In addition, the position of the

distinctive item within the list does not seem to impact the effect of primary distinctiveness. That is, the distinctive item is well recalled even when it is the first item in the list (Hunt, 1995). In this list position, the distinctiveness advantage must have resulted from the item standing out in memory rather then during its initial presentation. In contrast, with secondary distinctiveness (e.g., bizarre sentences), the presence of the distinctive items appears to impair memory for the common items, and list position is an important factor. For example, McDaniel and Geraci (2006) found that bizarre and common sentences were recalled equally well if the bizarre sentence appeared in the first half of a list. The typical bizarreness advantage was found when the bizarre sentences appeared in the second half of the list. These results demonstrate the importance of encoding processes in supporting the effects of secondary distinctiveness. Retrieval processes also play an important role. For example, the relatively good memory for bizarre sentences is often found in free recall but not cued recall (Einstein and McDaniel, 1987) or serial-order recall (McDaniel et al., 2000).

Despite McDaniel and Geraci hypothesis, few would argue that encoding and perceptual processes are not important in primary distinctiveness. After all, the green leaf on a red tree will not stand out if one is colorblind. That is to say, the features that support trace distinctiveness must be encoded. For example, consider the experiment reported by Van Overschelde et al. (2005). Research participants viewed names of American football teams (e.g., Texas Longhorns). In one condition, this list contained only the names of college teams, and in a second condition, the list contained one college team isolated in a group of professional teams. Only participants with a high knowledge of football, who were thus able to discriminate between professional and college teams, showed a memory advantage for the isolated name. Hunt and Lamb (2001) asked participants to name either a similarity or a difference between adjacent words in a list. In the control list, all the words belonged to the same conceptual category (e.g., tools). In the isolation list, one word was from a different conceptual category (e.g., vegetables) than the other words on the list. The typical isolation effect was found following the same judgments, but not following the difference judgments. Clearly, prior knowledge and encoding strategies play an important role in producing the effects of primary distinctiveness.

2.09.3 Theoretical Perspectives

From the discussion in the preceding section, we can see that the domain, the type of experimental design, and the locus of the effect are all central to studies concerning the impact of distinctiveness on memory. In addition, researchers have offered numerous definitions of distinctiveness (see Schmidt, 1991, and Hunt, 2006, for reviews). These include the ideas that distinctiveness is a property of an item, an item in context, a property of a memory trace, a property of a retrieval cue, a property of a cue in a context, a type of processing, and the response of an individual to a stimulus. Definitions of distinctiveness are typically tied to theoretical perspectives, and numerous theories have been proposed. The theories can be grouped into three primary classes: organizational theories, representational theories, and those theories focusing on the affective response of the perceiver. Each of these perspectives is outlined below.

2.09.3.1 Organizational Theories

Organizational theories can trace their roots to von Restorff, and thus to Gestalt approaches to psychology. Within this framework, a distinctive item is apart, or in a different category, than its physical or temporal neighbors. Thus, in a list-learning task, the distinctive item changes the organizational structure of the list. Bruce and Gains (1976) used this account to explain clustering of related isolated items in free recall. These results could not easily be explained by increased attention to or rehearsal of (Rundus, 1971) the distinctive items.

Reed Hunt and his associates have offered several elaborations on organizational approaches. These are variously referred to as the distinctiveness hypothesis (Hunt and Mitchell, 1978; Hunt and Elliot, 1980; Hunt and Einstein, 1981; Hunt and Mitchell, 1982), the organization and distinctiveness view (Hunt and McDaniel, 1993), and most recently and generally, as distinctive processing (Hunt, 2006). A common theme running through this research is the focus on individual item and relational processing in memory. For this reason, I refer to this approach as the individual item/relational processing view. Individual item (distinctive) processing serves to distinguish each item form other items in a set. Relational processing highlights features shared by items within a set, helping to delineate the search set. Depending on the nature of the set of items and the

retrieval context, each kind of processing can be beneficial to memory performance. Within this framework, "distinctiveness is the processing of difference in the context of similarity" (Hunt, 2006: p. 22). So, for example, in a group of relatively homogeneous items individual item (distinctive) processing would serve to differentiate the items and greatly improve recall (see the discussion of the Hunt and Lamb, 2001 study in section 2.09.2). However, in a list of unrelated items, relational (organizational) processing would benefit recall more than individual item processing.

Organizational approaches have numerous strengths. These include their application to both recognition and recall performance and their explanation for the effects of distinctive items on organizational processes in free recall. In addition, the general framework is applicable to a wide range of phenomena, including the isolation effect, the bizarre imagery effect, the humor effect, the word frequency effect, and the concreteness effect. Each of these effects, and their relation to distinctiveness, is discussed in section 2.09.4. The drawback of this approach is that it treats distinctiveness as an explanation, or a theoretical construct, rather than as an independent variable (Hunt, 2006). As such, Hunt's ideas can be used to explain why a distinctive item is well remembered in a given situation. However, the approach does not specify a direct measure of the distinctiveness of an experience. That is to say, it cannot predict what conditions will produce mnemonic isolation.

2.09.3.2 Representational Theories

According to a representational approach, the distinctive item has a memory representation that shares few features with other items in memory. That is, distinctiveness is the converse of similarity as defined by researchers such as Tversky (1977) and Shepard (1987). Thus, unlike Hunt's individual item/relational approach, this approach starts with a specification of what makes an item distinctive, and the focus is less on the processing or organization of an experience and more on static/mathematical ratios. Representational approaches can be traced to Murdock (1960) and his conveyor belt model (Murdock, 1972, 1974). In Murdock's approach, each item is assigned a value along some dimension (e.g., time or size). The distinctiveness of an item is then defined as a function of the sum of the difference between that item and other items on a list. Relative distinctiveness of a set of items is then defined as the ratio of each item's distinctiveness value to the sum of the distinctiveness values of other items in the set. This model has been most successfully applied to the serial position curve (see Neath, 1993), where each item's temporal position was used to calculate item distinctiveness.

Eysenck (1979) also described a representational view of distinctiveness, wherein distinctiveness was defined in terms of sets of overlapping information. Within this framework, three sources of information contributed to recognition memory: information from previous encodings, information from the study-trial encoding, and information in the test trial encoding. "[T]he most important factor in recognition memory is the extent to which the test-trial encoding contains information that is unique to the study-trial encoding" (Eysenck, 1979: p. 111). Recognition performance should then be a ratio of this distinctive information to shared information.

Whereas the Eysenck (1979) and Neath (1993) treatments focused on recognition performance, Nairne et al. (1997; see also Nairne, 2006) extended the framework to recall. Nairne et al. combined uncertainty (perturbation) in serial position information with a metric of positional distinctiveness to predict primacy and recency as a function of presentation rate and retention interval. Nairne (2006) applied the framework to the isolation effect in free recall, providing what is perhaps the most comprehensive coverage of distinctiveness within the representational perspective. This model might be seen as a more formal development of Eysenck's (1979) ideas. Nairne argued that recall is a function of the extent to which a retrieval cue uniquely matches a recall target. Such matches were determined by item similarity, which was defined in terms of shared semantic features. Item distinctiveness was the ratio of the similarity of the cue to the target item to the sum of the similarity of the cue to other possible candidates for recall.

Representational models are often presented in formal mathematical descriptions, providing some precision to their predictions, a true advantage over the verbal descriptions offered by the organizational approach. However, as noted above, most of the formal treatments have been applied to the serial position curve. Applications to other phenomena that fall under the distinctiveness rubric are rare. Schmidt (1996) applied the Murdock (1960) and Neath (1993) frameworks to the typicality effect in free recall and found that they made inaccurate

predictions. For example, Murdock's model incorrectly predicted that both very typical and atypical items embedded in a list of moderately typical items should be distinctive and thus well recalled or recognized. In addition, these theories fail to take into account recent developments concerning similarity (e.g., Gati and Tversky, 1984; Murphy and Medin, 1985; Medin et al., 1993). Items should not be thought of as containing a fixed set of features, with each feature having a fixed contribution to item similarity. Rather, feature selection and feature weights are determined by the context in which an item appears, and by the processing strategies brought to the task (see Hunt and McDaniel, 1993; Schmidt, 1996). Given the lack of a firm metric of similarity, it is difficult to construct a ratio of distinctive to shared features.

The representational approaches seem to have particular difficulty dealing with secondary distinctiveness. For example, within Murdock's (1960) theory, the distinctiveness of an item is calculated relative to other items in a list, not relative to the set of all items stored in memory. Yet, secondary distinctiveness is defined in terms of this larger set of information. How then, for example, could one calculate the relative distinctiveness of, say, high- and low-frequency words? Within Eysenck's framework, independent of experimental design, semantic processing leads to more distinctive memory traces than phonetic processing. As predicted, semantic processing leads to better memory than phonetic processing in both within- (Craik and Tulving, 1975) and between-subjects designs (see Johnston and Jenkins, 1971). Similarly, Valentine (1991) described a representational theory in his explanation of a good memory for distinctive faces. Within this view, faces are represented in multidimensional feature space. Typical faces fall in relatively crowded regions of this space containing many exemplars, whereas atypical faces fall in relatively empty regions. Item discrimination processes are thus relatively easy for atypical compared with typical faces. As a result, the discrimination advantage enjoyed by atypical faces should be present independent of experimental design. Thus, from a representational view, one can define distinctiveness in relative terms, in which case the theory cannot be applied to secondary distinctiveness. Or, one can define distinctiveness in absolute terms, in which case distinctiveness should aid memory independent of experimental design. Thus, representational views stumble over the fact that the effects of secondary distinctiveness are confined to within-subjects designs (see Section 2.09.4).

2.09.3.3 Affective Response Theories

One can imagine the landscape of everyday memories painted in neutral affective tones. However, some experiences would be painted in the vivid colors of strong emotional responses. These emotionally laden experiences should stand out in memory. The distinctive item, within this framework, is one that leads to a heightened physiological state of arousal or emotion. In Schmidt's (1991) classification, experiences that stood out because of their emotional content were classified as examples of emotional distinctiveness. For example, Hirshman et al. (1989) argued that the bizarreness effect resulted from the surprise experienced by participants at finding a bizarre sentence in a mixed list of bizarre and common sentences. Conversely, some researchers have argued that the effects of emotion on memory should be thought of as von Restorff effects (Loftus and Burns, 1982) or as attributable to distinctiveness (McCloskey et al., 1988; Dewhurst and Perry, 2000).

Numerous researchers have tied von Restorff, isolation, or distinctiveness effects to physiological correlates of increased attention, surprise, or emotion. Researchers within this tradition can probably trace their ideas to the work of Sokolov (1963), James (1890), and ultimately Darwin (1872). The nervous system ignores steady-state information and responds to change. From a Jamesian perspective, our perceptions of these nervous system responses 'are' the emotions we feel. These responses can be seen in indices of the orientation reflex (e.g., increased skin conductance, Gati and Ben-Shakhar, 1990), in the N400 and the P300 cortical responses (Fabiani et al., 2000), and in the release of stress-related hormones (Gold, 1992). Exactly how these responses translate into memory effects varies from theory to theory.

James (1890) argued that an emotional experience may be "accompanied by an extraordinary degree of attention" (James, 1872: p. 671). Similarly, Easterbrook (1959) argued that arousal may lead to a narrowing of attention. Bower (1992; see also Rundus, 1971) argued that an emotional event would disrupt rehearsal processes, leading to decreased rehearsal of surrounding material and increased rehearsal of the emotional item. Burke et al. (1992) suggested that an emotional experience would lead to a shift in the priority of information

processing. Thus, one explanation within affective theories of distinctiveness is that outstanding events (Ellis et al., 1971) receive extra attention and processing, and that this extra processing may be at the expense of other stimuli.

Gold (1987, 1992) focused on the neurobiological processes supporting memory. He argued that information storage was influenced by neuroendocrine responses that may promote memory coding. Specifically, stress releases epinephrine, which in turn leads to increased levels of blood glucose in the brain. Serum glucose levels were thought to regulate memory storage processes in an inverted-U-shaped fashion.

Brown and Kulik (1977) developed their flashbulb memory hypothesis, borrowing the potent mechanism from Livingston's (1967a,b) Now Print! theory. According to this view, the response to a significant event follows a three-stage process. First there is the recognition of novelty or unexpectedness (distinctiveness), followed by a test for biological significance, and then the "permanent registration of not only the significant novelty, but all recent brain events" (Brown and Kulik, 1977, p. 76). This is accomplished, according to Livingston, through diffuse action of the reticular formation. MacKay's binding hypothesis (MacKay et al., 2004) provides yet another perspective: in some of the other views expressed above, emotional stimuli soak up encoding resources. Specifically, through the action of the amygdala, the hippocampus binds word meanings to the context in which they occur.

Several researchers have proposed multistage processing of emotional stimuli. For example, Christianson (1992a,b) proposed a two-stage model wherein an emotional stimulus may automatically receive preferential processing, followed by elaborative processing of the stimulus. Fabiani and Donchin (1995) argued that working memory maintains a model of recent experience or context. The P300 response to distinctive stimuli reflects the updating of that model resulting from the necessity reorganizing the experience. If a stimulus requires this update, then it is marked, and the marking can be used to aid retrieval processes.

An obvious criticism of the emotional distinctiveness view is its extensive coverage of responses from the mild surprise of encountering a red word printed in a list of black words to the shock and horror one experiences at the loss of a loved one. Are the mechanisms proposed above (e.g., attention focusing, brain glucose, contextual binding) qualitatively the same across a continuum of emotional reactions, perhaps differing only in magnitude with changes in emotional intensity? Or are qualitatively different memory processes invoked by each of the many emotions? Some researchers have attempted to separate manipulations associated with distinctiveness into different groups. Michelon and Snyder (2006) argued that the N400 cortical response was seen with manipulations of secondary distinctiveness, and the P300 associated with primary distinctiveness. However, only the P300 was consistently associated with good memory performance. Schmidt (2006, 2007) has argued for a categorization of stimuli into those that are distinctive and those that are significant. Within this view, distinctiveness is the result of a mismatch between an experience and memory representations, whereas the significance of an experience results from a match between the experience and previous significant experiences. Only significant stimuli were thought to lead to strong emotional responses and poor memory for surrounding stimuli.

2.09.3.4 Hybrid Theories

Although there is considerable divergence across the three theoretical perspectives described above, there is also commonality. For example, theories of stimulus orientation (e.g., Ohman, 1979) often invoke a feature-matching process similar to representational theories of distinctiveness. Fabiani (2006) and Christianson (1992b) both describe poststimulus elaboration and rehearsal processing that are reminiscent of organizational theories of distinctiveness. Schmidt (1991; Schmidt and Saari, in press) developed the incongruity hypothesis, which contains elements of all three approaches.

According the incongruity hypothesis, each presented item is compared with the contents of working memory. If an item is encountered that contains features that substantially differ from the weighted features in working memory, then the observer orients to the item. This orientation is automatic and leads to the increased storage of features extracted from the item. Because the process is automatic, the incongruent item will not rob attentional resource from other items with the typically slow rates of presentation used in many memory experiments. Some effect may be observed, however, in RSVP tasks (Raymond et al., 1992). In a modification of this view, significant stimuli hold attentional resources during an emotional appraisal process

(Schmidt, 2002b; Schmidt and Saari, in press), disrupting processing of material in the spatial or temporal proximity of the significant item. Further processing of distinctive and significant experiences may occur as a result of controlled elaboration and organizational processes. The first two phases in the incongruity hypothesis clearly place the locus of the effects of distinctiveness on encoding processes. However, Schmidt (1991) also included a retrieval component: "during the memory test (Phase 3), the effect of incongruity on memory will depend on the degree to which stored information supports good memory performance" (p. 537).

There are several strengths of the incongruity hypothesis. First, the framework incorporates research concerning the effects of stimulus contrast on physiological measures of orientation and attention. Second, the framework includes an explanation of the effects of distinctiveness on organization in recall (e.g., Bruce and Gains, 1976). Third, the framework explains why the effects of secondary distinctiveness may be limited to within-subjects designs. However, some have argued that the incongruity hypothesis incorrectly predicts that the effects of primary distinctiveness should be reduced if the distinctive item is in the first serial position of the list (Hunt, 1995; McDaniel and Geraci, 2006). According to this view, the first item in a list cannot be incongruent, and thus should not stand out at retrieval. However, this interpretation of the incongruity hypothesis is overly simplistic. Increased attention to an item through incongruity has its impact on memory by increasing individual-item processing of the item. This increased processing then supports retrieval and discrimination processes, which lead to enhanced memory for the distinctive item. The first item in a list, because of its primacy position, should also lead to orientation and increased attention (Oberauer, 2003) relative to other items on the list. Thus, it is reasonable to assume that the same critical features will be encoded for the distinctive first item as are encoded for a distinctive middle item. In addition, feature selection during the encoding of subsequent list items will be aligned with the contrast to the first item. Memory advantages enjoyed by a distinctive first item should thus be similar to those of a distinctive middle item.

2.09.4 Empirical Phenomena

As indicated above, a large number of phenomena have been offered as examples of the effects of distinctiveness on memory, been explained by reference to the von Restorff effect, or been described as the result of distinctive processing. In the following section, I provide a brief description of many of these phenomena and attempt to cast them within the theoretical frameworks outlined above.

2.09.4.1 von Restorff's Original Work

von Restorff's (1933) original paper has been summarized elsewhere (Hunt, 1995); in addition, there is a very readable and accessible translation of her paper available online (see Hunt, 1995). However, given that her research is very unlike what most people think of as the von Restorff effect (e.g., Wikipedia, 2006), it is worth recounting some of her original work.

von Restorff described a series of experiments investigating proactive and retroactive interference effects in recall and recognition. In some of her experiments, she employed a collection of diverse objects (e.g., a number, a green square, a letter, a nonsense syllable). Some of the objects were massed, that is, four objects of the same type (e.g., four nonsense syllables) appeared in the list, and others were isolated, in that only one object of a specific type appeared in the list. She demonstrated that memory for isolated items exceeded memory for massed items. In another of her experiments, von Restorff contrasted memory for three lists: one number with nine nonsense syllables, one syllable with nine numbers, and a list of ten heterogeneous items (a number, a syllable, a button, a chemical compound, etc.). The proportion of isolated items recalled greatly exceeded the proportion of common items from the same list. She also demonstrated that isolated numbers or syllables were more likely to be recalled, and were more quickly recalled, than were numbers or syllables from the heterogeneous list. However, the difference between recall of an isolated item and recall of the same item from the heterogeneous list was smaller than the difference between the isolated item and the common items in the same list. Across experiments, she varied the structure of the lists, as well as the position of the isolated item. She concluded that similar list items were absorbed into the memory trace field, whereas isolated items were not. von Restorff reported these effects in recall and recognition, but the effects were larger in recall. Finally, she concluded that both proactive and retroactive interference were the result of these same field effects.

2.09.4.2 The Isolation Effect

Numerous researchers have adapted the design of the second von Restorff experiment described in the preceding section. In these experiments, a single isolated item (e.g., a word printed in red) is placed in a list of otherwise homogeneous items (printed in black). Memory for this item is then compared to memory for the same item in a list of homogeneous items (all words printed in black). Typically, recall of the isolated word would exceed recall of the same word printed in black (Rabinowitz and Andrews, 1973). This design focuses on one of the more striking findings reported by von Restorff and provides a purer measure of isolation. That is, in von Restorff's design, one cannot separate the positive effect of isolation on memory for the isolated item from potential negative effects of isolation on memory for the remainder of the list. In a well-designed experiment, memory for a physically identical stimulus can be either assessed with that stimulus isolated or not, and potential positive and negative effects of isolation can be identified (e.g., Schmidt, 1985). Wallace (1965) provided a detailed review of early isolation effect experiments; Schmidt (1991) provided a brief review of more recent research. Good memory for isolated items can be found with both physical and semantic isolation and is observed on recall, recognition, and even implicit tests (Geraci and Rajaram, 2004) of memory. The presence of an isolated item in a list impairs recall but not recognition of other items on the list (Schmidt, 1985). Because of its central place within distinctiveness research, all three theoretical perspectives described here have been applied to the isolation effect.

2.09.4.3 Bizarre Imagery and the Bizarreness Effect

Memorists have long believed that bizarre imagery is an effective mnemonic device (Yates, 1966). However, experimental tests of the benefits of bizarre imagery led to an inconsistent and confusing array of results. This confusion was greatly alleviated when McDaniel and Einstein (1986) conclusively demonstrated a bizarreness advantage in the recall of sentences. They demonstrated that the bizarreness effect was only obtained when participants experienced both bizarre and common sentences within the same experimental context. Thus was born the distinctiveness interpretation of the bizarre imagery effect.

It is beyond the scope of this chapter to review the vast literature on bizarre imagery effect. Additional coverage can be found in the Mental Imagery chapter in this volume (*See* Chapter 2.08), and a recent review of the bizarreness effects can be found in Worthen (2006). Bizarreness appears to have both positive and negative effects on memory, aiding memory for the bizarre material but interfering with sentence integration (McDaniel and Einstein, 1986) and retention of order information (DeLosh and McDaniel, 1996). As a result, the positive bizarreness effect is usually confined to free-recall tests and not found on cued-recall (see Einstein and McDaniel, 1987, for a review), ordered-recall (DeLosh and McDaniel, 1996), or recognition (McDaniel and Einstein, 1986) tests of memory performance. There are currently two prominent explanations for these effects, one from the organizational and one from the representational perspectives outlined in section 2.09.3. From the organizational perspective, bizarreness encourages individual-item processing at the expense of relational processing and the processing of order information (DeLosh and McDaniel, 1996). In contrast, McDaniel et al. (2000, 2005) have argued against differential processing interpretations of the bizarreness effect. Rather, the bizarreness benefit occurs at retrieval when the set of features used during retrieval "are functionally distinctive in the context of the retrieval set"(McDaniel et al., 2005, p. 271). It is unclear how to reconcile this position with the list order effects reported by McDaniel and Geraci (2006), described in Section 2.09.2.

2.09.4.4 The Humor Effect

Humorous material is often remembered better than nonhumorous material matched in content (Kaplan and Pascoe, 1977; Kintsch and Bates, 1977; Schmidt, 1994; Schmidt and Williams, 2001). Like the bizarreness effect, humorous material only has a mnemonic advantage in mixed-lists designs. Also, similar to the bizarreness effect, the humor effect is eliminated if the humorous material is presented in a block prior to the nonhumorous material in the same list (Guynn et al., as reported in McDaniel and Geraci, 2006). Unlike the bizarreness effect, the humor effect is obtained in cued recall (Schmidt, 1994). This is probably because humorous sentences are easily integrated, whereas bizarreness disrupts sentence continuity. Schmidt (2002a) provided a hybrid explanation of the humor effect. He concluded that humorous cartoons received increased

attention and discrimination processes in a mixed list and were given retrieval priority during the memory test.

2.09.4.5 The Serial Position Curve and Temporal Distinctiveness

Memory performance for a series of presented items is often a U-shaped function of input position, with participants showing good memory for the beginning and ending of the series and relatively poor memory for the middle positions. This ubiquitous phenomenon has led to numerous explanations, but it is most often cast within the modal model (i.e., Atkinson and Shiffrin, 1968) and the fate of items in sensory, short-term, and long-term memory (Crowder, 1976). However, Murdock (1962) suggested a different cause in his seminal paper on the shape of the serial position curve. He argued that the shape of the curve resulted from retroactive and proactive interference between adjacent items on the list. In addition, Murdock (1960) applied his distinctiveness model to the bowed serial position curve. Recently, researchers have returned to the distinctiveness explanation of the serial position curve (Nairne et al., 1997; Neath, 1993) within the representational view described above. Surprenant (2001) applied this approach to the memory of tonal sequences and, true to the von Restorff tradition, argued that a common theory of relative distinctiveness could be applied to both perceptual and memory phenomena.

The distinctiveness interpretation of the serial position curve also has its opponents. Rouder and Gomez (2001) argued that the temporal distinctiveness parameters used to model the serial position curve were arbitrary. Oberauer (2003) argued that an attentional gradient was necessary to account for the primacy effect. Lewandowsky et al. (2006) argued for an event-based rather then a temporal-based account of the curve. Within this view, the temporal structure of the list (beginning, end, inserted pauses, etc.) provides opportunities for consolidation, rehearsal, and organizational processes. These processes are responsible for the shape of the serial position curve, not temporal distinctiveness per se. Note that one could still use the concept of distinctiveness to explain the serial position curve within this framework. However, item distinctiveness would be event or item based and not tied to the memory representation of the temporal gradient.

2.09.4.6 Orthographic Distinctiveness

Zechmeister (1972) was one of the first researchers to report that orthographically uncommon words (e.g., 'llama') are remembered better than words that conform to common English orthography. This effect has been found on recall (Hunt and Mitchell, 1982), recognition (Zechmeister, 1972), and word fragment completion (Hunt and Toth, 1990) tests of memory. However, like many of the effects of distinctiveness, the orthographic distinctiveness effect appears to be confined to mixed-list designs (Hunt and Elliot, 1980). Interestingly, the effects of orthographic distinctiveness are additive with the effects of conceptual distinctiveness (Hunt and Mitchell, 1982; Kirchhoff et al., 2005), and thus orthographic and conceptual distinctiveness may be mediated by different mechanisms (Kirchhoff et al. 2005).

Geraci and Rajaram (2002) argued that the orthographic distinctiveness effect is only obtained on implicit memory tests if the participants are aware of the relation between the test and the encoding task. In addition, orthographic distinctiveness effects were reduced if the participants' attention was divided between two tasks. They suggested that conceptual processing of the relation between the distinctive and common items is important to produce the effect. Geraci and Rajaram concluded that their results were consistent with Hunt's distinctiveness hypothesis, as well as Schmidt's (1991) incongruity idea, and the Fabiani and Donchin (1995) framework described above.

2.09.4.7 The Word Frequency Effect

Recall of frequent words usually exceeds recall of infrequent words. In contrast, recognition memory for infrequent words usually exceeds that for frequent words (Gregg, 1976). The word-frequency effect in recognition is often cast within a representational view of item distinctiveness. That is, low-frequency words appear in fewer preexperimental encodings than high-frequency words. In terms of Eysenck's framework described above, it should be relatively easy to discriminate between the low-frequency word's experimental and preexperimental encodings (Brown, 1976; Eysenck and Eysenck, 1980), leading to higher hit rates and lower false alarm rates for low- than for high-frequency words (Rao and Proctor, 1984). Superior recognition of low-frequency words is found in both homogeneous and mixed lists (see Gregg, 1976, for a review), as well as

with implicit memory tests (MacLeod and Kampe, 1996). Eysenck and Eysenck (1980) provided support for the distinctiveness interpretation by demonstrating that distinctive processing (e.g., producing an infrequent modifier for the nouns in the study) reduced the word-frequency effect in recognition.

The word-frequency effect in recall has been cast within the individual item/relational processing view of distinctiveness (DeLosh and McDaniel, 1996; Dobbins et al., 1998; Saint-Aubin and LeBlanc, 2005). DeLosh and McDaniel (1996) argued that memory for word order plays an important role in many recall tasks. In addition, encoding "resources are lured to processing and interpreting the individual and idiosyncratic features of unusual items" (DeLosh and McDaniel, 1996, p. 1137). This shift in encoding robs resources from processing order information. In a pure list of high-frequency words, there are greater resources to devote to order processing than in a pure list of infrequent words, leading to the typical recall advantage found for the high-frequency words. However, in a mixed list of high- and low-frequency words, increased processing of the low-frequency words takes place at the expense of the order encoding of both types of words. The low-frequency words received increased individual item processing relative to the high-frequency words, and both types of items suffer from a disruption of order encoding or relational processing. The result is that in the recall of mixed lists, low-frequency words are sometimes recalled better than high-frequency words (Delosh and McDaniel, 1996; Saint-Aubin and LeBlanc, 2005). DeLosh and McDaniel demonstrated this reversal, as well as the predicted effect of mixed lists on memory for order information.

Saint-Aubin and LeBlanc (2005) also compared memory for pure and mixed lists of high- and low-frequency words. They employed relatively short lists of words and a serial order recall task. In their mixed lists, a single high- or low-frequency word was isolated in lists of five words of the other type. My interpretation of the order-encoding hypothesis leads me to predict that a single high-frequency item should be poorly recalled in the context of infrequent words. That is, the infrequent words should rob encoding processes from the high-frequency word. However, the high-frequency words were recalled better than the low-frequency words in this list structure. In addition, there was not a significant effect of word frequency when the lists contained primarily high-frequency items. These results seem to challenge the order-encoding interpretation of the word-frequency effect. Interestingly, Saint-Aubin and LeBlanc (2005) argued that their results supported a distinctiveness interpretation, citing Hulme et al.'s (2004) explanation of the word-length effect. According to Saint-Aubin and LeBlanc, high-frequency words were more distinctive than low-frequency words.

2.09.4.8 The Word Length Effect

On immediate-recall tests, recall of short words often exceeds recall of long words. This finding is often interpreted within a working memory model and the role of the phonological loop in immediate recall (Baddeley et al., 1975). However, the word-length effect is also found with delayed tests, and with lists that should exceed the memory span (Russo and Grammatopoulou, 2003), challenging the working memory interpretation of the effect. Three different distinctiveness interpretations of the word-length effect have been offered as alternatives to the working memory hypothesis. Hulme et al. (2004) argued that the word-length effect was in reality an effect of item complexity. Short items are less complex than long items and, thus, are less susceptible to memory errors (Neath and Nairne, 1995). In addition, because short items contain fewer features than long items, they will share fewer features across other list items. As a result, short items have greater item distinctiveness within the Neath representational framework. Hulme et al. used this framework to explain the absence of a word-length effect in mixed lists of long and short words alternating across input positions.

Cowan et al. (2003) also evaluated the word-length effect in mixed lists, but they varied the number of long and short words in the list and compared memory with and without articulatory suppression. The word-length effect was either reversed (without articulatory suppression) or eliminated (with articulatory suppression) when the list was composed of primarily short words. The typical word-length effect was obtained when the list was predominately long words. They concluded that in mixed lists, organizational factors play an important role in recall, invoking the organizational view of distinctiveness.

Hendry and Tehan (2005) investigated the word-length effect in mixed lists and employed both serial recall and recognition measures of memory performance. They observed the typical short word advantage on the recall task, but long words were recognized better than short words. They interpreted

their results with DeLosh and McDaniel's (1996) order-encoding hypothesis (see section 2.09.3.1). That is, long words rob encoding resources from the processing of order information, impairing serial recall, but, long words also benefit from increased item processing relative to short words, leading to greater recognition of long words than short words. From this perspective, long words are more distinctive than short words.

2.09.4.9 The Concreteness Effect

"Concrete language is remembered better than abstract language in a wide variety of tasks" (Paivio et al., 1994, p. 1196). The concreteness effect is most often interpreted within Paivio's (1971) dual-coding theory, according to which imaginal and verbal processing independently contribute to memory for concrete words, whereas only verbal processing is usually possible for abstract material. The concreteness effect is found in both within- and between-subjects designs (Marschark and Hunt, 1989). Nonetheless, Marschark and Hunt (1989) noted that the concreteness effect was greatly attenuated in free recall and argued that this challenged the dual-coding interpretation. Instead, they cast the concreteness effect within the individual item/relational-processing framework (see also Marschark, 1985, and Marschark and Surian, 1992). Within this view, concrete materials encourage encoding of perceptual attributes of the material that can serve a distinctive function at retrieval.

It is worth noting that both views of the concreteness effect include the role of item distinctiveness (Paivio et al., 1994). What appears to be at issue is whether or not separate memory codes for verbal and imaginal processing is a necessary component of an explanation of the concreteness effect. According to the dual-coding theory, the additive effects of concreteness and relatedness on memory performance implicate independent contributions of the two systems. In contrast, Marschark and Hunt (1989) argued that concreteness effects in recall should only be observed in the presence of relational processing. That is, the distinctive memory representations of concrete words cannot contribute to good memory performance if the search set cannot be identified by appropriate relational information. Paivio et al. (1994) and Richardson (2003) have challenged this assertion by reporting additive effects of concreteness and relatedness. However, ter Doest and Semin (2005) found a concreteness effect on an explicit word stem completion test for a list of related words but not for a list of unrelated words, providing support for the Marschark and Hunt position. Nonetheless, a concreteness effect was observed for the unrelated word list in their free-recall test. Clearly, neither dual coding nor distinctiveness by themselves provides complete explanations of the concreteness effect. Both perspectives rely on other mechanisms (e.g., task-appropriate processing, Hamilton and Rajaram, 2001) to handle the full range of phenomena.

2.09.4.10 The Picture Superiority Effect

Under many conditions, including in both mixed- and between-list designs, people remember pictures better than they remember words (see Paivio, 1971, 1986). Research concerning this picture superiority effect parallels research on the concreteness effect in several ways. Like the concreteness effect, early explanations of the picture superiority effect were cast within Paivio's (1971) dual-coding hypothesis. The dual-coding explanation was then challenged by a distinctiveness explanation (Nelson et al., 1976; Nelson, 1979), and the distinctiveness explanation was then given further support by studies employing implicit memory tests (Weldon and Coyote, 1996; Hamilton and Geraci, 2006). Despite these similarities, the concreteness effect has been cast within an individual item/relational processing view of distinctiveness, whereas the picture superiority effect has been cast within a representational view of distinctiveness.

Nelson's (1979) conceptualization of distinctiveness is very similar to that of Eysenck (1979) and other representational views of distinctiveness. "Retention level is assumed to be a direct function of the relatively unique and unified nature of the study trial encoding *and* [author's emphasis] the degree to which the retrieval environment recapitulates this encoding" (Eysenck, 1979: p. 49). From this view, pictures have more unique (distinctive) features than words. Words presented in a list are limited by font and letter constraints that render them visually similar. In contrast, a picture of an object may contain many features that help distinguish it from pictures of other objects appearing on a list. Evidence for this perspective was found in the fact that the picture superiority effect could be diminished, or even reversed, by employing pictures that were visually similar to one another (Nelson et al., 1976). This explanation of the picture superiority effect has

faired better than empirical tests of the individual item/relational explanation of the concreteness effect. Perhaps the concreteness effect should be recast within the Nelson/Eysenck view of distinctiveness. That is, perhaps concrete words encourage visual imagery processes, and the resulting images are more distinctive than the verbal representations of abstract words (Hamilton and Rajaram, 2001).

2.09.4.11 False Memory and the Distinctiveness Heuristic

Research concerning the distinctiveness heuristic trades heavily on the picture superiority effect. Israel and Schacter (1997) investigated memory within the Deese-Roediger and McDermott false memory paradigm (Roediger and McDermott, 1995; *See* Chapter 2.14). In this paradigm, a list of related words, for example, bed, rest, awake, and dream, is followed by a memory test. Of interest is participants' false memory for a related target word, such as 'sleep' Israel and Schacter compared memory for words spoken and written to words spoken and depicted in pictures. Picture presentation led to lower false recognition than written presentation. The authors argued that distinctive perceptual qualities of the pictures, features not available following written word presentation, served to reduce false memory. Schacter et al. (1999) later developed the idea of a distinctiveness heuristic: "a mode of responding based on participants' metamemorial awareness that true recognition of studied items should include recollection of distinctive details" (Schacter et al., 1999, p. 3). Schacter, Dodson, and associates have provided impressive support for the use of the distinctiveness heuristic (e.g., Schacter et al., 2001; Dodson and Schacter, 2001, 2002).

Hege and Dodson (2004) provided an alternative explanation for the lower false memories for pictures than for words. According to this view, the distinctive nature of pictures leads to impaired relational processing relative to the processing of printed text. As a test of this hypothesis, Hege and Dodson compared both recall and recognition memory following picture and word presentations. On the recall test, participants were asked to report any items related to the studied list, presumably bypassing any use of the distinctiveness heuristic. In support of the individual item/relational view, participants were still less likely to commit false recall of the target items following the picture than following the word presentation. On a follow-up recognition test, participants were instructed to put check marks next to items that actually appeared on the memory lists. Support for the distinctiveness heuristic was found when false recognition was lower for pictures then for words. Additional support for the distinctiveness heuristic was reported by Dodson and Hege (2005). In this study, the researchers varied the rate of presentation of test items on the recognition test. On self-paced tests, pictures lead to lower rates of false memories than words. However, on fast-paced tests (750 ms/item), the pictures and words led to comparable false recognition rates. In contrast, true recognition rates for words and pictures were similar, and both declined equally as the test pace increased. These results provide evidence against the idea that the low rate of false recognition of pictures results from reduced relational processing relative to words. Rather, the authors invoked two-stage theories of recognition (e.g., McElree et al., 1999; *See* Chapter 2.23) and argued that the distinctiveness heuristic is a time-consuming retrieval processes. As a result, lower false memories for pictures than for words only occurs during slow-paced recognition tests.

The distinctiveness heuristic should confound those of us studying distinctiveness and memory for several reasons. First, the idea presupposes that participants have the metamemorial abilities to discern which mnemonic variables are likely to increase and decrease the distinctiveness of memories. Given the literature reviewed here, it is clear that memory researchers do not agree on how variables impact item distinctiveness. Attributing this knowledge to the typical research participant is questionable at best. Second, the argument begins with the explicit assumption that pictures are more distinctive than words. Whereas there is research to support this claim, it is not an incontrovertible fact and, according to Nelson et al. (1976), depends on the pictures. Third, the Dodson and Hege (2005) results seem to undermine the whole enterprise. Not only do these results challenge the idea that words receive greater relational processing than pictures but they challenge the idea that the memory representations of pictures are more distinct than the memory representations of words. More distinctive memories should lead to greater true and lower false recognitions independent of the pace of the recognition test (from a representational view of distinctiveness). Nonetheless, the proportion of both hits and false alarms for pictures were equal to those of words on the fast-paced test. Perhaps the picture superiority effect results from dual coding, and the distinctive memory traces

referred to by the distinctiveness heuristic are stored in Paivio's imagery system. During the recognition test, participants may need to access this visual code to aid memory discrimination processes. It is well established that accessing a visual code for verbally presented materials requires time (Paivio and Csapo, 1969). If this dual-coding interpretation is correct, then the picture superiority effect on both true and false memories may tell us more about dual coding than about item distinctiveness.

2.09.4.12 Face Recognition

Recognition memory for unfamiliar faces is greatly influenced by face uniqueness (Going and Reed, 1974), distinctiveness (Cohen and Carr, 1975), or typicality (Light et al., 1979). Going and Reed (1974) attributed this effect to the fact that a greater number of eye fixations were devoted to unique than to common visual stimuli. Cohen and Carr (1975) argued that the effect was akin to the von Restorff effect, whereas Light et al. (1979) attributed the effect to interitem similarity. The most comprehensive treatment of the effect of facial distinctiveness on recognition has been offered by Valentine and his associates (Valentine and Bruce, 1986; Valentine, 1991; Valentine and Ferrara, 1991).

Valentine has argued that faces are represented in a multidimensional space, with typical faces represented near the conceptual core of the category and atypical faces located at the categorical fringe. Valentine and Bruce (1986) argued that participants detected that an atypical face was different from the category norm, and this led to distinctive encodings of these faces. However, they concluded that "[t]he exact nature of a mechanism which may give rise to the effect of distinctiveness of encoding is unclear" (Valentine and Bruce, 1986, p. 304). Valentine and Ferrara (1991) were more specific in that they modeled facial recognition within both the McClelland and Rumelhart (1985) distributed memory theory and Nosofsky's (1986) model of item recognition. In the McClelland and Rumelhart theory, connection weights were determined by the difference between an input face and the facial prototype. Within the Nosofsky framework, the atypical face is not given special treatment at encoding. Rather, the distinctive memory representation of the atypical trace aids discrimination processes in recognition memory. Valentine (1991), again adopted a representational view, and argued that distance in the multidimensional space supported discrimination processes in face recognition. Typical faces fall in a crowded region of space, whereas atypical faces fall in less densely populated regions, aiding item discrimination processes in recognition.

It is worth noting that research concerning facial memory relies on subjective ratings of facial typicality or distinctiveness. Memory performance is usually tested over a range of stimuli differing in distinctiveness, and then either memory is correlated with the measure of distinctiveness (e.g., Valentine and Bruce, 1986) or a median split is used to group faces into distinctive and common groups (e.g., Newel et al., 1999). In other words, researchers always seem to employ a mixed-list presentation of common and distinctive items. Valentine's (1991) theory implies that the effects of facial distinctiveness should occur in between-list manipulation of facial type as well. It would be nice to see an empirical demonstration of this prediction.

2.09.4.13 The Modality Effect

Auditory presentation of verbal material often leads to superior memory performance than visual presentation. This effect is more robust on tests of immediate memory than on tests of delayed memory (Penney, 1989). The immediate memory modality effect is often attributed to the beneficial effects of a separate auditory sensory store (Crowder and Morton, 1969). However, both the immediate-memory modality effect (Nairne, 1990) and the long-term modality effect (Conway and Gathercole, 1987) have been attributed to item distinctiveness. Conway and Gathercole have shifted their view of the role of distinctiveness in the long-term modality effect. Conway and Gathercole (1987) and Gathercole and Conway (1988) argued that auditory stimuli were temporally more distinct than visual stimuli. Conway and Gathercole (1990) argued for a translation processes, wherein translation from one input domain to another (e.g., voicing a visually presented word) led to a more distinctive memory representation than processing in one domain. Nairne (1990) argued that visual presentation led to primarily modality-independent memory representations, whereas auditory presentation created both modality-independent and modality-specific memory representation. The modality-specific representations available following auditory presentation provide distinctive features to aid memory performance. Nairne's model has been used to explain an impressive range of phenomena, including modality, suffix, and serial position effects,

as well as the impacts of articulatory suppression and irrelevant speech on memory performance (see Neath and Surprenant, 2003).

Recent research into the phenomenon of false memory (*See* Chapter 2.14) has led to an interesting twist in the interpretation of the modality effect. Smith and Hunt (1998) found that visual presentation led to fewer false memories in the Deese-Roediger and McDermott paradigm (see Roediger and McDermott, 1995) than did auditory presentation. They also demonstrated that a task designed to increase distinctive processing (pleasantness rating) reduced false memories. They concluded that visual presentation led to more distinctive memory representations than auditory presentation. Gallo et al. (2001) replicated and extended these findings to both within- and between-subject manipulations of modality. Furthermore, auditory presentation only led to greater false memories on visual tests of recognition memory. They concluded that participants use a list-specific heuristic, wherein distinctive visual cues retained from visually presented words aid discrimination between old and new items on the memory test.

It is hard to reconcile these views of the modality effect in false memory with the Conway and Gathercole (1990) and Nairne (1990) explanations of the modality effect in immediate recall. The false memory research is more consistent with the view of the modality effect developed by Penney (1989). She argued for separate streams of processing for visually and auditorily presented information. Visual information led to a rapidly fading visual code and a phonological code, whereas auditory information led to a more persistent acoustic code plus a phonological code. As a result, visual inputs are associated based on simultaneous presentation, whereas auditory information is integrated across time. Similarly, Arndt and Reder (2003) argued that auditory presentation encouraged relational processing across items, whereas visual presentation encouraged individual item (i.e., distinctive) processing (see also, Pierce et al., 2005). Thus, in order to explain enhanced memory following auditory presentation, and lower false memory following visual presentation, the modality effect has been recast from the original representational view to the individual item/relational processing view of distinctiveness.

2.09.4.14 Emotional Words

Researchers have long argued that emotional material is remembered better than neutral material (Kleinsmith and Kaplan, 1963, 1964; Maltzman et al., 1966). However, several researchers have noted that the effect may depend on experimental design (Walker and Tarte, 1963). Most of the early research investigating memory for emotional material employed mixed lists of emotional and neutral words. When memory for a homogeneous list of emotional words has been compared to a memory for a homogeneous list of neutral words, the emotional memory effect sometimes disappears (Dewhurst and Parry, 2000; Hadley and MacKay, 2006). Dewhurst and Parry (2000) argued that the mixed-list presentation enhanced the distinctiveness of the emotional words; however, they do not specify how this happens. Perhaps they have in mind a trade-off between individual-item and relational processing.

However, a distinctiveness interpretation of the emotional memory effect is complicated by the fact that not all emotional words have the same impact on memory and attention processes. Words associated with sex and the bathroom have a greater impact on memory than do less offensive words (Manning and Goldstein, 1976), and the emotional memory effect is larger for negative than for positively valenced emotional words (McNulty and Isnor, 1967; Dewhurst and Parry, 2000). Furthermore, Saari and Schmidt (2005) found an emotional memory effect for taboo words in both within- and between-subjects designs. In contrast, with negative affect non-taboo words, an emotional memory effect was only found when mixed-list designs were employed (Schmidt and Saari, in press).

The complex relation between word emotion and memory will likely require a hybrid explanation that includes both a representational view of distinctiveness and shifts in attentional resources. Schmidt and Saari (in press) noted that emotional words often lead to increased attention as measured by the Stroop color-naming task (see Williams et al., 1996, for a review). However, Schmidt and Saari found that emotional Stroop effect was modulated by both list structure and the type of emotional words employed. With taboo words, the emotional Stroop effect was found in both mixed- and pure list designs and was of equal magnitude in both designs. In addition, the memory advantage for taboo words occurred in both list structures but was larger in the mixed list design. This suggests that taboo words attract extra encoding resources in both experimental designs but benefit from item distinctiveness in a within-list design. A different pattern of results was found with nontaboo emotional words. With nontaboo words, the Stroop

effect was only found with relatively short interstimulus intervals (ISIs) and when the emotional and neutral words were presented in blocks. The Stroop effect for these nontaboo words was thus probably the result of carryover in the processing of one emotional word to the processing of the next word in the series (see also McKenna and Sharma, 2004). In contrast with the Stroop effect, enhanced memory only occurred in mixed list with these emotional nontaboo words, and the memory effect was independent of ISI or blocking. Thus, increased attention was not related to good memory for the nontaboo emotional words. Instead, these words gained their mnemonic salience from a distinctive retrieval context. Schmidt and Saari concluded that increased attention and a distinctive retrieval context work together to produce enhanced recall of taboo words, whereas with nontaboo emotional words, the emotional memory effect is the result of item distinctiveness. Thus, the results were compatible with the Schmidt (1991) incongruity hypothesis and the Fabiani and Donchin (1995) orientation-distinctiveness view.

2.09.4.15 Odor

Several researchers have reported that odor is a very effective retrieval cue (the so-called Proust phenomenon; Chu and Downes, 2002; Herz and Schooler, 2002). However, some researchers have failed to find that odor cues facilitate memory (Bjork and Richardson-Klavehn, 1989). Herz (1997) argued that for an odor cue to be effective, the odor must be salient in the environment. That is, the odor cue must be distinctive, or contextually inappropriate, in the experiment. Thus, the positive effects of odor on memory may be tied to cue-distinctiveness within an Eysenck framework of the effects of distinctiveness on memory.

2.09.5 Summary and Conclusions

Based on this review, one is tempted to conclude that the concept of distinctiveness in memory research is amorphous and has been utilized to explain such a wide range of phenomena that it is nearly bankrupt. Within the same phenomenon, completely opposing roles of distinctiveness have been proposed. For example, some researchers have argued that low-frequency words are more distinctive than high-frequency words, whereas others have argued that high-frequency words are more distinctive than low-frequency words (see also the word-length effect). Auditory presentation apparently leads to a more distinctive memory representation than visual presentation, unless of course you are discussing false memory, in which case visual presentation leads to more distinctive memory representations than auditory presentations. Within the same phenomenon, many different distinctiveness interpretations have been offered (e.g., the bizarreness effect, the word length effect, the concreteness effect). And, with conceptually and empirically similar phenomena (e.g., the concreteness effect and the picture superiority effect), distinctiveness explanations have taken different forms.

Schmidt (1991) also noted the varied forms of the distinctiveness hypothesis, leading him to ask: "Can we have a distinctive theory of memory?" (Schmidt, 1991, p. 523). There are several answers to this question. One answer is that the concept of distinctiveness can be used heuristically, or descriptively. In this approach, good memory implies distinctiveness. That is, distinctiveness is not really a theory of good memory but a description of why memory is good. Unfortunately, many researchers continue to use distinctiveness in this manner (see Hunt, 2006, for a similar complaint).

Alternatively, one can look for a coherent structure in the phenomena reviewed in this chapter and use that structure to narrow the theoretical and empirical fields. With very few exceptions, the effects of distinctiveness are modulated by experimental design. The notable exceptions include the word frequency effect in recognition, the concreteness effect, the picture superiority effect, and memory for taboo words. These phenomena occur in both between- and within-subject designs. It is tempting to attribute these select phenomena to mechanisms other than distinctiveness (i.e., familiarity, dual coding, and emotional processing, respectively). The remaining phenomena, those more naturally tied to distinctiveness, either disappear in between-list designs (e.g., bizarre imagery effect) or simply must be studied in within-subjects designs (e.g., the isolation effect and the serial position curve). Any successful theory of distinctiveness must explain the impact of experimental design on the pattern of results.

As another general observation, encoding as well as retrieval processes are nearly always a part of successful explanations of distinctiveness. The word-frequency effect in recognition is the only notable exception to this rule. However, Chunyan

et al. (2004) noted an association between the late-positive ERP and the correct recognition of low-frequency words – implicating encoding processes in the word-frequency effect. Diana and Reder (2006) also provided support for the role of encoding processes in the word-frequency effect in recognition. These researchers compared memory following either single-task (study the list) or dual-task (study the list while engaging in an addition task) encoding conditions. Performing the dual task during encoding eliminated the effect of word frequency on hit rates and reduced the effect on false alarm rates relative to single-task performance.

Of the 15 different memory phenomena reviewed herein, ten have been explained by specific reference to representational models. I caution against this view as an exclusive explanation of the effects of distinctiveness on memory. Representational views generally overlook encoding processes, and as I have argued, encoding plays an important role in modulating many of the effects of distinctiveness. Representational theories have defined distinctiveness in terms of a memory (or memory trace plus retrieval cue) representation that shares few features with other memory representations. Support for this assumption is found in the fact that recognition of distinctive items exceeds memory for typical items. Without some independent index of item distinctiveness, this explanation provides little insight into memory processes. (Note that this criticism does not apply to the distinctiveness explanation of the serial position curve, where position or time serves as a parameter in calculating item distinctiveness.) Schmidt (1995) provided evidence against representational approaches to category typicality effects on memory, and his criticism applies to representational approaches to secondary distinctiveness effects in general. Finally, the representational view implies a strong causal link between distinctiveness and memory. According to this view, the psychological space is relatively empty around distinctive items, and each distinctive item in a list should also be off by itself within this space. As a result, it should not matter if a list has 20 such distinctive items or ten common items and ten distinctive items. In both cases, each distinctive item should fall in a relatively empty psychological spatial region, leading to enhanced item discrimination processes relative to the common items found in crowded regions. Thus, distinctive memory representations should enhance memory in both mixed-list and between-subjects designs. In addition, distinctive memories should aid performance on intentional, incidental, and implicit tests of memory. Of the phenomena reviewed above, only the concreteness effect, the picture-superiority effect, and the word-frequency effect in recognition meet these standards. All of the other phenomena require additional explanatory mechanisms.

In order to avoid tautological explanations of memory performance, specific definitions of distinctiveness must be employed. These definitions must be supported by converging evidence. That is, if I say that A is more distinctive than B, and that item distinctiveness supports better memory for A than B, then I must independently demonstrate both halves of this assertion. In addition, theories of distinctiveness should provide specific mechanisms whereby distinctiveness aids memory performance. From the above review, we can see that the list of potential mechanisms is not long. The list includes increased attention (as indexed by neurological or behavioral correlates), increased item processing at the expense of relational processing, feature sampling, ease of discrimination, and retrieval priority.

These observations lead me to conclude that adequate theories of distinctiveness will necessarily be hybrid models of the kind proposed by Schmidt (1991), Christianson (1992a,b), Fabiani and Donchin (1995), Worthen et al. (1998), and some instantiations of the individual item/relational processing framework. Within each of these approaches, there is a clear definition of what is "distinctive" – they describe specific mechanisms whereby distinctiveness enhances memory, they include both encoding and retrieval components, and they can be applied across a range of empirical phenomena. In addition, these theories help to integrate physiological measures of attention and arousal with behavioral measures of attention and memory. The extended power of these approaches is also their drawback, for it is hard to make predictions concerning how distinctiveness should influence memory performance in specific situations.

In summary, the concept of distinctiveness has become chameleonic, as researchers color and stretch it in attempts to gain insight into a broad range of memory phenomena. Three different theoretical perspectives concerning distinctiveness can be identified: organizational theories, representational theories, and affective theories. The definition of distinctiveness, and the explanations for how it influences memory, vary across these perspectives and from one empirical phenomenon to another. As a result, there is a danger that distinctiveness will be relegated to little more than a description of good memory performance.

However, theories that describe specific mechanisms whereby distinctiveness influences performance have been proposed and do explain an impressive range of findings. Hybrid models that include organizational processes, emotional processes, and encoding and retrieval processes, as well as incorporating some of the ideas from representational views, appear the most promising.

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2.10 Mnemonics: Underlying Processes and Practical Applications

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In modern society, we rely on accurate memories to perform a variety of tasks throughout the day. We must remember passwords and user names while using the Internet, account and personal identification numbers for business transactions, and myriad telephone numbers for use both at work and at home. Even a mundane event such as a trip to the grocery store is dependent on accurate memory. Such a trip involves not only remembering what to buy at the store but also how to get to the store, the name and a few personal details of the chatty neighbor who will invariably end up next to us in the checkout line, and where the car is parked when we have completed our shopping. Clearly, we rely heavily on our memory system to negotiate the modern world. However, the reliance on memory for successful adaptation to the environment is not a product of modernity at all. In fact, the development of strategies to enhance memory – referred to as mnemonics – dates backs thousands of years. When all communication relied primarily on the oral tradition, the ancient Greeks and Romans developed strategies for remembering lengthy speeches and poems (Yates, 1966), and medieval lawyers used mnemonics to memorize entire sets of codes and laws (Fentress and Wickham, 1992). In fact, good memory was considered a virtue in medieval Europe, and mnemonic training was fundamental to medieval education (Carruthers, 1990). Thus, it appears that the desire to improve one's memory has a history that long predates the formal study of psychology. In this chapter, we describe some of the most popular and enduring techniques for enhancing memory and discuss the cognitive principles that are believed to underlie each technique.

2.10.1 Mnemonic Processes

Although numerous techniques and strategies have been offered to improve memory, the vast majority of mnemonics represent simple applications of basic research findings in cognitive psychology. In fact, the most popular mnemonic techniques are designed to capitalize on a few well-researched psychological processes: organization, elaboration, and mental imagery. Because of their fundamental importance to mnemonic strategies, we briefly discuss each of these processes next.

2.10.1.1 Organization

A major function of mnemonics is organization of to-be-remembered information. It has been clearly established that organized information is easier to remember than unorganized information (Jenkins and Russell, 1952; Bower et al., 1969; Broadbent et al., 1978) and that instructions to organize information enhances memory even in the absence of instructions to memorize (Mandler, 1967). It is also widely accepted that both episodic and semantic information is stored in long-term memory in an organized fashion (e.g., Smith et al., 1974; Collins and Loftus, 1975; Raaijmakers and Shiffrin, 1981; Anderson, 1983; Tulving, 1983). Moreover, examinations of recall output indicate that learners will subjectively organize information that is presented randomly (Bousefield, 1953), even if that information is, on the surface, unrelated (Tulving, 1962). Thus, it is likely that the organization function of mnemonics decreases storage demands by arranging the stimuli in a fashion that more closely matches the preexisting organizational structure of long-term memory. Furthermore, mnemonics that link the to-be-remembered information to the organizational structure of long-term memory may also facilitate retrieval (Baddeley, 1990).

2.10.1.2 Elaboration

A second major process used in the context of mnemonics is elaboration. Although the term is sometimes used synonymously with semantic analysis in textbooks (e.g., Matlin, 2005), an accurate definition describes elaboration more generally as a process of embellishment with additional information (Baddeley, 1990; Anderson, 1995). The advantage of the latter definition is that it allows for a distinction to be made between meaningful and nonmeaningful elaboration. A good deal of research (e.g., Craik and Tulving, 1975; Rogers et al., 1977; Nelson, 1979) supports the notion that meaningful elaboration enhances memory. However, nonmeaningful elaboration can also enhance memory, and under certain conditions, it can do so better than meaningful elaboration (Slamecka and Graf, 1978; Kolers, 1979; Pressley et al., 1987). Thus, it appears that it is the degree of elaboration induced by a mnemonic rather than its meaningfulness that facilitates memory (cf. Craik and Tulving, 1975).

It is also important to note how elaboration facilitates memory. It is believed that elaboration facilitates memory by making memories more discriminable at retrieval by virtue of distinctive processing (Craik, 1979; Lockhart et al., 1976). Distinctive processing is defined as the processing of difference in the context of similarity (Hunt, 2006). As it relates to mnemonics, this suggests that, by way of elaboration, a mnemonic adds uniquely identifying or item-specific information to to-be-remembered items. As a result of additional item-specific information, mnemonic-enhanced memories are more discriminable than memories of unelaborated items at retrieval.

2.10.1.3 Mental Imagery

Some of the most enduring mnemonics involve the use of mental imagery as a form of elaboration. The term mental imagery can be used to refer to any type of mental representation (e.g., Kosslyn et al., 1990; Intons-Peterson, 1992), but in the context of mnemonics, mental imagery is usually visual. A wealth of research (e.g., Paivio et al., 1968; Paivio, 1969; Bower, 1970; May and Clayton, 1973; Richardson, 1978) supports the notion that visual mental imagery enhances memory, and this effect is especially robust when interactive imagery is used (Wollen et al., 1972; Yesavage et al., 1983). Mental imagery is an effective component of mnemonics because it has the potential to enhance both the organization and elaboration of to-be-remembered information. Thus, the use of interactive visual imagery in mnemonics may serve to facilitate the encoding and storage of to-be-remembered information as well as it making the information more discriminable at retrieval.

The use of bizarre mental imagery as a component of mnemonics has been advocated by Greek and Roman orators (Yates, 1966), professional mnemonists (Lorayne and Lucas, 1974; Lorayne, 1990, 1998), and educators (Tess et al., 1999). As a result, the relative effectiveness of bizarre and common imagery has received a good deal of attention from psychological researchers. Empirical research suggests that instructions to use bizarre imagery can lead to better memory than instructions to use common imagery when both types of imagery are used (McDaniel and Einstein, 1986; Cornoldi et al., 1988; Worthen and Marshall, 1996) and either free recall or recognition is tested (Worthen, 2006). However, additional research (Anderson and Buyer, 1994; Weir and Richman, 1996; Worthen, 1997) indicates that the bizarreness advantage is due to the more

general process of bizarre elaboration rather than mental imagery *per se*. Regardless, it is likely that the use of bizarre elaboration in the context of a mnemonic makes the to-be-remembered information more discriminable at retrieval.

2.10.1.4 Retrieval Cues

As described earlier, organization, elaboration, and mental imagery are often treated as part of the encoding process. However, one could just as easily suggest that these processes are part of the retrieval process. Indeed, as Tulving (1983) convincingly argued, any distinction between encoding and retrieval processes is purely heuristic. Processes occurring at the time of learning exert their effect on the retrieval process, either as facilitation or interference, and thus no meaningful theoretical dichotomy between encoding and retrieval remains coherent. The challenge is to explain the influence of processing at the time of initial experience on processes required for successful test performance. To this point, we have hinted at the importance of developing cues that are diagnostic of the to-be-remembered information. A more elaborate rendition of this idea is that the processing of the original experience, when reinstated at testing, constrains production to a limited set of items. This idea has been suggested to explain the effect of various encoding manipulations.

An example of this type of approach within the context of mnemonics is research (i.e., Wallace and Rubin, 1991; Rubin, 1995) that has examined the use of rhyme and meaning to cue memory for narratives in the oral tradition. Such research suggests that rhyme and meaning cues work in concert to facilitate memory by constraining the number of stored choices available at retrieval (cf. Rubin and Wallace, 1989). More generally, this notion of constraining choices at retrieval is at the heart of the effectiveness of distinctive processing. As noted by Hunt and Smith (1996), organization serves to specify the episodic context in which to-be-remembered information was embedded. The addition of item-specific information (e.g., a unique cue) along with organizational processing limits the retrieval set to items that both share the unique feature and were present in the specified context. Thus, processes that ostensibly occur during encoding may very well exert their influence by providing diagnostically precise cues at retrieval.

2.10.2 Formal Mnemonic Techniques

In this section, we discuss strategies for improving memory that involve highly prescribed instructions. Typically, formal mnemonics involve instructions for associating to-be-remembered information with more well-established stored memories.

2.10.2.1 The Method of Loci

With evidence of its use dating back to circa 500 BC (Yarmey, 1984), the method of loci is perhaps the oldest enduring mnemonic. Believed to have been used extensively by Greek and Roman orators, the method of loci is designed to facilitate serial recall by organizing to-be-remembered information within the context of a well-established visual mental image. As an example, consider a herpetologist who wishes to use the method of loci to help remember the topics to be covered in a talk about venomous snakes in Texas. Specifically, the herpetologist wishes to discuss the five most common venomous snakes of Texas in order of potential dangerousness. From least to most dangerous, the snakes discussed in the talk would include the copperhead, the cottonmouth, the coral snake, the timber rattler, and the diamondback rattler. To use the method of loci to remember the list of species to be discussed, the herpetologist would first form a mental image of a very familiar place such as the layout of his home. The next step would be to form associations between the familiar image and the species of snakes to be discussed by mentally placing a cue representing each species in a separate part of the imagined location. In our example, the herpetologist might imagine the normally blue wooden front door of his house to be made of shiny copper to cue the memory of copperhead. Next, he might imagine a carpet of cotton balls (to cue cottonmouth) leading from the front door into the foyer. Similarly, to cue his memory for the coral snake, timber rattler, and diamondback rattler, the herpetologist could imagine a coffee table made of coral in the living room, a stand of small pine trees lining the stairwell, and an oversized diamond blocking passage at the top of the stairs. Finally, when the topics of the talk need to be recalled, the herpetologist would simply mentally revisit his familiar place and pick up the cues he left behind.

In theory, the method of loci should be an effective mnemonic because it represents an application of

all three basic mnemonic processes. First, the to-be-remembered information is organized in a serial fashion by association with a well-established memory. In our herpetologist example, the necessary serial order of the list is maintained by associating the least dangerous species with the entrance to the home and the more dangerous species with more interior locations within the home. Thus, when the herpetologist mentally revisits his mental image at the time of his talk, he will encounter cues in the specified order simply by following the natural layout of his home.

Second, the method of loci provides elaboration of to-be-remembered information via the development of associated cues (e.g., pine trees for a timber rattler) and the use of visual mental imagery. One should also note that imagery used in our example was interactive and bizarre by design. The interactive nature of the imagery is important because it enhances the link between the well-established memory of the familiar place and the less-well-established memory of the to-be-remembered information. The bizarreness of the imagery allows the to-be-remembered information to stand out against the common backdrop and should ultimately lead to a more discriminable memory trace.

The effectiveness of the method of loci as mnemonic technique is supported by anecdotal evidence from professional mnemonists (Lorayne and Lucas, 1974), case studies of exceptional memories (Luria, 1968), and empirical evidence. Regarding the latter, research indicates that the method of loci indeed enhances serial recall (Ross and Lawrence, 1968; Christen and Bjork, 1976; Wang and Thomas, 2000), even after a substantial retention interval (Groninger, 1971; Wang and Thomas, 2000). Furthermore, research has demonstrated that the same loci can be used to learn several different lists without proactive interference (Christen and Bjork, 1976; de Beni and Cornoldi, 1988; Massen and Vaterrodt-Plunnecke, 2006). Some evidence also suggests that mnemonic training with the method of loci can curb some age-related memory differences in elderly adults (Hill et al., 1991; Brooks et al., 1999). Overall, the method of loci is considered along with the peg-word method (described next) to be one of the most effective mnemonics for learning lists (Roediger, 1980). However, when used to learn more complicated verbal material (e.g., prose, discourse), the method of loci is effective when to-be-remembered information is presented orally, but not when information is presented in a written format (Cornoldi and de Beni, 1991; Moe and de Beni, 2005). Additionally, because of its complexity, the method of loci is generally considered an unsuitable mnemonic for the rehabilitation of memory for those suffering from brain injury (McKinlay, 1992; Richardson, 1995).

2.10.2.2 The Peg-Word Method

A mnemonic also designed to enhance serial learning, but with a less-storied history, is the peg-word method. Although its exact origins are unclear, the peg-word method may be a simplification of Grey's (1730) very complicated mnemonic system that involved the transformation of numbers to letters and sounds. Loosely similar to Grey's system, the peg-word method involves learning a list of words that rhyme with numbers to be used as framework with which to organize to-be-remembered items. In a typical rendering, the list of peg words includes bun, shoe, tree, door, hive, sticks, heaven, gate, wine, and hen to represent the numbers 1–10, respectively. To use the peg-word method, one first commits the list of peg words to memory. Then, when a list of items needs to be learned, an interactive visual image is formed between each to-be-remembered item and a peg word. As an example, suppose that an outdoor enthusiast wishes to remember a list of the most crucial items that one would need to survive in the wilderness. According to Bradley Angier's (1956) timeless book *How to Stay Alive in the Woods*, a minimal survival kit would include matches, a compass, a knife, a mirror, and maps. One could remember these items using the peg-word method by creating the following images: numerous matches protruding between two buns like a sloppy match sandwich, an animated compass wearing shoes (perhaps hiking boots!), a tree with a knife embedded in its trunk, a door with a mirror where the window should be, and an animated bee reading a map in front of a hive. When the items need to be remembered, one would simply recall the list of peg words in order. Just as the numbers 1–5 will cue memory for the peg words, the peg words should cue the list of needed items in a specific order.

Like the method of loci, the peg-word method makes use of organization, elaboration, and mental imagery. The association of to-be-remembered items to numbers allows the information to be organized in a specified sequence. The association of the to-be-remembered items to the peg words and the interactive mental image representing that association provides a good amount of elaboration to enhance the discriminability of the memory trace. Moreover,

discriminability of the memory trace may be also be enhanced by the nearly inevitable use of bizarre elaboration when using the peg-word method.

Despite the apparent counterproductivity of learning one list (peg words) to remember another list (to-be-remembered items), the usefulness of the peg-word method has received a good deal of empirical support. Research has demonstrated the effectiveness of the method when used by normal and learning-disabled children (Veit et al., 1986; Krinsky and Krinsky, 1996), normal and learning-disabled adolescents (Elliot and Gentile, 1986), college-age adults (Wood, 1967; Bugelski, 1968; Bugelski et al., 1968; Johnson, 1970; Wood and Bolt, 1970; Wang and Thomas, 2000), and older adults (Wood and Pratt, 1987). The peg-word method has also been found to be effective after both short and lengthy retention intervals (Wang and Thomas, 2000). Furthermore, the same list of peg words can be used for numerous lists without interference (Morris and Reid, 1970; Massen and Vaterrodt-Plunnecke, 2006). However, the peg-word method has been demonstrated to be ineffective when used to learn information that is high in category relatedness (Reddy and Bellezza, 1986) or for information that is presented very rapidly (Bugelski et al., 1968). Also, like the method of loci, the peg-word method may be too complex for use with brain-injured learners in rehabilitation settings.

The relative effectiveness of the peg-word method, the method loci, and rote rehearsal in determining serial recall is depicted in **Figure 1**.

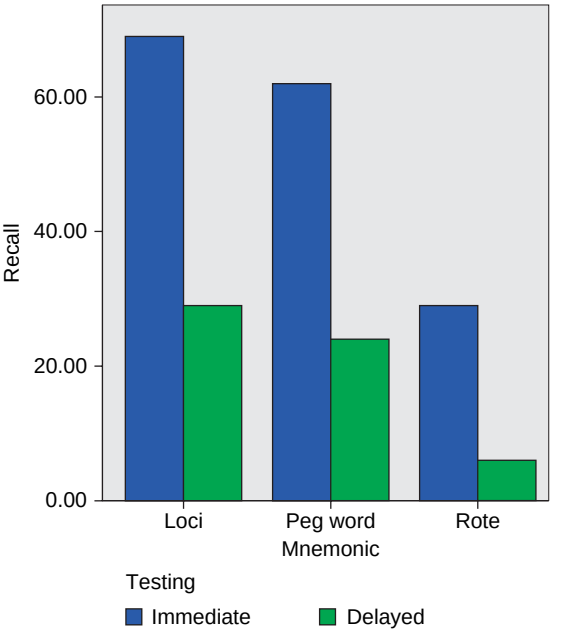

Figure 1 Percentage of correct serial recall as a function of mnemonic method and retention interval. Adapted from Roediger HL (1980) The effectiveness of four mnemonics in ordering recall. *J. Exp. Psychol. Hum. Learn. Mem.* 6: 558–567.

2.10.2.3 The Keyword Method

The keyword method was originally developed by Raugh and Atkinson (1975) as a procedure to facilitate second-language acquisition. The keyword method involves making an association between a to-be-remembered term's meaning and what the term sounds like in one's primary language and then using interactive mental imagery to elaborate on that association. For example, consider an English speaker who is trying to learn the Spanish term queso, which means cheese in English. When pronounced, queso sounds like 'CASE-OH.' Thus, the learner could use the English word case as the keyword for remembering that queso means cheese. Forming a mental image of a briefcase made of cheese (or a briefcase full of cheese) could embellish the association between the keyword and the translation. At recall, the Spanish term queso should cue the keyword (case) and, subsequently, the interactive mental image that combined the meaning of the to-be-learned term and the keyword (i.e., briefcase made of cheese). From a theoretical perspective, the keyword method should be an effective mnemonic because it encourages elaboration during the production or processing of the keyword (depending on whether the learner generates the keyword) and because of its use of interactive mental imagery.

Empirical research has demonstrated the keyword method to be effective in facilitating second-language acquisition both in children (Pressley, 1981; Pressley et al., 1981) and adults (Atkinson and Raugh, 1975; Raugh and Atkinson, 1975; Beaton et al., 2005). The keyword method has also been found to facilitate primary-language vocabulary learning in learning-disabled children (Cundus et al., 1986; Mastropieri et al., 1990), normal children (Levin et al., 1992), and normal adults (Pressley, 1982; Sweeney and Bellezza, 1982; Troutt-Ervin, 1990). Additionally, versions of the keyword method have been used to effectively enhance children's learning of music history (Brigham and Brigham, 1998), adult's memory for artists and their work (Carney and Levin, 1991), the quality of college students' written essays (Rummel et al., 2003), and

memory for prose in young, middle-aged, and elderly adults (Dretzke, 1993). However, a growing body of research (Wang et al., 1992, 1993; Wang and Thomas, 1995; Thomas and Wang, 1996) suggests that keyword method does not enhance recall after lengthy retention intervals (for contrary results, see McDaniel and Pressley, 1984; McDaniel et al., 1987). Research also indicates that the keyword method may not be effective for second-language acquisition among experienced foreign-language learners (van Hell and Mahn, 1997). Finally, regarding the keyword method, research (Campos et al., 2004; Beaton et al., 2005) suggests that the effectiveness of the method is dependent on mental-image quality.

2.10.3 Organizational Mnemonic Techniques

Organizational mnemonic techniques serve mainly to organize information such that all to-be-remembered information is linked together in memory. Unlike formal mnemonic techniques, organizational mnemonic techniques do not emphasize the establishment of associations between to-be-remembered information and previously stored memories to aid retrieval. Instead, organizational mnemonic techniques emphasize intralist associations and are thus less dependent on extralist cues for successful retrieval. Successful use of an organizational technique should consolidate all to-be-remembered information such that retrieval of one item cues memory for other items. Thus, like a connected chain of paper clips, successful retrieval of one item should result in successful retrieval of all items. However, a major disadvantage of this dependence on intralist cues is that the failure to retrieve a single item of the to-be-remembered information may result in the failure to recover all items cued by the forgotten item (cf. Bellezza, 1981, 1996).

2.10.3.1 Acronyms (Linking by Initial Letter)

The use of acronyms to enhance free or serial recall is a popular mnemonic among college students (Gruneberg, 1973; Stalder, 2005). This method simply involves making a word or pseudo word out of the initial letters of to-be-remembered information. For example, a biology student might use the acronym IPMAT to remember the five stages of cell division (interphase, prophase, metaphase, anaphase, and telophase). Perhaps due to its simplicity and popularity among students, the use of acronyms has been advocated for learning a variety of information, including assessment criteria for psychological disorders (Short et al., 1992; Pinkofsky, 1997; Pinkofsky and Reeves, 1998).

Despite its simplicity and popularity, empirical research investigating the effectiveness of the use of acronyms as a mnemonic has provided minimal support for the method. Although a few studies (Nelson and Archer, 1972; Kovar and Van Pelt, 1991; Stalder, 2005) suggest limited effectiveness of acronyms as mnemonics, the bulk of research (Boltwood, and Blick, 1970; Waite et al., 1971; Perewiznyk and Blick, 1978; Carlson et al., 1981) suggests that acronyms do not enhance recall, especially when they are self-generated (Kibler and Blick, 1972).

2.10.3.2 Linking by Story

Another way to organize to-be-remembered information such that all items are linked together is to form a story that incorporates each item. This method has the same organizational advantage of using acronyms but also includes a high degree of self-generated elaboration. Thus, unlike the use of acronyms, linking by story should result in a memory trace that both consolidates to-be-remembered items and is highly discriminable (cf. Bellezza, 1986).

Early empirical research investigating the linking-by-story mnemonic demonstrated the method to be effective for enhancing both serial (Bower and Clark, 1969) and free recall (Herrmann et al., 1973) of verbal material in college-age adults. Subsequent research has found the method to be useful for improving recall in elderly participants (Hill et al., 1991; Drevenstedt and Bellezza, 1993), memory-impaired participants (Wilson, 1995), and mildly retarded participants (Glidden, 1983). A variation of the linking-by-story method has also been found to improve memory for long strings of digits (Bellezza et al., 1992). Additionally, a study comparing the method of loci, the peg-word method, and the linking-by-story method found linking by story to be the most effective method to enhance free recall (Herrmann, 1987).

2.10.3.3 Categorical and Schematic Organization

The use of categories or schemas to organize to-be-remembered information is often advocated as a

mnemonic in textbooks (i.e., Solso, 2001; Matlin, 2005). A category organization involves organizing information in a taxonomic hierarchy whereby abstract category labels are used to organize subordinate exemplars. As an example, consider an angler who wishes to remember a list of items to needed for a fishing trip. The angler must remember the following items: rod and reel, motor key, filet knife, landing net, battery for boat, and plastic bags for storage of fish filets. These items could be organized into three separate categories, each of which subsumes two items. The battery and key would be subsumed under the category label boat items, the rod and reel and landing net would be subsumed under fishing tackle, and the knife and bags would be subsumed under fish-cleaning supplies. With this type of organization, one would need only to remember the category labels, which should cue the specific items to be remembered. As such, the organization of the to-be-remembered items should reduce cognitive load, and with this type of application, the intrusion of categorically related associates would not be necessarily problematic.

A schematic organization involves organizing to-be-remembered information such that spatial relations among items are maintained (Nakamura et al., 1992). Using our fishing-trip example, the needed items could be organized according to where they are to be placed in a boat. For example, the angler might note that the key and battery are in the stern of the boat, the fish-cleaning supplies in the main hatch, and the rod and reel and landing net in the bow of the boat. Thus, the stern, hatch, and bow of the boat would serve to organize and cue to-be-remembered items.

Empirical research using college-age adults (Nakamura et al., 1992) has demonstrated that information organized by taxonomic category and information organized into scene schemas is better recalled than unorganized information. Other research also using college-age adults (Khan and Paivio, 1988) has demonstrated that category organization and script-schema organization leads to equivalent levels of recall. However, it should be noted that categorical and schematic organization is unlikely to enhance recall in young children (Yoshimura et al., 1971).

2.10.4 Summary of Mnemonics and Mnemonic Processes

The mnemonics discussed in this chapter represent simple applications of well-established mnemonic processes. All of the mnemonics designed to enhance memory for lists included a procedure designed to enhance the organization of the to-be-remembered information. Moreover, the most effective of these mnemonics involve a combination of organization and at least one form of elaboration (see **Table 1** for a summary). The sole mnemonic discussed here that did not emphasize organization (the keyword method) was designed to enhance memory for paired associates rather than lists and thus would incur fewer benefits from organization. Nonetheless, even this method imposes some degree of organization on to-be-remembered information via the use of interactive mental imagery.

The importance of a combination of organization and elaboration to mnemonic effectiveness is attested by research that has shown that memory is enhanced by procedures that combine relational and item-specific processing (Einstein and Hunt, 1980; Hunt and Einstein, 1981). Relational processing refers to the processing of fundamental similarities among to-be-remembered items. Thus, relational processing is, in essence, organizational processing. Moreover,

Table 1 Mnemonic Effectiveness as a Function of Processes Involved

Mnemonic	*Organization*	*Elaboration*	*Imagery*	*Effectiveness*
Method of Loci	Yes	Yes	Yes	High
Peg-Word Method	Yes	Yes	Yes	High
Keyword Method	No	Yes	Yes	High
Acronyms (without imagery)	Yes	No	No	Low
Acronyms (with imagery)	Yes	No	Yes	Limited
Linking by Story	Yes	Yes	No	High
Category Organization	Yes	No	No	Limited
Schema Organization	Yes	No	No	Limited

Note: Imagery has both organizational and elaborative properties.

relational processing is believed to enhance memory by specifying a common context in which all to-be-remembered items are embedded. On the other hand, item-specific processing – the processing of unique characteristics of individual items – can be induced by elaboration. Thus, by specifying both a common context and uniquely identifying characteristics of individual items, a combination of relational and item-specific information should enhance the discriminability of list items in memory (Hunt and McDaniel, 1993; Hunt, 2006).

Applied to mnemonics, research on relational and item-specific processing suggests that any mnemonic technique that involves a combination of organization and elaboration should facilitate memory. However, a mnemonic that emphasizes only organization or only elaboration is unlikely to result in strong memorial benefits. In support of the latter claim, the use of acronyms may be an ineffective mnemonic because it mainly emphasizes organizational processing with little emphasis on elaboration. As such, acronyms may serve to link to-be-remembered items together, but, without elaboration, individual items are not particularly discriminable in memory. However, the simple addition of elaboration (i.e., mental imagery) to compliment the already existing process of organization significantly increases mnemonic effectiveness of an acronym (Wilding et al., 1986). Similarly, research investigating the mnemonic effectiveness of bizarre imagery as a method of elaboration has found that, in the absence of an organizational scheme, bizarre imagery is no more effective than using common imagery when only one form of elaboration (bizarre or common) is used during learning (Wollen and Cox, 1981; McDaniel and Einstein, 1986). However, if bizarre elaboration is complemented by an organizational scheme (i.e., the method of loci and the peg-word method), the result is a successful mnemonic. Thus, it is likely that the exact nature of the organization and elaboration comprising a mnemonic is far less important than the requirement that some form of both organization and elaboration is used.

2.10.5 Practical Issues

A major issue in the application of mnemonic techniques is whether the main components of a given mnemonic should be provided to or generated by the learner. Although basic research (e.g., Bowbrow and Bower, 1969; Slamecka and Graf, 1978) supports the notion that generation enhances memory, the application of this finding within the context of mnemonic research is not without qualification. In terms of simple mnemonic processes (e.g., mental imagery), research (Jamieson and Schimpf, 1980; Ironsmith and Lutz, 1996; Kuo and Hooper, 2004) indicates that self-generated elaboration is more effective than elaboration that is provided. However, provided elaboration can be more effective than learner-generated elaboration if it is in the context of a mnemonic that is difficult to use (Patton et al., 1991) or if it is to be used with learning-impaired populations (Swanson et al., 1988; Canellopoulou and Richardson, 1998).

A more general issue related to self-versus-other generation is whether self-generated mnemonic strategies are more effective than mnemonics devised by others. If, as we have argued, a few basic cognitive processes can account for the effectiveness of formal mnemonics, then one may be inclined to abandon formal mnemonics in favor of self-generated applications of mnemonic processes. This sentiment is reflected in the results of a study by Park et al. (1990), which suggests that memory researchers and other psychologists are more likely to both use and recommend the use of general mnemonic processes (e.g., organization and elaboration) than formal mnemonics.

Although spontaneous use of formal mnemonics is infrequent (Intons-Peterson and Fournier, 1986; Soler and Ruiz, 1996), this does not mean that self-generated mnemonics are more useful. For example, Wang and Thomas (2000) examined the effectiveness of the method of loci, the peg-word method, self-generated organizational and imagery mnemonics, and rote rehearsal in determining serial recall. They found self-generated strategies to be least effective when recall was tested immediately, but as effective as formal mnemonics after a 48-h delay. Similarly, research with elderly participants (Derwinger et al., 2003, 2005) has indicated only minimal advantages of self-generated strategies compared to other-generated strategies even after training. Apparently, the benefits of generation and ease of use associated with self-generated mnemonics is offset by the effort involved with devising a personal strategy.

Taking into consideration research examining self-generated versus other-generated elaboration and, more generally, self- versus other-generated strategies, the most important point seems to be that mnemonics are quite flexible. That is, as long as an

appropriate combination of cognitive processes is involved, a mnemonic can be effective regardless of whether it is fully self-generated or other-generated or whether it contains self- or other-generated components. Thus, the decision to use a self-generated or other-generated mnemonic may simply boil down to personal preference. As noted previously by Bellezza (1996), individual differences may play a large role in determining the effectiveness of any mnemonic. Considering that research also indicates that the effectiveness of a mnemonic depends on the demands of the learning situation (Roediger, 1980; Herrmann, 1987), it is likely that there is no single best mnemonic for a given person or even a given situation. As such, the best approach to improving memory across a variety of situations may be to have an assortment of mnemonic techniques at one's disposal.

2.10.6 Conclusions

Although a variety of strategies for enhancing memory have been offered throughout history, the most effective mnemonics involve some combination of organization and elaboration. Thus, it is likely that the specific means of encouraging organization and elaboration are less important than the requirement that both processes utilized. Similarly, when a mnemonic emphasizes both organization and elaboration, it can be effective regardless of whether it is devised by or provided to the learner. However, the appropriateness of any given mnemonic will be determined by characteristics of both the learner and the learning situation.

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2.11 Human Spatial Memory and Navigation

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2.11.1 Introduction

Effective navigation abilities are crucial for the survival of almost every living mobile species. They are essential, for instance, for finding the way back to a previously discovered source of food or water, for safely returning home after a sudden change of weather, and for not getting lost in a complex environment such as a cave. For most modern humans, effective navigation skills have become less critical for daily survival, but many common activities, such as getting to work and back home, are nevertheless still characterized by the need to navigate successfully between places.

Successful navigation relies on two capabilities. First, the organism needs to be able to construct enduring internal representations of the locations and identities of significant objects or landmarks in the environment. Second, the organism needs to be able to stay oriented with respect to these represented elements. As the organism moves, the spatial relations between the organism and the elements in the environment constantly change. To remain oriented and to avoid getting lost, spatial updating processes need to be invoked to compensate for those changes.

The goal of this chapter is to review empirical and theoretical advancements in the scientific understanding of human spatial memory and navigation. Our focus is on memories acquired from direct experience, such as vision and locomotion, and on spaces sufficiently large to afford movement, such as translation and rotation, although we also refer to some findings obtained from studies investigating memories of tabletop-sized environments. We are especially interested in the ways memories of familiar environments are used to guide locomotion, reorientation, and wayfinding. Our decision to focus on these topics should not be interpreted to imply that we believe that other types of spatial memories,

such as those obtained from language or indirect sources such as maps, are not important or not interesting. Indeed, for modern humans, navigation based on maps and written works, such as guidebooks, may be at least as important as navigation using one's sense of direction and knowledge of the relations between visible and invisible parts of an environment. Because of space limitations, we were forced to trade breadth against depth of coverage and opted to explore a smaller set of topics in detail, at the expense of several topics equal in importance to those covered here (for reviews of greater scope, see Golledge, 1999; Montello, 2005).

This chapter is divided into nine primary sections. We begin by discussing the elemental types of spatial knowledge: object identity, routes, environmental shape, and survey knowledge. In the second section, we investigate classical and current theories of the acquisition of spatial knowledge. The third section discusses properties of spatial knowledge, such as its hierarchical structure and orientation dependence. In the fourth section, we examine the concept of spatial reference systems and the nature of the spatial reference systems used in spatial memory and navigation. We then turn our attention to the processes and representations that underlie the abilities to guide locomotion and to avoid getting lost. In the sixth section of the chapter, we review contemporary theories of spatial memory and navigation, with an eye for similarities. The seventh section examines the development of spatial memory and navigational capabilities. The eighth and penultimate section looks at the brain networks underlying spatial memory abilities. We close the chapter with a summary and prospectus for future research on human spatial memory and navigation.

2.11.2 Types of Spatial Knowledge

2.11.2.1 Object Identity

The most elemental type of spatial knowledge may be knowledge of the identities and appearances of objects or environmental features (a hill, an intersection of paths, etc.). We use the term object identity to refer to this type of knowledge, recognizing that many entities in an environment that are important for navigation may not be objects in the narrow sense of the term (e.g., path intersection, saddle between two hills). This type of knowledge is sometimes referred to as landmark knowledge (e.g., Siegel and White, 1975), although landmark knowledge is a special case of object knowledge. People know the identities of many objects in their environments that may not serve as landmarks. Landmarks are objects of special significance to spatial memory and navigation (e.g., Couclelis et al., 1987): They are used to indicate the locations of other objects (e.g., the restaurant is in the basement of the Maxwell House Hotel); they may be the goals of navigation (e.g., I am going to Ryman Auditorium); they mark the locations of changes of direction (e.g., turn right at the Gaylord Entertainment Center); and they are used to maintain course (e.g., you will pass Tootsies Orchid Lounge on your right). According to Siegel and White's (1975) theory of the acquisition of spatial knowledge (discussed in the section titled 'Microgenesis of spatial knowledge'), landmark knowledge is the first to be acquired and is the building block of other types of spatial knowledge.

2.11.2.2 Route Knowledge

Route knowledge consists of knowledge of sequences of landmarks and associated decisions and actions. Actions specify the steps needed to get to the next landmark on the route (e.g., turn right at the post office and drive three blocks to the Laundromat). According to Siegel and White's theory, route knowledge does not represent distance, temporal duration, or turning angles early in acquisition. Such metric properties are acquired gradually with experience in an environment.

2.11.2.3 Environmental Shape

The importance of knowledge of environmental shape was discovered relatively recently. Cheng (1986) found that when rats searched for the known location of food in rectangular enclosures they often committed rotational errors in which they searched the correct location and the incorrect location differing from the correct one by 180° of rotation. For instance, if the correct location was in one of the corners, the rotational error would be the corner diagonally opposite to the correct corner. These errors occurred even when nongeometric featural cues, such as visual or tactile patterns, were available that would allow the rat to distinguish the correct location from the rotational error. Similar findings have been observed in many species, including humans (for a review, see Cheng and Newcombe, 2005). There is ample evidence that people are sensitive to environmental geometry when they learn a new environment (e.g., Shelton and McNamara, 2001;

Schmidt and Lee, 2006) and when they reorient and navigate (e.g., Sandstrom et al., 1998; Hartley et al., 2004; Ruddle and Péruch, 2004).

2.11.2.4 Survey Knowledge

Knowledge of the overall configuration of an environment, including knowledge of Euclidean (straight-line) distances and of interpoint directions, defined in a common reference system, makes up survey knowledge. A key feature of survey knowledge is that the spatial relations between locations can be retrieved or inferred even if the organism has never traveled between the locations. Survey knowledge of an environment is often referred to as a cognitive map (a term coined by Tolman, 1948) and likened to physical maps, although such language and parallels imply isomorphisms between the mental and the physical that do not exit. Survey knowledge is considered to be the most sophisticated type of knowledge obtained about an environment (e.g., Siegel and White, 1975). Behaviors taken to be the signature of survey knowledge include the abilities to create efficient routes (e.g., taking shortcuts), to point directly to unseen locations, and to estimate Euclidean distances.

2.11.3 Microgenesis of Spatial Knowledge

The process of the acquisition of spatial knowledge of a new environment has been referred to as microgenesis. The classical theory of the microgenesis of spatial knowledge was proposed by Siegel and White (1975) and it remains the dominant theory in the field (Montello, 1998). According to this theory, the identities and appearances of landmarks are learned first, followed by routes between landmarks. Route knowledge is primarily nonmetric early in acquisition, consisting of the order of landmarks and the appropriate actions to be taken at each one in the sequence. Through experience, route knowledge can acquire metric, or at least approximately metric, properties, such as distance, temporal duration, and turning angles. The most sophisticated form of spatial knowledge is survey knowledge, which is assumed to be derived from accumulated route knowledge (e.g., Thorndyke and Hayes-Roth, 1982).

Although this theoretical framework has been enormously influential, it has not received a great deal of empirical support (for reviews, see Montello, 1998; Ishikawa and Montello, 2006). The limitations of the classical theory are apparent in the findings of a recent study published by Ishikawa and Montello (2006). Participants in this experiment were passively transported by automobile along two routes in a private residential area. The routes passed around and over many hills, and afforded few views of distant landmarks. Learning took place over 10 days (once a week for 10 weeks); on the fourth and subsequent days, participants were transported along a connecting route between the two routes and encouraged to learn the spatial relation between them. Participants' knowledge of the routes and their interrelations was tested using landmark recall, direction estimates, route and Euclidean distance estimates, and map drawing.

Performance was above chance on all tasks after the first session, and near perfect on some, such as landmark sequence recall and route distance estimation. Direction estimates and more difficult distance estimates (e.g., Euclidean estimates within the more complex route) were only moderately accurate and improved modestly over the course of learning. However, substantial individual differences were observed. Some participants performed very well after only one or two sessions and maintained high performance levels on all tasks across all sessions. Another subgroup of participants performed poorly throughout the experiment and showed very little learning on the more challenging tasks, even after 12–14 h of exposure to the routes. Only about half of the participants improved monotonically over the course of learning, and those gains were not large.

These findings largely validate the theoretical distinction between route and survey knowledge, as tasks sensitive to route information, such as landmark sequence recall and route distance estimation, produced similar patterns of results, and tasks sensitive to the layout of the routes, such as Euclidean distance estimation, direction estimates, and map drawing, produced results similar to each other but different from the route tasks. However, these results contradict several key predictions of the classical theory. Landmark knowledge and route knowledge were acquired almost simultaneously. Route knowledge seemed to contain some quantitative information from the very beginning. Even at the earliest stages of learning, participants had some knowledge of the spatial layout of the routes. Finally, although some participants gained more accurate knowledge of the layouts of the routes over the course of learning, few of them could be characterized as having gained accurate survey knowledge of the environments (see also, Gärling et al., 1981; Golledge, 1993).

The evidence on spatial knowledge acquisition is most consistent with Montello's theoretical framework (Montello, 1998; Ishikawa and Montello, 2006). According to this theory, the process of acquiring knowledge of the spatial structure of large-scale environments consists of incremental accumulation of metric knowledge, instead of stage-wise transitions between qualitatively distinct types of spatial knowledge. Spatial knowledge is never limited solely to nonmetric information. This theory emphasizes the importance of knowledge integration – combining knowledge about separately learned places into more complex hierarchically organized representations – in spatial knowledge acquisition. However, even this theoretical framework does not predict or explain the large individual differences observed by Ishikawa and Montello.

2.11.4 Nature of Spatial Knowledge

2.11.4.1 Fragmented

Spatial knowledge is typically fragmented, in the sense that it consists of a patchwork of detailed knowledge of some areas and only sparse knowledge of other, possibly neighboring, areas (e.g., Lynch, 1960; Appleyard, 1970). Survey knowledge never has the property of being of uniformly high fidelity for all familiar areas.

2.11.4.2 Distorted

A second key property of spatial knowledge is that memories of spatial relations, such as distances, angles, and orientation, often differ from the physical values in systematic and predictable ways (e.g., Tversky, 1992, 2000). As discussed in several sections of this chapter, such distortions have played a prominent role in the development of theories of spatial memory.

Estimates of Euclidean distances are greater when locations are separated by a barrier or boundary (e.g., Kosslyn et al., 1974; Newcombe and Liben, 1982; McNamara, 1986) and tend to increase with the clutter between the locations (e.g., Thorndyke, 1981). Boundary effects occur even when the boundaries are subjective (e.g., McNamara et al., 1989; Carbon and Leder, 2005). Estimates of route distance increase with the number of turns (e.g., Byrne, 1979; Sadalla and Magel, 1980) and the number of intersections (e.g., Sadalla and Staplin, 1980). Distance estimates are also asymmetric under certain circumstances (e.g., Sadalla et al., 1980; McNamara and Diwadkar, 1997; Newcombe et al., 1999). In particular, distances from less salient locations or objects to more salient locations or objects (i.e., landmarks or reference points) are underestimated relative to the reverse. Angles of intersection between roads are remembered as being closer to 90° than they are in reality (e.g., Byrne, 1979; Tversky, 1981; Moar and Bower, 1983; Sadalla and Montello, 1989). Disparate regions of space, such as states or continents, are remembered as being aligned with each other, and individual regions of space are remembered as being oriented with canonical reference axes (e.g., Stevens and Coupe, 1978; Tversky, 1981). For instance, people believe that North America and South America are vertically aligned, even though the east coast of the U.S. is roughly aligned with the west coast of South America, and that the Bay Area of Northern California is oriented north-south, even though it actually is oriented along a north-west/south-east axis (Tversky, 1981). These biases produce systematic errors in judgments of the relative directions between objects and cities.

2.11.4.3 Hierarchical

There is strong evidence that memories of the locations of objects in the environment are organized categorically and hierarchically, such that a region of space may be represented as a whole, containing other regions and locations, and as a part, contained in larger regions. One indication that spatial memories are hierarchical is that judgments of the spatial relations between cities or objects are affected by the spatial relations between superordinate regions (e.g., Stevens and Coupe, 1978; Tversky, 1981; McNamara, 1986). For instance, in Stevens and Coupe's (1978) experiments, Reno was judged to be northeast of San Diego, even though it is actually northwest. According to hierarchical models of spatial memory, this error occurs, at least in part, because people represent Reno in Nevada, San Diego in California, and Nevada east of California. These spatial relations imply that Reno should be east of San Diego. Other evidence consistent with the hierarchical representation of space includes the effects of boundaries on distance estimations (cited previously), the effects of region membership on judgments of orientation (e.g., Wilton, 1979; Maki, 1981) and proximity (e.g., Allen, 1981), and errors in estimates of latitude, bearing, and distance at global scales (e.g., Friedman and Brown, 2000; Friedman et al., 2002; Friedman and Montello, 2006).

Even stronger evidence for hierarchical representations can found in studies in which task performance is shown to depend on the structure of explicit hierarchical models of spatial memory (e.g., Hirtle and Jonides, 1985; Huttenlocher et al., 1991; McNamara, 1986; McNamara et al., 1989). For instance, McNamara et al. (1989, Experiment 1) required subjects to learn the locations of objects in a large room; the objects were unrelated, and there were no physical or perceptual boundaries in the space. After learning, subjects were asked to recall all of the objects several times, to estimate distances between pairs of objects, and to take part in an item recognition test in which the measure of interest was spatial priming (e.g., McNamara et al., 1984). The latent hierarchical structure in each subject's recall protocols was modeled with the ordered-tree algorithm (e.g., Reitman and Rueter, 1980). An example is illustrated in **Figure 1**. Distance estimations and spatial priming were conditionalized on whether pairs of objects were in the same or different subtrees (e.g., ruler–coin vs. envelope–truck), controlling for Euclidean distance. Different subtrees were assumed to correspond to different subjective regions of space. Subjects underestimated distances between pairs of objects in the same subjective region relative to pairs of objects in different subjective regions, and spatial priming was greater between pairs in the same subjective region than between pairs in different subjective regions. Additional analyses showed that spatial priming increased with the depth at which object pairs were clustered (e.g., ruler–coin vs. ruler–pen vs. ruler–screw). These findings provide strong evidence that spatial memories are organized hierarchically, even when the layout lacks explicit perceptual organization.

The hierarchical structure of spatial memory affects navigation behavior, at least in virtual environments. Wiener and Mallot (2003) found that people minimized the number of region boundaries

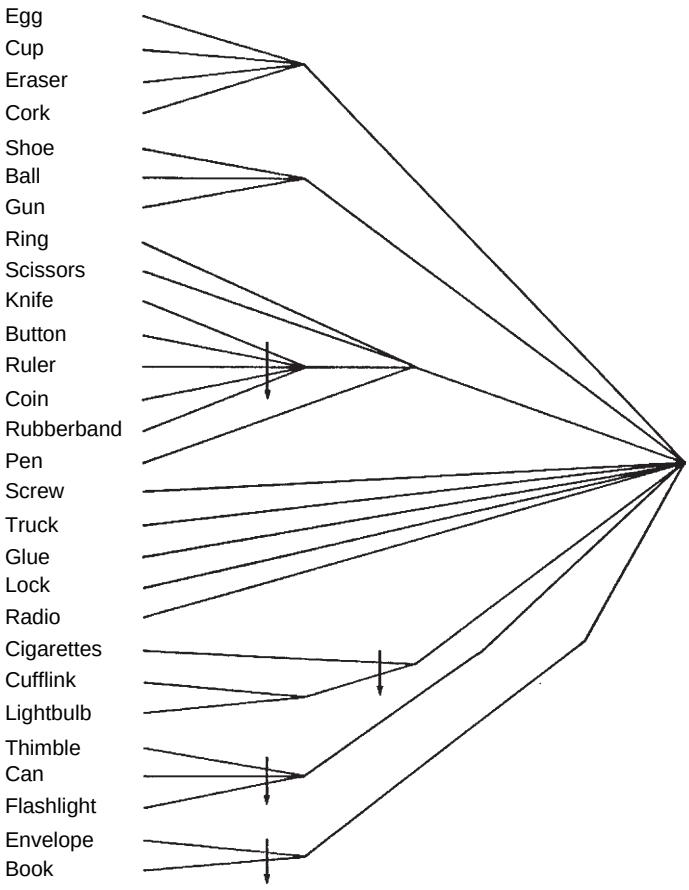

Figure 1 Ordered tree generated from recall protocols for a single participant. Reprinted with permission from McNamara TP, Hardy JK, and Hirtle SC (1989) Subjective hierarchies in spatial memory. *J. Exp. Psychol. Learn. Mem. Cogn.* 15(2): 211–227.

crossed when navigating to a goal location and that they tended to choose paths that permitted the quickest access to the region containing the goal location. Wiener et al. (2004) showed further that subjects learned environments faster and searched more efficiently when environments were divided into regions than when they were not. This improvement was on the order of a factor of 2. Their results also revealed that navigation strategies seemed to depend on the alignment of the dominant reference directions between different levels of the hierarchical mental representation (see also, Werner and Long, 2003; Werner and Schindler, 2004). (The concept of spatial reference directions and axes will be explored in detail in the section 'Spatial reference systems'.)

2.11.4.4 Orientation Dependent

It is well documented that long-term spatial memory is orientation-dependent (see McNamara, 2003, for a review). People recall and recognize interobject spatial relations more efficiently from some perspectives than from others. These privileged perspectives are usually aligned with (parallel or orthogonal to) experienced points of view (e.g., Shelton and McNamara, 2001) but also may be aligned with salient intrinsic axes of the array of objects (e.g., Mou and McNamara, 2002; Mou et al., 2007). Typical results are illustrated in **Figure 2**. There is evidence that spatial memories also may be viewpoint-dependent (e.g., Easton and Sholl, 1995; Waller, 2006; Valiquette and McNamara, 2007). Behaviorally this means that performance is better when the test perspective matches the location of the observer at the time of learning in addition to his or her orientation.

Orientation-independent performance has been observed in several published investigations of spatial memory (e.g., Evans and Pezdek, 1980; Presson and Hazelrigg, 1984; Presson et al., 1989; Sholl and Nolin, 1997, Experiments 3 and 4; Richardson et al., 1999, real-walk condition). McNamara (2003) discusses possible limitations of these studies in some detail. One important feature of those studies (with the exception of Evans and Pezdek's) is that only two orientation conditions were compared: The perspective parallel to and in the same direction as the learning view (0°) and the perspective differing by 180°. This fact may be important because task performance for the imagined heading of 180° is often much better than performance for other novel headings, and can be nearly as good as that for the learning view (e.g., Hintzman et al., 1981; Mou and McNamara, 2002). The cause of this effect is not known, but people may sometimes represent, at least partially, the spatial structure of the layout in the direction opposite to the learning view (Mou et al., 2004). It is also possible that people are able to capitalize, under certain conditions, on the fact that arrays of objects may have high self-similarity under rotations of 180° (e.g., Vetter et al., 1994). Investigations of the orientation dependence of spatial memories are at a distinct disadvantage if only the learning view and its opposite are compared.

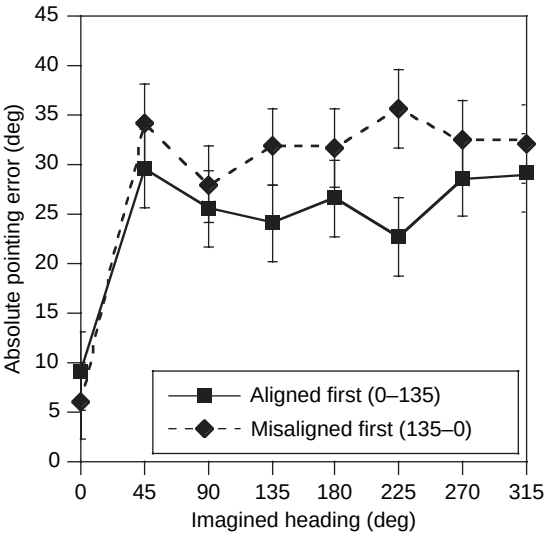

Figure 2 Results of Shelton and McNamara's (2001) third experiment. Participants learned the layout of seven objects in a room from two points of view (counterbalanced across participants): One view (0°) was aligned with salient environmental reference frames (e.g., walls of the room, square mat on which the objects were placed), and the other (135°) was misaligned with these features. Absolute pointing error in subsequent judgments of relative direction is plotted as a function of imagined heading, separately for the two learning-order groups. Results show that participants represented the layout using a single reference direction parallel to the aligned view and demonstrate the importance of environmental frames of reference in the selection of reference directions in memory. From Shelton AL and McNamara TP (2001) Systems of spatial reference in human memory. *Cogn. Psychol.* 43(4): 274–310.

2.11.5 Spatial Reference Systems

Spatial reference systems are essential for the specification of location and orientation in space. The location of Murfreesboro, Tennessee, for example,

can be specified by describing its position with respect to the boundaries of the state (e.g., Murfreesboro is in the center of Tennessee), by providing coordinates of latitude and longitude on the surface of the earth (e.g., Murfreesboro is located at 35°55′ N and 86°22′ W), or by describing its position relative to an observer (e.g., Murfreesboro is 31 miles to the first author's left as he writes this paragraph). People represent in memory the spatial properties of many familiar environments. Just as spatial reference systems are required to specify the locations of objects in physical space, so too spatial reference systems must be used by human memory systems to represent the remembered locations of objects in the environment.

A spatial reference system is a relational system consisting of reference objects, located objects, and the spatial relations that may exist among them (e.g., Rock, 1973, 1992; Talmy, 1983). The reference objects may be any objects whose positions are known or established as a standard and may include the observer, other objects in the environment, abstract coordinate axes, and so forth. Note that, according to this definition, a reference frame consisting of orthogonal axes is just one of many types of spatial reference systems. Many schemes for classifying spatial reference systems have been proposed (e.g., Hart and Moore, 1973; Paillard, 1991; Pani and Dupree, 1994; Levinson, 1996; Tversky et al., 1999). For the purposes of understanding the use of spatial memories in navigation and other actions in space, it is useful to distinguish egocentric and environmental reference systems (e.g., Klatzky, 1998). In this chapter, we consider environmental and allocentric reference systems to be equivalent.

Egocentric reference systems specify location and orientation with respect to the organism, and include eye-, head-, and body-based coordinate systems (e.g., Andersen et al., 1997). Returning to the previous example, the description of Murfreesboro's location relative the first author of this chapter uses an egocentric reference system.

Environmental reference systems define spatial relations with respect to elements of the environment, such as the perceived direction of gravity, the sun's azimuth, landmarks, or the walls of a room (e.g., Wehner et al., 1996). Abstract reference systems, such as coordinates of latitude and longitude, also qualify as environmental reference systems. An important subcategory of environmental reference systems are intrinsic reference systems. Intrinsic reference systems can be centered on an object (e.g., Rock, 1973; Marr, 1982). In such cases, the objects usually have inherent facets, such as natural fronts, backs, tops or bottoms, that can be used to define reference axes. The human body is a paradigmatic example. Intrinsic reference systems can also be defined by features of a collection of objects (e.g., Tversky, 1981; Mou and McNamara, 2002). The rows and columns formed by chairs in a classroom constitute an intrinsic reference system. Intrinsic reference systems also may be defined by less explicit perceptual organization, such as an axis of bilateral symmetry or the mutual alignment of several objects (e.g., Mou et al., 2007). An example is illustrated in **Figure 3**.

The primate brain represents the locations of objects in space using egocentric and environmental reference systems (e.g., Andersen et al., 1997; Snyder et al., 1998; Matsumura et al., 1999), and human navigation depends on both egocentric and environmental representations of the environment. Actions such as walking through doorways and other apertures, staying on paths, and avoiding obstacles require the computation of precise self-to-object spatial relations to guide locomotion (e.g., Rieser and Pick, 2006). But planning a route to a distant goal, and maintaining a sense of orientation in large-scale environments, would seem to require enduring representations of the locations of objects relative to other objects (e.g., Loomis and Beall, 1998). Contemporary theories of human spatial memory and navigation specify roles for both egocentric and environmental representations of space, and will be reviewed in detail in the section titled 'Models of spatial memory and navigation'.

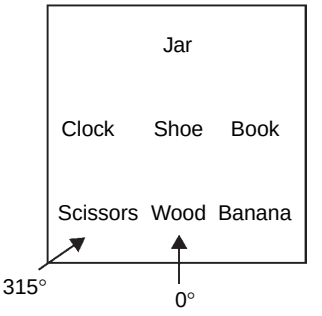

Figure 3 Layout used by Mou and McNamara (2002). Zero degrees is an axis of bilateral symmetry, increasing the salience of that axis and therefore the probability that it will be selected as a reference direction, even if participants study the layout from a different direction such as 315°. Reprinted with permission from Mou W and McNamara TP (2002) Intrinsic frames of reference in spatial memory. *J. Exp. Psychol. Learn. Mem. Cogn.* 28(1): 162–170.

The concept of spatial reference systems proves useful for accounting for two key properties of spatial knowledge. The orientation dependence of spatial memories indicates that the spatial layout of an environment is mentally represented using a dominant reference direction (e.g., Shelton and McNamara, 2001). Interobject spatial relations that are specified with respect to this reference direction can be retrieved, whereas other spatial relations must be inferred (e.g., Klatzky, 1998), introducing costs in latency and errors. The preferred directions in judgments of relative direction, for example, correspond to intrinsic directions in the layout that are experienced or are highlighted by instructions or layout geometry (e.g., Shelton and McNamara, 2001; Mou and McNamara, 2002; Mou et al., 2007). These preferred directions correspond to the dominant reference directions. A simple model of this form that accounts for orientation dependence in judgments of relative direction is illustrated in **Figure 4**.

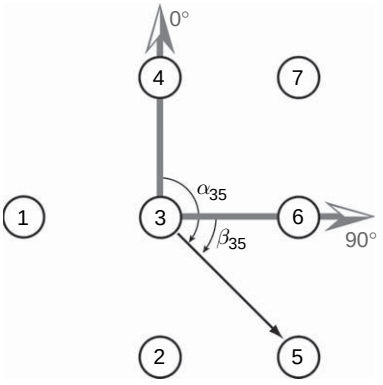

Figure 4 Schematic depiction of an orientation dependent model of enduring spatial memory. Circles symbolize the represented objects. Interobject spatial relations are symbolized by vectors; for simplicity, only the spatial relation between objects 3 and 5 is represented. Grey arrows symbolize reference directions in the representation. The angular relation from object 3 to object 5 is represented with respect to the 0° (α_{35}) and the 90° (β_{35}) reference directions. Because the direction from object 3 to object 5 relative to 0° is explicitly represented in memory, a task such as "Imagine you are standing at 3 and facing 4. Point to 5" is relatively easy, because that direction can be retrieved from memory. A task such as "Imagine you are standing at 3 and facing 7. Point to 5" in contrast, is relatively difficult, because the spatial relation between objects 3 and 5 is not represented relative to 45° and, therefore, must be inferred, which produces measurable performance costs. An important feature of the model is that it uses an environmental reference system, yet produces orientation-dependent performance.

The second key property explained by spatial reference systems is the hierarchical structure of spatial knowledge. This property may result from the use of spatial reference systems at multiple scales (e.g., Poucet, 1993). A region of space that can be viewed in its entirety from a single vantage point with minimal locomotion (vista scale as defined by Montello and Golledge, 1999) may be represented in a locally defined spatial reference system. Spatial reference systems used in neighboring regions of space may be interrelated in higher-order reference systems in which the local reference systems serve as elements. For instance, the spatial layout of each of the rooms in a house may be specified in a spatial reference system unique to the room. These spatial reference systems may serve as elements in a higher-order reference system defining the spatial relations among the rooms.

Reference systems within the same level and between levels of the hierarchy need not use common reference directions; that is, conceptual north in one region may or may not correspond to conceptual north in a neighboring region. The acquisition of skills attributed to the possession of survey knowledge, such as pointing accurately to unseen targets, may occur when the reference directions in such locally defined reference systems become integrated in such a way that all are aligned (Montello and Pick, 1993). Werner and colleagues (Werner and Long, 2003; Werner and Schindler, 2004) have shown that misalignment of reference directions in such a reference system hierarchy impairs way-finding performance and produces less accurate knowledge of interobject directions. Experiments reported by Wang and Brockmole (2003a) provide evidence that people maintain orientation with respect to a single reference system as they navigate. They had participants walk from a room in a building on a college campus to the outdoors and then back inside to the room. When oriented with respect to the room, participants lost track of their orientation with respect to the campus, and when oriented with respect to the campus, they lost track of their orientation with respect to the room (see also, Wang and Brockmole, 2003b).

2.11.6 Navigation and Spatial Updating

The processes and representations used in human navigation can be divided into three categories. Navigation that depends solely on the organism's

history of self-movement is referred to as path integration, or dead reckoning. By integrating velocity or double integrating acceleration with respect to time, an organism can estimate its displacement from a starting location (e.g., Gallistel, 1990). Cues to velocity or acceleration can be internal (e.g., proprioception, efference copy, vestibular cues) or external (e.g., optic or acoustic flow); however, to the extent that external cues are used, path integration is limited to situations in which external cues do not provide information about the organism's position in the environment (Philbeck et al., 2001). Wayfinding (or piloting), by contrast, refers to navigation that depends jointly on an enduring external or internal spatial representation (a map or cognitive map, respectively) and the observation of objects whose locations are specified in the spatial representation (e.g., Gallistel, 1990). The key feature of wayfinding is that the organism uses a representation of the layout of an environment and its perception of objects in that environment to find or orient with respect to objects that cannot be observed. Both path integration and wayfinding may require the organism to pass through apertures (e.g., doorways), follow paths, avoid obstacles and hazards, and so forth. This form of navigation has been referred to as steering (e.g., Rieser and Pick, 2006). Steering requires an organism to guide locomotion in relation to the perceived locations of objects but does not rely on an enduring external or internal spatial representation of the environment.

In general, as an organism locomotes through an environment, it must keep track of its location with respect to objects in the immediate environment and to unseen portions of the environment, to avoid obstacles, to remain oriented, and to navigate to distant goals (Loomis and Beall, 1998; Montello, 2005). These processes are referred to as spatial updating. Experimental research on human navigation has typically been aimed at trying to uncover the mental representations and processes used in spatial updating more broadly, and with the exception of work on path integration, does not divide neatly into categories corresponding to the three types of navigation.

Path integration is often investigated with triangle completion, or return-to-home tasks. An illustrative study was reported by Klatzky et al. (1990). Blindfolded participants were guided along paths of varying complexity and then required to walk back to the starting points. The paths consisted of one to three linear segments, separated by turns. The dependent variables were the angular turn participants made toward the origin and the distance they walked toward it. Participants' errors on both measures were low for the one-leg path and increased with the increasing number of segments. In general, path integration in humans is only moderately accurate and becomes less so as path complexity increases (e.g., Loomis et al., 1993, 1999; Cornell and Heth, 2004; Foo et al., 2005).

Nonvisual spatial updating has also been investigated in tasks that require the participant to keep track of multiple objects simultaneously. For example, Rieser et al. (1986) asked participants to study the locations of five objects in a room and then point to them while blindfolded. Participants were subsequently escorted to a novel position from which they had to point to the objects again. Locomotion resulted in small, nonsignificant updating costs relative to baseline (e.g., Rieser, 1989).

The results from these and similar paradigms suggest that humans are capable of relatively efficient updating when they move without vision, provided that the movement trajectory is not very complex. The increase in error for more prolonged movement is compatible with the assumption of an updating process that does not operate with perfect accuracy and accumulates error over the course of movement.

Spatial updating necessarily involves computations that compensate for the changes in observer–environment relations caused by locomotion. A number of studies have been conducted to identify which of the cues that are normally associated with physical locomotion are sufficient for efficient spatial updating. Purely imaginary locomotion typically produces inefficient spatial updating (e.g., Rieser et al., 1986; Rieser, 1989; but see, Wraga, 2003). Optic flow appears to be insufficient for efficient spatial updating (e.g., Chance et al., 1998; Klatzky et al., 1998; Péruch et al., 1997; but see Kearns et al., 2002; Riecke et al., 2002). A prerequisite for efficient updating seems to be that the person's physical position changes (Ruddle and Lessels, 2006). Whether this position change is accomplished through passive transport, which primarily provides vestibular cues, or through active movement, which provides additional proprioceptive and efferent cues, does not matter in many circumstances (e.g., Wang and Simons, 1999; Wraga et al., 2004). There is, however, evidence that those additional cues become beneficial when the movement trajectory is more complex (e.g., Sholl, 1989; Yardley and Higgins, 1998; Waller et al., 2004).

Evidence indicates that spatial updating during physical locomotion has two properties commonly attributed to automatic processes. First, it seems to require little attentional effort (e.g., Amorim et al.,

1997; Wang, 2004). Second, the changes in observer–environment spatial relations produced by physical locomotion are difficult to ignore (e.g., Farrell and Robertson, 1998; Farrell and Thomson, 1998; Wang and Simons, 1999; May and Klatzky, 2000; Waller et al., 2002). For example, in Farrell and Robertson's experiment, participants were required to rotate to a novel orientation, but point to objects as if they were still facing their initial orientation. Performance was as poor in this ignore-rotation condition as in the imagined rotation condition, indicating that participants were unable to voluntarily refrain from updating.

Another important property of automatic processes is their relative insensitivity to processing load. The evidence on the capacity limits of spatial updating is mixed. Results of at least one study indicate that spatial updating deteriorates in accuracy as the number of objects increases (Wang et al., 2006), whereas findings from other studies indicate that spatial updating is capacity-free (Rieser and Rider, 1991; Hodgson and Waller, 2006). This pattern of results has led some researchers to distinguish two forms of updating, one that occurs on-line and relies on working memory and another that occurs off-line and relies on long-term memory (e.g., Amorim et al., 1997; Cornell and Greidanus, 2006; Hodgson and Waller, 2006). This distinction is embodied in several theories of spatial memory and navigation (discussed in the section titled 'Models of spatial memory and navigation').

A common interpretation of the advantage in spatial updating produced by physical locomotion in the absence of vision (e.g., walking while blindfolded) relative to imagined locomotion is that body-based information facilitates the transformations needed to update observer–environment spatial relations (e.g., Rieser, 1989; Chance et al., 1998; Farrell and Robertson, 1998). This facilitation may result from the transfer of learned relationships between action and perception to relationships between action and representations (e.g., Rieser et al., 1995; Pick et al., 1999; Rieser, 1999; Rieser and Pick, 2006). The idea is that people learn the consistent covariations between their actions and the resulting changes in the appearance of the environment. This tight coupling of action and perception is proposed to be the basis for a coupling of action and representation. When a person moves without vision, he or she can use the learned covariation between biomechanical cues from locomotion and the changes in environmental flow to update the self-to-object relations at a representational level. It is argued that, by utilizing this learned covariation during locomotion, people can access the changing self-to-object relations directly rather than having to go through effortful cognitive computations.

An alternate account posits that the difficulty of updating after imagined movements results from interference that is caused by a conflict between the awareness of one's physical position in an environment and the discrepant position one has to adopt in imagination (e.g., May, 1996, 2004). May (2004) has proposed that interference arises from conflicts between object location codes at the sensorimotor level, which are specified relative to the physical position, and object location codes at the cognitive level, which are specified relative to the imagined position. Consistent with this hypothesis, pointing to objects from imagined facing directions is worse when people are oriented than when they are disoriented (e.g., May, 1996; Waller et al., 2002). The interference hypothesis is also supported by the finding that performance in both imagined rotations and imagined translations degrades monotonically as a function of object-direction disparity (May, 2004), which is defined as the magnitude of the difference between (1) the direction of the to-be-retrieved object relative to the imagined position and (2) the direction of the to-be-retrieved object relative to the physical position. To account for the finding that imagined rotations are more difficult than imagined translations (e.g., Rieser, 1989; Presson and Montello, 1994), even when object direction disparity is equated, May (2004) proposed a second source of interference that only applies to imagined rotations. This second source of interference, referred to as head-direction disparity, reflects conflicts that arise from having to specify an object direction relative to an imagined heading that is different from one's physical heading (e.g., Mou et al., 2004).

May (2004) has also shown that providing participants with additional time between the presentation of the to-be-imagined position and the presentation of the target object improved overall performance, but did not mitigate the effect of object-direction disparity in either imagined rotations or imagined translations. This finding indicates that the spatial transformations required for effective spatial updating cannot be performed efficiently in working memory, even if they are somehow facilitated by physical locomotion.

In summary, extant findings indicate that spatial updating during imagined locomotion is difficult in part because knowledge of object locations relative to the actual position interferes with knowledge of object locations relative to the imagined position.

But in addition, imagined spatial updating does not benefit from facilitative transformations provided by physical locomotion.

2.11.7 Models of Spatial Memory and Navigation

Cognitive models of spatial memory and navigation attempt to explain how the spatial structure of an environment is represented in memory and how memories of familiar environments are used to guide navigation. All of the models employ both egocentric and environmental representations of space, and although there are important differences between models in the nature of those representations and in the ways they are used to maintain orientation and guide navigation, the models are fundamentally quite similar.

These models include an egocentric system that computes and represents self-to-object spatial relations needed for spatially directed motor activity, such as walking, reaching, and grasping. In the models proposed by Burgess and colleagues (e.g., Burgess, 2002; Burgess et al., 2001; Byrne et al., 2007), Mou and colleagues (Mou et al., 2004, 2006) and Waller and Hodgson (2006), spatial relations represented in this system are transient and decay rapidly in the absence of perceptual support or deliberate rehearsal. In Sholl's model (Sholl and Nolin, 1997; Sholl, 2001; Holmes and Sholl, 2005) and in Wang and Spelke's model (2002), this system is dynamic but can represent more enduring egocentric self-to-object spatial relations. Recent evidence implicates the role of a transient egocentric system in spatial updating, but this evidence is far from definitive (Mou et al., 2006; Waller and Hodgson, 2006).

The second major system in all of the models is an environmental (allocentric) system. Wang and Spelke's model is perhaps the most unusual, in that the environmental system in this model only represents environmental shape. It is difficult to reconcile this aspect of the model with the abilities of people to judge interobject distances and directions using long-term memories of the layouts of environments. The other major difference among models, at least among those which specify the nature of the reference systems used in the environmental system, is whether the spatial reference system is orientation-dependent or -independent. Mou and McNamara argue that the environmental system uses an intrinsic reference system (as discussed in the section titled 'Spatial reference systems'). Sholl, in contrast, claims that an orientation-independent reference system is used, at least in well-learned environments. Sholl's model would seem to have difficulty accounting for the large body of evidence demonstrating orientation-dependent performance in spatial memory tasks, even for well-learned environments, as reviewed previously.

Finally, Wang and Spelke's model includes a third system in which the appearances of familiar landmarks and scenes are represented. These representations are viewpoint-dependent and can be conceived of as visual-spatial snapshots of the environment (e.g., Diwadkar and McNamara, 1997; Wang and Simons, 1999; Burgess et al., 2004). Valiquette and McNamara (2007) recently attempted to find evidence for such a system and to determine whether it could be distinguished from an environmental system. They asked participants to learn the locations of objects in a room from two points of view, one of which was aligned with salient environmental frames of reference (the mat on which the objects were placed and the walls of the room), and the other of which was misaligned with those same frames of reference (i.e., a view from the corner of the room). Participants then took part in judgments of relative direction (e.g., 'Imagine you are standing at the shoe, facing the lamp; point to the banana') and old–new scene recognition. Performance in judgments of relative direction was best for the imagined heading parallel to the aligned learning view and no better for the imagined heading parallel to the misaligned learning view than for unfamiliar headings. This pattern of orientation-dependent performance replicates previous findings (e.g., Shelton and McNamara, 2001; Valiquette et al., 2007). Performance in scene recognition, however, was equally good for the two familiar views and better for familiar than for novel views (see also Waller, 2006). These findings are consistent with a model in which interobject spatial relations are represented in an environmental system using intrinsic reference systems, as specified in Mou and McNamara's model, and visual memories of landmarks and scenes are stored in a viewpoint-dependent system, as specified in Wang and Spelke's model.

This viewpoint-dependent system may account for the effectiveness of the look-back strategy in wilderness navigation (e.g., Cornell et al., 1992). Routes often look quite different coming and going, leading to navigational errors on the return trip. The look-back strategy involves occasionally stopping and turning around to view one's route in the opposite direction while navigating in unfamiliar wilderness environments. These look-back views

may be stored in the viewpoint-dependent system and support place recognition when returning.

Spatial updating in these models takes place at two levels. Self-to-object spatial relations are continuously and efficiently updated in the egocentric system as a navigator locomotes through an environment. This updating process supports steering and interactions with objects in the environment. At the same time, the navigator must update a representation of his or her position in the environment, to remain oriented and to locate distant goals. This updating process takes place in the environmental system. According to Mou and McNamara, navigators update their position with respect to the intrinsic reference system used to represent the spatial structure of the local environment. Sholl's model is the most explicit about the environmental updating process. In this model, the egocentric system is referred to as the self-reference system, and it codes self-to-object spatial relations in body-centered coordinates, using the body axes of front–back, right–left, and up–down (e.g., Franklin and Tversky, 1990; Bryant and Tversky, 1999). The engagement of the self-reference system with the physical environment determines the position of a representation of the self-reference system in the environmental system. As a person moves in the environment, the axes of the representational self-reference system are moved to the corresponding new position in the environmental system representation.

To a significant degree, these models primarily describe the perceptual-cognitive architecture of the human spatial memory and navigation system. For this reason, they have varying amounts to say about the various topics covered previously in this chapter. All are intimately concerned with object location, survey knowledge, spatial reference systems, and spatial updating. But none of these models has much to say about route knowledge, the microgenesis of spatial knowledge, or the nature of spatial knowledge (e.g., distortions). An important direction for future research will be to extend these models to account for a broader array of findings in the spatial memory and navigation literature.

2.11.8 The Developmental Foundations of Navigation

Decades of research have revealed a host of burgeoning spatial abilities during the first few years of life. These developments are most likely intimately coupled with changing motor abilities (for discussion, see Campos et al., 2000) and, toward the end of infancy, symbolic capabilities such as language. In this section, we focus on the development of rudimentary abilities necessary for navigation (for a recent and more comprehensive review, see Newcombe and Huttenlocher, 2006). Specifically, we discuss the development of two of the elemental types of spatial knowledge: object location (including landmarks) and environmental shape. Route and survey knowledge follow later in development (e.g., Allen et al., 1979) and most likely depend on these earlier abilities. In addition, we consider what children's responses in various situations reveal about early use of egocentric and environmental spatial frames of reference.

2.11.8.1 How Children Use Objects and Landmarks

In the first months of life, infants can locate objects through response learning, which involves learning the association between a bodily response (e.g., an eye movement or a reach) and a particular position in space. For example, an infant may learn that lying in her crib she can turn her head to the left to see a colorful toy. Response learning illustrates a very simplistic egocentric reference system – one that does not take self-movement into account. In order to locate objects after movement through space, infants must be capable of what Rieser (2000) has called dynamic spatial orientation, which requires awareness of one's changing orientation with respect to the world.

Early studies have suggested limitations on the infants' ability to keep track of an object's location during self-movement. Acredolo (1978) examined 6-, 11-, and 16-month-olds in the following task. Infants first learned that an auditory cue signaled an interesting event in one of two windows (either on the left side of the room or on the right side of the room for each infant). Infants were then carried on a semicircular path to the opposite side of the room. Only 16-month-olds looked toward the correct window when the cue sounded, whereas younger infants continued to look toward the egocentric side on which the event had occurred earlier. Similar results were obtained by Bremner and Bryant (1977), who found that 9-month-olds continued to search for an object on the egocentric side of a table after being moved to the opposite side of the table.

These initial studies seemed to suggest that sometime during the second year there is a transition from response learning (not taking movement into account) to spatial updating (taking movement into account). However, it is likely that even very young infants use spatial updating when simple forms of movement, such as rotation about the trunk or tilting with respect to gravity, are involved (e.g., Rieser, 1979; Landau and Spelke, 1988). Furthermore, Newcombe and Huttenlocher (2000) have argued that spatial development is most likely characterized by an increased weighting of relevant cues rather than by the appearance of wholly new abilities (for evidence in older children, see Hund and Spencer, 2003). As infants become more mobile and can perform more complex actions in larger environments, cues such as self-movement and landmarks become increasingly relevant.

Early studies have also examined infants' ability to use landmarks as direct cues to locating objects. A direct landmark is one that is either contiguous with or adjacent to some target, thus serving as a beacon for the target location. (Because no coding of distance or angular information is necessary, use of direct landmarks is technically associative rather than spatial in nature.) In contrast is an indirect landmark, which is distant enough from a target that both are not visually available at the same time; consequently, in order to use an indirect landmark a viewer must represent the spatial relations between it and the target location. Acredolo and Evans (1980) explored the landmark use of 6-, 9-, and 11-month-olds. The task was similar to that used by Acredolo (1978) in that infants were carried to the opposite side of the room before searching for an event in a left or right window. Nine- and 11-month-olds clearly benefited from the presence of a landmark that surrounded the correct window, whereas 6-month-olds did not. A consistent finding was that of Bremner (1978), who found that 9-month-olds who moved to the opposite side of the table were more likely to search on the correct side for an object if the left and right hiding places were noted by a black cover and a white cover. Such findings seemed to indicate that, before they are capable of spatial updating, infants are able to use direct landmarks to locate targets. Additionally, when landmarks are highly salient, even 6-month-olds sometimes use them in locating target objects or events (Rieser, 1979; Lew et al., 2004).

Toward late infancy, humans show evidence of using landmarks in the surrounding environment in complex ways. Newcombe et al. (1998) examined children between the ages of 16 and 36 months in a task that required them to locate a toy in a long rectangular sandbox. Success required distance coding (in the continuous space of the sandbox) rather than the categorical coding involved in many earlier studies (e.g., at the left or right window). The children searched either with a circular curtain surrounding them (thus, with no indirect landmarks visible) or without the curtain (thus, in full view of surrounding landmarks in the room). After children watched an experimenter hide a toy in the sand, they moved to the opposite side of the box to perform the search. Children older than 22 months were more accurate when indirect landmarks were visible, whereas the youngest children performed the same whether the landmarks were visible or not. These data suggested that toward the end of infancy children begin to use indirect landmarks to guide navigation (see also DeLoache and Brown, 1983; Bushnell et al., 1995).

While Newcombe et al. (1998) argued that the indirect landmarks aided children's search, it is also possible that children were using the shape of the room (see discussion in section titled 'How children use environmental shape'). Consistent with this latter argument is a recent study by Nardini et al. (2006), who found that 3-year-olds were able to use the shape of the room during a search task that involved indirect landmarks, but not until 5 years did children seem to use the actual landmarks. In fact, there is recent evidence to indicate that young children do not represent landmarks in an environmental reference system.

In a series of experiments, Gouteux and Spelke (2001) examined preschoolers' ability to search for a target that was hidden inside one of several identical landmarks within a room. When landmarks were identical, the configuration (a triangle in some experiments and a rectangle in others) was the only available spatial information. The critical trials took place after children were disoriented within the search space. Across all experiments, children failed to use the configuration specified by the arrangement of landmarks. In contrast, when landmarks were differentiated, children were successful in locating the target. Gouteux and Spelke (2001) noted that the landmarks could have served as beacons for the target location rather than as cues to reorientation within the space.

Lee et al. (2006) explored this latter possibility. Four-year-olds searched for an object among three landmarks that formed an equilateral triangle – thus,

the geometric information alone was uninformative; two of the landmarks were identical. As in Gouteux and Spelke's (2001) experiments, children were disoriented before beginning their search. Children successfully retrieved objects that were hidden at the distinctive landmark; however, when objects were hidden at one of the two identical landmarks, children searched at each of those two landmarks with equal frequency. Lee et al. (2006) argue that children can use landmarks as beacons for target locations, but do not use them to reorient to the locations of other landmarks (see also, MacDonald et al., 2004). These findings are consistent with researchers who have argued that humans keep track of discrete objects egocentrically (Wang and Spelke, 2000; Wang et al., 2006). Once these egocentric relations are disrupted, humans cannot use individual objects to reorient to the locations of other objects. The validity of this claim has been a matter of dispute in the adult literature (see discussion in section titled 'Models of spatial memory and navigation'). However, children at least do seem to have difficulties remembering the locations of objects with respect to other objects.

2.11.8.2 How Children Use Environmental Shape

When toddlers and older children see an object hidden in one corner of a rectangular space and then undergo a disorientation procedure, they search equally in the correct corner and in the geometrically equivalent corner (Hermer and Spelke, 1994; Learmonth et al., 2001, 2002; see for a discussion Cheng and Newcombe, 2005). Since there is no spatial information available other than the shape formed by the walls of the room, these data clearly demonstrate that, by the time they can walk, humans use the shape of extended surfaces to reorient when lost and to locate desired objects. Furthermore, children's use of geometric information in extended surfaces generalizes to situations in which they are translated outside of the space before searching (Lourenco et al., 2005) and to spaces that are not rectangular (Huttenlocher and Vasilyeva, 2003; Hupbach and Nadel, 2005). Finally, the knowledge of geometric shape must be stored in an environmental reference system, since the disorientation would have disrupted any self-to-surface representations. The shape of extended surfaces, in contrast to object location, seems readily represented in an environmental reference system early in development.

When geometric information is ambiguous, combining that information with other sources of information can be a powerful tool. One question is whether children can combine information about the shape of a room with featural information, unlike rats and other species, which cannot combine these two sources of information (see discussion in the section titled 'Environmental shape'). Hermer and Spelke (1994) examined 3- and 4-year-old children in the following task. Children watched as an object was hidden in a corner of a rectangular room, were disoriented, and then were allowed to search for the object. The researchers found that when one of the walls was blue, making the correct choice of corner unambiguous, young children did not search with greater frequency in the correct corner. Since adults have no difficulty combining the geometry of the room with landmark information, Hermer and Spelke (1996) hypothesized that humans use language capabilities to solve such a task, a hypothesis supported by Hermer-Vasquez et al. (2001). In this study, adults who performed a verbal shadowing task while searching for an object that was hidden in one of four corners were less likely to use relevant landmarks in the room.

There is some controversy over the claim that geometric shape of space cannot be used in combination with landmarks without the aid of language (Learmonth et al., 2001, 2002; Hupbach and Nadel, 2005; see for a discussion Cheng and Newcombe, 2005). However, there is a considerable amount of support for the claim that geometric information, at least in some situations, is processed independently from other spatial cues. One particularly important variable seems to be the size of the room (Learmonth et al., 2002). In spaces that afford only minimal locomotion, children are more likely to ignore featural information and rely solely on the shape of the room. The reasons for this finding, whether they relate to limited locomotion, the proximity of landmark information, or both, are not yet clear (see for discussion Newcombe and Huttenlocher, 2006).

2.11.9 Cognitive Neuroscience of Spatial Memory

Our goal in this section is to review some of the primary findings that have emerged from decades of research on the neural bases of spatial memory. Recently there has been a growing focus on understanding how egocentric and environmental reference

systems operate in parallel and interact with each other. First we discuss how the hippocampal and parietal cortices subserve spatial memory. Next we turn to a discussion of the parahippocampal cortex, which has been the focus of recent growing interest in its role in navigation and its possible role in hippocampal–parietal interactions.

In now classic research with rats, O'Keefe and Dostrovsky (1971) demonstrated the existence of place cells in the hippocampus, which fire selectively based on the position in the environment that the animal occupies, independently of the animal's facing direction. O'Keefe and Nadel (1978) argued that these cells serve as the basis for an environmental spatial reference system, or the cognitive map. Ekstrom et al. (2003) have provided the first demonstration of place cells in the human hippocampus, confirming what was long hypothesized from several lines of research with humans. This literature has shown that the human hippocampus is involved in performance on a variety of spatial tasks (e.g., Maguire et al., 1997; Holdstock et al., 2000; Kesner and Hopkins, 2001; Stepankova et al., 2004). In particular, the hippocampus seems to be crucial for performance on spatial tasks that require learning the relations among external landmarks, i.e., tasks that cannot be solved using egocentric responding (Astur et al., 2002; Bohbot et al., 2004; Parslow et al., 2004; Shelton and Gabrieli, 2004).

Recently discovered grid cells in adjacent entorhinal cortex (Hafting et al., 2005) may serve a function complementary to place cells. Grid cells respond whenever the animal is in a position that coincides with a vertex in a grid of equilateral triangles that spans the surface of the environment. The grid is initially anchored to landmarks in the environment, although the cells continue to fire even in the dark. Thus, the cells may serve as the neural basis for an environmental reference system, in conjunction with the place cells, and also facilitate path integration within that environment.

In contrast to individuals who have endured damage to hippocampal regions, those with lesions to parietal regions sometimes exhibit severe difficulty navigating through immediate space, often failing to avoid obstacles (e.g., Stark et al., 1996). Such findings have led researchers to postulate that the parietal cortex is critically involved in action and, specifically, in representing self-to-surface relations (see for discussions Andersen et al., 1997; Colby and Goldberg, 1999).

Recently there has been a growing emphasis on how the parahippocampal cortex (PHC) serves spatial functioning. PHC is ideally situated for combining information from parietal and other temporal areas and also projects to entorhinal cortex, a primary input region for the hippocampus. As noted by Epstein:

> . . . the anatomical data suggest that a pathway from parietal cortex to parahippocampal cortex to the hippocampus may be critical for processing navigationally relevant spatial information. (Epstein, 2005: 971)

Neuroimaging studies have shown the PHC to be involved in a wide range of navigation tasks (e.g., Aguirre et al., 1996; Maguire et al., 1996; Mellet et al., 2000; Shelton and Gabrieli, 2002). In addition, humans who have endured damage to this area exhibit impairments in spatial tasks such as route learning and scene recognition (e.g., Bohbot et al., 1998; Aguirre and D'Esposito, 1999; Barrash et al., 2000; Luzzi et al., 2000; Epstein et al., 2001).

The posterior region of the PHC has been the focus of increasing interest due to its dedication to the perception of spatial scenes. In a functional magnetic resonance imaging (fMRI) investigation, Epstein and Kanwisher (1998) found that this area responds more to scenes than to houses, faces, or objects, even during passive viewing. Further experiments revealed that this area responds just as strongly to empty rooms as to scenes with multiple objects. Additionally, this region responds more to coherent scenes than to those in which the component parts are fractured and rearranged. Based on this set of findings, Epstein and Kanwisher called this region of cortex the parahippocampal place area (PPA). Both neuroimaging and lesion studies suggest that the PPA's role is one of encoding (Brewer et al., 1998; Epstein et al., 1999, 2001). Epstein et al. (2003) conducted a study indicating that the region processes geometric information in background elements, in particular. These researchers found that the PPA responds as much to changes in entire scenes as it does to changes in viewpoint of the same scene, suggesting that the PPA processes scene information in a viewpoint-dependent (egocentric) manner. However, there is evidence that over time the way the PPA processes particular scenes may become more viewpoint-independent (Epstein et al., 2005).

One notable finding that has contradicted studies on the PPA was that by Maguire et al. (1998). These

researchers found activations of the right PHC when subjects navigated through and learned a series of rooms with salient objects in a virtual reality environment. However, they did not find any medial temporal involvement when participants performed the same task with a series of empty rooms distinguished from each other only by their different shapes. They hypothesized that the parahippocampal region is involved in object-location binding, not analysis of the geometry of the scene. Consistent with this view is the recent finding that monkeys with lesions to the PHC are impaired in the formation of object–place associations (Malkova and Mishkin, 2003; see also Parkinson et al., 1988) and the finding by Bohbot et al. (1998) that humans with lesions to the right PHC are impaired in a spatial task that requires memory for object locations. The contradictory findings may have to do with functional differentiation within the PHC, with the PPA serving a specialized purpose of geometrical analysis and other regions involved in binding object information to the geometry.

2.11.10 Summary and Prospectus

Learning a new environment typically begins by learning routes from place to place; even in large-scale outdoor environments, navigation usually takes advantage of trails of some kind. People quickly acquire knowledge of the identities of important objects, or landmarks, and the sequential order of landmarks on routes. Route knowledge has at least quasi-metric properties very early during acquisition. Humans and many other organisms seem to be very sensitive to the shape of the immediate environment and to depend on environmental shape to reorient. With extensive experience in an environment, people sometimes acquire knowledge of its overall layout, or survey knowledge. The acquisition of spatial knowledge is best characterized as the incremental accumulation of quantitative spatial relations. Spatial knowledge does not seem to be limited to qualitative, nonmetric information at any point during acquisition.

Humans represent the locations of objects in space using egocentric and environmental (i.e., allocentric) reference systems, and navigation almost certainly depends on both egocentric and environmental representations of the environment. There is evidence that the process of learning a new environment involves interpreting the spatial structure of that environment in terms of an environmental spatial reference system. Interobject spatial relations seem to be specified with respect to a small number of reference directions. This aspect of the mental representation produces one of its key properties, orientation dependence: interobject spatial relations can be utilized more efficiently from perspectives aligned with the dominant reference directions in memory. These reference directions are typically parallel to points of view experienced during learning, but also may be determined by instructions and by properties of the environment, such as the mutual alignment of several objects or geographical slant. The use of spatial reference systems at multiple scales may explain why spatial knowledge is hierarchically organized.

Effective navigation in a familiar environment depends on the abilities to avoid obstacles and stay on course, to use one's history of self-movement to keep track of one's position, and to use mental representations of the layout of the environment to estimate the positions of objects that cannot be observed. Collectively, these abilities – steering, path integration, and wayfinding, respectively – are referred to as spatial updating. A prerequisite for efficient updating seems to be that the navigator's position in space changes. Imagined spatial updating is difficult and error-prone. An important source of this inefficiency seems to be conflicts that are created by having to imagine a position in the environment that is different from one's physical position in that environment. Physical locomotion in the absence of vision mitigates this interference and also seems to benefit from body-based information, which facilitates the transformations needed to update observer–environment spatial relations. But even physical nonvisual updating breaks down with prolonged movement over complex trajectories.

Contemporary models of spatial memory and navigation specify roles for three types of spatial memories: Egocentric self-to-object spatial relations used for steering and path integration, viewpoint-dependent representations of landmarks and scenes used for place recognition, and environmental representations of object-to-object spatial relations used for wayfinding and some forms of path integration. There are differences among the models in the properties of each of these representational systems and in the manner in which they are used in navigation. For instance, in some models, the egocentric system computes and represents transient representations, whereas in other models, these representations are more enduring. In one model, the environmental

system only represents the shape of the environment and is used for reorientation, whereas in the others, it represents object-to-object spatial relations and is used for virtually all locomotion in familiar environments. Despite these differences, however, the models are quite similar in terms of their overall architecture.

The development of these capabilities begins with simple forms of egocentric spatial coding, such as learning the association between a bodily response and a location in space, and of spatial updating, such as compensating for trunk rotation. During the second year of life, children begin to be able to use landmarks in more sophisticated ways and to update after complex movements, developments that are coincident with (and certainly related to) their increased mobility. By the time children can walk, they can use environmental shape, as defined, for example, by the shape of a room, to locate a desired object. This knowledge must be represented in an environmental frame of reference because it survives disorientation, which destroys self-to-object spatial relations. Toddlers appear to have difficulty under some conditions using featural cues or landmarks to find a desired object after having been disoriented. The ability to effectively use such cues does not develop until well into the school-age years.

Research on the neural basis of spatial memory and navigation in humans has isolated the hippocampus, the parietal cortex, and the parahippocampal cortex as especially important brain areas. The hippocampus seems to be critically involved in the formation of long-term representations of the spatial structure of the environment using environmental frames of reference. The parietal cortex is involved in representing the locations of objects in the egocentric reference systems needed for sensorimotor mappings and in coordinating these representations. The parahippocampal cortex is involved in navigation, and its posterior regions seem to play an important role in representing landmarks and scenes.

The scientific understanding of human spatial memory and navigation has advanced enormously since Tolman (1948) presaged the distinction between route and survey knowledge with his categorization of spatial memories into strip maps and comprehensive maps. Significant progress has been made in understanding the nature and acquisition of spatial memories, how remembered spatial relations are used to guide navigation, properties of spatial updating processes, the development of early navigational capabilities, and areas of the brain involved in spatial memory and navigation. But of course much remains to be discovered. Many important avenues of future research are indicated by the findings reviewed in this chapter. A few especially promising ones, to our minds, include the following.

There is abundant evidence of the hierarchical organization of enduring spatial memories, but the processes involved in the formation of such representations are not well understood. Of special interest are the mechanisms used to establish correspondences between representations that use different reference directions and the spatial updating processes used to switch from one hierarchical level to another. The relative importance of egocentric and environmental representations in various spatial tasks, their dynamical properties, and the processes by which egocentric representations in sensorimotor systems are transformed into environmental representations, and vice versa, are largely unknown. Much remains to be learned about how children come to represent spatial relations among landmarks in ways that effectively support navigation. Recent investigations of spatial updating in adults suggest that steering depends on a transient egocentric system, whereas wayfinding depends on an enduring environmental system. Relatively little is known about the nature and the development of these capabilities in children. Finally, research on the neural basis of human spatial memory and navigation has isolated a network encompassing, at minimum, the parietal cortex, the hippocampus, and the parahippocampal cortex. The nature of the representations in these areas and the interactions among them need to be explored in greater depth.

We look forward, with optimism, to seeing the empirical and theoretical fruits of these efforts to understand how people remember where they have been and how they find their way home.

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2.12 Forgetting

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2.12.1 Introduction

As Ebbinghaus observed in his famous monograph, "Left to itself every mental content gradually loses its capacity for being revived, or at least suffers loss in this regard under the influence of time" (1885/1964: 4). To most, forgetting is a scourge, a nuisance, a breakdown in an otherwise efficient mental capacity. The momentary loss of information is a regular part of the human experience, but normal forgetting, even when permanent, is misconstrued as a malfunction or breakdown. We forget for adaptive reasons, and understanding the characteristics of forgetting ultimately tells us a great deal about why and how we remember.

In this chapter we offer a general tutorial on the psychology of forgetting. Our focus will be on its empirical characteristics and proposed theoretical underpinnings, as revealed primarily through laboratory studies with healthy human participants. Excellent reviews on abnormal forms of forgetting, such as those that occur in brain-damaged patients, and on forgetting in nonhuman populations can be found elsewhere in this series. We begin the chapter with a brief discussion of the adaptive value of forgetting, followed by an examination of its functional and mathematical characteristics. Next, we discuss possible causal mechanisms in some detail – why do we forget? Finally, we end by reconsidering the meaning of forgetting and its proper role in modern memory theory.

2.12.2 Forgetting and Its Adaptive Value

At face value, memory seems to be about recovering the past, recapturing or reviving previous experiences. Yet, it is unlikely that memory actually evolved for this specific purpose. The past can never occur again, at least in exactly the same form, so there is limited adaptive value in developing a system that carries around intact records of prior experiences. Instead, memory has value because it allows us to use the past in the service of the present, to decide on an appropriate plan of action now or in the future (Suddendorf and Corballis, 1997; Tulving, 2002). Intact records of the past are relevant in some situations, but not in others. For example, we might need to remember the specific location of a food source, but we need not remember every instance in which a particular food type was consumed (we need to remember only that it was edible). As Lewis Carroll famously quipped, "it's a poor sort of memory that only works backwards."

Recognizing that memory's primary function is to deal with the present, or perhaps to anticipate the future, informs how we need to think about forgetting. Obviously, if nature did not 'design' memory to reproduce the literal past, then it is not surprising that we sometimes have difficulty reproducing it. The veridical details of an event tend to be ignored by our memory systems, which choose instead to process and store inferences or connections that are likely to benefit future responding (Bartlett, 1932; Schacter and Addis, 2007). Even when the details of an event are correctly stored, there is little reason to anticipate that those details will be stored indefinitely. In fact, it is easy to make the case that forgetting is a highly adaptive feature of cognition (Bjork and Bjork, 1988, 1996; Altmann and Gray, 2002). Having an intermediate, rather than complete, retention of the past may improve our ability to use inferential heuristics (Schooler and Hertwig, 2005), maximize our ability to detect causality (Kareev, 2000), and even maintain a sense of sanity in an ever-changing world (Luria, 1968).

More to the point, a well-designed memory system can be expected to show sensitivity to the likelihood that a past event will be needed, or appropriate, to a future situation. It makes no sense to retain long-defunct telephone numbers, or high school locker combinations: These are more apt to produce needless clutter than potentially useful records. Moreover, once an event occurs in the present, the likelihood that it will occur again (at least in a similar form) changes predictably with time. If a predator appears in your environment at time t, then the chances that it will appear again are usually greater at time $t+1$ than at time $t+2$. As it turns out, the function relating event recurrence with time typically takes a negatively accelerated form, just like the classic forgetting function (Anderson and Schooler, 1991). What we normally think of as forgetting, therefore, may simply represent memory's sensitivity to the statistical structure of events in the environment. We forget an item's occurrence with time because, in fact, that item is less likely to occur again with time.

2.12.3 The Characteristics of Forgetting

The fact that forgetting takes on a characteristic form is *prima facie* evidence that a psychological process is at work. We do not forget things randomly; rather, the loss of information proceeds in an understandable and predictable fashion. A typical retention function is shown in **Figure 1** and it contains a stark empirical regularity: There is a negatively accelerating downward loss in retention over time. We forget rapidly at first and then retention slowly levels off. This general pattern occurs regardless of the quantity or quality of the information learned and the retention measure employed.

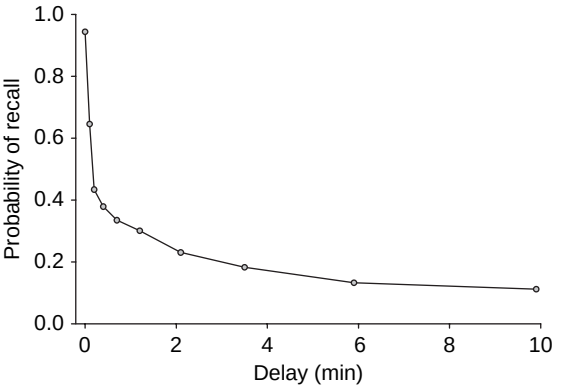

Figure 1 Probability of correctly recalling a word as a function of time. Data from Rubin DC, Hinton S, and Wenzel AE (1999) The precise time course of retention. *J. Exp. Psychol. Learn. Mem. Cogn.* 25: 1161–1176.

Ebbinghaus is usually credited with the first empirical demonstration of the forgetting function (although see Galton, 1879). Using his famous savings method, Ebbinghaus recorded the amount of time spent relearning an earlier memory series (usually consisting of nonsense syllables). In one case, he learned eight series of 13 syllables each (to a criterion of two errorless recitations) and then attempted to relearn the same material after one of seven delays ranging from 1 h to 31 days. The percentage savings, calculated as the difference in time spent during initial learning and relearning expressed as a percentage of the original learning time, dropped systematically over delay in a form resembling that shown in **Figure 1**. He expressed some surprise at the form of forgetting, particularly the lessening effect shown at the later delays. Forgetting "in the latter intervals is evidently so slow that it is easy to predict that a complete vanishing of the effect of the first memorization of these series would, if they had been left to themselves, have occurred only after an indefinitely long period of time" (Ebbinghaus, 1885/1964: 76). The question of whether information, once encoded, ever truly vanishes completely (in the absence of relearning or reexposure) remains an issue of concern today (Bahrick, 2000; Wixted, 2004a).

2.12.3.1 Forgetting's Mathematical Form

Psychologists have struggled to characterize the forgetting function in more precise mathematical terms. Ebbinghaus suggested a type of logarithmic function, but many candidates are viable. Linear functions can probably be ruled out, along with simple exponentials, but it is possible to salvage most functions with the right set of assumptions. Psychologists usually choose power – logarithmic, exponential power – or hyperbolic functions, but the decision is often driven by theoretical rather than empirical concerns. At present, despite over a century of effort, no firm consensus on the matter has arisen, although currently many researchers lean toward some kind of power function (e.g., Wixted and Carpenter, 2007).

The failure to reach consensus about forgetting's mathematical form is understandable; the enterprise is fraught with difficulties. For example, one needs criteria for choosing one function, or forgetting model, over another. It is possible to evaluate competing functions simply on the basis of a goodness-of-fit measure, which has typically been the criterion of choice (e.g., Anderson and Schooler, 1991; Wixted and Ebbesen, 1991; Rubin and Wenzel, 1996). However, goodness-of-fit measures usually ignore other important factors, such as the complexity of the function and its psychological viability (Roberts and Pashler, 2000; Pitt et al., 2002; Lee, 2004). There are also serious measurement concerns. The assessment of forgetting requires tracking performance through different points along the measurement scale. Can we really be certain that a drop in retention from, say, 90% correct to 80% correct means the same thing psychologically as a drop from 20% to 10%?

There is also enormous imprecision in the existing data. Forgetting experiments are hard to conduct. Longitudinal studies require testing the same individual at different retention intervals, which is practically difficult and can introduce a testing, or repeated retrieval, confound. In fact, repeated testing of the same information can actually improve overall performance under some conditions (e.g., hypermnesia, Roediger and Challis, 1989). Cross-sectional studies present similar practical difficulties and provide no assurances that the average retention estimates for the different groups accurately represent how forgetting proceeds in an individual (Rubin and Wenzel, 1996; Chechile, 2006). The net result is that most studies report only a handful of retention intervals and, for obvious reasons, the longest sampled retention interval rarely provides any true estimate of memory's permanence. Some memories may remain intact over a lifetime (Bahrick, 1984, 2000) which, in turn, places important constraints on the retention functions that can apply (Wixted, 2004a; Chechile, 2006). Similarly, one cannot ignore performance when the retention interval approaches zero (i.e., immediate testing); some functions are ill-defined at this point, and it is conceivable that special short-term or working memory systems complicate the retention function at very short retention intervals (Chechile, 2006).

There is also the issue of the proper retention measure. As noted, Ebbinghaus measured retention through savings in relearning, but there are many other measurement tools. One can assess memory through proportion correct recall, the d′ discriminability index in recognition, or through various indices of priming in implicit or indirect retention measures. The retrieval environment can also be enriched through the introduction of retrieval cues or degraded through the presence of other concurrent tasks. In addition, it is unclear whether delay should be defined as the simple passage of time, time calculated in terms of some kind of relative index (Bjork and Whitten, 1974; Baddeley, 1976), or perhaps the number or quality of intervening events (Waugh and Norman, 1965). Time and events are usually confounded because the passage of time is highly correlated with the number of intervening events (Chechile, 1987).

Despite these difficulties, when an empirical forgetting function is obtained it virtually always resembles the one shown in **Figure 1**. We forget rapidly at first and then retention slowly levels off. Empirically, the proportional rate of forgetting also slows over time, as aptly expressed in Jost's famous law of forgetting: "Given two associations of the same strength, but of different ages, the older falls off less rapidly in a given length of time" (Jost, 1897: 472). Younger memory traces, at least on average, are more vulnerable to the deleterious effects of time than older traces. Note that Jost's law conflicts with simple exponential forgetting functions, which assume constant proportional loss. In an exponential function, the proportional rate of loss remains constant (retention falls by 50% between t and $t+k$, where k corresponds to the function's half-life), so associations of equal strength, regardless of their age, should decline subsequently at the same rate. They do not, and this places important constraints on the possible mechanisms that underlie forgetting.

2.12.4 Determinants of Forgetting Rates

It is possible to reconcile Jost's law with exponential forgetting if we assume that forgetting rates vary with degree of original learning (Simon, 1966; Wixted, 2004a). For example, if well-learned information is forgotten more slowly than poorly learned information – that is, if the half-life of a memory trace varies with the degree of initial learning – then Jost's law still holds. The possibility that we lose information at a rate determined by its initial strength itself seems imminently reasonable, even intuitive, but it has received little empirical support in the laboratory. Variables that affect acquisition – e.g., word frequency, meaningfulness, similarity, and so forth – typically have little, if any, impact on subsequent forgetting rates (Underwood, 1964; Keppel, 1968).

Slamecka and McElree (1983) allowed the degree of original learning to vary and then assessed retention across several delays. In each case, despite wide differences in original acquisition level, nearly equivalent forgetting slopes were obtained. Importantly, this conclusion held across different retention measures, including free recall, cued recall, category recall, gist recall, and recognition (see **Figure 2**). The same conclusion holds when different mnemonic components or processes are assessed. McBride and Dosher (1999) used Jacoby's process dissociation technique to examine forgetting functions for conscious and automatic components of memory, as a function of the depth of initial processing (semantic vs. graphemic); similar forgetting rates were found for each component, despite differences in the overall level of availability. Even individual difference variables, such as age (Salthouse, 1991) and neurological status (Christensen et al., 1998), commonly fail to produce stark differences in either the form or the rate of forgetting.

These data, among many others, suggest that acquisition and forgetting are not merely two sides of the same coin; rather, variables that affect acquisition may not affect the forgetting process. Forgetting proceeds in its characteristic way, regardless of the retention measure or initial acquisition level, much like the action potential of a neuron, once generated, travels forward in a characteristic (all-or-none) fashion. Of course, this conclusion must be hedged a bit for all the methodological considerations that have been listed; plus, controversies have raged over how to measure the loss of information over time properly (e.g., Slamecka, 1985; Bogartz, 1990; Loftus and Bamber, 1990). Still, it is generally conceded that forgetting rates remain invariant as acquisition variables, mnemonic processes, and measurement vehicles are manipulated, although exceptions can be found in the literature. For example, McDonald and colleagues (2006) recently found evidence for acquisition-based heterogeneity in forgetting when a novel statistical procedure, multilevel modeling, was applied to the retention data and certain other methodological concerns, such as the mnemonic strategy adopted by the participant, were controlled. The debate continues, as it has for the past century.

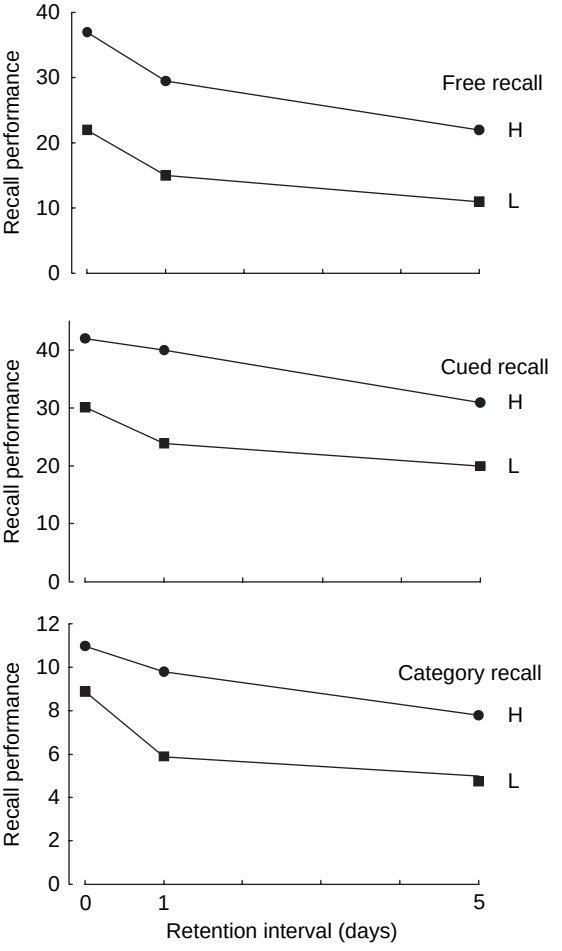

Figure 2 Forgetting functions obtained for high (H) and low (L) degrees of original learning as revealed for three different retention measures. After Slamecka NJ and McElree B (1983) Normal forgetting of verbal lists as a function of their degree of learning. *J. Exp. Psychol. Learn. Mem. Cogn.* 9: 384–397.

2.12.5 Mechanisms of Forgetting

It may be difficult to characterize the forgetting function completely, but every researcher recognizes that memory changes systematically with time. As noted earlier, there are excellent reasons to believe that forgetting is adaptive and, more importantly, that the forgetting function mimics the way events occur and recur in the natural world. Consequently, it is reasonable to search for a mechanism, or set of mechanisms, that affects the availability of learned material. Of course, any acceptable theoretical account of why we forget must come to grips with the regularity of the forgetting function itself. One cannot simply argue, for example, that forgetting is cue-dependent – that is, we forget in the absence of an appropriate retrieval cue – without also explaining why the form of forgetting is so regular and predictable.

Historically, researchers have appealed to three primary causal mechanisms to explain forgetting: autonomous decay, interference from other acquired information, and altered stimulus conditions (McGeoch, 1932; Bower and Forgas, 2000). There is a fourth mechanism, active inhibition induced by retrieval, that has been proposed more recently (Bjork and Bjork, 1992; Anderson, 2003), although it, too, has some interesting historical antecedents (e.g., Freud's concept of repression). Each of these mechanisms is discussed in more detail in the sections that follow.

2.12.5.1 Decay

When memory theorists use the term *decay*, they mean forgetting that occurs spontaneously with the passage of time. Decay is assumed to be autonomous, which means that its progression does not depend on some other active mnemonic process (such as the acquisition of new information). Of course, everyone believes there must be some kind of neurological underpinning for the decay process, such as a metabolic process that erodes or overwrites synaptic connections, but the mechanism itself is left unspecified. The natural process of radioactive decay is sometimes used as a rough analogy, in which a constant proportion of radioactivity is lost in a fixed unit of time.

To make a principled empirical argument for decay, it is necessary to show that forgetting proceeds in the absence of other activities, such as rehearsal or interference. As we discuss in the next section, newly established memories are quite susceptible to interference from other learned material, so it is necessary to control, if possible, for this factor. Rehearsal also needs to be ruled out, because repetition presumably counteracts the deteriorating effects of the decay process. One needs to create, in essence, a kind of mental vacuum in which time, and little else, is allowed to vary. If information is still forgotten in its characteristic way, then a time-based process, decay, must be responsible.

Various strategies have been employed to detect decay. Most have relied on a dual-task methodology in which the participant is asked to perform an attention-demanding task while simultaneously retaining critical target memories. For example, Reitman (1974) instructed participants to retain groups of words while detecting the occurrence of a tone signal in background noise. The tone detection task was assumed to be sufficiently dissimilar to the words to prevent interference, which typically depends on similarity, and sufficiently taxing to prevent rehearsal (although see Roediger et al., 1977). Similarly, Cowan and colleagues (1997) had people compare the frequencies of two tones separated by delays while performing a silent visual tracking task. It is difficult, perhaps impossible, to rehearse a pure tone, and the intervening visual distractor task seemed unlikely to produce interference, so any decline in the tone task was attributed to decay. In both of these cases, retention performance did decline with delay, implicating decay (although see Cowan et al., 2001).

Note that each of these paradigms investigated retention over the short term, that is, over a time-course of seconds. In fact, the concept of decay is rarely used to explain long-term forgetting, due in large part to the seminal arguments of John McGeoch (1932). Among other things, McGeoch noted that memories often remain highly available or even improve over time, a fact that seems fundamentally incompatible with decay. For example, when people are given repeated opportunities to recall earlier-presented material, they will often recall items on the second or third attempt that they failed to recall initially, a phenomenon known as reminiscence (Ballard, 1913). The Pavlovian phenomenon of spontaneous recovery, in which an extinguished conditioned response recovers after a period of rest, is another case in point (for empirical evidence of spontaneous recovery in human participants, see Wheeler, 1995).

McGeoch (1932) further noted that when the passage of time is held constant, the amount of

forgetting depends on the specific activities that occur during the delay. After learning a list of verbal items, if one group of participants rests and a second group learns another list, recovery of the initial list will be sharply impaired in the second group (Müller and Pilzecker, 1900). Moreover, the amount of forgetting will depend on similarity between the original material and the interpolated material (see Osgood, 1953). Forgetting is correlated with the passage of time, McGeoch argued, but the relationship is not causative. It is the events that happen 'in time' that produce forgetting, not time itself.

In the case of short-term retention, however, the situation is somewhat different. Decay remains quite popular among theorists in this arena because short-term retention is thought to tap special storage systems. In the working memory model of Baddeley and Hitch (1974), for example, information is retained for brief intervals in the service of more complex forms of processing (e.g., language), and special 'activity traces' are assumed to be stored in various loops, buffers, and sketchpads (Baddeley, 2000). In the absence of rehearsal, which serves a refreshing function, these traces decay autonomously. Most working memory theorists accept that short-term forgetting can occur as a consequence of other means, such as interference, but decay is assigned an important and even pivotal role (e.g., Page and Norris, 1998).

Memory traces may indeed decay in some circumstances and not in others. There could be something special about short-term activity traces, those engendered and maintained by special short-term memory systems, but the concept remains controversial. For example, Nairne (2002b) has shown that each of McGeoch's main arguments against decay in long-term memory apply equally well to immediate retention: Short-term retention can decline, remain the same, or even improve over time depending on the circumstance. To illustrate, in a study by Turvey et al. (1970), different groups of people were asked to count backward as a distractor activity for group-specific intervals prior to recall (e.g., one group counted for 10 s, another for 15 s, and another for 20 s). Equivalent amounts of forgetting were found across groups in this between-subject design (0.33, 0.30, and 0.30, respectively) (see also Greene, 1996). Moreover, on a critical trial all groups were switched to the same 15-s distractor period. Retention performance dropped in the 10-s group (from 0.33 to 0.20), stayed roughly constant in the 15-s group (0.30 to 0.28), and improved in the 20-s group (0.30 to 0.38). Note that the passage of time – and therefore the opportunity for decay – was equated across the groups on the critical 15-s trial, yet performance depended significantly on the timing of prior trials.

Space does not permit a complete review of all the relevant studies. Suffice to say that the correlation between time and forgetting is far from perfect even when retention is tested over intervals lasting seconds. Importantly, however, such findings by themselves do not rule out the concept of decay. It is still possible that memory traces decay with time, but particularly supportive retrieval environments can counteract the loss. In the Turvey et al. (1970) study, for instance, moving from a 20-s distractor-filled retention interval to a 15-s interval may have helped the participant discriminate to-be-remembered items from information recalled on prior trials (Nairne, 2002b). Similarly, the emergence of newly recalled items on a second or third recall attempt could simply reflect participants' ability to use recalled items as cues to help them recall new items. The state of the trace could still be degraded at time 2 relative to time 1, but a more supportive retrieval environment at time 2 nets improved recall.

At the same time, recognizing that retention cannot be predicted from the state of the memory trace, without considering the retrieval environment, seriously undermines the theoretical utility of decay. Appealing to decay as the source of forgetting is like appealing to strength as the source of remembering. As Endel Tulving (1983) has argued, memory traces "do not have strength independently of the conditions under which they are actualized" (Tulving, 1983: 240–241). Thus, losing trace features over time may or may not impair retention; it will depend on the particular features that are lost and their compatibility with the retrieval cues present at the time of retention testing (Nairne, 2002a).

2.12.5.2 Interference: Trace Degradation

The second, and more popular, interpretive tool used to explain forgetting is *interference*. The concept of interference is multifaceted, having several distinct meanings, but the important common denominator is the occurrence of other mnemonic events. We forget because other events interfere with the storage or recovery of target memories. Unlike decay, the interference perspective assumes that if one could create a mental vacuum – that is, if you could measure the state of a memory trace over time in the absence of other mnemonic events or activities – there would be no decline in the integrity of the trace. Forgetting

occurs because other events or activities, particularly ones that are memory-based, happen 'in time.'

There are two ways that interference is thought to operate. First, newly learned material can overwrite, erase, displace, or otherwise degrade an existing memory trace. The details are usually left unspecified, although it is generally assumed that similarity between original and new learning increases the extent of the interfering effect. As noted above, many studies have shown that if the retention interval is held constant, the nature of the activities that occur between study and test importantly determines what and how much is forgotten. In a classic study by Jenkins and Dallenbach (1924), for instance, people recalled more information if they slept through a retention interval than if they remained awake; the assumption here is that sleep protects one from the potentially damaging effects of interpolated interference. Comparable findings occur even for amnesic subjects: After hearing words or stories, if amnesic patients are allowed to spend a retention interval in a dark and quiet room, their subsequent retention is vastly improved relative to an interference control (Cowan et al., 2004).

Moreover, in an interesting parallel to decay, some researchers assume that the damaging effects of interference depend on the passage of time as well. Rather than exerting a negative effect, however, memory traces are assumed to become less vulnerable to the effects of interference with time because of a trace consolidation process. The notion that memory traces consolidate is widely accepted by neuroscientists, partly because retrograde amnesia, the loss of memories formed prior to brain damage, shows a distinct temporal gradient (Ribot, 1881). A blow to the head is more likely to lead to the loss of recently formed memories than to the loss of memories from the more distant past. Presumably this pattern occurs because the older traces have consolidated and, as a consequence, are less susceptible to interference. As Wixted (2004a,b) has recently observed, exactly the same reasoning can be applied to general mnemonic principles such as Jost's law: Given two traces of the same strength, but of different ages, retention of the older one will fall off less rapidly in a given length of time, presumably because the older trace has sufficiently consolidated.

In the laboratory, however, the concept of consolidation has a more checkered past. In fact, it has been largely rejected by memory theorists for decades because laboratory-based experiments typically fail to show convincing temporal gradients. It is possible to obtain robust retroactive interference – the term used to describe interference arising from events occurring after the target memory is established – when the interfering event occurs days or even weeks after the original encoding, a period far exceeding any reasonable consolidation time. Moreover, the interference that is obtained after a short delay, when consolidation processes are presumably active, is usually comparable in size to the interference obtained after lengthy delays (see Wickelgren, 1977, for a review).

In fairness to consolidation theory, though, the fact that robust retroactive interference occurs after a long delay does not rule out a consolidation process, for much the same reason that retention after a lengthy delay does not rule out a decay process. As discussed in the next section, interpolated learning can easily decrease the accessibility of a fully formed memory trace by impairing the diagnostic value of an associated retrieval cue. Moreover, Wixted (2004b) has recently challenged the accepted dogma concerning temporal gradients, arguing that traditional retroactive interference designs introduce methodological problems that cloud interpretation. Also, some clinical cases indicate that consolidation may last a relatively long time (Dudai, 2004).

Still, it is important to recognize that consolidation, even if empirically verified, can never stand as a completely adequate account of forgetting (see also Meeter and Murre, 2004). Consolidation theory, like decay theory, essentially reduces to a set of claims about how the integrity of a memory trace changes with the passage of time. As noted, memory traces do not have retention strength outside of the conditions under which they are accessed. For any given trace, degraded or otherwise, there are presumably retrieval conditions that will promote or hinder successful retrieval. To explain forgetting or retention adequately, one needs to consider the state of the memory trace as well as the conditions present at the time retrieval is attempted (Tulving, 1983).

2.12.5.3 Interference: Cue Impairment

We mentioned earlier that interference is thought to operate in two ways. The first, just discussed, is the degrading effect that new learning can have on the integrity of an existing trace. The second route places the locus of interference not in the trace itself, but in its eliciting retrieval cue. Psychologists generally assume that remembering is cue-driven. Memories are not thought to arise spontaneously; instead, they are activated by the presence of associated retrieval

cues. For decades, the empirical paradigm of choice among researchers was paired-associate learning in which participants are asked to associate target words (e.g., 'town') with cue words (e.g., 'cart'). The advantage of paired-associate learning is that it allows the experimenter to test the integrity of a specific memory by cuing the participant with its associated retrieval cue. The ability to reproduce the target word in the presence of its linked cue is used as the index of retention. When people fail to produce the appropriate response, given the presence of the retrieval cue, then obviously forgetting has occurred.

Once again, forgetting in such a context could occur because the integrity of the target memory has been degraded, either through a decay process or as a by-product of subsequent activity. However, it is also possible that the cue–target association is impaired, leaving the integrity of the target trace intact. In such a case, forgetting occurs because the retrieval cue is unable to elicit or reproduce a previously associated target memory. There are two reasons why the effectiveness of a retrieval cue can become impaired. First, as suggested by early interference theorists (e.g., Melton and Irwin, 1940; McGeoch and Irion, 1952), subsequent activity might lead to 'unlearning' of the cue–target association. Suppose, for example, that after learning an association between 'cart' and 'town', the cue 'cart' is subsequently associated with other targets (e.g., 'block' or 'train'). During this relearning phase, 'cart' occurs in the absence of 'town' and, therefore, the 'cart–town' association extinguishes, much like the process of extinction in Pavlovian conditioning (Pavlov, 1927).

The second and more commonly accepted mechanism for cue impairment is target competition. Even in the absence of unlearning, if a retrieval cue becomes linked to several targets, its ability to elicit any one of those targets lessens. So, if 'cart' is associated initially with 'town,' but then is later paired with 'block' or 'train,' the probability that 'town' will be produced with 'cart' on demand declines (see **Figure 3**). This characteristic of retrieval cues is known by several names, including *response competition, cue distinctiveness,* the *fan effect* (where 'fan' refers to the number of associated target responses), and *cue overload.* Historically the term 'response competition' was used in conjunction with 'unlearning' to form the two 'factors' of the famous two-factor theory of forgetting (McGeoch, 1942; Postman, 1961). Currently, the more popular moniker is *cue overload*, for reasons that will be discussed momentarily.

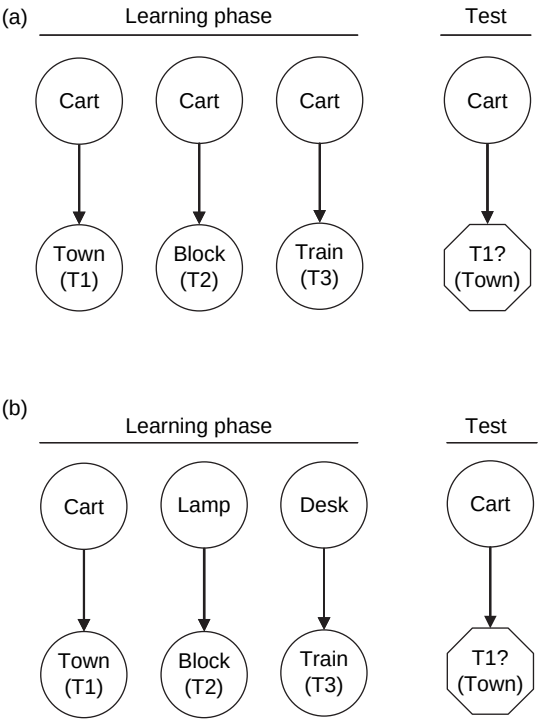

Figure 3 Illustration of a cue overload situation. In (a) the retrieval cue is linked with several targets, whereas in (b) it is associated only with one target. The predictive value of the cue in situation (a) is lower than in (b) due to cue overload.

The basic phenomenon of cue overload is well supported empirically. For example, as the number of study items from a particular category increases, the category name becomes a less effective cue for eliciting any one item in particular (Tulving and Pearlstone, 1966; Roediger, 1973). Cue overload explains the list length effect as well: recall of any given item from a memory list declines as the length of the list increases. The list length effect occurs, presumably, because people use some representation of the list itself as a cue and it becomes 'overloaded' (i.e., less diagnostic of any particular item) as the number of items subsumed under the list cue increases (Watkins and Watkins, 1975). More directly, it is well established that increasing the number of associated responses to a given target – that is, increasing the cue's associative 'fan' – slows down people's ability to verify any particular cue–target pairing (Anderson, 1974; Anderson and Reder, 1999).

Cue overload is the preferred term partly because the locus of interference is believed to lie primarily in the target selection phase rather than in competition among already selected responses. Although this distinction may seem somewhat arbitrary, or at least model-specific, its roots lie in classic work by

Barnes and Underwood (1959) on the modified modified free recall test (MMFR). In an MMFR test, people are asked to recall any or all response terms that have been associated with a cue. There is no requirement to recall a specific response term which, in turn, presumably eliminates any response competition (because any and all responses can be produced). Significant interference is still obtained in the MMFR test – that is, one's ability to recall 'town' to 'cart' remains impaired if other responses have been associated with the cue – and this fact was used by interference theorists to support the concept of unlearning (Postman, 1961). Modern theorists rarely invoke unlearning *per se*, choosing to argue instead that new associations essentially block or impair access to old ones during the target selection phase, or that the recall of one item leads to inhibition or suppression of the other (see Anderson and Neely, 1996, for a review).

Ascribing interference effects to cue impairment – specifically, the ability of a cue to produce a specific target – has considerable advantages. For example, it enables the theorist to explain both retroactive and proactive interference with a single mechanism. As discussed earlier, retroactive interference occurs when newly learned material acts retroactively to impair earlier learning; proactive interference occurs when information learned at time 1 interferes with the ability to access information learned at time 2 (see **Figure 4**). Underwood (1957) provided convincing evidence that much of what is forgotten in standard verbal learning experiments can actually be attributed to proactive interference (i.e., prior learning). **Figure 5**, which is based on data compiled originally by Underwood, shows proportion correct recall of a serial list, tested after an unfilled 24-h interval, as a function of the number of lists learned previously in the experimental context. When the critical list is the only list learned, about 80% of the material will be retained after 24 h; as the potential for proactive interference increases – that is, as the number of prior lists learned increases – delayed retention drops precipitously.

The robust nature of proactive interference is troubling for most of the forgetting mechanisms we have discussed. Why should learning 'cart–town' at time 1 impair one's ability to recover 'cart–block' learned at time 2? Certainly neither decay, nor overwriting, nor unlearning can explain the phenomenon because each is ascribed to things that happen after the point of acquisition. Significant proactive interference is found on an MMFR test as well (Koppenaal, 1963),

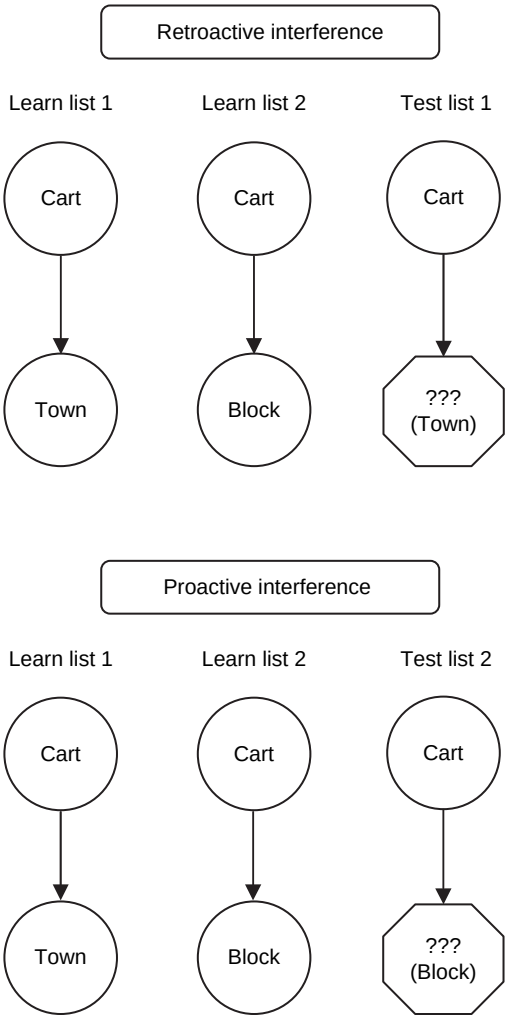

Figure 4 Representation of experimental settings revealing retroactive interference and proactive interference.

which rules out historical versions of response competition. The only viable forgetting mechanism is cue overload: As the number of targets associated with a cue increases, the ability of the cue to access any particular target declines. Note there is nothing about the order of acquisition that is inherent in the concept of cue overload, although the availability of particular cue–target associations does change in complex ways with time and testing method (see Postman and Underwood, 1973).

2.12.5.4 Cue Availability

In addition to interference from events that happen in time, forgetting can also arise from altered stimulus conditions, that is, when "the stimuli necessary to

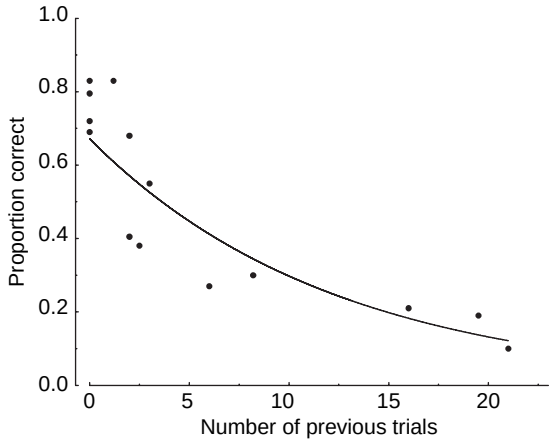

Figure 5 Proportion of correct responses plotted as a function of the number of previous trials. Data were collected from 14 different studies. After Underwood BJ (1957) Interference and forgetting. *Psychol. Rev.* 64: 49–60.

elicit the originally learned acts are not effectively present" (McGeoch, 1942: 501). Endel Tulving (1974) coined the term *cue-dependent forgetting* to describe the situation. Put simply, if you have learned to associate the target 'town' with the retrieval cue 'cart,' then in the absence of 'cart' you are unlikely to remember 'town.' In this case, there is no degradation or impairment of the target trace, nor any impairment in the cue-target association: We forget simply because we lack the right retrieval cue.

Cue-dependent forgetting helps to explain why memories that seem to have been lost can reappear at a later time. To use the terminology of Tulving and Pearlstone (1966), information can be 'available' in memory, in the sense that the encoded information remains intact somewhere in the memory system, but not 'accessible.' Accessibility requires an appropriate retrieval cue which may be absent at time 1 but may reappear at time 2. Of course, the fact that forgetting proceeds in such a regular fashion, with such a characteristic retention function, suggests that the appearance of retrieval cues may be time-locked. Indeed, psychologists have used the concept of context, which is assumed to change systematically over time, to explain the regularity of forgetting. During presentation, information becomes associated with the context which then changes in accordance with the flow of normal activities (see Estes, 1955; Mensink and Raaijmakers, 1988; Brown et al., 2000, for some specific theories on how context changes). (Note that the examples discussed so far involve external cues (e.g., presenting a cue previously associated with a target), but cue-dependent forgetting can also be demonstrated with internal cues (e.g., mood state; Bower, 1981). In this case, an internal stimulus (such as a mood state) acts as the functional retrieval cue.)

Accepting that remembering (and forgetting) is cue-dependent encourages us to specify the conditions that determine (1) when relevant retrieval cues will be available and (2) the effectiveness of those cues when present. Psychologists have spent decades investigating the second point and have reached consensus that retrieval cues are effective to the extent that they 'match' the contents of the original encoding. The encoding specificity principle, first articulated by Tulving and Thomson (1973), states that retrieval cues will be effective in eliciting targets if and only if the information about them and their relation to the to-be-remembered target is stored at the time of encoding. Thus, the conditions of encoding will uniquely determine whether any given cue will be effective in recovering a prior target episode. Retrieval cues will work if and only if they match, and consequently are a part of, the original encoding complex.

The encoding specificity principle asserts that preexisting associations between cues and targets, such as the semantic relationship between 'bloom' and 'flower,' cannot be used *a priori* to predict the effectiveness of one cue for another. This runs counter to intuition because one would normally expect a strong associate to elicit the target naturally, allowing it to be confirmed easily as a member of the study list. Yet, Tulving and Thomson (1973) showed that a weak associate to the target 'flower,' such as 'fruit,' can actually be a better cue than the strong associate when conditions promote the encoding of the weak associate during study. It follows as well that retention will depend on the match between the retrieval cue and the target memory, as encoded, rather than on the cue and target as originally presented. Presenting the same nominal cue at test will not necessarily be effective. It will depend on whether the participant interprets the retrieval cue at test in the same way that he or she interpreted the cue during the original encoding.

Virtually all psychologists recognize the importance of matching the encoding and retrieval environments, to assure the availability of an appropriate cue, but some have questioned the role of the match *per se*. For example, Nairne (2001, 2002a) has argued that it is the diagnostic value of the retrieval cue that really matters. Rather than a passive matching process, retrieval is better characterized as an active selection process wherein cues are used to

pick and choose from among viable retrieval candidates. Matching the retrieval cue with the original encoding context, as encoded, can be expected to increase the diagnostic value of the cue in most situations, but it is easy to conceive of situations in which increasing the match will not improve retention, or perhaps even lower it. For example, if features are added to the cue that match the target exactly, but also match additional non-target items, then the difficulty of the target selection process can increase and performance decline.

The situation is somewhat akin to the relationship between stimulus intensity and the perception of brightness. It is generally the case that increasing the intensity of a light source makes things look brighter, but what mainly determines brightness perception is relative intensity information. It is the number of photons falling in a given spot relative to the number falling in surrounding spots that determines how bright the central spot appears. In fact, it is possible to increase the absolute light intensity falling on the spot and make it look darker (as long as light intensity in the surround is greater still). In discussing brightness perception, it is misleading to focus on light intensity *per se* because our visual system tends to throw away absolute information in favor of relative comparisons. Similarly, for retrieval, it is misleading to focus on the absolute match between a retrieval cue and an encoded target when it is actually the diagnostic value of the cue – the relative match – that really matters (see Nairne, 2002a).

2.12.5.5 Retrieval-Induced Inhibition

One interesting feature of the forgetting mechanisms that we have discussed so far is their passive nature. Things happen to the memory trace which yields it less recoverable: It decays, it is degraded by subsequent events, the cue–target association is extinguished by new learning, or the memory target exists in an unrecoverable state because an appropriate retrieval cue is lacking. Yet from an adaptive perspective, it seems likely that our memory systems may have evolved active mechanisms to inhibit or suppress information in specific situations in which that information is not needed. Inhibitory processes certainly play an important role in the nervous system, particularly in neural communication, so it is not a stretch to assume that inhibition is vital to memory processing as well. We might also assume that the effects of inhibition, when it occurs, must be temporary given the continuously changing nature of our processing goals (MacLeod and Macrae, 2001).

To be clear, in modern memory theory the concept of inhibition is synonymous with suppression. When a memory trace is inhibited, it is not degraded, damaged, or impaired, it is simply rendered temporarily unavailable by an active suppression process. Importantly, suppression of this sort differs from the retrieval blocking produced as a consequence of cue overload. In cue overload, competition among viable targets produces a 'winner' and unselected targets suffer as a consequence, but there is no need to assume suppression of the 'losers.' Likewise, recall is often claimed to have inhibitory properties (Roediger, 1974, 1978) because the act of recalling one item can lower the probability that other items will also be recalled; however, this kind of 'output interference' is generally assumed to result from biased sampling rather than from suppression. Once an item is recalled, the probability that it will be sampled again increases (it is primed) which, in turn, lowers the probability that other targets will be sampled. Note that inhibition might well occur as a by-product of cue overload, or biased sampling in recall, but it is not needed to produce forgetting in these instances.

As it turns out, the best evidence for inhibition comes from an empirical procedure known as the *retrieval practice paradigm* (Anderson et al., 1994). Here, people first learn lists of category-exemplar pairs (e.g., 'fruit–banana,' 'drink–scotch') and are then asked to practice retrieval of half of the exemplars from half of the list categories. Practice takes the form of completing stem-recall tests ('fruit-or____?') which people are required to complete several times. Last, after a short delay, a final category cued recall test is given for all of the exemplars (see **Figure 6**). There are two main findings of note: First, recall of the practiced exemplars is superior to that of the unpracticed exemplars; second, recall of the unpracticed exemplars from the practiced categories is impaired relative to exemplars from the unpracticed categories. Thus, practicing the recall of 'fruit–orange' impairs recall of the unpracticed exemplar 'fruit–banana,' below the baseline recall level for exemplars from the unpracticed categories. This impairment is known as *retrieval-induced forgetting* and is thought to accrue from an active inhibitory process.

Of course, there are other interpretations of these findings. For instance, one could appeal simply to retrieval blocking. Practicing 'fruit–orange' increases the strength of the cue–target association which, in turn, should bias the system to sample 'orange' in the

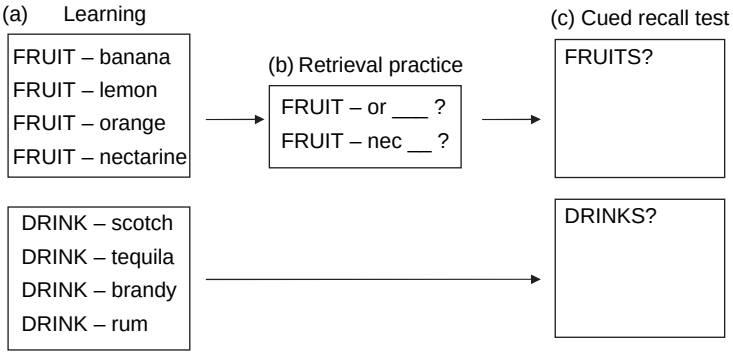

Figure 6 A simplified version of the retrieval practice paradigm. (a) Elements of two different categories are learned. (b) Half of the elements from one category are subject to a retrieval practice phase–cued word stem completion. (c) After a distractor period, participants respond to a cued recall test with the category name; participants are required to recall all the information learned in (a).

presence of 'fruit' to the exclusion of other exemplars. What supports the presence of active inhibition is the finding that the impairment is cue independent, that is, recall of 'orange' is impaired even when queried with cues unrelated to the category cue 'fruit' (see Anderson, 2003). In retrieval blocking, the impairment results from competition among targets elicited or matched by a given cue which, in turn, should lessen if 'orange' is cued by a target that is less overloaded. As a result, the retrieval practice paradigm seems to create a target that is truly suppressed, leading to impairment regardless of how the target is queried at the point of test.

As noted earlier, the idea that our memory systems may have developed mechanisms for actively suppressing information makes considerable adaptive sense. For any given constellation of retrieval cues, there is likely to be a wide array of potentially recallable responses, so it is in our interest to reduce irrelevant clutter. A telephone, for example, potentially elicits dozens of numbers to call, but we focus on the number at hand and push the remaining numbers out of mind (Levy and Anderson, 2002). A similar task faces us in perception: We must focus our attention on relevant portions of the sensory/perceptual message and block out the irrelevant ones. In memory, as in perception, it is adaptive to exercise cognitive control in our efforts to prioritize functioning. Inhibition – i.e., active suppression – is undoubtedly a useful weapon in the arsenal of cognitive control.

2.12.5.6 Motivated Forgetting

Proposals about inhibitory control in memory retrieval lead one naturally to the concept of repression, Freud's proposed defense mechanism (Freud, 1915). Although exactly what Freud meant by repression is open to some interpretation (Boag, 2006), it is generally conceived as a mechanism for preventing anxiety-inducing memories, usually traumatic, from entering the sphere of conscious awareness (Gleaves et al., 2004). Motivated forgetting of this sort seems adaptive: Unpleasant or traumatic memories can interfere with normal functioning, and the associated stress reactions can lead to long-term health consequences as well.

Adaptive speculations aside, is there solid empirical evidence for repression? Unfortunately, most of the relevant data are anecdotal. For obvious reasons, laboratory-based investigations of trauma-induced memory suppression are virtually impossible to conduct. The case for repression continues to rest largely on the many reports of trauma-based amnesia, and subsequent recovery during therapy, that have been obtained in clinical settings. Some relevant survey data and/or clinical cases exist as well, during which people with known histories of sexual abuse have reported periods of amnesia for their abuse (e.g., Williams, 1994; Schooler et al., 1997). However, perhaps not surprisingly, few in the scientific community find these data to be particularly convincing (e.g., Kihlstrom, 2004).

The clinical data are controversial for several reasons. First, in therapeutic settings it is often difficult to verify whether the traumatic event actually occurred or occurred in the form revealed by the recovered memory. Therapists usually do not seek independent corroboration of their clients' reports, again largely for ethical reasons (Shobe and Kihlstrom, 2002). Second, many psychologists believe that therapist–client interactions are particularly prone to the

inducement of false memories, perhaps because prior abuse is believed by many to be an important determinant of psychological problems. This is not to imply that false recovered memories are purposely implanted by the therapist, but they can occur as an unintentional by-product (e.g., Porter et al., 1999).

Finally, even if the traumatic event did occur, and the recovered memory is accurate in all details, this does not mean that an active repression process has produced the forgetting. As documented in this chapter, there are many reasons why people forget, and normal forgetting processes could easily account for many, if not all, of the verifiable cases of repressed memory. Just because a memory is traumatic does not mean that it is insensitive to decay, interference, or cue-dependent forgetting. In fact, given that many clinically relevant instances of abuse are thought to occur during childhood, or are accompanied by considerable emotional distress, it is perhaps not surprising that relevant retrieval cues are sometimes lacking at later points in time. Inhibitory mnemonic processes probably do exist, as evidence from the retrieval practice paradigm has shown, but whether there are special inhibitory processes (i.e., repression) that apply when memories are traumatic or emotionally tinged remains speculative at best.

2.12.6 Conclusions

Forgetting occurs when we fail to recover information that has been experienced previously. As noted initially, the common tendency is to label forgetting as a nuisance (or worse), but the process itself is actually quite adaptive. Imagine entering the parking garage after work and simultaneously recovering the locations of all of your previous parking spots. In his famous case study of the Russian journalist S., who was plagued by an inability to forget, Luria (1968) describes the torment S. experienced daily. S. had great trouble reading books, for example, because words and phrases so flooded his mind with previous associations that he was unable to concentrate. To avoid a truly cluttered mind, it is reasonable to assume that forgetting is a design feature of memory, that is, a cognitive capability that was selected for and maintained during the evolutionary history of our species.

Given the role that forgetting plays in normal functioning, it is reasonable to assume as well that there are many routes to forgetting. In the bulk of this chapter, we discussed a variety of forgetting mechanisms, everything from decay to interference to inhibition (summarized in **Table 1**). Each mechanism carries some weight of evidence and continues

Table 1 Summary of the mechanisms of forgetting presented in the chapter

Mechanism	*Source*	*Process*	*Effect*
Decay	Time	Autonomous process	Traces, or trace features, are permanently lost with time
Consolidation	Time	Autonomous process	Memories become more resistant to forgetting with time
Interference	Other acquired information	Trace degradation (Retroactive interference)	Newly acquired information damages the integrity of existing memory traces (e.g., overwriting)
		Cue impairment (Retroactive interference) (Proactive interference)	Other acquired information impairs the ability of a cue to produce a specific target due to the unlearning of preexisting associations between the cue and the target, or to target competition resulting from the association of the same cue with several targets (cue overload)
Cue availability	Altered stimulus conditions	Absence of an effective retrieval cue	The cue needed to elicit the originally learned information is not available
Retrieval-induced inhibition	Retrieval of other information	Active inhibition process	Temporary inhibition of information in situations in which it is not needed or when it competes with other target memories
Motivated forgetting	Repression	Active repression	Prevents anxiety-inducing memories, usually traumatic, from entering the sphere of conscious awareness

to have many advocates. It would be improper to conclude that any one of these mechanisms is 'the' forgetting mechanism because different situations will undoubtedly demand different forgetting solutions. In some circumstances, important information needs to be suppressed temporarily; in others it may be in our interest to forget things permanently (or nearly so).

Whatever the mechanism, however, it does remain a challenge for memory theorists to explain the regularity of the forgetting function. As documented earlier, the function relating recovery to time is very regular in form (see **Figure 1**). Attributing forgetting to interference from subsequent events, or to the action of cue overload, does little to explain why the forgetting curve is consistently negatively accelerated. Forgetting may indeed be cue-dependent, but then what determines the availability of appropriate cues? As the forgetting curve informs, forgetting is by no means a random occurrence.

Lastly, it is worth noting that forgetting can never be measured directly: We can only measure what has been 'remembered,' at a particular time, given a particular context. And, as we have described, what appears to have been forgotten may, in fact, turn out to be recoverable at another time or in a different context. To the extent that our memory systems are designed to use the past, in combination with the present, to generate adaptive behavior, then the variability of memory is hardly surprising. Stored information should be retrieved when it is necessary and not otherwise. In this sense it is a mistake to speculate about the ultimate 'cause' of forgetting, or to consider forgetting as a breakdown in normal functioning, because our memory systems are not designed to recover stored information at will: Recovery will depend on the situation and, more importantly, on the particular adaptive problem at hand.

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2.13 Inhibitory Processes

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2.13.1 Introduction

2.13.1.1 Retrieval Competition

In the course of a day, we encounter a huge number of experiences that we encode and store in our memory. Some of these experiences may be unique and lead to the encoding of very distinct features. Most of the experiences, however, will share certain features with other experiences, thus leading to the encoding of common features in our memory. In his morning lecture, a professor, for instance, may encode many distinct features of each participating student while, for all students, encoding the common feature of participating in this particular course of study. The encoding of this common contextual feature can create a problem for the professor's memory when later asked about the names of the participating students. Typically, the larger the number of students who were present in the lecture, the poorer will be the professor's recall for any one particular name. The reason for this retrieval problem is retrieval competition.

Retrieval competition means that memories that share a common cue – be it a contextual, semantic, or emotional cue – compete for conscious recall once the cue is provided and, as a result, show both reduced recall performance and increased response latencies (**Figure 1(a)**). Corresponding evidence has been provided by a number of studies in quite different experimental paradigms. In single-list paradigms, for instance, memory for target information has been found to decline and to be slowed down when the number of list items increases, which is known as the list-length effect (Watkins, 1975). In multiple-list

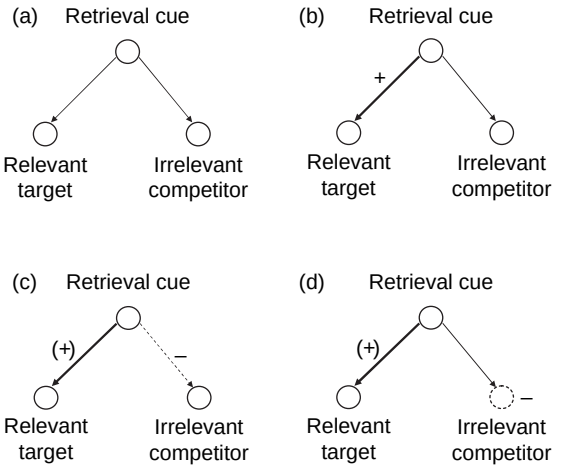

Figure 1 (a) Retrieval competition. Relevant target and irrelevant competitor material are connected to the same retrieval cue and compete for conscious recall once the cue is provided. For both materials, the competition reduces recall probability and increases response latency. (b) Blocking. Strengthened target information blocks access to nonstrengthened competitor information. Blocking occurs at test when the strengthened material is recalled first and hinders subsequent recall of the nonstrengthened material. Blocking does not affect the competitor's retrieval route and does not affect its memory representation. (c) Route deactivation. Deactivation of the retrieval route between cue and competitor information (with possible simultaneous enhancement of retrieval route between cue and target). Route deactivation takes place at a pretest encoding or a pretest retrieval stage and reduces chances of the retrieval cue to make competitor information recoverable. (d) Item suppression. Deactivation of the memory representation of the competitor information (with possible simultaneous enhancement of retrieval route between cue and target). Item suppression takes place at a pretest encoding or a pretest retrieval stage and reduces chances to recover the competitor information, regardless of which retrieval cue is provided.

paradigms, it has been shown that both the prior and the subsequent encoding of additional lists of items can impair memory for the target list, which is known as proactive and retroactive interference (Müller and Pilzecker, 1900; Underwood, 1957; for a summary, see Crowder, 1976).

Retrieval competition provides a challenge for the goal-directed use of memory. Indeed, in daily life, relevant and irrelevant information often share a common cue. This may be the case for the more relevant and more irrelevant things that occurred to us in the office, the expired and current passwords of our computer, or the past and current address of a friend's home. When trying to recall the relevant or current information, remembering then may fail because the irrelevant or out-of-date information is retrieved. Effective updating should reduce accessibility of the irrelevant memories and simultaneously enhance that of the more relevant information.

It is an old and prominent idea that, in our memory, inhibitory processes operate to serve the function of goal-directed remembering, reducing the accessibility of irrelevant information and enhancing that of the more relevant information. In this chapter, I summarize results from a number of experimental paradigms in which the action of inhibitory processes serving memory's goal-directed use has been suggested. While there is consensus in the literature that inhibitory mechanisms operate to overcome retrieval competition and enhance the processing of relevant information, different conceptions about inhibitory mechanisms exist. In fact, some researchers speak of inhibition whenever a mechanism reduces accessibility of irrelevant information, be it directly or indirectly. Others speak of inhibition only if a mechanism affects the irrelevant information directly, be it through deactivation of the information itself or through deactivation of some of its retrieval routes.

The goal of this chapter is not to discuss which mechanism reflects real inhibition and which does not. Rather, the goal is to indicate which of the suggested mechanisms operate under what conditions. As it turns out, different mechanisms operate in different experimental contexts, providing us with a detailed picture of how reduced accessibility of irrelevant information is achieved in memory. Before reviewing these results, some of the most important conceptions of inhibitory processes suggested in the past decades are outlined. After becoming familiar with these conceptions, I turn to determining which of the inhibitory processes operates in which experimental context.

2.13.1.2 Inhibitory Mechanisms

Three primary inhibitory mechanisms have been suggested to serve the goal of effectively separating relevant from irrelevant information and enhancing memory for the first at the expense of the second. The three mechanisms are blocking, route deactivation, and item suppression.

1. Blocking

As described in section 2.13.1.1, when memories share a common cue, they compete for conscious recall once the cue is provided and show reduced recall

performance (**Figure 1(a)**). Such retrieval competition has been shown to be strength dependent. That is, if material that is strongly represented in memory and material that is weakly represented share the same retrieval cue, there is a tendency for the stronger material to be recalled first (Rundus, 1973; Raaijmakers and Shiffrin, 1981; Wixted et al., 1997). Thus, if relevant material, like the current computer password, is represented more strongly in memory than irrelevant material, that is, the expired password, the difference can induce a competition bias at test favoring the early recall of the (stronger) relevant information – the current password. By involuntarily sampling the already recalled material repeatedly, this early recall of the relevant information then can block subsequent recall of the (weaker) irrelevant information – the expired password – and make it inaccessible (**Figure 1(b)**). Such blocking of irrelevant information has repeatedly been regarded as an important example of inhibition in human memory (Melton and Irwin, 1940; McGeoch, 1942; Rundus, 1973) and is a core feature of many computational models (Raaijmakers and Shiffrin, 1981; Mensink and Raaijmakers, 1988).

A crucial feature of blocking is that there is no direct effect of inhibition on the irrelevant material itself and no direct effect on the retrieval routes between the irrelevant material and its cue(s). Rather, the inaccessibility of the irrelevant material (the expired password) arises as a by-product of the strengthening of the relevant material (the current password), which, as a result of biased competition, favors recall of the stronger relevant material and blocks recall of the weaker irrelevant material. Because blocking typically operates at test, its effect should be visible in memory tests in which there is the opportunity for strength-dependent retrieval (i.e., in free recall tasks and in cued recall tasks in which more than one item is connected to the cue). In contrast, blocking should play only a minor role, if any, in memory tests in which item-specific probes are provided as retrieval cues, such as recognition tests or implicit memory tests. In all these tests, strength-dependent competition should be greatly reduced or eliminated.

2. Route deactivation

A more direct way to induce inaccessibility of irrelevant material would be to not only strengthen the relevant material but also weaken the retrieval route between the irrelevant information and its cue, so that retrieval of the irrelevant information becomes less effective (Melton and Irwin, 1940; Geiselman et al., 1983; **Figure 1(c)**). Applied to the computer password retrieval problem, this would mean that, rather than blocking retrieval of the expired computer password, an inhibitory mechanism would directly weaken the connection of the expired password's memory representation to the common password cue. Such route deactivation might operate while the new password is encoded, or it might operate after the encoding of the new password, for instance, when retrieving the current computer password. Because the retrieval routes to the irrelevant information would be affected directly, the effect of such a mechanism should be visible in a number of memory tests and should arise not just in free recall tasks but in all forms of cued recall as well. On the other hand, because the representation of the irrelevant material itself would not be affected, only minor, if any, forgetting of the irrelevant information should be observed in recognition tasks.

The strengthening of relevant information creates a difference in relative strength between relevant and irrelevant information and thus can lead to blocking. Route deactivation also creates a difference in relative strength, which may be particularly strong, if not only the retrieval routes for the irrelevant material are reduced but retrieval routes for the relevant material are simultaneously enhanced. Therefore, route deactivation may trigger blocking at test as an additional mechanism, an effect that should be largely restricted to free recall tasks.

3. Item suppression

Blocking and route deactivation reflect mechanisms that, following Tulving and colleagues' terminology (Tulving and Pearlstone, 1966; Tulving and Psotka, 1971; Tulving, 1974), result in loss of retrieval access to inhibited items rather than in loss of the items' availability. A loss in availability would imply that the memory representation of material is affected itself so that memory for the material is impaired regardless of which retrieval cue is provided. It has repeatedly been suggested that inhibitory processes may affect the item representation itself (Postman et al., 1968; Anderson and Spellman, 1995; **Figure 1(d)**). Applied to the computer password retrieval problem, for instance, this would mean that, rather than blocking the expired computer password or reducing its connection to the common password cue, the memory representation of the expired password would directly be suppressed. Such suppression might operate while the new password is encoded, or it might operate after the encoding of the new password, for instance, when retrieving the current computer password. Due to the direct effect

on the memory representation of the irrelevant information itself, item suppression would reflect a strong form of inhibition and should be visible over a wide range of memory tests, including recognition tasks and tasks that employ so-called independent probes, that is, probes not used until the test phase of an experiment.

Like route deactivation, a side effect of item suppression is that a difference in relative strength between relevant and irrelevant material is created, particularly if there is simultaneous strengthening of the relevant information. In memory tests that are sensible to strength-dependence effects, this difference can trigger blocking as an additional inhibitory mechanism. In free-recall tasks, item suppression, therefore, may reduce accessibility of irrelevant material very effectively.

In sum, although blocking, route deactivation, and item suppression can all serve the goal of reducing accessibility of irrelevant material and enhancing that of relevant information, the three mechanisms differ in the way the inaccessibility is achieved. In blocking, the inaccessibility arises because of the difference in relative strength between target and competitor information, with early recall of the stronger target information blocking recall of the weaker competitor information; in route deactivation, inaccessibility arises because of the direct deactivation of the retrieval route between the cue and the irrelevant information; and in item suppression, inaccessibility arises because of the direct deactivation of the memory representation of the irrelevant material itself, which, following Tulving's terminology, represents some form of information unavailability.

The difference in how inaccessibility is achieved in the three inhibitory mechanisms has implications for the range of memory tests in which the effects of inhibition can be observed. Blocking represents the weakest form of inhibition. Because it does not affect the irrelevant material directly, its effects should arise only in memory tests that leave sufficient room for strength-dependent retrieval, like free-recall tasks. By directly affecting the retrieval routes to the irrelevant material, route deactivation represents a stronger form of inhibition, the effects of which should be observable in free-recall and cued-recall tasks. Item suppression, finally, represents the strongest form of inhibition that affects the memory representation of the inhibited item itself and thus should create effects across a wide range of memory tests. Also note that route deactivation and item suppression can trigger additional blocking at test, thus creating a situation in which two inhibitory processes may operate in concert.

The next section examines the role of the three suggested inhibitory mechanisms in experimental paradigms that are often assumed to involve inhibition: strength-induced forgetting, retrieval-induced forgetting, directed forgetting, think/no-think impairment, and part-list cuing impairment. At the end of the section, current knowledge about the developmental trajectory of inhibition in the single paradigms is reviewed. In the conclusion, finally, the results are summarized and a taxonomy of the inhibitory paradigms is suggested.

2.13.2 Inhibitory Paradigms

2.13.2.1 Strength-Induced Forgetting

A simple way to emphasize memory for relevant material is to present the relevant information repeatedly or longer and the less relevant information less often or for a shorter time period. This is common use in textbooks or talks, in which important information is typically repeated in several places, and it is typically employed by students preparing for an exam, when they spend more time on the seemingly relevant material than on the seemingly irrelevant material.

Strengthening the memory representation of relevant information – be it through repeated or longer study – should lead to different memory performance for the relevant and irrelevant information, simply because of the resulting difference in (absolute) memory strength between the two types of information. The effect of strengthening or inhibition that we observe in experiments, however, is often larger than we might expect on the basis of the encoding difference, suggesting that additional processes enhance accessibility of the relevant material and reduce it for irrelevant material. This point has been demonstrated in a number of experimental paradigms, discussed next. In these paradigms, a subset of the material to be learned is strengthened, and its effect on later memory for the strengthened and nonstrengthened material is examined.

2.13.2.1.1 The mixed-list paradigm

In the mixed-list paradigm, participants are presented a list of items such as unrelated words. Strengthening effects are then examined by providing a longer presentation time for a subset of the material or by presenting a subset of the material

repeatedly (e.g., Tulving and Hastie, 1972; **Figure 2**). For instance, half of the items of a list may be shown for 6 s and the other half for 2 s, or half of the items may be shown twice and the other half once. Memory performance for the two types of items is compared with two pure-list baseline conditions in which there is no such partial strengthening and all material is studied in the same way (i.e., all items are studied for 6 s or all items are studied for 2 s). Memory is typically assessed by means of a free-recall test, a cued-recall test, or a recognition test.

On the basis of the encoding difference between strong and weak items, strong items should show better memory than weak items. In particular, strong items in mixed lists should show the same memory performance as strong items in pure lists, and weak items in mixed lists should show the same performance as weak items in pure lists. The general idea of retrieval competition and blocking, as outlined above (in sections 2.13.1.1 and 2.13.1.2), however, suggests otherwise, at least in free-recall tasks. Partial strengthening in the mixed-list paradigm should introduce a competition bias, leading to early recall of the strengthened items, which then may block subsequent recall of the nonstrengthened items. As a result, on average, strong items in mixed lists should show better performance than strong items in pure lists, and weak items in mixed lists should show poorer performance than weak items in pure lists. Such effects should be present in free-recall tasks, but they should be absent in recognition tasks, in which no such competition bias is expected.

Indeed, free-recall experiments have shown that, in mixed lists, strengthened items are better recalled than nonstrengthened ones and that this effect is not only a result of the difference in the items' absolute memory strength but is also attributable to their difference in relative strength. Recall of strong items was consistently found to be higher in mixed lists than in pure lists, and recall of weak items was

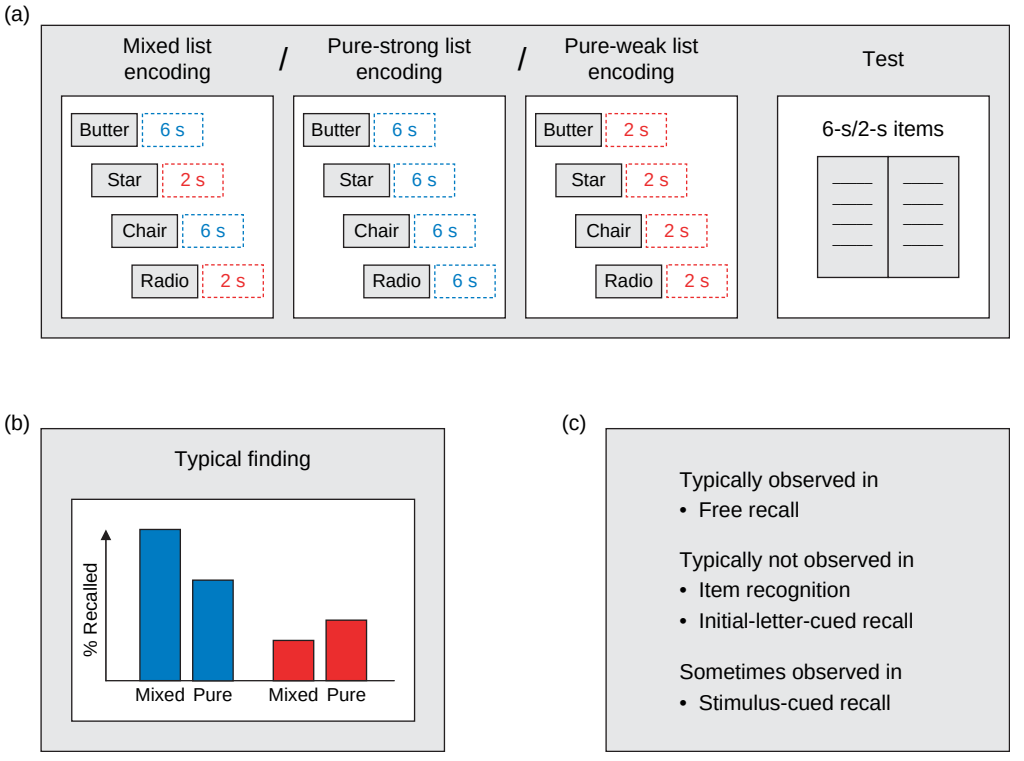

Figure 2 The list-strength effect. (a) The experimental paradigm. Participants study a mixed list consisting of strong and weak items. Memory for the two types of items on a later test is compared to two baseline conditions: a pure-strong list condition containing strong items only and a pure-weak list condition containing weak items only. Strengthening is accomplished by varying either the exposure time or the number of repetitions of the items to be strengthened. (b) The typical finding. Relatively more strong items are recalled from the mixed list than from the pure-strong list, and relatively fewer weak items are recalled from the mixed list than from the pure-weak list. (c) Memory tests. Examples of memory tests in which the list-strength effect typically arises and in which it typically does not arise.

found to be poorer in mixed lists than in pure lists (Tulving and Hastie, 1972; Ratcliff et al., 1990; see also Malmberg and Shiffrin, 2005). In contrast, such list-strength effects have typically been found to be absent in recognition tasks. Although recognition of strong items was found to be higher than recognition of weak items, performance for the two types of items did not vary with list composition (Ratcliff et al., 1990; Murnane and Shiffrin, 1991; Ratcliff et al., 1992).

A few studies reported reliable recognition effects in the mixed-list paradigm (see Norman, 2002, for item recognition, and Verde and Rotello, 2004, for associative recognition), which were used to draw inferences on whether the effects were caused by changes in recollection or changes in the familiarity of the items. Tulving (1985) distinguished between two bases for judging an item as 'old' on a recognition test: The participant specifically remembers the temporal and/or spatial context in which the item was studied (recollection), or the participant finds the item just familiar (familiarity; for a review, see Yonelinas, 2002). From the studies reporting reliable list-strength effects in recognition, evidence has arisen that the list-strength effect reflects mainly a modulation in recollection but does not affect familiarity.

A number of studies examined list-strength effects in cued recall. In paired-associate learning with stimulus-cued recall tests, a mixed picture arose with reliable effects in some experiments and nonreliable ones in others (Ratcliff et al., 1990). List-strength effects were also examined when categorized lists were studied. In this case, each category studied was a mixed category, with half of the items being strong (high-frequency) exemplars and half of the items weak (low-frequency) exemplars of the category, or a pure category, with all items being strong exemplars or all items weak exemplars. The items' category name and their unique initial letters were provided as retrieval cues at test. Performance of strong and weak items was compared between mixed categories and pure categories when the items were tested first within their categories, thus controlling for possible effects of output order. For both strong and weak items, no effect of category composition arose (Bäuml, 1997).

As a whole, these findings are largely consistent with the proposal that strengthening, as employed in the mixed-list paradigm, leads to blocking at test, which improves access to the relevant information at the expense of the access to the irrelevant material. Accordingly, the list-strength effect is typically present in free-recall tasks and is absent in recognition tasks. If output order is controlled, the effect is also absent in cued-recall tasks, which is consistent with the view that the inhibitory mechanism does not affect the items' retrieval routes or their memory representation.

2.13.2.1.2 Relearning and interference paradigms

Findings consistent with those from the mixed-list paradigm have been reported in two further strengthening paradigms, relearning and interference. In the relearning paradigm, strengthening effects are examined by presenting the relevant and irrelevant material together within one list and subsequently presenting the relevant material again for an additional study trial. This relearning condition is then compared with a condition in which there is no such reexposure of the relevant material.

Again, the idea of blocking suggests that, at least in free-recall tasks, retrieval competition may become biased because of the strengthening of a subset of the material and thus may increase recall of the relearned material at the expense of the material presented only once. Such effects, however, should not arise in recognition tests or tests using item-specific probes. A number of studies examined this latter prediction in cued-recall tests, in which the items' unique initial letters were provided as additional retrieval cues and output order was controlled by testing the target items first. In all these studies, relearning improved recall of the strengthened material but did not affect recall of the nonstrengthened material (Ciranni and Shimamura, 1999; Anderson et al., 2000a; Bäuml and Aslan, 2004). This finding is consistent with the assumption that strength-induced forgetting is mediated by blocking rather than by deactivation of retrieval routes or by deactivation of the item representation.

Both in retroactive and proactive interference, older studies had shown that strengthening of prior or subsequently encoded material can increase interference and thus increase forgetting of target material (for a review, see Crowder, 1976). Varying the degree of interpolated learning in a list-learning paradigm, Bäuml (1996) replicated this result and found that a higher degree of interpolated learning induced greater retroactive interference. In this experiment, output order was not controlled, and participants were free to recall the strengthened interpolated material prior to the (weaker) target material. In a second experiment, which was largely identical to the

first experiment, output order was controlled, and participants were asked to recall the (weaker) target material first. No effect of degree of interpolated learning was observed (for a related result regarding proactive interference, see DaPolito, 1966; for further investigation, see Delprado, 2005). Again, these findings point to the action of a blocking mechanism, which is activated in memory tests that permit recall of items in any order.

2.13.2.1.3 Summary

Results from paradigms investigating strength-induced forgetting indicate that the strengthening of relevant material at encoding can trigger inhibitory processes to improve memory for the strengthened material at the expense of that for the not strengthened or irrelevant material. Strength-induced forgetting is present in free-recall tests and absent in recognition tests or cued-recall tests, in which output order is controlled. These results are consistent with the proposal that the enhanced accessibility of strengthened material and the reduced accessibility of nonstrengthened material result from blocking at test, in which early recall of stronger items prevents subsequent recall of the weaker ones. The results do not support the idea that the memory effects in these paradigms are caused by direct inhibitory effects on the irrelevant material's representation or its retrieval routes.

2.13.2.2 Retrieval-Induced Forgetting

The strengthening of relevant material at encoding can trigger inhibitory processes on the nonstrengthened irrelevant material. Strengthening of relevant material, however, does not only occur at encoding but may happen through retrieval as well. In fact, while relearning is an often employed method to emphasize memory for relevant material, retrieval of previously studied material can serve the same goal. One may even expect to find strengthening through retrieval to induce the same inhibitory processes as strengthening at encoding.

Experimental studies have shown that retrieval typically enhances later recall of the practiced material (Hogan and Kintsch, 1971) and can even be more powerful in its effect than relearning is (Carrier and Pashler, 1992; Roediger and Karpicke, 2006). The question arises of whether strengthening through retrieval also induces inhibitory processes and, if it does, whether such inhibition is mediated by the same competition bias as the inhibition underlying strengthening through relearning. On the basis of many computational models of memory, this might be expected, given that retrieval has often been assumed to reflect some form of relearning (e.g., Rundus, 1973; Raaijmakers and Shiffrin, 1981).

2.13.2.2.1 Retrieval-practice paradigm

In the retrieval-practice paradigm, a subset of learned material is repeatedly retrieved, and the effect of this manipulation on later memory for the practiced and unpracticed material is examined (Anderson et al., 1994; **Figure 3**). Memory is typically assessed by means of free recall, cued recall, and recognition tests. In addition, so-called independent probe tests are conducted, in which memory is assessed using cues at test that were not employed in earlier parts of the experiment. On the basis of retrieval competition and blocking, it might be assumed that retrieval simply strengthens the practiced material and thus causes blocking for the unpracticed material. Such blocking would increase recall for the practiced material and decrease recall for the unpracticed material, relative to control items in a no-practice condition. If true, then the effect of retrieval would mimic the effect of relearning.

A large number of free- and cued-recall experiments have addressed this issue in recent years. In these experiments, participants often learned categorized item lists and then practiced half of the items from half of the categories. At test, the category names were then provided as retrieval cues and the task was to recall the previously studied items that belonged to the category name. Relative to the control items from the unpracticed categories, retrieval practice typically improved recall of the practiced items and impaired that of the unpracticed items from the practiced categories (Anderson et al., 1994; Anderson and Spellman, 1995; Macrae and MacLeod, 1999; MacLeod and Macrae, 2001; Bäuml and Hartinger, 2002). This pattern of results first of all mirrors the beneficial and detrimental effects of strengthening at encoding, suggesting that the effect may be a result of blocking.

If the effects of retrieval were equivalent to those of relearning and only reflected biased competition and blocking, the detrimental effects of retrieval practice should be eliminated once item-specific probes were employed at test and output order was controlled. The issue was examined in experiments in which the detrimental effects of relearning and retrieval were compared directly. Participants learned categorized lists. At test, the category names

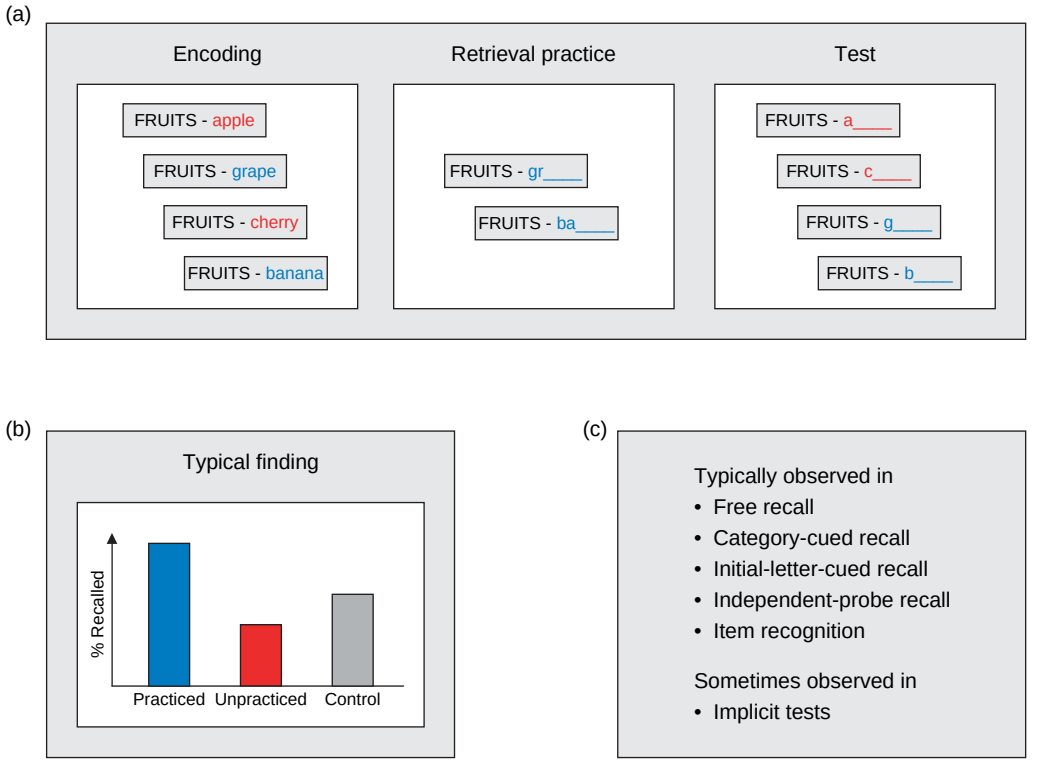

Figure 3 Retrieval-induced forgetting. (a) The experimental paradigm. Participants study a categorized item list. In a subsequent retrieval-practice phase, half of the items from half of the studied categories are repeatedly retrieved. On the final test, participants are asked to recall all previously studied items. (b) The typical finding. Practiced items show higher recall rates, and unpracticed items show lower recall rates relative to the control items from the unpracticed categories. (c) Memory tests. Examples of memory tests in which retrieval-induced forgetting typically arises.

and the targets' initial letters were provided as retrieval cues. To control for output order, the targets were always tested first within their categories. Consistent with the results from the strengthening paradigms, no detrimental effect of relearning arose. In contrast, reliable forgetting arose in the retrieval practice condition (Anderson et al., 2000a), indicating that retrieval-induced forgetting reflects a recall-specific effect and is not caused by blocking (for related results, see Ciranni and Shimamura, 1999; Bäuml, 2002).

Further studies support the proposal that retrieval-induced forgetting does not reflect blocking by showing that the effect is not only present in free and category-cued recall but occurs in other types of tests as well. Using the so-called independent probe procedure, for instance, retrieval-induced forgetting has been reported to be cue independent, that is, to generalize to cues other than those used at study or retrieval practice (Anderson and Spellman, 1995; Anderson et al., 2000b; Saunders and MacLeod, 2006; Aslan et al., 2007b). Accordingly, retrieval practice of 'fruit-apple' was found to induce forgetting of 'banana' not only when banana was tested with the same cue as was used at study and retrieval practice ('fruit') but also when it was probed with a new, independent cue (e.g., 'monkey'). This property of cue independence is taken as strong support for the view that retrieval-induced forgetting is caused by inhibition (Anderson, 2003; for failures to find cue independence, see Williams and Zacks, 2001; Perfect et al., 2004).

Retrieval-induced forgetting has also been found in recognition tests (Hicks and Starns, 2004; Verde, 2004; Gómez-Ariza et al., 2005; Spitzer and Bäuml, in press). As outlined in section 2.13.1.2, recognition can be based on recollective processes and/or familiarity processes (Tulving, 1985; Jacoby, 1991). In retrieval-induced forgetting, the recognition tests provided evidence that retrieval practice affects both recollective and familiarity processes of the unpracticed material. Studies in which associative recognition was employed reported a reduction in

recollective processes (Verde, 2004); studies in which item recognition was employed reported additional reductions in familiarity processes (Spitzer and Bäuml, in press). Regarding implicit memory tests, a mixed picture arises. Whereas some studies found reliable impairment in implicit tests (Veling and van Knippenberg, 2004), others failed to find an effect (Racsmany and Conway, 2006). Still others found effects in some tests but no effects in others (Perfect et al., 2002; see also Camp et al., 2005).

The results from all these studies are largely consistent with the assumption of an inhibitory mechanism that directly affects the representation of the unpracticed items itself. Because of such an impairment in item representation, all retrieval routes to the inhibited item should be less effective than without retrieval practice, and forgetting should be observed across a wide range of memory tests. The results from the studies employing recognition tests and tests using independent probes as cues support this view and are inconsistent with the hypothesis that the inhibition is the result of blocking or an effect on the retrieval routes between the inhibited item and its studied cue. Blocking assumes an inhibitory mechanism that operates at test. Here the proposal is that the inhibitory mechanism operates before the test in the retrieval practice phase of the experiment. In this phase, the not-to-be-practiced material is supposed to interfere and to be inhibited to reduce the interference and make retrieval of the target information easier (Anderson and Spellman, 1995; see also Anderson, 2003).

Two further lines of research support this view of retrieval-induced forgetting. First, response latency analysis sheds light on the dynamics of recall, allowing conclusions on the size of the underlying search set and the memory strength of the set's items (for a review, see Wixted and Rohrer, 1994). Applying such response latency analysis, Bäuml et al. (2005) found that retrieval practice reduces unpracticed items' recall probability but does not affect their response latency. This result mirrors typical effects of item strength manipulations as they occur as a result of variations in study time or study trials (Rohrer, 1996; Wixted et al., 1997), indicating that retrieval practice affects the memory strength of unpracticed items but does not prevent the items from being sampled. Second, a recent electrophysiological study reported retrieval-specific brain activities during the retrieval-practice phase, which were reflected in sustained prefrontal event-related potentials and correlated with the amount of forgetting in the later memory test (Johansson et al., 2007). The reported retrieval-specific effect indicates that retrieval-induced forgetting is the result of processes operating during retrieval practice and is not the result of blocking at test.

The inhibitory view of retrieval-induced forgetting presupposes some degree of retrieval competition and relational processing between single items. Consistently, Smith and Hunt (2000) reported reliable retrieval-induced forgetting if individuals encoded items in a relational way but not when they encoded them in an item-specific way, that is, by their features and distinctive qualities (regarding relational and item-specific processing, see Hunt and McDaniel, 1993). It is often assumed that positive moods encourage relational processing and negative moods encourage item-specific processing (e.g., Storbeck and Clore, 2005), thus raising the expectation that mood affects retrieval-induced forgetting. Bäuml and Kuhbandner (2007) examined the effect of positive and negative mood induction immediately before retrieval practice on retrieval-induced forgetting. Indeed, negative mood induction, but not positive mood induction, eliminated the forgetting.

Once material is processed in a relational way so that retrieval competition arises, the forgetting may be affected by the degree of interitem associations between the practiced and unpracticed material. Indeed, both instructions to interrelate to-be-practiced and not-to-be-practiced items in a meaningful way (Anderson et al., 2000b) and the use of strong pre-experimental associations between the two types of items (Bäuml and Hartinger, 2002; Bäuml and Kuhbandner, 2003) have been shown to eliminate retrieval-induced forgetting. Under conditions that simulate educational situations, Chan et al. (2006) even demonstrated that retrieval practice can benefit memory for the unpracticed material. These findings are consistent with a variant of item suppression in which items are represented as sets of features, and features that the unpracticed item shares with the practiced items are strengthened rather than inhibited. Because of this strengthening of some of the item's features, forgetting is reduced or eliminated and may even be reversed to show recall facilitation (for details, see Anderson, 2003).

At the core of the retrieval-practice paradigm is the action of an inhibitory mechanism that directly affects the representation of the irrelevant material. This effect is observable across a wide range of memory tests because the items' representation itself is affected. Note, however, that because item

suppression induces a difference in relative strength between practiced and unpracticed items, the inhibitory process should also create a competition bias at test, favoring early recall of practiced items at the expense of unpracticed items. Moreover, because there is not only suppression of unpracticed material but also strengthening of the practiced material, this bias should be particularly strong, triggering additional blocking in free-recall tasks. Results from several studies are consistent with this prediction (e.g., Anderson et al., 1994).

2.13.2.2.2 Output interference

If retrieval of material can cause forgetting of nonretrieved material, then retrieval-induced forgetting should also occur in the course of a recall test. In principle, recall of a first item should cause inhibition of the still-to-be-remembered items, as should recall of the second item, the third item, and so on. As a result, recall performance at test should decline as a function of testing position. This pattern is exactly what has been known as output interference for more than 30 years. Output interference has been demonstrated in a number of studies (Smith, 1971; Roediger, 1974; Roediger and Schmidt, 1980) and, among other factors, was taken as evidence that recall is a self-limiting process (Roediger, 1978).

Originally, output interference was explained in terms of retrieval competition, assuming that recall of a first item strengthens the item and thus, because of biased retrieval competition, makes retrieval of the remaining items harder. This blocking account obviously disagrees with the explanation of retrieval-induced forgetting as it occurs in the retrieval-practice paradigm (see section 2.13.2.2.1). Arguably, however, retrieval-induced forgetting as studied in the retrieval-practice paradigm and retrieval-induced forgetting as studied in the output-interference paradigm should be mediated by similar mechanisms and might even be equivalent.

A blocking account of output interference predicts that the forgetting should disappear once item-specific probes are presented at test. Thus, recall should not decline with testing position if, for instance, the items' unique initial letters were provided as cues (see section 2.13.2.1.1). In contrast, if output interference was mediated by the same mechanism as the forgetting in the retrieval-practice paradigm, recall should decline with testing position regardless of whether item-specific cues were provided or not (see section 2.13.2.2.1). As it turned out, output interference effects are maintained in the presence of item-specific probes (Anderson et al., 1994; Anderson and Spellman, 1995; Bäuml, 1997), which supports the suggested equivalence of effects in the two paradigms.

The relation between the two paradigms was further examined in two studies in which the role of item strength and item similarity in output interference were examined (Bäuml, 1998; Bäuml and Hartinger, 2002). The two studies found effects of item strength and item similarity in output interference that mirrored those known from the retrieval-practice paradigm, which is consistent with the view that the same inhibitory mechanisms operate in the two paradigms. Given the evidence for item suppression in studies using the retrieval-practice paradigm (see section 2.13.2.2.1), these results suggest a role of item suppression in output interference as well.

2.13.2.2.3 Summary

Like relearning of relevant material, retrieval of relevant material can impair memory for irrelevant material. Results from the retrieval-practice paradigm suggest that retrieval triggers two processes: one process strengthening the practiced material and a second process inducing inhibition of the unpracticed material. These two processes create some difference in relative strength between practiced and unpracticed items and thus leave room for blocking. Going beyond blocking, however, retrieval affects the unpracticed material's representation itself. Such item suppression is at the core of retrieval-induced forgetting and implies that the inaccessibility of the irrelevant material is not restricted to free-recall tasks but generalizes to a wide range of memory tests. The results also indicate that the detrimental effects of retrieval and relearning are mediated by different mechanisms.

2.13.2.3 Directed Forgetting

Relearning and retrieval practice may be regarded as forms of memory updating, in which part of previously studied material is reexposed or retrieved, suggesting that it is more relevant than the remaining material (Anderson and Schooler, 1991). Inhibitory processes then act on the seemingly less relevant material, either by blocking its access during recall or by directly affecting the material's representation itself. A different form of updating may arise in situations in which new information, such as the new computer password, has to displace old information, for example, the expired password. In this case,

memory for the new relevant information would benefit if memory for the irrelevant out-of-date information was reduced. Whether such updating is part of our memory and is mediated by inhibitory processes has been studied in list-method directed forgetting.

2.13.2.3.1 List-method directed forgetting

In list-method directed forgetting, participants learn two lists of items. After learning List 1, they receive a cue to either forget or continue remembering this list before studying List 2. After learning List 2, a recall or recognition test is conducted in which participants are asked to remember the previously studied items, including those the participants were originally cued to forget. Memory for List 1 and List 2 items is then compared between the two conditions (Bjork, 1970, 1989; **Figure 4**).

The results from numerous recall experiments show two effects of the forget cue: reduced recall of List 1 items, referred to as forgetting and improved recall of List 2 items, referred to as enhancement (for a review, see MacLeod, 1998). These effects provide the first evidence for memory updating in this paradigm, demonstrating reduced accessibility for the out-of-date information and enhanced accessibility for the new information. Arguably, the forgetting of List 1 items might be a result of demand characteristics, because participants may not try as hard to recover the to-be-forgotten List 1 items as they do for the to-be-remembered List 2 items. The effect of the forget cue, however, does not disappear if money is offered for recalled List 1 items (MacLeod, 1999), indicating that the effect probably is not a result of demand characteristics (for a recent variant of list-method directed forgetting, see Szpunar et al., in press).

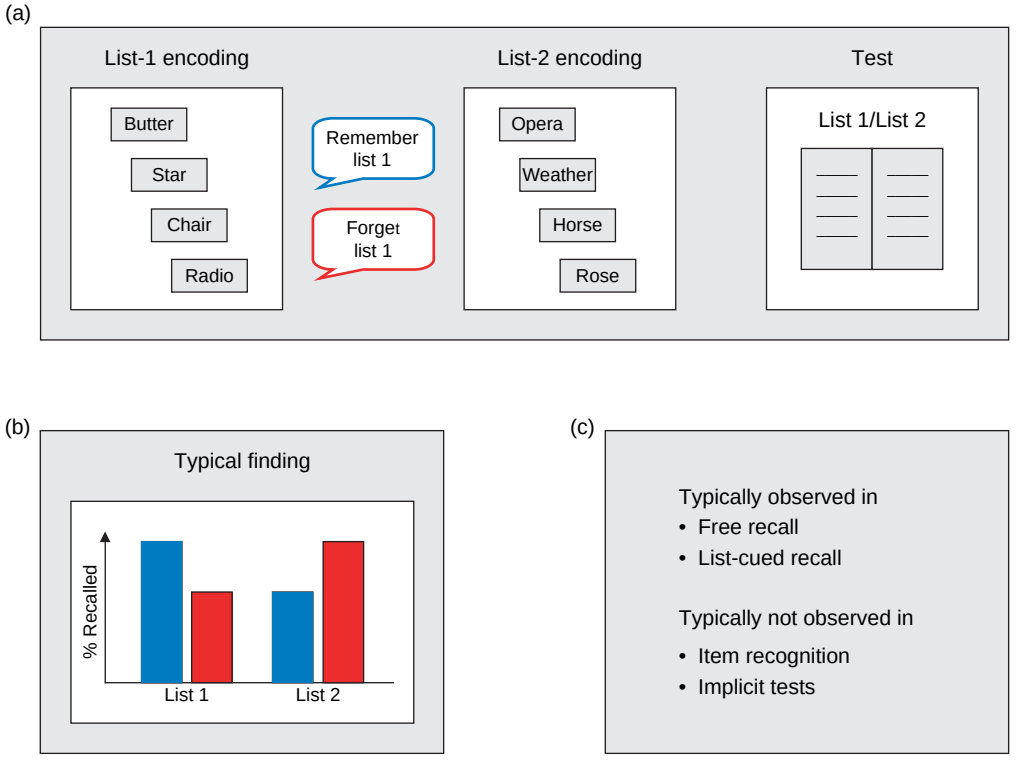

Figure 4 List-method directed forgetting. (a) The experimental paradigm. Participants study two lists of items and, after the presentation of List 1, receive a cue to either forget or continue remembering this list before studying List 2. After study of List 2, a recall test is conducted in which participants are asked to recall all of the previously presented items, including those the participants were originally cued to forget. (b) The typical finding. Compared with remember-cued participants, forget-cued participants typically show impaired recall of List 1 items and improved recall of List 2 items, referred to as the forgetting and the enhancement induced by the forget cue. (c) Memory tests. Examples of memory tests in which list-method directed forgetting typically arises and in which it typically does not arise.

In free-recall tests, participants in the forget condition show a tendency to recall List 2 items before List 1 items (Geiselman et al., 1983). As a result, the effect of the forget cue on List 1 items could be a result of blocking and output interference, in which early recall of List 2 items impairs subsequent recall of List 1 items. The two effects of the forget cue, however, have been found regardless of whether the two lists were recalled simultaneously in any order the participants wished (i.e., with a tendency to recall List 2 items before List 1 items) or whether they were recalled successively with List 1 items recalled prior to List 2 items (Geiselman et al., 1983; Zellner and Bäuml, 2006). This result indicates that list-method directed forgetting does not reflect effects of blocking and output interference.

List-method directed forgetting has also been assessed by means of recognition tests. In most of these studies, no effect of the forget cue emerged for either List 1 items or List 2 items (Geiselman et al., 1983; Basden et al., 1993; Sego et al., 2006; for an exception, see Benjamin, 2006). Impairments on List 1 recognition, however, were found when participants were required to make source memory judgments (Geiselman et al., 1983). On the basis of the recollection/familiarity distinction, this finding suggests that the forgetting in this paradigm reflects primarily a deficit in recollection and not in familiarity. No effects of the forget cue arose in implicit memory tasks (Basden et al. 1993; Basden and Basden, 1996).

Two particularly prominent accounts of list-method directed forgetting are selective rehearsal and inhibition. The selective rehearsal account assumes that during List 2 encoding participants in the remember condition rehearse both List 2 and List 1 items, whereas in the forget condition, the forget cue leads to selective rehearsal activities on List 2 items, thus improving later recall of List 2 at the expense of List 1 (Bjork, 1972). The inhibition account assumes that, by inhibiting List 1 items, the forget cue deactivates retrieval routes to List 1 items and, because of the resulting decrease in the items' interference potential, simultaneously improves access to List 2 items (Geiselman et al., 1983). Because the selective rehearsal hypothesis attributes directed forgetting to differences in encoding, it predicts effects on recall and recognition tests. The inhibition account attributes directed forgetting to effects on the List 1 items' retrieval routes, and thus the forgetting should be present in recall but should be absent in recognition. The above-mentioned failure to find directed forgetting on recognition tests supports the inhibition account.

Further evidence for the inhibition view arises from an experiment by Geiselman et al. (1983). They used a variant of the standard paradigm, in which participants alternately learned items intentionally and incidentally. Indeed, all participants were told to learn one item and judge the pleasantness of the next one. For both learn and judge items, the standard pattern of directed forgetting arose with reduced recall of List 1 items and improved recall of List 2 items. This result challenges the selective rehearsal hypothesis, because participants in the remember condition should not have tried to rehearse the incidental List 1 items and, rather, should have focused their rehearsal on the learn items. Incidental List 1 items thus should have shown the same performance in the remember as in the forget condition, which was not the case.

For most of the paradigms discussed in this chapter, there is broad consensus that inhibition operates on an item level. In contrast, in list-method directed forgetting it has been suggested that the inhibition operates on a list-level basis. According to this view, List 1 items form a unit, and the presence of the forget cue induces inhibition of the whole unit. Evidence in favor of this view comes from the Geiselman et al. (1983) observation that incidentally learned List 1 items share the same fate as intentionally learned List 1 items. Further support for the view arises from part-list reexposure studies. In these studies, after learning of the two lists, a subset of the List 1 items was reexposed as part of a recognition test before all previously studied items were to be recalled on a final recall test. The results provided evidence that part-list reexposure eliminates the forgetting of the remaining items (Bjork et al. 1973; Goernert and Larson, 1994; Bjork and Bjork, 1996), which is consistent with the view that the inhibition operates on a list-level basis. More recently, however, the findings were challenged by results suggesting that part-list reexposure reinstates mainly reexposed items and not the entire List 1 episode (Basden et al., 2003).

A priori, one might like to assume that the presence of the forget cue in the list-method directed forgetting procedure is sufficient to create the forgetting of List 1 items. As the results from two lines of research show, however, this is not the case. First, it has been found that the presentation of the forget cue creates forgetting only if it is presented before List 2 encoding, but not if it is

presented after the encoding (Bjork, 1970). Second, the effect of the forget cue arises only if there is additional List 2 encoding. That is, the forgetting of List 1 items disappeared if the forget cue was provided, but no additional List 2 learning took place (Gelfand and Bjork, 1985; Pastötler and Bäuml, in press). This finding indicates that the presence of the forget cue per se is not sufficient to create list-method directed forgetting. In particular, the result suggests that the inhibitory mechanism in this paradigm operates during List 2 encoding.

Accounts of directed forgetting are typically one-mechanism accounts according to which the same mechanism underlies the two effects of forgetting and enhancement. Although enhancement and forgetting in directed forgetting often go hand in hand, recently some exceptions to this rule have been observed, and forgetting has been found without enhancement (Conway et al., 2000; Sahakyan and Delaney, 2003, 2005; Zellner and Bäuml, 2006), and enhancement obtained without forgetting (Macrae et al., 1997; Benjamin, 2006). These dissociations suggest the action of two separate mechanisms, one mediating the forgetting and the other mediating the enhancement. Consequently, a two-mechanism account was suggested, according to which the forgetting is caused by some form of inhibition, whereas the enhancement results from a change in people's List 2 encoding with more elaborate encoding in the forget than in the remember condition (Sahakyan and Delaney, 2003).

At the core of list-method directed forgetting is the action of an inhibitory mechanism that induces effects on the irrelevant material by affecting the items' retrieval routes to their cue. Such inhibition should also trigger blocking. The difference in retrieval strength between inhibited List 1 items and noninhibited List 2 items should create biased retrieval competition, with relevant items being recalled prior to irrelevant items. Although there is evidence for the predicted recall order (Geiselman et al., 1983), to date such blocking effects have not been uncovered (Geiselman et al., 1983; Zellner and Bäuml, 2006).

2.13.2.3.2 Item-method directed forgetting

In list-method directed forgetting, relevant new material is encoded after irrelevant old information, initiating updating processes on the out-of-date information. However, relevant and irrelevant material may also be presented interspersed. In this case, different processing of the two types of information might be achieved by triggering inhibitory processes on each single irrelevant item. Whether such processes can affect later accessibility of the irrelevant information has been studied in item-method directed forgetting.

In item-method directed forgetting, participants study a list of items in which a cue to remember or forget the item follows presentation of each single item. Later, a memory test is conducted in which both to-be-remembered (TBR) and to-be-forgotten (TBF) items have to be recalled or recognized (Woodward and Bjork, 1971; see **Figure 2(a)** for a formally related paradigm). Performance for the relevant and irrelevant material is directly compared without reference to any additional baseline conditions (for a review, see MacLeod, 1998). Results from free-recall experiments typically reveal a difference in performance between TBR and TBF items, with better recall for TBR than TBF items, thus showing that the cuing is effective (Davis and Okada, 1971; Woodward and Bjork, 1971; Basden et al., 1993). Moreover, as is true in list-method directed forgetting, the effect is probably not a result of demand characteristics, because it does not disappear if money is offered for recall of the TBF items (MacLeod, 1999).

List-method directed forgetting is present in recall but is absent on recognition tests. In contrast, the difference between TBR and TBF items in item-method directed forgetting has been observed in both recall and recognition tests (Davis and Okada, 1971; MacLeod, 1989; Basden et al., 1993). Regarding the contribution of recollection and familiarity on recognition performance, the effect of the forget cue seems to reflect a difference in recollective processes rather than in familiarity. This is indicated because the difference in performance has been found to be present in remember judgments but not in know judgments (Gardiner et al., 1994; Basden and Basden, 1996). A different performance for TBR and TBF items was also found in implicit memory tasks (MacLeod, 1989; Basden and Basden, 1996), although the effect does not seem to be present in all types of tasks (MacLeod and Daniels, 2000).

The simplest view of these findings is a strengthening view according to which TBF and TBR items only differ in the degree to which the single items are rehearsed and strengthened in response to the respective cue (Bjork, 1972; Basden et al., 1993; see also MacLeod, 1998). Such an interpretation is

consistent with the finding of differences between TBR and TBF items in most types of memory tests, including recognition and some implicit memory tasks. Following this view, the forget cue would not inhibit retrieval routes or the memory representation of the TBF items. Because of the induced difference in relative strength between the two types of items, the forget cue, however, might create some blocking at test, with early recall of (stronger) TBR items blocking access to the (weaker) TBF items. If true, item-method directed forgetting would be similar in character to what occurs in the list-strength effect (see section 2.13.2.1.1). Moreover, item-method directed forgetting would reflect an instructional variant of the list-strength effect.

Our knowledge of the role of inhibitory processes in the list-strength effect is largely based on comparisons between performance in the mixed-list condition and performance in two pure-list conditions that are used as baseline conditions (see section 2.13.2.1.1). Item-method directed forgetting may also create a form of mixed list, consisting partly of the stronger TBR items and partly of the weaker TBF items. In this case, however, performance is typically not compared with pure-list baseline conditions. Such comparisons would strongly improve the database to derive a more clearcut indication of the role of inhibitory processes in item-method directed forgetting. Until then, it seems that inhibition may play a role in item-method directed forgetting experiments, but that this effect is restricted to blocking and is not caused by direct effects on the items' retrieval routes or the items' memory representation.

2.13.2.3.3 Summary

The presence of a forget cue can induce forgetting of irrelevant material. Depending on whether the relevant material is presented subsequent to the irrelevant material (the list method) or relevant and irrelevant material are presented interspersed (the item method), different effects arise. In list-method directed forgetting, the effect of the forget cue is found in free-, and list-cued recall tests but not in recognition or implicit memory tasks. The effect is likely to be the result of some deactivation of the retrieval route between the TBF items and their cue. The representation of the TBF items itself, however, does not seem to be affected. In item-method directed forgetting, the effect of the forget cue can be found across a wide range of memory tests, including free recall and recognition tasks. The results are largely consistent with a strengthening view, according to which the two cues lead to items of different memory strength because of selective rehearsal. While this difference in relative strength may create blocking in recall tests, no clearcut evidence for other forms of inhibitory processes, such as direct effects on retrieval routes or item representations, has yet been identified. It thus seems that quite different mechanisms are at work in list-method and item-method directed forgetting.

2.13.2.4 Think/No-Think Impairment

In item-method directed forgetting, a forget cue is provided after presentation of an item to inform participants that the item is irrelevant and will not be tested later. As summarized in the previous section, the results from a number of studies have shown that, in response to such a cue, rehearsal of an item can be stopped, thus demonstrating that forget cues at encoding can be effective. The question arises of whether a forget cue can not only stop rehearsal but may stop retrieval as well (Weiner and Reed, 1969). Evidence for this type of proposal arises from studies using the think/no-think paradigm.

The think/no-think paradigm is a memory adaptation of the go/no-go task, which is typically used to study control of prepotent motor responses. In the think/no-think paradigm, participants study weakly related word pairs (e.g., butter-opera) and are trained to answer with the appropriate associate (target) upon presentation of its counterpart (cue). After the training, participants engage in a think/no-think task. In each trial of the task, the cue word (butter) is provided and participants are required either to remember (think) or to actively suppress any thought (no-think) about its corresponding target (opera) and not let it enter consciousness. A large number of such trials is typically conducted. In a final cued-recall test, participants are then asked to recall the targets in response to the original stimulus cue, regardless of whether in the intermediate phase they were instructed to think about the item or to suppress it. As a variant of this testing procedure, a semantically related word (music) may be presented as cue rather than the original stimulus cue, thus permitting a test of cue-independent forgetting in this paradigm (Anderson and Green, 2001; **Figure 5**).

Results from several studies showed improved recall of think items and impaired recall of no-think items relative to baseline items, which were neither remembered nor suppressed in the intermediate think/no-think phase (Anderson and Green, 2001;

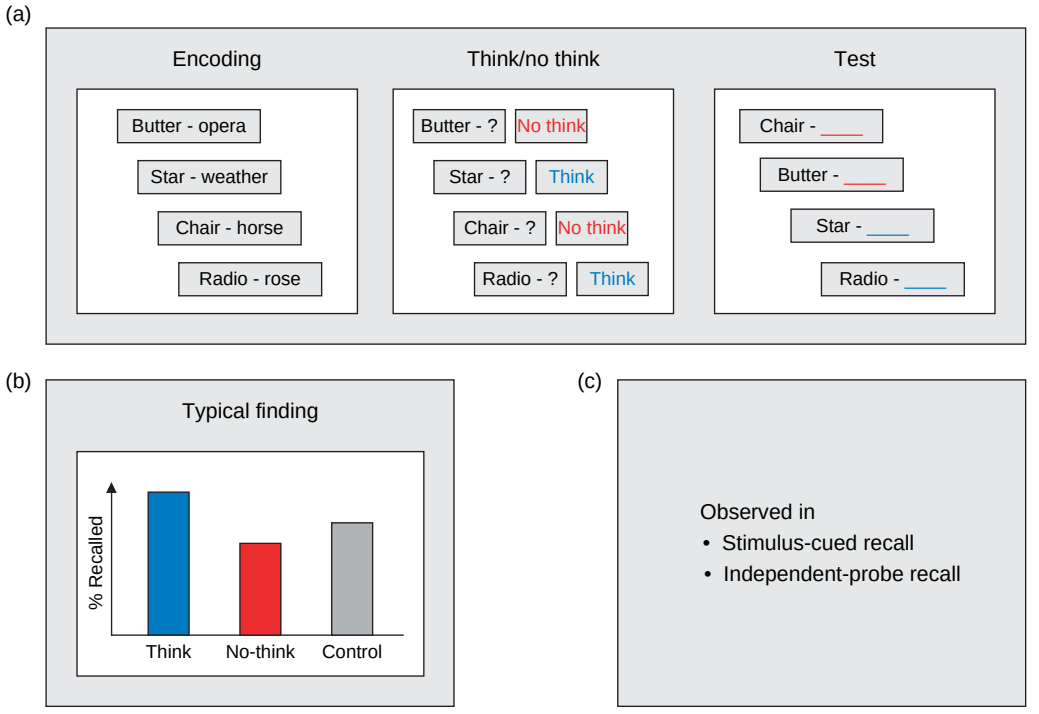

Figure 5 Think/no-think impairment. (a) The experimental paradigm. Participants study weakly related cue–target pairs and are trained to answer with the target upon presentation of its cue. After the training, participants engage in the think/no-think task. In each trial of the task, they are provided with a cue word and are required to either remember (think) or actively suppress any thought (no-think) about its corresponding target. On a later test, all targets are tested given their associate as the retrieval cue. (b) The typical finding. Think items are recalled better and no-think items are recalled worse than control items, which were neither remembered nor suppressed in the intermediate think/no-think task. (c) Memory tests: Examples of memory tests in which think/no-think impairment has been observed.

Anderson et al., 2004; Hertel and Calcaterra, 2005; Depue et al., 2006). In particular, the effect did not only arise if at test the original stimulus cue was presented, but was also present if a semantically related independent probe was provided (Anderson and Green, 2001; Anderson et al., 2004). Again, the effect does not seem to be a result of demand characteristics, as the forgetting has been found to be still present if money was offered as a reward for each recalled no-think item (Anderson and Green, 2001).

The few studies that to date reported successful forgetting in the think/no-think paradigm indicate that the forgetting in this paradigm arises if the number of no-think trials is high (16 trials) but is not present if the number of such trials is relatively low (one or four trials). Moreover, even with a high number of no-think trials, the effects are typically small, although the forgetting can increase somewhat if emotional material rather than neutral material is employed in the experiment (Depue et al., 2006). Researchers also failed to replicate the finding, despite several careful attempts to do so (Bulevich et al., in press), which suggests that the forgetting in this paradigm may not be very reliable.

Anderson and Green (2001) argued that the forgetting in the think/no-think paradigm is caused by inhibition. According to this account, during no-think trials, the memory representation of the targets is reduced so that accessibility is lowered regardless of which cue is provided and which retrieval route is used. In consequence, the forgetting should be observed across a wide range of memory tests, including independent-probe tasks, recognition tasks, or implicit memory tasks. Although there is evidence for cue independence in this paradigm (Anderson and Green, 2001; Anderson et al., 2004; but see Bulevich et al., in press, for failures to get the effect), no studies have yet been reported examining think/no-think impairment in recognition or implicit memory tests. Using imaging techniques, Anderson et al. (2004) examined neural correlates of the forgetting during no-think trials. They identified an interaction between

prefrontal cortex and hippocampus, which was related to the forgetting of no-think items on the final recall test and was interpreted as evidence for inhibition. However, because there was also increased activity in other brain regions during no-think trials, it was argued that the forgetting might also be a result of subjects' attempts to think about something else rather than to inhibition (Hayne et al., 2006).

Indeed, rather than reflecting item suppression, the forgetting in this paradigm might also be caused by some form of inaccessibility created through associative interference. This might occur if, for instance, participants adopt a strategy of thinking about other, distracting words during no-think trials, thereby building new associations to the cue and increasing (retroactive) interference (Hertel and Calcaterra, 2005; Bulevich et al., in press). Because interference effects are typically restricted to situations in which the original cue is provided at test, such noninhibitory accounts of the phenomenon would not predict forgetting when independent probes are provided. The reported failure to find forgetting when independent probes are provided as cues (Bulevich et al., in press) thus supports the associative interference account of the phenomenon. In contrast, Anderson and colleagues' reports of cue independence (Anderson and Green, 2001; Anderson et al., 2004) challenge the interference account and support the suppression account. Obviously, further research is warranted.

The results from the think/no-think paradigm suggest that cues to stop retrieval of an item can cause later forgetting of the item. Item-method directed forgetting shows that cues to stop rehearsal of an item can also be effective. While these lines of evidence converge on the view that cues to stop the processing of items, be it at encoding or retrieval, can be successful, there is evidence that the two forms of stopping are nonetheless different. First, stopping rehearsal seems to be relatively easy for participants, typically inducing strong performance differences between TBR and TBF items. In contrast, stopping retrieval seems to be hard, and participants may even fail to show forgetting in this task. Second, the stopping of rehearsal does not seem to be inhibitory in the sense that a TBF item is inhibited in its representation or its retrieval routes. In contrast, the stopping of retrieval has been argued to induce deactivation of the item representation, so that the item becomes unavailable. If true, stopping rehearsal and stopping retrieval may be quite different things in memory.

2.13.2.5 Part-List Cuing Impairment

What is common to the paradigms described earlier (in the subsections in 2.13.2 to this point) is that inhibitory mechanisms operate to emphasize accessibility of relevant material at the expense of that for irrelevant information. Inhibition thus seems to serve an adaptive goal and to support memory to function efficiently. On the other hand, there is evidence that inhibition is not always adaptive and goal directed. This is indicated by an example from the memory literature in which inhibitory processes emphasize accessibility of irrelevant material at the expense of that for relevant information. The example is part-list cuing impairment.

2.13.2.5.1 Beneficial and detrimental effects of cuing

There is broad agreement in the literature that the presence of adequate retrieval cues is crucial for successful episodic memory (e.g., Tulving, 1974). Consistently, cuing has been found to facilitate recall in many situations. For instance, if a categorized list with several items from each category is presented to participants and, at test, the category names or one item from each category are provided as retrieval cues, then such cuing typically enhances recall compared to unaided free recall (Tulving and Pearlstone, 1966; Tulving and Psotka, 1971).

Cuing, however, is not always facilitatory and can even be detrimental. If participants learn a categorized list and, at test, receive several items from each category as retrieval cues, then such part-list cuing often reduces recall performance for the remaining items, compared with the condition in which just one category exemplar is provided as a retrieval cue (Slamecka, 1968; Roediger, 1973; for reviews, see Nickerson, 1984, or Roediger and Neely, 1982). In general, if more part-list cues are provided than necessary to remind participants of the various categories, or subjective units, cuing can be detrimental (Basden and Basden, 1995).

Prior work assumed that part-list cuing impairment is caused by blocking (Roediger, 1973; Rundus, 1973). The idea was that reexposure of items as cues strengthens these items' representation. During attempts to recall the noncue items at test, this strengthening of the cue items then should lead participants to covertly retrieve cue items before noncue items and thus block recall of the noncues. Part-list cuing, therefore, should mimic the effects of

relearning, in which reexposure has been shown to block recall of the target material (see section 2.13.2.1.2).

Prior work demonstrated that the detrimental effects of relearning disappear once item-specific probes, such as the targets' unique initial letters, are provided to aid recall of the items. Thus, if part-list cuing impairment, like the detrimental effects of relearning, was caused by blocking, then part-list cuing impairment should also be absent if item-specific probes were provided. The issue was directly addressed in an experiment by Bäuml and Aslan (2004). Participants learned category exemplars consisting of target and nontarget items. In a subsequent phase, the nontarget items were reexposed, either for relearning (i.e., for a second study trial) or for use as retrieval cues at test. This reexposure occurred immediately before test, mimicking the typical part-list cuing procedure, or separated from test by a distractor task, mimicking typical part-list relearning. At test, the category-plus-first-letter cues of the target items were presented, and participants were instructed to recall the target items.

As expected from the relearning literature, part-list relearning had no detrimental effect on the target material. In contrast, part-list cuing had a detrimental effect. This held true both when the reexposure occurred immediately before test and when reexposure was separated from test by a distractor task. This finding indicates that part-list cuing differs from relearning and that its detrimental effects are not caused by blocking. In particular, it shows that part-list cuing impairment reflects an instructional effect. Reexposure induces forgetting when participants are oriented to use the reexposed items as retrieval cues (part-list cuing) but does not induce forgetting when the reexposed items are presented for an additional study trial (part-list relearning).

Results from recognition studies support the view that part-list cuing differs from relearning and other strengthening effects. Indeed, while strength-induced forgetting is typically absent in recognition tests, reliable part-list cuing impairment was found when memory for the noncues was assessed by means of a recognition task (Todres and Watkins, 1981). Part-list cuing impairment also arose in speeded recognition (Neely et al., 1983; Oswald et al., 2006). Because recognition performance is assumed to rely more on familiarity than recollection when participants are required to make recognition decisions very quickly (e.g., Yonelinas, 2002), this finding suggests that part-list cuing does not only affect recollective processes but affects the familiarity of the noncue items as well.

There is evidence that part-list cuing impairment is also present in recall tasks that employ independent probes (see section 2.13.2.6.1). Aslan et al. (2007a) reported a repeated-testing experiment in which, in the first test, participants were provided with part-list cues and were asked to recall half of the target items when cued by the items' unique initial letters. After a delay, a second test was conducted in which no part-list cues were provided and participants were asked to recall the remaining targets by means of independent probes, that is, probes that were not used in a previous phase of the experiment. Part-list cuing impairment was present in both tests, indicating that independent probes do not eliminate the forgetting.

The results from all these studies are consistent with an inhibitory view of part-list cuing impairment according to which part-list cuing triggers inhibitory processes that directly affect the item representation of the noncues. In this sense, the effect may mimic the effect of retrieval practice in retrieval-induced forgetting (see section 2.13.2.2). Indeed, several studies compared the detrimental effects of retrieval practice and part-list cuing directly within a single experiment (Bäuml and Kuhbandner, 2003; Bäuml and Aslan, 2004; Zellner and Bäuml, 2005). In all these cases, the same qualitative and quantitative effects arose. These findings agree with the view that part-list cuing leads to instructed covert retrieval of cue items and causes inhibition of noncue items very similar to how overt retrieval in retrieval-induced forgetting inhibits nonretrieved items (Bäuml and Aslan, 2004).

Retrieval-induced forgetting has been shown to be lasting and to still be present when item-specific probes are provided (see section 2.13.2.6.1). Consistent with the inhibitory view of part-list cuing impairment, part-list cuing impairment can also persist, even with item-specific probes (Bäuml et al., 2002; Bäuml and Aslan, 2004, 2006). On the other hand, there are demonstrations that, under certain conditions, the cuing effect can disappear with a delay (Basden and Basden, 1995; Bäuml and Aslan, 2006) and can be absent in the presence of item-specific probes (Aslan and Bäuml, in press). Bäuml and Aslan (2006) identified associative relations at encoding as a crucial factor in part-list cuing impairment. Data suggest that the detrimental effect of part-list cues is mediated by inhibition in situations with a relatively low level of interitem associations and is mediated by noninhibitory mechanisms in situations with a relatively high

level of interitem associations. Thus, apparently more than one mechanism is involved in this form of forgetting.

2.13.2.5.2 Summary

Providing a subset of studied material as retrieval cues for recall of the remainder often does not enhance but rather reduces accessibility of relevant targets. Such part-list cuing impairment reflects an instructional effect, with reexposure of items being detrimental only if participants are oriented to use the items as retrieval cues. Part-list cuing differs from part-list relearning and, in many situations, is the result of the action of an inhibitory mechanism that affects the noncue items' representation directly. Part-list cuing impairment thus mirrors retrieval-induced forgetting, in which retrieval practice affects the representation of the nonretrieved items. In contrast to retrieval-induced forgetting, however, part-list cuing is not adaptive or goal directed but, rather, provides an example in which inhibitory processes can impair access to relevant information rather than enhance it.

2.13.2.6 Developmental Trajectory of Inhibitory Processes

The role of inhibition in cognition is of central importance in the literature on cognitive development. This stems in part from findings reporting poor performance of young children and older adults in a number of inhibition tasks (Simpson and Foster, 1986; Tipper et al., 1989; Hartman and Hasher, 1991; Hasher et al., 1997). In particular, it is attributable to the hypothesis that young children and older adults suffer from a general deficit in inhibitory function (Hasher and Zacks, 1988; Bjorklund and Harnishfeger, 1990). Such a general deficit in inhibitory function might also apply to memory and be at the heart of the reduced memory performance of young children and older adults. It thus is important to examine the performance of young children and older adults in the inhibitory paradigms addressed earlier (in the subsections in 2.13.2 to this point).

The hypothesis of a general inhibitory deficit in young children and older adults indicates that the two age groups show problems across the whole range of inhibitory paradigms reviewed above. This holds while the results reported in the subsections in 2.13.2 to this point suggest the action of quite different inhibitory mechanisms in the different paradigms. Knowledge on the developmental trajectory in the single paradigms would improve our understanding of memory development and would also improve our understanding of the development of cognitive inhibition in general. In recent years, a number of results emerged regarding young children's and older adults' retrieval-induced forgetting, directed forgetting, and part-list cuing impairment. These results are reviewed in the next sections.

2.13.2.6.1 Retrieval-induced forgetting

In children, retrieval-induced forgetting has been studied using both cued recall and recognition tasks at test. Zellner and Bäuml (2005) reported two experiments using verbal categorized lists and category-cued recall tasks at test. First graders, second graders, fourth graders, and young adults were tested. All four groups of participants showed the standard pattern of retrieval-induced forgetting with improved recall of practiced items and impaired recall of unpracticed items. In particular, there were no differences in the amount of forgetting across participant groups, suggesting that the inhibition was effective in young children (**Figure 6(a)**).

Using pictorial material, Ford et al. (2004) examined retrieval-induced forgetting in 7-year-olds by means of a yes/no recognition task in the practice phase and a category-cued recall test and a recognition test in the final test phase. In both cases, robust retrieval-induced forgetting was found. Furthermore, the magnitude of the effect did not differ between children and young adults. Analogous results were reported by Lechuga et al. (2006), who examined retrieval-induced forgetting in 8- and 12-year-old children. Related results were obtained in studies using the selective postevent review (questioning) procedure with 5- and 9-year-olds (Conroy and Salmon, 2005) and 5- to 6-year-olds (Williams et al., 2002). Review of some events impaired memory for nonreviewed events with comparable impairment in all age groups.

Only very few studies exist to date in which retrieval-induced forgetting was studied in older adults. In a nondevelopmental study, Moulin et al. (2002) found retrieval-induced forgetting in Alzheimer disease patients and healthy age-matched older adults in both a standard category-cued recall and a category generation task. While this study demonstrated reliable forgetting in older adults, it left open the question of whether the effect differs quantitatively from that in younger adults. Aslan et al. (2007b) examined retrieval-induced forgetting in younger and older adults and found equivalent amounts of

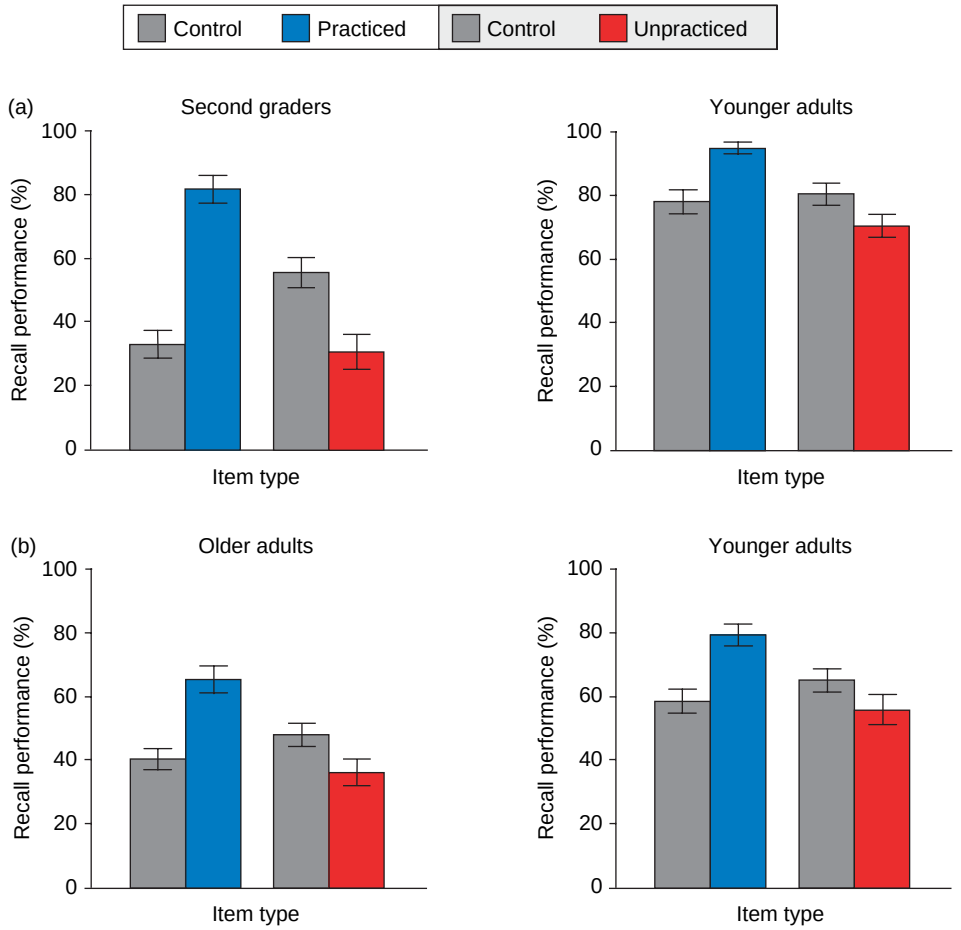

Figure 6 Retrieval-induced forgetting in young children and older adults. (a) Recall percentage (and standard error) of retrieval-practiced and not-retrieval-practiced items and of their (separate) control items in second graders and younger adults. For both participant groups, the data show beneficial effects of retrieval practice on practiced items and detrimental effects of retrieval practice on unpracticed items. From Zellner M and Bäuml K-H (2005) Intact retrieval inhibition in children's episodic recall. *Mem. Cogn.* 33: 396–404. Psychonomic Society, Inc., Experiment 1. Adapted with permission. (b) Recall percentage (and standard error) of retrieval-practiced and not-retrieval-practiced items and of their (separate) control items in younger and older adults. For both participant groups, the data show beneficial effects of retrieval practice on practiced items and detrimental effects of retrieval practice on unpracticed items. From Aslan A, Bäuml K-H, and Pastötter B (2007) No inhibitory deficit in older adults' episodic memory. *Psychol. Sci.* 18: 111–115. Blackwell Publishing, Experiment 1. Adapted with permission.

forgetting in the two age groups. This result held both when category names and when independent probes were provided as retrieval cues, suggesting that, in both age groups, the inhibition affects the items' memory representation itself (**Figure 6(b)**). Related results were again obtained when using the selective postevent review procedure (Koutstaal et al., 1999).

Thus, retrieval-induced forgetting seems to be present over most of the lifespan and to differ hardly, if at all, between young children, younger adults, and older adults. In particular, the results suggest that, in all these age groups, the effect is caused by the same inhibitory mechanism, which affects the nonretrieved items' representation itself. Thus, no evidence for an inhibitory deficit in young children or older adults arises in this type of task.

2.13.2.6.2 Directed forgetting

1. List-method directed forgetting

A number of studies examined list-method directed forgetting in young children (e.g., Bray et al., 1983; Harnishfeger and Pope, 1996). The results from these studies suggest that young children show problems in this type of task. In the study by Harnishfeger and Pope (1996), for instance, first, third, and fifth graders and young adults were compared. First and third graders

failed to show directed forgetting and showed hardly any effect of the forget cue at all. Normal directed-forgetting performance, however, was present from fifth grade on. The inhibition mechanism apparently develops over the elementary school years (**Figure 7(a)**).

There are three published studies to date that have examined list-method directed forgetting in older adults. In the first study, Zacks et al. (1996) used a variant of the task in which several short lists had to be studied and recall performance was measured cumulatively after presentation of all lists. A greater amount of forgetting was found for younger than for older adults. The results, however, were affected by floor effects. In a second study, Sego et al. (2006) followed previous work by Geiselman et al. (1983) and let younger and older adults alternately learn items intentionally and incidentally. For both types of items, largely identical forgetting was found in the two age groups. Zellner and Bäuml (2006) compared younger and older adults' directed forgetting in three experiments, in which the forget cue was varied within and between participants, the two lists were unrelated or related to each other, and recall of the lists was required simultaneously or successively. No age-related difference in directed forgetting performance emerged in any of the three experiments (**Figure 7(b)**).

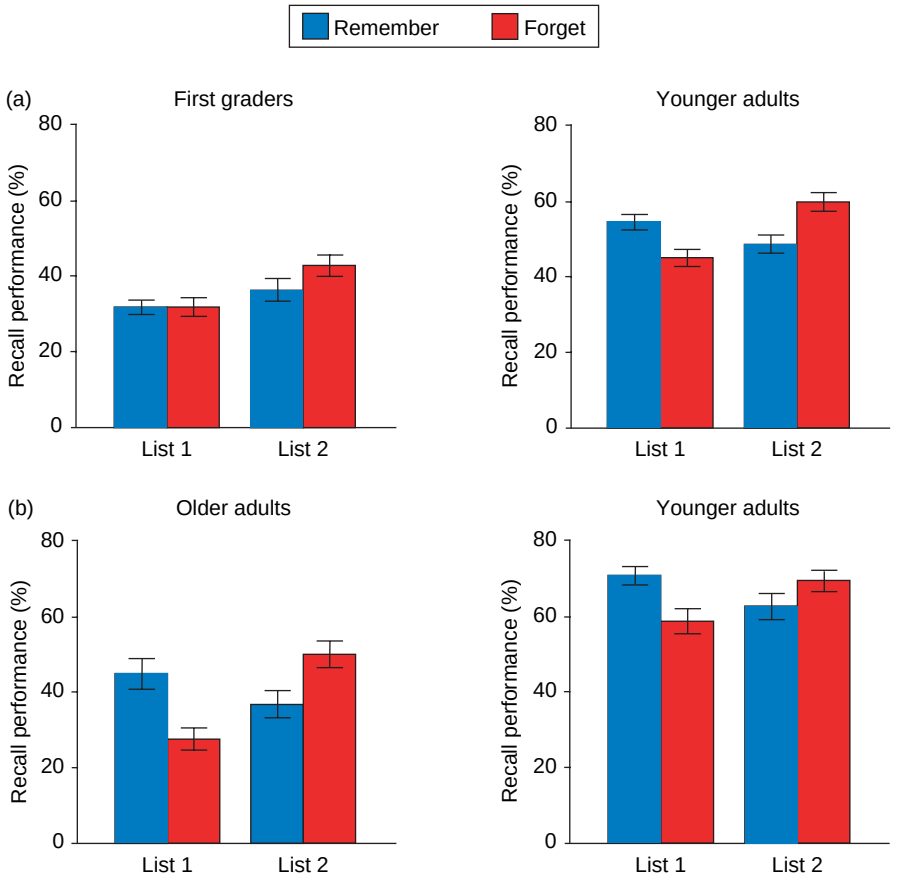

Figure 7 List-method directed forgetting in young children and older adults. (a) Recall percentage (and standard error) of List 1 and List 2 items as a function of whether a remember or forget cue was provided between learning of the two lists. While younger adults show the standard pattern of directed forgetting, for young children no reliable effect of the forget cue arises. This holds for both List 1 and List 2 items (K.-H. Bäuml, M. Zellner, and A. Aslan, unpublished data). (b) Recall percentage (and standard error) of List 1 and List 2 items as a function of whether a remember or forget cue was provided between learning of the two lists. Both younger adults and older adults show the standard pattern of List-method directed forgetting. From Zellner M and Bäuml K-H (2006) Inhibitory deficits in older adults – List-method directed forgetting revisited, *J. Exp. Psychol. Learn. Mem. Cogn.* 32: 290–300, American Psychological Association, Experiment 3. Adapted with permission.

Thus, while young children fail to show list-method directed forgetting, older adults seem to show intact forgetting. This result suggests that the underlying inhibitory mechanism develops in later childhood. Once developed, however, it remains intact with increasing age. The inhibitory mechanism underlying list-method directed forgetting thus differs in its developmental trajectory from that underlying retrieval-induced forgetting.

2. Item-method directed forgetting

Few studies have examined item-method directed forgetting in young children. Posnansky (1976) found better recall of TBR items than TBF items in both third and seventh graders, with no difference in the effect of the forget cue between the two age groups. Foster and Gavelek (1983) reported that even first graders recalled more TBR than TBF items, although the effect of the forget cue was smaller for first than for fifth graders. Regarding older adults' directed forgetting, several studies found a reliable difference between TBR and TBF items with increasing age. The difference, however, was smaller in older adults than in younger adults (Zacks et al., 1996; Earles and Kersten, 2002; Dulaney et al., 2004), which indicates that older adults show deficient directed forgetting in this type of task.

Together the results suggest that both young children and older adults show deficits in item-method directed forgetting. Following the strengthening view of item-method directed forgetting, these findings, however, do not imply inhibitory deficits in young children and older adults but, rather, may indicate differences in the degree to which the two age groups are able to strengthen relevant material.

2.13.2.6.3 Part-list cuing impairment

There seems to be only one study in the literature that examined part-list cuing impairment in young children. Zellner and Bäuml (2005) examined the detrimental effect of part-list cues in first graders, fourth graders, and young adults. All three groups showed reliable part-list cuing impairment with no difference in amount of forgetting across the three age groups. Moreover, in this experiment, part-list cuing impairment was directly compared with retrieval-induced forgetting. None of the three age groups showed any reliable difference between the detrimental effect of retrieval practice and the detrimental effect of part-list cuing.

In older adults, Marsh et al. (2004) found robust part-list cuing impairment in both younger and older adults across three experiments. If anything, the older adults showed stronger detrimental effects than the young adults and were disproportionately slow in the presence of part-list cues. This result suggests that part-list cuing impairment is not reduced in older adults. Part-list cuing impairment, like retrieval-induced forgetting, thus may be intact across most of the lifespan.

2.13.2.6.4 Summary

To date, relatively few studies have addressed the development of inhibition in human memory. From these studies a fair amount of knowledge has been gained regarding the development of inhibitory mechanisms as they occur in retrieval-induced forgetting, directed forgetting, and part-list cuing impairment. Unfortunately, there are no published studies to date in which the development of inhibitory processes involved in strength-induced forgetting and think/no-think impairment has been addressed, so that the current picture on the development of inhibition in memory is only fragmentary.

Still, current results clearly challenge the hypothesis of a general inhibitory deficit in young children and older adults by showing that both age groups show intact inhibition in some memory tasks. It thus seems that the picture of a general inhibitory deficit needs to be updated in favor of the picture of task-dependent inhibitory function. Specifying the exact nature of the inhibitory mechanisms that are intact in young children and older adults and of those that are deficient is a high priority for future research on the development of inhibitory function.

2.13.3 Conclusions

In the introduction, three inhibitory mechanisms were suggested to reduce accessibility of irrelevant memories: blocking, route deactivation, and item suppression (**Figure 1**). These mechanisms differ in whether they affect memories indirectly (blocking) or directly (route deactivation, item suppression), and whether they affect memories' retrieval routes (route deactivation) or their representation itself (item suppression). As a result, the three mechanisms also differ in the range of memory tests in which the effects of inhibition can be observed. While effects of blocking manifest themselves mainly in free-recall tests and effects of route deactivation in free- and cued-recall tests, effects of item suppression are

present over a wide range of memory tasks, including recognition and independent-probe tests.

The results on the experimental paradigms reviewed in section 2.13.2 suggest that each of the three mechanisms plays a role in reducing irrelevant memories' accessibility. However, the results also suggest that none of the three mechanisms is responsible for the effects in all the paradigms. Rather, it seems that a multiplicity of mechanisms are at work to induce inaccessibility of irrelevant material across a wide range of situations. In strength-induced forgetting, for instance, inhibition seems to be realized by means of blocking, in which early recall of strengthened (relevant) material hinders subsequent recall of nonstrengthened (irrelevant) material. Consistently, forgetting is present mainly in free recall tasks and is absent in recognition tasks. Strength-induced forgetting thus is mediated by a relatively weak form of inhibition that affects the irrelevant material only indirectly. The same mechanism is likely to be involved in item-method directed forgetting, at least when following the strengthening view of this form of directed forgetting.

In list-method directed forgetting, a stronger form of inhibition is at work in which the retrieval routes between the irrelevant material and its cue(s) are affected directly. Accordingly, forgetting in this paradigm can be observed in free and cued recall tasks while no effects arise in recognition tests, which rely mainly on the items' representation itself. In strength-induced forgetting, the effects on the relevant and irrelevant material's accessibility are mediated by the same mechanism. In list-method directed forgetting, there is evidence for two separate processes, one process reducing accessibility of the irrelevant material and the other process enhancing accessibility of the relevant information. When operating in concert, these two processes can create very effective memory updating.

In retrieval-induced forgetting, inhibition is realized by suppressing the representation of the inhibited items themselves, thus making retrieval less effective regardless of which retrieval cue is employed. Consistent with this strong form of inhibition, the forgetting in this paradigm can be found across a wide range of memory tests, including recognition and independent probe tests. As in list-method directed forgetting, there is evidence for the action of two processes, a forgetting mechanism directed on the irrelevant material and an enhancement mechanism directed on the relevant information. Together, they induce a strong difference in accessibility between relevant and irrelevant material and thus induce effective memory updating. There is also evidence that the same inhibitory mechanism underlies the forgetting in the think/no-think paradigm and in part-list cuing, because in both cases the forgetting has been found not only to arise in free- and cued-recall tasks but to generalize to other tasks as well.

The evidence that different mechanisms mediate inhibition in the single paradigms motivates a taxonomy of the paradigms, in which the paradigms are partitioned into three subsets, one in which the forgetting is caused by blocking (strength-induced forgetting, possibly item-method directed forgetting), one in which the forgetting is caused by route deactivation (list-method directed forgetting), and one in which the forgetting is caused by item suppression (retrieval-induced forgetting, think/no-think impairment, and part-list cuing impairment; **Figure 8**). Although currently there is only restricted knowledge regarding the developmental aspects of inhibition in the single paradigms, the suggested taxonomy is at least consistent with current knowledge. Current knowledge suggests comparable developmental trajectories for retrieval-induced forgetting and part-list cuing impairment and a different trajectory for list-method directed forgetting. Item suppression and route deactivation thus may follow different developmental paths.

Besides the differences in underlying mechanism, the single paradigms also differ regarding the stage at which the inhibition takes place. In list-method directed forgetting, inhibition operates before the test during the encoding of the new relevant material. In retrieval-induced forgetting and think/no-think impairment, inhibition also operates before the test, either while selectively retrieving relevant information (retrieval-induced forgetting) or while trying to stop retrieval of irrelevant information (think/no-think impairment). In strength-induced forgetting, item-method directed forgetting, and part-list cuing impairment, the inhibition operates at test, either by blocking recall of irrelevant material (strength-induced forgetting, item-method directed forgetting) or by suppressing relevant material through covert retrieval of cue items (part-list cuing impairment).

It is the general goal of inhibition in memory to enhance accessibility of relevant material at the expense of the accessibility of the irrelevant material. This goal is realized very differently in different situations. The differences are reflected in the

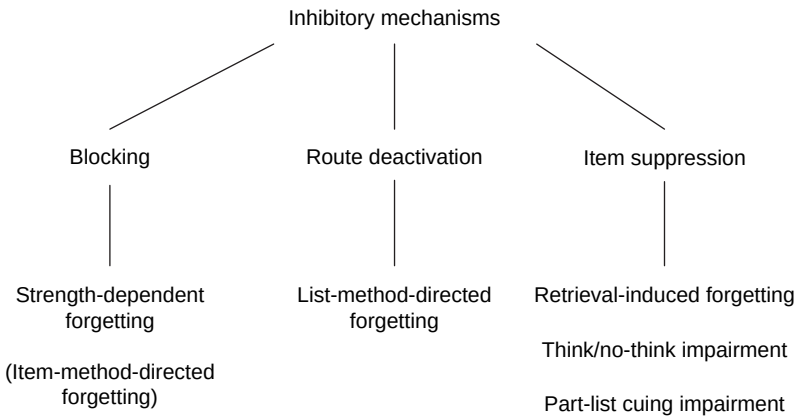

Figure 8 Taxonomy of inhibition paradigms. The taxonomy lists experimental paradigms in which some form of inhibitory mechanism is assumed to be crucially involved. The paradigms are partitioned according to which of the three mechanisms – blocking, route deactivation, and item suppression (**Figure 1**) – is supposed to mediate the inhibition.

diversity of mechanisms that mediate the effect in the single situations, and they are reflected in the varying stages at which the inhibition takes place. Together, the picture of a very flexible and goal-directed updating system arises, in which a multiplicity of inhibitory mechanisms operate at very different processing stages to overcome the problem of retrieval competition and interference and thus help memory function effectively. At the end of the nineteenth century, Ribot wrote that "Forgetfulness . . . is not a disease of memory, but a condition of its health and life" (Ribot, 1882: 61). The results reviewed in this chapter provide a vivid and detailed demonstration of the adequacy of this early view.

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2.14 False Memories

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Memory is impressive. People can recognize hundreds of pictures seen only once (Shepard, 1967) and recall hundreds of words in response to cues (Mäntylä, 1986). Memory's feats are not limited to short delays or to remembering simple materials in laboratory settings. People remember their high school classmates 15 years after graduation (Bahrick et al., 1975) and recall details about the German invasion of Denmark 50 years after they experienced it (Berntsen and Thomsen, 2005). This short list could easily be expanded.

And yet memory's failures can be equally impressive. For example, people's recognition memory for a penny is actually quite poor, even though they have likely handled hundreds (if not thousands) over the years (Nickerson and Adams, 1979). Similarly, the majority of people fail when asked to draw the layout of the number keys on a calculator, even though they could easily use such a device (Rinck, 1999). Memory failures are not limited to mundane objects, of course. Consider just a few examples: Parents misremember the way they raised their children (Robbins, 1963), eyewitness misidentifications occur (Wells et al., 2006), and people falsely remember being abducted by aliens (Clancy, 2005).

To understand human memory, we must understand memory's failures as well as its successes. What is more interesting than the fact that memory is fallible is that the errors are systematic. By systematic, we simply mean that the errors are not random. We understand something about the conditions under which errors are more or less likely; for example, delay is a manipulation that often increases memory errors. This systematicity occurs because errors are often byproducts of mechanisms that normally aid memory, meaning that memory errors can provide a window into the mechanisms of memory.

One of the classics in this tradition is Bartlett's 1932 study in which participants read and retold a Native American story entitled 'The War of the Ghosts.' When participants retold the story, they made systematic errors. They changed the unfamiliar Native American tale so that it made more sense to them and so that it fit better with their English culture. For example, in the retellings 'canoe' became 'boat' and the more supernatural parts of the story either disappeared or changed to be more consistent with a typical English story.

Bartlett concluded that our memories are reconstructive. We do not recall exactly what happened; rather, we reconstruct events using our knowledge, culture, and prior beliefs about what must have occurred. In other words, we use schemas to help reconstruct our memories. A schema is a knowledge structure that organizes what one knows and expects about some aspect of the world. Schemas are useful heuristics that allow us to fill in the gaps and to make predictions. Bartlett's participants possessed a schema about what happens in a typical story and they used this schema to reconstruct the atypical story that they had read.

Bartlett's ideas about reconstructive memory and the influence of one's prior knowledge have been modified only slightly through the years and are still thought to be the backbone of how our memory functions. Schemas have been repeatedly shown to have large effects on later memory. For example, consider a classic study in which participants read short passages, including one about an unruly child.

When told that the story was about Helen Keller, participants later falsely recognized sentences such as 'She was deaf, dumb, and blind.' When the protagonist was labeled as Carol Harris, however, participants rarely falsely recognized the same sentences (Dooling and Christiaansen, 1977). The familiar label presumably activated participants' prior knowledge of Helen Keller, which participants used to make sense of the passage and to fill in gaps in the story. One's schema of Helen Keller, for example, might include information about her childhood in Alabama and her disabilities, as well as how she blossomed into a successful speaker and writer with the help of her teacher Anne Sullivan. While this background knowledge is likely to aid comprehension of the passage, it also sets up the need to later discriminate between what was read in the passage versus what was inferred.

Schemas provide one example of a memory mechanism that can both help and hurt memory. Most of the time, schemas support accurate memory; however, in some instances (such as the Helen Keller example), they can lead us astray. In this chapter, we will consider several different memory mechanisms that, like schemas, can sometimes lead our memories astray. We will focus on memory errors that meet Roediger's (1996) definition of memory illusions. Specifically, the focus will be on "cases in which a rememberer's report of a past event seriously deviates from the event's actual occurrence" (Roediger, 1996: 76). We will place a particular emphasis on memory errors that are made with high confidence, are labeled as remembered, or otherwise appear phenomenologically real. To preview a few of the vivid memory errors we will discuss: they include high-confidence errors in eyewitness testimony, never-presented words 'remembered' as spoken by a specific person, and ease of processing mistaken for fame. In each case, we will describe a prototypical experiment and the results and discuss possible underlying mechanisms.

2.14.1 False Memory for Words: The Deese-Roediger-McDermott Paradigm

As already described, Bartlett emphasized the use of meaningful materials when examining reconstructive memory, to avoid studying memory that was "primarily or literally reduplicative, or reproductive . . . I discarded nonsense material because, among other difficulties, its use almost always weights the evidence in favor of mere rote recapitulation, and for the most part I used exactly the type of material that we have to deal with in daily life" (Bartlett, 1932: 204). Consistent with Bartlett's ideas, most of the studies we will describe in this chapter involve remembering videos, stories, slide shows, or personal memories. While words and nonsense syllables were frequently used in verbal learning experiments, Bartlett did not believe they would be useful in studying reconstructive memory since they did not encourage elaboration nor the use of schemas.

However, words have many properties that make them handy tools for the experimental psychologist. Tulving (1983) has made this argument eloquently: "words to the memory researcher are what fruit flies are to the geneticist: a convenient medium through which the phenomena and processes of interest can be explored and elucidated. . . words are of no more intrinsic interest to the student of memory than *Drosophila* are to a scientist probing the mechanisms of heredity" (Tulving, 1983: 146). Tulving goes on to point out that words have well-defined boundaries and are easily perceived, and that memories for words can easily be checked for accuracy. The point is that using word stimuli to study false memories would be very useful, if word stimuli could be selected that would encourage elaboration and the use of schemas. The argument is that the Deese-Roediger-McDermott (DRM) stimuli fit these requirements, and allow a simple and robust paradigm for studying false memories.

In a typical DRM experiment, participants learn lists of words, each related to a central nonpresented word, the critical lure. For example, participants hear or see 'nurse, sick, lawyer, medicine, health, hospital, dentist, physician, ill, patient, office, stethoscope, surgeon, clinic, cure.' Even though the critical lure 'doctor' was never presented, subjects are likely to include it when recalling the list items. They are also likely to incorrectly call it 'old' on a recognition memory test. The DRM paradigm appeals to experimenters because of the incredibly high rates of false memories observed in both free recall and on recognition measures. For example, in one of Roediger and McDermott's (1995) experiments, participants recalled the critical lures 55% of the time, a rate similar to recall of studied items presented in the middle of the list! False recognition was also very robust; Roediger and McDermott observed a false alarm rate of 76.5% for critical lures as compared to a hit rate of 72% for studied items. Similarly high levels of false memories have

been observed in dozens, likely hundreds, of experiments using this methodology.

Not only are DRM errors frequent, they are also phenomenologically compelling to the rememberer. Roediger and McDermott asked participants to label each word called 'old' as either 'remembered' or 'known.' 'Remembering' was defined as vividly recollecting details associated with a word's presentation (e.g., where it occurred on the list, what it sounded like, what one was thinking during its presentation), whereas 'knowing' meant simply knowing a word had been presented even though one could not recall the details of its presentation. As shown in **Figure 1**, the proportion of remember and know responses was very similar for the studied words and for the critical lures (Roediger and McDermott, 1995). That is, people were just as likely to claim they remembered the critical nonpresented lures as the studied words. People will also describe their false memories in some detail, attributing them to locations in the study list (Read, 1996) and to a particular speaker (Payne et al., 1996). They are also willing to estimate how frequently they rehearsed each false memory (Brown et al., 2000). In general, the false memory effect is very robust, persisting even when participants have been forewarned about the nature of the illusion (McDermott and Roediger, 1998).

Given the strength of the illusion, it is intriguing that not all lists of related words yield false memories (Deese, 1959; Gallo and Roediger, 2002). Listening to 'sour, candy, sugar, bitter, good, taste, tooth, nice, honey, soda, chocolate, heart, cake, tart, pie' is likely to yield a false memory for 'sweet,' whereas listening to 'sweet, sour, taste, chocolate, rice, cold, lemon, angry, hard, mad, acid, almonds, herbs, grape, fruit' is very unlikely to yield a false memory for the critical lure 'bitter.' Both lists were constructed from the same free-association norms, but only one yields high levels of false memories. A key difference between the lists involves backward associative strength (BAS); this is a measure of how likely the list items are to elicit the critical item in a free association task. In other words, BAS measures how likely participants are to report the critical lure as the first word that comes to mind in response to list items. Participants are likely to respond 'sweet' but not 'bitter' in response to words like 'sugar, sour, taste,' meaning that BAS is very high for 'sweet' but very low for 'bitter.' This difference is crucial; BAS is a major predictor of false recall ($r = 0.73$, Roediger et al., 2001b).

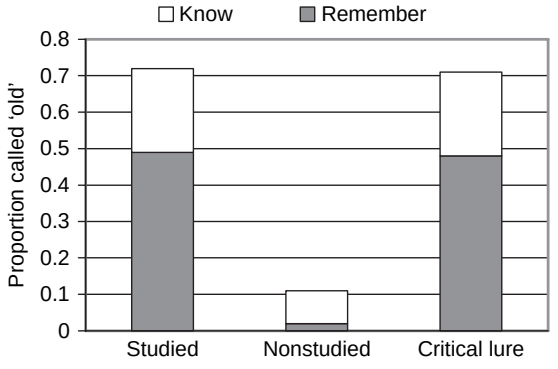

Figure 1 Recognition results for studied items and critical lures. Data from Roediger HL III and McDermott KB (1995) Creating false memories: Remembering words not presented in lists. *J. Exp. Psychol. Learn. Mem. Cogn.* 21: 803–814, Experiment 2.

In the activation monitoring framework's explanation of the DRM illusion, activation at encoding spreads through a preexisting semantic network of words, and the source of this activation is monitored at test. Hearing 'sour, candy, sugar' in the study list activates those nodes in the network. This activation spreads through the network (Collins and Loftus, 1975), activating related nodes. Because the critical lure is associated with so many study items (as indicated by its BAS value), it is activated from many different directions, leading to its heightened activation. If the participant fails to correctly monitor the source of that activation, a false memory will result.

According to the activation monitoring framework, manipulations that increase the amount of activation spreading to the critical lure should result in higher rates of false memories. Consistent with this, false memories increase as the study list increases in length, as longer lists mean that activation from a greater number of words spreads to the critical lure (Robinson and Roediger, 1997). Similarly, activation can spread from phonological associates. Listening to a list of words like 'bite, fight, rut, sprite, slight, rye' yields false memories for phonologically related nonpresented words such as 'right' (Sommers and Lewis, 1999). Intriguingly, lists that combine phonological and semantic associates (e.g., 'bed, rest, awake, tired, dream, scrub, weep, wane, keep') led to even higher rates of false memories than did purely semantic or purely phonological lists (Watson et al., 2003).

Activation alone cannot, however, explain all of the data. An interesting experiment on the effects of

presentation rate highlights the need for both activation and monitoring components. McDermott and Watson (2001) presented DRM lists at five different presentation rates: 20, 250, 1000, 3000, and 5000 ms per word. As expected, veridical recall of list items increased with longer presentation rates. More interesting were the false recall data. When the presentation rate increased from 20 to 250 ms, false recall increased from 0.14 to 0.31. However, when the presentation rate was further increased, the rate of false memories decreased, from 0.22 at 1000 ms to 0.14 at 3000 or 5000 ms. The argument is that semantic activation is increasing as the presentation rate increases, hence the jump in false memories observed at 250 ms. However, with the longer presentation rates, participants encode more information about studied words, allowing them to invoke monitoring strategies during retrieval that help them to judge the source of the activation.

Monitoring is necessary to explain other DRM data, such as the finding that on average older adults remember fewer studied words but falsely remember just as many critical lures (or even more) as do college students (e.g., Balota et al., 1999). That is, because older adults have relatively preserved semantic memory, there should not be age differences in the activation of the critical lure. Rather, what is affected is the ability to monitor the source of activation, as older adults typically have difficulty on source-monitoring tasks (Hashtroudi et al., 1989). More direct support for the monitoring explanation comes from a study linking the age effect to problems with frontal functioning (Butler et al., 2004). In this study, older adults were classified as high versus low functioning on tasks known to require frontal functioning (e.g., the Wisconsin card sort task). Importantly, older adults who scored high on frontal tasks performed similarly to young adults in a typical DRM paradigm. Only older adults who scored poorly on frontal tasks showed reduced true recall and increased false recall. Because frontal areas are often implicated in monitoring tasks (e.g., Raz, 2000), these data suggest it is monitoring ability, not age, that is critical for avoiding false memories.

Even young adults can be placed in situations that make monitoring difficult, forcing them to rely on activation. Consider Benjamin's (2001) study in which he repeatedly presented the DRM lists. Young adults were less likely to incorrectly endorse critical lures from lists presented three times, presumably because they were able to monitor the source of that activation. However, when participants were required to respond quickly at test, they falsely recognized more critical lures from the lists presented three times. Repeating the list presumably increased the activation of the critical lures. When time was plentiful during the recognition test, participants used monitoring processes to correctly attribute the source of the activation (and thus reduce, but not eliminate, the illusion). When retrieval time was short, monitoring was not possible, and the increased activation resulted in high false alarm rates (see also Marsh and Dolan, 2007).

The distinctiveness heuristic is one monitoring strategy that has been investigated in detail. Schacter and colleagues defined the distinctiveness heuristic as "a mode of responding based on participants' metamemorial awareness that true recognition of studied items should include recollection of distinctive details" (Schacter et al., 1999: 3). Anything that makes DRM stimuli more distinctive should increase participants' standards for what they consider to be old. Thus, picture lists yield lower rates of false memories than do word lists (Israel and Schacter, 1997), and pronouncing and hearing the words at study lowers the false alarm rate as compared to only hearing the words (Dodson and Schacter, 2001).

Activation monitoring is the preferred explanation of many researchers, but certainly not all. Other explanations share in common a mechanism for the lures being encoded, and then a monitoring function at test. For example, fuzzy trace theory (Brainerd and Reyna, 2002) proposes that both verbatim and gist traces are encoded for events. Verbatim traces reflect memories of individual events, while gist traces reflect the extraction of meaning across experienced events. During the presentation of a DRM list, verbatim traces would be encoded for the individual words, while at the same time the meaning of the entire list would be extracted and encoded into a gist memory. Later, retrieval of the gist trace could drive false memory effects.

We turn now from false memories of never-presented words to errors when remembering events such as crimes or traffic accidents. More important than the switch in what is being remembered, though, is that different memory mechanisms likely underlie the two types of errors.

2.14.2 Eyewitness Suggestibility: The Misinformation Paradigm

Psychologists have long been interested in the reliability of witnesses. Early in the twentieth century, researchers such as Hugo Münsterberg and William Stern were publishing on the unreliability of testimony. The major methodological breakthrough in this area, though, did not appear until the 1970s when Elizabeth Loftus published her seminal work. She developed the misinformation paradigm (also known as the post-event information paradigm) that involves a twist on the basic retroactive interference paradigms that were popular during the verbal learning era (McGeoch, 1932). In retroactive interference studies, researchers examine the effect of a second, interfering event on memory for an original event (as compared to a control group that was not exposed to the interference). The typical design is shown in the top part of **Table 1**. In verbal learning terms, all participants study paired associates A – B in the first phase of the experiment (e.g., Table – Radio). Next, participants in the experimental group learn A – D associations (e.g., Table – Pencil), whereas participants in the control group rest or learn C – D (e.g., Purse – Pencil). Finally, all participants are tested on A – B (e.g., Table – ?), and memory is poorer in the group that learned two different associations in response to A. What does this have to do with eyewitness memory? The bottom portion of **Table 1** shows the connection between the standard retroactive interference design and eyewitness memory. The witness views an event (A – B), such as a traffic accident (A) occurring near a stop sign (B). After the event, the police will repeatedly interview the witness, the newspaper will publish accounts of the crime, and the witness will talk about the event with other people. All these have the potential to provide interfering information. For example, the police might erroneously suggest that the accident (A) occurred near a yield sign (D) when really it occurred near a stop sign (B). Later, when the witness tries to remember the details of the original event (A – ?), he or she may recall the interfering misinformation instead of what was actually witnessed. In contrast, misinformation production would be low for subjects in a control condition who heard a neutral reference to a traffic sign.

One of the most classic laboratory demonstrations comes from Loftus et al. (1978; see also Loftus and Palmer, 1974). All participants viewed a slide show depicting a traffic accident; in the critical slide, a red Datsun was approaching an intersection with a traffic sign. One-half of participants saw a stop sign; the other participants saw exactly the same slide except that the intersection was marked with a yield sign. After seeing the slides, all participants answered a series of questions about the accident. Embedded in one of the questions was a reference to the traffic sign; half of participants were asked 'Did another car pass the red Datsun while it was stopped at the stop sign?' whereas the others answered 'Did another car pass the red Datsun while it was stopped at the yield sign?' Twenty minutes later, participants examined pairs of slides and determined which one had been presented in the original slide show. The critical pair required participants to pick between the Datsun at a stop sign versus a yield sign. When participants had answered the question containing misinformation, they selected the correct slide 41% of the time (below chance), as compared to 75% when the question had referred to the correct sign.

Numerous studies have since replicated the basic finding: Information presented after an event can change what the eyewitness remembers. The original event may take the form of a film, slide show, staged event, written story, or a real event. The misinformation may be delivered in the form of presuppositions in questions, suggestive statements, photographs (e.g., mugshots), or narrative summaries. It can come from the experimenter, a confederate, or the witness herself. The misinformation effect qualifies as a false

Table 1 Experimental designs for studying retroactive interference (RI) and eyewitness suggestibility

	Condition	*Study target (A – B)*	*Interference (A – D) or (C – D)*	*Test target (A – B)*
RI				
	Experimental	Table – Radio	Table – Pencil	Table – ?
	Control	Table – Radio	Purse – Pencil	Table – ?
Eyewitness				
	Misled	Accident – Stop sign	Accident – Yield sign	Accident – ?
	Control	Accident – Stop sign	Accident – Traffic sign	Accident – ?

memory since participants generally endorse the misinformation quickly and with high confidence (Loftus et al., 1989). When participants described their erroneous memories, undergraduate judges were at chance at differentiating between real and suggested memories (Schooler et al., 1986).

One prerequisite for suggestibility is that participants fail to notice any problem with the misinformation when it is presented. This is called the discrepancy detection principle (Loftus, 1992). Participants are more likely to accept and reproduce misinformation about peripheral details than central characters or details (e.g., Christianson, 1992). In contrast, blatant misinformation not only is rejected, it also increases resistance to other peripheral misinformation (Loftus, 1979). Blatant misinformation may serve as a warning that the source is not to be trusted. This would be consistent with findings that warnings given before encoding of misinformation successfully reduce suggestibility, probably because warned participants read more slowly as they search for errors (Greene et al., 1982). In general, slow readers are more likely to notice (and resist) misinformation (Tousignant et al., 1986).

Given that participants do not detect the misinformation, manipulations that are generally known to enhance remembering lead to increased suggestibility, presumably because they increase memory for the misinformation. For example, suggestibility is greater if participants generate the misinformation (Roediger et al., 1996) and if the misinformation is repeated (Mitchell and Zaragoza, 1996; Zaragoza and Mitchell, 1996). Participants may also be more likely to rely on the misinformation if they have poor memory for the original events. For example, dividing attention during study (but not during the post-event information phase) increases suggestibility (Lane, 2006).

One important question is what happens to the original memory. It is easy to imagine the practical implications: If the original and post-event misinformation coexist in memory, it suggests the usefulness of developing strategies to help witnesses retrieve the original event. However, if the misinformation overwrites the original memory, it suggests that no retrieval strategy will allow access to the original event. Originally, there was much debate over this issue, but several lines of evidence suggest that the two memories may coexist. For example, consider what happens when misled participants are allowed to make a second guess after producing misinformation. If the original memories were completely unavailable, second-chance responses should be at chance (as what would they be based on?). Instead, second-chance guesses of misled participants are above chance (Wright et al., 1996), suggesting that some information about the original event is still available.

Compelling data for the coexistence hypothesis comes from experiments using source monitoring tests rather than recognition tests. Typically, in the 1970s and 1980s participants were required to make 'old/new' judgments about items. However, an 'old' judgment does not necessarily imply that participants remember seeing the misinformation in the original event. For example, participants may remember reading the misinformation in a post-event narrative and assume that remembering it from the narrative means it must have been in the video as well. To test these ideas, Lindsay and Johnson (1989) compared two groups of participants, all of whom studied the same photograph of an office. Afterward, half of participants read a narrative that mentioned eight office-related objects that were not actually in the original picture. Control participants read an accurate narrative description of the scene. The novel manipulation was at test; half of participants took a standard 'yes/no' recognition test, and half took a source monitoring test. For each item on the recognition test, participants indicated 'yes' if the object had been in the photograph and 'no' if it had not. On the source test, participants indicated whether each test object had been only in the picture, only in the text, in both the picture and the text, or in neither the picture nor the text. The results were dramatic: The misinformation effect was eliminated in the source condition! In later experiments, the advantage of the source test was replicated, although suggestibility was reduced rather than completely eliminated (Zaragoza and Lane, 1994).

Recent research on the misinformation effect has moved from the debate about the fate of the original memory trace to other interesting questions. One current trend is the examination of the effects of social context on suggestibility. This includes both the social context in which participants are exposed to misinformation, as well as the social context in which participants first intrude errors. For example, researchers are examining the effects of receiving misinformation from other people as opposed to reading it in narratives or embedded in questions (e.g., Roediger et al., 2001a; Gabbert et al., 2004; Wright et al., 2005). A related question involves the response the witness receives from other people after she (the witness) makes a mistake. The question of how feedback affects a witness' memory is an

important one, as incorrectly telling the witness 'Good, you identified the suspect' can have many negative consequences (see Douglass and Steblay, 2006, for a review; *See* Chapter 2.44).

2.14.3 Verbal Overshadowing

Rehearsal (especially elaborative rehearsal) can be a useful mnemonic for remembering word lists and prose. But what happens when a rehearsal fails to adequately capture the original experience? For example, words rarely capture the richness of our perceptions. What are the memorial consequences of a description (a rehearsal of sorts) that is inadequate or even inaccurate?

Questions about the effects of language and memory are not new ones. Many undergraduates are familiar with a classic study in which labels influenced memory for pictures. A picture of two circles joined by a line was labeled as either 'glasses' or 'barbell,' and participants later redrew the pictures to be similar to the label (Carmichael et al., 1932). In the 1970s, there was much interest in how participants integrated verbal and visual information in memory (e.g., Pezdek, 1977; Gentner and Loftus, 1979). Depending on the study, opposite conclusions were reached. Sometimes labeling pictures and objects led to enhanced memory (e.g., Santa and Ranken, 1972), but other times labeling was associated with difficulty on later memory tests (e.g., Gentner and Loftus, 1979).

More recently, Schooler and Engstler-Schooler (1990) sparked interest in the question by contextualizing it within the eyewitness memory domain. After watching a 30-s video of a bank robbery, participants in their Face Verbalization condition wrote a description of the thief's face (participants in the control condition did an unrelated task during that time). At test, all participants saw eight similar faces (including the thief) and were asked to select the perpetrator from the video or to indicate if he was absent from the line-up. The intriguing finding was that 64% of control participants selected the target, as compared to 37% in the face verbalization condition. Schooler and Engstler-Schooler labeled their finding verbal overshadowing.

Verbal overshadowing is not limited to faces; it extends to other types of perceptual information. Describing a voice reduces the ability to later identify that voice from among six options (Perfect et al., 2002). The typical wine drinker shows verbal overshadowing for wines, as they are unable to verbalize the nuances of wine in the vocabulary of experts (Melcher and Schooler, 1996). After memorizing a map of a small town, participants who wrote about it later performed worse on distance estimation tasks than did control participants (Fiore and Schooler, 2002). That is, having described one's spatial mental model of the town led to confusion about the distances between the landmarks.

Several different explanations have been proposed. One possibility involves recoding (*See* Chapter 2.07). Specifically, when participants describe a visual stimulus from memory, they are effectively recoding it from a visual representation to a verbal one, and the more recent recoded memory then interferes with the original visual memory. Consistent with an interference account, inserting a delay between the description and the final test reduces verbal overshadowing (Finger and Pezdek, 1999), in the same way that a delayed test can reduce retrieval blocking in other interference situations (e.g., Choi and Smith, 2005).

The recoding account would predict that the quality of the new verbal representation (as measured by the description) should predict the effects of verbalization on later memory tasks. Although Schooler and Engstler-Schooler (1990) did not find a relationship between the quality of the descriptions and the ability to recognize the perpetrator, this may be because of the way the descriptions were scored. Descriptions were considered better if they described more features of the target; however, this dependent measure is not ideal, as face recognition depends on configural information rather than on recognition of individual features (e.g., Diamond and Carey, 1986). That is, while people may only be able to verbalize individual facial features (e.g., she has big eyes and she has freckles on her nose), face recognition depends upon hard-to-verbalize configural information about the relationship of features to one another (e.g., the relationship between the eyes and the nose).

Support for the recoding hypothesis comes from a meta-analysis of the literature about the type of instructions given to witnesses. Meissner and Brigham (2001) coded each study's instructions to participants as either standard or elaborative. Instructions were considered elaborative if "the authors explicitly encouraged their participants to go beyond their normal criterion of free recall and to provide more elaborative descriptions" (Meissner and Brigham, 2001: 607). Presumably, elaborative descriptions led

to less accurate recodings; consistent with this, elaborative descriptions were more likely to lead to verbal overshadowing than were descriptions resulting from standard free recall instructions (Meissner and Brigham, 2001). One study published since the meta-analysis deserves mention here. MacLin (2002) compared the effects of several different types of instructions on the verbal overshadowing effect. When participants were told to describe facial features, the standard effect occurred: On a later test, participants were less likely to identify the target than were control participants who did not describe the target. However, when participants were told to write a description comparing the target to a famous person such as Julia Roberts (the exemplar condition), verbal overshadowing was reduced. The effect disappeared in a prototype condition in which participants described "what type of person you think he most looks like" (MacLin, 2002: 932) in terms of occupation and personality. Thus, verbal overshadowing was most likely in the condition in which recoding emphasized facial features rather than more holistic information about the target face.

A second explanation of verbal overshadowing also hinges on the fact that descriptions often emphasize individual facial features rather than configural information. However, rather than proposing that a feature-based description interferes with retrieval of the original memory, the argument is that verbalization induces a processing shift at test (Dodson et al., 1997; Schooler, 2002). That is, because descriptions of faces emphasize individual features (as it is hard to verbalize relations between features), the participant carries over this type of processing to test. This is considered a processing shift, as face identification is normally based on configural information rather than features; carrying over a featural orientation would constitute inappropriate processing. One interesting finding is shown in **Figure 2**. Dodson and colleagues had participants view a target face and then do one of three tasks: Describe the target face, describe a parent's face, or list U.S. states and capitals (a control condition). As shown in the figure, describing any face (e.g., a relative's) reduced participants' ability to identify the target (Dodson et al., 1997). This is hard to reconcile with the idea that a recoded representation (of the target) is interfering with access to the original memory. Rather, it suggests that anything that emphasizes featural processing will encourage that same type of processing at test.

Similar conclusions were reached by Finger (2002), who added a second factor to the typical verbal

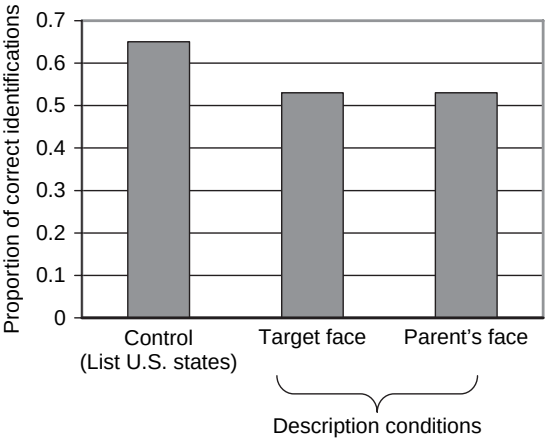

Figure 2 Recognition of target face after describing target face, parent's face, or listing U.S. states and capitals. Data from Dodson CS, Johnson MK, and Schooler JW (1997) The verbal overshadowing effect: Why descriptions impair face recognition. *Mem. Cogn.* 25: 129–139, Experiment 2.

overshadowing experiment. She crossed description (describe *vs.* control) with a post-description task (verbal *vs.* mazes). When solving mazes followed the face description, verbal overshadowing disappeared. In a second experiment, Finger replicated the effect with a second nonverbal task, namely listening to music. Engaging in holistic processing can change the processing set from one that emphasizes individual features to one that does not, with consequences for face identification.

Recent research suggests a number of relatively simple solutions to minimize the effects of verbal overshadowing of faces, such as inserting a delay between description and test (Finger and Pezdek, 1999) and preceding the test with a task that encourages configural processing (Finger, 2002). It remains to be tested whether these solutions are equally effective at reducing verbal overshadowing of other types of perceptual stimuli such as voices, wines, and maps.

2.14.4 Misattributions of Familiarity

Thus far, we have discussed misremembering laboratory events – be it misremembering a word that was never presented in a study list (in the DRM paradigm), incorrectly recalling a detail of a slide show (in the misinformation paradigm), or misidentifying a person from a video (in verbal overshadowing experiments). In contrast, in the next paradigm

we will review, the memory error involves misattributing something learned in the laboratory to preexperimental experience. More specifically, the paradigm is a recipe for fame; Larry Jacoby used straightforward experimental manipulations to make ordinary names appear famous.

The names Brad Pitt, Mark McGwire, and Sandra Day O'Connor are likely recognizable to you. In addition to agreeing that you have heard of these people before, you can probably justify your response by telling us Brad Pitt is an actor, Mark McGwire is an athlete, and Sandra Day O'Connor is a retired Supreme Court justice. You can also tell me whether or not other names are the names of famous people, even if you cannot say exactly why each person is famous. For example, try to identify the three famous people in the following list of six names: Zoe Flores, Minnie Pearl, Jessica Lynch, Joanna Emmons, Summer Foster, Hattie Caraway. Hopefully, at least one or two of the names will seem familiar to you, even if you do not know what accomplishments to associate with each name. Quite simply, the false fame paradigm increases the familiarity of nonfamous names (like Zoe Flores, Joanna Emmons, and Summer Foster) and places the respondent in a situation where familiarity is interpreted as fame.

In the typical paradigm, participants read a list of names explicitly labeled as nonfamous. In a second phase, participants judge the fame of each of a series of names; the test list includes moderately famous names like Minnie Pearl, new nonfamous names, and old nonfamous names that were read in the first part of the experiment. Critically, half of the participants are required to do a secondary task (e.g., monitoring an auditory stream of numbers for a series of three odd numbers in a row) at the same time as the fame judgment task. In the full-attention (control) condition, old nonfamous names are less likely to be judged famous than are new nonfamous names; in this condition, if participants can remember a name is old, then they can assume it is not famous. In contrast, in the divided-attention condition, participants are more likely to call old nonfamous names famous ($M = 0.28$) than new nonfamous names ($M = 0.14$) (Jacoby et al., 1989b). The logic is that under divided attention, participants are forced to base their judgments on the familiarity of a name, and that the cognitive load interferes with their ability to recollect whether names were presented in the first part of the experiment.

The false fame effect requires conditions that force participants to rely on familiarity rather than recollecting information about the names. For example, the false fame effect also occurs when attention is divided during encoding, as presumably that prevents encoding of item-specific information (Jacoby et al., 1989a). Similarly, under conditions of full attention, the illusion requires a delay between study of the nonfamous names and the fame judgments. Consistent with the idea that the false fame effect is familiarity driven, the effect is stronger in populations that are more likely to rely on familiarity, such as older adults (Bartlett et al., 1991; Multhaup, 1995).

This illusion is related to a more general framework on how people interpret feelings of familiarity. Vague feelings of familiarity are not specific to names; there are many situations in which familiarity is experienced and the perceiver must attribute that familiarity to something. In an impressive series of studies, Jacoby has shown that how that familiarity is interpreted depends on the experimental context. Familiarity can be interpreted as fame, but it can also lead to illusions of duration and noise level, for example. At test, previously studied words are judged to be presented longer than are new words (Witherspoon and Allan, 1985) and background noise is judged to be quieter for old sentences than for new sentences (Jacoby et al., 1988). The familiarity of the items causes them to be processed fluently, and in the context of perceptual judgments, this fluent processing is interpreted as perceptual conditions that aid identification of the items.

Familiarity may also play a role in the déjà vu experience (Brown, 2003, 2004). In the prior examples in this section, familiarity was successfully attributed to a source, albeit incorrectly: Familiarity was misinterpreted as fame and longer presentation durations, among other things. In contrast, déjà vu occurs when something feels familiar but the familiarity cannot be attributed to any prior experience. It is this unexplained familiarity with a situation that yields the puzzling déjà vu reaction. One hypothesis is that the individual previously experienced all or part of the present situation or setting, but cannot explicitly remember it. Thus implicit memory yields a familiarity response that is puzzling given the lack of episodic memory. Because déjà vu is a relatively infrequent phenomenon (Brown, 2003, 2004), it is difficult to capture in the laboratory. Some support for the implicit memory hypothesis, however, has been found in a laboratory paradigm (Brown and Marsh, in press). In this study, students from Duke University and Southern Methodist University

viewed photos of the away campus in an initial exposure phase (none of these students reported having visited the other campus in real life). During the initial session, participants made a simple perceptual judgment about each of 216 photos, which included the target away-campus photos as well as many filler photos. One week later, participants made judgments about whether or not they had visited each of a series of test photos. Critically, in addition to familiar places from their home campus, participants judged photos from the prior session. Prior exposure to away-campus scenes boosted participants' beliefs that they had visited the places in real life. Intriguingly, almost half of participants reported experiencing something like déjà vu in the study. In this case, familiarity with a scene influenced belief that the place had been visited in real life, and sometimes this familiarity was puzzling enough to be labeled as déjà vu (*See* Chapter 2.21).

In this section, we described how familiarity could be interpreted as fame as well as perceptual attributes such as the volume of noise. In the next section, we will consider whether familiarity with an event can increase people's beliefs that an event happened in their pasts.

2.14.5 Imagination Inflation

The relationship between imagery and perception has a long intellectual history, reaching back to philosophers such as Hume and Mills. In the 1970s, the key question involved the nature of the representation underlying images. In this context, Johnson and colleagues asked how we separate memories for images from memories based on perception. More generally, reality monitoring involves deciding whether a memory originated from an internal or external source, with internal sources being cognitive processes such as imagery, thought, and dreams. Johnson argued that internally generated and externally presented memories tend to differ in prototypical ways, and that these differences in qualitative characteristics were the basis for attributing memories to thought versus perception (e.g., Johnson and Raye, 1981). Compared to memories based on perception, memories of images were postulated to be less vivid and to be associated with the cognitive operations involved in their generation. Reality monitoring errors occur when memories contain characteristics atypical of their class. For example, easily generated images are more likely to be misattributed to perception than are difficult-to-imagine objects. Easily generated images are likely atypically vivid; in addition, their easy generation means they are not associated with a record of cognitive operations (Finke et al., 1988).

Misattributions of imagined events to perception have been documented with many different kinds of stimuli, including imagined voices (Johnson et al., 1988), imagined rotations of alphanumeric characters (Kahan and Johnson, 1990), and imagined pictures (Johnson et al., 1982). But can imagery cause confusions beyond these types of simple laboratory stimuli? That is, if you imagine an event, will you later come to believe that it really happened?

Garry and colleagues (1996) created a three-stage procedure to answer this question. In the first part of the experiment, participants rated the likelihood that they had experienced each of a series of life experiences (the Life Events Inventory; LEI), including winning a stuffed animal at a fair and breaking a window with one's hand. Two weeks after reading descriptions of the target events, participants imagined both the setting and the action of events in response to specific prompts. For example, in the broken window event, participants spent 20–60 s imagining the following setting: "It is after school and you are playing in the house. You hear a strange noise outside, so you run to the window to see what made the noise. As you are running, your feet catch on something and you trip and fall" (Garry et al., 1996: 210). After the imagination phase was finished, the experimenter pretended to have lost the original LEI and asked participants to fill out the questionnaire for a second time.

There were eight critical events judged unlikely to have occurred for a majority of the participants, and each participant imagined four of those during phase 2. Of interest was whether participants were more likely to change their beliefs about events they had imagined in phase 2, as compared to the control events not imagined. Garry et al. examined the percentage of critical items that were rated as more likely to have happened at time 2 (after the imagery phase) than at time 1. Increases in likelihood ratings were more common for imagined events than for control events. For example, consider the effect of imagining on people's beliefs that as a child they broke a window with their bare hand. The likelihood ratings increased from time 1 to time 2 for 24% of participants in the imagery condition, as compared to only 12% of control participants.

It is possible, of course, that participants had actually experienced these unusual events and that imagining them helped to cue the previously forgotten memories. One solution to this criticism is to control the original events in the laboratory, to allow certainty about what actually occurred. Because this is not possible with childhood memories, Goff and Roediger (1998) brought the encoding phase into the laboratory. The experiment had three sessions; during the first session, participants enacted, heard, or imagined simple events. For example, when the experimenter read aloud the sentence 'bounce the ball,' one participant would simply listen; another would imagine bouncing the ball, and a third would actually bounce the ball. Twenty-four hours later, participants returned for a second session in which half of participants imagined events and half did math problems. In the imagery condition, participants were guided to imagine each event zero, one, three, or five times; the events included ones from the first session as well as completely new events. Participants in this condition rated the vividness of each image. Finally, 2 weeks after the initial session, participants were given recognition and source monitoring tests. Participants were explicitly told that their memory was being tested for the first day only. They were first asked if they remembered hearing certain events. If they answered no, they gave a confidence rating in their answer. If they answered yes, they specified the format of the remembered event (heard and enacted, heard and imagined, or heard only) and rated their confidence in that judgment. Of interest was whether imagining new events in session 2 would increase beliefs that the events had been performed in session 1. Replicating findings from studies using LEI measures, Goff and Roediger found that events that were only imagined during the second session were later misremembered as having been performed during the first session. Imagining a bouncing ball in the second session increased participants' beliefs that they had actually bounced a ball in the first session. Furthermore, as the number of imaginings in session 2 increased, participants were more likely to incorrectly label a never-performed action as having been performed in the first session, as shown in **Figure 3**.

The finding of imagination inflation for laboratory events supports the idea that imagination can yield false memories and that the effects observed with the LEI cannot be attributed solely to recovery of previously forgotten events. Why do these effects occur? In their original demonstration of imagination

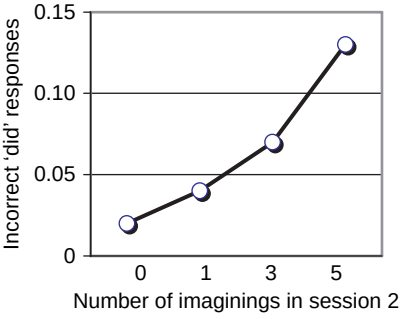

Figure 3 False 'did' judgments for never-performed actions as a function of number of imaginings in session 2. Data from Goff LM and Roediger HL III (1998) Imagination inflation for action events: Repeated imaginings lead to illusory recollections. *Mem. Cogn.* 26: 20–33.

inflation, Garry and colleagues favored a reality monitoring explanation, whereby an imagined memory was misattributed to perception. Specifically, Garry et al. argued that imagination increased the perceptual information associated with the events, thus increasing the similarity of these imagined memories to performed events. This account predicts that imagination inflation should be greater when images are detailed, as they will be more readily confused with perception. Consistent with this hypothesis, Thomas and colleagues (2003) found that elaborative imagery instructions increased the imagination inflation effect, as compared to standard imagery instructions. Like Goff and Roediger, Thomas' participants completed an initial encoding phase and returned a day later for the imagination phase. Instructions in the simple imagery condition paralleled Goff and Roediger; for example, participants were asked to 'imagine getting up and opening the door.' Participants in the elaborative imagery condition were to imagine two additional statements, which included two sensory modalities; for example, 'Imagine getting up and opening the door. Imagine how the door handle feels in your hand. Imagine how the door sounds as you open it.' If the event was not imagined in the middle session, participants were very good at identifying new events. However, imagining events in the middle session led to imagination inflation, and this effect was bigger (12%) following elaborative imagery than simple imagery (7%).

To recap, imagining events may increase their vividness, a key characteristic of perceived memories. This is not the only explanation for the imagination inflation effect, however. Imagining events may also

increase their familiarity, which can also lead to memory misattributions (as described in the previous section of this chapter). The imagination scripts used to guide the imagery also usually contain a lot of suggestive information over and above the vivid images generated by the participant. In short, does imagination underlie the effect, or is the effect at least partly driven by familiarity (as discussed in the section on false fame), as opposed to imagination?

Several data points suggest that imagining vivid details is not necessary to increase beliefs that events occurred in childhood. For example, similar effects are observed when participants paraphrase the script normally used to guide imagery (Sharman et al., 2004). The data also look similar when participants explain how the events might have happened in one's childhood (Sharman et al., 2005). Of course, in both of these cases, it is possible that participants might spontaneously generate images even though they were not explicitly directed to do so. However, Bernstein and colleagues observed inflation in a study in which spontaneous generation of images was quite unlikely. Their study extended the revelation effect to autobiographical memory (Bernstein et al., 2002). The revelation effect is the finding that requiring participants to unscramble a stimulus (to reveal it) increases the likelihood that it will be judged 'old' (Westerman and Greene, 1996). Bernstein et al. found that participants were more likely to believe childhood events had in fact occurred if they had to unscramble the events before judging them (e.g., 'broke a dwniwo playing ball'). Unscrambling presumably does not encourage imagery, and thus it suggests that LEI ratings can be based on factors other than image vividness, such as familiarity.

It should be clear that the just-described results do not negate the role of imagination in false memory creation. Finding that explaining, paraphrasing, and unscrambling events can all inflate confidence in remembered events does not preclude imagination also playing a role. Rather, such results emphasize the importance of isolating the contribution of imagination, as imagination is often combined with other factors that yield false memories.

2.14.6 Implanted Autobiographical Memories

It is possible to make a person remember a word that was never presented, to misjudge the fame of a name, or to misremember a detail from a witnessed event. But do people ever falsely remember entire events? The answer is yes. Consider the case of Shauna Fletcher, who came to believe her horrible memories of childhood sexual abuse were false memories (Pendergrast, 1996). How could this happen? Shauna traced her memories to several different sources, blaming her therapist for suggesting that the events occurred, and books and movies for providing the images she remembered. Shauna's experiences parallel the findings from laboratory studies: Implanting memories is possible, but not simple. A single misleading statement does not yield the kind of false memories experienced by Shauna. Correspondingly, the laboratory procedures for implanting entire memories tend to be much more complicated than those described earlier in the chapter, oftentimes combining multiple suggestive techniques.

Loftus and Pickrell (1995) demonstrated that false autobiographical memories can be implanted using laboratory techniques. The critical false memory involved being lost in a shopping mall as a child. To camouflage the purpose of the experiment, participants were also interviewed about childhood events that had actually occurred; a close relative of the participant provided the true memories. The relative also provided plausible details to aid in constructing the false memory (e.g., stores the family shopped, other family members likely to have been present, etc.) and verified that the participant had not been lost in a shopping mall around the critical time period (age 5).

Participants reviewed four events: three that were true and the critical false event. Each event was described in a booklet, and participants were instructed to remember the events and to write about the specific details of each. If participants did not remember the event, they were to indicate that on the form. Approximately 1–2 weeks later, participants were interviewed about the events. In addition to recalling details of the events, participants rated each memory for clarity (1 = not clear; 10 = extremely clear) and confidence that additional details could be remembered later (1 = not confident; 5 = extremely confident). A second interview, conducted 1–2 weeks later, was similar to the first interview.

Did participants come to remember being lost in the shopping mall at age 5? Critically, seven out of 24 participants claimed to remember the false memory (fully or partially) while writing about it in the initial booklet. Although their descriptions of the false events were shorter than those of true memories, the clarity ratings given to these false memories

increased across interviews. At the end of the experiment, five participants were unable to pick out the false event and instead guessed that one of the true events had never happened.

The reader may be wondering why we consider the Loftus and Pickrell (1995) study to be an example of successful memory implantation. After all, most participants never believed the lost-in-the-mall memory and were able to identify it as the false event. What is crucial is that the implantation rate was above zero. That is, to argue that implanting false memories is possible, one only needs to show one successful implantation.

False memories are not limited to erroneous memories of being lost in the mall as a child. Experimenters have been successful at implanting many different types of events in participants. Participants have come to falsely remember participating in a religious ceremony (Pezdek et al., 1997), riding in a hot air balloon (Wade et al., 2002), putting the gooey toy Slime in an elementary school teacher's desk (Lindsay et al., 2004), and being admitted to the hospital (Hyman et al., 1995). Different approaches have been taken to ensure a false memory was in fact implanted, as opposed to a true memory being recovered. One is to confirm events with parents, as Loftus and Pickrell (1995) did. Another is to choose events that are very implausible, or even impossible. Braun and colleagues (2002) used the latter approach, implanting false memories for meeting a Warner Brothers character, Bugs Bunny, at Disneyland.

The procedures for implanting false memories are often elaborate, far beyond the simple suggestions typical of eyewitness misinformation studies. Successful studies typically follow three rules of thumb (Mazzoni et al., 2001; Lindsay et al., 2004). First, the target event must be deemed plausible. For example, it is easier to implant a false memory for being lost in the mall than it is to implant a false memory of an enema (Pezdek et al., 1997; see also Hart and Schooler, 2006). Second, the target event must be elaborated upon. For example, suggestibility was greater for participants who were required to imagine and describe the target events, probably because the guided imagery task led to more detailed memories (Hyman and Pentland, 1996). Third, the products of this elaboration must be attributed to memory, as opposed to other sources.

Although this framework is generally useful for thinking about memory implantation, one difficulty is that many manipulations likely affect more than one process. Consider, for example, Pezdek and colleagues' difficulty in implanting a false memory involving a Catholic ceremony (the Eucharist) in Jewish participants. Were the Jewish participants able to reject the event because it was implausible to them or because they were not familiar enough with the event to elaborate upon the suggestion? Similarly, consider what happens when a participant sees a doctored photograph depicting her engaged in the target false event. In this type of study, after a relative verifies that the participant has never ridden in a hot air balloon, Photoshop is used to insert a real childhood photo into a photograph depicting a hot air balloon ride (Wade et al., 2002). Such a procedure yields false memories in about half of participants (a high rate) – but is unclear at a cognitive level how the photograph has its effect on memory. The very existence of a photograph of the event increases the plausibility of the event, as well as providing vivid details about the supposed event.

The aforementioned examples illustrate the challenge of doing research in this area, namely the difficulty of linking manipulations to specific cognitive processes. We do not, however, intend to be pessimistic. The demonstrations of memory implantation were critical first steps, and they are being followed by systematic manipulations aimed at better elucidating the underlying cognitive processes. Rather than trying to equate different events with different levels of an independent variable, one approach is to try to implant the same event while experimentally manipulating a variable that affects only one possible factor, such as plausibility. Mazzoni and colleagues took this approach when examining memories for demonic possession (Mazzoni et al., 2001). Keeping the target event constant, they showed that reading articles about possession dramatically increased later beliefs that one had witnessed a demonic possession (as compared to the control group).

One of the major puzzles in this research area is why vivid false memories can be successfully implanted in some participants but not others. For example, across eight well-cited studies, Lindsay et al. (2004) observed that the implantation rate ranged from 0% to 56% of participants! We know of no study in which the false memory was successfully implanted into 100% of participants. Thus we predict one fruitful avenue for future research will be investigating individual differences in suggestibility. In the best study to date, Hyman and Billings (1998) looked for relationships between rates of false

memory implantation and scores on four cognitive/personality scales. Two interesting results emerged. First, false memory scores were higher for participants who scored higher on the Creative Imagination Scale (CIS), a scale that measures imagery ability as well as suggestibility. In other words, participants who were better able to elaborate upon the suggestion were more likely to come to remember the false event. Second, false memory scores were higher for participants who scored higher on the Dissociative Experiences Scale (DES), a scale that measures both normal experiences such as distraction as well as less normal experiences such as hearing voices. Scoring higher on the DES may be related to difficulties with source monitoring.

In short, implanting detailed false memories is a complex process. It combines many of the techniques described earlier in the chapter in the context of other false memory paradigms, including imagery instructions, misleading suggestions, and a test situation that does not encourage participants to evaluate the source(s) of their memories. In this context, we turn to a discussion of how the various memory errors relate to one another.

2.14.7 Connections Across False Memory Paradigms

We have described six different paradigms that yield memory errors: The DRM paradigm, the eyewitness misinformation paradigm, verbal overshadowing studies, misattributions of familiarity, imagination inflation, and implanted autobiographical memories. What is the relationship between these very different paradigms?

We linked each memory error to possible mechanisms: Spreading activation (and monitoring of that activation) in the DRM paradigm, interference and failure to monitor source in the misinformation paradigm, an inappropriate shift in processing at test in the verbal overshadowing paradigm, a misattribution of familiarity in the false fame effect, increased familiarity and vividness (and possibly reality monitoring failures) in imagination inflation, and elaboration and source misattribution in the implanted memory studies. Sometimes, the same mechanism is implicated across illusions; for example, source monitoring failures are implicated in the misinformation effect and in implanting false autobiographical memories. Imagination inflation likely involves reality-monitoring errors, a specific type of source error. Misattributions of activation (in the DRM paradigm) and familiarity (as observed in the false fame paradigm) can also be interpreted as source errors. In other cases, the mechanisms appear qualitatively different, as in the case of the transfer inappropriate processing shift in verbal overshadowing studies. Of course, one issue is that likely more than one mechanism is involved in each illusion (and the convergence of mechanisms is probably why the errors are so robust). For example, imagination inflation likely depends on both vivid encoding (which may also increase familiarity) and some kind of monitoring failure at test. One other point worth noting is that even if the same mechanism is implicated in two different illusions, the instantiations of that mechanism may be quite different. For example, even though source errors are implicated in both the DRM illusion and the misinformation effect, giving participants a source test has very different effects in the two cases. As already mentioned, a source test can reduce susceptibility to post-event information (e.g., Lindsay and Johnson, 1989; Zaragoza and Lane, 1994). However, source tests yield more puzzling results when used in the DRM paradigm; depending on the features of the source test, the rate of false memories may be higher (Hicks and Marsh, 2001), lower (Multhaup and Conner, 2002), or similar (Hicks and Marsh, 1999) to that observed on item memory tests.

More generally, comparing the effects of standard manipulations on the different measures of suggestibility is a useful way of examining similarities and differences across false memory paradigms. For example, many researchers are interested in differences in suggestibility between children and college students. This comparison has been made in at least three of the six paradigms we described – DRM, eyewitness misinformation, and implanted memories – and the conclusion about age is not the same across paradigms. For example, younger children are normally more suggestible in eyewitness misinformation paradigms than are older children (Bruck and Ceci, 1999), but older children are more suggestible than younger children in the DRM paradigm (e.g., Brainerd and Reyna, 2007). That is, even though there are clear age differences in source monitoring abilities (e.g., Lindsay et al., 1991), with older children doing better than younger, older children are more suggestible in the DRM paradigm. Why is this, given that we already alluded to the role of source monitoring in the DRM paradigm? The paradox can be resolved by attributing the key age

difference to encoding, rather than to retrieval-based processes such as source monitoring. Specifically, because younger children have difficulty noting semantic relations between items (Brainerd and Reyna, 2007), they may be less likely to encode the critical lure. In the terms of activation-monitoring theory, activation will be less likely to spread to the critical lure from related studied items; in the terms of fuzzy trace theory, younger children will be less likely to extract the gist of the list. By either account, the result is the same: It does not matter if younger children are poor at source monitoring if there is no trace for them to attribute to a source! Again, this example highlights the inadequacy of simply attributing DRM and eyewitness errors to difficulties with source; the full picture is more complicated.

There are at least two other approaches for connecting false memory paradigms. One is to test the same participants in multiple paradigms, and another is to link false memory in different paradigms to the same standardized measures of individual differences. The logic is that if comparable mechanisms underlie the errors, then the same individuals (or the same types of people) should perform similarly across paradigms. For example, Clancy and colleagues (2002) examined suggestibility in the DRM paradigm in control participants and in people who believed aliens had abducted them. Memories of alien abduction are of interest since the scientific community views alien abductions as impossible occurrences, leading these memories to be classified as false memories (although not implanted in the laboratory, of course). Interestingly, false recognition of nonpresented words was higher for people with alien abduction memories ($M = 0.67$) than for control participants ($M = 0.42$). In this same study, correlations between false memory and scores on individual difference scales were also observed. The rate of false memories was greater for individuals who scored highly on scales measuring absorption and dissociative experiences (DES) and reported more symptoms of post-traumatic stress disorder. The reader will recall that the DES is a scale that measures both normal experiences such as distraction as well as less normal experiences such as hearing voices, and that higher scores on the DES may be related to difficulties with source monitoring. Higher DES scores predicted implantation of a false childhood memory for spilling punch on the mother of the bride, although absorption did not (Hyman and Billings, 1998). Scores on the DES have also been related to imagination inflation (Paddock et al., 1999), and pathological scores on this scale have been linked to suggestibility in the eyewitness misinformation paradigm (Eisen et al., 2001). However, DES scores are not related to susceptibility to the false fame illusion (Peters et al., 2007). Understanding such individual differences will likely be an important part of future research on memory errors and suggestibility.

We end with a note on another approach we believe will help elucidate the relationships between different false memory paradigms: neuroimaging. Consider a study by Cabeza and colleagues (2001), in which participants watched two very different sources (a Caucasian male and an Asian female) read DRM-like lists, followed by a recognition memory test. At test, studied words and critical lures yielded similar activation in anterior medial temporal lobe (MTL) areas, but activation in posterior MTL differentiated true and false memories. Cabeza et al. associated anterior MTL with retrieval of semantic information and posterior MTL with perceptual information. What would the pattern be like for familiarity-driven illusions, such as false fame? To the extent that the same mechanisms underlie different memory errors, similar patterns of activation should occur.

2.14.8 Conclusions

In this chapter, we reviewed just six of the many published paradigms for creating false memories. Together, the data highlight the constructive nature of memory, as proposed by Bartlett (1932). We have also tried to stress that not all memory errors are equal. Not surprisingly, given the complexity of memory, there are many different ways that error can enter the system, from encoding to retrieval.

While we have focused on errors, we would be remiss not to point out that reconstructive memory is often very useful. For example, familiarity often is an excellent cue that something has been experienced before, and it is only in certain situations that this heuristic leads to error. More generally, errors are often the by-product of processes that support veridical memory. Memory errors are more than intriguing illusions. A thorough understanding of memory's errors will provide insight into the processes that normally aid memory.

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2.15 Memory in and about Affect

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Philosophers, politicians, and playwrights alike have recognized for centuries the capacity of moods to color the way people remember the past, experience the present, and forecast the future. Psychologists, however, were relatively late to acknowledge this reality, despite a number of promising early leads (e.g., Rapaport, 1942/1961; Razran, 1940). Indeed, it is only within the past 30 years that empirical investigations of the interplay between affect and cognition have been published with regularity in mainstream psychology journals (see LeDoux, 1996).

Psychology's late start in exploring the affect–cognition interface reflects the fact that neither behaviorism nor cognitivism – the two paradigms that dominated the discipline throughout the twentieth century – ascribed much importance to affective phenomena, whether in the form of specific, short-lived emotional reactions or more nebulous, long-lasting mood states (for detailed discussion of affect-related concepts, see Russell and Feldman Barrett, 1999; Russell and Lemay, 2000).

From the perspective of the radical behaviorist, all unobservable mental events, including those affective in nature, were by definition deemed beyond

the bounds of scientific psychology. Though early behaviorist research examined the environmental conditioning of emotional responses, later studies focused mainly on the behavioral consequences of readily manipulated drive states, such as thirst or fear. In such studies, emotion was instilled in animals through crude if effective means, such as electric shock, and emotionality was operationalized by counting the number of fecal boluses deposited by small, scared animals. As a result, behaviorist research and theory added little to our understanding of the interrelations between affect and cognition.

Until recently, the alternative cognitive paradigm also had little interest in affective phenomena. To the extent that the cognitive revolutionaries of the early 1960s considered affects at all, they typically envisaged them as disruptive influences on 'proper' – read 'emotionless' or 'cold' – thought processes. Thus, the transition from behaviorism to cognitivism allowed psychology to reclaim its head, but did nothing to recapture its heart.

Things are different today. Affect is now known to play a key role in how information about the world is processed and represented. Moreover, affect underlies the cognitive representation of social experience (Bower and Forgas, 2000), and emotional responses can serve as an organizing principle in cognitive categorization (Niedenthal and Halberstadt, 2000). Thus, the experience of affect – how we feel about people, places, and events – is central to people's cognitive representations of themselves and the world around them.

When it comes to memory, a major theme of this book, two overarching questions are of interest. One of these concerns memory 'in' affect: How do affective states or moods influence the acquisition and retention of information? The other question involves memory 'about' affect: What determines the accuracy and other attributes of memory for emotionally charged events? Over the past 30 years, both questions have been pursued with a wide array of subject species (humans, mice, mollusks, etc.), scientific approaches (experiential, physiological, neuroimaging), and memory methods (different materials, tasks, and measures). In addition, each of these highly general questions subsumes a host of more specific issues, including such varied topics as the consistency of flashbulb recollections (Talarico and Rubin, 2003), cognitive and clinical investigations of mood-dependent memory (Eich and Macaulay, 2006), dissociable influences of affective valence and arousal on memory vividness (Kensinger and Corkin, 2004; Kensinger and Schacter, 2006), and neural systems underlying the encoding and retrieval of emotional events in animals and humans (LaBar and Cabeza, 2006; Phelps, 2006).

This chapter surveys only a small segment of the vast affect/memory literature and its scope is limited to human cognitive and social research. Whereas the first part of the chapter covers mood-congruent cognition, a concept that is central to understanding how affective states influence memory, the second part focuses on memory for trauma, a controversial topic with important implications both for cognitive theory and for clinical practice. Given that mood congruence has had little overlap, conceptually or methodologically, with research on memory for traumatic events, our approach will be to treat memory in and about affect as distinct topics. Nevertheless, consideration of these topics together invites exploration of the possible empirical and theoretical issues that might unite them. To this end, we close with a discussion of potential ways in which the principles and findings of mood congruence might apply to understanding the processes leading to reports of recovered memories of trauma.

2.15.1 Memory in Affect

People often acquire, remember, and interpret information about themselves, and the world around them, in a manner that matches their current state of affect or mood. However, these mood-congruent effects are not universal, but depend for their expression on a variety of task-, person-, and situation-specific variables (Bower and Forgas, 2000). Since the early 1980s, a great deal of effort has gone into explaining why mood-congruent effects are robust and reliable under certain circumstances, but weak or nonexistent under others. The fruits of this effort are the focus of discussion in this part of the chapter.

We begin by introducing the concepts of affect priming and affect-as-information, both of which are central in understanding the impact of moods on the substance of cognition, or what people think. Attention then turns to the processing consequences of affect, that is, the impact of moods on cognitive style, or how people think. These opening remarks on cognitive substance versus style will set the stage for discussion of an integrative theory – Forgas's (1995, 2002) affect infusion model (AIM) – that seeks to specify the ways in which affect influences cognition in general and social cognition in

particular. Next we consider the critical part that different information processing strategies play in the occurrence of mood congruence, and we conclude this section by summarizing some of the strengths and shortcomings of the AIM.

2.15.1.1 Affect Priming and Affect-as-Information

Several theorists maintain that moods influence the content of cognition because they influence the memory structures people rely on when processing information. For example, Wyer and Srull (1989) suggested that recently activated concepts are more accessible because such concepts are returned to the top of mental storage bins, which in turn means that subsequent sequential searches are more likely to access the same concepts again. As affective states facilitate the use of positively or negatively valenced mental concepts, this could account for the greater use of mood-congruent constructs in subsequent tasks.

A more comprehensive explanation of this effect was provided by Bower's (1981) associative network model. On this view, the observed links between affect and cognition are neither motivationally based (cf. the psychoanalytic theory of Feshback and Singer, 1957), nor are they the result of merely incidental, spatiotemporal associations (cf. the conditioning theory of Byrne and Clore, 1970). Instead, Bower (1981) argued that affect is integrally linked to an associative network of mental representations. Accordingly, the activation of an affective state should selectively and automatically prime associated thoughts and representations previously linked to that affect, and these concepts should be more likely to be used in subsequent constructive cognitive tasks.

Consistent with the network model, early studies provided strong support for the concept of affective priming, indicating mood congruence across a wide range of cognitive tasks. For example, people induced to feel good or bad tend to selectively remember more mood-congruent details from their childhood and more of the real-life events they had recorded in a daily diary for a week (Bower, 1981). Mood congruence was also observed in subjects' interpretations of social behaviors and in their impressions of other people (Bower and Forgas, 2000).

However, subsequent research showed that mood congruence is subject to several boundary conditions (see Blaney, 1986; Bower, 1987; Singer and Salovey, 1988). Difficulties in demonstrating reliable mood-congruent effects were ascribed to such varied causes as the lack of sufficiently strong or intense moods (Bower and Mayer, 1985), the subjects' inability to perceive a meaningful, causal connection between their current mood and the cognitive task they are asked to perform (Bower, 1991), and the use of tasks that prevent subjects from processing the target material in a self-referential manner (Blaney, 1986). Interestingly, mood-congruent effects tend to be more reliably obtained when complex and realistic materials are used in conjunction with tasks (e.g., association generation, impression formation, or inference making) that require a high degree of open, constructive processing (e.g., Bower and Forgas, 2000; Mayer et al., 1992). Such tasks provide people with a rich set of encoding and retrieval cues and thus allow affect to more readily function as a differentiating context (Bower, 1992). A similar point was made by Fiedler (1991), who suggested that mood congruence may obtain only in constructive cognitive tasks – those that involve an open-ended search for information (as in recall tasks) and the active elaboration and transformation of stimulus details using existing knowledge structures (as in judgmental and inferential tasks).

It appears, then, that affect priming occurs when an existing affective state preferentially activates and facilitates the use of affect-consistent information from memory in a constructive cognitive task. The consequence of affect priming is affect infusion: The tendency for judgments, memories, thoughts, and behaviors to become more mood congruent (Forgas, 1995, 2002). But in order for such infusion effects to emerge, it is important that subjects adopt an open, elaborate information processing strategy that facilitates the incidental use of affectively primed memories and information. Thus, the nature and extent of affective influences on memory and cognition should largely depend on what kind of information processing strategy people employ in a particular situation. Later we will review the empirical evidence for this prediction and describe an integrative theory that emphasizes the role of information-processing strategies in moderating mood congruence.

Alternatively, the affect-as-information (AAI) model of Schwarz and Clore (1983, 1988) suggests that "rather than computing a judgment on the basis of recalled features of a target, individuals may . . . ask themselves: 'How do I feel about it? [and] in doing so, they may mistake feelings due to a preexisting state as a reaction to the target" (Schwarz,

1990: 529). Thus, the model implies that mood congruence in judgments is due to an inferential error, as people misattribute a preexisting affective state to a judgmental target.

The AAI model incorporates ideas from three past research traditions. First, the predictions of the model are often indistinguishable from earlier conditioning research by Clore and Byrne (1974). Whereas the conditioning account claimed that spatiotemporal contiguity is chiefly responsible for linking affect to judgments, the AAI model posits an internal inferential process as producing the same effects (see Berkowitz et al., 2000). A second tradition that informs the AAI model comes from research on misattribution, according to which judgments are often inferred on the basis of salient but irrelevant heuristic cues – in this case, affective state. Thus, the AAI model also predicts that only previously unattributed affect can produce mood congruence. Finally, the model also shows some affinity with research on judgmental heuristics, insofar as affective states are thought to function as heuristic cues in informing people's judgments.

People typically rely on affect as a heuristic cue when they lack either or both the motivation and the cognitive resources to process information more extensively. This happens when "the task is of little personal relevance, when little other information is available, when problems are too complex to be solved systematically, and when time or attentional resources are limited" (Fiedler, 2001: 175). For example, some of the earliest and still most compelling evidence for the AAI model came from an experiment (Schwarz and Clore, 1983) that involved telephoning respondents and asking them unexpected and unfamiliar questions. In this situation, subjects have little personal interest or involvement in responding to a stranger, and they have neither the motivation, the time, nor the cognitive resources to engage in extensive processing. Relying on prevailing affect to infer a response seems a reasonable strategy under such circumstances. In a conceptually similar example, Forgas and Moylan (1987) asked people to complete an attitude survey on the sidewalk outside a cinema in which they had just watched either a happy or a sad movie. The results showed strong mood congruence: Happy theatergoers gave much more positive responses than did their sad counterparts. In this situation, as in the study by Schwarz and Clore (1983), respondents presumably had insufficient time, motivation, or capacity to engage in elaborate processing, and hence they may well have relied on their temporary affect as a heuristic cue to infer a reaction. Thus, depending on the task, situation, and resources at hand, either affect priming or AAI can take the lead in coloring or infusing cognition with current affect.

2.15.1.2 Processing Consequences of Affect

Affective states or moods shape not only the substance of cognition but also its style. It has been proposed that positive affect recruits less effortful and more superficial processing strategies; in contrast, negative affect seems to trigger a more analytic and vigilant processing style (Clark and Isen, 1982; Schwarz, 1990; Mackie and Worth, 1991). However, more recent studies have shown that positive affect can also produce distinct processing advantages: Happy people often adopt more creative and inclusive thinking styles and display greater mental flexibility than do sad subjects (Bless, 2000; Fiedler, 2000; Isen, 2004).

Several theories have sought to explain affective influences on processing strategies. One suggestion is that the experience of a negative mood, or any affective state, gives rise to intrusive, irrelevant thoughts that deplete attentional resources, which in turn leads to poor performance in a variety of cognitive tasks (Ellis and Ashbrook, 1988; Ellis and Moore, 1999). An alternative hypothesis points to the motivational consequences of positive and negative affect: Whereas people experiencing positive affect may try to maintain a pleasant state by refraining from any effortful activity, negative affect may motivate people to engage in vigilant, effortful processing (Isen, 1984). In a variation of this idea, Schwarz (1990) has suggested that affective states have a signaling or tuning function, informing the person that relaxed, effort-minimizing processing is appropriate in the case of positive affect, whereas vigilant, effortful processing is best suited for negative affect.

These various accounts all assume that positive and negative affect decrease or increase the effort, vigilance, and elaborateness of information processing, albeit for different reasons. Recently, Bless and Fiedler (2006) have conjectured that the evolutionary significance of positive and negative affect is not simply to influence processing effort, but to trigger two fundamentally different processing styles. They suggest that positive affect promotes a more schema-based, top-down, assimilative processing style, whereas negative affect produces a more bottom-up, externally focused, accommodative processing strategy.

These strategies can be equally vigilant and effortful, yet produce markedly different cognitive outcomes by directing attention to internal or external sources of information.

These affect-induced processing differences may well have evolutionary origins, consistent with the idea that the basic function of affective states is to rapidly trigger cognitive strategies most likely to produce adaptive responses to a situation (Frijda, 1986). In other words, affect may operate like domain-specific adaptation that meets the requirements for special design (Haselton and Ketelaar, 2006; also see Forgas et al., 2007).

2.15.1.3 Cognitive Benefits of Mild Dysphoria for Eyewitness Memory

Another perspective on the processing consequences of affect is provided by recent research showing that affect-induced differences in processing style have major implications for memory and memory-based social cognitive tasks, including some surprising cognitive advantages associated with mild dysphoria.

For example, a recent series of studies revealed a beneficial effect of negative affect on eyewitness memory (Forgas et al., 2005). Affect can impact eyewitness memory at any or all of three distinct stages: (1) when the event is first witnessed (encoding stage), (2) when misleading information is encountered later on (post-event stage), and (3) when the information is retrieved (retrieval stage). Several experiments examined mood effects at Stage 2 and found that positive affect promoted, and negative affect inhibited, the incorporation of false details into eyewitness memories (Forgas et al., 2005), consistent with the more attentive, accommodative processing style associated with negative affect that may have helped witnesses to identify misleading details when exposed to them (Bless and Fiedler, 2006).

In one study (Forgas et al., 2005, Experiment 1), participants viewed pictures showing a car crash scene (negative event) and a wedding party scene (positive event). One hour later, following the induction of a happy, sad, or neutral mood, participants completed a questionnaire about the scenes that either contained or did not contain misleading information. In this particular study, moods were induced by asking participants to reflect upon, write about, and emotionally relive either a positive, neutral, or negative experience from their personal past. In addition to this life-events technique, many other methods of mood modification (involving videos, music, guided imagery, etc.) are available to investigators in the affect/cognition area (see Coan and Allen, 2007). After an additional interval filled with other tasks, the accuracy of their eyewitness memory for the scenes was tested. As predicted, and as indicated in **Figure 1**, exposure to misleading information significantly reduced eyewitness accuracy, an effect that, remarkably, was increased by positive mood and decreased by negative mood. In fact, negative mood almost completely eliminated the familiar misinformation effect.

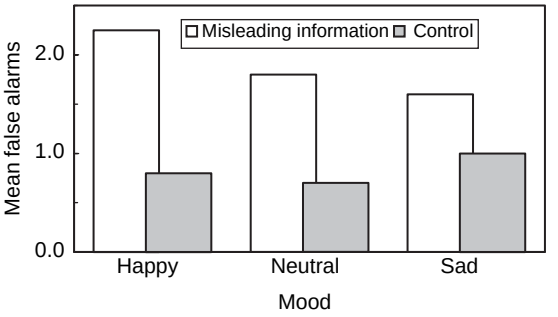

Figure 1 Mean false alarms to misleading postevent information as a function of participants' mood (happy, neutral, or sad) and condition (misleading information previously presented versus control). From Forgas JP, Vargas P, and Laham S (2005) Mood effects on eyewitness memory: Affective influences on susceptibility to misinformation. *J. Exp. Soc. Psychol.* 41: 574–588; Experiment 1; used with permission from Elsevier.

In a second study (Forgas et al., 2005, Experiment 2), students in a lecture hall witnessed a staged aggressive encounter between a lecturer and a female intruder. One week later, eyewitnesses to this episode were induced into a positive or negative mood and then completed a questionnaire about the episode that either did or did not contain planted, misleading information. When the accuracy of their eyewitness memory for the episode was subsequently tested, negative affect again seemed to have all but eliminated this source of error in eyewitness memory. Signal detection analyses confirmed that negative affect actually improved eyewitnesses' ability to discriminate between correct and misleading details.

Can people suppress the impact of their moods on their thinking when instructed to do so? In a third study (Forgas et al., 2005, Experiment 3), participants watched a videotape of a complex event (a wedding or a convenience-store robbery). After viewing a happy or sad videotape, they completed a short questionnaire that either did or did not contain

misleading information. Some participants were also instructed to disregard and control their current affective state. Exposure to misleading information again reduced eyewitness accuracy, and did so most when people were in a happy rather than a sad mood. Instructions to control affect did not reduce this mood effect, but rather, produced an overall conservative response bias.

These experiments offer convergent evidence that negative moods can have significant adaptive effects on memory and cognitive performance, by promoting a more focused, accommodative processing style that reduced people's susceptibility to misleading information and thus improved eyewitness memory. These results are consistent with theories that predict that good and bad moods should have an asymmetric effect on processing strategies and outcomes (Forgas, 1995, Bless, 2000; Forgas, 2002).

2.15.1.4 Mood Congruence and the Affect Infusion Model

We have seen that affective states have clear if complex effects on both the substance of cognition (i.e., the contents of one's thoughts) and its style (e.g., whether information is processed systematically or superficially). It is also clear, however, that affective influences on cognition are context-specific. A comprehensive explanation of these effects needs to specify the circumstances that promote or impede mood congruence, and should also define the conditions likely to trigger either affect priming or affect-as-information mechanisms.

The AIM (Forgas, 1995) seeks to accomplish these goals by expanding on Fiedler's (1991) idea that mood congruence is most likely to occur when circumstances call for an open, constructive style of information processing. Such a style involves the active elaboration of the available stimulus details and the use of memory-based information in this process. The AIM thus predicts that (1) the extent and nature of affect infusion should be dependent on the kind of processing strategy that is used, and (2) all things being equal, people should use the least effortful and simplest processing strategy capable of producing a response. As this model has been described in detail elsewhere (Forgas, 1995, 2002), only a brief overview will be provided here.

The AIM identifies four processing strategies that vary according to both the degree of openness or constructiveness of the information-search strategy and the amount of effort exerted in seeking a solution. The direct access strategy involves the retrieval of preexisting responses and is most likely when the task is highly familiar and when no strong situational or motivational cues call for more elaborate processing. For example, if you were asked to make an evaluative judgment about a well-known political leader, a previously computed and stored response would come quickly and effortlessly to mind, assuming that you had thought about this topic extensively in the past. People possess a rich store of such preformed attitudes and judgments. Given that such standard responses require no constructive processing, affect infusion should not occur.

The motivated processing strategy involves highly selective and targeted thinking that is dominated by a particular motivational objective. This strategy should be impervious to affect infusion (Clark and Isen, 1982) and may produce mood-incongruent outcomes when the motivation is to control or reverse affect congruence (Forgas and Ciarrochi, 2002). For instance, if in a job interview you are asked about your attitude toward the company you want to join, the response will be dominated by the motivation to produce an acceptable response. Open, constructive processing is inhibited and affect infusion is unlikely to occur. The consequences of motivated processing should depend on the particular processing goal and may also produce a reversal of mood-congruent effects (Berkowitz et al., 2000).

The remaining two processing strategies require more constructive and open-ended information search strategies, and thus facilitate affect infusion. Heuristic processing is the kind of superficial, quick processing style people are likely to adopt when they lack motivation or resources to process more extensively (Schwarz and Clore, 1983; Forgas and Moylan, 1987). Heuristic processing can lead to affect infusion as long as people rely on affect as a simple inferential cue and depend on the 'how do I feel about it' heuristic to produce a response (Schwarz and Clore, 1988; Clore et al., 2001).

When simpler strategies such as direct access, motivated processing, or heuristic processing prove inadequate, people need to engage in substantive processing to satisfy the demands of the task at hand. According to the AIM, substantive processing should be adopted when (1) the task is in some ways demanding, atypical, complex, novel, or personally relevant, (2) there are no direct-access responses available, (3) there are no clear motivational goals to guide processing, and (4) adequate time and other

processing resources are available. Substantive processing is an inherently open and constructive strategy, and affective states may selectively prime or enhance the accessibility of related thoughts, memories, and interpretations. The model makes the interesting and counterintuitive prediction that affect infusion – and hence mood congruence – should be increased when extensive and elaborate processing is required to deal with a more complex, demanding, or novel task. This prediction has been borne out by several studies that will be summarized shortly.

The AIM also specifies a range of contextual variables related to the task, the person, and the situation that jointly influence processing choices. For example, greater task familiarity, complexity, and typicality should recruit more substantive processing. Personal characteristics that influence processing style include motivation, cognitive capacity, and personality traits such as self-esteem (Smith and Petty, 1995; Rusting, 2001). Situational factors that influence processing style include social norms, public scrutiny, and social influence by others (Forgas, 1995).

An important feature of the AIM is that it recognizes that affect itself can also influence processing choices. As noted earlier, Bless and Fiedler (2006) have proposed that positive affect typically generates a more assimilative, top-down, schema-driven processing style whereby new information is assimilated into what is already known. In contrast, negative affect often promotes a more accommodative, piecemeal, bottom-up processing strategy in which attention to external events dominates over existing stored knowledge.

The key prediction of the AIM is the absence of affect infusion when direct access or motivated processing is used and the presence of affect infusion during heuristic and substantive processing. The implications of this model have been investigated in many studies involving several substantive areas in which mood congruence has been demonstrated, including affective influences on attention, learning, memory, and social cognition. The following subsections present a snapshot of some of these studies and areas.

2.15.1.4.1 Mood congruence in attention and learning

Many everyday cognitive tasks are performed under conditions of considerable information overload, when people need to select a small sample of information for further processing. Affect may have a significant influence on what people will pay attention to and learn (Niedenthal and Setterlund, 1994). Due to the selective activation of an affect-related associative base, mood-congruent information may receive greater attention and be processed more extensively than affectively neutral or incongruent information (Bower, 1981; Bower and Cohen, 1982). Several studies have shown that people spend longer reading mood-congruent material, linking it into a richer network of primed associations; as a result, they are better able to remember such information (Bower and Forgas, 2000).

These effects occur because "concepts, words, themes, and rules of inference that are associated with that emotion will become primed and highly available for use … [in] … top-down or expectation-driven processing … [acting] … as interpretive filters of reality" (Bower, 1983: 395). Thus, there is a tendency for people to process mood-congruent material more deeply, with greater associative elaboration, and thus learn it better. Consistent with this notion, depressed psychiatric patients tend to learn and remember depressive words particularly well, a cognitive bias that disappears once the depressive episode is over (Bradley and Mathews, 1983; Watkins et al., 1992). However, mood-congruent learning is seldom seen in patients suffering from anxiety (Watts and Dalgleish, 1991; Burke and Mathews, 1992), perhaps because anxious people tend to use particularly vigilant, motivated processing strategies to defend against anxiety-arousing information (Mathews and MacLeod, 1994; Ciarrochi and Forgas, 1999). Thus, as predicted by the AIM, different processing strategies appear to play a critical role in mediating mood congruence in learning and attention.

2.15.1.4.2 Mood congruence in memory

Several studies have shown that people are better able to consciously or explicitly recollect autobiographical memories that match their prevailing mood (Bower, 1981). Depressed patients display a similar pattern, preferentially remembering aversive childhood experiences, another kind of cognitive bias that disappears once depression is brought under control (Lewinsohn and Rosenbaum, 1987). In line with the AIM, these mood-congruent effects also emerge when people try to recall complex social stimuli (Fiedler, 1991; Forgas, 1993).

Research using implicit tests of memory, which do not require conscious recollection of past experience, also provides evidence of mood congruence. For

example, depressed people tend to complete more word stems (e.g., 'can') with negative than with positive words they have studied earlier (e.g., 'cancer' vs. 'candy'; Ruiz-Caballero and Gonzalez, 1994). Similar results have been obtained in other studies involving experimentally induced states of happiness or sadness (Tobias et al., 1992).

2.15.1.4.3 Mood congruence in associations and interpretations

Cognitive tasks often require people to go beyond the information given, forcing them to rely on associations, inferences, and interpretations to construct a judgment or a decision, particularly when dealing with complex and ambiguous social information (Heider, 1958). Affect can prime the kind of associations used in the interpretation and evaluation of a stimulus (Clark and Waddell, 1983).

The greater availability of mood-congruent associations can have a marked influence on the top-down, constructive processing of complex or ambiguous details (Bower and Forgas, 2000). For example, when asked to freely associate to the cue 'life,' happy subjects generate more positive than negative associations (e.g., 'love, freedom' vs. 'struggle, death'), whereas sad subjects do the opposite (Bower, 1981). In a related vein, mood-congruent associations emerge when emotional subjects daydream or concoct stories about fictional characters depicted in the Thematic Apperception Test (Bower, 1981). Mood-primed associations also play an important role in clinical states: Anxious people tend to interpret spoken homophones such as pane/pain or dye/die in the more anxious, negative direction (Eysenck et al., 1987), consistent with the greater activation these mood-congruent concepts receive.

Such mood-congruent effects can have a marked impact on many types of social judgments, including perceptions of human faces (Schiffenbauer, 1974), impressions of people (Bower and Forgas, 2000), and self-perceptions (Sedikides, 1995). However, several studies have shown that this associative effect is diminished as the targets to be judged become more clear-cut and thus require less constructive processing (Forgas, 1995). Such a diminution in the associative consequences of mood with increasing stimulus clarity again suggests that open, constructive processing is crucial for mood congruence to occur. This same mechanism also leads to mood congruence in more complex and elaborate social judgments, such as judgments about the self and others, as the results sketched in the following section suggest.

2.15.1.4.4 Mood congruence in self-judgments

Affective states have a strong assimilative influence on memory-based judgments about the self: Positive affect improves and negative affect impairs the valence of self-conceptions. In one study (Forgas et al., 1990), happy or sad students who had scored well or poorly on a recent exam were asked to rate the extent to which their test performance was attributable to factors that were internal in origin and stable over time. Compared to their negative mood counterparts, students in a positive mood were more likely to claim credit for success, making more internal and stable attributions for high test scores, but less willing to assume personal responsibility for failure, making more external and unstable attributions for low test scores.

Of related interest is a study by Sedikides (1995), who asked subjects to evaluate a series of self-descriptions related to their behaviors or personality traits while they were in a happy, sad, or neutral mood. Based on the AIM, Sedikides predicted that highly rehearsed core conceptions of the self should be processed quickly using the direct-access strategy and hence should show no mood-congruent bias; in contrast, less salient, peripheral self-conceptions should require more time-consuming substantive processing and accordingly be influenced by an affect-priming effect. The results supported these predictions, making Sedikides' (1995) research the first to demonstrate differential mood-congruent effects for central versus peripheral conceptions of the self.

Affect also appears to have a greater congruent influence on self-related memories and judgments made by people with low versus high self-esteem, which may reflect a parallel difference in the stability of their respective self-concepts (Brown and Mankowski, 1993). For instance, Smith and Petty (1995) observed stronger mood congruence in the self-related memories reported by low rather than high self-esteem individuals. As predicted by the AIM, these findings suggest that low self-esteem people need to engage in more open and elaborate processing when thinking about themselves, increasing the tendency for their current mood to influence the outcome.

Affect intensity may be another moderator of mood congruence. One study showed that mood

congruence is greater among people who score high on measures assessing openness-to-feelings as a personality trait (Ciarrochi and Forgas, 2000). However, other studies suggest that mood congruence in self-related memories and judgments can be spontaneously reversed as a result of motivated processing strategies. Sedikides (1994) observed that after mood induction, people initially generated self-statements in a mood-congruent manner. However, with the passage of time, negative self-judgments spontaneously reversed, suggesting the operation of an automatic process of mood management. Research by Forgas and Ciarrochi (2002) replicated these results and indicated further that the spontaneous reversal of negative self-judgments is particularly rapid and pronounced in people with high self-esteem.

In summary, moods have been shown to exert a strong congruent influence on self-related memories and judgments, but only when some degree of open and constructive processing is required and when there are no motivational forces to override mood congruence. Research to date also indicates that the infusion of affect into self-judgments is especially likely when these judgments (a) relate to peripheral in contrast to central aspects of the self, (b) require extensive, time-consuming processing, and (c) reflect the self-conceptions of individuals with low rather than high self-esteem.

2.15.1.4.5 Mood congruence in person perception

The AIM predicts that the more people need to think in order to compute a response, the greater the likelihood that affectively primed ideas will influence the outcome. To test this prediction, several researchers have manipulated the complexity of the subjects' task in order to create more or less demand for elaborate processing.

In one set of studies (Forgas, 1992), happy and sad participants were asked to read and form impressions about fictional characters who were described as being highly typical or highly atypical and having an odd combination of attributes (e.g., an avid surfer whose favorite music is Italian opera). The expectation was that when people have to form an impression about a complex, ambiguous, or atypical individual, they will need to engage in more constructive processing and affectively primed associations should thus have a greater chance to infuse the judgmental outcome.

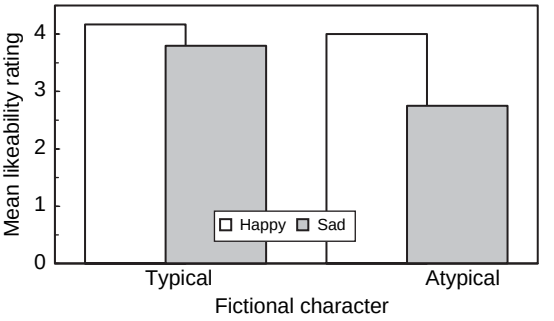

Figure 2 Impact of participants' mood on their ratings of the likeability of typical or atypical fictional characters. From Forgas JP (1992) On bad mood and peculiar people: Affect and person typicality in impression formation. *J. Pers. Soc. Psychol.* 62: 863–875; Experiment 3; used with permission from the American Psychological Association.

Consistent with this reasoning, participants took longer to read about unusual as opposed to conventional characters. Moreover, mood-congruent effects were more pronounced when happy and sad participants judged the likeability of atypical in contrast to typical fictional characters, a finding depicted in **Figure 2**. Similar results were found in a follow-up study in which the to-be-judged targets were odd versus ordinary couples rather than individuals (Forgas, 1993).

Research investigating the impact of mood on judgments and inferences about real-life interpersonal issues paints a similar picture. For example, partners in long-term, intimate relationships showed clear evidence of mood congruence in their memories and attributions for actual conflicts, and paradoxically, these effects were greater when thinking about more serious conflicts that required more extensive processing (Forgas, 1995). These results provide direct evidence for the process dependence of affect infusion into social judgments and inferences. Even judgments about highly familiar people are more prone to affect infusion when a more substantive processing strategy is used.

Some individual-difference or personality characteristics, such as trait anxiety, can also influence processing styles and thereby significantly moderate the influence of negative mood on intergroup judgments (Ciarrochi and Forgas, 1999). Low trait-anxious whites in the United States reacted more negatively to a threatening black out-group when experiencing negative affect. Surprisingly, high trait-anxious individuals showed the opposite pattern: They went out of their way to control their negative

tendencies when feeling bad and produced more positive judgments. Thus it appeared that low trait-anxious people allowed affect to influence their judgments, while high trait-anxiety combined with aversive mood triggered a more controlled, motivated processing strategy designed to eliminate socially undesirable intergroup judgments.

2.15.1.5 Strengths and Shortcomings of the Affect Infusion Model

To recap, the AIM attempts to account for mood-congruent effects in social cognition (Forgas, 1995). It provides a means of integrating two explanations of mood effects, namely AAI (Schwarz and Clore, 1988) and affect priming (Bower, 1981). It outlines the situations in which each process dominates and therefore is the primary method for affect to influence people's thoughts and behaviors. The model accounts well for mood effects on learning and memory (Bower and Forgas, 2000) and a wide array of affective influences on social cognition, including perceptions of others as well as oneself.

The AIM also casts light on the conditions that are more or less likely to evidence mood-congruent effects. Specifically, when processing is easy and familiar, current mood has less of an impact on task performance than when processing is more demanding, more difficult, and more unusual. Hence, it is precisely when people are paying greater attention, considering carefully, and exerting more cognitive effort that they are likely to be biased by their current, and often unrelated, mood state (Forgas, 1995, 2002).

This pattern of results has an important implication, namely, that such performance differences are more than mere responses to demand characteristics created by experimental mood manipulations, one of the oldest and thorniest issues in contemporary cognition/emotion research (see Polivy and Doyle, 1980; Bower, 1981; Ingram, 1989). Since any demand characteristics that exist should be constant across easy and difficult processing conditions, they cannot be responsible for any behavioral differences that are found between these conditions. Consequently, the greater conceptual precision provided by the AIM makes it a more parsimonious explanation of the data set as a whole.

Though the AIM connects and clarifies data from many domains, several findings are difficult to reconcile with the approach. For instance, the model suggests that negative affect encourages bottom-up, externally focused processing, but the literature on self-focused attention in depression (Pyszczynski and Greenberg, 1987) indicates that negative affect leads to more internally focused processing, proposals that are clearly in conflict.

Another troublesome subject for the AIM is mood *in*congruence, a curious phenomenon that has been seen in several studies involving autobiographical memory, person perception, and other social cognitive tasks. Parrot and Sabini (1990, Experiment 2), for instance, found that college students tended to feel happier on clear than on cloudy days – no surprise there. Yet when asked to recollect a salient experience from their high-school years, the students recalled mostly pleasant events on gloomy days and mostly unpleasant events on sunny days.

The causes of such counterintuitive results remain uncertain. The AIM is chiefly concerned with either the presence or absence of mood-congruent effects, not with the reverse. As mentioned earlier, several researchers have suggested that mood incongruence may be related to an individual's ability and desire to strategically regulate his or her mood (e.g., Sedikides, 1994; Forgas and Ciarrochi, 2002), but other factors may also play an important role.

One such factor was discovered accidentally in research (Eich, 1995) dealing with the mood-mediation theory of place-dependent memory: The idea that how well memorial information transfers from one physical environment (e.g., a sunny courtyard) to a different setting (e.g., a dimly lit room) depends not on how similar the two places look but rather on how similar they feel. On this view, place-dependent effects in human memory represent a special, and rather subtle, form of mood-dependent memory (Eich, 2007).

Participants in two studies (university undergraduates) were asked to recollect or generate as many as 16 specific episodes or events, from any time in the personal past, that were called to mind by neutral noun probes, such as ship and street. After recounting the gist of a given event (what happened, who was involved, etc.), students rated the incident along several dimensions, including its original emotionality: How pleasant or unpleasant the event seemed when it took place. Participants completed this task of autobiographical-event generation in one of two environments: either a small, dark, and spartanly furnished basement office or in a warm, inviting, and exquisitely scenic Japanese garden. The expectation, which was confirmed in both studies, was that

students would generally feel happier (more pleasant, more energized) when tested in the latter locale.

Nonetheless, neither experiment provided any evidence of an overall mood-congruent effect: Mean ratings of event emotionality were statistically the same for events that had been generated in the garden versus the office. However, a different picture developed when participants were divided into two groups – aware versus unaware – based on statements they made about the aims and methods of the research in an in-depth postexperimental interview. Whereas participants in the aware group recognized that different environments might evoke different moods, those in the unaware group seemed not to appreciate the possibility of an affect/environment connection.

Among aware subjects, there was clear evidence of mood congruence: Averaging across the two studies, ratings of event emotionality were higher (i.e., more positive) for autobiographical events that had been generated in the garden than in the office. Among unaware subjects, however, the tendency was toward mood incongruence: Events generated in the happy garden were rated as being somewhat less pleasant than those that had been recollected in the sad office.

Additional evidence relating to the awareness factor comes from two recently completed studies (Eich et al., unpublished observations) that were methodologically similar to those outlined earlier in all major respects but one: The frequency with which participants were asked to reflect upon and rate their current mood before, during, and after the task of autobiographical-event generation (which again was carried out in either the garden or office locales). Following the lead of Berkowitz et al. (2000), we reasoned that the very act of assessing one's current mood would enhance awareness of a connection between that mood and the environment in which the assessment is made. In line with this reasoning, the percentage of subjects classified as aware was much higher (63% vs. 29%) in the experiment in which the students evaluated their moods repeatedly than in the otherwise identical experiment in which moods were rated infrequently.

Though it appears that awareness of an affect/environment connection helps determine whether autobiographical memories coincide or contrast with a person's current mood, exactly how and why this happens remains to be explained. The search for a theory would be aided by answers to a host of novel questions. For instance, what role does affect/environment awareness play in free association, self-judgment, person perception, and other social cognitive tasks that, like autobiographical memory, are known to be highly sensitive to mood effects? Also, is there a theoretically meaningful nexus between the concept of awareness, as it applies to mood congruence, and its applicability to other aspects of social cognition, including the influence of explicit versus implicit attitudes on behavior (Greenwald and Banaji, 1995; Greenwald et al., 2002) and the conscious versus nonconscious priming of stereotypes (Bargh and Chartrand, 1999; Bargh and Ferguson, 2000)? And is affect/environment awareness relevant not only to mood-congruent memory, but to mood-dependent memory as well (Eich and Macaulay, 2006)?

This concludes our brief look at the concept of mood congruence and of some of the ways in which affective states influence cognition in general and memory in particular. Now we turn attention from memory 'in' affect to memory 'about' affect, and take up the complex, challenging, and controversial matter of memory for traumatic events.

2.15.2 Memory about Affect

The effects of emotion on memory for personal events is one of the most controversial issues in all contemporary cognition/emotion research. This issue has been studied in different contexts, and in every case the only conclusion upon which everyone agrees is that the impact of emotion on memory is an extremely contentious topic. For example, in research investigating flashbulb memories for salient news events, some have proposed that emotion enhances event recollection (Conway, 1995), whereas others have argued that flashbulb memories are not especially accurate (McCloskey et al., 1988; Neisser and Harsch, 1992). Similarly, in studies involving eyewitness memory, whereas some have claimed that emotion promotes eyewitness performance (Yuille and Cutshall, 1986), others have maintained that emotion impairs eyewitness memory (Loftus and Burns, 1982). While discussion of the role of emotion has been contentious in both the domains of news events and eyewitness memory (for a review, see Schooler and Eich, 2000), in no domain are the paradoxical claims regarding the effects of emotion on memory more evident than in the territory of trauma. The remainder of the chapter will focus on memory for traumatic events.

2.15.2.1 The Memory Wars

How people remember and forget trauma has been among the most polarized, controversial debates in the history of psychology and psychiatry (Loftus, 1997; McNally, 2003). Especially bitter has been the controversy regarding the authenticity of reportedly repressed and recovered memories of childhood sexual abuse (CSA). This controversy has sometimes been dubbed the memory wars (Crews, 1995; Schacter, 1995).

Some scholars believe that the mind protects itself by repressing or dissociating traumatic events from awareness, making it difficult for victims to remember their most horrible experiences until many years later. As Brown et al. (1998: 97) have argued, "when emotional material reaches the point of being traumatic in intensity – something that cannot be replicated in artificial laboratories – in a certain subpopulation of individuals, material that is too intense may not be able to be consciously processed and so may become unconscious and amnesic." Conversely, many psychologists hold that abuse, combat, and other overwhelmingly horrifying events are ostensibly imprinted in memory and are seldom, if ever, truly forgotten (Pope et al., 1999; McNally, 2003; Kihlstrom, 2004). For example, Roediger and Bergman (1998: 1095) remarked that it is "mysterious how painful events, banished to an unconscious state for years through mechanisms of dissociation or repression, could be brought back to consciousness and recollected with great fidelity."

Additionally, skeptics have warned that memories may be susceptible to distortions (Schacter, 1999) and that therapeutic interventions such as hypnosis, dream interpretation, and imagination – intended to recover memories of CSA – may unintentionally foster pseudo-memories of CSA (Loftus, 1993; Lindsay and Read, 1994). Thus, McNally (2005: 815) maintained that "the movement to help survivors recall these allegedly repressed memories resulted in the worst catastrophe to befall the mental health field since the lobotomy era."

2.15.2.2 Remembering and Forgetting Trauma

Since the onset of the memory wars, a multitude of studies have addressed whether traumatic memories can be forgotten. A number of retrospective and prospective studies of CSA have found a nontrivial proportion of victims saying that they at some point in their life had not remembered their abuse. For example, in one of the most widely cited retrospective studies, Briere and Conte (1993: 24) asked 450 patients in treatment for CSA the following question: "During the period of time between when the first forced sexual experience happened and your eighteenth birthday, was there a time when you could not remember the forced sexual experience?" Fifty-nine percent of the patients answered that there had been such a time. Accordingly, Briere and Conte concluded that a substantial number of survivors experience sexual abuse-related repression of their traumatic memories prior to recovering the memories later in life.

However, due to methodological limitations, this study cannot be taken as support for massive repression. First, participants in the study were patients possibly exposed to therapeutic techniques likely to foster memories of abuse (Poole et al., 1995). Also, as in many studies in this domain, it was not established whether the recalled abuse had actually happened. Moreover, the duration of amnesia for trauma was unspecified. Perhaps the most important issue concerns the question that respondents were given. In a way, this question was formulated in an ambiguous way. Thus, McNally and colleagues pointed out that participants were more likely answering a different question: "Was there ever a time that you did not think about having been abused?" (McNally et al., 2004: 131). That is why an affirmative answer to this question does not necessarily provide solid evidence for the type of massive repression put forward by CSA researchers. Instead, such a positive reaction might simply mean that those who experienced CSA can sometimes manage not to think about the abuse. These and other critical points have also been made with regard to similar retrospective studies published in the last 15 years.

A much smaller number of studies have used a prospective methodology to assess whether traumatic events can be forgotten. In an influential study by Williams (1994), 129 women with previously documented histories of CSA were interviewed. Of these, 38% failed to report the index event of abuse for which Williams had a record. Some authors have interpreted these data as showing that massive forgetting of trauma is not only possible, but even very common. However, there are several other, more likely explanations. A majority of the participants, 68%, who had apparently forgotten the index event of abuse did report other abuse events, suggesting that the index event may have merely been less traumatic or less important to them than other

instances of CSA. Given that several women had been abused when they were younger than 5, not remembering the abuse might be the result of childhood amnesia. Moreover, one can argue that the younger the child at the time of the index event, the more likely she is to fail to understand the abuse as sexual at the time. Also, other women may not have wanted to label themselves as abused and/or disclose such personal matters to the interviewer. Thus, a failure to disclose cannot be regarded as evidence of repression (Loftus et al., 1994).

A study by Goodman and colleagues (2003) provides further data on failure to disclose abuse. They assessed 168 persons who had been involved in legal proceedings concerning sexual abuse. These proceedings occurred when the persons were approximately 9 years old. A survey was administered 13 years after the persons had been involved in the legal proceedings. Questions about sexual abuse were inserted in a longer survey concerning legal attitudes and experiences. Results revealed that about 16% failed to report the target incident during a telephone interview conducted approximately 13 years after the events in question. Nondisclosure dropped to 8% after follow-up by a mailed questionnaire and a telephone interview. Moreover, an in-depth analysis by Goodman and Paz-Alonso (2006) yielded a reduced estimate of 4% for the incidence of traumatic amnesia. As these studies indicate, claims of widespread repression and recovery of childhood abuse have been exaggerated. Accordingly, Goodman et al. (2003) concluded that the findings, rather than supporting the existence of special memory mechanisms unique to traumatic events, instead imply that normal cognitive operations underlie long-term memory for CSA.

2.15.2.3 False and Recovered Memories

Another possibility for the impression that one has harbored repressed memories is that a failure to remember traumatic events took place because such events did not actually occur in the first place (Loftus, 1998). On first impression, the idea that someone might remember having experienced a trauma that never took place seems an unlikely account for repression. Yet, people have recollected atrocities that never happened and have been experiencing the emotional pain paralleled with their belief in the authenticity of their memories. Some of the improbable traumatic events for which people claim to have recovered memories in recent years involve satanic ritual abuse (Scott, 2001) and abduction by space aliens (Mack, 1994; Clancy, 2005), memories which are occasionally 'recovered' during psychotherapy. In reviewing the influence of psychotherapy, Lindsay and Read (1994: 304) concluded that "there are good reasons to believe that: (1) some recollections produced by intensive memory recovery may be false; and (2) when such techniques are used it is very difficult to discriminate between clients who are remembering accurately and clients who believe they are remembering accurately but are not."

The fact that a growing number of former patients have retracted their claims of CSA also suggests that false CSA memories can be induced by therapists. Most retractor cases involve adults who had sought psychotherapy for depression or related complaints. During therapy, memories of CSA were recovered. However, later patients come to believe that the 'recovered memories' were only products of therapeutic suggestion (e.g., Ost et al., 2002).

In the 1990s, the experimental research community responded in earnest to these frequent memory reports by patients claiming their experiences had been previously repressed. If these memories were not authentic, where could they have come from? If they were false, how could they develop? With these questions in mind, several lines of research on the development of false beliefs and memories began to flourish (for reviews, see Laney and Loftus, 2005; Smeets et al., 2005). One of the best-known tasks that has been strikingly successful in creating pseudo-memories in the laboratory is the Deese-Roediger-McDermott (DRM) paradigm (Deese, 1959; Roediger and McDermott, 1995). In this task, participants often falsely recall and recognize a nonpresented word or critical lure (such as 'sleep') following presentation of several of its strongest associates ('bed, rest, awake, tired,' and the like).

The DRM paradigm relies on semantic material. Yet, apart from semantic material, research has demonstrated that techniques such as imagination inflation (Garry et al., 1996), dream interpretation (Mazzoni et al., 1999), and suggestions containing incorrect feedback (Crombag et al., 1996; Hyman and Billings, 1998; Jelicic et al., 2006) may create false beliefs and pseudo-memories. Moreover, recent studies have successfully employed doctored photographs (e.g., a youngster riding in a hot-air balloon) to suggest childhood events that never happened to the child or adult participants (Wade et al., 2002). Additionally, studies have shown that experimental

manipulations intended to implant pseudo-memories may have overt behavioral consequences (Bernstein et al., 2005).

2.15.2.4 Recovered Memories in the Laboratory

Remarkably, until recently no studies had been conducted on the cognitive functioning of people in the center of this recovered-memory debate: those who report repressed and recovered CSA memories. This state of affairs could be due to the fact that few clinicians have expertise in laboratory research and few cognitive psychologists have access to trauma populations (McNally et al., 2004). In fact, Richard McNally and Susan Clancy of Harvard University were the first to apply experimental methods to investigate memory functioning in people reporting repressed and recovered memories of CSA. By doing so, their studies have tested hypotheses relevant to mechanisms implicated in the ability to repress and recover traumatic memories, as well as mechanisms relevant to forming pseudo-memories of trauma (McNally, 2003). For example, they examined whether individuals reporting recovered CSA memories are more prone to false memory effects induced in the laboratory (Clancy et al., 2000). In one of their studies, they used the previously described DRM paradigm to show that, relative to individuals with continuous memories and controls with no history of abuse, individuals reporting recovered CSA memories more often falsely recognized the nonpresented critical lures. Subsequently, these findings were extended to trauma-related material (Geraerts et al., 2005). That is, besides neutral DRM lists (e.g., critical lure 'sleep'), trauma-related lists (e.g., critical lure 'assault') were employed. It was found that individuals reporting recovered abuse memories are more prone to falsely recalling and recognizing neutral and trauma-related words that were never presented.

Several researchers have argued that such susceptibility to false memories may be due to a source-monitoring deficit, that is, incorrect judgments about the origin or source of information (Johnson et al., 1993). For example, subjects may think of the nonpresented lure at study, so then at test they must differentiate between memories of internally generated thoughts versus memories of the studied words. Results reported by Clancy et al. (2000) and by Geraerts et al. (2005) suggest that individuals reporting recovered CSA memories may have a source-monitoring deficit for all types of material, whether the content is neutral or trauma-related. It can be speculated that these individuals have a tendency to adopt an internally generated thought as being a genuine memory. This could have important implications, both in terms of the development of false memories *per se* and in terms of the development of mistaken beliefs. Thus it may be that a subsample of those with recovered memories developed false memories via a subtle interaction between intrinsic source-monitoring difficulties and suggestive therapeutic techniques.

2.15.2.5 Underestimation of Prior Remembering

Although the research above suggests that recovered memories are likely to be false memories, Schooler and coworkers (e.g., Schooler et al., 1997; Shobe and Schooler, 2001) described several case studies of individuals who experienced the discovery of apparently long-forgotten memories of abuse, memories for which corroborative information could be found. Interestingly, in two of the cases the partners of the women who reported full-blown recovered-memory experiences said that the women had talked about the abuse before they had the recovered-memory experience. In both cases, the women seemed to be surprised to discover that they had talked about the abuse prior to their recovered-memory experiences. Schooler and colleagues proposed that these cases illustrate a forgot-it-all-along (FIA) phenomenon, which at its core entails the underestimation of prior recollections of past events.

Recent studies have provided elegant laboratory analogs of this FIA phenomenon. For example, a series of experiments by Arnold and Lindsay (2002, 2005) required participants to recall material in qualitatively similar versus different ways on two occasions. They argued that if the retrieval of CSA memories in qualitatively different ways can lead to the underestimation of previous CSA recollections, then this mechanism should transfer into the lab. In the basic procedure, participants studied a list of homographic target words, each accompanied by a biasing context word (e.g., hand: PALM). In Test 1, participants were tested on a subset of the study list, with some of the target items being cued with the studied-context word (e.g., hand: P–M) and the rest of the items cued with another-context word (e.g., tree: P–M). In the final test, participants were tested on all of the studied items, and the studied-

context cues were always given as recall prompts. Additionally, after recalling each word, participants were required to judge whether they had recalled that word on Test 1. The key result was that participants more often forgot their prior recall of the words when they had been cued with the other-context cue than with the studied-context cue on Test 1. Hence, these results provided compelling evidence that remembering a past event in a different way can result in a failure to remember a prior instance of recalling that event.

Recently, the link between the FIA effect and recovered memories has been studied in the laboratory by Geraerts et al. (2006). The issue of interest was whether individuals reporting recovered CSA memories are more prone to underestimating their prior remembering, relative to individuals with continuous CSA memories and controls reporting no history of abuse. Using Arnold and Lindsay's (2002) FIA test, Geraerts et al. (2006) found that participants with recovered CSA memories were found to be more prone to forget that they had previously recalled a studied item when they had been cued to think of it differently on two recall tests. That is, the FIA effect was larger in those who reported recovered memories.

In a related study, Geraerts et al. (2006) asked participants to recall autobiographical events (e.g., being home alone as a child) in an emotionally negative or positive framing across three test sessions over a period of 4 months. Given the cue 'being home alone as a child,' for example, a participant assigned a positive framing for that event might recall enjoying the feeling of freedom of having the house to himself/herself; the same participant assigned a negative framing for that event in session 2 might reminisce about feeling lonely after a while.

In the first session, participants were instructed to recall 25 selected events in either a positive or negative frame. After 2 months, participants were asked to recall 16 of the target events a second time. For half of the trials, the framing cue presented with the events corresponded to the negative/positive framing cue presented with the autobiographical events during the first session, whereas for the remaining trials the framing was the opposite from the framing cue presented in the first session (i.e., positive framing if the framing on the first session had been negative, and vice versa). In session 3, again 2 months later, participants were tested on all the target events, accompanied by the framing cues that were presented with the targets during session 1. Again, individuals reporting recovered CSA memories showed an enhanced FIA effect relative to individuals with continuous abuse memories and controls, even when mildly emotional autobiographical material was used over a period of 4 months, conditions that more closely mirror everyday life (if not memories of trauma). These findings imply that some of the participants' recovered CSA memories may be fundamentally accurate, but that these individuals may have underestimated their prior memories for the abuse.

2.15.2.6 Discovered or False Memories?

The two basic findings discussed above – source monitoring deficits and the FIA effect – suggest radically different interpretations of recovered memories. On the one hand, studies by Clancy et al. (2000) and Geraerts et al. (2005) show that reports of recovered memories are associated with false memory effects as measured by the DRM task. Conversely, the results reported by Geraerts et al. (2006) indicate that recovered memory reports are intimately related to underestimation of prior remembering. However, it seems implausible that one and the same report of a recovered memory could be linked both to false memory effects and to the underestimation of prior remembering. How can these phenomena be integrated? Careful inspection of the precise types of recovered memory experiences may provide an answer to this question.

Two clearly distinguishable types of recovered memory experiences have been documented in the literature (e.g., Shobe and Schooler, 2001). In one type, people come to believe that they are abuse survivors, commonly attributing their current life difficulties to their repressed memories of abuse. Here, abuse events tend to be recalled gradually over time, often by suggestions of a therapist. People usually indicate that they have 'learned' (e.g., through hypnosis) that the abuse occurred to them. In the other type of recovered memory experience, people are suddenly reminded of events they believe they had not thought about for many years. They are shocked and surprised by their recollection, but not by the content of the memory as such. This kind of recollection differs from the one in which the person is gradually recalling the abuse, often in the course of therapy. For this reason, Schooler and coworkers (Schooler et al., 1997; Schooler, 2001) referred to these suddenly recovered memories as discovered memories, reflecting situations "in which

individuals sincerely perceive themselves to have discovered memories of experiences of which they think they had previously been unaware" (Shobe and Schooler, 2001: 100). This term keeps open the possibility that individuals could have discovery experiences corresponding to memories that were not completely forgotten.

Given these two types of recovered-memory experiences, it is not too farfetched to speculate that people who report CSA memories recovered during therapy may score high on tasks yielding false memory effects, like the DRM task. Yet, they may perform similarly to control participants on tasks tapping the FIA effect. Conversely, one would expect that people with spontaneously recovered memories would be especially prone to the FIA effect, whereas they would score similarly to controls on false memory tasks, such as the DRM. Preliminary analyses of the data collected in several studies with individuals reporting recovered CSA memories indicate that this is the case (Geraerts, 2006).

2.15.2.7 Corroborative Evidence of Abuse

Recent research supports the view that CSA memories discovered outside of therapy are more likely to reflect genuine events relative to memories recovered in therapy (Geraerts et al., 2007b). In this study, people with recovered CSA memories responded to an extensive memory questionnaire. Participants were asked to characterize their prior degree of forgetting, the quality of their memory recovery if they had one, the nature and context of the abuse, and the qualities of their current memory. Moreover, information was sought to verify or corroborate the CSA memories. Memories were characterized as corroborated if one or more of the following three criteria were met: (a) another individual reported learning about the abuse soon (i.e., within the next week) after it occurred, (b) another individual reported having also been abused by the alleged perpetrator, or (c) another individual reported having committed the abuse him/herself. The presence of corroborative evidence was evaluated by two raters blind to any additional information associated with each case.

Results revealed that memories recovered unexpectedly, outside of therapy, were significantly more verifiable than memories that were reported to have been gradually recovered within the context of therapy. As indicated in **Table 1**, abuse events recovered during therapy could not be verified, while 37% of the CSA memories discovered outside of therapy were independently corroborated; the latter figure is similar to the 45% verification rate found for continuously accessible memories. These results support the view that memories recovered unexpectedly outside of therapy (i.e., discovered memories) are more likely to correspond to genuine abuse events, relative to memories recovered in therapy.

Table 1 Percentage of memories of childhood sexual abuse that could or could not be corroborated

	Corroboration	
Participant group	*Yes*	*No*
Continuous recollection	45% (32)	55% (39)
Recovered out of therapy	37% (15)	63% (26)
Recovered in therapy	0% (0)	100% (16)

Number of participants per condition is enclosed in parentheses.
Source: Geraerts E, Schooler JW, Merckelbach H, Jelicic M, Hauer BJA, and Ambadar Z (2007b) The reality of recovered memories: Corroborating continuous and discontinuous memories of childhood sexual abuse. *Psychol. Sci.* 18: 564–568; used with permission from Blackwell Publishing.

Moreover, in this study, 85% of participants reporting recovered memories failed to appreciate their abuse as traumatic at the time it occurred, in part due to lack of understanding the nature of the event (for related results, see Clancy and McNally, in press). In fact, many of them rated the abuse as being more traumatic now than it was at the time of the abuse. This was especially the case for participants who suddenly recalled long forgotten and often corroborated episodes of abuse. Several of them were exposed to one or sometimes more episodes of abuse that were nonpenetrative (e.g., fondling). Such events were experienced as confusing or distressing but not essentially frightening. Individuals reporting them might have managed not to think about these experiences, particularly if retrieval cues were absent (e.g., in cases in which the victim or the perpetrator had moved away). Years later, appropriate retrieval cues might be encountered, triggering the recollection of the long-forgotten abuse experiences, which the person now correctly understands to be sexual abuse. This realization often is accompanied by an onrush of emotions which is interpreted as the impact of remembering something for the first time.

Although such cases undoubtedly qualify as recovered/discovered memories of sexual abuse,

they cannot be taken as evidence for amnesia. Contrary to the standard view of repression, people do not forget their abuse in the strict sense of the word, because the abuse was neither perceived as traumatic nor recognized as abuse. No special mechanisms, such as repression or dissociation, have to be put forward to clarify why these misapprehended abuse experiences did not come to mind for many years. Also, no special mechanisms such as repression are needed to explain reports of CSA memories recovered during therapy. Memories recovered during therapy, as well as discovered memories, both render a scenario in which a false impression of previous nonavailability of abuse memories arises, while in fact, no special mechanisms such as dissociation or repression are needed to account for these impressions of repression.

2.15.2.8 Mechanisms of Traumatic Memory

Does traumatic memory involve special mechanisms? According to one popular view known as the trauma-memory argument, memories of traumatic events have special properties that distinguish them from ordinary memories (for a critical discussion, see Kihlstrom, 1996). In this view, traumatic memories are qualitatively different (i.e., processed and stored differently) from other types of memories, thereby involving mechanisms different from those associated with general memory functioning (van der Kolk, 1996). This view asserts that many survivors of a trauma invoke mechanisms such as repression and dissociation, which result in dissociative amnesia for the stressful event itself. Moreover, it is contended that survivors of a trauma suffer from intrusions with strong sensory qualities. This dissociative style of processing would also create a substantial overlap between dissociative and posttraumatic stress disorder symptoms. There are several versions of this theoretical stance (Brewin et al., 1996; Ehlers and Clark, 2000), but the core assumption they have in common is that trauma has a special impact on the way in which memories of the traumatic event are organized (for discussions, see Kihlstrom, 1996; Shobe and Kihlstrom, 1997; Kihlstrom, 2006; for a reply, see Nadel and Jacobs, 1998).

Although the trauma-memory argument has gained popularity among many clinicians, some findings argue against this view. Systematic studies suggest that only a small minority of war victims report dissociative amnesia. For example, Kuch and Cox (1992) studied 124 Holocaust survivors and found that dissociative amnesia, with an estimated lifetime prevalence rate of 3%, was quite rare in this group. Likewise, Merckelbach and colleagues (Merckelbach et al., 2003) found in a group of 29 Dutch concentration camp survivors only one survivor reporting mnemonic experiences that might be taken as evidence for dissociative amnesia. The authors noted that in this case there was a serious possibility that drug abuse contributed to the poor memory of the traumatic episode. Similarly, Geraerts and colleagues (Geraerts et al., 2007a) found that in a sample of Croatian war veterans who had been confronted with extremely aversive events during the Balkan wars, dissociative amnesia was rarely reported. In sum, several recent findings do not support the existence of special memory mechanisms that are unique to traumatic events.

2.15.3 Integrating Memory in and about Affect

In the preceding pages, we have treated memory in and about affect as distinct topics. Such a treatment was possible because research on mood-congruent cognition has had relatively little overlap with research on memory for traumatic events. Nevertheless, consideration of these topics together invites exploration of the possible empirical and theoretical issues that might unite them. To this end, we close with a discussion of potential ways in which the principles and findings of mood congruence might apply to understanding the processes leading to reports of recovered memories of trauma.

2.15.3.1 Connections between Mood Congruence and Traumatic Memory

According to the AIM, the motivational and resource demands of the situation determine which of two distinct processes – affect priming or AAI – mediate mood-congruent effects. Given the variable conditions under which individuals can think about traumatic experiences, it seems likely that each of these processes might influence recovered memory reports under different circumstances.

2.15.3.1.1 Affect priming

When individuals are highly motivated, have sufficient resources, and are elaborating on self-relevant information, they are likely to experience affective

infusion, whereby the information that is generated/attended to is shaped in accordance with the present mood, presumably through a process of affect priming. When individuals are in therapy they are talking about issues of immense self-relevance. They experience powerful emotions. They are highly motivated to think through their experiences. And with the therapists' support, they are likely to have adequate resources to engage in elaborative systematic processing. Thus therapy potentially provides an extremely fertile ground for the priming of affect-related memories, thoughts, perceptions, and other cognitive constructions. From this perspective, it seems possible that affect priming, when combined with a therapist's suggestions, could spawn false memories. For example, if therapy invokes emotions of betrayal and trauma, then affective infusion might facilitate the adoption of suggested memories that are consistent with those emotions.

Admittedly, negative emotional states can minimize susceptibility to suggestion, whereas the above characterization proposes that therapy-induced negative emotions might enhance suggestibility. Importantly, however, the reduced susceptibility to misinformation reviewed in this chapter involved minor details of little self-relevance, and not in accord with the induced negative affect participants were experiencing. In contrast, therapy-suggested experiences of abuse would be highly self-relevant and likely in accord with the emotional state that the patient is experiencing at the time. Thus, in the context of therapy, the capacity for affect priming to generate affectively matched cognitions may outweigh the capacity for negative emotions to reduce suggestibility, thereby leading to a net increase in false memories.

Though speculative, the suggestion that affect priming could be a source of therapy-induced false memories might be empirically explored by examining whether the match between an affective state and a memory suggestion affects the generation of false memories. For example, in the imagination inflation paradigm (Garry et al., 1996), imagining events, such as putting one's hand through a window or finding a 10-dollar bill, increases the perceived likelihood that these events occurred. If affect priming enhances false memories in therapy, then it seems likely that imagination inflation might similarly be associated with affect-infusion (or mood-congruent) effects. Accordingly, participants may be more likely to believe they had once found a 10-dollar bill if they imagine this in a good mood, or more likely to believe they put their hand through a window, if they imagine this in a bad mood. Such a findings would suggest that affect priming could be an even greater source of false memories in the substantially more emotional and self-relevant context of therapy.

2.15.3.1.2 Affect-as-information

In the secure atmosphere of therapy, individuals are likely to have the resources to think about traumatic experiences using elaborative systematic processing. However, when memories of abuse arise unbidden and out of therapy, the emotional onrush can be overwhelming. Individuals reporting memory discoveries outside of therapy describe their experience with terms such as stunned, chaos in my emotions, overwhelmed, and like a ton of bricks just hit me (Schooler, 2001).

According to the AIM, in a situation in which cognitive resources are overwhelmed by emotion, it is likely that AAI processes would take place. In keeping with this view, Schooler (2001) speculated that AAI may lead individuals to infer, based on their profound affective experience of discovery, that they must be remembering the abuse for the first time. According to this discovery misattribution account, individuals confuse the emotion associated with discovering a new interpretation of the experience with that of discovering the memory itself.

Several strands of evidence support a discovery misattribution account whereby individuals use the affect associated with discovering a new understanding of their experience to falsely infer that they have discovered a forgotten memory. First, both of the original cases of misconstrued forgetting involved individuals who reported experiencing an overwhelming onrush of emotion after reinterpreting their abuse experiences (Schooler, 2001). For example, one case involved a woman who reported having been raped while hitchhiking. In her recounting of her memory, she reported that originally she had thought of the experience as a sexual experience gone awry, indicating that she had "made such a mess of it . . . by resisting what I thought was supposed to be a sexual experience" (Schooler, 2001: 120). However, following the onrush of emotions associated with her memory discovery, she reported thinking "my God . . . I had been raped! . . . that's a crime! I was 16, just a kid" (Schooler, 2001: 121). Similarly, in the large-scale corroborative effort by Geraerts et al. (2006), change in interpretation was

one of the best predictors of memories being characterized as previously forgotten.

Laboratory research also provides evidence for discovery misattribution. For example, the experience of discovering the solution to an anagram can be confused with the experience of remembering having seen the word corresponding to the anagram's solution (S. Dougal and J. W. Schooler, unpublished observations). Together these strands of evidence suggest that the reduced resources associated with the emotional onrush of realizing that one was the victim of abuse, could enable an AAI process whereby individuals misattribute the emotion of discovering a new understanding of the event to that of discovering the memory itself.

2.15.3.2 Final Thought

Memory research has come a long way since the time that it shunned emotion. Our review of the role of emotion in and about memory reveals that there is much that simply could not have been known about memory, were memory researchers to have remained limited to the random-word-list paradigms that were the bread-and-butter of memory experiments for so many years. Not only did such paradigms lack the emotional manipulations that have proven to be so informative, but by ignoring elaboration and self-relevance, these procedures were inherently insensitive to many of the consequences of affect. Moreover, understanding memory for emotional events necessarily requires researchers to leave the confines of their laboratories and explore the far more complex situations in which traumatic memories actually take place.

Nevertheless, consideration of the relations between traumatic-memory reports and performance on basic word-list paradigms has yielded critical insights into the processes underlying the formation of recovered-memory reports. Thus, while memory in and about affect illustrates just how far memory research has come, its also illuminates the value of remembering its roots.

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2.16 Retrieval Processes in Memory

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2.16.1 Retrieval Processes in Memory

A dominant framework during the past four decades has postulated three critical components to understanding memory – encoding, storage, and retrieval. But the relative importance that researchers have attributed to each of these components has varied over the course of history. In his 2000 chapter for the Tallinn Conference, Roediger traced this history by taking as his departure point Endel Tulving's remark, "the key process in memory is retrieval." Roediger noted that, despite the fact that the role of retrieval had been emphasized since the writings of Wolfgang Kohler (1947) and Richard Semon (1921; see Schacter, 1982, 2001; Schacter et al., 1978), the belief that encoding and storage processes are the key components of memory has persisted through recent history. Roediger then elaborated both the logical and empirical arguments to demonstrate why retrieval is the key process for understanding human memory (Roediger and Guynn, 1996; Roediger, 2000). The purpose of the current chapter is to reinforce and expand upon this argument.

It is reasonable to assume that without encoding and storage of information there can be no retrieval. But the converse is truer – without retrieval there is no evidence that either encoding or storage ever occurred. Furthermore, retrieval can occur even in the absence of encoding, as in the case of false memories. Retrieval processes thus provide a measure of not only what was encoded and stored but also of what constitutes memory from the perspective of the rememberer – regardless of the reality of that memory. In essence, retrieval then is the measure of memory.

In emphasizing the importance of retrieval processes, Tulving and Pearlstone (1966) proposed a critical distinction between the availability and the accessibility of information. Importantly, they proposed that what is available in memory cannot be known unless that information is accessed. If accessibility is the key to determining the availability of memory, then how can we determine what is accessible? The answer to this question depends on our understanding of the retrieval conditions that can successfully detect encoding and storage. In other words, our measure of what is available in memory is contingent on being able to arrange retrieval conditions that can elicit available memories. It follows then that an understanding of retrieval processes is crucial for understanding the nature of memory.

In this chapter, we have organized the discussion of retrieval processes into six sections: (1) task

differences – the role of retrieval cues, (2) encoding and retrieval interactions, (3) retrieval mode, (4) repeated retrieval, (5) retrieval in a social context, and (6) retrieval errors and other retrieval effects. Many of these sections are the very topics of some of the other chapters in this volume and are covered comprehensively in those chapters. We bring together these topics here to evaluate their significance specifically in the context of retrieval and to determine how these phenomena and processes reveal something about the nature of retrieval process *per se*.

2.16.2 Task Differences – The Role of Retrieval Cues

Numerous studies have now amply demonstrated the critical role retrieval cues play in revealing availability of memory. These studies were inspired by a wide variety of theoretical frameworks, and as such they can be organized under a number of different subtopics. Regardless of the theoretical perspectives that inspired these studies – and we will discuss some of these perspectives in the course of this chapter – we include them here because they also underscore the importance of retrieval cues.

Some retrieval cues are internal, as in the case of the most quintessential of all memory tasks, free recall. In this task, participants are given no cues and are asked to write down all the studied information that they were presented earlier. As such, participants rely on their own internal resources – strategies, organization, and cues – to report studied information while performing the free recall task. Retrieval cues can also be external, and in this case the variety of retrieval tasks designed with different cues can span a wide realm, depending on the experimenter's theoretical needs. The most common of such tasks are cued recall and recognition, and we will discuss some findings that show the efficacy of these tasks in improving accessibility to learned information.

In their seminal study, Tulving and Pearlstone (1966) reported the extent to which accessibility can differ just between free-recall (where the cues are internal) and cued-recall (where the cues are external) tasks. In this experiment, participants studied categorized word lists, which consisted of a category name followed by a list of words (of varying length – 12, 24, or 48 words per list) that represented instances of that category. Later the participants were given either a free-recall test or a cued-recall test, the difference between these two tests being that the cued-recall test provided participants with the category names of each word list. Results indicated that participants were able to recall many more words under cued-recall conditions than under free-recall conditions. Furthermore, the benefit of cued recall over free recall increased as the number of words on the to-be-remembered list increased. These results have been interpreted as evidence that not all information that is available is also accessible. Accessibility depends upon the type of cues provided at test.

This demonstration can be expanded by adding tasks that provide even more external cues than those used in the category/cued-recall task. Under such conditions, we would expect memory output to increase as the information provided by the retrieval cues increases – as long as the encoding conditions across these memory tasks remain the same. This scenario can be found in another landmark paper by Tulving (1985), where he introduced the remember–know paradigm. We will discuss this paradigm in a later section on 'Retrieval mode,' but for now, we focus on the inclusion of a third task in Tulving's study. Subjects first studied category names and exemplars (musical instrument–viola) and later completed three successive memory tasks – free recall, category-cued recall (where the category name served as the retrieval cue, e.g., musical instrument–_____), and category plus letter recall (where, in addition to the category name, the first letter of the exemplar was also presented as the retrieval cue, e.g., musical instrument–v_____). As the retrieval cues increased, so did the memory output. A recent study in our lab (Hamilton and Rajaram, 2003) replicated and extended this pattern by adding a fourth memory task to the mix – the recognition-memory task. In this task, the test cues completely recapitulate the study cues and by so doing provide maximal assistance for retrieval. We changed the design further by conducting these tests in a between-subjects design such that successive retrieval was not required and the efficacy of different cues could be assessed without contamination from the other retrieval tasks. Our aim in expanding and changing the design concerned issues of memory experience that we do not discuss here. Instead, we focus here on the overall memory performance across the four tasks. With our design, we replicated and expanded on Tulving's (1985) results such that memory output increased as retrieval cues increased,

with the highest level of performance occurring in the recognition task (mean proportions of total items correctly retrieved: free recall = .21; category-cued recall = .40; category plus letter-cued recall = .56; recognition = .87 (Hamilton and Rajaram, 2003, Experiment 2), collapsed across the levels of processing manipulation discussed in the next section). Our results further illustrate how retrieval cues can change accessibility. We will return to role of retrieval cues in a later section on retrieval modes.

To summarize, as the number of cues available at retrieval increases, so does the memory output. This fact is important in distinguishing between memories that are available but simply inaccessible. Information that cannot be recalled during a free-recall task may be available, but currently inaccessible. That same information may be recalled during a recognition task.

2.16.3 Encoding and Retrieval Interactions

Studies discussed in the previous section show how increased retrieval cues can improve memory performance. This conclusion rests on the assumption that, when retrieval cues are varied, the encoding conditions are held constant. This assumption is the converse of a popular approach where encoding conditions are varied while the retrieval task is held constant. One of the most robust and enduring examples of the latter approach is the levels-of-processing paradigm. In their classic paper, Craik and Lockhart (1972) presented the levels-of-processing framework in which information encoded for its meaning is predicted to be more memorable than information processed at more 'shallow' levels such as focusing on the sound of the word or the letter patterns. This pattern of performance has now been replicated hundreds of times and is routinely observed in the standard memory tasks (Lockhart and Craik, 1990). For example, in the second experiment of our study described in the previous section (Hamilton and Rajaram, 2003), we observed the levels-of-processing effect within each of the four retrieval tasks – free recall, category-cued recall, category-plus-letter recall, and recognition. That is, within each task the level of memory output was consistently higher for items encoded for meaning than for items encoded at a shallow level. Even so, as we noted before, memory output increased as retrieval cues increased, and this pattern was true both for items that were encoded at a deep level and for items encoded at a shallow level.

Yet task differences can change memory accessibility in another way – different retrieval cues can interact differently with the encoded information when the encoding is also varied. Such encoding–retrieval interactions change memory performance in specific ways in contrast to the general effects illustrated in the empirical example above. The idea of encoding–retrieval interactions is embodied in the encoding specificity principle (Tulving and Osler, 1968; Tulving and Thomson, 1973; Tulving, 1974) and the transfer-appropriate processing framework (Morris et al., 1977; Roediger et al., 1989) – two theoretical approaches that inspired extensive research and have unraveled yet another layer of mystery about memory functions.

2.16.3.1 The Encoding Specificity Principle

Studies inspired by the encoding specificity principle have shown findings that seem counterintuitive in the context of the memory effects we discussed in the previous section. It turns out that, contrary to general expectations, an increase in retrieval cues or the provision of strong retrieval cues does not always produce the best memory performance. This is because strong cues are not always the best match for the study material. In a now classic study, Thomson and Tulving (1970) reported the highly counterintuitive finding that even the absence of retrieval cues can sometimes produce better memory than the presence of retrieval cues. Furthermore, strong retrieval cues can be sometimes *less* effective than weak retrieval cues. The design of this study went like this. During encoding, participants studied a list of words that were presented either alone (e.g., BLACK), with a weak associate (e.g., train – BLACK), or with a strong associate (e.g., white – BLACK). Later participants were given one of three types of recall tests – a free-recall test, a cued-recall test with weak associates serving as the recall cues, or a cued-recall test with strong associates serving as the recall cues. Results of this study indicated that it was the degree of match between cues at study and test (rather than the strength of preexisting associations between the cue and test word) that determined recall (**Table 1**). Strong associates aided recall if they were also given at encoding. However, if weak associates were given at encoding, strong associates as test cues hurt recall. In other words, a retrieval cue is effective if and only if it reinstates the original encoding (see also Tulving and Thomson, 1973).

Table 1 Mean number of words recalled across various encoding and retrieval conditions

	Retrieval condition		
Encoding condition	*Free recall*	*Cued recall with weak associate*	*Cued recall with strong associate*
Word only	14.1	11.1	19.0
Word with weak associate	10.7	15.7	13.9
Word with strong associate	12.2	9.2	20.2

Adapted from Experiment 1 in Thomson DM and Tulving E (1970) Associative encoding and retrieval: Weak and strong cues. *J. Exp. Psychol.* 86(2): 255–262.

As Tulving (1983) pointed out, the locus of the memory effect then is neither at encoding nor at retrieval *per se* but in the interaction between the two. It is reasonable to wonder at this point whether such an interaction undermines the case that retrieval is the key process for understanding memory. To the contrary, such findings underscore the importance of arranging the retrieval conditions that maximally exploit the features of the encoding conditions. This requirement becomes increasingly important as researchers explore the effects of increasingly complex variables – both at encoding and at retrieval – such as place and the internal state of the individual. We review a selection of studies here to illustrate this point and refer the reader to a comprehensive review by Roediger and Guynn (1996) on this subject.

A number of these studies have used the encoding–retrieval paradigm that Tulving (1983) proposed, where both encoding and retrieval conditions are experimentally manipulated. In its most basic form, it involves an encoding experiment with two (or more) encoding conditions (e.g., A and B) and a retrieval experiment with two (or more) retrieval conditions (e.g., X and Y) being conducted simultaneously. By examining only how A differs from B we are able to determine the influence of encoding. Similarly, by examining only how X differs from Y we are able to determine the influence of retrieval. By manipulating both encoding and retrieval conditions simultaneously we are able to examine the interaction between encoding and retrieval conditions. For example, in the study by Thomson and Tulving (1970) just described, encoding conditions were manipulated such that the words were studied under one of three circumstances (word alone, word paired with a weak associate, or word with a strong associate). Similarly, retrieval conditions were also manipulated. Participants were given either a free-recall test, a cued-recall test with the weak associates as cues, or a cued-recall test with the strong associates as cues. It is only by examining the interaction between encoding and retrieval conditions that Thomson and Tulving (1970) were able to observe support for the encoding specificity principle.

2.16.3.1.1 Place-dependent memory

The introduction of the encoding–retrieval paradigm inspired several studies that focused on two sets of variables – place and internal state – to test the encoding specificity principle. These variables carry wide appeal because they are complex and close to real life. In a frequently cited study, Godden and Baddeley (1975) examined the effects of matching or mismatching the place – including the environment – on memory in a rather interesting way. In their study, subjects studied a list of 36 words either on dry land or under water (**Table 2**). These encoding conditions were later crossed with two retrieval conditions in a 2×2 factorial design such that subjects performed a free recall task either in the same place/environment as the encoding condition (dry land–dry land or underwater–underwater) or in a different place/environment (dry land–underwater or underwater–dry land). The findings revealed what is known as place-dependent memory and were consistent with the encoding specificity principle; recall was best when the place/environment matched across study and test regardless of whether the place was on land or underwater.

Researchers have also examined place-dependent memory using more common places such as classrooms

Table 2 Mean number of words recalled in Expt. 1 as a function of learning and recall environment

	Recall environment	
Learning environment	*Dry*	*Wet*
Dry	13.5	8.6
Wet	8.4	11.4

Adapted from Experiment 1 in Godden DR and Baddeley AD (1975) Context-dependent memory in two natural environments: On land and underwater. *Br. J. Psychol.* 66(3): 325–331.

(Smith et al., 1978). In a series of experiments, Smith et al. showed that the environmental context effects (manipulated in their study by changing or keeping constant the classroom in which study and test took place) occur reliably. Furthermore, these context effects emerged even if the subjects did not perform the retrieval task in the same room as long as they imagined being in the same room while taking the test. This finding is intriguing because it suggests a strong role of the internal resources in mediating effects related to the external environment.

This implication – that internal resources play an important role in encoding–retrieval interactions – is consistent with the observations that the effects of the encoding–retrieval match are often task dependent. It turns out that place- and context-match effects occur more reliably in free recall but rarely in recognition (see Smith, 1988). This pattern makes sense if internal resources are critical for the encoding–retrieval interactions to occur, because free recall requires internal generation of context, associations, and thoughts, whereas recognition is driven at least in part by the external cues provided to the subject. A number of studies have since reinforced the importance of internal resources in mediating the place-dependent memory effects (Eich, 1985; Fernandez and Glenberg, 1985; McDaniel et al., 1989). As we will see shortly, task selection at retrieval seems to play a significant role in mood-dependent memory as well. Once again, this pattern points to the role of internal origins in mediating the encoding–retrieval interaction effects.

2.16.3.1.2 State-dependent memory

The impact of two interrelated factors we have just discussed – type of retrieval tasks and the involvement of internal resources – has also emerged in two other domains of encoding–retrieval interactions, both of which can be subsumed under the construct of internal states. One concerns state-dependent memory and the other concerns mood-dependent memory. The effects of state-dependent memory have been reported in studies that involved the administration of drugs such as alcohol (e.g., Lowe, 1982) or marijuana. For example, in a study that administered marijuana (e.g., Eich et al., 1975; Eich, 1980), participants encoded information either in a drug state (20 minutes after smoking a marijuana cigarette) or in a sober state (20 minutes after smoking a cigarette that only tasted like a marijuana cigarette). Later, there were four possible recall conditions such that type of test (either free-recall or a category-name cued-recall test) and physiological state (either same as encoding or different from encoding) were crossed with one another. The results indicated that a change of pharmacological state from encoding to retrieval impaired performance on a free-recall test but not on a cued-recall test. Further, even with free recall, it is important to note that drug states, even when matched across study and test, are not the best for improving memory because the best recall was observed when information was both encoded and retrieved in a sober state. Returning to the comparison between free- and cued-recall tasks, the general conclusion of these results was that internal state can sometimes serve as a memory cue (as is the case with the free-recall results of this experiment). However, when there are more effective external cues (such as category names) people do not use the less-effective internal cues (as in the case with the cued-recall results of this experiment).

2.16.3.1.3 Mood-dependent memory

We now turn to mood-dependent effects on memory. These effects are especially intriguing because people have an intuitive sense that memory must be sensitive to how we feel when we learn and when we retrieve the learned information. As we discussed earlier, internal context seems to be quite important for understanding encoding–retrieval interactions, and mood certainly provides a prototypical example of internal context (see Eich, 1985). Yet it turns out that findings in this area of research reveal a complex relationship between mood and memory.

Mood is usually manipulated in studies by using hypnotic suggestion, happy/sad music, or comic/sad video clips, and rating scales are often used to measure the attainment of mood. Early studies reported promising results in that mood match across study and test produced better memory performance. For instance, one study reported such effects in endogenously occurring mood states where psychiatric subjects reproduced more free associations if their mood (manic or normal) matched across the first and second attempts than if it mismatched (Weingartner et al., 1977). In another study, Bower et al. (1978) manipulated happy or sad mood through hypnotic suggestion and observed substantially higher recall of common words had they been studied and tested in the same mood than in different moods. But the empirical story got murky thereafter and led researchers to question mood-dependent

memory effects (see Blaney, 1986). In fact, in a later study, Bower and Mayer (1989) failed to replicate the mood-dependent memory effect, and similar failures to replicate started to accumulate in the literature (see Eich, 1995b, for a review).

Two phenomena subsequently clarified when the mood-dependent memory effect is likely to occur, and both of these phenomena are consistent with the notion that internal context (or internal state) is important for observing the expected encoding–retrieval interaction in mood studies. In one study, Eich and Metcalfe (1989) asked subjects to either read the to-be-recalled targets (cold) or generate them from semantically related cues (hot-???). Subjects performed this task in either pleasant or unpleasant moods induced through different types of music. Subjects were later induced to experience the same mood or a different mood before recalling the word pairs under conditions of free recall. Mood-dependent effects appeared only for items that were generated during study and not for items that were simply read (**Table 3**). The authors replicated these findings in other experiments within the series, and this effect has since been replicated by others as well (Beck and McBee, 1995).

Taking a different approach, Eich et al. (1994) investigated this question by asking subjects to generate events from their own lives. The experimenters manipulated mood by inducing either a pleasant or an unpleasant mood while subjects performed this task. Later, subjects were asked to recall the gist of events they had generated earlier while experiencing either the same mood as before or a different mood. Two interesting effects emerged: (1) in the first session, subjects generated events that were consistent with their mood (either pleasant or unpleasant), producing a mood congruency effect (Blaney, 1986); (2) in the second session, subjects were better at recalling those events that matched their mood at retrieval than they were at those that mismatched, thereby producing a mood-dependent memory effect. These studies emphasize the special role of internal states in producing mood effects in line with Eich and Metcalfe's arguments that mood-dependency effects only occur for self-generated activities (i.e., for internally generated thoughts) and not for externally produced events. Furthermore, the role of internal resources becomes even more important when we consider the nature of the retrieval task that effectively produces these effects. As with place-dependent memory, mood-dependent memory effects are also observed more reliably in free recall than in recognition (Eich and Metcalfe, 1989). At a broader level, this cluster of findings from manipulations of place, state, and mood lends further support to the theme that internal resources play an important role in mediating encoding–retrieval interactions.

Table 3 Probability of recall as a function of item type and encoding/retrieval condition

	Test condition			
	Read words		*Generated words*	
Encoding condition	*Happy*	*Sad*	*Happy*	*Sad*
Happy	.09	.04	.32	.17
Sad	.05	.07	.17	.27

Adapted from Experiment 1 in Eich E and Metcalfe J (1989) Mood dependent memory for internal versus external events. *J. Exp. Psychol. Learn. Mem. Cogn.* 15(3): 443–455.

The place-, state-, and mood-dependent memory effects reviewed so far show the complexities involved in studying variables that are multidimensional. Their complexities pose a challenge to researchers in being able to reinstate the exact conditions across study and test. In fact, some of these variables can sometimes be confounded with each other such that one variable (e.g., mood) can mediate the effects of another variable (e.g., state) and further complicate our understanding of encoding–retrieval interactions. For example, Eich (1995a) has argued that place-dependent memory is actually just a special case of mood-dependent memory. In an experiment examining this hypothesis (Eich, 1995b, Experiment 3), participants generated autobiographical events in a pleasant environment and in a pleasant mood. Later, they were asked to recall this information in one of four distinct conditions defined by the 2×2 factorial combination of (1) same versus different place and (2) same versus different mood. In this study, it made no difference whether participants were tested in the same versus a different place. However, there was a significant difference when participants were tested in the same mood (55% recall) versus a different mood (45% recall). Based upon these results, it is possible to conclude that how well information transfers from one place to another depends not on how similar the two locations look, but rather on how similar the two locations feel. A similar argument has been set forth regarding state-dependent memory effects. The drugs that most reliably produce state-dependent retrieval effects (such as alcohol and amphetamines) are

accompanied by large mood changes. This led Bower (1981) to conclude that state-dependent effects are achieved as a result of the confounded mood-dependent effects.

In brief, thus far in this section, we have reviewed classic and representative studies that show how encoding and retrieval interactions reveal effects of specificity in memory. Together, studies on place-dependent, state-dependent, and mood-dependent memory also show the importance of the types of cue and the task selection at retrieval for detecting these patterns of specificity.

2.16.3.2 The Transfer-Appropriate Processing Framework

The encoding specificity principle proposes that memories are associated with particular cues, and recall is predicted to be enhanced if the cues at retrieval are the same as those that were encoded in the memory traces formed at encoding. We now turn to a discussion of another influential approach – known as the transfer-appropriate processing framework (Roediger et al., 1989; Roediger, 1990) – that is similar to the encoding specificity principle but emphasizes the processes and procedures of mind (Kolers and Roediger, 1984) rather than its structural contents to explain the interactions between encoding and retrieval. In this processing approach, recall is predicted to be enhanced when the processing at retrieval is the same type of processing as encoding. For example, this approach predicts superior memory for 'shallow' encoding of items if the retrieval task capitalizes on the processing of shallow aspects of the study material (Morris et al., 1977; Roediger et al., 1989). This prediction is at odds with the classic and robust demonstrations of the levels of processing effect we discussed earlier, where information processed for meaning is retrieved better than information processed for its shallow aspects such as phonemic details (Craik and Lockhart, 1972; Craik and Tulving, 1975). But the two phenomena can be reconciled if we take into account the critical role retrieval processes play in tapping the encoded information. In their study, Morris et al. (1977) factorially varied the type of encoding task with the type of retrieval task. In particular, during encoding, participants were asked to determine either whether a given word fit into a sentence (a deep semantic encoding task) or whether it rhymed with another word (a shallow phonetic encoding task). Later, participants were either given a standard recognition task or a recognition task involving rhymes (i.e., "does this word rhyme with a previously seen word?"). When the encoding rhyme questions required 'yes' judgments ("does dog rhyme with hog"?), a very interesting pattern of results emerged on the later memory tasks; while semantic acquisition was superior to rhyme acquisition when tested using a standard recognition task, the converse was true when tested using the rhyme recognition task. Based upon these results, Morris and colleagues concluded that shallow encoding is not necessarily inferior to deep encoding and that the effectiveness of any given encoding task depends upon the relationship between the encoding task and retrieval task.

In the late 1980s and early 1990s, Roddy Roediger and his colleagues (Roediger et al., 1989; Roediger, 1990) published a comprehensive framework for testing the principles of transfer-appropriate processing. This framework inspired an empirical revolution that sought to specify the nature of encoding and retrieval processes and the ways in which the selection of these processes can impair or maximize memory performance. A full description of this framework, its tenets, and the major findings are beyond the scope of this chapter, but we recommend several comprehensive reviews to the reader on the theoretical and empirical developments in this area of research (Roediger et al., 1989, 1990; Roediger, 1990; Roediger and McDermott, 1993). In brief, this framework states that memory performance improves to the extent that cognitive processes engaged during test match the processes that were engaged during study. Consistent with this main tenet, extensive research has now shown that subtleties in the match or mismatch of processes during encoding and retrieval can produce large effects on memory performance. As a result, it is critical to select retrieval tasks that closely match the encoding task in their processing requirements. We will discuss some empirical illustrations of this key conclusion in the next section, where we describe the impact of retrieval mode on memory performance.

To briefly summarize the arguments thus far, the encoding specificity principle postulates that memory is enhanced when the cues at recall match the cues at encoding (as is the case in place-, state-, and mood-dependent memory). In a similar vein, the transfer-appropriate processing approach postulates that memory is enhanced when the processes engaged during recall match the processes engaged during encoding. Together, these studies demonstrate the importance of

arranging the retrieval conditions so that we can optimize accessibility to learned information.

2.16.4 Retrieval Mode

In his chapter for the Tallinn conference, Roediger (2000) discussed the concept of retrieval mode and its power to reveal aspects of memory that might otherwise remain concealed. We expand upon this notion in this section. We will consider the significance of retrieval mode from two perspectives that Roediger noted: (1) explicit and implicit modes of retrieval and (2) remembering and knowing information from the past. Each perspective has brought into focus many important questions concerning retrieval and has led to substantial empirical and theoretical developments on these issues. We refer the reader to two directly relevant chapters in this volume that tackle each of these topics in depth – one by D. L. Schacter on implicit memory (*See* Chapter 2.33) and another by J. M. Gardiner on remembering and knowing (*See* Chapter 2.17). In this section, we discuss a few examples to demonstrate how a change in the retrieval mode can bring about changes in accessibility to studied information.

2.16.4.1 Explicit versus Implicit Memory

The relevance of task differences and encoding–retrieval interactions in improving accessibility comes together in rather dramatic ways when we consider the explicit versus implicit modes of retrieval in which people engage while doing memory tasks. In their seminal papers, Warrington and Weiskrantz (1968, 1970) reported findings that nicely illustrate how retrieval mode can affect the memory product. In these studies, amnesic subjects performed poorly, as would be expected, when asked to think back on the study episode and recall what was studied (as in free recall). But these subjects exhibited, rather surprisingly, normal memory performance when they were asked to complete a memory task with the first response in the manner of problem solving (for example, complete the physically impoverished cues in a word fragment completion task with the first response that comes to mind). Graf and Schacter (1985) introduced the distinction between explicit and implicit memory to respectively capture this difference between a mode of retrieval where people think back on the study episode (all the memory tasks discussed in the other sections of this chapter) and a mode where no reference is made to study episode during test performance.

These differences in the retrieval task instructions can change memory performance of not only individuals who have amnesia but also individuals who possess intact memory functions (see Schacter, 1987, 1990; Roediger et al., 1989, 1990; Roediger, 1990; Roediger and McDermott, 1993, for representative reviews.) We will discuss some findings observed in individuals with intact memory to elaborate this point. In the previous section, we discussed the classic levels of processing effect on tasks such as free recall and recognition (Craik and Tulving, 1975) and the reversal of this effect when the recognition task provided phonemic cues as opposed to the standard cues (Morris et al., 1977). Interestingly, the conceptual advantage of levels of processing disappears if test conditions require implicit retrieval in response to perceptual test cues. In other words, on tasks such as word identification (reading words presented rapidly at threshold durations), word fragment completion (presenting test cues with some letters missing, e.g., _ t r _ _ b _ r _ _), or word stem completion (str_____), completing the cues with the first solution that comes to mind confers little advantage for words studied for meaning compared to words studied for their perceptual features (e.g., Jacoby and Dallas, 1981; Graf and Mandler, 1984; Roediger et al., 1992). Thus, the levels of processing effect can vary as a result of the tasks used; it occurs on free recall and recognition (Craik and Tulving, 1975), reverses on a phonemic cued-recall task (Morris et al., 1977), and disappears on implicit tasks that rely on perceptual processes for completion. These findings once again highlight the importance of retrieval cues in accessing learned information.

The disappearance of study differences on implicit tasks can change the way we theorize about the significance of encoding (or storage) versus retrieval. For instance, the advantage in free recall for concrete words (such as table, bus, strawberry – words that can be imaged or represented as objects) over abstract words (such as pledge, destiny, care) has been attributed to dual storage of concrete words in verbal and image codes compared to single representations of abstract words (only verbal code) (Paivio et al., 1968; Paivio, 1969). Yet, in a study from our lab (Hamilton and Rajaram, 2001) we found that on implicit tests such as word fragment completion (_ t r _ _ b _ r _ _) and implicit general knowledge test (what fruit wears its seeds on its skin?), there was an equivalent

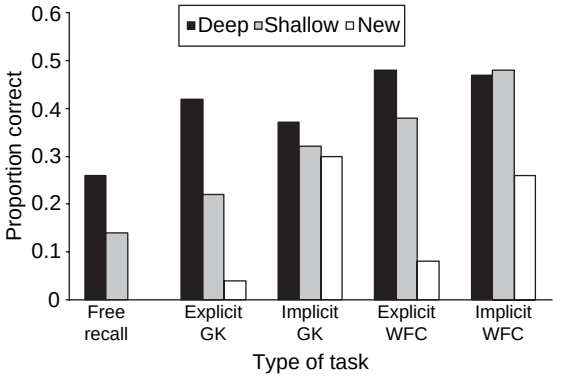

Figure 1 Mean proportion of correct responses for each item type for each of the five tasks used (GK, general knowledge task; WFC, word fragment completion task). Adapted from Experiment 2 in Hamilton M and Rajaram S (2001) The concreteness effect in implicit and explicit memory tests. *J. Mem. Lang.* 44(1): 96–117.

advantage for producing studied concrete words and studied abstract words over their nonstudied counterparts (**Figure 1**). In other words, on an implicit memory task there was no memorial benefit of concrete words over abstract words. Therefore, the concrete/abstract distinction in memory cannot be discussed only in the context of differential encoding or storage. This distinction demands a more complex explanation because this effect is not ubiquitous – it can be specific to a particular mode of retrieval.

A converse pattern can also emerge by changing the retrieval mode such that some study differences do not affect explicit memory but produce changes in implicit memory. For example, changing the modality of presentation at study – presenting words either in the auditory or the visual modality – does not change the level of free recall (Blaxton, 1989; Srinivas and Roediger, 1990; Rajaram and Roediger, 1993), and this null finding suggests that modality of presentation at study does not matter. However, this conclusion is only partly correct as we (Rajaram and Roediger, 1993) found in our study with four different implicit tasks involving perceptual cues (see **Figure 2**). When subjects were presented with impoverished cues such as word fragments, word stems, rapidly presented words in the word identification task, or anagrams (brtaserwyr) to solve in the anagram solution task (strawberry) and were asked to perform these tasks with the first solution that comes to mind, performance improved on these implicit tests if the study and test materials were presented in the same modality compared to different modalities (see also Jacoby and Dallas, 1981; Kirsner et al., 1983; Graf and Mandler, 1984; Roediger and Blaxton, 1987; Blaxton, 1989; Srinivas and Roediger, 1990; Weldon, 1991). In other words, the impact of a study variable is sometimes detectable only when subjects used the implicit retrieval mode. (As an aside, but consistent with the general argument about the impact of retrieval cues in modulating memory performance, we also found that studied pictures produced the worst performance on these implicit tasks that provided word-based cues. This, of course, is contrary to the pattern that is typically observed in free recall and recognition, where memory for pictures is better than that for words (Paivio et al., 1968; Madigan, 1983; Weldon and Roediger, 1987; Rajaram, 1993).)

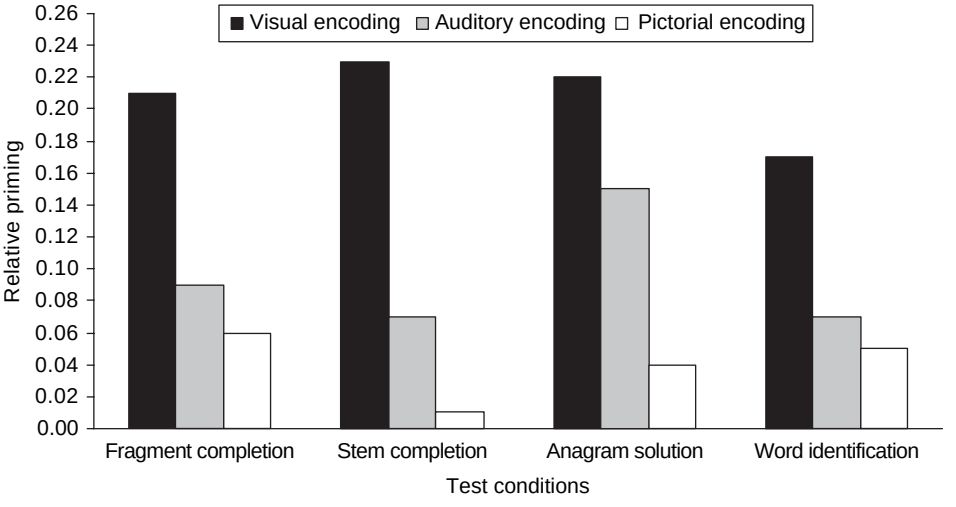

Figure 2 Relative priming for the four implicit memory tasks across different encoding conditions. Adapted from Rajaram S and Roediger HL (1993) Direct comparison of four implicit memory tests. *J. Exp. Psychol. Learn. Mem. Cogn.* 19(4): 765–776.

The impact of retrieval mode on memory performance can be seen even when the retrieval cues themselves are held constant and only the mode of retrieval is varied through instructions, or when the retrieval intentionality criterion is met by the experimental conditions (Schacter et al., 1989). For example, when performance on free recall is contrasted with performance on a task such as implicit word fragment completion, the retrieval mode changes, but so do the test cues (no test cues in free recall and perceptually degraded cues in fragment completion, e.g., _ t r _ _ b _ r _ _). But the role of retrieval mode in detecting memory would be more persuasive if dissociations between explicit and implicit memory could be observed even when the same test cues were used in both conditions. Graf and Mandler (1984) reported such a dissociation between an explicit word stem cued-recall task and an explicit word stem completion task where the same test cues (e.g., ele_____) were used, and the levels of processing effect occurred on the explicit but not the implicit version of the task. In a large-scale study, Roediger et al. (1992) reported similar patterns of performance when they contrasted explicit and implicit versions of the test using identical stem cues (e.g., ele_____) as well as explicit and implicit versions of the test using identical fragment cues (e.g., _ l _ p _ a n _). Along the same lines, in our study with concrete and abstract words we just described, we found that the presence of the concreteness effect in free recall also extended to the explicit retrieval version of the general knowledge test (complete the following question with a studied word: "What fruit wears its seeds on its skin?") but, as noted earlier, not to the implicit version of the general knowledge test (Hamilton and Rajaram, 2001). In other words, we demonstrated dissociative effects on explicit and implicit versions of a different type of task, namely general knowledge, while holding the test cues constant (see again **Figure 1**).

2.16.4.2 Differentiating between Conceptual and Perceptual Retrieval Cues

While explicit and implicit retrieval modes can produce various dissociations of theoretical significance such as the ones just described, dissociations can also occur *within* one retrieval mode – for example, with differences in implicit retrieval cues provided to the participants. In previous sections, we described how differences in retrieval cues can bring about changes in memory performance within the context of explicit memory tasks such as free recall, cued recall, and recognition. The tenets of the transfer-appropriate processing framework (Roediger et al., 1989; Roediger, 1990) predict systematic differences even within implicit memory tasks, depending on the type of process demanded by the tasks. If the implicit memory task largely depends on perceptual processes for its successful completion – as in the cases of word fragment completion, word stem completion, perceptual identification tasks – then encoding orientation produces one type of effect. But if the implicit task mainly relies on conceptual processes – as in the cases of the general knowledge test described earlier and also tests such as implicit category association test (given a category name, participants are asked to produce all the exemplars that come to mind within 30 s) – then the same encoding orientation produces the opposite effect. So, for example, the modality effect observed across different *perceptual* implicit tasks described in the study by Rajaram and Roediger (1993) disappears on *conceptual* implicit tasks because changes in modality of presentation are not important for accessing meaning (e.g., Blaxton, 1989; Srinivas and Roediger, 1990). Encoding variables that differentiate the extent to which meaning (rather than surface information as in the modality manipulation) is varied during study produce the opposite effect: As we described earlier in this section, the levels-of-processing effect that reflects changes in conceptual processing of information disappears on *perceptual* implicit tasks. But this effect is reliably observed on *conceptual* implicit tasks such as the implicit general knowledge test (Hamilton and Rajaram, 2001) and the implicit category association test (Hamann, 1990; Srinivas and Roediger, 1990). Together, these patterns show that, while retrieval modes of explicit and implicit retrieval change accessibility in dramatic ways, the nature of the process demanded by the retrieval task (conceptual versus perceptual) can also change accessibility in a manner that is powerful and that can be orthogonal to the retrieval mode itself.

2.16.4.3 Remembering and Knowing

We now turn to a brief discussion of another type of distinction between two retrieval modes – remembering versus knowing one's past. Unlike the explicit–implicit distinction, where people are, respectively, either aware or not aware of the connection between the past and present, the remember–know distinction is made when people are aware of

the connection. This distinction instead concerns the quality of retrieval experience that accompanies retrieval (Tulving, 1985). A person is said to engage in remembering if the retrieved memories are vivid and detailed. Remembering involves being able to think back to episodes and mentally reliving the past event, and in this way it is also said to involve mental time travel (Tulving, 2002). In brief, remembering is considered to be the purest measure of episodic memory. The experience of knowing is associated with semantic knowledge. Sometimes, retrieved information is associated with the past (unlike the case of implicit memory), but its rooting in the past lacks a sense of immediacy or detail such that one cannot tell when and where this information was encountered before. Despite having confidence in this type of memory, one experiences less personal connection and more of a generic sense about this information. In brief, Tulving proposed that the experience of knowing provided a measure of semantic memory. Tulving (1985) introduced the remember–know paradigm to enable quantitative measurements of these qualitative distinctions in retrieval.

The remember–know paradigm has been used widely to study the nature of retrieval experience and has produced both a large body of systematic findings and considerable debate (see Jacoby et al., 1997; Rajaram and Roediger, 1997; Gardiner and Conway, 1999; Rajaram, 1999; Gardiner and Richardson-Klavehn, 2000; Roediger et al., 2007, for reviews). For present purposes, we emphasize the dissociations and associations that systematically occur between these two distinct retrieval experiences. We have already discussed ways in which the levels-of-processing effect can vary as a function of encoding–retrieval interactions and explicit–implicit retrieval instructions. The presence of the levels of processing effect in explicit memory retrieval suggests that this effect should manifest itself in both remember and know judgments. However, the findings show that items studied for their meaning are given more remember judgments than items studied for their surface features, but this pattern is not observed for know judgments (Gardiner, 1988; Rajaram, 1993). Thus, retrieval can vary for the same set of encoding conditions even within the domain of explicit memory, once again emphasizing the important role that retrieval probes play in revealing the nature of memory.

In summary, we use the term retrieval mode to refer to distinct methods and experiences of retrieving information. Explicit retrieval refers to the conscious and intentional recall of previous experiences. In contrast, implicit retrieval refers to performance changes that are a result of prior experience but are unaccompanied by intentional or conscious recall of previous learning. Dissociations can occur between these two retrieval modes such that some factors (e.g., concrete/abstract words) can influence explicit memory but not implicit memory, while other factors (e.g., modality of presentation) can influence implicit memory but not explicit memory. Interestingly, the distinction between explicit and implicit memory retrieval is modified by the processing demands of these retrieval tasks such that dissociations can also occur *within* a particular mode of retrieval if the retrieval cues rely on different types of processes. As a result of this, *conceptual* implicit cues reveal the levels-of-processing differences but remain insensitive to modality changes, whereas *perceptual* implicit memory cues produce a reverse pattern of memory performance.

The notion of changes in the retrieval mode can also be applied to a distinction within explicit memory in terms of remembering and knowing – a distinction that is based on the quality of the information that is recalled. Remembering refers to recall that is accompanied by vivid details and a sense of mental time travel. In contrast, knowing refers to recall without specific details or a sense of when the information was encountered before. A dissociation also occurs between these two retrieval modes such that some factors (such as depth of processing) can influence remembering but not knowing. A variety of studies have also shown reverse dissociations and some associations as well between these two experiential modes of retrieval.

2.16.5 Repeated Retrieval

In previous sections, we focused on changes in the retrieval context – task differences, the match between encoding–retrieval interactions, and changes in the retrieval modes – to explore how retrieval processes affect detection of memory. We now review a class of retrieval phenomena that are quite different from the preceding ones but are equally important in revealing the nature of memory. These phenomena have to do with repeated attempts at thinking about a particular event. It is common experience to repeatedly try to recall something from the past that simply eludes us at a given moment. Are such efforts useful? In a research context, we might ask, do repeated

attempts at recall improve memory performance? The brief answer to this question is yes. As Roediger and Karpicke (2006b) recently noted in their comprehensive review of research on testing effects,

> . . . testing not only measures knowledge, but also changes it, often greatly improving the retention of the tested knowledge. (Roediger and Karpicke, 2006b: 181)

Improvement in memory performance through repeated retrieval attempts can be understood by examining two related but distinct phenomena – hypermnesia and repeated testing. Hypermnesia refers to an improvement in the total amount of material recalled across repeated attempts, and it is usually obtained with free recall and not so often with other tasks. Repeated testing benefits occur when having taken a prior test – either recall or recognition – improves performance on a later test – again, either recall or recognition. We will review selective studies on both these phenomena as they reveal the importance of retrieval attempts.

Systematic efforts toward understanding the positive effects of repeated attempts – or hypermnesia – can be traced back to Ballard's (1913) and W. Brown's (1923) classic papers. Ballard proposed the concept of reminiscence and defined it as, "the remembering again of the forgotten without re-learning" (Ballard, 1913: 17). W. Brown introduced the phenomena of inter-test forgetting – the number of items that were recalled on the first attempt but not on the second – and inter-test recovery – the number of additional items recalled on the second attempt – to capture the effects of repeated recall on memory output. W. Brown's findings showed that repeated attempts at recalling a list of studied words (or recall of states) resulted both in inter-test forgetting and inter-test recovery, but there was an overall improvement in memory performance such that inter-test recovery exceeded inter-test forgetting across recall attempts. Erdelyi and Becker (1974) termed this reliable net gain across repeated attempts at recall as hypermnesia. Modern interest in research on hypermnesia can be traced back to the findings that Erdelyi and colleagues reported in the 1980s (see Erdelyi and Kleinbard, 1978; Erdelyi, 1984; Erdelyi et al., 1989).

Interestingly, for memory to improve with repeated testing, recall attempts do not have to occur necessarily in the form of consecutive and distinct recall tests. Roediger and Thorpe (1978) asked subjects to study 60 words or 60 pictures and attempt recall either in three successive recall tests (each lasting 7 min) or a single recall test that lasted 21 min. In both testing conditions, subjects were asked to draw a line after each minute of recall. Pictures produced greater hypermnesia than did words, a finding that seems to hold in other studies as well (see also Erdelyi and Becker, 1974; Payne, 1986), and this was true for three successive recalls as well as for one long recall that was equal in duration to three successive recalls. More relevant to the present point is the finding that recall increased over time in both retrieval conditions, and it did so at the same rate. An important implication of this finding for educational purposes is that having more time to retrieve information benefits performance even when the study efforts remain the same.

The presence of hypermnesia in Roediger and Thorpe's (1978) design shows that repeated retrieval effort over an extended period of time is the key to improving memory. But could memory improve simply by increasing the time that elapses between study and recall? This is, of course, a counterintuitive possibility because we expect delay to worsen memory, not improve it. However, in a standard repeated-testing design, the delay between study and a given recall test is confounded with the timing of multiple tests. That is, the second test comes much later in time than the first, and so on. Also, Shapiro and Erdelyi (1974) found that recall of studied pictures improved when the delay between study and recall was 5 min compared to when it was 30 s. The key to understanding this unexpected outcome may lie in the instructions subjects received during the 5-min delay; subjects were asked to covertly review the materials they had studied earlier. As Roediger and Payne (1982) argued, when subjects engaged in thinking about the study materials in Shapiro and Erdelyi's study, this act amounted to repeated retrieval practice and produced memory benefits despite the delay between study and recall.

To address this possibility, Roediger and Payne systematically examined the selective influence of delay between study and test and of the number of prior recall tests in a repeated testing design. In their study, all the subjects performed three recall tasks, but one group started the sequence after a short delay, the second group started the sequence at the time when the first group performed the second recall test, and the third group started the sequence at the time the first group performed the third recall test (**Table 4**). As the recall findings from this study show, recall was equivalent on the first recall test

Table 4 Mean recall on the three successive tests for each delay condition

Condition	*Items recalled*				
Immediate	Test 1 25.6	Test 2 27.9	Test 3 30.1		
Short delay		Test 1 25.1	Test 2 27.5	Test 3 29.8	
Long delay			Test 1 25.6	Test 2 28.9	Test 3 31.3

Adapted from Roediger HL and Payne DG (1982) Hypermnesia: The role of repeated testing. *J. Exp. Psychol. Learn. Mem. Cogn.* 8(1): 66–72.

regardless of when the first test occurred (short delay, 25.6; intermediate delay, 25.1; long delay, 25.6). In contrast, recall increased during the same temporal window if the number of prior recall tests increased (the first test for the long-delay group, 25.6; the second test for the intermediate-delay group, 27.5; and the third test for the short-delay group, 30.1). Interestingly, many of these findings in hypermnesia studies have been secured with the study of pictures but not always with the study of words. We refer the reader to comprehensive reviews by Payne (1987) and Roediger and Challis (1989) for discussions on this and other complicating issues as well as for theoretical considerations in hypermnesia research. Regardless, these studies decisively point to the critical and specific role played by repeated attempts at retrieval in improving memory.

Repeated retrieval improves access to studied material in yet another way. Sometimes, the differential effects of different study methods that do not emerge on the first recall test are revealed on a later second test. For instance, in a study by Wheeler and Roediger (1992), subjects studied 60 pictures either presented one by one and accompanied with auditory presentation of the names or presented in the same manner visually but accompanied with an auditory presentation of a story. Shortly after completing a distractor task, subjects recalled the names of the pictures on one, two, or three successive tests. All the subjects returned 1 week later and also completed a final free-recall test. Their performance on this final test distinguished between the benefits of multiple retrievals for different study methods. Subjects' final recall was substantially higher for pictures that were embedded in a story than pictures that were presented without a story during study, and this difference became increasingly pronounced as the number of prior recall tests increased. Once again, we see the power of retrieval processes in that repeated retrieval can increase not only memory output when the study conditions remain the same; it can do so by bringing out the differential efficacy of study methods that might otherwise remain obscure.

As the preceding discussion shows, repeated retrieval clearly increases accessibility and produces improvements in memory. But in a discussion that emphasizes the importance of retrieval, it is important to ask what is more effective – repeated retrieval or repeated study? After all, a vast empirical literature in cognitive psychology shows that repeating information at study reliably improves recall and recognition (*see* the chapter by R. L. Greene in this volume on repetition and spacing effects; Chapter 2.06). There are many interesting phenomena associated with repetition at study, including the nearly ubiquitous demonstration that spaced repetition at study – that is, repeating items with one or more intervening trials in the study list – produces better memory compared to massed presentation – that is, repeating items twice or more in consecutive trials in the study list. Given the beneficial effects of repeated study, it is logical to ask how repeated retrieval fares in comparison. This question has been tested in many ways and from different theoretical as well as applied perspectives. Yet, the answer is impressively consistent. Repeated retrieval not only benefits memory, it does so to a greater extent than does repeated study. We recommend Roediger and Karpicke's (2006b) review that we earlier referenced for an in-depth discussion of different theoretical and empirical issues related to this broad question, and for the practical implications from this research for improving educational practices. Here, we will review a selection of studies that demonstrate this conclusion. In these studies, the focus is not on hypermnesia that arises when the same test is taken multiple times but on benefits of repeated testing where the successive tests are not necessarily identical, but having taken the prior tests nevertheless improves performance on the later tests.

As early as the first quarter of the twentieth century, two studies demonstrated many of the key findings from repeated-testing designs. In one study, Gates (1917) varied the amount of time given to subjects for only studying versus for recalling while being able to refresh memory for the forgotten material. On a final (serial) recall test of the studied

material (nonsense syllables or biographies), Gates found that spending more time on recall with opportunities to refresh the forgotten information produced better final recall than more time devoted only for study – provided that a certain, minimum amount of time was first spent for study in the former condition (see also Thompson et al., 1978).

In another study, Spitzer (1939) found that two attempts at retrieval improved memory on a multiple-choice recognition memory test. Furthermore, the sooner the first test was administered, the less was the forgetting and the higher was the performance on a later second test. The inoculating effects of the first test, and of its timing, have received considerable attention in the repeated-testing literature. While the inoculating effects of the first test on later memory seem secure (Wheeler and Roediger, 1992), the specifics concerning the optimal timings of multiple tests continue to be investigated (Landauer and Bjork, 1978; Balota et al., 2006, 2007; Logan and Balota, in press).

The early intimations of a relative advantage of increased testing over increased studying in the studies by Gates (1917) and Spitzer (1939) have been systematically tested from various perspectives in the modern literature and have produced a very nice body of data. A key factor in predicting the relative advantage of repeated study versus repeated retrieval concerns the delay between study and the final memory test. In a seminal study, Tulving (1967) used a comprehensive design that included comparisons of various study–test schedules for a total of 24 trials each and had subjects study a total of 36 words – alternating study and test (STST, STST, and so on), three study and one test (SSST, SSST, and so on), and one study and three tests (STTT, STTT, and so on.) Also critical for present purposes, subjects were given 1 s per item to study and an equivalent total of time for test (so, subjects received 36 s to recall all the studied items.) The results showed that recall performance was comparable over the 24 trials across the three different study-test schedules. In other words, repeated testing did not improve memory over repeated study.

Tulving's findings were surprising in light of the general conclusion we have already stated, but these findings were replicated by others (e.g., Lachman and Laughery, 1968; Rosner, 1970; Birnbaum and Eichner, 1971; Donaldson, 1971). Also, these findings make sense if we consider them in light of Roediger and Thorpe's (1978) findings we discussed earlier; recall improves as subjects receive additional time to do the task. However, in Tulving's experiment subjects were given only 36 s to recall 36 words. Even if the words had been perfectly learned, this is a very short time period with which to recall them. Initial support for this possibility was found in a study where subjects either studied a list of 40 words four times or studied it once and recalled it three times (Hogan and Kintsch, 1971; see also Thompson et al., 1978). In a final recall test conducted 2 days later, prior training with multiple tests produced 5% better recall than prior training with multiple study.

Roediger and Karpicke (2006a, Experiment 2) recently published a study using educational materials that provides an impressive resolution to the question concerning the relative importance of repeated study versus repeated tests. Subjects studied prose passages on scientific topics and were tested on a recall test that was similar to essays in its format. In one condition, subjects studied the passage four times (SSSS). In a second condition, subjects studied the passages three times and were tested once (SSST), and in the final condition, subjects studied the passages once and were tested three consecutive times (STTT). Subjects also took a final recall test either 5 min or 1 week after this learning sequence. As can be seen in **Figure 3**, more idea units were recalled following repeated study if the final recall test occurred after a short delay of 5 min. But the final recall performance was better after repeated testing if

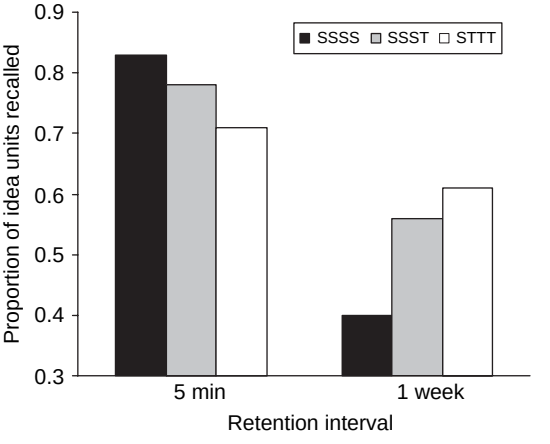

Figure 3 Mean proportion of idea units recalled on the final test after a 5-min or 1-week retention interval as a function of learning condition (SSSS, SSST, or STTT). The labels for the learning conditions indicate the order of study (S) and test (T) periods. Adapted from Experiment 2 in Roediger HL III and Karpicke JD (2006a) Test-enhanced learning: Taking memory tests improves long-term retention. *Psychol. Sci.* 17(3): 249–255, with permission from Blackwell Publishing.

the final test occurred after a long delay of 1 week. In summary, these findings pinpoint the key conditions that are responsible for when study and retrieval repetitions can produce differential benefits in memory; repeated study improves memory in the short term, but repeated testing produces improvements in the long term. This long-term advantage of repeated testing over repeated study seems secure because it has been shown to also occur with word lists (Wheeler et al., 2003).

A general explanation for why repeated retrieval benefits long-term retention more than repeated study harkens back to the principle of transfer-appropriate processing we discussed in earlier sections on encoding–retrieval interactions and retrieval mode (Morris et al., 1977; Roediger et al., 1989). Roediger and Karpicke (2006b) recently noted that one reason for the superiority of repeated retrieval is that the same processes are engaged when people retrieve information again, whereas, as McDaniel (2007) has argued, different processes are often engaged across learning and testing situations. The match in processes in the former condition illustrates the operation of transfer-appropriate processing.

Another explanation of the benefit of repeated testing is that initial testing results in the creation of multiple retrieval routes to the to-be-remembered item, thus making recall more likely at a later test. In a study by McDaniel and Masson (1985), subjects were given either a phonemic or a semantic encoding task followed by a cued-recall test with either semantic or phonemic cues. Thus, half of the subjects received the same type of information at encoding and the first test, and half of the subjects received a different type of information at encoding than at the first test. Later, subjects were able to recall more information on a second test when the cues from the first test had not matched the original encoding. This finding is consistent with the idea that the initial test improves recall on a later test if it is able to produce an elaboration of the existing memory trace by increasing the variability of the encoded information (see also McDaniel et al., 1989). From a retrieval point of view, this means not only that retrieval is changing the existing memory representation, but that varied changes make subsequent retrieval more and more likely to occur.

Roediger and Karpicke (2006b) have reviewed a sizeable literature that points to yet another related but distinct basis for benefits from repeated retrieval, namely the process of generation. Briefly, just as the generation processes at encoding improve memory such that items generated from semantic cues are later recalled and recognized more often than items that were simply read, generation of studied items during first recall improves performance on a following test (see Jacoby, 1978; Bjork and Bjork, 1992; Bjork, 1994, 1999; Roediger and Karpicke, 2006a). Consistent with this idea, a prior recall test that requires generation of studied information (such as providing short answers) improves performance on later recall (short answers) as well as recognition (multiple-choice format), whereas a prior recognition test (that does not require generation because the studied items are presented again) does not produce comparable benefits on later tests (Kang et al., 2007). Recent work (McDaniel et al., 2007) has also demonstrated this effect in a college course. In this study a benefit was found for short-answer quizzes (a recall test) over additional study but not for multiple-choice quizzes (a recognition test) over additional study on a final exam. This is particularly impressive given not only the variability of additional studying and motivation of the students within the class, but also the fact that the quizzes were administered up to 5 weeks prior to the final exam. Findings such as these reveal the specificity of effects that prior retrieval produces on later retrieval and point to the underlying processes that mediate such patterns.

In conclusion, testing – or retrieval – not only has a powerful influence on long-term retention but is also an effective learning device with important educational implications. The judicious use of testing in educational settings should benefit students' performance. This goal is embodied in recent cognitive research that aims to identify optimal retrieval practices for improving retention and academic performance. We refer the reader to M. A. McDaniel's chapter on 'Education and Learning' in this volume (*see* Chapter 2.43) for an in-depth discussion of these important issues.

2.16.6 Retrieval in a Social Context

For decades now, experimental studies on memory have typically focused on the individual, and the study of retrieval processes has been no exception. But just as we retrieve the past not just once but often repeatedly, we also retrieve the past not just alone but often in collaboration with others. People recall the past in dyads (e.g., friends and couples), triads (e.g., friends, colleagues), or in larger groups. It was

only around the mid- to late 1990s that research on the effects of social context on memory started to gain momentum. We review some of the core findings from this area of research as relevant to the process of retrieval here and refer the readers to scholarly reviews by Weldon (2001) for the historical antecedents and the emerging research on social processes in memory, and by M. Ross (*see* Chapter 2.47) and J. V. Wertsch (*see* Chapter 2.48) in this volume on the nature of social memory processes and collective memory, respectively.

Both the early neglect of group processes and the recent focus on group processes make sense on theoretical and empirical grounds, because assessment of how social processes influence retrieval first requires a clear understanding of how individual memory processes work in isolation. Now that a substantial body of evidence and major theoretical frameworks are in place on the nature of individual memory, researchers have the necessary empirical and theoretical bases against which the social influences on individual memory can be measured. Similarly, researchers can also test for potential similarities and differences between individual memory and group memory processes.

We first focus on group memory because retrieval processes appear to play a central role in mediating group memory effects. Studies that report group retrieval – or collaborative memory – effects typically compare collaborating groups to nominal (or control) groups. In collaborative groups, members collaborate during retrieval. In nominal groups the nonredundant responses of an equal number of individuals who worked alone are pooled together (Basden et al., 1997; Weldon and Bellinger, 1997). Collaboration in the experimental group is instantiated by asking subjects to contribute their retrieved responses in any order or in a turn-taking order. Results do not seem to change as a function of the particular procedure used for collaboration (Basden et al., 1997; Weldon and Bellinger, 1997; Weldon et al., 2000; Wright and Klumpp, 2004).

The central finding in group retrieval studies turns out to be counterintuitive. Collaborating groups recall significantly fewer studied items than nominal groups (Basden et al. 1997; Weldon and Bellinger, 1997), a phenomenon that Weldon and Bellinger (1997) call collaborative inhibition. In Weldon and Bellinger's experiment, participants encoded a list of words alone. Later, they were asked to recall the information either individually or in a collaborative group of three individuals. When the participants recalled individually, a nominal group score was created that consisted of counting up all the nonredundant answers of three individuals. The results indicated that, while collaborative groups recalled more than the average individual, the nonredundant responses of three individuals recalling alone (i.e., nominal groups) exceeded collaborative group performance.

Collaborative inhibition appears to be largely a retrieval phenomenon, as this effect is reliably observed when the encoding conditions are held constant across the nominal and collaborative groups. The retrieval basis of this effect is further supported by the proposal that collaborative inhibition is similar to another well-known retrieval phenomenon, namely, the part-list cuing inhibition effect (Slamecka, 1968; Basden et al., 1977; Roediger and Neely, 1982; Basden and Basden, 1995). The part-list cuing inhibition refers to yet another counterintuitive phenomenon in memory; when subjects are presented with a partial list of studied words during recall and are asked to recall the remaining studied words, their recall is poorer for the remaining subset compared to a condition where none of the studied items are provided during recall. Thus, having access to a part of the studied lists inhibits the recall of the remaining words. The locus of this effect appears to be at retrieval because recall for the remaining subset improves on a later trial if the partial list is no longer provided. Thus, the dip in recall during the first trial turns out to be temporary and does not reflect poorer encoding or storage in the part-list condition. This finding then begs the question – why do partial lists inhibit recall? Evidence shows that individuals develop their own idiosyncratic organization of the studied material and use it during recall (Tulving, 1962; Roenker et al., 1971; Rundus, 1971). The presence of a subset of items during recall disrupts such organizational and retrieval strategies and leads to suboptimal recall performance (Basden and Basden, 1995).

B. H. Basden and colleagues extended the logic of retrieval disruption to the collaboration situation and tested the idea that collaborative inhibition observed in group memory is similar to the part-list cuing inhibition effect in individual memory. The logic behind this theoretical extension goes like this. Recall of a given member is reduced in a collaborative group because responses produced by other group members serve as part-list cues and disrupt the idiosyncratic retrieval strategies on which each individual member relies during group recall. Such retrieval

disruption – resulting from the input of other group members – reduces the individual contributions from each member and leads to lowered group recall.

B. H. Basden et al. (1997) reported evidence that supports the retrieval disruption hypothesis. In this series of experiments, B. H. Basden and colleagues also reported the same pattern of results as Weldon and Bellinger (1997), showing that nominal recall was greater than collaborative recall. However, they also showed that this effect was mediated by the extent to which collaboration disrupts the individual's organizational structure. For example, in their first study, participants were given one of two types of encoding tasks. Some participants were asked to learn many (15) instances of a few (6) categories. Other participants were asked to learn few (6) instances of many (15) categories. D. R. Basden and Draper (1973) had previously argued that within-category organization is more likely to occur with large categories. As a result, each individual's retrieval strategy should be at more variance from another individual's retrieval strategy when the categories are large. If collaborative inhibition is due to retrieval disruption, there should be greater collaborative inhibition for participants who studied large categories (15 instances of 6 categories) than for participants who studied small categories (6 instances of 15 categories). Consistent with this hypothesis, the magnitude of collaborative inhibition varied as a function of list structure. In fact, collaborative inhibition was found only for participants who studied large categories and not for participants who studied small categories.

The collaborative inhibition effect in group retrieval and the part-list cuing inhibition effect in individual retrieval are related in yet another interesting way. As we have described, both phenomena are said to occur because of retrieval disruption during retrieval. It turns out that in both cases retrieval disruption does not impair individual memory beyond the conditions where it operates. As we noted earlier, in the case of part-list cuing inhibition, there is evidence that, if the part-list cues are removed during subsequent individual recall, subjects elicit previously blocked studied words (Basden et al., 1977). Similarly, after the completion of the group retrieval session, if each group member individually recalls studied items, the 'lost' items during collaboration resurface in later individual recall (Finlay et al., 2000; see also Weldon and Bellinger, 1997). These interesting parallels suggest that group retrieval can be sensitive to the same cognitive mechanisms that mediate individual retrieval.

In an earlier section on task differences, we discussed the critical role retrieval cues play in determining the accessibility of studied information. Initial evidence from studies on collaborative memory suggests retrieval cues also modulate the key finding on which we have focused here – collaborative inhibition in group retrieval. Collaborative inhibition typically occurs when a collaborative group engages in free recall (Basden et al., 1997; Weldon and Bellinger, 1997). This outcome is consistent with the retrieval disruption account because, as we have noted, free recall relies heavily on the internal organization and strategies of each participant, and the disruption of this strategy lowers each member's contribution to the group product.

This logic predicts that, if more cues are provided as external aids during retrieval, each participant group member would need to rely much less on internal resources. As a result, disruption is less likely to be a factor during the process of collaboration when retrieval cues are present. Current evidence supports this argument. While collaborative inhibition consistently occurs in free recall, this effect disappears in a paired-associate recall task that provides partial study cues, and even reverses in a recognition memory task that recapitulates the entire study item. Finlay et al. (2000) reported a study in which subjects studied pairs of weakly related words. At test, subjects received the first word of the studied pair and recalled the second pair either individually or in pairs. Nominal dyad performance (nonredundant, pooled recall of two individuals who worked alone) did not differ from that of the collaborating dyads even though the typical collaborative inhibition effect occurred with the free-recall task.

Clark and colleagues (2000) used the recognition memory task in their study on collaborative memory with the aim of elucidating the nature of the collaboration process from a different perspective. But their findings are interesting in the present context for yet another, related, reason – the effects of maximal retrieval cues on mediating collaborative memory. Subjects studied a list of unrelated words and were later tested on a recognition memory task that consisted of an intermixed list of studied and nonstudied words. These researchers assessed the performance of three-member collaborative groups against three measures derived from the nominal groups of same size – the best group member, the majority vote, and the average of the group. In all three comparisons, the recognition performance of the collaborative groups exceeded that of the

nominal groups. In other words, Clark et al. reported that there was a collaborative facilitation effect in recognition memory.

We have focused on group retrieval to discuss the role of retrieval in assessing memory in a social context. We close this section by briefly describing evidence that has just started to emerge on how social processes can affect individual retrieval. As we noted earlier, the disruptive effects of collaboration are temporary, and later individual recall shows recovery of studied items that were not produced during collaboration.

In a study from our own lab, we examined this effect in recognition. We presented subjects with a list of unrelated words to study and later gave a recognition task in which we assessed effects of collaborative discussion on individual memory (Rajaram and Pereira-Pasarin, 2007). We found that collaborative discussion just prior to making individual recognition responses led to more accurate performance (in both d′ and hits–false alarm measures) than in a retrieval condition that required no prior collaboration. The beneficial effects of collaboration here are impressive because collaboration can potentially affect individual recognition in a negative or a positive direction. It can increase an individual's propensity to go along with the group's input regardless of whether or not it is correct. Individuals can accept nonstudied items as studied, reject studied items, or do both, thereby producing lower memory accuracy. Or, individual subjects can reject more nonstudied items or accept more studied items, or do both, thereby increasing memory accuracy. Yet, group input in our study enhanced individual recognition, and this advantage persisted up to 1 week (see **Figure 4**). As we discuss in a later section titled 'Retrieval errors and other retrieval phenomena,' such benefits do not always occur, because other researchers have shown that input from other group members can sometimes increase memory errors. Regardless, this cluster of findings shows that encoding in and of itself does not determine how much or what an individual will ultimately remember. Retrieval processes – either inherent in the individual or modified by social input – play a large role in determining the final memory output.

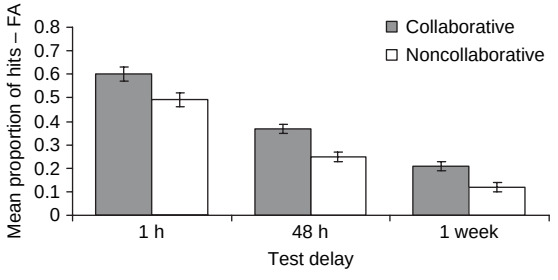

Figure 4 Mean proportion of hits minus false alarms (FA) as a function of collaborative and noncollaborative conditions and retention interval. Adapted from Experiment 2 in Rajaram S and Pereira-Pasarin L (2007) Collaboration can improve individual recognition memory: Evidence from immediate and delayed tests. *Psychon. Bull. Rev.* 14: 95–100.

As Gardner (1985) noted, researchers have tended to set aside social, emotional, and cultural processes in the pursuit of understanding cognition. In other words, researchers have typically viewed social, emotional, and cultural processes as contextual factors whose influences need to be controlled for rather than as key components in understanding cognition. But memory – or more specifically, retrieval – is usually a social process (Weldon, 2001). Not only do people often recall with others (as discussed in this section, and to some extent also in a later section on retrieval errors), but how the recalled information is interpreted is often a function of the person's sociocultural environment. Theories of memory that are based only on the individual are therefore incomplete. The initial evidence (briefly presented earlier; *see* Chapter 2.47) provides initial support for these conclusions and also suggests that future research should focus on social processes of memory as a factor rather than a confounding variable.

To summarize, collaborative inhibition is the counterintuitive finding that collaborative groups are able to recall less information than the pooled nonredundant responses of nominal groups of equal size. This is theorized to be a retrieval effect similar to the part-list cuing effect. Retrieval disruption (created by the recall products of other group members in the case of collaborative retrieval) disrupts individuals' idiosyncratic retrieval strategies and causes poorer overall memory performance. However, this disruption does not seem to impair subsequent individual memory, and in some cases (for instance, as with recognition memory) may actually enhance individual memory.

2.16.7 Retrieval Errors and Other Retrieval Phenomena

Earlier, we reviewed research that shows that repeated retrieval improves memory. As Bjork (1975) noted, retrieval is a memory modifier. Interestingly, the act

of retrieval can also reduce memory accuracy. People recall emotional, significant, or entertaining events from their lives often, and it is all too common to embellish the events from one telling to the next or from one audience to another. Bartlett's (1932) classic study is often cited to illustrate how memory output can change from one recall to the next. In this study, people were asked to read a Native American story called 'The War of the Ghosts.' Importantly, the significance of many details in this story was not apparent to people of different cultural backgrounds. The more times that people were asked to retell the story, the more the stories became distorted such that subjects omitted unfamiliar details and inserted materials to make the story consistent with their schemas. These dramatic changes across repeated retrievals have since been replicated in a study by Bergman and Roediger (1999).

Bartlett's (1932) study and Bergman and Roediger's (1999) critical replication show that individuals modify story output even without any intervention from outside sources. It is easy to imagine then that the social situations individuals encounter can change the contents of what individuals might retrieve from one occasion to the next. For example, social situations often dictate whether a story should be told in an accurate or entertaining fashion. In a study by Dudukovic et al. (2004) participants were asked to either retell a story with a goal to be accurate, or with a goal to be entertaining. While the participants did not differ on a later recognition test, they did differ on a later recall test. The participants who had originally told the story accurately recalled more information with less exaggeration than the participants who had originally told the story for entertainment. Thus, the way that we recount information to others influences the way that information is later recalled. As we will describe next, retrieval errors can also creep into individual performance when others provide input during retrieval.

In a previous section on retrieval in a social context, we discussed evidence that shows positive influences of input from others. But individuals also make more retrieval errors under certain conditions if they previously received erroneous input from others. In a study that assessed the effects of social contagion, Roediger et al. (2001) presented subjects with everyday scenes (e.g., a kitchen) during the study phase. Later, subjects recalled the scenes along with a confederate who inserted related but nonstudied items during recall (e.g., toaster). On a final test where subjects engaged in recall alone, they falsely recalled related but nonstudied items more often if they had been inserted by a confederate in the earlier recall phase than if no mention of them had been made.

Basden et al. (2002) reported similar effects of social input in a study where they presented semantically related 'DRM' (Deese-Roediger-McDermott) lists during study and constructed a perceived group-recall situation with the use of interconnected computers. DRM lists consist of thematically related words such as 'dream,' 'bed,' 'night,' etc., where a critical word such as 'sleep' is missing. In individual memory studies, subjects erroneously recall these critical nonpresented words at levels as high as true recall and also give 'remember' responses indicating vivid memory for having seen them before (Roediger and McDermott, 1995). In Basden et al.'s study, subjects engaged in perceived group recall followed by individual recall. During perceived group recall, the subjects were led to believe that they viewed the responses of other group members on the computer screen during recall but in fact the generated responses were controlled by the experimenter. In one condition of perceived group recall, subjects saw the critical, nonpresented lures, and in another condition these items were not included in the supposed responses from other members. Subjects included more erroneous responses in their final individual recall protocols if they had previously participated in one of the two perceived group-recall phases than if they had not participated in the perceived group-recall phase. In this way, individual retrieval can be socially influenced. The process of collaboration can lead to individual retrieval benefits as discussed in the previous section but also to retrieval errors, as these studies show (also see Basden et al., 2000, 2002; Reysen, 2005, for related findings).

Another topic of considerable interest in memory retrieval focuses on the subjects' ability to identify the source of information they recall – a phenomenon called reality monitoring (Johnson and Raye, 1981) The general approach here is to ask subjects whether the item they recalled (or recognized) was presented to them (i.e., the item originated from perception) or was internally generated (i.e., the item was something they imagined or dreamed). According to this framework (e.g., Johnson, 1991; Johnson et al., 1993), people do not explicitly tag memories with source information. Rather, they typically make source attributions based on a generalized evaluation of whether a memory's qualities match

expectations. These judgments capitalize on the average differences of the characteristic qualities of memories from different sources. For instance, perceived events tend to include more information about perceptual, temporal, spatial, and affective characteristics and less information about cognitive processes than imagined events. A judgment of 'perceived' rather than 'imagined' should therefore be given if the evaluation of a memory's qualities results in a great deal of information about perceptual and spatial details, accompanied by little information about the cognitive processes that took place during encoding. Attributing a memory to the source for which that memory's qualities are most characteristic maximizes the odds of accurately judging the memory's source. Reality-monitoring failures occur when people falsely claim either that something was perceived when it was actually internally generated or that something was internally generated when it was actually perceived. A detailed review of this topic can be found in a chapter on source monitoring by S. Lindsay (*see* Chapter 2.19) in this volume.

These processes in reality monitoring constitute yet another form of retrieval, one that is characterized by metamemory judgments, because subjects make judgments about information retrieved from memory. In an earlier section on retrieval mode, we described remember and know judgments, which can also be considered metamemory judgments because subjects report the quality of memory for the information they retrieve (see Rajaram and Roediger, 1997; Rajaram, 1999; Roediger et al., 2007). There are also other well-known metamemory judgments such as feelings of knowing (see Koriat, 1995) and the tip-of-the-tongue state (see Brown, 1991; Schwartz et al., 2000) that researchers study to find out subjects' sense of what they can retrieve even when recall does not succeed. These judgments reveal interesting – metacognitive – aspects of the retrieval process as subjects make judgments about the likelihood of retrieval under certain circumstances. In the feeling-of-knowing state (see Koriat, 1995) subjects can reliably report whether they can recognize an item on a multiple-choice test even though they were unable to recall that item, and in the tip-of-the-tongue state, people can reliably indicate whether or not the information they are trying to retrieve is on the tip of their tongue and could be retrieved. We recommend chapters by A. Koriat on control processes in remembering (*see* Chapter 2.18) and by A. S. Brown on the tip-of-the-tongue states (*see* Chapter 2.22) for detailed discussions of these topics.

In conclusion, the ways in which retrieval conditions are arranged to a large extent determine how much memory accessibility can improve. But in many situations, retrieval can also act as a memory modifier and can do so in systematic ways. Such situations can lead to systematic errors in retrieval, as revealed by the DRM effect. Furthermore, when recalling information, people are motivated not only to present a coherent story, but also to tell the story with a particular purpose (for example, to be entertaining). Both of these motivations can serve to lower overall memory accuracy. Retrieval errors can also be the result of social influences (believing that you saw something that someone else endorsed seeing), or reality monitoring errors (believing that you saw something that you only imagined seeing). Finally, many meta-memory processes such as the tip-of-the-tongue phenomenon also modulate the success of retrieval.

2.16.8 Concluding Comments

As we noted in the introduction, much of memory research has been guided by a focus on three putative components – encoding, storage, and retrieval. But from the perspective of the rememberer, it is the act of retrieval that constitutes memory. In this chapter, we have taken this perspective to explore various phenomena in memory research that tell us something important about the process of retrieval and ways in which this process enables access to what we have learned. The phenomena and findings reviewed in this chapter point to the unique role that retrieval processes play in modifying the effects of different encoding processes. Above all, these findings show that, without a proper understanding of the nature and power of retrieval, our understanding of how memory works is not only incomplete but also flawed.

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2.17 Remembering and Knowing

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> This ultimate puzzle, the subjective experience of consciousness, is perhaps a good place for any purely scientific survey, namely one of objective facts, to cease.
>
> (Greenfield, 1997: 192)

> . . . the peculiarity of individual experience does not place the subject of individual experience outside the realm of scientific inquiry. Any explanation of consciousness must account for subjective states of awareness. . .
>
> (Searle, 1997: 49–50)

2.17.1 Introduction

The mystery of consciousness seems to have resisted scientific investigation for at least two main reasons, first, because consciousness is apparently unitary and, second, because it is subjective. It is difficult for science to deal with phenomena that cannot be broken down into constituent parts that can be investigated separately, as well as in conjunction. Thus, little theoretical progress was made in memory research for so long as memory was thought of as being a single mental faculty – memory is memory – and significant progress has been made only since memory began to be classified into various systems and component processes. Similarly, little progress can be made in understanding consciousness for so long as consciousness is thought of just as consciousness. Consciousness has to be broken down and classified into different natural kinds as a precondition for scientific inquiry. This chapter concerns one such classification, a simple classification of consciousness in relation to memory that was proposed by Endel Tulving, along with a paradigm for investigating the different kinds of consciousness (Tulving, 1983, 1985).

Tulving's (1983, 1985) original proposal was of three kinds of consciousness, each reflecting one of three memory systems. The three kinds of consciousness were autonoetic, noetic, and anoetic, which respectively mean self-knowing, knowing, and not knowing. The three corresponding memory systems were episodic, semantic, and procedural memory. Only autonoetic and noetic consciousness involve awareness of memory. Anoetic consciousness is bound to the present, not the past, and involves awareness of the current situation. Autonoetic consciousness allows awareness of oneself in a previous situation, in the sense of what has been called mental time travel. It allows one to reexperience previous events and to relive them mentally, including one's own part in them. It is characteristic of episodic memory, which therefore entails something more than, or other than, autobiographical memory.

Autobiographical memory includes facts known about one's past in an abstract, historical sense, without any ability to reexperience those autobiographical events or relive them mentally. It is noetic consciousness that gives rise to awareness of this kind, and it is characteristic of semantic memory, which also includes all the general knowledge that one has acquired about the world.

The remember-know paradigm was introduced by Endel Tulving (1985) as a procedure for obtaining what have been called subjective (or first-person) reports of autonoetic and noetic awareness. In this paradigm, for each item retrieved participants are instructed to report "whether they actually 'remember' its occurrence in the list or whether they simply 'know' on some other basis that the item was a member of the study list" (Tulving, 1985: 8). The proportion of remembered items gives a direct measure of autonoetic awareness, whereas the proportion of known items reflects noetic awareness. It follows that remembering, as a state of awareness, gives a better measure of retrieval from episodic memory than other more traditional measures such as recall or recognition.

Since the introduction of this paradigm, it has been significantly modified and very widely used, such that there is now a substantial body of research in which participants have been required to make remember and know responses at the time of retrieval. A large number of empirical facts about autonoetic and noetic awareness have been established. Other theoretical accounts than that provided by the distinction between episodic and semantic systems have also been invoked to account for these facts. These include dual-process models of recognition that distinguish between two independent processes, recollection and familiarity, which are assumed to respectively give rise to corresponding recollective experiences (that is to remembering) and to feelings of familiarity (Mandler, 1980; Jacoby, 1991). Another processing account distinguishes between the distinctiveness and the fluency of the processing involved, with distinctiveness giving rise to remembering and fluency giving rise to knowing (Rajaram, 1996). A third approach has been to try to model remember and know responses using signal detection measures of trace strength and response criteria in recognition tests. According to some of these models, remembering simply represents the adoption of more stringent response criteria than that adopted in deciding whether the item was a member of the study list (Donaldson, 1996; Hirshman, 1998; Inoue and Bellezza, 1998).

Studies using the remember-know paradigm, particularly the early ones, were widely criticized because of the so-called subjectivity of remember and know responses, which to some critics puts the paradigm outside the realm of scientific inquiry. However, such criticism has become more muted of late, mainly by virtue of the empirical facts that have been discovered and the theoretical understanding of them that has been achieved. There are also purely rational arguments against the overly narrow view of scientific method and explanation that this criticism embodies.

The aim of this chapter is to survey what has been achieved by studies that have made use of the remember-know paradigm. To that end, the paradigm itself is described in more detail in the next section of the chapter. This is followed by a section that argues, on rational grounds, for the objectivity of the subjective experiences of consciousness measured by remember and know responses. The next section of the chapter surveys many of the empirical facts that have been gleaned experimentally, especially from earlier studies. After that, the major theoretical accounts are briefly outlined. Next follows a lengthy section concerned with more recent empirical findings and current theoretical issues. The chapter concludes with a critical theoretical evaluation.

2.17.2 The Paradigm

Although in introducing the paradigm Endel Tulving (1985) used free recall, cued recall, and recognition tests, nearly all the subsequent studies that made use of it used recognition tests. The two most significant modifications to the paradigm in part reflect this rather restricted usage. The first modification was to identify know responses in the instructions given to participants closely with familiarity rather with some other basis. This modification has occurred gradually, over a period of years, in different studies. Instructions often emphasize the importance of having strong feelings of familiarity or knowing, or being highly confident of such feelings. The second modification was to add a guess response (Mantyla, 1993; Gardiner et al., 1996). Both these modifications have some theoretical importance. Identifying know responses with familiarity links those responses more directly to semantic memory and to the familiarity process in dual-process models. The addition of a guess response has implications both for a familiarity process and for signal detection models. But the primary reason for allowing this response was

methodological. It allows for recognition decisions that are more strategically based and not associated either with experiences of remembering or with knowing. Guess responses therefore remove a potential confounding of such strategically based recognition decisions with remember or know responses. They also provide a default response if participants realize that their initial recognition decision was mistaken. There are thus good methodological reasons for recommending that the paradigm should usually include guess responses (Gardiner and Conway, 1999). This paradigm is summarized schematically in **Table 1**. For a full version of typical written test instructions that includes those for guess responses, see Gardiner and Richardson-Klavehn (2000).

It is also good practice to check the validity of remember, know, and guess responses by having participants provide descriptions of the reasons underlying a random selection of them after the test is over. It is important that participants provide evidence of contextually relevant mental details for remember responses and do not provide such evidence for know or guess responses. And such descriptions can be of considerable interest in their own right (Gardiner et al., 1998a). Neither the use of guess responses nor that of posttest checks of the validity of all three kinds of responses has become universal practice, however, even in more recent studies, despite the increased risk of obtaining potentially misleading results (see, e.g., Gardiner et al., 1996, 1997).

Table 1 The usual remember-know (guess) paradigm in recognition memory tests

Procedure
Study tasks
Retention interval
Recognition tests (Old/New)
If Old, then Remember, Know, or Guess
Response definitions
Old/New: Test item occurred/did not occur in the study list
Remember: Test item brought back to mind some specific recollection of something you thought about when it occurred in the study list
Know: Test item does not bring back to mind something you thought about when it occurred in the study list, but it seemed strongly familiar in the experimental context
Guess: Test item did not give rise either to experiences of remembering or of knowing it occurred in the study list, but you have other reasons for guessing that it might have done

2.17.3 The Objectivity of Subjective Experiences of Consciousness

Criticism that the subjectivity of the states of awareness reported by remember and know responses means that those responses do not yield scientific data is misguided. In effect, this criticism argues that a recognition response is objective but a remember or know response is subjective. But remember and know responses (guess responses, too) are simply a partitioning of the recognition response into some constituent components, and so, by the same token, the recognition response is simply based on the sum of those components. A recognition response also reflects subjective states of awareness. It is difficult, looking at the four response definitions given in **Table 1**, to make a convincing case for there being between the first definition and the three definitions that follow it a categorical shift from objectivity to subjectivity.

Remember and know responses, respectively, reflect distinct populations of experiences of autonoetic and noetic awareness, and it is critical for a scientific approach that these natural kinds of subjective experiences are treated at the population level, not at the level of individual instances of such experiences, which are inevitably idiosyncratic (Gardiner, 2001). Psychology is a biological science, not a physical one, and biology is characterized by population thinking, not by essentialism (Mayr, 1982). Every individual instance of remembering may be unique, just as every individual member of a biological species is unique. In each case, that which is uniquely individual may be impenetrable to science, but the population to which individual instances conform is not impenetrable to science, whether of a biological species or of a mental state of awareness. Therein lies the importance of a conceptual classification of so-called subjective consciousness.

But, as the saying goes, the proof of the pudding is in the eating. For data to be amenable to science, those data must yield phenomena that are systematic, replicable, and intelligible theoretically. It was by no means a foregone conclusion that this would turn out to be the case using the remember-know paradigm, and early studies using the paradigm were primarily concerned with seeing whether such a case could be established empirically. That is, they were concerned initially with whether or not, and then under what circumstances and for what reasons, functional dissociations might be observed between the reported states of awareness. Functional dissociation refers here to the discovery of

dissociative effects of experimental manipulations on the reported states of awareness or of dissociative effects between them in comparing different subject populations. The first such study was by Gardiner (1988), who found that the beneficial effects both of deeper levels of processing and the detrimental effects of longer retention intervals were essentially confined to remembering. This kind of outcome was soon replicated by other studies that included the effects of word frequency (Gardiner and Java, 1990), undivided versus divided attention, use of a digit-monitoring task (Gardiner and Parkin, 1990), and the picture superiority effect (Rajaram, 1993). The first study reporting dissociative effects among different subject populations was by Parkin and Walter (1992), who found that reports of remembering were greatly reduced in older compared with younger adults. This kind of outcome that was soon replicated by other studies that included patients with Alzheimer's disease (Dalla Barba, 1997), patients with schizophrenia (Huron et al., 1995), and amnesic patients (Knowlton and Squire, 1995).

2.17.4 Functional Dissociation

It is important to appreciate that functional dissociation between remembering and knowing cannot reliably be inferred from more familiar measures of recognition memory such as old versus new. To illustrate this, each of the following subsections of the chapter begins with a figure that simply shows overall proportions of correct responses from four different studies, and then goes on to show how those proportions were partitioned between remembering and knowing. This initial survey ignores false alarm rates, not because they are unimportant but because they do not much affect the conclusions to be discussed.

2.17.4.1 Experimental Manipulations

Figure 1 shows the proportions of study list items that were correctly identified as old from each of four studies involving different experimental manipulations. The first example is taken from a levels-of-processing study (Gardiner et al., 1996) in which participants had to either report a semantic associate for a presented word or to report any two letters not present in the word, and it shows a large levels-of-processing effect. In the second example, study list words were presented rapidly with the instruction to monitor the number of words that contained letters that were blurred (there were none), a task intended to encourage perceptual processing and discourage conceptual processing (Gregg and Gardiner, 1994). At test, half the words were presented in the same visual mode, and half were presented auditorily. Recognition was much more likely when study and test modes were the same. In the third example, participants studied a mixed list of words and pronounceable nonwords (Gardiner and Java, 1990), but this material manipulation had little effect on recognition. And in the final example, participants heard a set of musical phrases taken from folk songs in a culture with which they were unfamiliar (Polish, for English participants, and vice versa) either just once or on three successive study trials before the test (Gardiner and Radomski, 1999). Not surprisingly, the musical phrases were much more likely to be recognized following three study trials than following only one.

Table 2 summarizes the full partitioning of these recognition data between remember and know responses, and it illustrates four different kinds of outcome. The levels-of-processing effect occurred in remember but not know responses. The effects of study/test mode occurred in know but not remember responses. For words and nonwords, there was a crossover effect such that word recognition was accompanied by more remember than know responses, whereas nonword recognition was accompanied by more know than remember responses. But the effect of study trials on the recognition of musical phrases was similar for both responses, with increased remembering and increased knowing.

What is important about the pattern of results illustrated in **Table 2** is that it demonstrates what has been termed functional independence between remembering and knowing (Gardiner and Conway, 1999). That is, there are variables that affect remembering but not knowing; variables that affect knowing but not remembering; variables that have opposite effects on remembering and knowing; and variables that have similar effects on remembering and knowing. Experimental conditions can affect the two states of awareness, separately or jointly, in ways that cannot be inferred from the overall proportions of items that are correctly recognized.

These kinds of outcomes have been replicated many times in various different studies. Among many other examples, selective advantages to remembering have been found for intentional versus incidental learning (Macken and Hampson, 1993), for slow versus fast presentation rates (Dewhurst and

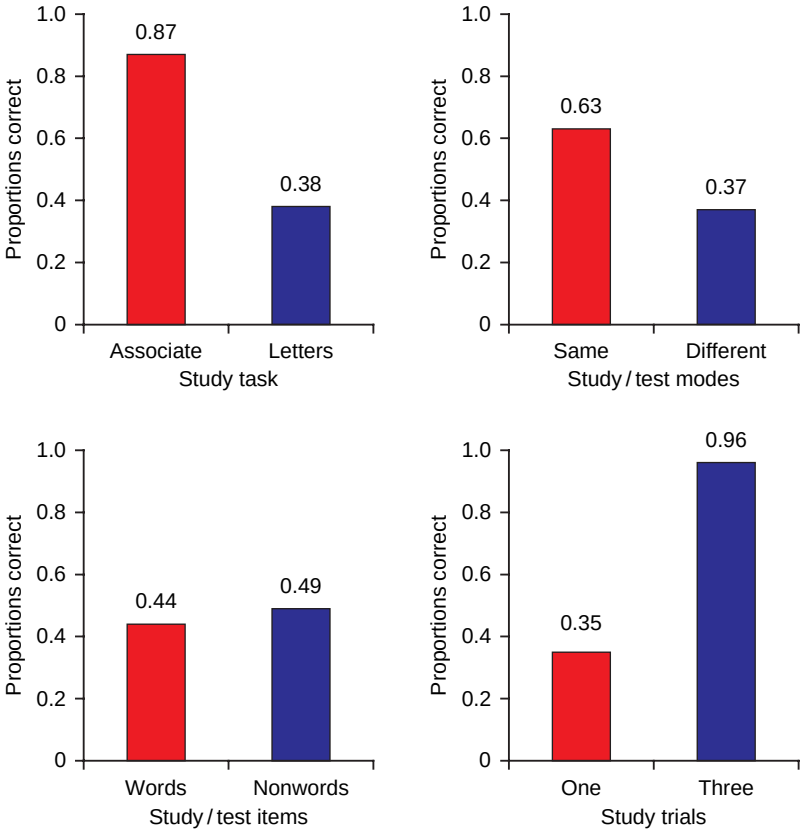

Figure 1 Effects of some experimental manipulations on overall recognition (correct 'old' response proportions). These examples, going clockwise from the top left-hand corner, are taken, respectively, from experiments by Gardiner JM, Java RI, and Richardson-Klavehn A (1996) How levels of processing really influences awareness in recognition memory. *Can. J. Psychol.* 50: 114–122; Gregg VH and Gardiner JM (1994) Recognition memory and awareness: A large effect of study-test modalities on "know" responses following a highly perceptual orienting task. *Eur. J. Cognit. Psychol.* 6: 137–147; Gardiner JM and Java RI (1990) Recollective experience in word and nonword recognition. *Mem. Cognit.* 18: 23–30; and Gardiner JM and Radomski E (1999) Awareness of recognition memory for Polish and English folk songs in Polish and English folk. *Memory* 7: 461–470; all figures used with permission.

Anderson, 1999), and for orthographically distinctive versus orthographically common words (Rajaram, 1998). Serial position effects occur in remembering and not in knowing, and prior recall tests boost remembering but do not boost knowing (Jones and Roediger, 1995).

Selective increases in knowing but not in remembering have been found to result from presenting identical (Rajaram, 1993) or associatively related (Rajaram and Geraci, 2000) test primes and from cohort activation in a preceding lexical decision task (Dewhurst and Hitch, 1997). Dividing attention by suppressing the processing of foveal information also selectively facilitates knowing (Mantyla and Raudsepp, 1996). Opposite effects on remembering and knowing have been found for massed versus spaced repetitions of study list items (Parkin and Russo, 1993); for the revelation effect, which occurs when gradually revealing words at test compared with presenting them normally (LeComte, 1995); and from encoding faces with respect to their similarity versus encoding them with respect to their distinctiveness (Mantyla, 1997). Parallel increases in both remembering and in knowing have also been found when manipulating response deadlines in recognition tests to compare speeded with unspeeded recognition decisions (Gardiner et al., 1999).

2.17.4.2 Special Populations

Figure 2 shows the proportions of study list items that were correctly identified as old from each of four studies involving different special populations. The first example is taken from a study involving amnesic

Table 2 Effects of some experimental manipulations on remembering and knowing (for correct 'old' response proportions)

Manipulation	*Condition*	*Remember*	*Know*
Study tasks	Associate	.72	.15
	Letters	.18	.20
Study/test modes	Visual/visual	.11	.52
	Visual/auditory	.10	.27
Study/test items	Words	.28	.16
	Nonwords	.19	.30
Study trials	One	.14	.21
	Three	.37	.32

These examples are taken, respectively, from experiments by Gardiner JM, Java RI, and Richardson-Klavehn A (1996) How levels of processing really influence awareness in recognition memory. *Can. J. Psychol.* 50: 114–122; Gregg VH and Gardiner JM (1994) Recognition memory and awareness: A large effect of study-test modalities on ''know'' responses following a highly perceptual orienting task. *Eur. J. Cognit. Psychol.* 6: 137–147; Gardiner JM and Java RI (1990) Recollective experience in word and nonword recognition. *Mem. Cognit.* 18: 23–30; and Gardiner JM and Radomski E (1999) Awareness of recognition memory for Polish and English folk songs in Polish and English folk. *Memory* 7: 461–470.

patients (Schacter et al., 1997a). In the second example, the study compared the performance of younger with older adults (Perfect et al., 1995). The third example is taken from the study by Huron et al. (1995) involving schizophrenic patients. And the final study involved high-functioning adults with autistic spectrum disorders or Asperger's syndrome (Bowler et al., 2000). In the first three of these special populations, recognition performance was impaired to varying degrees, but there was little difference between the performance of adults with autistic spectrum disorders and that of an appropriately matched control group.

Table 3 summarizes the full partitioning of these recognition data between remember and know responses. The pattern of results here is rather different from that in **Table 2**, but it is clear that the two states of awareness differ in different populations in ways that cannot be inferred from the overall proportions of items that are correctly recognized. Remembering but not knowing was greatly reduced in the amnesic patients. Remembering was also greatly reduced in older compared with younger adults, but this reduction was largely offset by increased knowing. Schizophrenic patients also remembered less than an appropriately matched control group, but there was little difference in the amount of knowing. And, though overall recognition was much the same for adults with autistic spectrum disorders as for the control group, this masked a trade-off between reduced remembering and increased knowing.

Although these kinds of outcomes have also been replicated in other studies, there is considerable variability among some of these studies. Other studies involving amnesic patients have found reduced knowing as well as reduced remembering (Knowlton and Squire, 1995). Other studies involving older adults found increased knowing, as well as reduced remembering (Parkin and Walter, 1992), particularly when encoding was not controlled by specific study tasks (cf. Perfect et al., 1995). Further studies have confirmed the selective deficit in remembering in schizophrenic patients (Danion et al., 1999) and in adults with autistic spectrum disorders, though in the latter case this deficit is not always accompanied by increased knowing (Bowler et al., 2007).

Other special populations in which remembering and knowing have been investigated include epileptic patients with temporal lobe lesions. Blaxton and Theodore (1997) found that patients with left temporal lobe lesions reported far more knowing than remembering, whereas patients with right temporal lobe lesions reported far more remembering than knowing. There is also some evidence of reduced remembering in recognition memory for threat-related words in clinical anxiety states (Mogg et al., 1992). Alcohol (Curran and Hildebrandt, 1999) and other drugs such as lorazepam (Curran et al., 1993) and midazolam (Hirshman et al., 2002) can also adversely and selectively affect remembering, but emotionally negative stimuli tend to be better remembered than positive or neutral stimuli (Ochsner, 2000).

2.17.5 Major Theories

Such findings have attracted considerable theoretical interest, and at least four major theories were initially advanced to account for them.

2.17.5.1 Episodic and Semantic Memory Systems

According to Tulving (1983, 1985), remembering is an expression of autonoetic consciousness and hence retrieval from episodic memory, and knowing is an expression of noetic consciousness and hence retrieval from semantic memory. Thus, retrieval from both systems contributes to performance in recognition

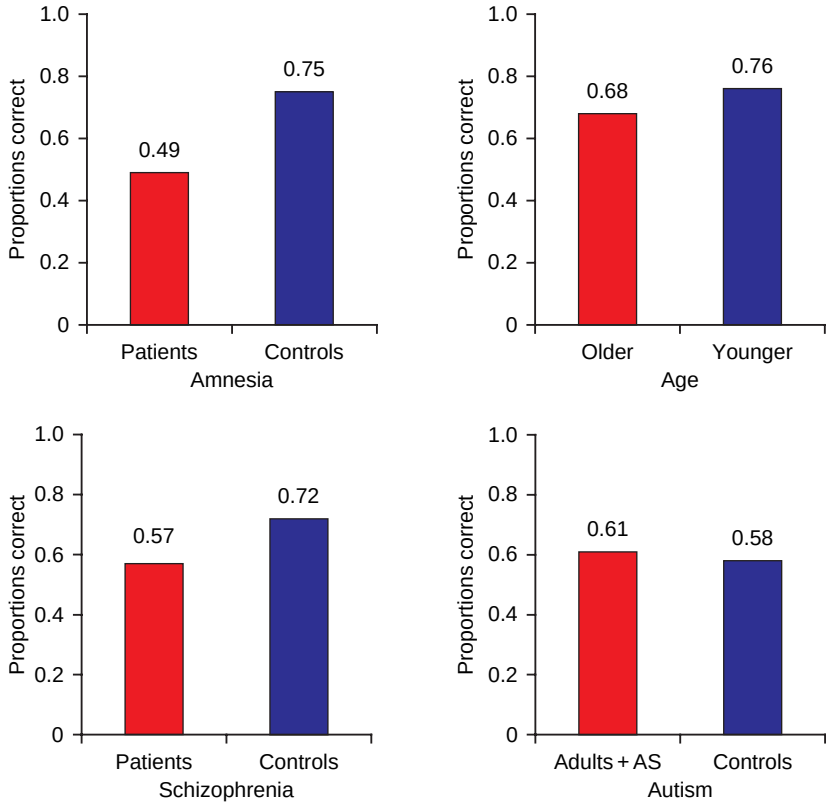

Figure 2 Differences between recognition (correct 'old' response proportions) in some special populations. AS stands for Asperger's syndrome, a mild form of autism. These examples, going clockwise from the top left-hand corner, are taken, respectively, from experiments by Schacter DL, Koutstaal W, Johnson MK, Gross MS, and Angell KE (1997a) False recollection induced by photographs: A comparison of older and younger adults. *Psychol. Aging* 12: 203–215; Perfect TJ, Williams RB, and Anderton-Brown C (1995) Age differences in reported recollective experience are due to encoding effects, not response bias. *Memory* 3: 169–186; Huron C, Danion JM, Giacomoni F, Grange D, Robert P, and Rizzo L (1995) Impairment of recognition memory with, but not without, conscious recollection in schizophrenia. *Am. J. Psychiatry* 152: 1737–1742; and Bowler DM, Gardiner JM, and Grice S (2000) Episodic memory and remembering in adults with Asperger's syndrome. *J. Autism Dev. Disord.* 30: 305–316; all figures used with permission.

tests, among others. Tulving (1995) additionally proposed an SPI model of relations between these systems such that that encoding into semantic and episodic systems is serial (S), storage is parallel (P), and retrieval is independent (I). Given some minimal registration of the occurrence of an event, that event may only be stored in the semantic system. Given more attention at encoding and more conscious control, the event may be further encoded into episodic memory. Events may be stored in both systems but retrieved independently from them. Tulving (1993) also proposed a coordination hypothesis, which concerns the relation between awareness at encoding and awareness at retrieval. According to this hypothesis, the retrieval of information from a system is possible only at a level of awareness that does not exceed the level of awareness achieved at encoding. In other words, it is not possible for the retrieval of information that has only been encoded into the semantic system to give rise to autonoetic awareness, however much time and conscious effort goes into the retrieval attempt.

Much of the evidence is consistent with this account, but more critical to it is recent evidence concerning the underlying neuroanatomical substrates of episodic and semantic systems. For example, in an event-related potentials (ERP) study, Mangels et al. (2001) showed that whereas some minimal level of encoding (with divided attention) is sufficient to lead to knowing in recognition memory, remembering depends on more

Table 3 Differences between remembering and knowing in some special populations (for correct "old" response proportions)

Condition	*Group*	*Remember*	*Know*
Amnesia	Patients	.21	.28
	Controls	.50	.25
Age	Older adults	.17	.51
	Younger adults	.53	.23
Schizophrenia	Patients	.23	.34
	Controls	.39	.33
Autism	Adults with Asperger's	.36	.25
	Controls	.47	.11

These examples are taken, respectively, from experiments by Schacter DL, Koutstaal W, Johnson MK, Gross MS, and Angell KE (1997a) False recollection induced by photographs: A comparison of older and younger adults. *Psychol. Aging* 12: 203–215; Perfect TJ, Williams RB, and Anderton-Brown C (1995) Age differences in reported recollective experience are due to encoding effects, not response bias. *Memory* 3: 169–186; Huron C, Danion JM, Giacomoni F, Grange D, Robert P, and Rizzo L (1995) Impairment of recognition memory with, but not without, conscious recollection in schizophrenia. *Am. J. Psychiatry* 152: 1737–1742; and Bowler DM, Gardiner JM, and Grice S (2000) Episodic memory and remembering in adults with Asperger's syndrome. *J. Autism Dev. Disord.* 30: 305–316.

extensive brain activity, including sustained interaction of frontal and posterior regions. Other studies (to be discussed later) have provided evidence that implicates hippocampal as well as frontal regions of the brain in remembering (Eldridge et al., 2000; Wheeler and Stuss, 2003), and there is increasing evidence of functional dissociations between remembering and knowing at the level of the brain (Wheeler and Buckner, 2004). As Tulving (2002) put it in the title of a recent review, episodic memory now extends "from mind to brain."

2.17.5.2 Memory Process Accounts

Among the most prominent alternative theories are those that distinguish between two kinds of processes, rather than two memory systems.

2.17.5.2.1 Recollection and familiarity

Dual-process models of recognition memory distinguish between recollection and familiarity processes, each of which is assumed to give rise to corresponding experiences of recollection and familiarity (Mandler, 1980; Jacoby, 1991). These two processes are assumed to be independent and to vary in the extent to which they are consciously controlled. Recollection is generally thought to be a relatively slow, effortful process that depends on conscious control, whereas familiarity is thought to be a relatively fast, automatic process that does not depend on conscious control. Because experiences of recollection and familiarity are indicated by remember and know responses, the remember-know paradigm is also a procedure that can be used to test the relative contributions these two processes make to recognition. The independence assumption means that some recognition responses are based jointly on recollection and familiarity, and so an independent remember-know model is used to provide estimates of the two processes (Yonelinas and Jacoby, 1995). In this model, remember responses are taken to provide a relatively direct estimate of the recollection process, but the familiarity process is estimated by dividing the proportions of know responses by one minus the proportions of remember responses. For a version of the paradigm that allows independence between remember and know responses, see Higham and Vokey (2004).

The use of this procedure to provide such process estimates has proved to be quite controversial (Richardson-Klavehn et al., 1996; Jacoby et al., 1997). Nonetheless, in a comprehensive review Yonelinas (2002) showed that there is quite good agreement between the conclusions drawn from this procedure and from other procedures used to provide estimates of recollection and familiarity, namely, the process-dissociation procedure (Jacoby, 1991) and receiver operating characteristics (ROCs). Moreover, although the processes of recollection and familiarity offer an alternative theory to that offered by the distinction between episodic and semantic memory systems, there is in broad terms a great deal of convergence between these two accounts. Even know responses, which have generated more controversy, have a parallel meaning from each theoretical perspective. In dual-process models, they indicate familiarity in the absence of recollection. In the systems approach, they indicate semantic memory in the absence of episodic memory. But theorists who have adopted this process account are primarily concerned with the underlying processes rather than with remembering and knowing *per se*, whereas theorists who have taken the systems approach focus more on the actual states of awareness. So one important difference between the two approaches is whether the primary data are regarded as estimates inferred from remember and know responses or remembering and knowing as such.

2.17.5.2.2 *Distinctiveness and fluency*

Results of earlier studies using the remember-know paradigm suggested that remember and know responses were influenced, respectively, by conceptual and perceptual variables and hence by conceptual and perceptual processes (Rajaram, 1993), thereby linking them with the transfer-appropriate processing framework (Roediger et al., 1989). However, results from more recent studies forced a revision of this view, as some perceptual variables were found to influence remembering but not knowing, and some conceptual variables were found to influence knowing but not remembering. Rajaram (1996), for example, found that a size congruency effect, that is, superior recognition memory for pictures presented in the same size at study and test rather than in alternative sizes, occurred only in remembering. And Mantyla (1997) found that grouping faces that seemed similar into several conceptual categories, a relational task that depends on the use of schema in semantic memory, increased knowing. He also found that rating the facial distinctiveness of different faces increased remembering.

Such findings led to the proposal of a distinctiveness/fluency framework according to which remembering benefits from the distinctiveness of the processing and knowing benefits from its fluency, regardless of whether that processing is conceptual or perceptual (Rajaram, 1996, 1999). It is important to note that in this approach, the distinctiveness or fluency of processing is not inferred from remember and know responses but independently based on other theoretical considerations. Though this approach offers a different theoretical perspective, nonetheless in some respects it complements memory systems and dual-process models. Distinctiveness implies greater attention and more elaborative processing, which will increase encoding into episodic memory or enhance a recollection process. Fluency implies less attention and minimal processing, which may increase encoding into semantic but not into episodic memory or enhance a familiarity process.

2.17.5.3 Signal Detection Models

The three foregoing theories all converge on the conclusion that remembering and knowing reflect two distinct underlying components of memory, though they differ in their characterization of those components. The possibility that remembering and knowing might simply reflect a single memory component has been raised by signal detection models. That remembering and knowing might simply map onto single-trace strength, with higher or lower degrees of confidence, had been discounted on the basis of some early studies in which sure versus unsure recognition responses were shown to yield different patterns of results to remember versus know responses (Gardiner and Java, 1990; Parkin and Walter, 1992; Rajaram, 1993). However, Donaldson (1996) showed that a single-trace signal detection model could provide an approximate fit to results from the remember-know paradigm, and this approach has recently been followed up by other theorists (Hirshman and Henzler, 1998; Dunn, 2004; Wixted and Stretch, 2004). The essential claim is that remembering and knowing reflect decision processes rather than memory and are simply a matter of confidence. A more stringent response criterion is set for remember responses, and a more lenient criterion, corresponding with the overall criterion for recognition, includes know responses.

This approach has been supported by meta-analyses of results from many different studies as well as by results from individual experiments, but it has also been strongly criticized on various grounds, and there are quite technical arguments involving the appropriateness of the various different assumptions and measures that can be used in signal detection models. The assumptions and measures used by Donaldson (1996) have subsequently been shown to support a dual-component interpretation, not his original model, by yielding higher estimates of trace strength when those estimates are derived from both know and remember responses rather than from remember responses alone (Gardiner and Gregg, 1997; Gardiner and Conway, 1999; Gardiner et al., 2002). If remembering and knowing merely reflect different response criteria such estimates should be the same. But the assumptions and measures that yield those outcomes have been discredited in favor of others that have been shown to support a one-dimensional signal detection model (Dunn, 2004; Macmillan et al., 2005).

2.17.6 Further Empirical Extensions and Theoretical Issues

In recognition memory, noetic awareness usually corresponds with familiarity in the sense of some recent but unremembered encounter with the test item. But with respect to general knowledge in semantic memory, noetic awareness does not refer

to some recent but unremembered encounter but rather to "just knowing" that something is so. Conway et al. (1997) gave UK undergraduate students forced-choice tests of knowledge acquired in various courses immediately at the end of the courses and again some months later at the end of the academic year. In these tests, students reported whether they remembered their answer, just knew it, chose it because it seemed more familiar (in the sense of having been encountered recently), or had simply guessed. In the initial tests, top-scoring students reported more remembering than students with lower scores. In the final tests there was a 'remember-to-know' shift. Those same top-scoring students reported more just knowing the answers than students with lower scores. Lower-scoring students showed a similar remember-to-know shift, but it was less pronounced. There were no such trends in reported familiarity or guessing. These findings have been replicated in a similar study carried out in Australia (Herbert and Burt, 2001). They illustrate the role of remembering in the acquisition and schematization of knowledge. Initially, remembering the learning episodes is helpful, but over time, with further study and coursework, the ability to remember the original learning episodes is lost and knowledge acquired from them becomes schematized in semantic memory.

But remembering may not be necessary for the acquisition of knowledge. There are amnesic patients who seem to have acquired normal semantic memory knowledge despite showing little or no evidence of any experiences of remembering. One such case, initially reported by Vargha-Khadem et al. (1997), is that of Jon, a young adult with early-onset developmental amnesia caused by selective bilateral damage to the hippocampus. Jon has above-average intellectual abilities, and he has acquired good general knowledge, including good language skills. Nonetheless, though he understands the distinction between remembering and knowing and will follow instructions for remember and know responses in recognition tests, there is no evidence that he experiences remembering in such tests (Baddeley et al., 2001; Gardiner et al., 2006a). When asked to describe what it was that he remembered about those items he claimed to remember, Jon could only say again that he remembered them. His recognition performance was not enhanced by task enactment compared with reading a phrase that described an action task. Task enactment normally boosts remembering (Engelkamp, 1998). Nor did Jon's ERPs show the normal late positive component (LPC) that has been associated with remembering; they did show an earlier negative component (the so-called N400 effect) that has been associated with knowing (Rugg et al., 1998). Jon also claimed to remember general knowledge facts that he knew prior to a laboratory study of how he acquires novel facts, unlike participants in a control group who (correctly) claimed just to know them. Jon did successfully learn quite a few of the novel facts that he knew prior to an unpublished study, albeit at a greatly reduced rate compared with that of the control group. This reduced learning performance reflects Jon's inability to use remembering of the learning episodes as an aid to knowledge acquisition.

At the other extreme, knowledge of an event that has occurred recently but which cannot be remembered also gives rise to noetic awareness and represents a minimal level of encoding in semantic memory. Encoding at this level can be fostered by having very rapid, perceptually oriented study conditions of the sort used by Gregg and Gardiner (1994; see also Gardiner and Gregg, 1997) or by having divided instead of full attention at study (Mangels et al., 2001). Under these conditions, there are at least some effects in memory that occur in knowing instead of remembering.

Shown in **Figure 3** is an example of the size congruency effect in picture recognition memory with either full attention or divided attention at study (Gardiner et al., 2001). Divided attention reduced recognition performance but did not influence the size congruency effect. **Table 4** includes the partitioning of these data between remember and know responses. With full attention, the size congruency effect occurred in remembering, replicating results first reported by Rajaram (1996; see also Yonelinas and Jacoby, 1995).

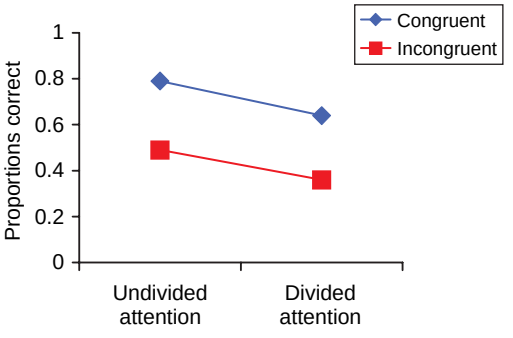

Figure 3 Effects of attention and picture size congruence on overall recognition (correct 'old' response proportions). Adapted from Gardiner JM, Gregg VH, Mashru R, and Thaman M (2001) Impact of encoding depth on awareness of perceptual effects in recognition memory. *Mem. Cognit.* 29: 433–440, Table 1, with permission from The Psychonomic Society.

Table 4 Further effects (shown in bold) of some experimental manipulations on remembering and knowing (for correct 'old' response proportions)

Manipulations	*Study/test congruency*	*Remember*	*Know*
Full attention	Same size	**.53**	.26
	Different size	**.37**	.27
Divided attention	Same size	.15	**.34**
	Different size	.12	**.22**
Full attention (with words)	Same voice	**.44**	.31
	Different voice	**.34**	.33
Divided attention (with nonwords)	Same voice	.18	**.38**
	Different voice	.16	**.28**
Longer study time	Same size	**.42**	.14
	Different size	**.32**	.14
Shorter study time	Same size	.22	**.28**
	Different size	.19	**.18**

These examples are taken, respectively, from experiments by Gardiner JM, Gregg VH, Mashru R, and Thaman M (2001) Impact of encoding depth on awareness of perceptual effects in recognition memory. *Mem. Cognit.* 29: 433–440; Karayianni I and Gardiner JM (2003) Transferring voice effects in recognition memory from remembering to knowing. *Mem. Cognit.* 31: 1052–1059; and Nega C (2005) Perceptual effects and recollective experience in face recognition. *Exp. Psychol.* 52: 224–231.

With divided attention, however, the effect occurred in knowing. Similar results, also shown in **Table 4**, obtain for congruency of male or female speaker's voice (Karayianni and Gardiner, 2003) and, with a manipulation of study time duration rather than of attention, for facial size congruency (Nega, 2005). For reasons that remain unknown, other similar conditions do not reveal this transfer of effects from one state of awareness to the other. Curran and Hildebrandt (1999), for example, found that although alcohol selectively reduced remembering, the generation effect (better memory for study list words participants generate rather than read) remained in remembering. It did not emerge in knowing.

That following relatively impoverished study conditions some effects occur in knowing instead of in remembering supports the SPI model of relations between semantic and episodic systems (Tulving, 1995). Impoverished encoding conditions reduce the more elaborative and consciously controlled encoding necessary for episodic memory, but do not much affect the more automatic and less consciously controlled encoding sufficient for semantic memory. By the same token, these findings support the distinctiveness/fluency framework (Rajaram, 1996) and confirm that the distinction between conceptual and perceptual processing is orthogonal to the distinction between the two states of awareness, because similar perceptual effects can occur in either state. Impoverished encoding conditions presumably reduce the opportunity for more distinctive processing without much affecting processing fluency.

Another general theoretical issue concerns the relations between consciousness of memory, in the sense of remembering versus knowing, and consciousness of task control, whatever the task may be. It has been rather generally assumed that whereas remembering is consciously controlled, knowing is not. These assumptions have not only been central to dual-process models, according to which recollection is a controlled process and familiarity is an automatic process, but they have also been made in relation to episodic and semantic memory systems. Access to episodic memory usually requires conscious effort, whereas access to semantic memory tends to be automatic (Tulving, 1983).

Conscious control at encoding can easily be manipulated by comparing full with divided attention, as in **Table 4**. Such manipulations are less effective for retrieval in recognition tests but conscious control there can be effectively manipulated by using a response deadline procedure that compares speeded with unspeeded recognition. Because more automatic retrieval is thought to occur more rapidly than consciously controlled retrieval, fast recognition decisions are assumed to be more automatic and slower ones more controlled. The data summarized in **Table 5** show the effects of fast (under 700 ms) compared with slow (over 3000 ms) recognition decisions on voice congruence effects following either full attention or divided attention at encoding. In the preceding examples of results, false alarms, and also guess responses (if included in the paradigm), were omitted, but in **Table 5** the complete results are shown.

With full attention at encoding, the voice congruence effect occurred in remembering, and with divided attention at encoding the effect occurred in knowing, as found by Karayianni and Gardiner (2003). In neither case was the voice congruence effect influenced by response deadline. This suggests that though the state of awareness with which the voice congruence effect is associated depends on the degree of conscious control at encoding, it does not depend on the degree of conscious control at retrieval. That the effect in knowing remained in knowing even with slower more effortful recognition decisions is consistent with

Table 5 Mean proportions of responses for fast and slow recognition as a function of voice congruence and following full or divided attention at study

	Congruent		*Incongruent*		*New items*	
Attention/response category	*Fast*	*Slow*	*Fast*	*Slow*	*Fast*	*Slow*
Full attention						
Old	.48	.73	.36	.61	.11	.16
Remember	.29	.45	.16	.28	.03	.04
Know	.16	.24	.15	.28	.05	.07
Guess	.03	.04	.04	.05	.03	.06
Divided attention						
Old	.46	.69	.36	.62	.13	.17
Remember	.13	.24	.13	.24	.03	.03
Know	.31	.41	.21	.31	.07	.08
Guess	.02	.04	.03	.07	.03	.05

Adapted from Gardiner JM, Gregg VH, and Karayianni I (2006b) Recognition memory and awareness: Occurrence of perceptual effects in remembering or in knowing depends on conscious resources at encoding, but not at retrieval. *Mem. Cognit.* 34: 227–239, Tables 2 and 4. with permission from The Psychonomic Society.

Tulving's (1993) coordination hypothesis, according to which however much time and effort goes into the retrieval attempt, the resulting state of awareness cannot exceed the level of awareness achieved at encoding. That the effect in remembering remained in remembering, even with faster more automatic recognition decisions, is consistent with the view that remembering can be more automatic, as well as being more effortful and consciously controlled. Finally, that knowing, as well as remembering, generally increased with slow recognition decisions is consistent with the view that knowing, as well as remembering, can be more effortful and consciously controlled, as well as being more automatic. Similar results were obtained by Konstantinou and Gardiner (2005) in a recognition study of famous faces (see too, Gardiner et al., 1999). There is also evidence that when response times are measured, instead of being manipulated, recognition decisions associated with remember responses are made more rapidly than those associated with know responses (Dewhurst and Conway, 1994; Dewhurst et al., 2006). Those results imply that, if anything, it is remembering that is more automatic and knowing that is more consciously controlled.

There is other evidence that remembering and knowing may both be more consciously controlled, or more automatic, depending on encoding and retrieval conditions, from studies in which word-stem cued recall (e.g., DEF. . . .: What study list word does this remind you of?) is compared with incidental word-stem completion (e.g., DEF. . . .: Complete this word stem with the first word that comes to mind). In such comparisons, generating compared with reading words at study leads to superior recall (the generation effect), but the words that were read at study are more likely to be those that come to mind in incidental word-stem completion (the priming effect). Java (1994; see also Java, 1996) replicated this reversal of the generation effect and showed that both tests gave rise both to remembering and to knowing. Moreover, though the generation effect in recall occurred in remembering, the read superiority effect in incidental word-stem completion was restricted to words participants reported as not being in the study list. Such results underscore the need for a distinction between the voluntary (intended) or involuntary (unintended) retrieval of study list words, as evidenced by the reversal of the generation effect, and awareness of memory, as evidenced by remember and know responses. This distinction between retrieval volition and awareness of memory has been discussed in some detail by Richardson-Klavehn et al. (1996) and it has been supported by the results of other empirical studies (e.g., Richardson-Klavehn and Gardiner, 1996, 1998; see also Richardson-Klavehn et al., 2002).

The guess responses shown in **Table 5** are fairly typical, especially when, as here, the instructions discourage guessing. Guess response rates to study list items have generally been found to be little different from those for unstudied items, that is, false alarms (Gardiner and Conway 1999; Gardiner et al., 2002). Hence, when participants report guessing, they do seem to be guessing. There are also usually more know false alarms than remember false alarms, again as shown in **Table 5**. False alarms

are by definition inaccurate with respect to identifying previously studied items but they are not necessarily inaccurate with respect to indicating states of awareness, nor should they be if reports of those states of awareness are valid. It is perfectly natural that participants may have some genuine experiences of remembering and some genuine experiences of knowing when deciding about test items that did not occur in a study list. Moreover, the extent to which people have such genuine experiences will naturally vary depending on experimental conditions.

Experimental conditions have been deliberately designed to manipulate the extent to which people have such 'false' memories, but genuine experiences include those intended to foster illusions of memory. Roediger and McDermott (1995) reintroduced such experimental conditions in revising a converging associates paradigm originally used by Deese (1959). In this paradigm, participants study lists of words that are all highly associated with target associates that are not presented (e.g., study 'bed, rest, awake, tired, dream, . . . etc.,' for the target associate 'sleep'). In one of their experiments, Roediger and McDermott (1995) gave participants either immediate free recall tests or an arithmetic filler task before giving them a recognition test that included the target associate (termed the critical lure) as well as other lure items unrelated to the study lists.

Participants recalled the critical nonpresented word for 55% of the lists, which proportionally approximated the rate of recall for the words from the middle of the studied lists. The recognition results are shown in **Table 6**. The critical lure was recognized to practically the same extent as were the study list words. Moreover, recognition of the critical lure was associated primarily with remember responses, though know responses too were somewhat greater for the critical lure than for other unrelated nonstudied words.

Table 6 Mean proportions of responses for studied words, critical lures, and nonstudied words

Item type and condition	*Old*	*Remember*	*Know*
Studied			
Study + recall	.79	.57	.22
Study + arithmetic	.65	.41	.24
Nonstudied	.11	.02	.09
Critical lure			
Study + recall	.81	.58	.23
Study + arithmetic	.72	.38	.34
Nonstudied	.16	.03	.13

Reprinted from Roediger HL and McDermott KB (1995) Creating false memories: Remembering words not presented in lists. *J. Exp. Psychol. Learn. Mem. Cognit.* 21: 803–814, Table 2, with permission from The American Psychological Association.

Such striking illusions of memory have since been replicated many times. For example, Düzel et al. (1997) investigated the ERP correlates of remembering and knowing using the converging associates paradigm. They found that the patterns of neural activity for remembering and knowing when recognizing studied words and when falsely recognizing critical lures were indistinguishable. The patterns of neural activity were predicted by the states of awareness not by the accuracy of the recognition judgment. Schacter et al. (1997a) extended the paradigm by also presenting lists of perceptually similar words (words that all looked and sounded alike). They found that with perceptually rather than conceptually induced false recognition, the recognition of critical lures was mainly associated with knowing, rather than with remembering. And in a long-term diary study, Conway et al. (1996) also found that false recognition of lures that were plausible diary entries (altered or false records of events and thoughts) was associated mainly with knowing, especially for thoughts. Other memory illusions in knowing, as well as in remembering, have been reported (see, e.g., Dewhurst and Hitch, 1997).

Illusions of memory can be understood within an attributional approach to memory (Jacoby et al., 1989). Experiences of remembering or of knowing are attributed to particular circumstances or situations, sometimes correctly, sometimes not. Thus, illusions of memory, and false alarms generally, may reflect genuine experiences of memory that are then misattributed to the prior occurrence in a studied list of the items that gave rise to those experiences. Such attributions implicate source memory and reality monitoring, and they have been widely studied within a source-monitoring framework (Johnson et al., 1993). A number of these studies have used memory characteristics questionnaires to provide a more fine-grained analysis of differences between remembering and knowing, both for veridical and for illusory memories (Mather et al., 1997; Schacter et al., 1997b). In general, remembering when veridical seems characterized by the availability of sharper perceptual detail and sometimes by less affect than when illusory. A multinomial model of multidimensional source information that incorporates remember and know responses has been proposed by Meiser and Broder (2002), who replicated previous findings that memory for source is more accurate with

remembering than with knowing (Conway and Dewhurst, 1995; Dewhurst and Hitch, 1999). They also found that source memory for different contextual features was stochastically related for remembering but independent for knowing. Creating an episode that will be remembered involves the binding together of different contextual features, but the occurrence of each of those individual features may be known without retrieving the episode. In a somewhat similar vein, Sikstrom and Gardiner (1997) found that whereas the words that were remembered from the same study list in successive tests of recognition and cued recall (the 'recognition-failure' paradigm) were stochastically related, there was no such relationship between the words that were known in each test. People can also predict which state of awareness they will experience when recognizing items that they cannot recall (Hicks and Marsh, 2002).

There is evidence that remember and know responses are influenced by test–list context. Bodner and Lindsay (2003) found that how much people reported remembering and the relative weighting they assigned to different aspects of what they remembered were affected by whether word lists that had been studied at a medium level of processing were tested along with other words that had been studied at either a shallow or deep level of processing. When asked to report their strongest recollections, the main recollections were of list source (the level of processing), of some thought or association, and of some visual image. The proportions of these different recollections given as the strongest recollection accompanying remember responses varied considerably as a function of test–list context. Importantly, performance in direct tests of memory for source was not influenced by study–test context. These findings support a functional view that places some emphasis on the uses to which mental experiences of memory are put, and how people come to define remembering and knowing under various task demands and conditions, as well as on the experiences as such (see also Whittlesea, 2002a,b).

Clearly, people make decisions about their experiences of memory, but the idea that remembering and knowing just reflect decision making and differ only quantitatively, not qualitatively, is misconceived. This is the claim made in a signal detection model that assumes a single trace strength with different response criteria for remembering and for knowing (Donaldson, 1996; Hirshman and Master, 1997; Hirshman, 1998; Inoue and Bellezza, 1998). This model can simulate dissociative effects between remembering and knowing by assuming that different experimental conditions affect the placement of the response criteria. Bodner and Lindsay (2003) are among others (e.g., Gardiner et al., 1998b) who have strongly criticized this model on the grounds that it provides no explanation as to why the placements of response criteria are affected by different experimental conditions and in different populations in the ways that they are. Nor do changes in criteria provide any account of how such different experiences of consciousness come to mind, only of how, once they have come to mind, decision processes may operate on responses based on them. Moreover, experimental manipulations of response criteria run the risk of invalidating the responses. Participants are given somewhat contradictory instructions when told to respond with very lenient or with very strict response criteria but at the same time only to respond according to the definitions given in **Table 1**. This may be partly why false alarm rates have been exceptionally large in some studies that have manipulated response criteria and why one such study found effects of response criteria on both know and remember responses (Hirshman and Henzler, 1998), another study on know but not remember responses (Strack and Forster, 1995), and other studies only on guess responses (Gardiner et al., 1997, 2002). There is also some evidence of small effects on response criteria depending on whether remember and know responses are given after an old/new responses, or in parallel, that is, remember or know or new. The latter, one-stage procedure, leads to a more lenient response bias (Hicks and Marsh, 1999; Eldridge et al., 2002; Gardiner et al., 2005).

Though studies in which confidence judgments have been directly compared with remember and know responses as, for example, by substituting sure and unsure judgments for those responses, have consistently found that the confidence judgments yield different patterns of results (e.g., Gardiner and Java, 1990; Perfect et al., 1995; Mantyla, 1997; Holmes et al., 1998; Gardiner and Conway, 1999; Rajaram et al., 2002), both Dunn (2004) and Wixted and Stretch (2004) have shown how differences between remember/know responses and sure/unsure judgments are not inconsistent with the signal detection model, which can fit these data too. Wixted and Stretch (2004) suggested that rather than representing a single process, the strength dimension might represent the sum of recollection and familiarity. They also listed various findings that are consistent with this model, such as the finding that

in meta-analyses of different experiments, remember and know hit rates and false alarm rates are correlated. But one could list other findings that are inconsistent with this model, which include evidence that remembering and knowing may differ substantially in the absence of any differences in trace strength, as measured from overall recognition scores (Conway et al., 2001; cf. Hirshman and Lanning, 1999) and evidence that the ROC curves predicted by this model have the wrong slope (Rotello et al., 2004).

A two-dimensional signal detection model proposed by Rotello et al. (2004) is more in keeping with Tulving's (1985) original proposal. According to their sum-difference (S) theory (T) of remembering (RE) and (A) knowing (K), both remembering and knowing contribute to the sum of the overall trace strength, on which old-new judgments are based. Remember and know responses, however, are based on a weighted difference between the two contributing dimensions. Rotello et al. showed how STREAK can account for new ROC curves, including those derived not only from confidence in old-new judgments but also confidence *in* remember and know responses (see too, Rotello and Macmillan, 2005).

Word frequency effects in recognition memory have also proved controversial theoretically. The discovery that the superior recognition of low-compared with high-frequency words occurred in remembering, not in knowing (Gardiner and Java, 1990), ruled out earlier suggestions that this effect was due to greater increments in familiarity (Mandler, 1980). Since then, other kinds of dual-process accounts of word frequency effects using the remember-know paradigm have been developed (Guttentag and Carroll, 1997; Joordens and Hockley, 2000; Reder et al., 2000). These accounts are specifically directed at the word-frequency mirror effect, that is, the finding that whereas there are more correct old judgments for low- than for high-frequency words, there are also more incorrect new judgments for high- than for low-frequency words. According to these theories, the low-frequency advantage in correct old judgments arises from their greater distinctiveness in the experimental context, which boosts recollection and hence occurs in remembering. In contrast, the finding of more incorrect new judgments for high- than for low-frequency words is attributed to greater semantic activation from their greater preexperimental familiarity. Hence this effect should occur in knowing. Moreover, for the same reason, old high-frequency words should also give rise to more know responses than the old low-frequency words. Reder et al. (2000) found good support for these predictions, and they developed a computational model (SAC: Source of Activation Confusion) to account for them. This model distinguishes between word or concept nodes and episode nodes, the activation of which respectively gives rise to familiarity and to recollection, hence to know and remember responses. See Diana et al. (2006) for further discussion of this model.

Joordens and Hockley (2000) also proposed a similar dual-process account, though without the aid of a computational model. Some of the effects they found are illustrated in **Table 7**. Furthermore, Gregg et al. (2006) found that with a divided attention task at study there was a high-frequency advantage not simply in the number of know responses (i.e., both hits and false alarms), as found in previous studies, but in their accuracy (i.e., in corrected recognition scores). This outcome suggests that under at least some circumstances that reduce remembering, the high-frequency advantage in knowing can be driven more by experimental than by preexperimental familiarity.

Further support for this kind of dual-process account of word frequency effects was found by Hirshman et al. (2002), who used midazolam to induce amnesia with the assumption that this drug would have larger effects on recollection than familiarity. The effects of the drug were to remove the usual low-frequency advantage in remembering the old words but to leave the high-frequency advantage to both old and new words unaffected, with the result that the traditional word frequency effect – higher hit rates for the low frequency words – was reversed (see also Balota et al., 2002).

Table 7 Mean proportions of responses for low- and high-frequency words

	Old items		*New items*	
Response category	*High freq.*	*Low freq.*	*High freq.*	*Low freq.*
Immediate test				
Old	.83	.90	.12	.08
Remember	.60	.68	.04	.02
Know	.23	.22	.08	.06
Delayed test				
Old	.63	.68	.26	.18
Remember	.36	.47	.08	.06
Know	.27	.21	.19	.12

Adapted from Joordens S and Hockley WE (2000) Recollection and familiarity through the looking glass: When old does not mirror new. *J. Exp. Psychol. Learn. Mem. Cognit.* 26: 1534–1555, Table 1, with permission from The American Psychological Association.

However, Malmberg et al. (2004) have shown that these results, and by implication other findings taken to support dual-process accounts, are also consistent with a variety of single-process, retrieving effectively from memory models (Shiffrin and Steyvers, 1997) that, rather like signal detection models and other global models of memory, assume that recognition is based on a continuous random variable that may be conceptualized as trace strength or familiarity. Thus, here too data that seem to support dual-process accounts may also be consistent with single-process accounts, though, as Malmberg et al. (2004) pointed out, that does not necessarily mean that dual-process models are incorrect. There continues to be controversy about the extent to which such effects in remembering and knowing are best explained by dual-process accounts or by global models of memory (see, e.g., Park et al., 2005).

Studies involving amnesic patients have yielded a similar kind of theoretical problem. Yonelinas et al. (1998) reported a convergence of remember-know, process dissociation (Jacoby, 1991) and ROC data from amnesic patients and matched controls and concluded that these data all supported a dual-process account, rather than a single-process one, partly on the grounds that the patients' ROC curves were symmetrical, whereas the ROC curves for the controls were asymmetrical, as is more usual. But there is also evidence that once differences in the strength of memory are taken into account, ROCs for patients and for controls are similar (Wais et al., 2006). Such findings are relevant to debate about the role of the hippocampus with respect to remembering and knowing in amnesic patients and about whether the hippocampus supports both states of awareness or selectively supports remembering, with the implication that other parts of the medial temporal lobe may support knowing. Although there are studies that strongly imply a selective role for the hippocampus in remembering (e.g., Aggleton et al., 2005; Gardiner et al., 2006a), others find that patients with selective hippocampal damage are similarly impaired in both remembering and knowing (e.g., Manns et al., 2003). At the moment, it remains unclear how this important issue will be resolved.

Further evidence relevant to this issue comes from studies of brain imaging in normal adults. For example, in a functional magnetic resonance imaging (fMRI) study Eldridge et al. (2000) showed that the hippocampus is selectively active when recognition is accompanied by remembering but not when it is accompanied by knowing. In another fMRI study, Henson et al. (1999) found several brain regions that were differentially activated when remembering or when knowing, and that greater activation in anterior left prefrontal, left parietal, and posterior cingulate regions was associated with remember responses. Wheeler and Buckner (2004) also used fMRI, and they too found functional dissociations between remembering and knowing. Lateral parietal regions responded preferentially with remembering, whilst other medial regions responded strongly both with remembering and with knowing.

In discussing implications for theories of remembering, Wheeler and Buckner (2004) pointed out that the evidence, particularly from parietal regions, suggests at least a partially shared neural basis for remembering and for knowing. But as well as sharing certain memory-related neural processing with knowing, remembering has additional and distinct neural correlates. This interpretation runs counter to the assumption of independence between recollection and familiarity as conceived in some dual-process models, and it seems more consistent with the SPI model (Tulving, 1995), according to which events are encoded serially into semantic and episodic systems. Moreover, evidence that remembering involves distinct neural processes that lead to the retrieval of the content of qualitatively distinct phenomenal experiences is quite beyond the scope of single-process theories, according to which remembering falls along a continuum of familiarity and is simply a matter of decision criteria or confidence.

Other brain regions that seem crucial for remembering include the frontal lobes. Some studies, though not all, have found correlations between measures of frontal lobe function and the amount of remembering reported (e.g., Parkin and Walter, 1992). Wheeler and Stuss (2003) compared patients with injuries restricted to the frontal lobes that were either centered in the frontal poles or confined to the dorsolateral prefrontal context. Overall recognition performance in the two patient groups was very similar to that in a matched control group. But although patients with the dorsolateral injuries were unimpaired either in remembering or in knowing, patients with polar injuries were selectively impaired in remembering. Some of these results are summarized in **Table 8**. This dissociation links remembering to other cognitive functions that seem to depend on polar regions of the frontal lobes, such as theory of mind and the concept of self, self-monitoring, and planning for the future. These broader implications of remembering were also emphasized by Levine

Table 8 Mean proportions of responses for patient and control groups

	Studied			*Unstudied*		
Response category	*Old*	*Remember*	*Know*	*Old*	*Remember*	*Know*
Dorsolateral	.70	.52	.18	.04	.01	.03
Polar	.60	.16	.44	.02	.01	.01
Patient mean	.65	.34	.31	.03	.01	.02
Control mean	.67	.36	.31	.03	.01	.02

Adapted from Wheeler MA and Stuss DT (2003) Remembering and knowing in patients with frontal lobe injuries. *Cortex* 39: 827–846, Appendix B, with permission from Masson SPA.

et al. (1998; see also Levine, 2000) in their investigation of remembering and knowing in another patient with brain injuries to the frontal cortex, particularly the right ventral frontal lobe. This patient too showed similar levels of recognition performance to that observed in a control group, but with selectively impaired remembering. Levine et al. also found that their patient was significantly impaired in self-regulation and suggest that his behavior generally is driven by generic knowledge in semantic memory, rather than by goals and intentions that arise from a sense of his own identity.

Thus, although there is still a great deal to be learned about the brain mechanisms underlying experiences of remembering or of knowing, it has already become clear that the extended networks likely to be involved are at least partially distinct in critically important ways and in ways that relate the two states of awareness to much broader aspects of cognitive function, especially those related to the sense of self. Other recent studies that converge on this conclusion include those concerned with normal aging (e.g., Bunce and Macready, 2005), during which autonoetic awareness diminishes, and those concerned with autism (e.g., Bowler et al., 2007) and schizophrenia (e.g., Tendolkar et al. 2002; Danion et al. 2003), two disorders that are also associated with reduced autonoetic awareness and, to widely varying extents, an altered sense of self.

2.17.7 Theoretical Evaluation

Although all of the theories that have been put forward to account for remembering and knowing help elucidate these states of awareness, none provides an entirely satisfactory account of them. The distinction between episodic and semantic systems is in some respects compelling, and there has to be at least a partially distinct and dissociable neural basis for remembering and knowing, even if it is not yet entirely clear what this basis is. One major problem for this theory concerns the interface between episodic remembering and autobiographical memory (see Conway and Pleydell-Pearce, 2000; see also Rubin et al., 2003), which may be a part of the semantic system that includes not only facts known about oneself but also a more generic kind of remembering and which, indeed, can perhaps simulate remembering. The distinctiveness/fluency framework continues to provide useful guidelines with respect to which variables are likely to influence each state of awareness (e.g., Brandt et al., 2003; Dewhurst et al., 2005) but does not take us very much further. The earlier dual-process models continue to provide a reasonably good account for much of the evidence (see Yonelinas, 2002), but there is other evidence against some of their commonly held assumptions, such as those about conscious control and independence. Other dual-process models have been developed, initially in relation to word frequency effects (e.g., Reder et al., 2000; Diana et al., 2006). The attributional approach and the source-monitoring framework offer a more functional view, but perhaps have limited scope. Signal detection models are overly focused on modeling responses rather than on understanding the states of awareness that give rise to them and, as Dunn (2004) pointed out, the challenge for this approach is to develop more psychologically meaningful accounts.

There has recently been a spate of formal quantitative models. But the increasing technical sophistication and complexity of some of these models and the rather general ability of most of them to provide a reasonably good fit to the data make it increasingly difficult to see how to distinguish between them empirically (see, e.g., Rotello and Macmillan, 2005; Macmillan and Rotello, 2006; Murdock, 2006). Confronted by a plethora of

alternative versions of such models, it is hard (despite the claims sometimes made for this approach) to see any great advantage of quantitative modelling over a less mathematically, more conceptually driven approach. Nor should it be forgotten that many of the most important advances made in the last 50 years or so were spearheaded by the introduction and empirical refinement of new concepts, concepts such as those of retrieval, levels of processing, and memory systems.

Remembering and knowing are natural mental phenomena that evolved for some purpose and so have adaptive significance, both for the species and for the individual. Individuals make judgments, reach decisions, and take action on the basis of these states of awareness. So in one sense their true significance, with respect to adaptation and behavior, if not with respect to memory theory, lies in the personal and social uses to which they are put. This is another reason why it has been important to study these states of awareness experimentally and to discover how they are influenced by different conditions and what their neural correlates are. Gaining a better understanding of remembering and knowing theoretically will depend on further evidence that links these states of awareness not only with behavior but also with the brain. The most promising new cognitive theories are likely to be those that have some conceptual correspondence with what is known about neuroanatomical function (see Roediger et al., 2007), and no theoretical assumptions that seem inconsistent with neuroanatomical function should be seriously entertained.

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2.18 Controlled Processes in Voluntary Remembering

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2.18.1 Introduction

The focus of this chapter is on voluntary remembering, in which memories are retrieved through a deliberate, goal-directed search process. Voluntary remembering occurs either in response to an external query or to a query that is generated internally by the person, usually in order to achieve some higher-order goal. For example, a person may try to recall the name of a person, to answer an exam question, or to recount an entire episode to a friend.

This type of remembering can be contrasted with involuntary memory, in which past events come to mind spontaneously and automatically, without any conscious intention to conjure them up. Involuntary memory often occurs during routine daily activity, without any apparent cue (Berntsen, 1996, 1998; Kvavilashvili and Mandler, 2004). An important subclass of involuntary memory that has received special attention is that of intrusive memories. Such memories, typically of traumatic events, occur not only in the absence of an intention to retrieve the events but also against the person's will (Koutstaal and Schacter, 1997; McNally, 1998). Intrusive memories reflect a failure of control over retrieval, because the person is unable to prevent these memories from arising, or fails to terminate them once they arise.

Although we concentrate here on voluntary remembering, we stress that the distinction between voluntary and involuntary memory processes is not sharp, and that any particular act of remembering may involve a mixture of these types of processes. For example, during the deliberate scrutinizing of one's memory for a particular detail, various memory fragments may suggest themselves, diverting the search in new directions. Sometimes, such fragments may even 'intrude' against the rememberer's will, blocking access to other, desired pieces of information.

2.18.2 Processes Involved in Remembering

We begin by outlining some general memory principles. In particular, we discuss (1) the role of retrieval cues and retrieval-encoding interactions in determining the accessibility of stored information and (2) the role of metamemory processes in monitoring and

controlling the retrieval and reporting of that information. We then integrate these elements within a schematic framework that will guide a more detailed treatment of controlled processes in remembering.

2.18.2.1 Retrieval Cues and Retrieval-Encoding Interactions

The amount of information stored in memory exceeds by far the amount of information that can be accessed at any given point in time. In the terminology introduced by Tulving and Pearlstone (1966), much more information is *available* in memory than is *accessible* at any moment. Thus, although we may momentarily fail to retrieve the name of an acquaintance, we may still be able to recall it on some later occasion or recognize it from among several alternatives. The discrepancy between the availability of information and its accessibility to consciousness testifies to the critical role of retrieval processes in bringing stored information to mind (*See* Chapter 2.16; Roediger, 1999).

What prevents all of the available information from being accessed? What is the process by which people search for and recollect stored information from long-term memory?

Tulving (1983) promoted the now-accepted idea that memory is a joint product of stored memory traces and the cues that are present when retrieval is carried out. Thus, given the same conditions of study, retrieval success can vary greatly depending on the conditions of testing. For example, memory is generally better under cued than under uncued recall testing (Tulving and Pearlstone, 1966). The conditions that instigate retrieval often provide many useful retrieval cues. In externally posed queries, some of the cues can be found in the query itself, whereas others may be available in the more general retrieval context. Even when these cues are not sufficient to directly elicit the target item, they can help delimit the memory regions in which that item is likely to be found.

Cues differ considerably in their effectiveness for aiding retrieval. Research examining the effectiveness of extralist words in prompting the recall of studied words (Nelson et al., 2005) indicates that retrieval success varies with a large number of associative properties of the cue and of the target. For example, the larger the number of words that a cue word elicits in word association norms, the lower its effectiveness in facilitating the retrieval of a studied word. The most effective cues for retrieving an event are personal cues associated with the encoding of that event, because these cues are well integrated into the memory trace of the event (e.g., Mantyla, 1986). Many standard mnemonic techniques have people encode the target information together with specific cues that can later be used to prompt retrieval.

In a landmark article, Tulving and Thomson (1973) formulated the *encoding specificity* principle, which states that a cue presented during testing will be effective in aiding retrieval to the extent that it has been encoded together with the solicited memory target at study. A large amount of research has provided evidence for this principle (Tulving, 1983). It has also been extended in the form of the more general principle of *transfer-appropriate processing,* according to which retrieval is effective to the extent that the processing that occurs during retrieval reinstates the processing that took place during encoding (Kolers and Roediger, 1984; Srinivas et al., 1998).

In line with these principles, retrieval efficiency depends on the extent to which the testing conditions reinstate the overall conditions of study. Thus, retrieval is *context dependent,* in that memory is best when testing occurs in the same physical environment in which learning took place. For example, Godden and Baddeley (1975) found that divers who studied a list of words, either on land or underwater, performed better when tested in the same environment as at study rather than in the other environment. Participants have also been found to recall a larger number of words when tested in the same room in which they studied the words than when tested in a different room (Smith et al., 1978). Context-dependent effects are more likely when the environmental contexts differ substantially and when participants deliberately associate the studied material with features of the study environment (Smith and Vela, 2001). These effects are generally obtained for recall but not for recognition (Eich, 1985), suggesting that context reinstatement specifically facilitates retrieval.

Similar evidence exists for the state dependency of memory, indicating that memory performance is best when learning and testing occur under the same internal state. For example, what participants learn while drunk, they remember better while drunk than while sober, and vice versa (Goodwin et al., 1969). A similar pattern has been observed for the effects of marijuana (Eich et al., 1975) and mood (Eich and Metcalfe, 1989). Like context dependence, state-dependent memory benefits are more clearly observed for free recall than for recognition or cued recall (Eich, 1980).

2.18.2.2 Metacognitive Monitoring and Control Processes

Much of the work on the effects of cueing and retrieval-encoding interactions has been conducted within a conceptual framework that views the rememberer as a passive conduit through which information flows. For example, the work reviewed in the previous section has mainly emphasized the automatic effects of external and internal retrieval cues and retrieval-encoding interactions on memory performance. In recent years, however, there has been an increased emphasis on the active role of the rememberer in strategically regulating the process of remembering. This new emphasis is most prominent in the area of metacognition research, in which monitoring and control processes have been shown to play a critical role throughout the various phases of remembering (Barnes et al., 1999; Koriat, 2007): They are involved in deciding whether to initiate a memory search, what type of search and retrieval process to use, where in memory to search, when to terminate the search, whether or not to report the retrieved information, and at what level of precision or coarseness to report it. Such decision processes are integral components of remembering – influencing its course and the quality of its products. Traditional memory research has generally avoided the investigation of rememberer-controlled memory processes, perhaps because the operation of these processes was seen to conflict with the desire to achieve strict experimental control (Nelson and Narens, 1994; Koriat and Goldsmith, 1996a).

In the following section, we introduce a schematic framework to help identify and conceptualize the memory and metamemory processes involved in remembering, taking into account the critical role of retrieval cues and encoding-retrieval interactions, just described. This framework will guide the discussion of controlled processes in remembering throughout the remainder of this chapter.

2.18.2.3 A Schematic Framework

Let us consider the simple case in which a person is presented with a memory query in the form of a question. How does one come up with an answer to that query? **Figure 1** presents a schematic framework for the processes involved in remembering. Broadly speaking, we first *search* our memory for the best answer we can find and then decide whether and how we want to *report* it. For simplicity, we describe the processes involved in remembering sequentially, although we assume that they are actually somewhat overlapping and parallel.

Memory search is conceptualized here as an iterative process. First, the rememberer sets parameters that define what he or she is looking for in memory and determine broadly the manner in which that information will be accessed. The search parameters include cues that are provided explicitly in the memory query and additional cues that are available in the overall retrieval context or generated by the rememberer in response to the query (cf. target descriptions in Norman and Bobrow, 1979). The parameters also include search criteria that define what will be considered a satisfactory answer to the query (verification criteria in Norman and Bobrow, 1979) and a rough metacognitive assessment of the accessibility of the answer. Another important parameter is the search strategy that will be invoked.

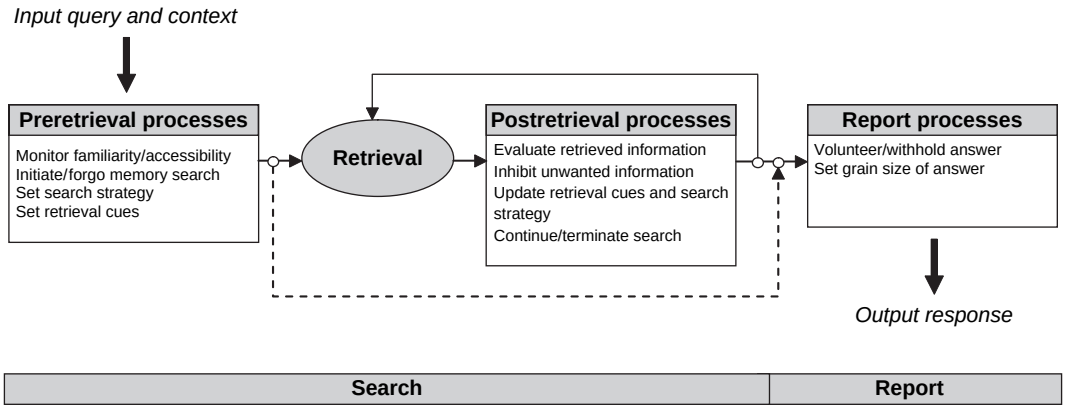

Figure 1 A schematic framework for the memory and metamemory processes involved in remembering (dashed line represents the decision to forgo a memory search).

These parameters determine the initial course of the retrieval of information from memory, as well as whether retrieval will be attempted at all. Because it appears to capture much of the mainstream thinking about memory retrieval, we adopt Tulving's (1983) concept of *ecphory* to describe the specific operation of retrieval during a (sometimes) more prolonged memory search process. According to this concept, when an item of information is encoded, a memory trace (engram) is created that includes not only the item itself but also other information from the cognitive context at the time of encoding (related thoughts, for instance). During retrieval, parts of the encoded engram combine synergistically with the search cues to produce "a conscious memory of particular aspects of the original event" (Tulving, 1976, p. 40). Consequently, the retrieved (ecphoric) information that comes to mind is actually a combination of the search cues and stored information. We assume that, although rememberers cannot control the process of ecphory itself, which is conceptualized here as an automatic, ballistic operation (Moscovitch, 1994; Guynn, 2003; but see Naveh-Benjamin et al., 2000), they can influence the outcome of a memory search by controlling the parameters that are used for the individual operations of retrieval and the overall strategy that determines the number and nature of these operations.

A very different conception of remembering is offered by the reconstructive approach (Bartlett, 1932; Neisser, 1967; Barclay, 1986), in which remembering is assumed to involve reconstructive inferences that may supplement the retrieval process. In terms of the framework presented here, however, it should not matter much whether a candidate answer is produced by a retrieval process such as ecphory or, instead, by some type of inferential, schema-based reconstruction process; much of the surrounding control processes would remain essentially the same. In any case, there has been very little work, if any, detailing the processes involved in reconstructive remembering.

The results of each retrieval (ecphory) attempt are evaluated by the rememberer to determine whether the sought-for information has been reached. If not, the search parameters may be refined, and a fresh retrieval attempt is made. Because of the critical role that search parameters play in retrieval, the metacognitive control exerted in the evaluation of results and in the consequent updating of these parameters has a high impact on remembering. The iterative search process is terminated either when the rememberer gives up (e.g., after drawing a blank or running out of time) or when a retrieved answer is identified as the best one that can be found.

Once a best-candidate answer has been reached, other factors now come into play in converting that answer into an overt memory response (Tulving, 1983). For example, the decision whether to report the best answer or withhold it and respond "don't know" (Koriat and Goldsmith, 1996b), and the decision regarding the level of generality or precision (grain size) at which to report the answer (Goldsmith et al., 2002), are both under the strategic control of the rememberer.

In what follows, our discussion of controlled processes in remembering will be divided in terms of the processes that take place before retrieval, those that take place after the retrieval of some candidate answer, and finally, the processes that take place in deciding what to report, and how.

2.18.3 Controlled Preretrieval Processes

2.18.3.1 Deciding Whether to Initiate or Forgo a Memory Search

When confronted with a memory query, one does not always proceed immediately to initiate retrieval. Rather, in many cases a preliminary feeling of knowing (FOK) may signal that it is not worthwhile to search for the answer, either because it is not in memory or because it might require more time and effort than is warranted under the circumstances. Thus, a preliminary monitoring stage may be postulated in which one makes a rough assessment regarding the availability of the answer in memory and the effort needed to access it. The initial FOK is assumed to rely on the overall familiarity of the query (Schwartz and Metcalfe, 1992; Nhouyvanisvong and Reder, 1998) and the extent to which it brings to mind some fragmentary clues (Koriat, 1993, 1995). Reder (1987) argued that a fast, preretrieval FOK is routinely and automatically made in response to the familiarity of the terms of a memory query. She found that the latency of making a fast FOK is shorter than that of accessing the answer, suggesting that preliminary FOK is not based on the retrieval of an answer. If the question does not produce a feeling of familiarity, chances are that one will not initiate a deliberate search for the answer. Glucksberg and McCloskey (1981; see also Kolers and Palef, 1976), for example, showed that people answer "I don't know" more rapidly when no potentially relevant

information is accessible ("Does Margaret Thatcher use an electronic toothbrush?") than when some information can be retrieved ("Is Kiev in the Ukraine?"). They proposed that low preliminary FOK can cause people to forgo a memory search. Note, however, that a preliminary 'Don't Know' response appears not to prevent automatic activations that may ultimately evoke the solicited target (Koriat and Lieblich, 1977).

Using an episodic cued recall task, Malmberg (in press) has recently shown that enhanced cue familiarity increases the time participants search for the answer before giving up and also increases the likelihood of retrieving the correct answer. Familiarity, however, appeared to have little effect when participants were led to believe that familiarity was not correlated with the memorability of the target. Thus, it would seem that the effects of preliminary FOK on the initiation of a memory search are at least partly strategic. In fact, Reder and her associates argued that preliminary FOK can guide the choice of question-answering strategy, as discussed in the next section. Note also that cue familiarity may affect not only the initiation of the search for the target but also the continuation of the search after it has been initiated, as suggested, perhaps, by the results of Malmberg.

2.18.3.2 Choosing a Search Strategy

Several strategies of memory search have been discussed in the literature. The strategy used to search memory determines in part the context of retrieval, the generation of additional retrieval cues, and the ways those cues are used to retrieve information from memory. By controlling the choice of search strategy, either initially or after a previous strategy has failed, the rememberer can influence the course of remembering as well as its results.

One prominent strategy is embodied in the classic two-stage generate–recognize model (Bahrick, 1969, 1979). In this strategy, the rememberer uses the available cues to define a region in memory in which the solicited item is likely to reside (e.g., "vegetables," "words strongly related to *doctor*," "Spanish family names"). Candidate items are then generated, and a subsequent monitoring process is used to select (recognize) the target from among them. For example, when trying to recall the name of an old acquaintance, one might run through a number of female names in one's head and hope that one of the names will be recognized as the target. In response to theoretical and empirical challenges (e.g., Thomson and Tulving, 1970; Tulving and Thomson, 1973; Wiseman and Tulving, 1976), more recent versions of the generate–recognize model (Jacoby and Hollingshead, 1990; Weldon and Colston, 1995; Higham and Tam, 2005, 2006) acknowledge that generated candidates may be a joint product of semantic and episodic influences (*See* Chapter 2.27). Nevertheless, these models continue to embody a memory search strategy that might be portrayed as 'casting a wide net' rather than trying to retrieve the target item directly.

Metacognitive knowledge about subtle characteristics of the encoding and retrieval contexts can guide the controlled use of the generate–recognize strategy. For example, Higham and Tam (2005) found that participants were sensitive to the strength of the semantic cue–target relations in studied lists of paired associates, and that this awareness influenced the set of plausible candidates that were generated during a cued-recall test: When participants expected weak cue-to-target relations, they were not likely to generate targets strongly related to the retrieval cues. Koriat and Lieblich (1974) also observed that participants' guesses of a target word while in a tip-of-the-tongue (TOT) state are sensitive to the specific definition of the population from which the target is said to have been drawn.

Clearly, however, rememberers do not always resort to a generate–recognize strategy. As Bahrick (1979) has observed, one does not recall the name of his wife by generating a series of female names and selecting the correct name. Instead, in this case and many others, a direct-retrieval process is invoked, in which relatively specific and constrained retrieval cues allow one to 'home in' directly on the target representation in memory. This process is assumed to be automatic and effortless. In fact, Bahrick (1979) suggested that only when direct retrieval fails do people resort to other strategies. Guynn and McDaniel (1999) proposed that, when a large amount of contextual information has been encoded along with the target, rememberers prefer direct retrieval over the generate–recognize strategy because the contextual information facilitates a narrowly focused ecphory operation. Higham and Tam (2005) suggested that direct-retrieval and generate–recognize strategies can be conceived as lying along a continuum representing the degree to which retrieval is constrained.

Jacoby and colleagues proposed a controlled mode of retrieval that they call source-constrained retrieval – the deliberate use of target-source

information to constrain what comes to mind during retrieval. In a series of experiments (Jacoby et al., 1999, 2005a,b), participants studied a list of words under shallow or deep encoding and were tested using an old/new recognition test. When they were later tested for their memory of the foils that appeared on the first test, their performance was better for the foils that had appeared on a test of deeply encoded study items than for those that had appeared on a test of shallowly encoded study items. This result was taken to suggest that the participants had used their metacognitive knowledge of the original encoding operations to constrain their retrieval on the first test by applying these same operations to the test probes. The same pattern was not found for elderly participants, presumably because elderly people fail to take advantage of their knowledge about encoding operations to constrain their retrieval.

The next strategic choice to be considered involves basing one's answer on reconstructive inference rather than on the reproductive retrieval of stored information (Neisser, 1984; Hall, 1990). Several researchers have proposed that the choice between reconstructive and reproductive remembering is, at least partly, under the control of the rememberer (Reder, 1987; Ross, 1989). Reder (1987) showed that, when the familiarity of the question is low, people tend to answer the question by making plausible inferences about the answer on the basis of a variety of cues, rather than by attempting to retrieve the answer directly from memory. She also suggested that the tendency to rely on plausible inference increases in old age (Reder et al., 1986). Similarly, Ross (1989) proposed that, when accuracy motivation is low, people tend to utilize a schema-based reconstruction strategy rather than engaging in an effortful reproductive retrieval. For example, in attempting to recall one's past attitudes, a person might use his or her present attitudes as a benchmark against which to reconstruct the past attitudes in light of an implicit theory of stability or change. To reconstruct how one felt 5 years ago, one might ask oneself: Is there any reason to believe that I felt differently then than I do now (Ross, 1989)? Several studies have shown that people tend to exaggerate the similarity between their present and past attitudes (e.g., Bem and McConnell, 1970).

Finally, a fourth general strategy can be identified that might be called 'mediated' retrieval, in which one initially sets out to retrieve contextual information that may then assist in generating further cues to guide more direct retrieval attempts (e.g., Williams and Hollan, 1981; Reiser et al., 1985). For example, when trying to remember the gifts one received at one's last birthday party, a person might first try to retrieve the general party context, including the friends who attended, in order to make the subsequent retrieval of the gifts themselves more efficient.

2.18.3.3 Specifying the Initial Context of Search and Generating Internal Retrieval Cues

As discussed earlier, retrieval cues play a critical role in the efficient retrieval of information from memory. That role begins with the cues that are presented explicitly in the memory query and those that are available implicitly in the more general retrieval context. Such cues may aid retrieval either automatically or in a more deliberate and controlled manner. The controlled exploitation of cues is particularly transparent when retrieval is difficult and prolonged.

One searches one's memory in a controlled manner by specifying certain characteristics of the solicited information as retrieval cues. Norman and Bobrow (1979) termed such specifications 'descriptions.' Descriptions may include the context of the solicited event (e.g., time, place) and additional information. Norman and Bobrow suggested that the descriptions are continually updated after each retrieval attempt. Following up on these ideas, Burgess and Shallice (1996) proposed a controlled descriptor process that is responsible for translating memory queries into a form that corresponds to the way the relevant information is stored in long-term memory. They suggested that one of the causes of clinical confabulation disorder is impaired descriptor processes.

Other researchers have put forward similar ideas. Norman and Schacter (1996; Schacter et al., 1998), for example, used the term 'focusing' to describe the preliminary stage in retrieval in which the rememberer refines the description of the characteristics of the sought-for episode. Similarly, Moscovitch and Melo (1997) suggested that confabulators might be impaired in the strategic use of general and personal knowledge to constrain their memory search so as to home in on the target. Dab and colleagues (Dab et al., 1999) described a patient whose confabulations apparently stem from deficient cue setting. In contrast to other confabulators, this patient had

preserved memory and postecphoric verification abilities but exhibited a selective impairment of the search descriptor process. Finally, the work of Jacoby and colleagues on source-constrained retrieval, mentioned earlier (Jacoby et al., 2005a,b), suggests that rememberers use contextual knowledge to constrain their retrieval queries, and that elderly people may be particularly deficient in this type of retrieval control.

Once an initial search description has been formed, further cues may be recruited during the search. Indeed, several studies have identified a reiterative pattern that occurs in the course of arduous remembering. Williams and Hollan (1981), for example, proposed that remembering consists of a series of kernel retrieval processes, each including three stages: a memory region is specified in which a search is to be conducted, that region is searched for additional clues, and the information retrieved is evaluated. Information that passes the evaluation is then used to guide the next retrieval attempt. This cycle is repeated, gradually refining the description of the information to be searched, until the search closes in on the target. Thus, in attempting to retrieve the names of high school classmates, participants in Williams and Hollan's (1981) study produced an enormous amount of information that was incidental to the task of recalling the names, including details about the school, about where people lived, and so forth. Examination of this information suggested that its main function was to probe one's memory for additional clues that could better specify a new context for search.

Similarly, Reiser and his associates (Reiser et al., 1985, 1986), in studying the recall of autobiographical episodes, also emphasized that one memory retrieval can be undertaken in order to provide cues for a subsequent retrieval. According to their context-plus-index model, specific personal episodes are recalled by first recovering the general context in which they were likely to have been encoded and then specifying the features that uniquely distinguish these experiences from others in that context. They proposed that scripts (e.g., 'eating in restaurants'; Schank, 1982) typically serve as convenient retrieval contexts. Burgess and Shallice (1996) also noted that participants did not always retrieve the target memory record directly but sometimes recovered a useful cue first. For example, it was not uncommon for participants to answer the question "What was the weather like yesterday morning?" by trying to remember first what they were wearing.

Similar processes appear to take place in retrieving information from semantic memory. A study by Walker and Kintsch (1985) suggests that retrieving the members of natural categories also relies on the recovery of context. Verbal protocols suggested a series of two-stage cycles: generating a context in which category members are likely to be found, and then using that context as a retrieval cue to produce the category members themselves. Interestingly, most of the contexts generated were episodic rather than abstract-semantic (e.g., in searching for automobiles, one might visualize the cars in a parking lot or in front of one's dormitory).

We noted earlier that retrieval is more efficient when the retrieval context closely matches the encoding context. Rememberers can take advantage of this principle by deliberately attempting to reinstate the encoding context. Thus, for example, a study by Smith (1979) suggests that mental reinstatement of the learning environment may be almost as beneficial for retrieval as actual, physical reinstatement. Notably, mental context reinstatement has been incorporated into the Cognitive Interview (Fisher and Geiselman, 1992) as a means of facilitating witness recollection; prior to answering specific questions about a past event, witnesses are instructed to mentally recreate the context or state that existed at the time of the original event. Another memory principle that can be taken advantage of in a controlled manner is the effect of schema activation on retrieval. For example, in Anderson and Pichert's (1978) classic experiment, participants read a story about two boys playing in a house from one of two perspectives, that of a home buyer or that of a burglar. After a standard recall task, participants were asked to recall the story again, now adopting the other perspective. The participants could now recall additional details that were related to the new perspective.

So far we have emphasized the deliberate use of retrieval cues in remembering. However, throughout the search, automatic activations can bring to mind a variety of associations and memories. Thus, retrieval often involves a complex interplay between a controlled process and the automatic involuntary emergence of ideas and associations (Collins and Loftus, 1975; Nelson et al., 1998) that emanate from the retrieval context or from the information already recovered (Moscovitch, 1989; Jacoby, 1991). Sometimes the controlled process will seize onto ideas that emerged involuntarily and use them as intermediate cues on the way to the sought-for target. In other cases they may be

recognized as unwanted 'interlopers,' and effort will be exerted to oppose their interfering influence (section 2.18.5.3; Jones, 1989).

2.18.4 Retrieval (Ecphory)

As explained in section 2.18.3.2, in this chapter the retrieval-ecphory operation is treated as an automatic, ballistic process whose course is not under the control of the rememberer. Understanding the nature of this process has been one of the long-standing goals of memory research, and many formal models have been proposed to describe it (e.g., Raaijmakers and Shiffrin, 1980, 1981; Hintzman, 1987; Murdock, 1993). We assume that rememberers can exert control over retrieval only by affecting the input to the retrieval operation. Such control, as the preceding discussion suggests, can have a very large impact on the outcome of the retrieval operation in particular, and on the search process generally. In addition, rememberers also make use of the retrieval output to guide subsequent retrieval operations and to convert the retrieved information into an overt response. These aspects of postretrieval control are covered in the following sections.

2.18.5 Controlled Postretrieval Processes

As noted earlier, search and retrieval can be conceptualized as a reiterative process in which a description is formed, cues are recruited to facilitate the search, candidate answers are evaluated, and – depending on the results – the search may be terminated or the cycle may continue. In this section we focus on processes that take place following the retrieval of candidate answers. These include monitoring and control processes that aid in achieving one's goals. First, rememberers monitor whether the search is on the right track and, if necessary, refine and reformulate the memory description or change the retrieval strategy. Second, they evaluate the correctness of retrieved candidate answers in deciding whether or not the target has been reached. Third, inhibition may be applied to reduce the interference from items of information that come to mind but are judged to be incorrect. Finally, in deciding whether to continue or terminate the search, rememberers may assess the likelihood of success and the additional time and effort needed to reach the target. Such an assessment may be particularly important when remembering is done under pressure, for example, when a lecturer quickly decides to settle for 'several researchers have shown' instead of continuing to search for the specific names of the researchers. We examine each of these processes in turn.

2.18.5.1 Updating and Refining the Search Strategy and Internal Retrieval Cues

In the previous section we emphasized the control exerted by rememberers in setting up the initial search parameters (internal retrieval cues and overall search strategy). We also noted, and reemphasize here, the reiterative-cyclical nature of the search process. After each retrieval attempt, these search parameters may be refined and reformulated in light of the information that has been retrieved. As observed by Norman and Bobrow (1979) and by several researchers subsequently, the 'descriptions' of the sought-for information are continuously updated during the retrieval cycle, based on newly retrieved information.

Search strategies may also be changed in light of the retrieved information. For example, participants may abandon one strategy in response to the retrieval of information that appears to be particularly useful in the context of a different strategy (Williams and Santos-Williams, 1980). When a controlled, deliberate search proves unsuccessful, however, rememberers may decide to relinquish strategic control altogether, adopting a passive-receptive attitude. Nickerson (1981) noted that, in retrieving words from lists, participants often begin with a passive attitude and then switch to an active, systematic search when the passive approach no longer yields a satisfactory return (see also Walker and Kintsch, 1985). Koriat and Melkman (1987) observed a similar pattern and also showed that, when attentional resources are diverted, the retrieval of words from a list becomes less controlled, moving along associative links between the words rather than along conceptual-logical relations.

2.18.5.2 Evaluating the Correctness of Retrieved Information

A great deal of work emphasizes the importance of postretrieval monitoring processes that evaluate the relevance and correctness of retrieved information (e.g., Burgess and Shallice, 1996; Kelley and Jacoby, 1996; Schacter et al., 1998; Koriat, 2000; Mitchell

and Johnson, 2000). On the basis of these processes, one decides not only whether each piece of information that comes to mind is correct or not but also whether the search is on the right track, whether to continue searching for additional candidate responses, and which of the many candidates that came to mind is the best candidate answer. In a later section we discuss the further crucial role of monitoring processes in deciding whether or not to report the best candidate answer, and in what form. The operation of these processes is particularly important in real-life situations (e.g., eyewitness testimony) in which a premium is placed on accurate reporting.

Discussions of metacognition generally distinguish between two basic types of monitoring processes (Koriat and Levy-Sadot, 1999). Information-based processes involve analytic, deliberate inferences in which beliefs and knowledge in long-term memory are consulted and weighed to reach an educated judgment. Experience-based processes, in contrast, are sensitive to online mnemonic cues, such as retrieval fluency, that derive from the experience of remembering itself. These cues give rise to subjective feelings (e.g., a sense of conviction), which then serve as the bases for metacognitive judgments (Strack, 1992; Kelley and Jacoby, 1996; Koriat and Levy-Sadot, 1999).

As an example of information-based, analytic monitoring, rememberers may base their confidence in the correctness of a particular candidate response on the weight of the evidence that they can marshal in favor of that candidate relative to the evidence in support of the alternative candidates (e.g., Koriat et al., 1980; Griffin and Tversky, 1992; McKenzie, 1997; Yates et al., 2002). Rememberers may also base their confidence on metacognitive beliefs about their own competence and skills (Dunning et al., 2003; Perfect, 2004) and about the way in which various factors can affect memory performance (Dunlosky and Nelson, 1994; Mazzoni and Kirsch, 2002).

In contrast to this type of analytic and deliberate evaluation, experience-based monitoring relies on mnemonic cues that derive from the online processes of remembering. Such cues as the ease with which information comes to mind, or its vividness, may contribute implicitly to the subjective confidence in the correctness of that information. For example, it has been observed that the more effort and the longer the deliberation needed to reach an answer, the lower is the confidence in that answer (e.g., Nelson and Narens, 1990; Robinson et al., 1997; Koriat et al., 2006). Kelley and Lindsay (1993) showed that when priming speeds up the emergence of an answer, confidence judgments also increase accordingly. This effect occurred even for plausible but incorrect answers. Although typically correct answers are associated with shorter latencies than incorrect answers, so that response latency is diagnostic of the correctness of the answer that is retrieved or recognized, there are situations in which retrieval fluency can be misleading (Chandler, 1994). For example, asking participants to imagine some childhood events increased confidence that these events did indeed happen in the past (Garry et al., 1996). Merely being asked about an event twice also increased subjective confidence. Possibly, imagining an event or attempting to recall it increases its retrieval fluency, which in turn contributes to the confidence that the event has occurred.

A prominent theory that includes both automatic and controlled monitoring processes is Johnson's (1997) source monitoring framework. According to this framework, in discriminating the origin or source of information, people take advantage of the fact that mental experiences from different sources (e.g., perception vs. imagination) differ on average in their phenomenal qualities such as visual clarity and contextual details (*See* Chapter 2.19). Although these diagnostic qualities can support a rapid, heuristically based source monitoring, sometimes more strategic, deliberative processes may be applied. Both types of processes require setting criteria for making a judgment and procedures for comparing activated information to the criteria. Closely related processes have been discussed in the context of Jacoby and Kelley's attributional approach to memory (e.g., Jacoby et al., 1989; Kelley and Rhodes, 2002) and in Whittlesea's SCAPE framework (e.g., Whittlesea and Williams, 2001a,b; Whittlesea, 2002).

Many memory errors are the result of source confusions – the attribution of retrieved elements to the wrong context (Johnson, 1997). For example, the effects of misleading postevent information have been attributed, at least in part, to deficient source monitoring, by which the postevent misinformation is wrongly attributed to the witnessed event (see Lindsay, 1994; Mitchell and Johnson, 2000). Source confusions can arise when the activated information during retrieval is incomplete or ambiguous, or when the cues used in attributing information to sources are not diagnostic. Divided attention during encoding has been found to impair source monitoring (Craik and Byrd, 1982), presumably

because they disrupt contextual binding. High perceptual similarity between two sources, as well as similarity in the encoding processes, also increase source confusions (Ferguson et al., 1992; Dodson and Johnson, 1996). Although vividness and perceptual detail are generally diagnostic of actual memories (Conway et al., 1996), thinking about imagined events also increases their vividness, thereby impairing reality monitoring for these events (Suengas and Johnson, 1988).

Several mechanisms have been proposed that can help reduce source confusions and reject false memories (see Odegard and Lampinen, 2006). For example, distinctive encoding manipulations have been shown to reduce the occurrence of false recall and recognition. Such manipulations include presenting each word together with a picture representing it (Israel and Schacter, 1997; Schacter et al., 1999), visual rather than auditory presentation (Smith and Hunt, 1998), having participants say the words out loud at study (Dodson and Schacter, 2001), or having the participants rate the pleasantness of the words during study (Smith and Hunt, 1998). Schacter et al. (1999) have explained such findings in terms of a *distinctiveness heuristic,* a mode of responding based on participants' metacognitive belief that true memory of studied items should include recollection of distinctive details. Participants can use this heuristic to reject foils that evoke memorial experiences lacking the distinctive qualities known to be present at study. A similar metacognitive strategy has been suggested by Strack and Bless (1994) to underlie judgments of nonoccurrence. They showed that, if an event is judged to be memorable (salient) but elicits no clear recollection during testing, it can be rejected with high confidence as not having occurred. In contrast, in the absence of a clear recollection of a nonmemorable event, rememberers may infer that the event actually had occurred but had simply been forgotten. Also, studying material under conditions unfavorable for learning (or expecting fast forgetting, Ghetti, 2003) results in a relatively high rate of false alarms for nonmemorable distractors.

In the framework of Fuzzy Trace Theory, Brainerd et al. (2003) proposed recollection rejection as another mechanism for identifying and editing out false memories. By this mechanism, a distractor that is consistent with the gist of a presented item may be rejected when the verbatim trace of that item is recollected. Thus, participants can reject 'SOFA' as having occurred in the study list if they recall that the word 'COUCH' was in the list and if they have noticed that all words in the study list were unrelated to each other. Recollection rejection has been shown to operate in rejecting false narrative statements (Brainerd et al., 2006) and may also occur for self-generated candidate responses that emerge during recall.

Finally, Burgess and Shallice's (1996) model, mentioned earlier, also includes a mechanism for the screening of retrieved information. The model assumes that 'editor' processes are initiated whenever a descriptor is set. These processes check that retrieved memory items do not contradict previously retrieved elements of the event, and that they are compatible with the overall descriptor requirements. Evidence for the operation of such a mechanism comes from error corrections in verbal protocols obtained during autobiographical recollections of recent everyday events. One participant, who was asked to describe the first thing that came to mind that happened to him in January, was recorded thinking:

> Something that happened in January? . . . I completed a major sale. No! I didn't complete a major sale in January at all. I didn't sell anything at all in January because I remember looking at the board and that was blank." (Burgess and Shallice, 1996: 382)

Applying their model to the study of confabulations, Burgess and Shallice (1996) pointed to impaired editor processes, along with insufficiently focused retrieval descriptions, as two of their main causes.

2.18.5.3 Inhibiting Wrong/Irrelevant Information

As noted earlier, a great deal of unwanted information is retrieved during the search for a solicited target, which must be cast aside as the search continues. Therefore, a potentially important contributor to successful retrieval is the efficient inhibition of such incidental information and, in particular, the inhibition of rejected candidate answers that would otherwise keep coming to mind and interfering with the search. The effect of such interference has been emphasized in studies of the TOT phenomenon, in which the failure to retrieve the correct target while in the TOT state is attributed, in part, to the interfering effect of 'interlopers' – plausible but wrong candidate answers that share some features with the

target (Reason and Lucas, 1984; Jones, 1989; Burke et al., 1991).

It has been observed that retrieving some items of a studied list with the aid of category cues impairs the later recall of other studied items from the same category, but not of other unrelated studied items (Anderson et al., 1994; Anderson and Spellman, 1995; Anderson, 2003). This retrieval-induced forgetting has been attributed to inhibitory mechanisms that operate to suppress unwanted information in order to overcome retrieval competition (Anderson et al., 2000; Levy and Anderson, 2002). Hasher and her colleagues (Hamm and Hasher, 1992; Hasher et al., 1999) suggested that inhibitory processes are used to suppress goal-irrelevant information that has been activated in working memory, or to prevent candidate answers from being immediately reported, so that other candidates can also be retrieved and considered (Hasher and Zacks, 1988; Hasher et al., 1999; Radvansky et al., 2005). May and Hasher (1998) demonstrated that the controlled inhibition of the irrelevant contents of working memory is deficient in older adults, and in young adults during their off-peak time of the day.

Directed forgetting is another example of controlled inhibition in memory. Research indicates that, when people are instructed to forget a previously learned piece of information, they are often successful in reducing or eliminating the interference between that information and the subsequent retrieval of to-be-remembered information (Bjork and Woodward, 1973; Bjork, 1989). The underlying mechanism seems to involve inhibiting the retrieval of the to-be-forgotten information. Indeed, when memory is tested through recognition or relearning, or when it is tested through indirect measures of memory such as priming, performance on the to-be-forgotten items is typically comparable to that of to-be-remembered items (Basden et al., 1993; Bjork and Bjork, 1996).

2.18.5.4 Deciding Whether to Continue or Terminate the Search

We have characterized the search process as reiterative, but it is, of course, not endless. At some point, the memory search must terminate – either when no relevant information can be retrieved or after some information (correct or incorrect) has been retrieved, and the rememberer either believes that the target has been reached or has given up. The decision to stop the search is at least partially under the control of the rememberer and is based on such factors as level of confidence in the best candidate answer produced so far, the feeling that one knows the answer even though it has not (yet) been retrieved, the amount of time and effort invested so far, and the incentives for successful performance.

Whereas it is self evident that high confidence in a retrieved answer will induce the rememberer to terminate the memory search, there is also evidence that this decision is affected by the feeling of knowing (FOK) regarding answers that have not yet been retrieved. When FOK is high, participants spend more time searching for the target before giving up than when FOK is low (Nelson and Narens, 1990; Barnes et al., 1999).

The decision to continue the search is also affected by the expected reward for correct retrieval. Loftus and Wickens (1970) found that the larger the reward offered at the time of retrieval, the more time participants spent before terminating the retrieval, although this did not affect their performance. More direct evidence comes from Barnes et al. (1999) in examining the 'willingness to continue searching' component of their metacognitive retrieval model. They assumed that the willingness to continue searching depends on two conflicting incentives – the reward for finding the correct answer and the cost of spending additional search time. For example, in most exam situations, continuing to search for an answer to one question is beneficial to the extent that this allows the correct answer to be reached, but it is detrimental to the extent that this takes away from the time that can be spent on other questions. Manipulating the reward for each correct answer and the cost of additional search time on a cued-recall test, Barnes et al. (1999) found that both higher rewards and lower costs induced the participants to take longer before responding. This increased the number of correct responses and decreased the number of omission errors without increasing the number of commission errors – indicating that the additional retrieval effort was not in vain.

2.18.6 Controlled Report Processes

2.18.6.1 Deciding Whether or Not to Report an Answer

Much memory research has used forced-report testing procedures, such as forced-choice recognition or forced cued recall, in which the participant is required to select/provide an answer to each and

every test probe. In most everyday memory situations, however, as in many laboratory recall tasks, rememberers have the option of *free report;* that is, they are allowed to decide for themselves whether to answer a particular memory query, or instead to respond 'don't know' (or refrain from responding).

The option of free report is particularly crucial in situations, such as courtroom testimony, in which a premium is placed on accurate reporting. Koriat and Goldsmith (1994, 1996b) showed that, when participants are given the option of free report and a moderate incentive for accurate reporting (a penalty for each wrong answer equal to the reward for each correct answer), they are able to boost the accuracy of what they report substantially in comparison to forced-report testing. They do so by withholding best-candidate answers that are likely to be wrong. For example, in one study (Koriat and Goldsmith, 1994, Experiment 1), the option of free report allowed participants to increase their recall accuracy from 47.6% in forced report to 76.6%. Moreover, when given an even stronger accuracy incentive (a 10:1 penalty-to-reward ratio; Koriat and Goldsmith [1996b, Experiment 1], or the loss of all winnings if a single wrong answer is volunteered, Koriat and Goldsmith [1994, Experiment 3]), report accuracy was boosted even further. In each case, however, the increased report accuracy came at the price of a reduction in the quantity of correct information reported – that is, a quantity-accuracy trade-off (see also Barnes et al., 1999; Kelley and Sahakyan, 2003).

The existence of a quantity-accuracy trade-off means that rememberers must strive to find a compromise between these two conflicting aims in regulating their reporting. Consider, for example, a courtroom witness who has sworn "to tell the whole truth and nothing but the truth." Generally, it is not possible to fulfill both endeavors simultaneously. How, then, should the witness proceed?

Koriat and Goldsmith (1996a) proposed a model (for similar models, see Barnes et al., 1999; Higham, 2002), in which one first assesses the likelihood that one's best candidate answer is correct and then compares this assessment to a report criterion. The answer is volunteered if its assessed probability of being correct passes the criterion; otherwise, it is withheld. The setting of the criterion is assumed to depend on the relative incentives for accuracy and quantity; in general, report accuracy should increase, but the quantity of correct answers should decrease as the criterion level is raised.

In line with this model, a very strong relationship was found between the tendency to report an answer under free-report conditions and subjective confidence in the answer (assessed probability that the answer is correct). In one study, for example, the mean within-participant gamma correlation between confidence in the answer and the decision to volunteer it or withhold it on a recall test was .95 (Koriat and Goldsmith, 1996b, Experiment 1; see also Kelley and Sahakyan, 2003). In addition, manipulating the incentives for accurate reporting in the manner described earlier (by manipulating the relative rewards and penalties for correct and incorrect answers, respectively) induced rememberers to adjust their report criterion accordingly; higher levels of confidence were required for reporting answers under a strong accuracy incentive than under a more moderate accuracy incentive (Koriat and Goldsmith, 1996b, Experiment 1; Kelley and Sahakyan, 2003, Experiment 1). Finally, modeling the report decision in terms of a confidence criterion (cut-off), with the level of the criterion for each participant allowed to vary as a free parameter, yielded a very good fit with the data, accounting for about 94% of the participants' actual report decisions under recall testing (Koriat and Goldsmith, 1996b, Experiment 1). Similar levels of fit were found by Kelley and Sahakyan (2003).

The consideration of the role of metacognitive monitoring and control processes in reporting has yielded some interesting insights concerning variables that affect memory accuracy and quantity performance. One, of course, is the effect of accuracy motivation mentioned earlier. A second important variable is monitoring effectiveness, that is, the extent to which the rememberer can distinguish between correct and incorrect answers. On the one hand, as monitoring effectiveness increases, the option of free report allows one to screen out wrong candidate answers without also mistakenly screening out correct candidate answers, thereby reducing the rate of the quantity-accuracy trade-off. On the other hand, when monitoring effectiveness is impaired, the exercise of the option to withhold answers may yield little or no benefit in terms of report accuracy (Koriat and Goldsmith, 1996b; Rhodes and Kelley, 2005; Kelley and Sahakyan, 2003) and may simply reduce the quantity of correct information that is reported (Higham, 2002), compared with forced report.

A third important variable is test format with – recall versus recognition. This variable has been implicated in both traditional, quantity-oriented research and in more naturalistic, accuracy-oriented

research, with opposing implications. Whereas the general finding from decades of laboratory research (e.g., Brown, 1976) is that recognition testing is superior to recall testing in eliciting a greater quantity of correct information from memory, the established wisdom in eyewitness research, for example, is that recall is superior to recognition in eliciting accurate information from rememberers (e.g., Hilgard and Loftus, 1979; Neisser, 1988). Koriat and Goldsmith (1994), however, showed that this recall–recognition paradox actually stems from the common confounding between test format (recall vs. recognition) and report option (free vs. forced). Typically, recognition participants are forced either to choose between several alternatives or to make a yes–no decision regarding each and every item, whereas recall participants have the freedom to withhold information that they are unsure about. Comparing performance on a free-recognition test (in which participants had the option to respond 'don't know' to individual items), to a free-recall test, Koriat and Goldsmith (1994) found that recognition quantity performance was still superior to recall, but now recognition accuracy was as high or even higher than recall accuracy. An examination of the underlying memory and metamemory components of recall and recognition performance (*See* Chapter 2.20; Koriat and Goldsmith, 1996b) indicated that monitoring effectiveness was in fact somewhat lower for recognition than for recall testing, but that this disadvantage was more than compensated for by superior memory access and the adoption of a more conservative report criterion under recognition testing.

The consideration of the role of metacognitive monitoring and control processes in reporting has also yielded interesting insights with regard to other important topics and questions, such as developmental changes in memory accuracy (Koriat et al., 2001; Roebers et al., 2001), memory decline in the elderly (Jacoby, 1999; Pansky et al., 2002; Kelley and Sahakyan, 2003; Rhodes and Kelley, 2005), cognitive and metacognitive impairment in schizophrenia (Danion et al., 2001; Koren et al., 2006), psychometric and scholastic testing (Koriat and Goldsmith, 1998; Higham, 2007), and the classic encoding specificity principle (Higham, 2002; Higham and Tam, 2005). As just one example, there has been a question about the reliability of children's memory, particularly in the area of legal testimony, (e.g., Bruck and Ceci, 1999). Yet, Koriat et al. (2001) showed that children as young as 8 or 9 years old can regulate their memory reporting to produce a more accurate record of past events when they are allowed to screen out wrong answers and when they are explicitly motivated to do so. Furthermore, like adults, they are also sensitive to specific levels of accuracy incentive, increasing the accuracy of their reports further when a higher premium is placed on memory accuracy. However, the children in that study (see also Roebers et al., 2001) and elderly adults in other studies (Pansky et al., 2002; Kelley and Sahakyan, 2003; Rhodes and Kelley, 2005) were found to be less effective than young adults in utilizing the option to withhold answers to enhance their accuracy.

Of course, there may be variables whose influences are not amenable to control by way of report regulation. For example, Payne et al. (2004) observed that when participants were allowed the option of free report, they could enhance their overall memory accuracy, but the withholding of answers did not reduce stereotype bias. Their findings suggest that stereotypes distort memory through an unconscious-accessibility bias to which subjective confidence is insensitive. The implication is that any variable that affects memory performance without affecting subjective confidence (i.e., that cannot be monitored) will not be susceptible to report control.

2.18.6.2 Deciding on the Grain Size of the Reported Answer

In addition to the exercise of report option, another means by which rememberers regulate the accuracy and amount of information that they report is controlling the *grain size* of their report, that is, the precision or coarseness of their answers (Yaniv and Foster, 1995, 1997; Goldsmith and Koriat, 1999; Goldsmith et al., 2002, 2005). For example, when asked to specify what time an event occurred, a rememberer who is unsure might provide a relatively coarse response such as "in the late afternoon" or "between 5.00 and 6.00 p.m.," rather than venture a more precise response. In fact, Neisser (1988) observed that, when answering open-ended questions, participants tended to provide answers at a level of generality at which they were "not likely to be mistaken." Of course, more coarsely grained answers, while more likely to be correct, are also less informative. Thus, Goldsmith et al. (2002) proposed that the control of grain size is guided by an accuracy-informativeness trade-off (see also Yaniv and Foster, 1997), similar to the accuracy-quantity trade-off that guides the exercise of report option. They found that, when participants were allowed to

control the grain size of their report, they did so in a strategic manner, sacrificing informativeness (precision) for the sake of accuracy when their subjective confidence in the more precise-informative answer was low. The participants also took into account the relative payoffs for accuracy and informativeness in choosing the grain size of their answers; they tended to provide more precise answers (thus taking a greater risk of being wrong) when the relative payoff for informativeness was high than when it was low. The monitoring and control processes involved in the regulation of memory grain size appear to be similar to those underlying the decision to volunteer or withhold specific items of information, implying perhaps the use of common metacognitive mechanisms.

As in the case of report option, a consideration of the control of grain size in memory reporting has begun to shed light on other memory phenomena and issues. One example is the potential role of control over grain size in modulating the changes that occur in memory over time. Goldsmith et al. (2005) examined the regulation of report grain size over different retention intervals. Starting with the well-known finding that people often remember the gist of an event though they have forgotten its details, they asked whether rememberers might exploit the differential forgetting rates of coarse and precise information in regulating the accuracy of the information that they report over time. The results suggested that, when given control over the grain size of their answers, people attempt to maintain a stable level of report accuracy by providing coarser answers at longer retention intervals.

In this section we focused on the control of grain size that takes place at the reporting stage. There is evidence, however, that rememberers can also control the level of coarseness or precision at which they retrieve information (Anderson et al., 2001; Brainerd et al., 2002; Koutstaal, 2003; Koutstaal and Cavendish, 2006). Koutstaal (2003), for example, showed that rememberers can flexibly alternate between attempts to query memory at a highly specific level and attempts to query memory at a categorical level, and that this flexibility is somewhat impaired in older participants. Moreover, Koutstaal and Cavendish (2006) found that initially inducing participants to adopt and use a gist-based retrieval orientation can impair performance on a subsequent memory task that requires a more precise retrieval orientation.

2.18.7 Concluding Remarks

In this chapter, we examined the processes of voluntary remembering that are under the control of the rememberer. Such control is evident throughout the course of remembering, from the initial decision regarding whether and how to begin the memory search, until the final decision regarding how the retrieved information is to be reported. The investigation of self-controlled processes in remembering presents a methodological challenge to students of memory, because such processes are, by definition, less amenable to strict experimental control. Yet, as evidenced by the work reviewed in this chapter, recent years have seen a growing willingness to face this challenge. Clearly, however, much more work needs to be done to illuminate the underlying mechanisms of controlled remembering and clarify the intricate interplay between controlled and automatic memory processes. Ultimately, research should be targeted toward integrating these processes into more general theories of memory and remembering.

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2.19 Source Monitoring

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People sometimes experience difficulty identifying the origins of their thoughts, images, and feelings. You might, for example, find yourself wondering 'Where did I get the idea that the U.S. Speaker of the House is third in line to the presidency?' or 'Did I turn off the oven before I left the house, or did I only think about turning it off?' Moreover, people sometimes erroneously attribute thoughts, images, and feelings to origins other than the true sources. Victims of cryptomnesia (unconscious plagiarism), for example, experience memory-based thoughts as new ideas (Brown and Murphy, 1989; Marsh et al., 1997; Stark and Perfect, 2006). In déjà vu, in contrast, a person has the subjective experience of recognizing a current situation as familiar without having experienced a directly corresponding prior episode (Brown, 2004).

The source monitoring framework (SMF) is an evolving collection of ideas designed to explain the

mechanisms by which people attribute mental events to particular origins (Johnson et al., 1993; Johnson, 2006). It is referred to as a framework, rather than a theory or model, in acknowledgment of the fact that the approach stops far short of fully specifying or formalizing the mechanisms involved in identifying the sources of mental events. The core thesis of the SMF is that thoughts/images/feelings that come to mind do not include abstract tags or labels that name their sources, but rather have qualitative and quantitative characteristics that are more or less diagnostic of source. As elaborated in the following, mental events are said to be attributed to particular sources on the basis of their characteristics in the context of the individual's current orientation.

Source is a multidimensional construct that refers to the various dimensions that collectively specify how one came to have a particular mental experience. Dimensions of the sources of memories include spatial/environmental context, temporal context, modality of apprehension (e.g., whether a remembered sentence was heard or read or merely imagined), and agent (e.g., who said a remembered utterance). Thus the concept of memory for source is similar to what Brown and Kulik (1977), in their exploration of flashbulb memories, termed memory for circumstances of encounter (e.g., your recollections of learning of the 9/11 attacks on the World Trade Center).

The construct of source is also similar to (but more inclusive than) that of memory for context. Some models of memory make a sharp distinction between memory for content and memory for context, characterizing the latter as an abstract tag or label that is associated with but not intrinsic to the memorial representation of content (Anderson and Bower, 1974; Murnane et al., 1999). Such models constrain the range of potential contexts quite narrowly (e.g., List 1 vs. List 2). In the SMF, in contrast, the distinction between content and source is a blurry one, sources are thought to be inferred from multiple aspects of the accessed memorial information itself rather than read off from a tag, and the range of potential sources is unbounded.

2.19.1 Underlying Assumptions Regarding Basic Mechanisms of Memory

The SMF is not in itself an account of encoding/retention/revival of memory information, but it rests on certain assumptions about how memory works (see Johnson, 1985). Space constraints prohibit a detailed exegesis here, but it is worthwhile briefly summarizing some key points. One such assumption is that memory traces or records are by-products of the multiple cognitive operations that underlie and give rise to ongoing experience. It follows that memory traces for any given event are distributed across multiple processing subsystems. Reading a word, for example, involves a host of cognitive processes, from relatively low-level, data-driven, automatic, generic processes (e.g., figure/ground separation, identifying letter and word shapes, etc.) to higher-level, more conceptual, abstract, effortful, and instance-specific processes (e.g., noting a conceptual relationship between a study word and an earlier word on the list). All such processes have lasting effects on the processing subsystems that perform them (memory traces), as per connectionist models of memory (McClennand and Rumelhart, 1985; McClelland et al., 2003; see also Kolers and Roediger, 1984).

The SMF assumes that the revival of information from memory follows the transfer-appropriate processing (TAP; Morris et al., 1977) or encoding specificity principle (ESP; Tulving and Thompson, 1973), as in Tulving's ideas about synergistic ecphory (Tulving, 1984). Ongoing processes that are sufficiently and distinctively similar to past processes cue revival of those past processes. Importantly, some aspects or features of the processes that gave rise to and constitute a past episode can be revived without other aspects or features being revived. A cue might bring to mind information about the spatial location of a previously presented stimulus, for example, but not information about its color. Or one might recollect relatively abstract, conceptual aspects of a past experience without remembering surface-level details of that experience (e.g., one might remember that a previously presented word was a taboo word but not remember the exact word) (cf. Brainerd and Reyna, 1990).

Several factors influence which features of a past event are, versus are not, revived by a cue. For one, cognitive processes vary in the extent to which they produce distinctive traces of the sort likely subsequently to be experienced as recollections of a specific prior episode. Highly automatic, low-level, data-driven processes are rarely consciously experienced in ongoing experience, and they tend to be executed in much the same way each time they are engaged. Thus records of any particular instance of such processing cannot readily be cued (cue overload) and in any case their revival would not directly

give rise to thoughts or images per se because their initial performance did not give rise to thoughts or images. That is, processes that are tacit and unconscious in ongoing experience are tacit and unconscious when later cued, although the influence of such memory traces can be detected under some conditions, such as in measures of repetition priming involving transformed text or highly unusual fonts (Kolers and Roediger, 1984; Wiggs and Martin, 1994; Westerman et al., 2003).

Another determinant of which aspects of a past experience are versus are not revived by a cue is the nature of the cue, as per TAP/ESP. For example, memories of prior sensory processes are more likely to be revived by representation of the perceptual stimulus than by a more abstract cue. Being oriented toward remembering – what Tulving (1998) called being in retrieval mode – can be described in TAP terms as a matter of configuring aspects of current thought in ways that more effectively act as cues for past thoughts. Finally, which aspects of a past event are revived in response to a cue is influenced by the extent to which different aspects of the initial event were bound together with one another, which in turn reflects attention during and shortly after the event. Aspects of an event that are in the focus of attention and that are reflected upon as or immediately after they occur tend to be bound together in ways that support revival of aspect X in response to a cue that maps on to aspect Y (Johnson et al., 2005). Refreshing newly created memories by reflecting on recently experienced events may also be involved in memory consolidation (Wixted, 2004).

Cues rarely if ever revive only memory information from a single to-be-remembered prior episode. Rather, cues evoke episodic information from multiple past events that are similar in various ways to the cues (cf. Neisser's 1981 concept of "repisodes"), along with more abstract knowledge and beliefs conceptually related to the cues. Thus schema and scripts, biases, expectations, stereotypes, etc., are evoked by cues in parallel with episodic details when we remember the past. The memory system must work in such a way that cues evoke memory information from multiple episodes combined with more general knowledge, because otherwise we could only retrieve memories of event X by way of extremely X-specific cues. If the system gave us only exactly what we were looking for, we would have to be able to specify very precisely what it is we are looking for. Of course, if we knew exactly what information we sought from memory there would be no need to look for it. Thus we need a sophisticated set of source monitoring processes not only to specify the sources of a particular memory record but also to help us differentiate between recollections of the multiple episodes, repisodes, inferences, schemas, etc., that come to mind in response to internally and externally generated cues.

The SMF also assumes that reviving memory information itself leaves traces. That is, when a person recollects a past event she creates memories of that episode of recollection. Cues that are effective for reviving the event itself are also likely to be effective for reviving such memories of remembering (Lane et al., 2001). After multiple instances of recollecting a particular past event, the revival of memories of the prior recollections may come to dominate those of the event itself. Relatedly, the way a person talks about his or her memories of an event can influence subsequent recollections of that event (Higgins and Rholes, 1978; Marsh and Tversky, 2004; Echterhoff et al., in press). Reviving memories also appears to strengthen the binding between different aspects of the remembered event (i.e., those aspects that are revived; Johnson, 1994); again, binding is key to the episodic, autonoetic quality of remembering (i.e., the subjective experience of partially reliving a prior experience in one's personal past).

2.19.2 Johnson and Raye's Reality Monitoring Model

The SMF is an outgrowth and elaboration of Johnson and Raye's (1981) reality monitoring (RM) model. The RM model was primarily an account of how individuals differentiate between memories of actual perceptual events versus memories of thoughts, fantasies, or dreams (e.g., 'Did I lock the door, or did I only think about locking the door?'). The RM model emphasized the role of average quantitative differences between memories of actual versus imagined events. The model posited, for example, that memories of actual experiences tend, on average, to be more perceptually detailed than memories of imagined events and hence that amount of perceptual detail serves as a cue to a memory's reality status: Perceptually detailed memories probably really happened, whereas perceptually vague memories were probably merely imagined. As another example, the RM model held that memories of imagined events typically include more traces indicating effortful, internally generated cognitive operations (i.e., the mental processes involved in deliberately imagining the event), and hence that amount of memorial evidence of cognitive operations serves as a cue for

differentiating between memories of actual versus imagined events.

According to the RM model, perceptually rich memories with little indication of effortful cognitive operations are likely to be experienced as memories of actual events. Thus, for example, a memory of an unusually vivid and fluently generated fantasy is likely to be misidentified as a memory of an actual event. The RM model also includes a more reflective, systematic process that can be engaged when memories with intermediate quantitative characteristics come to mind. Those more analytical processes use knowledge and beliefs to make deliberative inferences about the reality status of a remembered event based on its content (e.g., 'It must be that I really did give that message to Sara, because if I hadn't she would have called me by now'). Such systematic processes may also be engaged when the qualitative content of a memory contradicts the reality status implied by its quantitative characteristics. A vivid memory of unaided flight, for example, might initially be classified as a memory of a real event by rapid, heuristic processes based on quantitative characteristics, but then be reclassified as a memory of a dream or fantasy based on the rememberer's belief that people cannot fly.

Johnson and her coworkers amassed a considerable body of evidence in support of the RM model. For example, participants rated their memories of past fantasies as less perceptually detailed than their memories of past real events, and when asked why they believed a particular memory was of a real versus imagined event, they often cited such characteristics (Johnson et al., 1988b). As another example, subjects were more likely to confuse memories of seeing line drawings with memories of imagining line drawings if the objects were easily imaged than if they were difficult to image (Finke et al., 1988).

The SMF incorporates the ideas of the RM framework, but as explained below it differs from it in two major ways. First, the SMF assumes that the quantitative characteristics of memories (e.g., amount of perceptual detail) constitute only a small subset of a broad range of memorial characteristics that can be used quickly and automatically to attribute thoughts, images, and feelings to particular sources of past experience. Second, rather than dichotomizing between internally generated and physically instantiated events, the SMF seeks to account for an unbounded range of finer-grained source identifications that, collectively, specify all dimensions of a mental event's origin.

2.19.3 Memory Source Monitoring

2.19.3.1 Basic Mechanisms

As noted, the key premise of the SMF is that the sources of memories are rarely abstractly specified (named or labeled) in the memorial contents whose revival is prompted by a cue. This follows from the assumption that memory traces are by-products of ongoing cognitive processes, and from the corollary that individuals only occasionally reflect on and label the various dimensions of the source of ongoing events. As you read the preceding paragraph, for example, you probably were not thinking, 'I'm reading Steve Lindsay's chapter in Roddy Roediger's handbook at time X on date Y in place Z.' What is tacit in ongoing experience will be tacit in memory records. Consequently, even if the full wealth of cognitive processes performed during a particular past event could be revived, they would probably not abstractly label or specify many source dimensions.

According to the SMF, the processes by which memories are attributed to sources are analogous to those by which aspects of perceptual events are attributed to particular sources in ongoing experience (see also Payne and Blackwell, 1998). If your friend Kathy calls you on the phone, when she says 'Hello' you recognize her voice; the auditory signal does not include any abstract designation of the speaker's identity, but processing the sounds evokes the information that leads you to hear it as Kathy's voice. In both the perceptual and memorial cases, source attributions are usually made very quickly, with little if any conscious awareness of a decision-making process and with very high levels of accuracy. But various conditions can undermine source attributions, making them more difficult and error prone.

One source of difficulty in memory source attributions is sparse revival of memorial information. This is analogous to the difficulty you might have in recognizing a friend's voice on the phone if the connection was bad. In the memorial case, sparse revival may be due, for example, to poor attention during the event itself or to poor cuing (e.g., cues that only partially map onto the to-be-remembered past event and/or that also map on to numerous other past events (cue overload)). Thus, various manipulations that impair encoding or retrieval of source-specifying aspects of an event tend to lower source monitoring performance. Troyer et al. (1999), for example, showed that performing a finger-tapping task during study substantially lowered SM accuracy (more than it lowered recognition). Similarly, Zaragoza

and Lane (1998) showed that subjects who encountered or retrieved misleading suggestions under divided attention were more likely to later make false-memory reports consistent with those suggestions than were subjects who encountered or retrieved them under full attention (see also Lane, 2006).

Memory source attributions are also compromised when two or more sources of prior experience are highly similar to one another. If your friends Kathy and Francine have very similar voices, then you may misidentify a recollected utterance of Kathy's as having been made by Francine, just as you might confuse Kathy's voice with Francine's on the phone. In a breakthrough study by Johnson et al. (1988b), subjects heard an experimenter say some words and imagined other words, and later attempted to remember which words had been spoken and which had been imagined. Subjects who had been instructed to imagine the words in their own voice were substantially more accurate than were those who had been instructed to imagine the words in the experimenter's voice (even though old/new recognition was equivalent in those two conditions). I term this a breakthrough study because, to the best of my knowledge, it was the first in which the sources could only be differentiated on the basis of qualitative content (e.g., remembered sound of voice) as opposed to quantitative characteristics (e.g., amount of sensory detail). As another example along the same lines, participants in a study by Lindsay et al. (1991) watched a video in which two individuals took turns telling a story about going to the circus; subjects were later tested on their ability to identify which of the storytellers had mentioned particular details. Performance was substantially poorer when both storytellers were teenaged girls than when one storyteller was a teenaged girl and the other was an elderly man. Presumably, memories of the appearance and sound of the two speakers were more diagnostic of source when the two storytellers were dissimilar on those dimensions.

Effects of source similarity on subsequent SM are not limited to perceptual similarity; semantic or conceptual similarity can also reduce SM discrimination. For example, in the Lindsay et al. (1991) study, subjects more often failed to remember which storyteller had talked about a particular detail (e.g., that the sword swallower wore black boots) if both storytellers had said something about that circus act than if only one of them had said anything about that act.

As one might expect given the analogy to perception, source monitoring attributions can be influenced by expectations and stereotypes held by the rememberer. For example, Marsh et al. (2006) found that stereotypically masculine statements were more likely to be attributed to a male speaker and stereotypically feminine statements to a female speaker. Similarly, Mather et al. (1999) found that subjects tended to attribute remembered utterances to speakers whose political views fit those utterances. Spaniol and Bayen (2002) found that SM judgments were more likely to be influenced by schemas when memory was relatively poor, just as expectations are more likely to distort perception of vague or ambiguous stimuli than strong and clear ones.

Another sort of bias, termed the it-had-to-be-you effect, is the tendency to attribute false memories to whichever source tends to give rise to weaker memories. In an experiment by Johnson et al. (1981), for example, subjects listened to the experimenter say some words and generated words of their own; on a later test, when they falsely recognized a word, they tended to say that the experimenter, rather than themselves, had generated that word. Presumably, memories of nonpresented words tend to be fairly vague and weak, biasing subjects toward assuming that they came from whichever source tends to give rise to weaker memories. In the first of a clever pair of studies, Hoffman (1997) set up a situation in which recognition memory was better for items that subjects had been asked to imagine in an initial phase than for items they had perceived; when subjects false-alarmed to a nonstudied item, they tended to classify it as having been perceived. In Hoffman's second experiment, phase 1 was changed in ways that led memory to be better for perceived than for imagined items, which in turn reversed the direction of the bias: Now when subjects false-alarmed they tended to attribute those memories to imagination rather than perception. Bink et al. (1999) provided evidence and arguments to the effect that such biases are not necessarily based on strength *per se*: Rather, subjects are biased to attribute false memories to whatever source has characteristics that resemble those of false memories.

In a related phenomenon, people often report phenomenological qualities of false memories that correspond to the characteristics of the source to which the person erroneously attributes those memories. For example, Mather et al. (1997) had subjects listen to audio recordings of Deese/Roediger/McDermott (DRM) lists read by different voices. Such lists consist of words that are all backward associates of a critical word that is not, itself, included in the list (e.g., bed, rest, awake, tired, etc., for the critical word sleep), and

subjects very often falsely remember the nonpresented critical lure. If one speaker read all of the words in each list, then when subjects false-alarmed to critical lures they were very likely to attribute their memories of the lure to the associated list (for analogous findings, see Lampinen et al., 1999; Gallo et al., 2001; Roediger et al., 2004).

As noted earlier, variables that impair encoding often compromise SM. But under some conditions superior encoding can promote SM errors. For example, Toglia et al. (1999) found that deep as opposed to shallow processing of DRM items increased correct recall but also increased false recall. Likewise, Gallo and Roediger (2003) found that elderly adults did more poorly than younger adults on remembering the source of studied DRM items, and that this age-related decline in source memory had the salutary effect of reducing the tendency to attribute false memories of critical lures to the associated list. Similarly, Lyle et al. (2006) found that elderly adults were less able to remember the spatial locations in which images had been presented for study and that they were less likely to falsely claim to have studied look-alike foils in those locations. In all of these cases, the processes that promote accurate recollection also tend to promote illusory recollection.

Under some conditions, processes that enhance memory for studied items also serve to differentiate memories of studied versus nonstudied items and hence to lower the incidence of false memories. Dodson and Schacter (2002), for example, had subjects study a list of words or a list of pictures, then tested their recognition memory with words. Some new (nonstudied) test words were repeated on the test with various lags (as per Jennings and Jacoby, 1997). Subjects who had studied items as pictures were substantially less likely to false-alarm to repetitions of new items on the test than were subjects who had studied items as words, presumably because having studied pictures led subjects to expect that they would be able to recollect pictorial information in response to test probes corresponding to studied words.

Instructions to attend to memory sources, or warnings about potential source monitoring confusions, usually reduce the likelihood of such errors. Presumably such instructions encourage individuals to engage more deliberative, systematic source monitoring processes, rather than relying on quick and easy but more error-prone source monitoring heuristics. Under conditions that encourage lax source monitoring, subjects may endorse almost any item that seems familiar, whereas under other conditions subjects may take care to disentangle different sources of familiarity. For example, Lindsay and Johnson (1989) tested subjects in a variant of Loftus's eyewitness misinformation procedure (e.g., Loftus et al., 1978). Subjects viewed an event, were exposed to misleading suggestions regarding some details in that event, and were then tested on memory for the event. Those tested with a yes/no recognition memory test very often falsely responded Yes to test items that referred to details that had been suggested but not witnessed. Subjects tested on a SM test, in contrast, rarely claimed to have seen in the event things that had merely been suggested, presumably because the SM test encouraged subjects explicitly to query their memories of each item to differentiate between different sources of familiarity (cf. Zaragoza and Koshmider, 1989; Echterhoff et al., 2005). Similarly, manipulations that make the source of misleading suggestions more salient and memorable tend to reduce SM errors (e.g., Sharman et al., 2005).

Source attributions can be made at a wide range of degrees of precision or grain size (e.g., Schacter et al., 1984; Dodson et al., 1998). You might, for example, remember that a statement was made by a woman rather than by a man, perhaps even that the statement was made by a woman student in one of your classes last week, without being able to identify the speaker. The specificity of source attributions is partly a matter of the accessible memory information; the information revived about a past event is often sufficient only for a relatively crude level of source monitoring. Also, within the limits of the accessible memorial information, the specificity of source attributions is flexibly tuned to the rememberer's current goals. Oftentimes people are not concerned about precisely specifying the sources of the thoughts and images that come to mind. In telling an anecdote at a social gathering, for example, one may babble along, interweaving recollections of the to-be-related episode with memories of other prior experiences and memories of stories told by others, filling in weak spots in the narrative with inferences, and enlarging the fish that got away without being aware of doing so, because one's objective is to be entertaining rather than to monitor the origins of one's material.

Most SM attributions are made quickly and without conscious reflection (again, just as is the case with most identifications in ongoing perception). But sometimes rapid, heuristic SM processes fail to produce a source attribution at the appropriate grain

size, and the rememberer has a subjective experience of being unable to specify a memory's source. In such cases the individual may bring more consciously controlled reflective strategies to bear. One such strategy is deliberatively cuing memory in different ways in an effort to retrieve additional source-specifying details. Another is to retrieve memories that are associated with the memory in question (e.g., memories of what happened before or after the event in question, or memories of other events involving the same agent or context as the memory in question). The use of memories of associated events to guide deliberative SM judgments has not received much study, but there is evidence that subjects report more memories of preceding and succeeding events for memories of actual events than for memories of imagined events (Johnson et al., 1988b). Intuition suggests that memories of associated events play major roles in resolving SM failures. Yet another deliberative SM strategy is reasoning (e.g., inferring when an event occurred on the basis of the idea that causes precede effects).

2.19.3.2 Source Monitoring Versus Old/New Recognition

As has long been noted, most laboratory studies of recognition memory are essentially tests of SM, because both studied and nonstudied stimuli are familiar to subjects from extraexperimental sources. Such tests require subjects to discriminate between items encountered extraexperimentally and in the study list versus items encountered extraexperimentally but not in the study list (Anderson and Bower, 1972). Moreover, even when novel stimuli are used, subjects at test must discriminate between reactions to stimuli that stem from having encountered those stimuli on the study list versus those that arise for other reasons (e.g., ease of processing the test probes; Whittlesea, 1993, 2002).

In a typical SM experiment, subjects study items from two sources and are later tested on a mixture of items from Source A, items from Source B, and new items. Thus performance can be assessed in terms of old/new discrimination (i.e., proportion old recognized as old regardless of source-identification accuracy) and in terms of SM accuracy (e.g., proportion of old items recognized as old correctly attributed to source). In most such situations, SM accuracy requires a finer grain of memory specificity than does old/new recognition, simply because Sources A and B are nested within the set of old items. Thus correctly recognizing an item as being from a particular source within the experiment generally requires a finer level of detail than does recognizing an item as having been presented in one source or another in the experiment.

Because of this characteristic difference in grain size, SM is sometimes sensitive to variables that do not significantly affect old/new recognition accuracy. For example, relative to healthy young adults' performance, poorer SM but equivalent old/new recognition may be observed in young children (Foley and Johnson, 1985; Lindsay, 2002), elderly adults (McIntyre and Craik, 1987; Hashtroudi et al., 1999), and amnesics (Shimamura and Squire, 1987). Similarly, under at least some conditions, dividing attention at study has larger effects on SM than on old/new discrimination (e.g., Frost et al., 2002; Castel and Craik, 2003).

It is possible to contrive situations in which conditions that lead to inferior old/new recognition lead to superior SM. Subjects in a study by Lindsay and Johnson (1991), for example, saw a series of words, some presented on the right and others on the left. Half the subjects performed a relatively deep orienting task for words on the right and left, whereas others performed a deep task for words on one side and a shallow orienting task for words on the other side. As one would expect, old/new recognition was poorer among subjects who studied half of the items with a shallow task than among subjects who studied all of the items with a deep task. But because memory for orienting task provided a potent cue for source discrimination among subjects who studied half of the items with a shallow task, those in that condition had higher SM scores than those who studied all of the items with a deep task.

Despite such dissociations, the SMF holds that old/new recognition judgments and SM judgments generally have much in common. In many laboratory tasks, memory information that indicates that an item came from source X also constitutes evidence that the item is old. To the extent that two judgments draw on the same information, performance on them will be correlated (Glanzer et al., 2004; Johnson, 2005).

2.19.3.3 Measures of Source Monitoring

In many studies, SM has been indexed as the proportion of old items recognized as old that are also correctly attributed to source (sometimes called an

identification of origin or IDO score). For example, given two sources, A and B:

$$(A|A + B|B)/(A|A + B|B + A|B + B|A)$$

where A|A means that the subject responds A when given an item from source A, etc. One limitation of this measure is that it is likely to be inflated by guessing. Another is that it assumes that SM is equivalent for items from sources A and B, which is not necessarily the case. Yet another concern is that the IDO score implies that SM discrimination and old/new recognition are independent; put differently, the IDO score may confound old/new discrimination and source discrimination.

As a solution to these problems, Batchelder and Riefer (1990) introduced a multinomial model of SM that yields measures of sensitivity and bias for both old/new recognition and source attribution. They and others subsequently elaborated on the multinomial approach, offering a variety of multinomial models for old/new discrimination and source discrimination (Batchelder et al., 1994; Bayen and Murname, 1996; Meiser, 2005). Taking a different approach to the same problems, Banks (2000) developed a multidimensional signal detection model to assess sensitivity and bias for both old/new discrimination and source attribution, since built upon and supported by others (Glanzer et al., 2004). Yonelinas (1999) proposed a model in which recognition without source identification (i.e., familiarity) is described as a signal detection parameter, whereas source identification is assumed to rely on a threshold recollection process (see Qin et al., 2001; Parks and Yonelinas, 2007; Wixted, 2007, for comments on the Yonelinas model). My hunch is that source identification or recollection tends to behave like a threshold process when the materials and procedures are such that source discrimination relies on generation of a very narrow range of kinds of memory information (i.e., on any given trial, a subject will either generate that information or not), whereas in situations in which source can be correctly identified on the basis of numerous different kinds of information, source identification will behave more like a signal detection parameter.

There are no theory-free measures of memory for source (nor, for that matter, of memory without source identification; cf Jacoby et al. 1997). Moreover, there is no one true measurement model that applies in all situations (Meiser, 2005). Rather, the best measure will rely on the specifics of the situation, depending on factors such as the extent to which identification of sources A and B relies on the same sorts of memory information. Pending a more complete understanding of memory, it may be that the best approach is to compare a variety of measures; often they converge quite closely, and when they do not, the disparities have the potential to be illuminating. Note that I am not suggesting that researchers try all measures and then report only the one that best supports their biases.

2.19.3.4 Time Course of Source Monitoring

On average, coarser source discriminations can be made more quickly than finer ones. When a recognition probe is presented, information that enables the subject to recognize the item as familiar from the experiment typically comes to mind more quickly than information that enables the subject to identify the specific source within the experiment (Johnson et al., 1994). This may simply be due to the fact, noted earlier, that specific within-experiment sources are nested within the larger category of items presented during the experiment, and hence on average require a finer grain size. These time course effects may contribute to the finding that various types of memory errors are more common when subjects are given little time to respond (Dodson and Hege, 2005; Jones, 2006). Such findings have sometimes been described as evidence for a sharp dichotomy between a fast familiarity process and a slow recollection process (McElree et al., 1999), but as noted earlier the SM perspective describes familiarity and recollection as *ad hoc* categories of memory influences rather than as discrete memory systems.

The SMF does not assume an invariant two-stage process in which items are first recognized as old and then attributed to particular sources. It sometimes occurs that an item is initially recognized as old and then attributed to a particular source, but on other occasions an item might first be identified as coming from a particular source (e.g., speaker A) and on that basis experienced as old. Multinomial models appear to imply a two-stage process, but such models are analytic tools, not processing models.

2.19.3.5 Temporal Source Monitoring

Among the most common real-world SM failures and confusions are those involved in situating a

remembered event in time. 'Was it yesterday or the day before that Justin dropped off the key? Was that before or after Myta called?' 'When I had my tonsils out and stayed home from school eating Jello, was it fall, winter, or spring?' There are literatures on various aspects of memory for temporal information, including a large body of work on serial recall (Anderson and Matessa, 1997), studies of memory for duration (Yarmey, 2000), and research on dating public and personal events (Brown et al., 1986; Burt, 1993; Berntsen and Rubin, 2004; Friedman, 2004; Lee and Brown, 2004). But as far as I know, there has been no empirical work on temporal memory explicitly grounded in a SM perspective.

The SMF suggests that qualitative and quantitative aspects of accessed memory information may provide cues as to when a remembered event occurred. Thus, a recollection of something happening while you were sitting at your breakfast table might be identified as an event that happened in the morning; memories of a snowball fight would likely be attributed to winter. Just as with other attributes of source, such cues can be misleading: perhaps, for example, the snowball fight took place in July in the mountains.

Dating remembered events poses special problems for SM because the contents of event memories usually provide only very indirect cues to the date. If, for example, you once had an accident driving to work, years later you might still be able to recall many details of that experience because of its distinctiveness and salience, and those memories might enable you to specify the location of the accident, the approximate time of day (e.g., driving to vs. from work, in light or darkness), and even perhaps the season (rain or snow), but the memory records probably will not provide direct cues to the date on which the accident occurred. The memories constrain the date (e.g., if you retrieve information about geographical location, and you traveled that route only during a particular period), but such constraints tend to be imprecise (except for memories of events intrinsically associated with particular dates).

Consistent with these ideas, people generally have difficulty dating autobiographical events. For example, Friedman (1987) interviewed people 9 months after a major earthquake: On average, respondents were correct to within 1 h in their judgments of the time of day the earthquake occurred but erred by nearly 2 months in their judgment of the month (see also Thompson et al., 1996; for work on the development of temporal SM, see Friedman and Lyon, 2005).

Repeated experiences of highly similar events increase the difficulty of specifying the date on which a particular instance occurred. On which birthday did you receive that blue sweater? Such a question is likely to cue multiple birthdays, each sharing numerous features and none easily dated, such that they tend to blend together in recollection (into what Neisser, 1981, termed repisodes). Relatedly, Connolly and Lindsay (2001) found that children were more susceptible to misleading suggestions regarding variable details about an event they had experienced on several occasions.

2.19.3.6 Affect and Source Monitoring

Emotional arousal tends to enhance memory for occurrence but to impair memory for source. For example, Johnson et al. (1996) showed subjects videos of individuals making emotionally evocative and neutral statements, with instructions that either oriented the subjects to their own affective responses or to those of the speaker. Focusing on one's own emotional responses improved recognition of spoken statements on a subsequent test, but it impaired the ability to remember which speaker had made which statements.

In a more recent study using a short-term source task, Mather et al. (2006) found better item recognition for emotional than for neutral pictures, but better memory for the pictures' spatial locations for neutral than for emotional pictures. Emotionally evocative materials may encourage a narrowing of attention that undermines the binding together of the evocative item and its surrounding context (as per weapon focus; Mitchell et al., 1998).

Orienting toward one's emotions during an event does not always impair subsequent SM. In the Johnson et al. (1996) study just described, for example, shifting the self-focus from how participants felt about the statements to how participants felt about the individual speakers eliminated the self-focus deficit. Although I am not aware of any study testing the hypothesis, it is likely that if a particular emotion was diagnostic of a source, then emotion would be a basis for veridical SM. Nonetheless, in many situations stimuli that evoke strong emotional responses shift attention away from external details that might subsequently be useful for SM.

2.19.3.7 Developmental Changes in Children's Source Monitoring

Children as young as 5 years (and probably younger) can do as well as adults on SM tasks in which the sources are quite dissimilar (even when performance for all age groups is below ceiling). But when the sources in a particular situation are highly similar, then younger children do more poorly than adults. For example, seminal studies by Foley and Johnson and coauthors showed that 5-year-olds were as accurate as adults at remembering which of two actors had performed particular actions, but had more difficulty than adults discriminating between memories of actions they had performed versus memories of actions they had imagined themselves performing (Foley et al., 1983; Foley and Johnson, 1985; Foley et al., 1989). Presumably the cognitive processes involved in performing and imagining oneself performing an action are highly similar, and hence memory records of those two types of events are difficult to discriminate. Consistent with this account, Lindsay et al. (1991) found that young children also had more difficulty than older children when discriminating between memories of what they had seen another person do and memories of what they had imagined that person do.

In more recent research, Foley and coauthors found that, after taking turns with the experimenter to add pieces to a collage or model, preschoolers showed a pronounced tendency to remember themselves as having made contributions that were actually made by the experimenter (Foley and Ratner, 1998; Foley et al., 2002). Foley and coauthors proposed that this is at least in part due to children spontaneously anticipating their collaborator's actions; memories of such self-generated anticipations would be highly similar to, and hence easily confused with, memories of having performed actions.

Why do preschoolers make more errors on difficult SM tasks than older children or adults? It is possible that young children imagine events more vividly than do older children, and hence that their memories of imagined and actual events are inherently more confusable than the memories of older children (especially when real and imagined events are performed by the same agent). It is also likely that the memorial information automatically generated in response to test probes becomes more source-specifying with age (i.e., older children recollect more details, including source-specifying ones; e.g., Sluzenski et al., 2006). My hunch, though, is that the primary source of this age difficulty interaction has to do with developmental improvements in strategically controlled SM. Older children and young adults take longer to respond when source discriminations are difficult than when they are easy, whereas my impression is that younger children often respond as quickly under difficult conditions as under easy ones. It may be that older children have better metacognitive insight into when they do versus do not have an adequate basis for making a source attribution and/or are more skilled at deliberately searching for additional source-specifying memory information when needed (Ackerman, 1985; Schacter et al., 1995). Also, preschoolers' memory-test responses seem to be driven largely by the semantic content or gist of the items, rather than recollections of episodic details or verbatim traces (Brainerd and Reyna, 1995). As noted, older children may also place greater reliance on heuristic biases that, while imperfect, often do lead to correct source attributions.

In a series of studies by Poole and Lindsay (1995, 2001, 2002), 3- to 8-year-old children experienced a series of interactive events and subsequently listened to a parent describe some of those events along with nonexperienced events (including an ambiguous instance of touching). Subsequently, when children received an optimal, nonleading interview, many of them reported having experienced events that their parent had described but that they had not really experienced (including a number of reports of the ambiguous touching event). In response to open-ended questions, the oldest children were just as likely as the youngest children to make false reports of suggested events, perhaps reflecting offsetting effects of age-related improvements in ability to remember and talk about the suggestions as well as age-related improvements in the ability to suppress such reports. Late in the interview, children were specifically asked to discriminate between events they remembered experiencing and those that they might merely have heard about. This SM test substantially reduced false reports of suggested events in older children, but had no such effect on younger children.

In Poole and Lindsay's 2002 study, half the children participated in a simple SM-training procedure at the beginning of the interview. In this procedure, the interviewer performed some actions (e.g., wiping off the tape recorder) and talked about performing other actions (e.g., pushing the button to reset the counter on the tape recorder). Immediately thereafter, children were asked whether the

experimenter had really performed each action, and they were given explicit corrective feedback (e.g., 'That's right, I really did wipe off the tape recorder; you know that because you saw me do it,' or 'Think hard – Remember when I said that I sometimes push the button to reset the counter on the tape recorder? But you didn't really see me push the button to reset the counter on the tape recorder, did you? No, you didn't, so "No" is the right answer'). This procedure substantially reduced, but did not eliminate, false reports of suggested details in response to direct questions in the main part of the interview for 7- and 8-year-old children; it had no impact on younger children (see also Giles et al., 2002; Bright-Paul et al., 2005; Thierry et al., 2005).

SM is not a single skill that children acquire at a specific age. Rather, SM involves inferences about numerous dimensions of source – remembering who, remembering where, remembering how, remembering when, etc. – and depends upon multiple kinds of mental activities (e.g., perceptual analysis and reflective integration during encoding, revival of memory records, and decision-making processes at test). Thus developmental changes in SM are gradual and situation-specific rather than sudden and global. These considerations also suggest that SM development is correlated with individual differences along a number of dimensions (Lorsbach and Ewing, 1995; Quas et al., 1997; Welch-Ross et al., 1997; Drummey and Newcombe, 2002; Roebers and Schneider, 2005).

2.19.3.8 Source Monitoring Performance in Old Age

Henkel et al. (1998) reviewed a wealth of evidence indicating that SM performance generally declines late in life. As with young children, elderly subjects can do well on SM tasks when the sources are highly discriminable, but their performance deteriorates sharply as source similarity increases. Henkel et al. (1998) argued that aging-related SM deficits may be mediated by reductions in the extent to which contextual details are encoded in ways that tightly bind them together with other aspects of an event (see also Lyle et al., 2006). Poorer encoding and integration of features means that older adults are less able to recollect such details later on, leaving them with more vague, abstract memories of experienced events. Such memories are difficult to discriminate from memories of internally generated events.

2.19.3.9 The Neuroscience of Source Monitoring

The hippocampus appears to play important roles in episodic memory. Johnson (2006) argued that the hippocampus is particularly important in binding together different aspects or features of an event to create complex, multifaceted memories which, among other things, afford SM attributions. Damage to the hippocampus and surrounding areas has profound debilitating effects on episodic memory (Milner, 2005). Mitchell et al. (2000) used functional magnetic resonance imaging (fMRI) in a short-term memory test in which young and old adults were either required simply to recognize items or to bind together items and locations. They found that younger adults exhibited greater hippocampal activity on binding trials than on item trials, whereas older adults did not (consistent with a selective age effect on performance of SM vs. old/new recognition tests). Johnson (2006) also argued that the prefrontal cortex (PFC) is likely to be involved in noting and reflecting on relationships between features of events, and that such processes, too, play important roles in creating highly source-specific event encodings.

There is also evidence for roles of sensory and motor cortex during encoding in laying the groundwork for subsequent SM performance. In an fMRI study by Gonsalves et al. (2004), for example, subjects saw some items and were asked to imagine seeing others. Activation in visual areas was greater for to-be-imagined items that subjects later erroneously claimed to have seen than for those that they correctly reported imagining, consistent with the idea that vivid and detailed images are more likely to be later mistaken as memories of perceptual events (see Leynes et al., 2006, for a related finding with event-related potential (ERP)).

Earlier, I noted that PFC is thought to be involved in discovering and maintaining attention to relations between different features or aspects of an event in ways that may be important for hippocampal consolidation of complex memories. It is also thought that the PFC plays important SM roles during remembering. Consistent with that claim, Johnson et al. (1997) found greater PFC activity on an SM test than on an old/new test for the same items. Johnson (2006) reviewed a number of ERP and fMRI studies whose findings suggest that the left PFC is particularly important for SM judgments.

2.19.4 Related Theoretical Perspectives

2.19.4.1 Jacoby's Memory Attribution Approach

Larry Jacoby and his coauthors noted that people sometimes use memory information from specific prior episodes without having the subjective experience of remembering (as in involuntary plagiarism), and that people can have the subjective experience of remembering specific prior episodes that they never in fact experienced (as in various forms of false memories; e.g., Schacter, 2001). Jacoby and coworkers argued that the subjective feeling of remembering arises from an unconscious attribution that is based on the fluency with which an item is processed. Specifically, when cognitive processing is surprisingly fluent one may attribute that fluency to the use of memory, especially if the situation highlights the past (i.e., memory) as a source of influence on current processing (Jacoby and Dallas, 1981; Jacoby and et al., 1989a).

Bruce Whittlesea's SCAPE model can be described as an elaboration of Jacoby et al.'s (1989a) ideas regarding fluency-based attributions to memory. Whittlesea has emphasized that it is unexpected fluency, not fluency *per se*, that leads to memory attributions (a point that was tacit in Jacoby's treatment; e.g., Jacoby and Whitehouse, 1989). Whittlesea and Williams (1998), for example, exposed subjects to words and nonwords and later tested them on a mix of studied and nonstudied words and nonwords. Subjects read each test word aloud before making a recognition judgment to it. Half of the nonwords were regular (e.g., hension), whereas the others were irregular (e.g., stofwus). The key finding was that reading times were fastest on words, but it was the regular nonwords that drew the highest rate of false alarms. Presumably, subjects tended to attribute the fluency with which they read words to their status as words. Regular nonwords may thus have been experienced as surprisingly fluent. It is only when the fluency is discrepant with the person's moment-by-moment impression of how fluent his/her processing should be, and when memory is a plausible source of that fluency, that the person is likely to attribute fluency to memory.

The question of what leads people to attribute thoughts, images, and feelings to memory versus to other sources can be described in terms of the SMF: Thoughts, images, and feelings that come to mind with characteristics typical of memories are likely to be experienced as memories, especially if the person is oriented to the past as a source of current mental events. Similarly, those with the characteristics of perception will tend to be attributed to sensory stimuli (sometimes giving rise to hallucinations; see Johnson, 1988), those with the characteristics of new ideas will be experienced as novel insights, etc. From this perspective, relative fluency is but one cue to source.

2.19.4.2 Dual-Process Models of Recognition Memory and the Remember/Know Distinction

Dual-process models of recognition memory hold that items can be correctly recognized as old on either of two independent and qualitatively different bases: (1) Familiarity, a rapid, automatic, undifferentiated feeling of having previously encountered a test item; and (2) recollection, a more deliberative and effortful process of retrieving episodic details regarding the prior encounter with an item (Mandler, 1980; Jacoby, 1991). That contrast is related to the distinction between Remember and Know judgments in the remember/know procedure, in which subjects are asked to indicate whether affirmative recognition judgments are based on episodic recollections of details of encountering the item on the study list or on an undifferentiated feeling of just knowing that the item was on the list.

According to the SMF, processing a test probe sometimes leads to the generation of sufficient source-specifying memory information to enable source identification at a particular grain size, and other times does not (as governed by the principles discussed earlier). The SMF also suggests that certain kinds of memorial information are relatively likely to give rise to a subjective experience of remembering a unique prior episode, whereas others are more likely to give rise to a less-differentiated sense of familiarity. Specifically, source identifications and reports of remembering are likely to arise from access to memories of relatively reflective, elaborative, integrative, distinctive processes. Reports of just knowing, in contrast, are likely to reflect memories of more automatic, data-driven, generic cognitive processes. The recollection/familiarity and remember/know contrasts refer to categorically distinct phenomenological experiences, but from the SMF they are thought to arise

from a continuum of memory specificity (Dodson and Johnson, 1996; Gruppuso et al., 1997; Bodner and Lindsay, 2003).

2.19.4.3 Constrained Retrieval

Can you recall an event that occurred when you were in high school that is somehow associated with fire? To generate such a memory, you might in principle first retrieve lots of fire-related memories and then check to see if any of them occurred in high school, but in practice we seem to constrain retrieval such that memories are more likely to come to mind if they are from the to-be-recalled source than if they are from other sources (although of course the constraint is imperfect). Jacoby et al. (2005) proposed that such constrained retrieval plays a central role in enabling individuals to remember material from the appropriate source. They also argued that people can constrain the ways they process recognition test probes so as to facilitate retrieval of memory information from the to-be-recognized source as opposed to memory information from other sources. These provocative new ideas valuably complement the SMF's emphasis on monitoring.

2.19.5 Empirical Phenomena Illuminated by the Source Monitoring Framework

The study of memory phenomena that can be described as SM failures or confusions far predates the development of the SMF itself. In this section, I provide brief reviews of a number of such phenomena; for a wider-range review, see Schacter (2001).

2.19.5.1 Verbal Learning Effects

Prior to the development of the SMF, phenomena involving SM had been investigated for many years in the verbal learning tradition. For example, studies of list differentiation assessed subjects' ability to attribute studied words to different study lists (Winograd, 1968; Abra, 1972). This research demonstrated the importance of factors such as semantic similarity and temporal separation of the lists. Such findings informed efforts to understand retroactive and proactive interference effects (Postman, 1975).

2.19.5.2 The Eyewitness Misinformation Effect

Studies of eyewitness memory, and of the effects of suggestive influences on eyewitnesses' reports, have featured prominently if sporadically in the history of psychology (for reviews of early psychological research and speculation on this topic, see Brigham and Grisso, 2003; Goodman, 2006). In the mid-1970s, Beth Loftus and coauthors reported studies that inspired interest in this domain that continues to the date of this writing. Loftus et al. (1978) introduced a three-phase procedure in which subjects first viewed a series of slides depicting an event, then were exposed to verbal information that included misleading suggestions regarding some details in that event, and later were tested on memory for the initially witnessed details. Their key finding was that subjects' answers were often based on the misinformation, rather than on what they had actually witnessed. For example, having seen a slide in which a traffic intersection was marked with a yield sign and then later being exposed to the suggestion that the intersection was marked with a stop sign, subjects quite often reported at test that the intersection had been marked with a stop sign.

Throughout most of the 1980s, debate on this eyewitness misinformation effect focused on the question of whether or not misleading suggestions regarding a witnessed detail impaired witnesses' ability to recall or recognize the witnessed detail (e.g., whether the stop sign suggestion impaired memory for the yield sign). McCloskey and Zaragoza (1985) considerably enlivened that debate with an article providing a cogent logical analysis of the various reasons that suggestions could lower accuracy even if they had zero effect on ability to remember the witnessed details (e.g., compared to control subjects who had never encoded the event detail, misled subjects who also had failed to encode the event detail would be less likely to guess correctly on the test), and six experiments whose results provided no support for any event-detail memory impairment phenomenon (but see Payne et al., 1994; Chandler et al., 2001; Eakin et al., 2003, for evidence that modest memory-impairment effects are obtained under some conditions).

In the late 1980s and throughout the 1990s, attention shifted from this memory-impairment issue to the question of whether or not misled subjects believe that they remembere witnessing details that had in fact merely been suggested to them. This question

falls squarely in the purview of the SMF, and the answer (as with any psychological question) is, it depends. As previously mentioned, under some conditions, misinformation effects obtained on a yes/no recognition test (i.e., subjects falsely responding Yes to items that were merely suggested to them) vanish when subjects are given a SM test that orients them toward scrutinizing the sources of their memories (Lindsay and Johsnon, 1989; Zaragoza and Koshmider, 1989). That might be because on the yes/no test subjects sometimes endorse items that they believe they remember from the misinformation (e.g., because they assume the misinformation was accurate). Alternatively, it might be that the SM test leads subjects to use more systematic SM procedures to avoid SM confusions that they would make using more heuristic processes on a yes/no test. Importantly, it has been amply demonstrated that misinformation effects can be obtained on SM tests if the conditions make SM difficult (the sources are highly similar and there is a delay between them and the test, the subjects are young children or elderly adults, etc.; see Lindsay, 1994; Zaragoza and Lane, 1998; Poole and Lindsay, 2001; Mitchell et al., 2003).

Even positive responses on an SM test are not definitive evidence that subjects genuinely believe that they remember witnessing suggested details. If subjects trust the source of the suggestions, they might be tempted to claim that they both remember encountering details in that source and witnessing those details. As a stronger test of the hypothesis that subjects are sometimes genuinely unaware of the source of their memories of suggested details, Lindsay (1991) applied Jacoby's opposition procedure (Jacoby et al., 1989b) in a misinformation paradigm. Subjects witnessed a theft depicted in a series of slides, and were later exposed to a narrative description of the theft that presented misleading suggestions regarding some details and control information about other details. In the difficult condition, the event and narrative were presented in immediate succession, with the test given 2 days later; for subjects in the easy condition, the event was presented on the first day and the narrative was presented 2 days later, immediately followed by the test. This latter condition was easy both in that it should be easy at test to remember the suggestions (which had just been presented minutes before) and it should be easy to differentiate memories of the suggestions from memories of the event (due to the large separation between the two sources). At test, subjects were given cued recall questions along the lines of, 'Under what sort of tool did the handyman hide the stolen calculator in his toolbox?' with half of the questions pertaining to items for which subjects had received misleading suggestions (e.g., hammer in event, wrench in narrative) and others pertaining to items for which no suggestions were given (e.g., see a can of Coca-Cola in the event, read it described as a can of soda in the narrative). Crucially, before taking the test subjects were emphatically told that if they remembered having heard something in the narrative that might be used as an answer to a question on the test they could know for certain that it was a false suggestion, and that they should therefore not report anything they remembered from the narrative. Subjects in the easy condition showed no tendency to report suggested details; given that these subjects were in a good position to remember those details, this indicates that subjects understood and followed the instruction not to report details from the narrative. Subjects in the difficult condition, in contrast, quite often reported suggested details. Significant suggestibility effects under opposition instructions provide powerful evidence that subjects are sometimes genuinely misled about the sources of their memories (see also Holliday and Hayes, 2002; Eakin et al., 2003; Price and Connolly, 2004).

2.19.5.3 False Memories Induced by Schemas, Scripts, and Associations

The SMF fits well with earlier research on schema-based memory errors, in which individuals' knowledge and beliefs were shown to distort their memory reports (Bartlett, 1932; Brewer and Treyens, 1981). That is, schemas support the fluent generation of inferences that may have many of the characteristics of memories. As a recent example consistent with this idea, Gerrie et al. (2006) found that subjects who had viewed slides depicting highly scripted events (e.g., making a peanut butter and jelly sandwich) very often falsely recognized script-typical slides that had been omitted from the studied series.

2.19.5.4 Other Fluency-Based False Memories

Similar to knowledge and beliefs, other variables that facilitate processing of recognition test probes can increase endorsement rates. For example, Jacoby and Whitehouse (1989) preceded recognition test probes with briefly presented primes that either matched or mismatched the probe. When prime

duration was very short, such that subjects were not consciously aware of the presentation of the prime, Yes rates to both old and new probes were higher when preceded by matching primes. Presumably, the brief prime facilitated processing of the test probe and that fluency was attributed to prior exposure on the study list. Of critical importance, when primes were presented for a slightly longer period, so that subjects were consciously aware of them, the data pattern reversed as subjects evidently overattributed the fluency with which they processed test probes to the preceding matching prime. Similarly, Whittlesea (1993) found that a variety of manipulations of the fluency with which test probes were processed affected recognition responses. Lindsay and Kelley (1996) demonstrated analogous effects in cued recall: A manipulation that enhanced the ease with which words popped to mind in response to recall cues increased both accurate and erroneous cued recall reports.

2.19.5.5 Veridical and Illusory Recovered Memories of Childhood Sexual Abuse

The 1990s saw a heated controversy regarding cases in which individuals reported that they had recovered long-forgotten histories of childhood sexual abuse. The debate focused on cases in which reports of recovered memories arose in the context of psychotherapy oriented toward fostering memory recovery. Critics of such therapies argued that they were dangerously suggestive and that they sometimes led clients to develop false beliefs or false memories of abuse that never really occurred (Loftus, 1993). Some proponents of trauma-memory-oriented therapies countered that such criticisms were anti-feminist, pro-perpetrator backlash against victims of childhood sexual abuse.

This is a tremendously complex, multifaceted, and emotionally explosive topic, with valid concerns on both sides (Read and Lindsay, 1997). Fortunately, although strenuous contentions still arise in this area (Wade et al., 2007), my perception is that a middle-ground position that acknowledges the likelihood that both essentially accurate and essentially illusory recovered memories occur has come to dominance (Lindsay and Briere, 1997).

In any case, the point for present purposes is that the SMF was of considerable value in understanding how a prolonged, socially influenced, multipronged (albeit well-intentioned) effort to foster the recovery of suspected hidden memories of abuse could, instead, lead individuals to develop false beliefs and memories of abuse (Lindsay and Read, 1994, 2006). There is, for example, some evidence that individuals who report recovered memories are more susceptible to SM confusions on laboratory tasks (McNally et al., 2005) and that they are more prone to forget prior instances of remembering events (Geraerts et al., 2006).

2.19.5.6 The Knew-It-All-Along Effect

The knew-it-all-along (KIA) effect, or hindsight bias, is observed when persons report that they possessed knowledge at a previous point of time that they in fact acquired subsequent to that time (Fischhoff, 1975; Wood, 1978; Hasher et al., 1981). Of particular interest here is the memory version of the KIA effect, in which subjects answer a set of questions in phase 1, are then exposed to the correct answers to some of those questions in phase 2, and in phase 3 are asked to re-answer the questions exactly as they did in the first phase. The standard finding in this procedure is that subjects' re-answers to items for which they had been shown the correct answers are often shifted in the direction of the correct answers.

When subjects demonstrate a KIA effect, do they have an (illusory) subjective experience of remembering themselves giving newly learned correct answers on the initial test? Or is their experience merely one of guessing or inferring their prior responses? There is evidence that, under at least some conditions, subjects fail to appreciate the extent to which their re-answers are influenced by the experimental exposure phase in KIA procedures (Begg et al., 1996) and in closely related procedures (e.g., Prentice and Gerrig, 1999; Marsh et al., 2003), but do subjects remember giving correct answers that they did not really give?

To explore this question, Michelle Arnold and I (Arnold and Lindsay, in press) conducted KIA experiments in which subjects were asked to report, for each re-answer, whether they: (1) remembered giving that answer initially, (2) knew they had given that answer without being able to recollect having done so, or (3) felt that they were merely guessing or inferring that they had given that answer. Under standard KIA procedures (passive exposure to the correct answers to trivia questions), when subjects showed a KIA effect they almost always reported guessing or inferring their prior answers. But when the materials were insight problems and the second phase involved providing subjects with sufficient

cues to solve the problems, then they quite often subsequently reported false memories of answering questions correctly in the first phase. Presumably in the latter procedure, memories of having been led to figure out a problem in Phase 2 were highly confusable with memories of having spontaneously solved that problem in phase 1.

2.19.5.7 The Forgot-It-All-Along Effect

Schooler et al. (1997) sought out cases in which adults reported having recovered long-forgotten memories of childhood sexual abuse for which there was evidence that the abuse had occurred. They reported two cases in which individuals had apparently told others about the abuse during the period of alleged amnesia. Schooler et al. speculated that these women had recalled the abuse in a qualitatively different way that was accompanied by strong emotions, and that they made an unconscious attribution along the lines of 'I must not have known about this before, lest I wouldn't be so emotionally affected by these recollections.' Schooler et al. termed this hypothetical phenomenon the forgot-it-all-along (FIA) effect, in reference to the aforementioned KIA effect.

Arnold and Lindsay (2002, 2005) developed a laboratory analogy designed to capture some aspects of this hypothesized FIA effect. Subjects were cued to remember items on two different occasions; for half of the items the cues were varied on the two occasions so as to shift the way the subjects thought about the recalled item. On the second test, after each item was recalled, we asked subjects whether they had also recalled that item in the first test. We found that when subjects had recalled the same item on each of the two tests, they were more likely to fail to remember their test-1 recall of the item if they had been cued to think of the item in different ways on the two tests (i.e., a FIA effect). Geraerts et al. (2006, Experiment 2) extended the procedure to memories of autobiographical events and, as mentioned earlier, found larger FIA effects among subjects who reported having recovered repressed memories of childhood sexual abuse than among control subjects.

2.19.5.8 Cryptomnesia

Cryptomnesia, also known as unconscious or inadvertent plagiarism, occurs when an individual mistakes memories of another's ideas as new ideas of his or her own. Brown and Murphy (1989) introduced a three-phase procedure for studying cryptomnesia. In an initial phase, subjects took turns (with one another or with the experimenter or computer) generating items that fit a specified constraint (e.g., names of musical instruments). In the second phase, subjects were asked to recall their own phase-1 contributions. In phase 3, subjects were asked to generate new items not previously generated by them or anyone else in the experiment. Cryptomnesia was often observed in phases 2 and 3, with subjects tending to claim that they recalled themselves generating items that others had in fact generated, and including in their 'new' phase 3 generations items that they or others had generated in phase 1.

As the SMF would lead one to expect, manipulations that increase the similarity between self-generated and other-generated ideas increase rates of cryptomnesia. For example, subjects tested in same-sex pairs show higher rates of cryptomnesia than those tested in different-sex pairs (Macrae et al., 1999), a finding that also emerged in a retrospective self-report survey of everyday cases of cryptomnesia by Defeldre (2005). Marsh et al. (1997) reported converging evidence for the idea that failures in SM processes underlie cryptomnesia. More recently, Stark and Perfect (2006) found that elaborating on another's idea substantially increased subsequent plagiarism, perhaps because the processes performed when elaborating an idea are very similar to and hence highly confusable with those involved in hatching the idea.

2.19.5.9 The Mere Exposure Effect

In a classic paper, Kunst-Wilson and Zajonc (1980) demonstrated that very briefly presented neutral stimuli were subsequently preferred over novel neutral stimuli in two-alternative forced-choice judgments, even though subjects were at chance when explicitly asked to discriminate between previously exposed and new stimuli on the same test pairs. Anecdotal reports (and my own experience) indicate that it is not easy to obtain above-chance preference coupled with at-chance recognition, but that pattern has been reported sufficiently often to compel the conclusion that it is a real albeit delicate phenomenon (Seamon et al., 1983a,b). Both aspects of this effect are interesting. First, it is interesting that influences of prior exposure can be experienced as preference. This is an SM failure of a sort, perhaps reflecting an inherent tendency to prefer stimuli that are easily processed (Winkielman et al., 2006). It is perhaps noteworthy

that to the best of my knowledge, the effect on preference in the absence of recognition has only been reported with stimuli that afford little in the way of strong preferences (e.g., random polygons). It is also intriguing that subjects at chance on recognition have been shown to select previously exposed items at above-chance levels on certain other kinds of judgments (e.g., brightness or darkness judgments in Mandler et al., 1987; see Seamon et al., 1998, for evidence that it is easier to obtain the dissociation pattern with affective judgments than other sorts of judgments).

Arguably more interesting than the above-chance performance on preference judgments is that, having memories sufficient to generate this preference effect, subjects nonetheless respond on the recognition test as though they had no such memories. Whittlesea and Price (2001) offered arguments and evidence to the effect that this dissociation arises because subjects tend to make preference judgments in a nonanalytic, holistic manner, whereas they tend to make recognition judgments in a more analytic, feature-based manner. Presumably, the latter orientation toward test stimuli reduces the extent to which subjects cue revival of the weak and poorly bound memory records of the prior exposure. This may also account for the evidence of Seamon et al. (1998), mentioned previously, that various judgment tasks are differentially sensitive under conditions that lead to chance-level recognition.

2.19.5.10 Déjà Vu

Most people report that they have had the uncanny experience of being in what they know to be a novel situation and yet feeling that they have previously been in that situation. If the mere exposure effect is tough to get in the lab, déjà vu is nigh unto impossible, so the latter effect has been studied with self-report measures. Brown (2004) summarized that research and offered three accounts of déjà vu: (1) a decoupling of streams of perceptual processing that normally progress in synchrony, such that one stream runs faster than the other with the later stream, then cuing memories of the (milliseconds old) faster stream; (2) a momentary lapse of attention, during which perceptual processes carry on automatically, with memories of those (poorly bound) perceptual processes being cued when attention returns to the ongoing situation; and (3) partial revival of memories of some similar past situation, giving rise to a strong feeling of familiarity without providing sufficient source-specifying information to enable the person to attribute that familiarity to its correct source. The last of these accounts is most amenable with a memory SM perspective, but as discussed in the next section, all three are in keeping with a broader approach to SM.

2.19.6 Challenges and Future Directions

2.19.6.1 Multidimensional Source Monitoring

Most studies motivated by the SMF have explored rememberers' ability to discriminate between memories from two sources (e.g., two external sources or an external source versus an internal source such as a spontaneous inference or a directed image), typically using forced-choice tests. In everyday life, SM is much less constrained. If, for example, you try to remember how you got the idea that polar bear hair is translucent and hollow, the range of potential sources is very wide. A number of recent studies have tested SM across two pairs of nested sources (e.g., identifying which of four individuals – two women and two men – had said particular words; Dodson et al., 1998). Some studies have involved simultaneous explorations of two different dimensions of source manipulated orthogonally (e.g., font size and location; Marsh et al., 2004; Starns and Hicks, 2005). I suspect that much more can be done to explore SM in situations in which the range of potential sources is broad.

2.19.6.2 Interpersonal Source Monitoring

In the course of conversation, auditors sometimes make inferences regarding the sources of their interlocutor's memory reports. You may, for example, have listened to someone relating an anecdote and thought to yourself, 'He's probably making that part up,' or 'I bet she's exaggerating a bit,' or 'I bet he got that from the *National Enquirer*.' The bases for such inferences are likely numerous and complex, particularly in cases in which the auditor has extensive prior experience with the storyteller or has independent knowledge of the content of the tale. Such inferences have as much to do with social and personality psychology as with cognition, but nonetheless the SMF may inspire hypotheses about at least some of the processes involved in making inferences about the accuracy and source of another person's verbal reports.

Even when listening to an unfamiliar person describing a novel event, auditors may make inferences about the accuracy and reliability of those reports. This is especially so when conditions foster concerns about lying (as in police investigations), and there is an extensive and fascinating literature on deception detection (Granhag and Vrij, 2005). Sporer (2004) has developed a deception-detection scale based in part on the SMF, and this approach appears to have substantial potential. Relatedly, jurors weigh the testimony of witnesses, evidently driven largely by the witnesses' apparent confidence (e.g., Brewer and Burke, 2002; Tetterton and Warren, 2005).

It is also interesting to consider interpersonal SM in situations in which lying is not at issue but in which storytellers might nonetheless be mistaken. Schooler et al. (1986) exposed subjects to misleading suggestions regarding a witnessed event, had them write descriptions of the event, and gave those descriptions to new subjects for evaluation; these evaluations were slightly but significantly above chance (see also Johnson and Suengas, 1989). Johnson et al. (1998) found that the more details an account contained, the more believable naive judges found that account to be. Lindsay et al. (2000) found that undergraduates role-playing as police officers were above chance at discriminating between accurate and inaccurate truthful witnesses, but that they did so less well than witnesses' own self-ratings of confidence (see also Dahl et al., 2006). Here again the SMF is a source of hypotheses as to how perceivers make such judgments and how their accuracy might be improved.

2.19.6.3 Falsifiability

The SMF has a great many degrees of freedom. For one thing, memory records are described as multifaceted, imperfectly bound constellations of numerous aspects or features, from low-level perceptual primitives to conceptual reflections. Thus, for example, two sources might be highly similar along some dimensions and quite distinct along others (e.g., Marisa and Jim might both have Spanish accents but very different pitches, whereas Marisa and Elke might have similar pitches but different accents). How do multiple dimensions of similarity interact? As another example, compared to generating an image of an item once, generating it several times may increase both (1) records of cognitive operations associated with generating an image of that item (which could be taken as evidence that the item was generated) and (2) the fluency and vividness with which the latter images were generated (which could be taken as evidence that the item was perceived). Without a theory to specify which aspects will be more or less accessible and more or less heavily weighted in a particular situation, it is not always obvious which conditions will lead to more or fewer SM failures.

Moreover, SM performance is said to depend not only on the characteristics of memory records but also on the rememberer's expectations, biases, stereotypes, current orientation, and goals. Variations along these higher-level dimensions can interact with variations in the characteristics of memory records. As an example, consider an eyewitness misinformation study by Bonto and Payne (1991), in which some subjects were exposed to the witnessed event and the postevent information in the same context, whereas others were exposed to the two sources in a different context. The SMF would predict that source discriminations would be more difficult in the same-context condition than in the different-context condition, but Bonto and Payne found equivalent (and substantial) influences of misinformation in both conditions. One possible account has to do with the fact that Bonto and Payne's procedure likely encouraged subjects to rely on memories from both sources. There was no warning about misinformation, so subjects may have assumed that the postevent information was a legitimate and reliable source of answers to test questions and hence not been concerned about discriminating memories from the two sources.

Some of the most clever SM research in recent years has come out of the labs of Rich Marsh and his coauthors, including several studies that further illustrate the difficulty of using the SMF to make specific predictions. In a study by Marsh et al. (2002), for example, subjects were presented with compound words (e.g., deadbolt, neckline) in two sources and were later tested on either a yes/no recognition test or on a SM test. Of central interest was the rate of falsely claiming to have studied conjunctions (e.g., deadline). One might expect that the SM test would lead subjects to scrutinize their recollections more carefully before responding and thereby lower the rate of such errors. Instead, Marsh et al. found that when the two sources were sharply dissimilar and when the 'parents' of a conjunction had both been presented in the same source, then subjects tested with the SM test were more likely to make

conjunction errors than were subjects tested with the recognition test (for related results, see Hicks and Marsh, 2001). Marsh, Hicks, and their colleagues have discussed these and similar results in terms consistent with the SMF, but the point for present purposes is that the framework does not always provide a clear and firm framework for predicting behavior in complex situations.

In many well-controlled and simple experiments, the SMF is falsifiable, but in more complex, less controlled situations it is often possible to fashion accounts consistent with the SMF for a variety of different empirical outcomes. Some theorists (e.g., Reyna and Lloyd, 1997) have strongly criticized the SMF for this limited falsifiability. This may partly be a matter of taste, with some theorists putting a premium on falsifiability and others esteeming the extent to which a theory serves to organize and inspire nuanced hypotheses regarding a wide range of phenomena. Of course, in the long run, proponents of the SMF hope to more precisely specify the interactions among the numerous variables involved in attributing mental events to particular sources.

2.19.7 Conclusion

Some theories describe remembering as a matter of using the episodic memory system, knowing as a matter of using the semantic memory system, skilled performance as a matter of using the procedural memory system, etc. Indubitably there are functional brain systems specialized for the sorts of cognitive processes that typically support remembering, knowing, doing, etc. But just as surely those brain systems do not operate in isolation from one another, and the thoughts, images, and feelings to which they give rise are products of multiple subsystems interacting. Because the implications of mental contents vary greatly as a function of their sources (e.g., remembering that one previously encountered a tiger near this water hole is more consequential than remembering that one previously dreamed of such an encounter and less consequential than currently sighting a tiger), we routinely monitor the sources of our thoughts, images, and feelings. The SMF provides a productive way of thinking about the processes by which such attributions are made.

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2.20 Metamemory

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Metamemory refers to the processes and structures whereby people are able to examine the content of their memories, either prospectively or retrospectively, and make judgments or commentaries about them. Thus, metamemory is not memory itself, although it may depend critically upon memory. Rather it is the judgments, assessments, or commentaries that are made about memories or learning. These kinds of self-reflective judgments have a long and controversial history. Presumably, for example, when Descartes was engaged in his famous doubting meditation – musing about how his memories or perceptions could have been different than they were, or how he could have been mistaken about them – he was engaging in metacognition. This kind of reflection was taken by him as the basis of all knowledge and the core of our phenomenological selves. Similarly, the introspectionists (with whom behaviorists later took such exception) were, presumably, engaging in what we would now call metacognition. The lack of reliability of their findings was a shortcoming that proved devastating for their method by opening the door for the behaviorists to oust the study of consciousness, at least temporarily, from the domain of respectable topics in psychology. However, the judgmental biases that were the bane of early twentieth century introspectionism are now being studied under the guise of the biases and framing effects that are both systematic and rampant in metacognitive judgments.

That these metamemory judgments can be studied objectively, and reliably, is now apparent, with many hundreds of studies having been directed at issues of human metacognition. Indeed, growing interest and research from a metacognitive perspective – with its emphasis on people's memory-based attributions – can be considered one of the most significant developments in the science of psychology in this new century. Both the processes that underlie the judgments themselves and the implications that these judgments have for self-guided control of learning are yielding to investigation. Current methods promise both enhanced understanding of impairments in metacognition and also the possibility of remedying certain biases to enable people to better assess and control their own learning.

How these judgments are made has been the focus of much research, and some of these processes are detailed shortly. Classically, three types of judgments

have formed the core of metamemory research: Feeling-of-knowing judgments, tip-of-the-tongue judgments, and judgments of learning. Although there may be some differences between feeling-of-knowing and tip-of-the-tongue judgments (see Schwartz, 2006), their similarities outweigh their differences, and we treat them together. However, the restriction to these so-called classic judgments is arbitrary, because metamemory refers to any judgment that is about a memory. The reflective quality is what is important in the definition. Thus, other judgments such as confidence judgments, source judgments, recognition judgments, and remember/know judgments are also properly considered to be metamemory. Indeed, any attribution about memory is properly considered to be metamemory, and one may even argue that all memory output relies at least partially on metamemory. For instance, if you covertly recall that the word needle was on a list of words that you just tried to memorize or that Dr. Case told me that the medication would have no side effect, you would likely not report the word needle or recommend the medication to a friend if you were not sufficiently confident in your memory (Koriat and Goldsmith, 1996). Accordingly, we also briefly discuss other memory judgments – in particular, source judgments and remember/know judgments – in the same context as the classical metamemory judgments.

Figure 1 provides an illustration of what is meant by metamemory. Nelson and Narens (1990), in a highly influential paper, argued that metacognition entailed two mental levels: an object level and a metalevel. The object level consists of the memories themselves. The metalevel involves monitoring the object level, such as reflecting upon memories and ongoing learning. When the object level is memory, such monitoring is measured by feeling-of-knowing judgments, judgments of learning, source judgments, or judgments about whether the individual remembers the event explicitly or only knows that it must have happened. The requirement is that the object of the judgment be a mental event, rather than something that is present in the environment. Many animals can make judgments about the world, but few are capable of reflecting on the objects of their minds, such as their memories (see Terrace and Metcalfe, 2005). The ability to so reflect indicates a fundamentally different kind of mental life for the animals that have it.

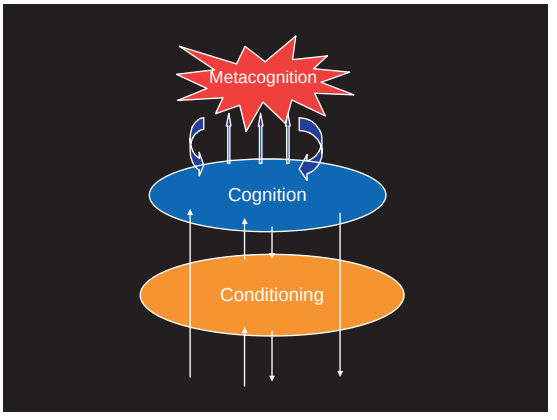

Figure 1 A model of the relations among metacognition, cognition, and conditioning. The model shows that the metacognitive level both monitors (up arrows) and controls (down, thick arrows) the contents of the cognitive level.

As can also be seen from the figure (arrows flowing from metacognition to cognition), metacognition is presumably necessary for high-level control of one's own mental processes and memories. Without knowledge of what one does not know, one could not be expected to take action to remedy the situation by, say, allocating differential study opportunities, rehearsal, or time. The metacognitive individual can choose to mould his or her own mind by self-initiated study processes, thereby learning things under self-control rather than only under stimulus control. To regulate effectively, such self-guided learning requires accurate metacognitions, of course, but it also depends on their appropriate use. If one's metacognitive judgments are inaccurate, self-regulated study could be suboptimal because the person does not know what he or she does not know. Such metacognitive failure could result because of immature metacognition capabilities or because of an impairment due to illness, stroke, or head injury. Distortions in metacognition also occur, even in normal and unimpaired people, because they are blinded by some illusion of metacognition due to the circumstances of the task at hand. Many metacognitive illusions – or biases – have now been documented by researchers (Bjork, 1994), and understanding and finding methods to debias them is fundamental if self-guided study is to succeed (Thiede et al., 2003). However, self-controlled learning and memory processing can also go awry even when a person's metacognitions themselves are excellent, if those metacognitions are not converted into optimal control strategies. One could know what one knows, but still do the wrong thing. Finally, even if one knows what one knows, and one knows what

to do about it, the actual implementation of the control knowledge could be faulty – leaving a fully metacognitive person still unable to effectively control their own learning and memory.

Whereas early research on metacognition and control focused almost exclusively on people's judgments about their memories, with the often-stated hope that this would lead to enhanced learning, recent research is increasingly aimed at the control aspect of meta-level awareness. In the section that follows, we focus on the judgments themselves, first, and on theories about how those judgments are made. We then turn to how those judgments are put to use in controlling learning and memory.

2.20.1 Metamemory Paradigms

2.20.1.1 Feeling-of-Knowing Judgments

The feeling-of-knowing judgment was the first to be systematically explored experimentally, by Joseph Hart, in 1965. Hart gave people a variety of general information questions to answer. When they could not answer a question, he asked them whether they felt they knew the answer anyway. The feeling that they knew it corresponded to their later choosing the correct answer on a recognition memory test. This paradigm posed a puzzle: How is it that people could ostensibly not know, as evidenced by their failure to produce the answer, and yet still be able to predict accurately whether they would know in the future, as evidenced by the correlations that were well above chance between their predictions and subsequent performance?

This finding of above-chance predictive accuracy has been replicated hundreds of times, so there is no doubt as to its reliability. The research in recent years has been directed not at establishing the predictive accuracy of feeling-of-knowing judgments but, rather, at understanding what cues people are using that give rise to it. Several theories have addressed this puzzle of seemingly not knowing and knowing at the same time, that is, how people are able to correctly predict what they will know in the future, when at the moment they are unable to retrieve the correct answer.

2.20.1.1.1 Theories

Whereas some early theories suggested that the person might have direct access to subliminal traces, all modern theories are basically heuristic in nature; that is, they assume that people have explicit access to some information that notably may be correct or incorrect, diagnostic or nondiagnostic, and that their feeling-of-knowing judgments are based on this information. Thus, while all current theories of this metamemory paradigm (and, indeed, of all metamemory paradigms) are heuristic theories, they differ in the exact heuristic that they propose people are using to make metamemory judgments.

2.20.1.1.1.(i) Domain and cue familiarity A logical possibility for the basis of feeling-of-knowing judgments is that people assess the familiarity of the cue (i.e., the question itself) or the domain of the question. Greater familiarity leads to higher judgments, that is, more confidence that a currently unretrieved answer will later be recognized. Concerning domain familiarity, even though people may be unable to immediately answer a question such as "Who painted *The Sunflowers*?," they may be able to assess how much they know about art and make a reasonable judgment on that basis. If they know something about art, they may be able to narrow down the field in a recognition test and eliminate incorrect alternatives. Thus, this kind of familiarity with the domain of the question may both be used to make a feeling-of-knowing judgment and be diagnostic, because, in general, strategic multiple-choice decision making will be better in well-known than in little-known domains. Thus, the person may not know who painted a particular painting but may nevertheless have a quite good idea of who did not do so, and such knowledge will help them on the test.

Glenberg and Epstein (1987) conducted an experiment in which people were selected for participation based on their expertise in various domains. They were then presented with texts to read that were either in their own domain or not. They found that people made higher judgments of knowing on those passages that were within their own area of expertise, thereby indicating that this kind of knowledge about the domain is one of the cues or heuristics that people use in making their judgments. Surprisingly, however, in this particular case, experts were not well calibrated when making judgments within their own domain. The mystery of this unexpected result remains unresolved even today. Finally, because many studies of feeling of knowing have been conducted with general information questions, and there are several domains of knowledge implicated in these questions (e.g., American history, old movies, sports, geology, capitals of various countries, etc.), knowledge of the types of general knowledge

one knows most about could be quite predictive of recognizing the correct answers.

Concerning cue familiarity, Reder and her colleagues (e.g., Reder, 1987; Reder and Ritter, 1992; Miner and Reder, 1994) conducted a series of experiments in which they showed that the familiarity of the cue influenced feeling-of-knowing judgments. For instance, Reder and Ritter (1992) presented participants with math problems (e.g., $113 + 29 = ?$) and had them quickly decide whether they wanted to retrieve or compute answers to each one. Prior to making a decision, the cue item was primed, without altering the target answer. Thus, in a math problem such as $113 + 29$, they would prime the cue by giving another problem such as $113 * 29$. When $113 + 29 = ?$ was presented, people then hit a button if they wanted to retrieve the answer as compared to compute the answer. If they already knew the answer, it would behoove them (because they would gain a greater reward) to hit the retrieve button, indicating that they could quickly retrieve the answer from memory. The interesting finding, from the perspective of the cue-familiarity heuristic, is that when the cues had been primed, people were more likely to indicate that they could retrieve the answer, even though such priming might even have hurt the retrieval of the correct answer. In a similar manner, Metcalfe et al. (1993) found that cue priming of verbal pairs influenced the feeling of knowing without altering target retrievability. In particular, they showed that the crucial factor influencing the magnitude of feeling-of-knowing judgments was the number of repetitions of the cue (which presumably would boost cue familiarity), rather than the retrievability of the sought-after target.

Whereas these and other studies (e.g., Maki, 1999; Eakin, 2005) clearly implicate the familiarity of the cue as one heuristic that people use in making metamemory judgments, evidence also suggests that partial information retrieved about the target is important.

2.20.1.1.1.(ii) Partial target accessibility The other main source of information for making feeling-of-knowing judgments is partial knowledge about the target. Perhaps one recalls that the answer to the sunflower question given earlier is an impressionist, and maybe even that there is a 'G' in the name. Even with this information, the person may be unable to give the answer. However, such partial target information may be sufficient that he or she will assign the item a high feeling of knowing. Such partial information, which is about the target itself, may be insufficient to allow the person to express the target item but may, nevertheless, indicate (often correctly) that he or she will be able to select the target in a multiple-choice test. (The only problem the person might experience in the present case could be in distinguishing Gauguin from Van Gogh, should both be present in the list). Thus, if partial or fragmentary target information is retrieved, it may be used to indicate that people will know the answer (and hence be related to high feeling-of-knowing judgments).

Koriat (1993) conducted experiments in which the to-be-remembered items were four-letter nonword strings. He showed a positive correlation between the number of letters the person could recall and their feeling of knowing rating. Of course, having three letters rather than just one was highly predictive of whether they would be able to pick the right answer from the set of alternatives offered, and so the predictive accuracy of this particular information-based metacognition was extremely high. The experiment was designed such that a 20-questions strategy was highly diagnostic, because one could eliminate half of the multiple-choice test alternatives with every letter correctly remembered. Playing 20 questions, and deliberatively assigning feeling-of-knowing judgments on the basis of the knowledge that partial information would allow them to eliminate alternatives in the test, is a logical possibility, and one that should work fairly well in the world. Phenomenologically, the judgments often feel more intuitive and less deliberative; however, even if people are less analytic about making these judgments than Koriat's experiments would suggest, if one has partial information, such as the first letter of the target, such information may give rise to a diffuse feeling that one knows more than nothing, and in many cases, one would be correct to inflate one's feeling of knowing.

It seems likely that the two mechanisms – cue familiarity and partial target information – account for most of the variability in feeling-of-knowing judgments. If so, hybrid models that describe how both cues combine (e.g., Leibert and Nelson, 1998; Koriat and Levy-Sadot, 2001) will likely fare well and are worthy of further exploration.

2.20.1.2 Tip-of-the-Tongue States

While overlapping in many respects with feeling-of-knowing judgments, tip-of-the-tongue judgments focus more directly on highly accessible partial information, and they appear less inferential in nature (for a general review, *See* Chapter 2.22). Nevertheless,

even if tip-of-the-tongue states merely represent very strong feelings of knowing, tip-of-the-tongue judgments have been investigated extensively (and separately from feeling-of-knowing judgments) because they occur so commonly in everyone's lives (Schwartz, 1999). In fact, well before the term 'metamemory' was coined, and before other metamemory judgments were scrutinized, the tip-of-the-tongue state captured the attention of William James (1890/1981). In his now-famous quote, James wrote: "Suppose we try to recall a forgotten name. The state of our consciousness is peculiar. There is a gap therein; but no mere gap. It is a gap that is intensely active. A sort of wraith of the name is in it, beckoning us in a given direction, making us at moments tingle with the sense of our closeness.… The rhythm of a lost word may be there without a sound to clothe it; or the evanescence sense of something which is the initial vowel or consonant may mock us fitfully, without growing more distinct" (James, 1890/1981: 243–244).

Schwartz (1999) has conducted a survey of 51 language groups and found that in the majority of them, there is an expression for what, in English, is called the tip-of-the-tongue state, though the exact expression varies slightly. In Korean, for example, this state is provocatively called "sparkling at the end of the tongue." This state seems to be almost universally experienced.

2.20.1.2.1 Theories

2.20.1.2.1.(i) Partial target access In a manner that is similar to the target access view of feelings of knowing, the dominant theory of tip of the tongues is that they reflect partial target access. In support of this view, a number of studies have shown that people are able to report the number of syllables in the to-be-retrieved word, some aspects of semantic content, or its first letter (for a review, see Schwartz, 2002).

2.20.1.2.1.(ii) Lexical access without phonological access Burke et al. (1991; see James and Burke, 2000) have proposed that a semantic level of representation of a sought-after word feeds to an articulatory/phonological level, which is necessary for word retrieval and output, and that the two representations can be dissociated. One dissociation is reflected by a tip-of-the-tongue state when the individual has complete or partial access at the semantic or lexical level, without being able to translate that activation into a phonological form that allows retrieval – or output – of the sought-after word. According to this model, the individual really can know an answer without being able to articulate it. Older adults seem to exhibit this phenomenon whereby an impairment occurs in phonological translation, which results in more tip-of-the-tongue states (for a recent review, see Schwartz and Frazier, 2005).

A prediction of this model is also supported by evidence from Metcalfe et al. (1995), who described an anomic patient who had difficulties retrieving words. In particular, this patient (HW), after experiencing a severe stroke, was able to converse intelligently but was unable to articulate the words for nearly all specific nouns, verbs, or adjectives when so requested. Thus, if asked to fill in the correct answer "One _____ the Thanksgiving turkey by brushing butter on while it is roasting," "The precious gem that is red is the _____," "The name of people who explore caves is _____," or even "Sirius is the _____ star in the sky excluding the sun," HW could not say bastes, ruby, spelunker, or brightest. However, he expressed a strong tip-of-the-tongue for these words. When he was later given a recognition test, he was able to pick the correct alternative with an accuracy better than that of Dartmouth College students, indicating that he knew the words he was seeking (i.e., he had semantic knowledge or lexical access) but could not articulate them. Burke et al.'s model eloquently explains HW's deficit.

2.20.1.2.1.(iii) Blocking One phenomenon seen in conjunction with tip-of-the-tongue states is that people often report that an incorrect response persistently comes to mind. This persistent alternative is usually called a blocker. We suspect that what makes tip of the tongues frustrating at times is that people in a blocked tip-of-the-tongue state know perfectly well that what keeps persistently coming to mind is wrong. Blocked tip of the tongues differ from nonblocked tip of the tongues insofar as people's phenomenology is different. In addition, it has been shown that blocked tip of the tongues tend to be more difficult to resolve than tip of the tongues without a blocker (Burke et al., 1991; Reason and Lucas, 1984). Researchers have thought that blockers actively keep people from accessing the correct answer. However, recent research by Kornell and Metcalfe (2007) indicates that this active blocking role of the so-called blockers is incorrect. In particular, they conducted an experiment to investigate the idea that blockers impaired performance, as is assumed both in the tip-of-the-tongue literature (Jones, 1989) and in the insight literature, where a similar phenomenon is thought to occur (Mayer, 1995). Theorists have stated

that people need to incubate (e.g., take a break to think about something unrelated to the problem) in both a problem-solving attempt or when attempting to retrieve a sought-after answer when in tip-of-the-tongue state. If a persistent alternative came to mind originally (which is supposed to be actively interfering with the generation of the correct solution), this break may allow one to forget it. If so, the off time will allow the problem-solver to overcome the harmful blocker and retrieve a correct solution.

To test this idea, Kornell and Metcalfe (2007) asked people to state whether their tip-of-the-tongue states included a blocker or not. The subjects then either continued to try to solve the problem or waited until the end of the experimental session for the additional minutes that they were assigned to attempt to solve the problem. As in the past literature, blocked tip of the tongues were resolved with a frequency that was lower than that of nonblocked tip of the tongues. Furthermore, consistent with the reminiscence literature, people answered more questions correctly at a delay than immediately. However, the delay interval did not particularly help the blocked tip of the tongues, as compared to the nonblocked tip of the tongues, as should have been the case had the blockers themselves kept the correct answer from appearing. Also, the blockers were forgotten over the delay interval. Thus, the delay interval did, effectively, get the blockers out of mind (as presumably should have been necessary to obviate their deleterious effect). But that made no difference for the rate of resolution, indicating that the so-called blockers do not really block. Kornell and Metcalfe (2007) favored a road sign view of blockers; they are in the person's semantic network, and the person might well articulate them in their quest for the correct answer, but they do not actively participate in the process. Whether they are accessed or not has no effect on the probability of retrieving the target.

2.20.1.2.2 Function of feeling-of-knowing and tip-of-the-tongue states

Little emphasis has been placed on the question of why people have feeling-of-knowing states or tip of the tongues. Perhaps the nagging emotional quality of the tip of the tongues is motivational and keeps people seeking an answer that otherwise they would not try to find. Similarly, Reder and Ritter (1992) have suggested that people's feelings of knowing indicate to them that there is something in memory to be found, and hence these feeling states – especially the fast feelings of knowing – provide information that people use to determine whether they will or will not attempt retrieval. Systematic research on whether and how feelings of knowing and tip of the tongues guide decision making and retrieval is needed.

2.20.1.3 Judgments of Learning

Judgments of learning are assessments that people make, either while in the course of learning, or afterwards, about how well they have learned the particular target materials under question. These judgments are thought to be of fundamental importance because the monitoring of study tapped by them is presumably used by a person to determine whether or not to study (e.g., Thiede and Dunlosky, 1999; Son and Metcalfe, 2000). Thus, if the judgments are faulty, so too will be people's subsequent study behavior. It is thought that with biased judgments, ultimately people's learning will be less than optimal.

Judgments of learning can be made in a cumulative manner, whereby the participant is asked to assess the degree of learning over an entire list or session, or they can be made on an item-by-item basis. For instance, when studying a list of 20 paired associates (e.g., dog–spoon), participants may be asked to predict how many out of 20 they will correctly recall when later tested (e.g., dog–?). While studying, they may make item-by-item judgments of learning, where participants are shown either only the cue (e.g., dog–?) or both the cue and response (e.g., dog–spoon) and are asked to predict the likelihood that the correct response (i.e., spoon) will be recalled. Item-by-item judgments of learning can be made either immediately while the person is learning or directly following that learning, or they can be made at a delay. As compared to aggregate judgments, the item-by-item judgments of learning currently have received the most empirical and theoretical attention in the field (for a comparison of the two judgments, see Dunlosky and Hertzog, 2000), so we shall largely restrict our review to them.

Two major findings have held up extremely well over the course of the last decade of research and have become the target of much further investigation. First, delayed, cue-only judgments of learning are highly accurate. The gamma correlations relating people's judgment-of-learning ratings to their later performance are often in the 0.90 range. In contrast, immediate judgments of learning and delayed judgments of learning when the cue and target are also given are often rather inaccurate, and it is not

uncommon to see the analogous gamma correlations being around +0.30. The reasons for these differences, which are tightly related to theories of how people make judgments of learning in these different conditions, are outlined below. The second major finding is that whereas first-trial immediate judgments of learning (and aggregated judgments) are often overconfident (i.e., their mean value is higher than the mean performance that people exhibit when they are tested), judgments of learning made on a second study-test trial over the same items are nearly always underconfident. Again, we discuss the explanations researchers have isolated (and those potential reasons that they have discredited) in the theoretical section that follows, titled 'Theories of the delayed-judgment-of-learning effect.'

2.20.1.3.1 Theories of the delayed judgment-of-learning effect

Four theories have been directed at the issue of why accuracy (as measured by resolution or the correlation relating judgments of learning to subsequent performance) is substantially greater for delayed than immediate judgments of learning, which has been dubbed the delayed judgment-of-learning effect (Nelson and Dunlosky, 1991). The first was the monitoring dual memories hypothesis, and the second is the transfer-appropriate processing framework. The third is the self-fulfilling prophecy hypothesis, whereby the judgment itself alters memory, and this alteration is responsible for the boost in accuracy for delayed judgments of learning. The fourth is a stochastic drift model.

2.20.1.3.1.(i) Monitoring-dual-memories hypothesis Nelson and Dunlosky's (1991) monitoring-dual-memories hypothesis assumes that judgments of learning are made by retrieving information from both short-term memory (STM) and long-term memory (LTM). In the immediate-judgment-of-learning condition, STM information is highly accessible, but it is transient and does not reflect what information will be available at final test. The presence of this STM information during the judgment, therefore, adds nondiagnostic information to the judgment, thereby reducing the accuracy of the judgments of learning. In the delayed-judgment-of-learning case, people are thought to base their judgments primarily on the retrieval of information from LTM. This retrieved information is more accurate in predicting final test performance, which is also based on LTM alone. This first explanation has a basic similarity to the second explanation – the transfer-appropriate processing explanation – insofar as both posit that the information that the person bases the judgment on is more similar to the information at time of test for the delayed than immediate judgments of learning.

2.20.1.3.1.(ii) Transfer-appropriate monitoring hypothesis The second explanation – a transfer-appropriate processing view – proposes that the delayed-judgment-of-learning effect occurs because of differences between the two judgment-of-learning conditions in the degree of contextual match from the time of the judgment to the time of the test (Begg et al., 1989; Dunlosky and Nelson, 1997). Making a judgment of learning in a situation that is as similar as possible to that of the test should maximize its accuracy. Insofar as the retrieval attempt, which is thought to be the critical information on which the judgment of learning is based, is more similar between a delayed test and a delayed judgment of learning than between a delayed test and an immediate judgment of learning, the delayed judgments are predicted to be more accurate.

2.20.1.3.1.(iii) Self-fulfilling prophecy hypothesis The third explanation locates the increase in gamma accuracy between immediate and delayed judgments of learning in a differential change in memory with immediate and delayed judgments of learning that comes with making the judgment itself (Spellman and Bjork, 1992; Kimball and Metcalfe, 2003). This third theory has been called a Heisenberg explanation or the self-fulfilling prophecy hypothesis. An assumption here is that people attempt retrieval to make their judgments of learning but, in the delayed-judgment-of-learning condition, are successful with only some of those attempts. The practice elicited by cue-only delayed judgments of learning enhances memory for retrieved items, but only some items are retrieved at the delay. Moreover, the items that receive this memory boost are not distributed randomly across the judgment of learning range, but rather are those given high judgments of learning, because the basis of the judgment is whether or not the person is able to retrieve. Those items that people fail to retrieve are given low judgments of learning and get no boost in study. Thus, the high-judgment-of-learning items benefit from an extra (spaced) study trial, while the low-judgment-of-learning items receive no additional practice and get no memory boost. This differential study has an effect on memory that bolsters the predictive

value of the ratings only in the delayed-judgment-of-learning condition. In the immediate-judgment-of-learning condition, virtually all items are recalled during the judgment (e.g., Nelson et al., 2004), which occurs immediately after study and has little memorial effect. In addition, being uniform across the entire judgment-of-learning range, this immediate retrieval does not make the high judgments of learning more memorable or the low judgments of learning less memorable.

2.20.1.3.1.(iv) Stochastic drift model Finally, Sikstrom and Jonsson (2005) propose (in a manner related to the monitoring dual memories hypothesis) that the accuracy difference is because memory strength for any given item can be decomposed into exponential functions with slow and fast components. The drift from these decay processes from time of judgments to time of test is large for immediate judgments of learning, resulting in low predictability, but is smaller for the delayed judgments, resulting in high predictability. This model is most welcome in the field for two reasons: First, because it is a much needed formal model of the processes thought to underlie the judgments and their consequences, and second, because it makes new predictions about outcomes.

2.20.1.3.1.(v) Status of theories for the delayed-judgment-of-learning effect Although considerable empirical work has been conducted to evaluate these theories (either in isolation or in competition), it is currently premature to declare one as a clear winner. Nevertheless, albeit intuitive, the transfer-appropriate monitoring hypothesis has been repeatedly disconfirmed (e.g., see Weaver and Kelemen, 2003; Dunlosky et al., 2005b). Moreover, recent modeling of the delayed-judgment-of-learning effect suggests that both a monitoring-dual-memories component and a Heisenberg-style component may be required to fully account for the effect (Jang et al., 2006).

All four of the theories explain the delayed judgment-of-learning effect by assuming that people make their judgments by using the heuristic of trying to retrieve the target, at least in the delayed case. None of these models take into account the possibility that other cues may be used to make the delayed judgments of learning. However, Son and Metcalfe (2005) have shown that people sometimes make very fast delayed judgments of learning and that these fast judgments of learning are probably not based on retrieval or attempted retrieval of the target. They showed that there were notable differences in the results when people were simply asked to make delayed judgments of learning as compared to when they were asked to attempt to retrieve the target immediately prior to making each judgment of learning (e.g., for detailed application of this method, see Nelson et al., 2004). In particular, the very fast judgments of learning drop out in the latter case, suggesting that normally people are doing something to produce these fast judgments of learning that they are not doing when they explicitly try to retrieve the target. They suggested that people are basing these fast low judgments of learning on a lack of familiarity with the cue, and that when the cue is unfamiliar, people do not bother to try to retrieve the target. In this way, they proposed a two-factor hypothesis in which familiarity and retrieval interact to influence people's judgments of learning.

Benjamin (2005) provided support for a two-factor hypothesis by showing that when people are time pressured, factors that affect cue familiarity come into play in their judgments of learning. When they are not time pressured, factors affecting the retrievability of the target are influential. Note that these are the same two cues that people use in making feeling-of-knowing judgments. With delayed judgments of learning, these cues appear to be used in a specific order. First, people assess the familiarity of the cue. If it is unfamiliar, they give a low judgment of learning. If it is familiar, they go on to the second stage, in which they attempt retrieval of the target. If they cannot do so, they give the item a relatively low judgment of learning; if they can do so, they give it a high judgment of learning. Given the evidence for the second factor in delayed judgments of learning, it appears that none of the four theories can fully account for the judgments. Regardless of its ultimate explanation, however, there is general agreement that delayed judgments of learning may be quite valuable in helping people both accurately monitor and effectively control their learning (Bjork, 1994).

The heuristics used when people make immediate judgments of learning are less straightforward than those used in making delayed judgments of learning. Data indicate that a variety of cues may play a role, such as the fluency of processing words during study (Begg et al., 1989), the fluency of generating study strategies (Hertzog et al., 2003), the relatedness of words within paired associates and across individual words (e.g., Koriat, 1997; Dunlosky and Matvey, 2001; Matvey et al., 2006), and memory for the outcome of previous tests (Finn and Metcalfe, 2007, 2008), among many others (for a review, see Koriat,

1997). Whereas some of these cues, clearly, must have some predictive value – the gamma correlations are nearly always greater than zero – they are typically less diagnostic than the cues used in delayed judgments.

2.20.1.3.2 Theories of the underconfidence-with-practice effect

The second major finding within the judgment-of-learning literature is that although people's judgments of learning tend to be overconfident on the first trial, by the second trial, there is a shift to underconfidence that persists on subsequent trials. Much research has focused on this underconfidence-with-practice effect, and a number of efforts to explain it, based on exactly how people make judgments of learning, have been proposed (as shown in **Figure 2**, from Koriat et al., 2002). Besides drawing attention to the underconfidence-with-practice effect, Koriat et al. (2002) demonstrated that it persisted despite a variety of experimental manipulations that might otherwise provide explanations of it. For example, feedback about performance on a prior trial had no effect. Both incorrectly and correctly recalled Trial 1 items showed underconfidence on Trial 2. Although this finding suggests that past test performance may not drive the effect, Finn and Metcalfe (2007) have shown that the underconfidence is significantly larger for items that were incorrect on Trial 1 than for items that were correct on Trial 1, qualifying the earlier conclusion that Trial 1 performance was irrelevant.

One possible explanation for the underconfidence-with-practice effect is that people are underconfident

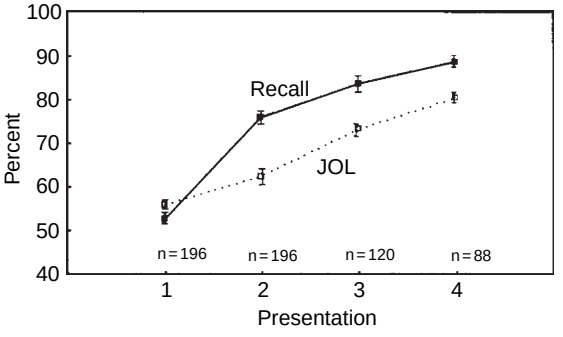

Figure 2 Illustration of the underconfidence-with-practice effect, with judgments of learning (JOL) showing overconfidence on an initial study trial and underconfidence on subsequent trials. From Koriat A, Sheffer L, and Ma'ayan H (2002) Comparing objective and subjective learning curves: Judgments of learning exhibit increased underconfidence with practice. *J. Exp. Psychol. Gen.* 131(2): 147–162.

because they have insufficient control over their own study to learn because the duration of study for each item is typically under experimental control. In contrast to this possibility, the underconfidence-with-practice effect was found when the study time allowed for each item was fixed or when it was self-paced (Koriat et al., 2002). Perhaps people just do not care and make the judgments without due consideration. However, even with incentives given for making accurate judgments – a manipulation that increases Trial 1 judgment of learning accuracy – the underconfidence-with-practice effect persisted. Thus, mere laziness on the part of participants does not appear to be the answer.

Numerous studies have shown that easy materials tend to result in less overconfidence than difficult materials (Lichtenstein and Fischhoff, 1977), so perhaps the underconfidence-with-practice effect is just another manifestation of item effects described in the confidence literature. Although possible, Koriat et al. (2002) reported that both easy and difficult items showed the underconfidence-with-practice effect. And it does not appear to be attributable to the undue effects of retrieval fluency from the first test trials (Serra and Dunlosky, 2005). Their idea was that people might assign low judgments of learning to items that were recalled on Trial 1, slowly or with great difficulty. The data, however, did not support this hypothesis.

One hint about the underconfidence-with-practice effect comes from the finding that immediate judgments of learning show the effect, whereas delayed judgments of learning do not (e.g., Meeter and Nelson, 2003; Koriat and Ma'ayan, 2005; Scheck and Nelson, 2005; Koriat et al., 2006; Finn and Metcalfe, 2007). In fact, early evidence relevant to the underconfidence-with-practice effect involved delayed judgments of learning and did not demonstrate the effect (Dunlosky and Connor, 1997). Meeter and Nelson (2003) showed only a 1% difference between delayed judgments and recall performance on Trial 2. Serra and Dunlosky (2005) showed underconfidence for both delayed and immediate judgments but a much greater shift toward underconfidence across trials for immediate judgments. Koriat et al.'s (2006) data showed overconfidence with delayed judgments of learning, though the difference from calibration was slight. Taken together, these reports suggest that delayed judgments of learning are not underconfident but, rather, are very close to being perfectly calibrated. Immediate judgments of learning, however, are nearly always underconfident after the first study-test trial.

As discussed, one difference between immediate judgments of learning and delayed judgments of learning is that people are very likely to rely on different heuristics in making the two different judgments. In the former case, as described earlier, they rely primarily on retrieval of the target item (with some reliance on familiarity of the cue). In the latter case, though, the heuristics are less clear. Finn and Metcalfe (2007) have proposed that use of the Memory for Past Test heuristic selectively in the immediate judgment of learning case, could account for much of the underconfidence-with-practice effect. The idea is that when people make second-trial judgments of learning they think back to whether they remembered that particular item in the past test. If they did, they give it a high judgment of learning. If they did not, they give it a low judgment of learning. If people were using this heuristic, they would tend to underestimate current trial performance insofar as it ignores the new learning in which the person has just engaged. Thus, they would be underconfident. The relationship between second-trial judgments of learning and Trial 1 performance would be expected to be stronger than the relationship between second-trial judgments of learning and Trial 2 performance, which it is (King et al., 1980). Furthermore, when Trial 1 test was manipulated independently of Trial 2 test, people's judgments of learning gravitated toward their manipulated first trial test performance (Finn and Metcalfe, 2008). And finally, when people were asked to simply report what they did to make the judgment, reliance on first trial test performance was a frequently given reason for the judgment given (Dunlosky and Serra, 2006). Thus, the use of this heuristic appears to be a viable candidate for explanation of the underconfidence-with-practice effect, though there are no doubt other factors that contribute to people's second-trial immediate judgments of learning (e.g., Kelley and Muller, 2006).

2.20.1.3.3 Function of judgments of learning

It is commonly believed that judgments of learning are of critical importance in learning insofar as they determine what people will choose to study and for how long they will persist (e.g., Nelson and Narens, 1990; Nelson and Dunlosky, 1991; Mazzoni and Cornoldi, 1993; Nelson and Narens, 1994; Benjamin et al., 1998; Koriat, 2000; Metcalfe, 2000). If these judgments of learning are accurate, then people will be in a position to choose to study the items that will result in optimal learning. If they are biased, or inaccurate, however, they will be unable to make such optimal choices.

Although the available evidence suggests that judgments of learning in part drive the allocation of study time, this evidence has been largely correlational, so direct experimental evidence is needed to more definitively establish that when metacognitions are manipulated people's study choice follows. Nevertheless, some demonstrations show that when people with inadequate metacognitions have been induced to make more accurate metacognitive judgments, their learning is improved. For instance, Thiede et al. (2003) had students study paragraphs and make a judgment of learning for each. Before making a judgment for a paragraph, participants were asked to generate five keywords about the paragraph that captured its essence. One group generated keywords (and made judgments) immediately after reading each paragraph, whereas another group did so after all the paragraphs were read. After reading and judging the paragraphs, (1) a test was administered about the content for each of the paragraphs, (2) participants were allowed to select paragraphs for restudy, (3) they restudied chosen texts, and (4) a final test was administered.

Several outcomes are notable. First, judgment-of-learning accuracy for predicting first-test performance was substantially greater for the delayed judgment ($+0.70$) than for the immediate group ($< +0.30$). Second, whereas first-test performance did not differ for the groups (both had a mean value a bit greater than $+0.45$ questions correct), the final test performance was much better for the delayed group (approximately 0.65 correct) than for the immediate group (approximately 0.50). Why such a difference? Fine-grained analyses showed that the delayed group, who had much better judgment accuracy, was more likely to choose paragraphs for restudy that they did not know well, and hence they made the greatest gains in learning during restudy. Without the ability to isolate these less well-known items, students' metacognitive judgments simply did not help them effectively regulate their learning. Thus, preliminary evidence is suggestive that people's metacognitions are used to allocate restudy and, more important, that at least one condition that boosts accuracy can also support more effective learning (for other relevant evidence, see Dunlosky et al., 2005a).

2.20.1.4 Source Judgments

Source judgments refer to attributions about the origins of our thoughts and memories (Johnson and Mitchell, 2002; for a review, *See* Chapter 2.19). As such, these judgments are metacognitive, being judgments about other cognitions. Such judgments are targeted when a person is asked who said a particular statement, where they heard something, whether they said something or someone else did, whether they saw the defendant rob the store or only saw him on the sidewalk afterwards, and so on.

Failures of source memory can have profound consequences. One such consequence is unconscious plagiarism. Another is a breakdown in reality monitoring, such as may be seen in psychiatric syndromes such as schizophrenia, in which a person cannot monitor whether the source is internal or external, and in which reality breaks down. Accurate source monitoring is critical for the eyewitness to a crime, but unfortunately, this kind of metacognition can be highly inaccurate.

2.20.1.4.1 Theories of source monitoring

Johnson and Raye (1981; Johnson, 1983; Johnson et al., 1993) have formulated a model, called MEM (for multiple-entry modular memory system framework), which brings together many of the findings from the source literature in a coherent and elegant form. The consensus view, articulated in the MEM model, of the mechanisms underlying source judgments is that they, like other metacognitive judgments, are based on heuristics. When asked to assess a source, people use what information comes to mind to make the judgments, and this information itself can vary radically depending upon a number of factors. For example, if two potential sources are highly similar to one another, the memory will be highly confusable and the resultant judgment will be more difficult and error prone. If they are quite different from one another, the task is easier. So, if one has to say whether Mary or Lynn said a particular sentence, if Mary is female and Lynn is male, the task is much easier than if both are female (Ferguson et al., 1992). If the two sources are spatially discrete, once again the task is easier than if they are overlapping (Ferguson et al., 1992). Physical differences of this sort have been well documented, are systematic, and conform very nicely to one's intuitions.

Interestingly, though, it is not only the conditions in the world that determine how confusable the sources of different events will be but also the individual's mental capabilities and mental operations that play a part. If a person is readily able to construct vivid images – being able to mentally see a turkey when the word turkey is read – and if he or she automatically encodes concrete nouns as images, then the source distinction of whether a word or a picture was presented will be more difficult than for a different person whose imagery capabilities are less well developed (Johnson et al., 1979). If a person is told to imagine words being spoken in a particular person's voice, which is similar to the speaker's, as opposed to imaging in a voice less similar, the source judgments will be affected (Johnson et al., 1979). The vividness of a person's imagination, then, can have a dramatic effect on whether things that actually happened are confused with those that were only imagined.

Since Johnson's seminal research in the field, the literature has grown extensively, with research involving everything from basic cognitive theory to the neurological underpinnings of source memory. Certainly, this literature is too broad to cover here (for a review, see Johnson et al., 1993; Mitchell and Johnson, 2000; Johnson and Mitchell, 2002), but in contrast to many other coverages of metamemory, we wanted to draw some attention here to this very important, and pervasive monitoring skill.

2.20.1.5 Remember/Know Judgments

People can distinguish between events or items that they remember (i.e., for which they have a clear and distinct recollection not only for the target material itself but also for the circumstances of having learned it) versus those that they only know. For example, one might remember one's first iPod, including the circumstances under which one obtained it, and so on, but only have a feeling that they know they saw such-and-such a person some time ago without being able to recall the specific episode. In typical experiments, participants will study a list of words (e.g., pencil, table, football, etc.). After study, the words are presented again mixed with new words, and participants are asked whether each item was originally presented (i.e., a standard recognition judgment), and then whether they recollect that it was presented or merely know that it was presented. In this example, you may state that you recognize that both pencil and football had been presented, but when asked for a remember/know judgment, you may recollect seeing football because you recollected that when it was

originally presented you thought of your favorite football team (e.g., the Denver Broncos), whereas you have no recollections about pencil but just have a diffuse feeling, knowing that it was presented.

Being able to tell the difference between remembering and knowing, that is, the ability to make this particular judgment about a memory, is a category of metacognition that is thought to have significance for our understanding of human consciousness (for a general review on remember-know judgments, *See* Chapter 2.17). Events that are recollected are thought to be true memories and to exemplify a special form of memory and consciousness called autonoetic consciousness (Tulving, 2005) or explicit memory (Graf and Schacter, 1985). Facts that are judged to be only known are thought to require only semantic knowledge or mere familiarity and are thought to require only primed noetic consciousness or implicit memory.

There have been many debates over the past decade about this distinction. People question whether it means that there are different systems of memory, or whether it might be due only to differences in the amount of information stored (e.g., with better-stored memories being judged as remembered and less well-stored memories being judged as merely known). One larger issue here is to whether the phenomenology of recollecting actually contributes to one's recognizing something as being previously studied versus whether this phenomenology is merely epiphenomenal; you have the experience of recollecting (e.g., that you recalled Denver Broncos when football had been presented), but this experience does not contribute to your ability to correctly recognize an item as previously studied. Advocates of dual-process models of recognition – which indicate that both familiarity and recollection influence recognition decisions – state that recollection has a causal influence on our recognition performance, whereas strength theorists claim that a single underlying memory dimension (e.g., familiarity alone) can adequately explain recognition. For the latter group, recollections merely arise from having strong memories, but the phenomenology itself is not important for understanding recognition *per se* (for competing views, see Yonelinas, 1994; Rottello et al., 2005).

Paradigms involving this distinction purportedly allow us to ascertain whether people are consciously aware of the memories. This particular metacognitive judgment, then, is one that has been extensively researched and debated. A detailed discussion of the remember/know literature is given in a separate chapter of this handbook, and so we do not elaborate further on it here. We include this section only to note that this particular judgment, like all of those outlined above, is a kind of metacognition because it involves an attribution about a memory, though one that may have considerable consequence for understanding human memory and consciousness.

2.20.2 Conclusion

Much progress has been made in understanding the mechanisms that underlie the judgments that people can make about their memories. There is considerable agreement that metacognitive judgments are heuristically based. People seem to rely on the information that they have at hand, and usually on a fairly shallow assessment of that information, to make these judgments. Because these judgments are heuristically based, systematic biases are observed. Under some circumstances, people will be underconfident or overconfident; in other situations, they can be misled. However, insofar as research is untangling those systematic biases and the reasons for them, we are increasingly in a position to help students improve their metacognitions, and hence base their learning on a firmer foundation.

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2.21 Déjà Vu

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The déjà vu phenomenon is one of the most intriguing illusions of memory. We automatically monitor the familiarity of experiences and surroundings. Nearly always, our subjective sense of familiarity corresponds to objective reality, but on rare occasions these clash, giving rise to a feeling of familiarity in the absence of objective evidence.

> We are in a strange place, perhaps on holiday for the first time at a hotel. Suddenly, without warning, a certain feeling of familiarity seems to create itself. At once we seem to know the whole scene, windows, doors, pictures, and view from the windows. We recognize the person with whom we are speaking, although . . . we have never seen him to this minute. We even recognize the words he is saying, though it is impossible to know what he is going to say. We have the feeling of having been through everything before! Then, in a flash, the illusion vanishes. (Humphrey, 1923: 137)

The standard definition for déjà vu, presented by Neppe (1983b: 3), is "any subjectively inappropriate impression of familiarity of a present experience with an undefined past" and a more recent reformulation that incorporates cognitive terminology is "an objective assessment of unfamiliarity juxtaposed with a subjective evaluation of familiarity" (Brown, 2003: 2).

2.21.1 Challenges of Déjà Vu Research

The literature on déjà vu extends back over 150 years, and interest in the phenomenon encompasses a wide range of disciplines from medicine to philosophy to psychology (Brown, 2004). Despite this plethora of attention, the phenomenon of déjà vu has struggled to make a solid connection with empirical research in psychology. Although some twentieth-century researchers studied memory errors (e.g., Bartlett, 1932), most followed the lead of Ebbinghaus (1885), where memory errors were ignored or controlled rather than examined as worthy phenomena in their own right (cf. Roediger, 1996). There has been a change over the past few decades, and cognitive scientists have begun to explore the relationship of the déjà vu experience to phenomena such as repetition priming (Schacter, 1996), source attribution (Hoffman, 1997), perceptual fluency (Jacoby and Whitehouse, 1989; Bernstein and Welch, 1991; Joordens and

Merikle, 1992; Roediger, 1996), and subliminal mere exposure (Seamon et al., 1983).

What makes research on déjà vu problematic is the lack of any clearly identifiable eliciting stimulus and objective behaviors. Not only have the causes been elusive; an objective observer may find it impossible to determine whether someone is experiencing déjà vu. Other cognitive phenomena, such as the tip-of-the-tongue experience (Brown, 1991; also *see* Chapter 2.22), have a clear stimulus (John Kerry's vice presidential running mate in 2000) and behavioral resolution (John Edwards). However, reports of déjà vu experiences rely on one's sensitivity to, and awareness of, their own cognitive functioning.

2.21.2 Defining Déjà Vu

Through the mid-1900s, researchers used over 30 different words and phrases to describe déjà vu (see Brown, 2004), including such colorful expressions as paramnesia (Burnham, 1889), diplopia (Taylor, 1931), perplexity psychosis (MacCurdy, 1925), promnesia (Myers, 1895), and prescience (Crichton-Browne, 1895). This diversity of terms reflects several things about the experience. First, it is inexplicable and difficult to construct a word or short phrase to appropriately label it. Second, developing a reasonable definition of the subjective experience has been problematic. Brown (2004) assembled over 50 different definitions that reflect this hegemony. Third, there is a lack of consensus among researchers and writers on the cause: Is it a physiological, memory, or perceptual problem? This diversity hampered early research, and settling on the common French language term was accompanied by considerable struggle and debate (Marková and Berrios, 2000).

2.21.3 Methods of Investigating Déjà Vu

The primary method for studying déjà vu has been retrospective questionnaire, usually involving either a brief inquiry about the incidence of déjà vu or a more extensive assessment of multiple dimensions of the déjà vu experience (setting, duration, etc.). The most significant problem with retrospective surveys of déjà vu stems from the rarity of the experience (Adachi et al., 2003). Given that most people who do have déjà vu only experience it one to two times per year, it poses a serious challenge to remember the details of an experience that probably happened many months ago, and in ordinary circumstances that may not be very memorable. Thus, survey data provide a conservative estimate, at best, of the actual frequency of déjà vu. Biases also exist in the sampling of respondents (cf. Brown, 2004), with many surveys based on a selection of individuals that are conveniently available (college students for research professors; hospital patients for research physicians).

2.21.3.1 Unfortunate Association with the Paranormal and Abnormal

A serious problem with déjà vu assessments is that about one-third of surveys imbed their déjà vu item among items inquiring about paranormal (extrasensory perception, haunting, poltergeist, unidentified flying object) phenomena (cf. Brown, 2004). Other questionnaires imply a relationship between déjà vu and dimensions of psychopathology such as agoraphobia, depersonalization, and derealization (Buck and Geers, 1967; Harper, 1969; Harper and Roth, 1962; Brauer et al., 1970; Buck, 1970; Myers and Grant, 1972). Thus, respondents are given the message that déjà vu is inherently abnormal, which may reduce the willingness of individuals to admit to having such experiences.

2.21.3.2 Prospective Surveys

A prospective survey can solve the memory problem inherent in retrospective surveys. However, only Heymans (1904, 1906; translated by Sno and Draaisma, 1993) has employed such a technique, and the lack of methodological clarity coupled with the disparity of incidence estimates in two separate samples (14% vs. 62%; see Brown, 2004) make these data difficult to interpret. One other published prospective report is a case study. Leeds (1944) experienced an extraordinarily high rate of déjà vu (once every 2–3 days), which motivated him to keep a remarkably detailed record of 144 of his own déjà vu experiences, including date, time, intensity, duration, physical setting, and his behaviors, as well as his psychological and physical state at the time. He discovered that the intensity and duration of the experiences were directly related, that longer inter-episode intervals resulted in more intense and longer experiences. Leeds further observed that déjà vu experiences come in clusters and occasionally occur in dreams. His record is fascinating in its remarkable detail, although these experiences may reflect pathological rather than normal déjà vu.

2.21.4 Incidence of Déjà Vu

Based on numerous surveys, déjà vu may not be a universal experience. Given this, it is important to separately examine two different dimensions – the incidence of the illusion and how often it occurs among those who have ever experienced it (experients). Across 57 outcomes from 42 published studies, Brown (2004) found that déjà vu is experienced by about two-thirds of those surveyed. The incidence varies considerably across surveys – ranging from 10–100% – and several factors account for this extreme variability. The item context within which the query is placed probably has a substantial impact. As noted earlier, when the déjà vu item accompanies items on paranormal phenomena, this most likely suppresses the reported incidence. The cultural acceptability and understanding of the experience has increased across time (cf. Brown, 2004), so more recent surveys tend to show a higher incidence. Finally, the age of the sample influences the incidence, and samples of older participants tend to show a lower incidence (cf. Brown, 2003).

How often does déjà vu occur among experients? Surveys suggest that it is not a singular event, with the vast majority of experients having more than one lifetime déjà vu (Palmer 1979; Kohr, 1980). About half of experients have had seven or more occurrences in their lifetime (Kohr, 1980). Using a Likert scale, around half of respondents rate déjà vu occurrences as 'seldom' (Leeds, 1944; NORC, 1984, 1988, 1989; McCready and Greeley, 1976), with a relatively small percentage (between 9% and 18%) rating it as 'frequent.' Data derived from more recent surveys indicate that déjà vu appears to occur at least once a month for most experients (Ardilla et al., 1993; Brown et al., 1994; Roberts et al., 1990).

2.21.5 Nature of the Déjà Vu Experience

2.21.5.1 What Triggers Déjà Vu?

Brown et al. (1994) discovered that the most important element in eliciting déjà vu is the general physical setting, with over half (54%) of experients claiming that this was always the cause (see Neppe, 1983b). Spoken words, actions, and objects are also noted as contributing to the déjà vu experience, although to a far lesser extent. Stress is mentioned in many anecdotal reports as a causative factor in déjà vu (see Brown, 2004), although Brown et al. (1994) found that only a small fraction of open-ended survey responses (about 20%) contained any such reference. The vast majority (three-quarters) of déjà vu experiences occur indoors, about evenly distributed between private and public buildings. Furthermore, about half of déjà vu experiences occur when one is engaged in recreational activities. Déjà vu rarely happens when one is alone and most typically occurs while in the company of one other person (usually a friend).

The déjà vu experience is relatively brief, with about half of survey respondents reporting that it lasts less than 5 s. Déjà vu is more likely to occur during the afternoon or evening (Heymans, 1904; Leeds, 1944) and late in the week (Thursday through Saturday) (Brown, 2004), and the sense of time seems to be momentarily slowed for many experients. It is difficult to precisely identify the emotional reaction accompanying déjà vu. Some characterize this as an essential part of the experience, but the most predominant reactions appear to have more of cognitive than emotional flavor (Brown, 2004), with those surveyed most often describing it as eerie, surprising, odd, confusing, exciting, and curious.

2.21.6 Physical and Psychological Variables Related to Déjà Vu

The most consistent finding in the déjà vu literature is that the incidence systematically decreases with age from the 20s through the 80s (Brown, 2003, 2004), although teenagers tend to experience déjà vu less often than those in their 20s. Data on changes across broad age ranges are found in two studies with hospital patients (Chapman and Mensh, 1951; Richardson and Winokur, 1968). A larger sample with a more representative cross section of individuals was conducted by the National Opinion Research Center (NORC) in their General Social Surveys conducted in 1984, 1988, and 1989. The data from these sources are summarized in **Figure 1**, and all three sources confirm the systematic decline in the incidence of déjà vu with age. In addition, significant negative correlations have been found between age and déjà vu experience, ranging from −.22 to −.38 (Chapman and Mensh, 1951; Kohr, 1980; NORC, 1984, 1988, 1989; Sno et al., 1994; Adachi et al., 2003). Given that older adults used to be young, the lifetime incidence of déjà vu should either remain steady or increase, rather than drop. However, this logically impossible age change is probably due to an increase

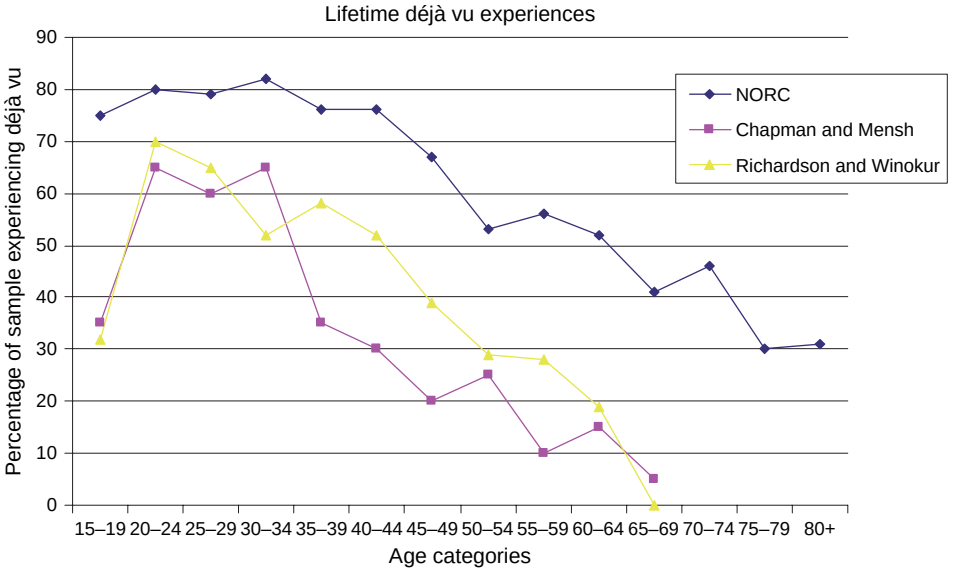

Figure 1 Percent of respondents experiencing déjà vu across age groups (from three surveys).

in the cultural awareness and acceptability of the experience across the past four or five decades (Brown, 2004).

Among experients, the incidence of déjà vu drops off dramatically across the adult age span (Chapman and Mensh, 1951; Richardson and Winokur, 1968), as reflected in **Figure 2**. Why do older experients report fewer incidents of déjà vu? Perhaps they are less in touch with the subtle qualities of their own cognitive experiences (Brown et al., 1995), are more settled in their physical routine and less likely to encounter new experiences to trigger déjà vu, or have a greater acceptance of incongruent memory experiences (Adachi et al., 2003). There has been no extensive survey on the minimum age at which déjà vu first occurs, although Neppe (1983b) discovered that some adults claim to have had their first déjà vu sometime in the teens or 20s. Fukuda (2002) found a

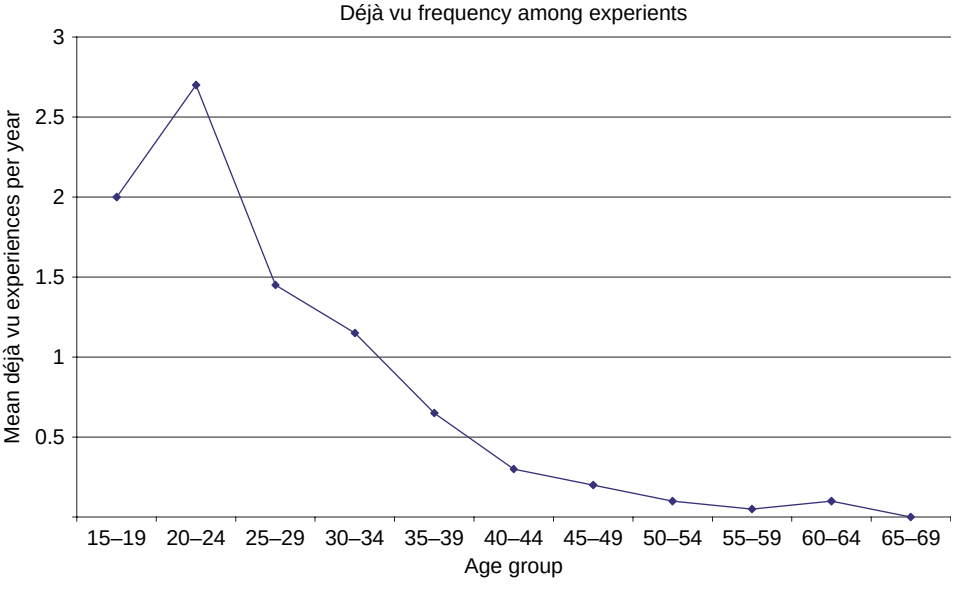

Figure 2 Yearly frequency of déjà vu across age groups among those who have the experience.

somewhat earlier estimate in a retrospective survey of college students, with most claiming a first experience between ages 6 and 10 (49%) or between ages 11 and 15 (33%).

There is a positive relationship between déjà vu incidence and education (Chapman and Mensh, 1951; Richardson and Winokur, 1967, 1968; Harper, 1969; Palmer, 1979; Kohr, 1980; Neppe, 1983b; NORC, 1984, 1988, 1989; Gallup and Newport, 1991; Adachi et al., 2003) and between déjà vu and travel frequency (Brown, 2004). Déjà vu incidence appears to be directly related to socioeconomic status (SES) (Crichton-Browne, 1895; Chapman and Mensh, 1951; Richardson and Winokur, 1967, 1968; Harper, 1969; Palmer, 1979; Gallup and Newport, 1991). Because socioeconomic status and academic achievement are closely intertwined, Brown (2004) attempted to extricate the separate contributions of each variable by comparing low versus high SES within each of four education levels within the NORC survey database. Déjà vu incidence was higher for low than for high SES within each education level, although the incidence systematically increased with each level of education. Thus, SES and education level are associated with déjà vu experience in opposite ways: Déjà vu is inversely related to SES but directly related to education level. Travel also appears to be associated with déjà vu. Those who do any travel are more likely to report déjà vu than those who don't, with little difference across various numbers of trips (Brown, 2004). Déjà vu incidence is unrelated to gender, race (black vs. white), religious preferences, or political affiliation (Brown, 2003, 2004), although there is a clear tendency for déjà vu incidence to be higher among those with a liberal versus conservative orientation.

There is some evidence that various prescription and nonprescription drugs have the potential to either trigger (amphetamines, Ellinwood, 1968; carbamazepine and clonazepam, Garbutt and Gillette, 1988; toluene, Takaoka et al., 2001; amantadine hydrochloride and phenylpropanolamine hydrochloride, Taiminen and Jääskeläinen, 2001) or reduce (clonazepam, Ide et al., 2000) déjà vu experiences, but this is based on selective data from case reports. There appears to be a tendency for déjà vu to be elevated in alcoholics (Turner, 1910), and NORC data reveal a positive association between alcohol consumption and déjà vu, with those who drink experiencing it more frequently than those who don't.

2.21.7 Physiopathology and Déjà Vu

Some of the early explorations of déjà vu were motivated by an apparent association between temporal lobe epilepsy (TLE) and déjà vu (Quaerens, 1870; Jackson, 1888; Maudsley, 1889; Crichton-Browne, 1895). A small percentage of TLEs experience déjà vu in the aura that immediately precedes their seizure, and one physician documented his personal experiences in a published report, claiming that his first epileptic attack was preceded by a series of increasingly frequent and intense déjà vu experiences (Quaerens, 1870). This issue has been debated for many decades, but most evidence suggests that déjà vu is not symptom or cause specific to epilepsy (Richardson and Winocur, 1967; Harper, 1969). However, research conducted on TLEs has contributed to our understanding of the experience. An examination of cortical activity in TLEs with both electrical stimulation and recording procedures (Mullan and Penfield, 1959; Cole and Zangwill, 1963; Penfield and Perot, 1963; Penfield and Mathieson, 1974; Gupta et al., 1983; Cutting and Silzer, 1990; Gloor, 1991; Palmini and Gloor, 1992; Fish et al., 1993; Weinand et al., 1994; Sengoku et al., 1997; Adachi et al., 1999) suggests that déjà vu is associated with activity in the right temporal lobe, specifically involving the hippocampus and areas immediately surrounding it (e.g., amygdala, parahippocampal gyrus, temporal isocortex) (although see Brown, 2004, for cautions in interpreting these data).

Some evidence suggests that déjà vu may be more common in those who have suffered head trauma, especially accompanied by amnesia (Weinstein et al., 1962) or disturbance/loss of consciousness (Harper, 1969; Weinstein, 1969). It has been suggested that déjà vu is associated with various moderate to severe psychological disturbances (Calkins, 1916; Pickford, 1944) such as schizophrenia (Neppe, 1983b; Sno et al., 1992; Sno, 2000), but there has been no definitive verification that déjà vu is associated with any form of psychopathology (Brown, 2004). There is, however, modest evidence that déjà vu may be associated with depersonalization and derealization (Roth, 1959; Buck and Geers, 1967; Harper, 1969; Myers and Grant, 1972), but others have failed to find support for such an association (Brauer et al., 1970; Dixon, 1971; Adachi et al., 2003). Some have argued that déjà vu should be included in the *Diagnostic and Statistical Manual* (DSM) of the American Psychiatric Association as a form of

psychopathology (Sno and Linszen, 1990; Sno et al., 1992), although this change has not garnered broad support (cf. Pagliaro, 1991).

2.21.8 Interpretations of Déjà Vu

Researchers have proposed over 40 different explanations for déjà vu (Brown, 2004), and Neppe (1983a: 33) cogently suggests that "... one single explanation for déjà vu is probably as untrue as one single cause for headache." Belief in the existence of parapsychological phenomena (mental telepathy, precognition, reincarnation) may have roots in déjà vu (Stern, 1938; Carmichael, 1957), given that it is a common experience that can easily feel supernatural. There has also been a considerable amount of published speculation about the psychodynamic underpinnings of déjà vu, suggesting that an individual attempts to subconsciously reduce situational anxiety by labeling it as 'familiar' (see Arlow, 1959). These two classes of interpretations will not be covered in this chapter, but the interested reader is directed to summaries found in Brown (2004).

2.21.9 Dual-Processing Explanations

Four different categories of scientifically oriented explanations of déjà vu are described in Brown (2004). Of these, the dual-processing explanations are perhaps more philosophical than scientific, yet draw on established cognitive phenomena. Each variety of explanation in this category assumes that two routine cognitive processes are momentarily out of normal synchrony with each other. For instance, retrieval and familiarity usually work jointly and in close coordination – when one recalls information it is accompanied by an assessment of familiarity. However, if familiarity assessment becomes activated spuriously and independently of recall, a situation may be incorrectly assessed as previously experienced when it was not (Claparède, 1951; Gloor, 1991). Similarly, if encoding and retrieval, which normally operate separately, are activated simultaneously, this could result in a misimpression that the present (new) experience has been retrieved from memory (de Nayer, 1979). Along similar lines, déjà vu has been interpreted as involving the merging of perception and encoding processes (Carrington, 1931), perception and retrieval (Ellis, 1911), implicit and explicit information processing (Wigan, 1844; Myers, 1895), and different states of conscious awareness (Jackson, 1888). Although all of these are logically engaging and theoretically possible, they do not readily lend themselves to empirical evaluation. In the following sections are three categories of déjà vu explanations that provide a more solid scientific foundation on which to evaluate the possible cause(s) of déjà vu.

2.21.10 Neurological Explanations

Déjà vu may result from a minimal biological dysfunction involving cortical information processing. This interpretation has its roots in the observation that some TLEs experience déjà vu prior to their seizure (see earlier section titled 'Physiopathology and déjà vu'). If a minor neurological misfiring, or small seizure, occurs in individuals without brain pathology, then this could possibly trigger déjà vu (Halgren et al., 1978; Bancaud et al., 1994). Elaborating on this concept, Spatt (2002) speculated that spontaneous activity in the parahippocampal area of the cerebral cortex, a region routinely involved in encoding and retrieval activities, could create a brief sense of inordinate familiarity that is disconnected from one's present objective experience.

A second variety of neurological explanation assumes that a brief and minimal change in the speed of neural transmission could create an illusion of 'pastness.' Our nervous system transmits the perceptual information we receive in a highly reliable and consistent fashion. Imagine, however, that this tightly formatted neural transmission is momentarily altered by an aberrant event – say, a deficiency or excess of a neurotransmitter at a particular synaptic juncture. This retarding (Grasset, 1904) or acceleration (Allin, 1896) of the message may be misinterpreted as inordinate familiarity (cf. Jacoby, 1988; Jacoby et al., 1988) with an objectively new experience (Burnham, 1889; Ellis, 1911; Schacter, 2001).

Extending this speculation, if two perceptual pathways are involved, any temporal disparity would be even more pronounced. It has been demonstrated that perceptual information is transmitted to cortical processing centers via multiple pathways (Schneider, 1969; Goodale and Milner, 1992; Milner and Goodale, 1995). Imagine that a slight interruption occurs to one of these two messages, but not the other. An additional separation of only a few extra milliseconds between the duplicate messages may create a perceptual echo sufficient to flummox the

interpretive centers of the cortex and lead to a misimpression that the second (delayed) perceptual event duplicates the first (leading) message. This possibility was proposed over a century ago and has been elaborated on and extended by many others since (Wigan, 1844; Osborn, 1884; Maudsley, 1889; Myers, 1895; Humphrey, 1923; Efron, 1963; Weinand et al., 1994). Some of the early versions of this dual-message interpretation were grounded in a communication problem between hemispheres and disparities regarding the timing of this information exchange (Wigan, 1844; Myers, 1895; Humphrey, 1923).

A frequently reported feature accompanying personal reports of déjà vu is a sense of precognition (cf. Brown, 2004), with an individual believing that they know exactly what is going to happen moments before it does. On the surface, this appears to challenge any scientific explanation of déjà vu. However, a neurological interpretation involving dual pathways may help explicate this unusual subjective experience. When an inordinate separation occurs between two neural messages, the brain could theoretically focus primarily on either the leading or the trailing version. If the trailing perception is central, then a sense of déjà vu results because the first message already arrived. In contrast, if the brain invests in the leading message, then a sense of precognition could result because the individual can literally foresee what will happen moments later via this brief preview (Efron, 1963; Kohn, 1983).

2.21.11 Implicit Memory Explanations

A déjà vu experience may result from an implicit memory for one or more aspects of the present situation (Schacter, 1987; Richardson-Klavehn and Bjork, 1988; Roediger and McDermott, 1993). When the present setting cues an implicit memory that is missing an associated episodic component, one could experience a sense of familiarity that is missing the recollective dimension. The simplest form of this interpretation is that our entire present experience duplicates something encountered earlier (Chapman and Mensh, 1951). Abercrombie (1836) and Osborn (1884) present case reports of déjà vu experiences in adulthood that were later traced to actual childhood events. Abercrombie (1836) described a woman who had a déjà vu experience when escorted to the room where her mother had died. She had no conscious recollection of having been there before, but found out later that as an infant she had been taken to visit her dying mother there. Osborn (1884) discussed a similar incident, where a man experienced a déjà vu at a castle entrance, only to find out later that he had visited that very location with his parents when he was one and a half years old.

Literary works also have the capacity to implant in memory detailed descriptions of scenes or settings that subsequently provide a striking fit to real experience (Hawthorne, 1863; Knight, 1895). Media exposure (television, movies, magazines) also has the potential to paint vivid mental images that can connect to subsequent real experiences, without a recollection of the source. Ellis (1897, 1911) described a déjà vu in a particular setting that he later traced back to a stereoscope picture that he had seen of that exact location. The Internet now provides a particularly rich set of images of real locations. In fact, a television ad by Hotels.com humorously highlights this possibility: A man walks into a room and breathlessly exclaims, 'I've been here before,' to which his partner replies that he saw it in a Web video preview prior to reserving the room online.

Rather than duplicating the entire previous experience, Osborn (1884) speculated that déjà vu may result if the manner in which the information is processed duplicates the way that a previous experience was processed (Kolers, 1973; Morris et al., 1977; Kolers and Roediger, 1984). Although more difficult to test, this processing duplication could allow a much broader generalization of familiarity from one setting to another. Also, the duplication could involve the general perceptual form of some visual or auditory event from our past (Grasset, 1904; Reed, 1974). This gestalt familiarity interpretation is deftly illustrated by Dashiell (1937) in his textbook on psychology (see **Figure 3**). He suggested that if an individual encounters setting A that is new, this may evoke a déjà vu due to its resemblance to the general form of setting B that is familiar. None of the individual elements are identical across settings, but the overall form of the new street scene strongly resembles the familiar scene – large building close on the right side of the street, a church with steeple down the left side of the street, and so forth.

Rather than duplicating the entire prior experience or its general form, perhaps one element in the present experience is old but not identified as such. This element elicits a strong familiarity response that we fail to attach to it, and so we

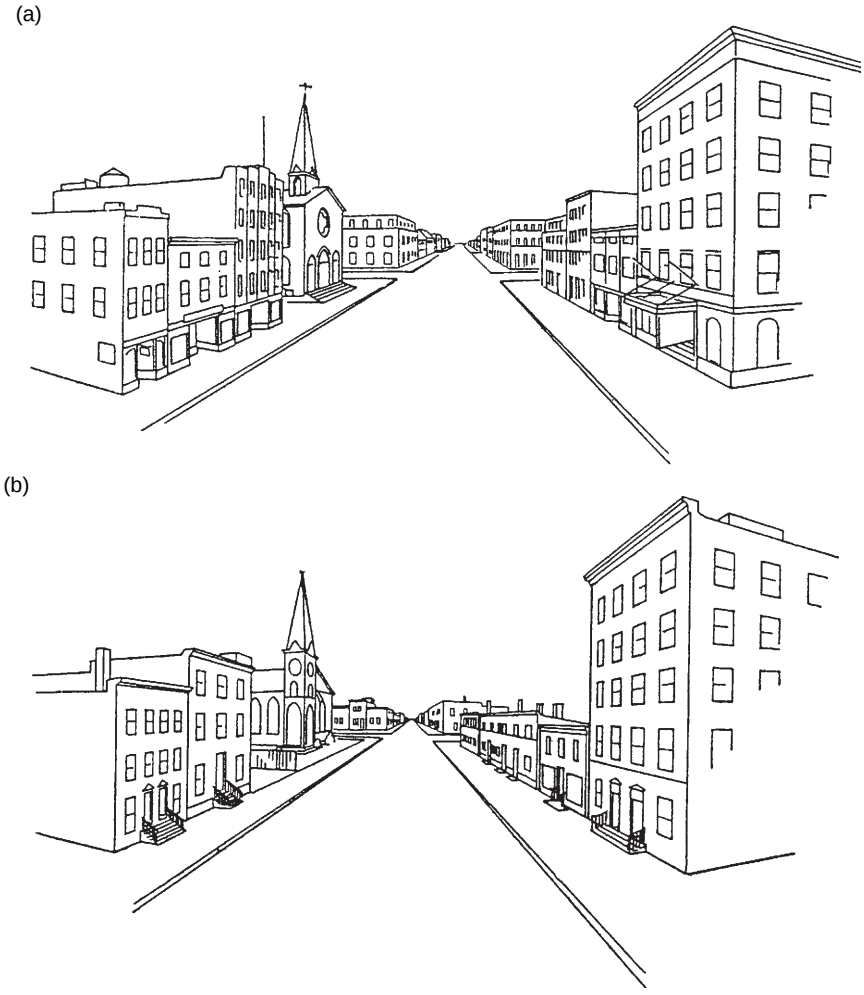

Figure 3 Illustration of gestalt familiarity explanation of déjà vu (from Dashiell, 1937).

misattribute the sense of familiarity to the entire setting:

> Suppose . . . that after I have visited the picture gallery I go to the next city where there is another gallery. Perhaps in some corner of a room there is some insignificant detail, such as a gilded cornice, that is the same as in the last gallery. This will be seen and recognized, but the feeling of recognition, instead of being confined to the one detail, may be spread over the whole room. (Humphrey, 1923: 140)

The familiar element may not be from objective experience but implanted via imagination or fantasy. MacCurdy (1925) described a déjà vu that he traced to memory fragments from a previous dream. This could also happen with daydreams (Chapman and Mensh, 1951), and Titchener (1928) asserted that our imaginings may leave an even stronger memory trace than real experiences. Sully (1887) also suggested that exquisite prose descriptions may paint a vivid mental picture of a single element – face, object, building façade – and this may have the power to elicit a later déjà vu when a real object resembles it. Multiple familiar elements in combination may have the same effect (Wohlgemuth, 1924; Fleminger, 1991; Findler, 1998; Lampinen, 2002) and may actually increase the probability of déjà vu by summating familiarity while simultaneously interfering with the retrieval of contextual elements necessary for explicit recall (Hintzman, 1988).

Whereas the aforementioned speculation revolves around familiarity associated with unrecognized and embedded stimulus element(s), déjà vu could also result from an affective response (positive or

negative) misinterpreted as familiarity (Baldwin, 1889; Allin 1896; Zeidenberg, 1973; Fleminger, 1991; Pagliaro, 1991). Perhaps the strange visceral reaction associated with déjà vu in personal descriptions is not a result, but rather a cause, of the experience (Angell, 1908; Reed, 1974). Seamon et al. (1983) specifically proposed that the type of positive affective response (liking) that is enhanced by mere exposure may underlie déjà vu, and that this may be especially likely with a subliminal exposure which precludes explicit recollection:

> The experience of déjà vu . . . is an expression of the familiarity of a similar stimulus without the retrieval of that earlier event or its context into conscious awareness . . . . Essentially, the same outcome was observed in this study: people liked familiar stimuli without recognizing the basis for their familiarity. In this respect, the finding of target selection by affect in the absence of recognition is similar to the well-known, but poorly understood, phenomenon. (Seamon et al., 1983: 188)

Brown and Marsh (2005) have experimentally evaluated the implicit memory interpretation of déjà vu. They had students superficially process briefly presented scenes of an unfamiliar college campus, and then presented both old and new scenes in a second session (1 or 3 weeks later). In order to model déjà vu, they asked students whether they had actually visited each pictured scene, not just whether it looked familiar. Brown and Marsh (2005) found that the previous presentation of a scene significantly increased mean visit ratings, thus demonstrating that a prior laboratory encounter with a stimulus has the capacity to influence the likelihood of a reaction similar to déjà vu in autobiographical memory.

Hypnosis may also hold promise in modeling déjà vu in the laboratory. Marcuse et al. (1945) reported that a sense of déjà vu accompanies some recollections of stimuli encountered previously under hypnosis. Banister and Zangwill (1941a,b) explored this technique more directly by presenting pictures and odors to hypnotized participants followed by a suggestion to forget. Later, participants were asked to evaluate these same stimuli during full consciousness awareness. Although they were able to elicit a sense of déjà vu in some individuals, they were only moderately successful. O'Connor et al. (2006) have recently applied a similar procedure. Hypnotized participants were told that they would later experience a sense of specific (episodic) familiarity for words with green borders and a sense of unspecified (déjà vu) familiarity for words with red borders. This technique precipitated a déjà-vu-like experience in some participants (5 of 18), although others interpreted the sensation as a tip-of-the-tongue experience (*See* Chapter 2.22). O'Connor et al. noted substantial individual differences in susceptibility to déjà vu, and this should be examined more carefully in future research.

2.21.12 Double Perception Explanations

The double perception interpretation of déjà vu rests on the possibility that when an ongoing stream of perception is momentarily dissected or disrupted, this creates the impression of duplicate events:

> . . . you are about to cross a crowded street, and you take a hasty glance in both directions to make sure of a safe passage. Now your eye is caught . . . by the contents of a shop window; and you pause . . . to survey the window before you actually cross the street . . . the preliminary glance up and down, that ordinarily connects with the crossing in a single attentive experience, is disjointed from the crossing; the look at the window, casual as it was, has been able to disrupt the associative tendencies. As you cross, then, you think 'Why, I crossed this street just now'; your nervous system has severed two phases of a single experience, both of which are familiar, and the latter of which appears accordingly as a repetition of the earlier. (Titchener, 1928: 187–188)

Similar speculation is more than a century old (Burnham, 1889; Grasset, 1904) and numerous versions of this explanation have been put forth. One variety proposes that the perceptual experiences both preceding and following the gap are at full awareness, with the ongoing stream of perception being fractured by distraction from either the environment (Conklin, 1935; Leeds, 1944; Tiffin et al., 1946) or our mental activities (Lalande, 1893; Allin, 1896). Allin (1896) suggested that this attentional break between the perception and the reperception moments later is made especially compelling because the reprocessing is faster due to enhanced perceptual fluency (Jacoby and Dallas, 1981).

A second version of the double perception explanation involves a diminished first perception (glance) followed by a second perception at full awareness. The

initial perception may be subpar because one's attention is momentarily degraded (1) due to fatigue or distraction (Allin, 1896; Dugas, 1902; West, 1948), (2) because a particular feature is first perceived peripherally and then focally, or (3) from inhibition by other elements in the perceptual array (Dixon, 1971). This diminished-to-full version of the double perception interpretation is lent credibility by several lines of cognitive research. Jacoby and Whitehouse (1989) conducted an investigation inspired by Titchener's (1928) description (see preceding quote). After studying a word list, participants took a recognition test where each word was preceded by a briefly flashed (subliminal) stimulus consisting of the word itself (match), an unrelated word (nonmatch), or a series of symbols (control). Jacoby and Whitehouse (1989) discovered that a new word preceded by itself (match) is more likely to be incorrectly evaluated as old, compared to a new word preceded by a nonmatch or control stimulus. They likened this outcome to déjà vu, and their findings have been replicated by Joordens and Merikle (1992) as well as by Bernstein and Welch (1991), who further suggested that the prime presentation does not have to be subthreshold to elicit this illusion of familiarity.

A second line of research lending credibility to this interpretation is inattentional blindness. Mack and Rock (1998) demonstrated that when searching for a target (+) in a visual display, if a distractor object is inserted along with the target cross after a number of simple trials, most participants will fail to report noticing it, even though the display is well above threshold. Interestingly, this obliviscence is greater when the distracting stimulus appears in the center of the display with the target off to one side. Participants who claim not to have noticed the distracting stimulus show evidence that it was perceived and stored in memory because priming for that distractor stimulus is enhanced on a subsequent indirect test of memory. This processing of unnoticed visual stimuli has also been found in research on cell phone use when driving (Strayer and Johnson, 2001; Strayer et al., 2003).

2.21.13 Summary and Future Directions

Déjà vu is a recognition illusion experienced by about two-thirds of individuals, and the incidence generally decreases with age. Most experients have had déjà vu multiple times, generally once every 1 to 6 months in a younger sample (cf. Brown, 2004). Déjà vu experiences are generally brief (several seconds long), triggered by the entire setting, and are more likely to occur indoors, while relaxing, and in the company of friends. The illusion is more likely to occur in individuals with more education, travel experience, and liberal attitudes. Given the rarity of déjà vu, the enigmatic nature of the causative factors, and amorphous response, eliciting a déjà vu experience in the lab may be problematic. However, attempts to model different aspects of the illusion may provide a creative source of research ideas on cognition in general (Seamon et al., 1983; Jacoby and Whitehouse, 1989; Bernstein and Welch, 1991; Joordens and Merikle, 1992; Brown and Marsh, 2005; O'Conner et al., 2006).

There is probably no single cause for déjà vu any more than there is one cause for headaches (Neppe, 1983a). Implicit memory and double perception hold explanatory promise, and evolving research technologies in brain recording and stimulation, psychopharmacology, and virtual reality may also prove useful in clarifying the external (stimuli) and internal (cognitive processing mechanisms) factors that have the capacity to elicit déjà vu (cf. Brown, 2004). Several issues require explication before any complete understanding of the phenomenon can occur, including (1) why déjà vu is less likely with older adults, (2) why déjà vu occurs in both mundane and unique settings, (3) why déjà vu is rarely reported by those with serious memory problems, and (4) why the illusion so often involves a sense of precognition or prior dream.

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2.22 Tip of the Tongue Experience

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Routine word retrieval is a remarkable accomplishment when it goes smoothly. We fluidly access and emit tens of thousands of words with hardly a ripple of conscious awareness. On rare occasions, this transition from word knowledge to word output fails and immediately grabs our attention. This tip-of-the-tongue (TOT) experience is common to us all – we are positive that we know the missing word, but are unable to produce it at the moment. William James (1893) provided a colorful and oft-quoted characterization of the TOT experience:

> Suppose we try to recall a forgotten name. The state of our consciousness is peculiar. There is a gap therein; but no mere gap. It is a gap that is intensively active. A sort of wraith of the name is in it, beckoning us in a given direction, making us at moments tingle with the sense of our closeness, and then letting us sink back without the longed-for term. If wrong names are proposed to us, this singularly definite gap acts immediately so as to negate them. They do not fit into its mould. And the gap of one word does not feel like the gap of another, all empty of content as both might seem necessarily to be when described as gaps. . . . The rhythm of a lost word may be there without a sound to clothe it; or the evanescent sense of something which is the initial vowel or consonant may mock us fitfully, without growing more distinct. (James, 1893: 163–164)

One of the primary goals of empirical research on TOTs is to provide a unique picture of an otherwise incredibly rapid and automatic behavior "... similar to how slow-motion photography clarifies the dimensions of a hummingbird's flight" (Brown, 1991: 204). Brown and McNeill (1966) were the first to apply a scientific approach to study this phenomenon. Their research was methodologically and analytically comprehensive and served as a prototype for subsequent investigations on this topic. They presented definitions of relatively rare words ("one who collects stamps" for philatelist; "a secretion of the sperm whale used in the manufacture of perfume" for ambergris) and elicited TOTs on 13% of such trials. Most surprising was how often information about the missing target word (first letter, number of syllables) was available during this experience, suggesting to Brown and McNeill (1966) the concept of generic recall, where the first stage in word retrieval involves accessing abstract

(syllables, syllabic stress) and partial (letter) information about the target word. Their investigation set the stage for a steady growth in research on this topic, with the number of articles approximately doubling every decade since their original publication (see summaries by Brown, 1991; Smith, 1994; Schwartz, 2002b). Whereas most investigators use TOTs as a springboard to address mechanisms of memory storage and retrieval, the TOT experience has also been applied to theories of language production (e.g., Caramazza and Miozzo, 1997; Faust et al., 1997; Askari, 1999; Beattie and Coughlan, 1999; Faust et al., 2003; Faust and Sharfstein-Friedman, 2003; Gollan and Acenas, 2004; Golan et al., 2005) and metamemory (Koriat, 1993; Metcalfe et al., 1993; Schwartz, 1998, 1999, 2001a, 2002b; Schwartz and Frazier, 2005; Schwartz and Smith, 1997).

2.22.1 Eliciting and Measuring TOTs

TOTs are a universal experience. In both laboratory and diary investigations, it is the rare individual who fails to experience a TOT, and the concept is universally recognized across individuals and cultures. In fact, the "tongue" metaphor characterizes this word generation difficulty across a wide range of languages (45 of 51 sampled by Schwartz, 1999) and is most likely attributable to the subjective impression that the problem is localized in the late stages of oral language production. People report that between one and two TOTs occur per week in everyday life, as reflected in diary studies of naturally occurring TOTs (Cohen and Faulkner, 1986; Reason and Lucas, 1984; Burke et al., 1991; Ecke, 1997; Heine et al., 1999; Schwartz, 2001b; Gollan et al., 2005), as well as in retrospective assessments of TOT incidence (Burke et al., 1991; Sunderland et al., 1986).

Most laboratory studies on TOTs use definitions as target word cues, following Brown and McNeill's (1966) lead. Others have successfully elicited TOTs using faces (Maylor, 1990; Burke et al., 2004; Cross and Burke, 2004), line drawings and pictures (Brown and Nix, 1996; Gollan and Acenas, 2004; Gollan and Brown, 2006), theme songs (Riefer et al., 1995), smells (Jönsson and Olsson, 2003), learned paired associates (Ryan et al., 1982; Metcalfe et al., 1993), and artificially constructed materials (Smith et al., 1991; Schwartz and Smith, 1997; Schwartz, 1998). TOTs elicited in the laboratory are similar to those that occur naturally (Ryan et al., 1982; Burke et al., 1991; Schwartz, 2001b), but the incidence varies considerably depending on the type of cue materials, procedures, and individuals. A summary of 188 outcomes from 72 published articles in **Figure 1** indicates that the incidence of TOTs is generally between 6% and 15% of items (cf. Brown, 1991).

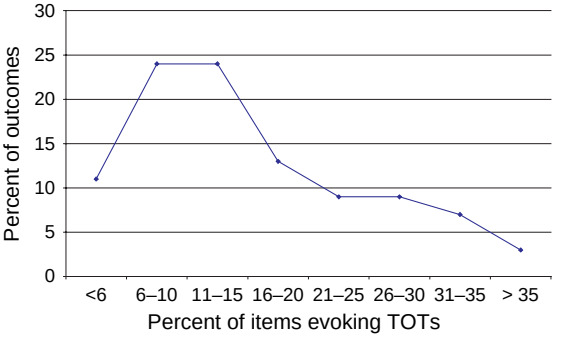

Figure 1 TOT rate in lab investigations.

Given that TOTs are relatively rare and depend on self-report, there are several measurement issues to consider in this research. First is the distinction between TOT and feeling-of-knowing (FOK) assessments (cf. Yaniv and Meyer, 1987). Typically, an FOK relates to whether one believes that they can later recognize the missing target word, whereas TOTs are triggered by a sense of imminent recall (Maril et al., 2001; Maril et al., 2005). In addition, FOK assessments are requested for all missing target words, whereas TOTs occur spontaneously on only select target words. Researchers also separate positive TOTs (TOT+), where the sought-after target word is the one intended by the experimenter, and negative TOTs (TOT−), where the elusive word differs from the intended target, a distinction made through a recognition test (Brown and McNeill, 1966; Burke et al., 1991). One could argue that a TOT− is a genuine TOT experience, but most exclude these items from analyses (Burke et al., 1991; Rastle and Burke, 1996; Harley and Bown, 1998). There is also the 'fragmentary data problem,' first identified by Brown and McNeill (1966), related to the considerable variation in TOT probability across both participants and stimuli. With each participant's TOTs elicited by a different subset of target words, standard statistical procedures are not well suited to analyzing such data sets.

Finally, there is the phenomenology of TOTs. Informal descriptions of TOTs include such information as James' (1893) observation of a 'tingling'

sensation during TOTs and Brown and McNeill's (1966) suggestion that we are 'seized' by, and fall 'under the spell' of, a TOT. Such subjective aspects of experience have received only cursory attention (Schwartz et al., 2000; Schwartz, 2002b), although there seems to be some validity to the sense of imminence, emotionality, and strength associated with TOTs.

2.22.2 Influencing TOT Probability

There have been a number of efforts to influence TOT probability through instructions, as well as with primes preceding, and cues following, the target word probe. These manipulations are motivated by efforts to evaluate the mechanisms underlying TOTs. If TOTs are based on a subjective sense of impending or imminent recall of the target word (inferential position), it seems logical that the incidence of TOTs could be manipulated through instructions or motivation. In line with this, Widner et al. (1996) told one group of participants that target words were relatively easy to retrieve, whereas another group was told that they were difficult (the same set of targets were used with both groups). The easy instructions elicited significantly more TOTs, either due to increased motivation to show retrieval competence or greater stress from the struggle to retrieve 'easy' targets (cf. Schwartz, 2002b). In either case, Widner et al. (1996) suggested that an important component of TOTs may be personal expectations about our knowledge base and retrieval competence.

Given that TOTs are sometimes accompanied by related words, it is natural to wonder whether presenting such words influences TOTs in either a positive or negative direction. These experimental manipulations have involved inserting related words either immediately following (cueing) or prior to (priming) the presentation of the target word probe. These efforts have been aimed primarily at differentiating two memory access interpretations of TOTs: blocking and incomplete activation (see discussion later in this chapter).

2.22.2.1 Cueing

Jones and Langford (1987) presented cue words that were either (a) phonetically related ('axial' for target 'alchemy'), (b) semantically related ('incubus' for target 'banshee'), (c) both phonetically and semantically related ('abnormality' for target 'anachronism'), or (d) unrelated ('opinionated' for 'cherubic') to the accompanying target word. Phonetically related cue words increased TOTs, whereas semantically related cue words did not, an effect replicated by both Jones (1989) and Maylor (1990a) (cf. Askari, 1999). Unfortunately, cues were not counterbalanced across definitions, and subsequent research suggests that this apparent difference was most likely due to the specific definitions and/or cue words used (Meyer and Bock, 1992; Perfect and Hanley, 1992). Thus, it appears that there is no clear evidence for a related cue word influencing TOT probability. However, this procedure is based on the unlikely assumption that retrieval is not initiated until the definition is completely processed. Rather, it seems more reasonable that retrieval starts well before the entire definition is read. Other investigations have also presented semantic and phonological information related to the target word following the target word probe (Heine et al., 1999; James and Burke, 2000; Abrams et al., 2004), but these generally are designed to influence the resolution rather than the incidence of TOTs, and this research will be covered later in this chapter.

2.22.2.2 Priming

A less ambiguous way to influence TOT probability is by presenting related words prior to the target word probe. Although this solves the previously noted problem, it creates another: Participants may become aware of the prime-target relationship and develop strategies to search for target words based on the prime words (cf. Jones, 1989). Rastle and Burke (1996) addressed this problem by embedding target words in an apparently unrelated task preceding the TOT probe session. Participants rated the pronunciation difficulty of a set of words, half of which were targets in the subsequent TOT task. Rastle and Burke (1996) found that there were significantly fewer TOTs on words appearing in the prior prime list, compared to targets that had not been previewed. They further discovered that both shallow (syllable count) and deep (pleasantness) processing of the targets reduced subsequent TOTs.

Several additional investigations have examined the effects of priming with related words, rather than with the target word itself. Burke et al. (2004) presented primes that were either phonologically related (cherry pit) or unrelated (cane) to a celebrity's name (Brad Pitt). Phonologically related primes

significantly reduced TOTs relative to unrelated primes, an outcome found with both younger and older adults. Some participants noticed the correspondence between prime and target (see preceding point), and analyses including only unaware subjects yielded a significant prime-related decrease in TOTs for older but not younger participants. Thus, phonological activation can reduce TOTs, but this effect appears to be more pronounced for older adults.

In a similar investigation, Cross and Burke (2004) used semantically (rather than phonologically) related prime words in a proper name retrieval task. Participants were primed with a famous name, either related (Eliza Doolittle) or unrelated (Scarlett O'Hara), to the subsequent target actor (Audrey Hepburn). In the related condition, the actor had actually portrayed the character whose name was primed earlier. There was a slight elevation in TOT rate following a semantically related versus unrelated prime, but this difference was nonsignificant.

Rather than priming the entire target word, James and Burke (2000) exposed participants to various phonological fragments from the target word embedded in a set of 10 prime words preceding the target word cue. With related prime sets, half (5) of the words shared some phonological component ('in*di*gent, *ab*stract, trun*cate*') with the target word ('abdicate'). This type of phonological priming reduced TOTs, relative to unrelated prime sets, where none of the 10 words shared a phonological relation with the target.

2.22.2.3 Target Word Characteristics

Brown and McNeill (1966) originally proclaimed that TOTs occur primarily with rarely used (low-frequency) words, and this assumption has permeated most subsequent research (Naito and Komatsu, 1989; Burke et al., 1991; Askari, 1999; James and Burke, 2000; Lesk and Womble, 2004). Significantly more TOTs have been found with low- versus high-frequency target words (Harley and Bown, 1998; Vitevitch and Sommers, 2003), and target word frequency and TOT probability have been shown to be negatively correlated (Gollan and Silverberg, 2001). Furthermore, Burke et al. (1991) found that about half of naturally occurring TOT target words were so rare that they did not appear in word-frequency norms, and those that did were substantially below the average frequency of occurrence. Smith et al. (1991) experimentally manipulated target word frequency by varying the number of study trials. They found that TOTs were reduced for words with greater frequency, although this difference was significant in only one of two outcomes. In contrast to this, participants rate their naturally occurring TOT targets (diary studies) as relatively familiar (Cohen and Faulkner, 1982; Reason and Lucas, 1984), although data are based on subjective impression rather than objective data.

Another dimension that appears to influence TOT rate is neighborhood density, or the number of words that are phonologically or orthographically similar to the target. More TOTs occur with targets from sparse compared to dense neighborhoods (Harley and Bown, 1998; Vitevitch and Sommers, 2003), suggesting that dense networks may facilitate retrieval. This intriguing finding is based on small and highly selective subsets of words, but this topic certainly warrants additional research. Finally, TOT incidence is considerably higher for proper names compared to other types of target words (common nouns, objects, verbs) in both diary (Burke et al., 1991; Cohen and Faulkner, 1986; Schwartz, 2001b; Gollan et al., 2005) and experimental (Rastle and Burke, 1996; Evrard, 2002) investigations. Interestingly, Gruneberg et al. (1973) found that when individuals are asked to generate their own TOTs ('prospect') they are most likely to search through sets of proper names.

2.22.3 Partial Target Word Information

One of the more striking aspects of the TOT experience is that while hanging in linguistic limbo, unable to pull up the elusive target, various aspects of this word often come to mind. In fact, this anecdotal observation was a primary motivation for Brown and McNeill's (1966) original investigation. The meaning of partial target information availability is limited by the fact that it accompanies only some TOTs, and there is considerable variability across studies in how this information is requested, recorded, and reported. Although Brown and McNeill (1966) reported that first letter guesses regarding the target word were correct over half of the time during TOTs, they did not indicate how often such guesses were made. Harley and Bown (1998) found that first letter guess were made on an unimpressive 5% of TOTs, and others don't even provide such information about the missing target word because it is volunteered too infrequently (Schwartz and Smith, 1997; Vitevitch and Sommers, 2003). Thus, one must be careful not

to overinterpret the significance of such findings based on low response rates for select items.

For naturally occurring TOTs, approximately two pieces of peripheral information are typically reported, and this rate is lower in lab studies, at less than one piece of information per TOT (Burke et al., 1991; Rastle and Burke, 1996). More than half of naturally occurring TOTs may be accompanied by peripheral information (Cohen and Faulkner, 1986), but this incidence appears to be substantially lower with lab-induced TOTs (Heine et al., 1999). The most salient type of partial target word information is the first letter. Brown and McNeill (1966) originally found that participants correctly guessed the first letter on 57% of attempts, and that 49% of similar sounding (SS) words accompanying TOTs share a common first letter with the intended target. Although subsequent research has yielded a wide range of correct guess accuracy (63%, Yarmey, 1973; 71%, Koriat and Lieblich, 1974; 68%, Rubin, 1975; 40%, Brown and Nix, 1996; 28%, Caramazza and Miozzo, 1997; 54%, Harley and Bown, 1998; 56%, Dahlgren, 1998), the average appears to be around 50% and definitely above chance levels. There is also evidence that individuals can correctly identify the final letter(s) or phoneme during TOTs (Koriat and Lieblich, 1974; Brown and Nix, 1996; Caramazza and Miozzo, 1997), although the accuracy is considerably lower.

The number of syllables in the missing target word also appears to be available on some occasions. Brown and McNeill's (1966) participants were correct 60% of the time guessing number of syllables (from 1 to 5), and later research has yielded somewhat lower rates, averaging around 50% (80%, Koriat and Lieblich, 1974; 35%, Brown and Nix, 1996; 37%, Caramazza and Miozzo, 1997; 54%, Dahlgren, 1998; 55%, Vigliocco et al., 1997; 45%, Gollan and Silverberg, 2001). Although less compelling than first letter guesses, given that the majority of English words are two or three syllables long, these data still suggest that individuals have access to word-form information at greater than chance levels.

Finally, target word gender (masculine, feminine) appears to be accessible during TOTs above chance levels in Italian (71%, Caramazza and Miozzo, 1997; 72%, Miozzo and Caramazza, 1997; 84%, Vigliocco et al., 1997) and French (85%, Ferrand, 2001), although not Hebrew (55%, Gollan and Silverberg, 2001). Thus, most evidence indicates that syntax (gender) as well as orthography (first letter) and general word form (syllables) is accessible during TOTs.

2.22.4 Words Related to the Target Word

Individuals often report that nontarget words come to mind when foundering in a TOT experience. These interlopers (Jones, 1989) are immediately identified as incorrect, yet persist in consciousness. They have been variously referred to as relatives (Astell and Harley, 1996), blocking intermediates (Reason and Lucas, 1984), intruders (Schwartz, 1994), candidates (Cohen and Faulkner, 1986), persistent alternatives (Burke et al., 1991; Heine et al., 1999), and ugly stepsisters (Reason and Lucas, 1984). The significance of these interlopers is the subject of a continuing debate that is primarily related to the etiology of TOTs (covered later in this chapter).

Some view interlopers as a byproduct of the TOT experience (cf. Cross and Burke, 2004), reflecting how close one is to the missing target word (Brown and McNeill, 1966). Others see interlopers as causing TOTs: Phonetically or semantically related words are inadvertently retrieved, and their presence hinders successful access to the target word (Jones and Langford, 1987; Jones, 1989; Brown, 1991). Finally, some speculate that these interlopers actually facilitate TOT resolution by sharpening the target word specification (Cohen and Faulkner, 1986).

The majority of naturally occurring (diary) TOTs are accompanied by related words (Reason and Lucas, 1984; Cohen and Faulkner, 1986; Burke et al., 1991; Heine et al., 1999), and their incidence in laboratory-induced TOTs is lower at approximately one-third of TOTs (Burke et al., 1991; Riefer et al., 1995; Brown and Nix, 1996; Harley and Bown, 1998; Riefer, 2002). Most find that semantically related interlopers are more common than phonologically (orthographically) related interlopers (Brown and McNeill, 1966; Riefer et al., 1995; Ecke, 1997; Harley and Bown, 1998), and most are from the same syntactic class as the target word (Burke et al., 1991; Harley and Bown, 1998; Ecke, 2001). It is also common for the interloper and target to share the same initial phoneme and number of syllables (Burke et al., 1991).

2.22.5 Resolving TOTs

2.22.5.1 Resolution Probability

The vast majority of naturally occurring TOTs are eventually resolved (92%, Burke et al., 1991; 78%, Cohen and Faulkner, 1986; 95%, Heine et al.,

1999; 89%, Schwartz, 2001b). About half are resolved within a minute, with most resolved within 1 h (Burke et al., 1991; Schwartz, 2002a). In laboratory research, resolution probability is more difficult to assess because the trial by trial procedure often limits opportunity. Given that most studies allow a maximum of 1 min per trial, any resolutions not occurring within this time frame may not be captured in the procedure. Thus, resolution rates are lower (and highly variable) in laboratory investigations, with an average at around half of TOTs (66%, Gruneberg et al., 1973; 48%, Finley and Sharp, 1989; 38%, Riefer et al., 1995; 22%, Brown and Nix, 1996; 54%, Brédart and Valentine, 1998; 90%, Dahlgren, 1998; 44%, Harley and Bown, 1998; 66%, Beattie and Coughlin, 1999; 50%, Gollan and Silverberg, 2001; 43%, Vitevitch and Sommers, 2003).

2.22.5.2 Resolution Process

There are several ways in which the target word can be recovered: mental search, where the individual continues an active memory search; external research, where reference sources or individuals are consulted; and pop-ups, where the word seems to come to mind spontaneously. The incidence of each resolution process varies widely (Read and Bruce, 1982; Reason and Lucas, 1984; Burke et al., 1991; Heine et al., 1999; Schwartz, 2002a) and may partially reflect variations in one's motivation to pursue the inaccessible target word, especially in laboratory TOTs, where one has little personal investment in recovering the elusive word (cf. Brown, 1991). Some controversy surrounds pop-ups because this superficially appears to rely on 'unconscious' mental processes. Whereas some find the incidence quite high, accounting for the majority of resolutions (Finley and Sharp, 1989; Burke et al., 1991; Heine et al., 1999; Schwartz, 2001b), others find the incidence trivial. More specifically, Read and Bruce (1982) found that 3% of TOTs were resolved through 'spontaneous retrieval' and suggested that the incidence in other studies is inflated because pop-ups are striking and hence selectively memorable. Others note that semantic or phonetic cues that one encounters in the environment may trigger the missing target word without conscious awareness of the connection between cue and missing target (Abrams et al., 2003).

Related to pop-ups is the possibility of persistent subthreshold activation of the target word immediately following the TOT. Yaniv and Meyer (1987) suggested that "...the memory trace of a currently inaccessible item may be at least partially primed for a period of time after information is processed from an initial probe question..." (p. 188). In support of such speculation, Yaniv and Meyer (1987) found that immediately after an unresolved TOT, lexical decision latencies were faster for TOT targets than for unrelated words, suggesting a heightened activation level. However, Connor et al. (1992) replicated Yaniv and Meyer (1987) with a 1-week separation between the TOT and lexical decision task, an interval that logically exceeds any continuing activation. Connor et al. (1992) suggested that rather than continuing subthreshold activation, words from well-known categories of information are more likely to lead to both higher TOT incidence and faster lexical decisions (cf. Naito and Komatsu, 1989).

2.22.5.3 Resolution through Cueing

Different procedures have been used to aid TOT resolution. Re-presenting the same target-word cue after a delay has been moderately successful (15%, Brown and Nix, 1996; 12%, Schwartz, 1998), as well as providing the first letter of the missing target word (Freedman and Landauer, 1966; Brennen et al., 1990; Heine et al., 1999). Others have used a more subtle manipulation: burying phonetic fragments of the target word in a set of words processed immediately following the TOT. This procedure is similar to one used to manipulate TOT probability (see earlier discussion). James and Burke (2000) found that processing five words sharing phonological components with the target word (embedded in a 10-word set) while in a TOT improved resolution probability, compared to an unrelated set of words. In a more focused effort to specify which phonological element(s) are important, White and Abrams (2002) found that only the first, but not the middle or last, syllable can enhance TOT resolution. Refining this even further, Abrams et al. (2004) discovered that the first letter alone is insufficient, but that the entire first syllable is needed. Finally, Abrams and Rodriguez (2005) found that the first phoneme facilitated TOT resolution only if the target ('rosary') is cued with a word from a different syntactic category ('robust') from the target. When cue word shares the same syntactic class ('robot') with the target, a common initial phoneme is of no assistance in TOT resolution. Thus, phonological activation has the capacity to add activation to the unavailable TOT target word, facilitating resolution of the TOT, but this occurs only if the cue word differs in syntax from the target.

2.22.6 Etiology of TOTs

2.22.6.1 Direct Access Explanations

Perhaps the liveliest issue in TOT research in the past decade involves identifying the cause of the experience. As noted earlier, Brown and McNeill (1966) originally proposed that TOTs reflect generic recall, where access to specific words involves first sorting through word sets with similar meaning or phonology and then narrowing this down to the particular target. Their ideas did not capture the imagination of researchers in the field, but other interpretations emerged based on the presumption that the TOT reflects a partial activation of the target word. These direct access interpretations fall into two different categories: blocking and incomplete activation. The blocking explanations assume that access to the missing target word is hindered by the presence of other words related to the target (interlopers) (Woodworth, 1938). This perspective motivated some to use related words in an attempt to precipitate TOTs (Reason and Lucas, 1984; Jones and Langford, 1987; Jones, 1989). However, this theory has not received clear empirical support (Meyer and Bock, 1992; Perfect and Hanley, 1992) and cannot easily account for the absence of interlopers for many TOT experiences.

A more likely cause of TOTs is incomplete activation, posited by Burke et al. (1991) and derived from research on language production. Under this interpretation, word production occurs in sequential stages, where activation is passed from a semantic to a phonological representation of the word (Node Structure Theory, or NST). On most occasions, sufficient activation is transmitted from the semantic to the phonological nodes, but on rare occasions the activation conveyed to the phonological nodes is inadequate for complete word production (Burke et al., 1991). Burke et al.'s (1991) version of NST is the transmission deficit hypothesis (TDH). This is the most prominent of incomplete activation theories applied to TOT research, and Burke et al. (1991) speculated that the three most important factors affecting the transmission of activation are (a) recency of word experience, (b) frequency of word experience, and (c) age of the individual. More specifically, a TOT is less likely if the target word has been recently experienced, the word is frequently experienced, and the individual is younger.

Several lines of evidence support TDH. First, individuals can access some aspects of the missing target word (e.g., first letter) because the activation passed along to the lexical nodes is sufficient to make accessible some aspects of the word form without activating the entire lexical entry. The occurrence of interlopers can also be accounted for by TDH. Phonological nodes are shared across multiple words (e.g., 'cha' is shared by 'charity' and 'chastity'), and the activation of a shared node can transmit priming backwards to the semantic level and supply sufficient activation to another word structurally related to the target. TDH is also supported by an age-related decrease in partial target word information (Burke et al., 1991; Rastle and Burke, 1996) and interlopers (Burke et al., 1991). The age-related decline in transmission of activation reduces phonological activation, making elements of the target word (first letter, etc.) less available and reducing the amount of backward activation to related words.

Experimental efforts to increase phonological activation of target word components appear to influence TOTs in a manner congruent with TDH. As described earlier in this chapter, James and Burke (2000) found that phonological components of the target not only reduce TOTs when presented as primes preceding the definition, but also increase TOT resolution when presented as cues following the definition. Rastle and Burke (1996) further found that presenting target words in a prior prime list decreases the number of TOTs, suggesting that recent encounter facilitates the transmission of activation, congruent with TDH (Heine et al., 1999; White and Abrams, 2002; Abrams et al., 2001, 2004).

2.22.6.2 Inferential Explanations

In contrast to direct access, an alternative interpretation is that TOTs reflect an individual's inference about their personal knowledge (cf. Schwartz and Smith, 1997), rather than conveying information concerning the partial activation of the target word. The most thoroughly detailed inferential interpretation is the metacognitive control theory of Schwartz (1999, 2001a, 2002b), which suggests that TOTs arise from an individual's inference about retrieval probability for that particular word. When retrieval fails, we evaluate how accessible a target word should be. If higher than a certain threshold, we experience a TOT. If lower, we have a 'don't know' (DK) response. This evaluation of word accessibility serves to maintain our retrieval effort longer, compared to words not so assessed, yielding higher recall rates following TOT versus DK states and more time

spent in the retrieval effort (Schwartz, 2001a). Koriat (1993) also suggested that a TOT does not reflect special access to the unavailable target word, but is based on partial information retrieved when searching for the word, whether or not these fragmentary data are related to the missing target. Thus, our sense of imminent recall derives not from how close we are to the target word, but how much partial information comes to mind.

Inferential theorists point to the fact that TOTs can occur for artificially constructed stimuli (TOTimals, or made-up animals) that have not been given a name (Schwartz, 1998), and that the likelihood of a TOT is directly related to the amount of target word information provided in the retrieval cue (Schwartz and Smith, 1997). Schwartz (1998) also argued that inferential theories receive support from the fact that we can experience TOTs for nonexistent target words. 'Illusory' TOTs for a fictitious fact ("What is the name of the legendary floating island in ancient Greece?"; "What is the capital of Bormea?") cannot be based on partial target word information because none exists. However, one difficulty with such a conclusion is that these stimulus materials were ineffective in eliciting TOTs with older adults because they universally recognized that the questions had no answer (Schwartz, 2002b). Others argue that illusory TOTs may actually be negative TOTs (TOT–) for real target words, with participants making perceptual or interpretative errors in reading the 'definitions' (Taylor and MacKay, 2002).

In summary, it is possible that both direct access and inferential components contribute to the TOT experience (Schwartz, 1994). Direct access is supported by our ability to correctly access parts of the missing word. And because every TOT relies, to some extent, on our personal cognitive evaluations of our knowledge store and retrieval capability, inferential theories have some place in the explanatory picture.

2.22.7 Individual Differences

2.22.7.1 Age

A ubiquitous finding in TOT research is that older adults experience more TOTs than younger adults. This difference has been found in both diary studies (Cohen and Faulkner, 1986; Burke et al., 1991; Heine et al., 1999) and laboratory investigations (Burke et al., 1991; Brown and Nix, 1996; Rastle and Burke, 1996; Dahlgren, 1998; Heine et al., 1999; James and Burke, 2000; Gollan and Silverberg, 2001; White and Abrams, 2002; Vitevitch and Sommers, 2003; Cross and Burke, 2004; Gollan and Brown, 2006). What is less clear is to what extent this reflects a verbal deficit (decrement theory) or a verbal surplus (incremental-knowledge theory) (Schwartz and Frazier, 2005), keeping in mind that these two positions are not necessarily mutually exclusive (Schwartz and Frazier, 2005; Gollan and Brown, 2006). Burke et al. (1991) suggested that aging naturally diminishes the amount of semantic to phonological priming that occurs during normal word production, and that an increase in TOTs reflects this growing deficit. Gollan and Brown (2006) argued that older adults also have more opportunities to experience TOTs because they have larger vocabularies than younger adults. When compared using a set of words that both groups know equally well, older and younger adults experienced similar numbers of TOTs. Gollan and Brown (2006) further suggested that the negative effects of aging (NST) are more pronounced on easier words because they are closer to ceiling levels of activation. Regardless of the position, any group comparison using laboratory investigations with a common set of target words is problematic. If TOTs are viewed as deficiency and analyzed relative to all unsuccessful items (cf. Brown, 1991), the more verbal group may show a greater deficit because they have fewer nonrecalled items in their baseline. If TOTs are viewed as a surplus and analyzed relative to correct retrievals, the group differences often disappear (cf. Gollan and Brown, 2006). Given that most lab studies use a fixed set of targets, one may or may not find group differences, depending on one's orientation (cf. Gollan and Brown, 2006). It is likely that both verbal deficit and surplus mechanisms contribute to age-related TOT differences, although it should be noted that proper names always show substantial age differences, even when knowledge levels are equated (Cross and Burke, 2004).

At the other end of the age spectrum, TOTs have also been documented among children, but this area remains relatively unexplored. Apart from informal documentation (Wellman, 1977; Elbers, 1985), there are only three published reports using standard laboratory designs. All involve a comparison of normal and language-disabled (LD) children (Faust et al., 1997; Faust et al., 2003; Faust and Sharfstein-Friedman, 2003) and suggest that the incidence of TOTs in normal children is in the same range as that found in adults (11% in grades 2 to 3; Faust et al., 1997; 19% in grades 3 to 4, Faust et al., 2003; 7% in

grades 7 to 8, Faust and Sharfstein-Friedman, 2003). Also similar to adults, partial phonemic and semantic information is often available during TOTs. This research also shows that TOT rates for LD children are twice that of normal children, and that partial information available during their TOTs suggest that LD children suffer deficiencies in phonetic but not semantic access to the target words.

2.22.7.2 Language Competence

The relationship between vocabulary ability and TOTs is ambiguous. Whereas Dahlgren (1998) found that those with high verbal ability have significantly more TOTs than those with low verbal ability, Heine et al. (1999) found a nonsignificant correlation between laboratory-induced TOT rate and vocabulary level. Burke et al. (1991) discovered no correlation between TOTs and vocabulary for naturally occurring TOTs. For lab-induced TOTs, Burke et al. (1991) found no correlation for younger adults but a significant negative correlation for older adults. The inconsistency in these outcomes may be due to the restricted range of vocabulary ability in the particular participant samples, or the selective nature of stimulus materials. An evaluation of the relationship of verbal ability to TOTs should be a routine fixture in future research because the interaction of verbal ability level and word frequency may be an important factor affecting TOT rate (cf. Gollan and Brown, 2006).

Another verbal ability difference examined in TOT research involves monolinguals versus bilinguals. This research has consistently shown that bilinguals experience more TOTs than monolinguals (Gollan and Silverberg, 2001; Gollan and Acenas, 2005; Gollan et al., 2005; Gollan and Brown, 2006), a difference most likely due to bilinguals' reduced use of words (on the average) in both languages (Gollan and Silverberg, 2001; Gollan and Acenas, 2005). This outcome is congruent with TDH (Burke et al., 1991) in that the level of phonetic activation for bilinguals' vocabulary words is less than that for monolinguals because the average usage per word is lower.

2.22.8 Summary

TOTs are a nearly universal experience across a broad range of cultures. TOTs are elicited in the laboratory for around 10–15% of targets, and such lab-induced TOTs have a reasonable similarity to those occurring naturally in diary studies. Proper names are especially likely to trigger TOTs. The missing target comes to mind on most occasions, although this rate is lower in laboratory than in diary investigations. During a TOT, related words (interlopers) come to mind frequently, and certain features of the missing target word (e.g., first letter, number of syllables) appear accessible even when the target word is not. Phonological information about the missing target word appears to reduce TOT incidence and increase TOT resolution. TOTs are more prevalent among older (versus younger) adults and among bilinguals (versus monolinguals). Although some have suggested that TOTs result from target word blocking caused by interlopers or inferences about target word accessibility, most research supports the idea that TOTs result from insufficient phonological activation of the target word.

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2.23 Theories of Recognition Memory

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2.23.1 Introduction

Current theories of recognition memory include quantitative models and detailed neural/computational models. In this chapter we review three general classes of quantitative memory models (i.e., threshold, signal detection, and hybrid models) and assess each model's ability to account for results from a variety of recognition paradigms. Implications of these findings for neural/computational models (e.g., network models of the medial temporal lobes) are then considered.

For each of the models, we first describe the core assumptions, point out the motivations underlying these assumptions, and describe their major predictions. We then evaluate each class of model in light of the empirical literature. In general, the results indicate that pure threshold and pure signal detection models are not consistent with the existing data, whereas several hybrid models (those that include both signal detection and threshold processes) are consistent with a majority of the results.

One of the most direct ways of testing quantitative recognition models is to use a receiver operating characteristic (ROC) analysis, and this method has been applied most extensively in studies of item recognition. Recently, ROC methods have also been applied to other types of recognition, such as relational (e.g., source and associative), remember/know, and exclusion recognition tests. Taken together, these various recognition tasks provide a rich set of data with which to evaluate these models. Neural data, including recent studies of amnesia, event-related potentials (ERPs), and functional magnetic resonance imaging (fMRI), also prove useful in assessing these models. We begin by describing exactly what ROCs are and why they are particularly useful in evaluating models of recognition memory.

2.23.1.1 What Is an ROC?

An ROC is a function that relates the proportion of correctly recognized target items (i.e., the hit rate) to

the proportion of incorrectly recognized lure items (i.e., the false alarm rate) across variations in response bias (i.e., the propensity to make a positive recognition response). In a test of item recognition memory the hit rate is the probability of correctly accepting an old (studied) item as old, and the false alarm rate is the probability of incorrectly accepting a new (unstudied) item as old. ROCs are based on signal detection theory, which assumes that recognition memory (standard item recognition in this case) can be described as a set of two overlapping distributions, one for old or studied items and one for new or unstudied items. The distributions reflect the variation in 'memory strength' (a term that has been interpreted in many different ways, but is commonly thought to be similar to familiarity) for these two sets of stimuli (see **Figure 1(a)** for an illustration). In order to make a binary old/new recognition decision, an individual is assumed to select some level of memory strength as a criterion; items with strengths greater than the criterion are then accepted as old, and items with strengths less than the criterion are rejected as new. However, ROCs contain multiple points collected under different levels of response bias, rather than just the one point generated in old/new paradigms.

The multiple points in ROCs can be obtained in various ways, but by far the most common is the confidence rating method (for comprehensive discussions of general ROC methods see Wickens, 2002; Macmillan and Creelman, 2005). For example, after studying a list of items, subjects are presented with a mixture of old and new items and are required to indicate how confidently they recognize each item on a confidence scale, such as the commonly used six-point scale, with 1 labeled 'Sure New' and 6 labeled

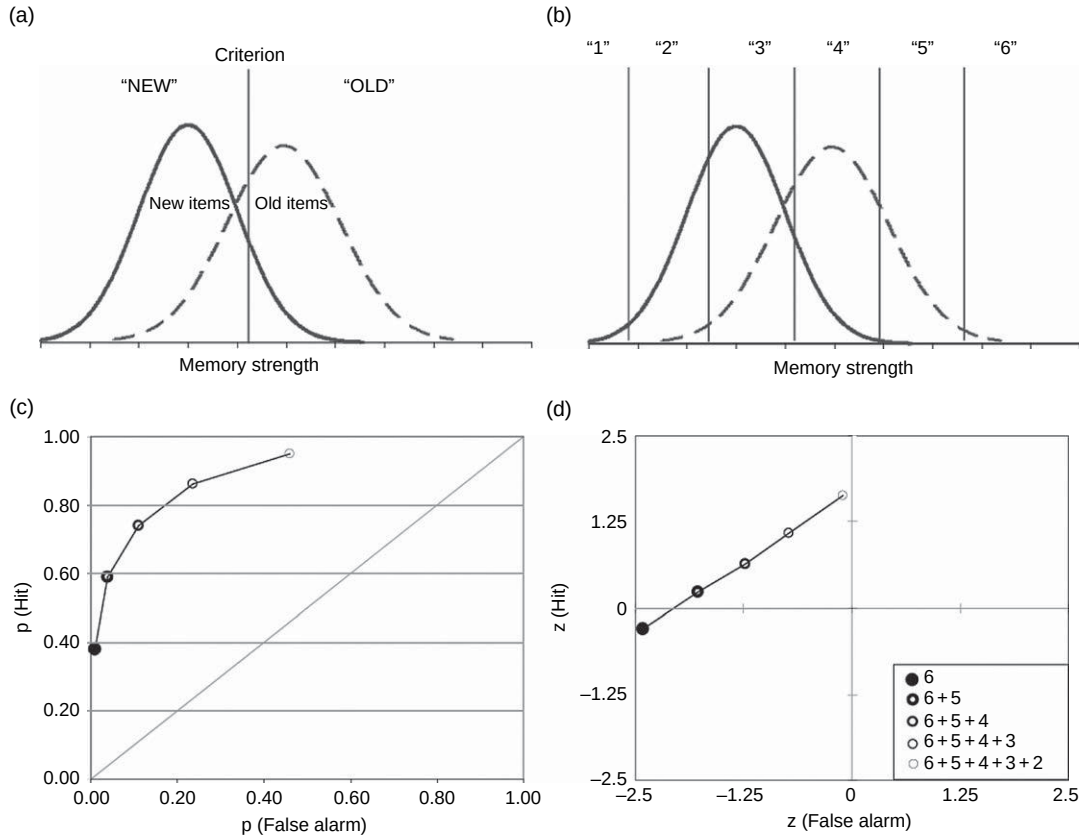

Figure 1 Example new and old item distributions in (a) a standard binary old/new item recognition paradigm and (b) a confidence rating paradigm for item recognition. Panels (a) and (b) are the same, except for the type of response(s) subjects are required to make. Example ROCs in (c) probability space and (d) z-space. The points in panels (c) and (d) represent pairs of hits and false alarms that are summed across a typical confidence scale ranging from 1 to 6, with 1 labeled 'Sure New' and 6 labeled 'Sure Old.' Thus, the first point represents hits and false alarms for items given a confidence rating of 6 or 'Sure Old.' The next point represents hits and false alarms for items given a confidence rating of 5 and 6, and so on down the confidence scale. Note that the final point (for 1 or 'Sure New') is not plotted because it is constrained to be (1,1).

'Sure Old' (e.g., Sure New 1 2 3 4 5 6 Sure Old). This confidence scale is a way of observing changes in response bias without actually having to manipulate bias experimentally. Thus we can observe how hits and false alarms change as a function of response criteria (i.e., how they change for different ratings on the confidence scale). High confidence ratings correspond to relatively conservative response biases (a low propensity to accept an item as old, or a strict criterion), and low confidence ratings correspond to relatively lax response biases (a high propensity to accept an item as old, or a lax criterion). Thus, subjects are assumed to make decisions by placing n–1 criteria for an n-point confidence along the memory strength axis, as illustrated in **Figure 1(b)** for the standard 6-point confidence scale. An ROC is actually constructed by plotting hit and false alarm pairs (hits on the y-axis, false alarms on the x-axis) beginning with the most confidently recognized items (e.g., hits = P('6'|old), false alarms = P('6'|new)), then repeatedly recalculating the values by including the next most confidently recognized items (e.g., hits = P('6'|old) + P('5'|old), false alarms = P('6'|new) + P('5'|new), etc). Thus, the left-most point on the ROC (**Figure 1(c)**) reflects the hit rate plotted against the false alarm rate when adopting the strictest response criterion (or the least amount of bias). Each subsequent point reflects performance at a more and more relaxed response criterion. Importantly, the function is cumulative, and so both the hits and false alarms are constrained to increase or remain constant as the scoring criterion is relaxed. The example ROC in **Figure 1(c)** shows how each point on the function is calculated from a standard six-point confidence scale. Note that chance performance would be reflected by a function lying on the diagonal (i.e., when hits = false alarms), and increasing accuracy is associated with a function moving toward the upper left, such that the greater the area under the curve, the greater the memory sensitivity or discriminability.

2.23.1.2 Why Bother with ROCs?

ROCs have been examined for several reasons. First, given that a subject must adopt some response criterion in order to make an old/new recognition memory judgment, theories of recognition performance must be able to characterize the relationship between accuracy and response criterion, and because ROCs are hit and false alarm rates under different criteria, they provide the opportunity to test a model's characterization of that relationship. Second, ROC results prove to be much more constraining than standard old/new recognition data. That is, memory studies that require subjects to make binary old/new decisions produce an ROC with only one point (a single hit rate and a single false alarm rate). To appreciate the utility of ROC studies, one need only consider how many different theories might account for such a single point – it is difficult to imagine any theory having any difficulty. However, true ROCs – that is, ROCs with multiple points – provide much greater constraint, and thus many fewer theories are able to account for the results. Third, if one can find a theory that adequately accounts for the relationship between accuracy and response bias, then it could be used to derive estimates of accuracy that are not distorted by the particular response criterion that the subject had at a given time. Without such a theory, it may be impossible to determine if an experimental manipulation has influenced accuracy or response bias, or both. So determining which theory can accurately account for ROCs has serious implications not only for theory development, but for measuring recognition memory performance itself.

The shape of an ROC is typically quantified by plotting the z-score (i.e., the inverse of the standard cumulative normal distribution, which assumes a mean of 0 and a standard deviation of 1) of each hit and false alarm rate to produce a zROC (see **Figure 1(d)**). The theoretical motivation for doing so is grounded in signal detection theory as discussed in detail in the sections that follow. Standard linear regression is used to estimate the slope and intercept of the function. If the zROC is linear, then the y-intercept is a rough estimate of recognition accuracy and the slope is an index of the asymmetry of the ROC in probability space. For example, the ROC in **Figure 1(c)** is asymmetrical along the diagonal (it is pushed up on the left side); the slope of the zROC on the right indicates how asymmetrical the ROC on the left is. If the ROC were perfectly symmetrical around the diagonal, the slope of the zROC would be 1.0, but when it is asymmetrical in the way shown in **Figure 1(c)**, the z-slope is less than 1.0. If it were asymmetrical in the other direction it would have a z-slope greater than 1.0, but this is almost never observed in recognition studies. Generally then, the greater the deviation of the slope in z-space from 1.0, the more asymmetrical the ROC is.

Although there are some very stark differences between some of the models reviewed here, and especially between the different classes of models,

there are also a few very basic assumptions that they all share. They all assume that recognition decisions require an evaluation of some kind of mnemonic evidence (i.e., memory strength, which may comprise various underlying components depending on the model) that varies for both target and lure items. Thus, all the models are depicted in terms of their probability density distributions of memory strength for target and lure items, such as those shown in **Figures 1(a)** and **1(b)** (which show an unequal-variance signal detection model). The x-axis always represents memory strength (or 'evidence,' more generally), and there will always be at least two probability distributions along that axis corresponding to the lure and target items (although sometimes more axes are introduced, and not all distributions necessarily lie along the same axes).

The models we review in this chapter all make predictions about the shape of zROCs. Therefore, the shape of zROCs found in different types of recognition tests serve as the real tests of the models we consider – that is, the primary question is whether a model is capable of predicting (or at least explaining) the shapes that ROCs and zROCs take on under the experimental conditions devised thus far. First, for each class of models, we review the most prominent models to date and their ROC/zROC predictions, with an eye toward understanding why the models make the predictions that they do. After describing the models and their predictions in each class, we then briefly review the empirical ROC and zROC findings, indicating which models have been best supported by data thus far.

2.23.2 Evaluating Theories of Recognition

Theories of recognition fall into three general classes: threshold, signal detection, and hybrid models (i.e., models including both threshold and signal detection assumptions). In the following sections, we describe the core assumptions of these theories, focusing on the most frequently used models within each class, and evaluating their ability to account for recognition data.

2.23.3 Threshold Models

Threshold theories are one of the simplest classes of recognition memory models, and they motivate the common practice of estimating memory accuracy by subtracting false alarms from hits. Although their origins are obscure, they can be traced back to the psychophysical work of Fechner (see Boring, 1929), in which it was proposed that there is some minimum sensory signal strength (i.e., the 'threshold' or 'limina') that must be attained before a subject is able to perceive a stimulus. Threshold models treat memory as probabilistic in the sense that only some proportion of the items will exceed the threshold. Thus, for all threshold models, memory is described essentially in terms of success and failure; there is some probability with which memory will succeed (strength exceeds the threshold) and some probability with which memory will fail (strength falls below the threshold).

2.23.3.1 High-Threshold Model

The high-threshold model (HT) is perhaps the simplest threshold model (see **Figure 2**) and is the one that is assumed when subtracting false alarms from hits to measure accurate recognition performance. It assumes distributions of old and new items, with old items falling farther to the right along the strength axis than new items. Note that the distributions for HT models are often represented as rectangular for the sake of simplicity, but they can actually take on various different shapes (see Macmillan and Creelman, 2005). The threshold is the point at which the old item distribution exceeds the new item distribution. The new item distribution falls below the threshold, suggesting that new items are never truly remembered in the same sense that old items are. The proportion of old items above the threshold is the probability R_T. Memory decisions are made by selecting some level of memory strength as a response criterion and accepting items that exceed that level of strength as having been studied. If the response criterion is set exactly at the threshold, then the hit rate will be equal to R_T, and the false alarm rate will be zero. For any given experimental condition the threshold is fixed, but the response criterion is free to vary in either direction of the threshold so the hit rate may exceed R_T (with a lax criterion) or may be less than R_T (with a strict criterion).

Critically, as the response criterion is relaxed (i.e., shifted to the left of the threshold), the hit rate and false alarm rate will increase at a constant rate until they reach 1.00, producing a linear ROC like that seen in **Figure 2**. When a linear ROC is plotted on z-coordinates, the resulting zROC is actually U-shaped. Thus, assessing the linearity of empirical ROCs and z-ROCs provides a direct test of this

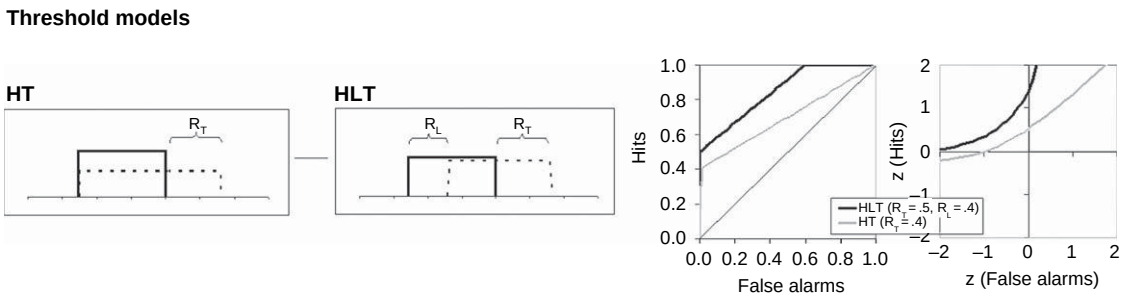

Figure 2 Strength distributions and predicted ROCs and zROCs for the high threshold (HT) and high-low threshold (HLT) memory models. Predicted ROCs on the left are plotted in probability space, and the ROCs on the right are plotted in z-space. Adapted from Yonelinas AP and Parks CM (2007) Receiving operator characteristics (ROCs) in recognition memory: A review. *Psychol. Bull.* 133: 800–832, with permission.

threshold model. Moreover, the model predicts that as performance increases the ROC should become more asymmetrical. That is, as memory increases, the left y-intercept will increase, and thus the slope of the ROC (and the z-ROC) will decrease.

2.23.3.2 High-Low Threshold Model

The high-low threshold (HLT) model is another common threshold model (sometimes referred to as the 'two-high threshold' or the 'double-high threshold' model), in which a second memory component is added to represent the probability that new, or lure, items can be recognized as new (R_L, e.g., "I would have remembered my name if it had been in the study list"). The HLT model is identical to the HT model except that the new item distribution extends further to the left than the old item distribution, creating a second threshold, which old, or target, items cannot cross (see **Figure 2**). The low threshold falls at the left-most point of the old item distribution, such that any new item falling below this threshold can be recognized as a new, or lure, item. As with the other threshold, the fact that old items cannot cross the low threshold suggests that they cannot be recognized as new in the same sense that truly new items can be. Although there are two thresholds, a single criterion is used in a typical old/new recognition experiment, just as it would be in the HT model (and in an ROC experiment, there would be the usual n–1 criteria for an n-point scale).

The HLT model generates ROCs very similar to those generated by the HT model, except that the right end of the ROC moves up and intersects the upper x-axis at a point that is R_L from the 1,1 intercept (see **Figure 2**). Thus, like the HT model, the HLT model predicts linear ROCs that are U-shaped in z-space, but it is not constrained to generate an asymmetrical ROC. In fact, the degree of ROC asymmetry can vary independently of overall performance. If R_T is greater than R_L, then the ROC will have a slope less than 1; if R_T is less than R_L, then it will have a slope greater than 1; and if the two parameters are equal, then the ROC slope will be equal to 1 (i.e., a symmetrical ROC).

Although the HT and HLT models predict linear ROCs, it is possible to produce various nonlinear ROCs by introducing additional parameters or thresholds. For example, Luce (1963) proposed a 'two-state high-threshold' model that produces an ROC made up of two joined linear segments (also see Green, 1960; Norman and Wickelgren, 1965). In fact, by adding multiple steplike thresholds or decision rules, one can produce an ROC that is effectively curvilinear (e.g., Krantz, 1969; Buchner et al., 1995; Malmberg, 2002). However, the latter approach is rarely adopted because it requires an additional free parameter for each new threshold (Hilford et al., 2002). Nonetheless, a consideration of these models is important because it shows that, although ROC experiments may prove useful in testing specific threshold models, results that disconfirm one threshold model may not be problematic for another. Here, we focus on the HT and HLT models because they are the two most commonly adopted threshold models.

There is one important caveat about using tests of linearity to assess the threshold models. Technically, the HT and HLT models (and models in which these theories are nested, such as some of the dual-process models discussed later) predict ROCs that are kinked at their extremes. That is, for both models, the ROC is linear until it intersects the y-axis, at which point it is forced to drop and approach the 0,0 intercept (see **Figure 2**). This occurs when the response criterion

moves to the right of the threshold (the high one for HLT), so that false alarms are at zero and the hit rate drops as the criterion becomes more and more strict (i.e., moves further to the right). For the HLT model, the same will also occur as the response criterion becomes very lax (i.e., moves to the left) – that is, the hit rate will reach 1.0 before the false alarm rate, and the false alarms will continue to increase until they reach 1.0 as well. This means that, if the extreme points on an ROC approach floor or ceiling levels, the ROC might appear curvilinear, even if it is perfectly predicted by a threshold model. Thus, when assessing threshold-based models it is important to determine if the extreme points in the average ROC, as well as the individual subject ROCs, are approaching floor or ceiling levels. In these cases, an evaluation of ROC linearity may not provide a valid assessment of threshold models.

Threshold theory, as a general class of models, has one core assumption: there is a sensory limit or threshold. With respect to memory, this means that, although there is a strength continuum, memory can fail. However, additional assumptions have sometimes been adopted by different theorists. Although these auxiliary assumptions do not always alter the predictions that the models make, in some cases they do. One very common assumption is that only old items can exceed the high threshold. This assumption is made in both the HT and HLT models (see **Figure 2**). Thus, the models assume that only items that have been studied can exceed the upper threshold, and that false alarms arise because the response criterion is set to the left of the threshold (likewise, only lures can cross the low threshold in the HLT model). In contrast, however, Luce's (1963) threshold model allows a portion of the new item distribution to exceed the high threshold, and thus it allows for the possibility that false alarms might arise even when the response criterion falls to the right of the threshold.

Second, it is sometimes assumed that the threshold marks a discrete boundary between conscious and nonconscious memory. For example, Fechner suggested that items falling below the threshold are not consciously perceived (i.e., they are subthreshold or subliminal) (as discussed in Boring, 1929). This assumption leads to the expectation that asking subjects to report when they are truly remembering a test item, or if they know it was studied on the basis of familiarity, such as in Tulving's (1985) remember/know (RK) procedure, might provide a way of determining the subject's memory threshold (e.g., Yonelinas and Jacoby, 1995). Thus, the R_T parameter (i.e., the left y-intercept of the ROC) might correspond to the proportion of remembered items. Alternatively, the threshold may correspond to the distinction between know and new responses, if both remember and know responses are treated as forms of conscious memory. In any case, the consciousness assumption has not been widely accepted, and many discussions of threshold theory – particularly those that appeared during the 1960s and 1970s – make no direct mention of conscious experience. Thus, even if a threshold does exist, subjects may not be able to determine exactly what level of memory strength corresponds to this threshold.

Third, it is sometimes assumed that memory strength is all-or-none in the sense that items are either in a discrete and homogeneous 'remembered' state, or they are in a distinct and mutually exclusive 'not remembered' state. Indeed, the typical equations of threshold theory represent memory as a simple probability, which is sometimes interpreted as indicating that all remembered items must be alike because there is no representation of the variance in memory strength. The implication of this assumption is that subjects either remember everything about an event or they remember nothing about the event. If this were true, though, it is not clear how subjects could make meaningful confidence judgments. For instance, if threshold theory is a state theory (the idea the there are mutually exclusive, discrete, homogeneous states of memory), the depiction of threshold theory in **Figure 2** and its description here would be misleading. In the figure, the x-axis would not represent strength, or if it did, then there would be no true distribution of items along that axis. Moreover, memory decisions would not be made by setting criteria based on different strengths, as different strengths would not exist. Instead, subjects would be forced into the awkward situation of randomly responding with different levels of confidence despite their experience of a uniform level of familiarity (and hence confidence) for all remembered items. As Wickelgren put it:

> If a subject truly had only two states in his [memory] system and were faced with the problem of choosing one of six rating responses, the subject would either think the experiment or the experimenter was pretty stupid, or else that there was something wrong with him or his understanding of the task. In either case, it is not clear what his decision rule would be. (Wickelgren, 1968: 129; but see Krantz, 1969, for an alternative view)

Thus, if threshold theory is indeed a state theory, it may not be useful to use confidence-based ROCs as a means of testing it. However, various theorists, including Fechner, have argued that sensation strength can vary continuously and be subject to a threshold (e.g., Fechner as described in Boring, 1929; Swets, 1961; Krantz, 1969), and confidence-based ROCs have been used extensively to test the threshold models (but see Malmberg, 2002, for an argument concerning the usefulness of ROCs in deciding between continuous and state theories). So although the all-or-none assumption is sometimes adopted, it is not a necessary assumption of threshold theory in general.

Fourth, threshold theory is sometimes interpreted as indicating that there is a non-mnemonic process such as 'random guessing' or 'noise' that contributes to performance in addition to a true memory retrieval process (e.g., Batchelder and Riefer, 1990). The idea is that if an item's strength exceeds the threshold, then memory is successful and the item will be remembered, whereas if its strength falls below the threshold, then memory fails and the item will not be remembered. In the latter case, the item might still be accepted as old on the basis of some non-mnemonic process such as a guess or a response error. Although it seems reasonable that subjects might guess or make response errors, the proposal that these errors are due solely to such non-mnemonic processes is not a necessary assumption of threshold theory in general. That is, all responses could be based on memory strength, but because the old and new item distributions can be completely overlapping at points below the threshold, accepting items below the threshold becomes functionally equivalent to guessing or random responding.

2.23.3.3 Evaluation

The threshold models provide a poor account of recognition memory. This evaluation is based on their predictions of linear ROCs and U-shaped zROCs. First, in tests of item recognition there is no evidence for the threshold that serves as the core of threshold theory. That is, item ROCs are not linear as predicted by the models, but are instead curvilinear and have an inverted U shape. This pattern was first reported by Egan (1958) (see **Figure 3**) and has now been observed in countless experiments (for earlier discussions of this finding see Egan, 1958; Murdock, 1974; Ratcliff et al., 1992, 1994; Glanzer et al., 1999). Moreover, item recognition ROCs are linear when plotted in z-space, in contrast to the U-shaped zROCs predicted by the threshold models. Although slight deviations from linearity in z-space have been reported in item recognition studies (e.g., Ratcliff et al., 1994; Yonelinas, 1997, 1999a; Heathcote, 2003), the zROCs are almost never as U-shaped as the threshold models predict.

There are, however, some aspects of the ROC data that are consistent with the threshold notion. For example, the HT model predicts that as performance increases the ROCs should become more asymmetrical (z-slopes should drop), and there are variables, such as levels of processing and list length, that do result in this pattern (see **Figure 4**; e.g., Yonelinas et al., 1996; Glanzer et al., 1999). However, other manipulations,

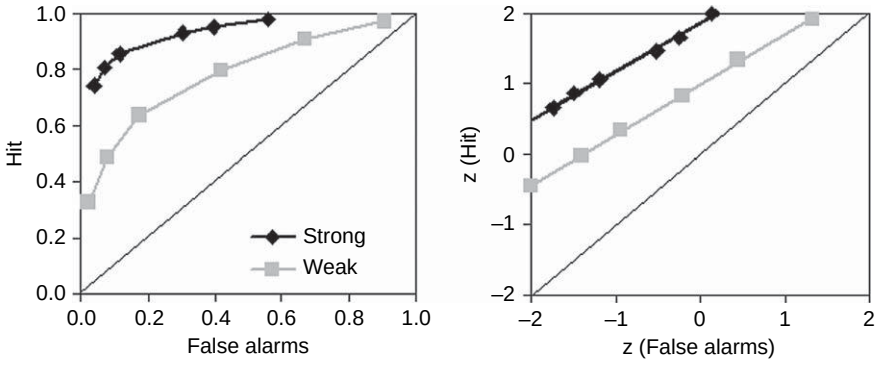

Figure 3 Item recognition ROCs for weak (one presentation) and strong (two presentations) items plotted in probability space (on the left) and in z-space (on the right). The figure illustrates that item ROCs are (a) curved downward in probability space, (b) linear in z-space, and (c) asymmetrical along the chance diagonal and thus (d) they have a slope in z-space of less than 1.0. Further, the slopes of the two zROCs are the same, but sensitivity differs between conditions. From Experiment 1 (Figure 20) in Egan JP (1958) Recognition memory and the operating characteristic. *USAF Operational Applications Laboratory Technical Note.* No. 58–51: 32. Hearing and Communication Laboratory, Indiana University.

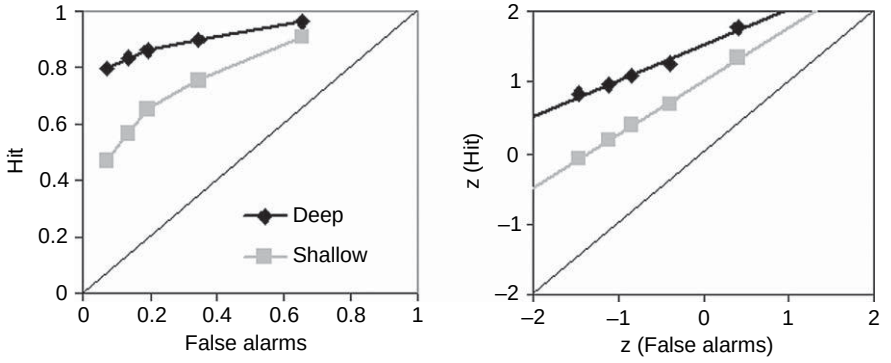

Figure 4 Item recognition ROCs and z-ROCs for items encoded deeply or shallowly. Both the z-slope and sensitivity differ between conditions. From Experiment 1, Yonelinas AP, Dobbins I, Szymanski MD, Dhaliwal HS, and King L (1996) Signal-detection, threshold, and dual-process models of recognition memory: ROCs and conscious recollection. *Conscious. Cogn.* 5: 418–441.

such as study repetition, increase performance but do not affect symmetry (e.g., Glanzer et al., 1999), which presents problems for the HT model. However, the HLT model, which includes two thresholds and so can vary the degree of asymmetry independently of overall performance, is consistent with these aspects of the recognition results.

In addition, linear ROCs have been reported in several studies of relational recognition tasks, such as test of source or associative recognition (e.g., Glanzer et al., 1999; Yonelinas, 1999a; Rotello et al., 2000; Arndt and Reder, 2002). These linear ROCs are consistent with the threshold predictions and suggest that, at least in relational tests, subjects do sometimes fail to retrieve the relevant information altogether (i.e., items fall below threshold). In direct contradiction to threshold theory predictions, though, curved ROCs have also been reported in other relational recognition experiments (e.g., Yonelinas, 1999a; Qin et al., 2001), and therefore these models do not provide an adequate account of relational recognition performance either.

There are various other ROC results discussed in the following sections that present additional problems for the threshold models, but the item and relational ROC results noted here have led most researchers to reject the threshold models as viable accounts of recognition memory.

2.23.4 Signal Detection Models

Signal detection theory (e.g., Tanner and Swets, 1954; Swets et al., 1961) is a statistical decision model that has been applied to studies of item recognition (e.g., Egan, 1958; Murdock, 1965; Parks, 1966; Banks, 1970) and source recognition (e.g., Marsh and Bower, 1993; Hoffman, 1997). In item recognition tasks, it is assumed that studied items have greater memory strength than nonstudied items, but there is variability in memory strength such that the old and new items form overlapping Gaussian (or normal) distributions as in **Figure 5**. The distance between the old and new distributions measured in z-scores is d', which represents how much stronger the studied items are than the new items. Recognition decisions in standard old/new tests are made by setting a response criterion equal to some level of memory strength and responding 'old' only to items exceeding that criterion; in confidence-rating tests, decisions are made by placing n–1 criteria along the memory strength axis for an n-point confidence scale.

The core assumption that all pure signal detection models share is that the strength distributions of old and new items are normal (or Gaussian) in shape. The Gaussian shape of these distributions gives rise to curved ROCs that are perfectly linear when plotted in z-space, and thus the linearity of empirical zROCs can be used to test this assumption (see **Figure 5**). The reason Gaussian distributions produce curvilinear ROCs is because changes in response criteria result in disproportional changes in the hits and false alarms. When the response criterion starts off as strict (very far to the right), changes (moving to the left) will have large effects on the proportion of the old item distribution that will be recognized, but relatively modest effects on the proportion of the new item distribution that will be recognized. In contrast, as the response criterion

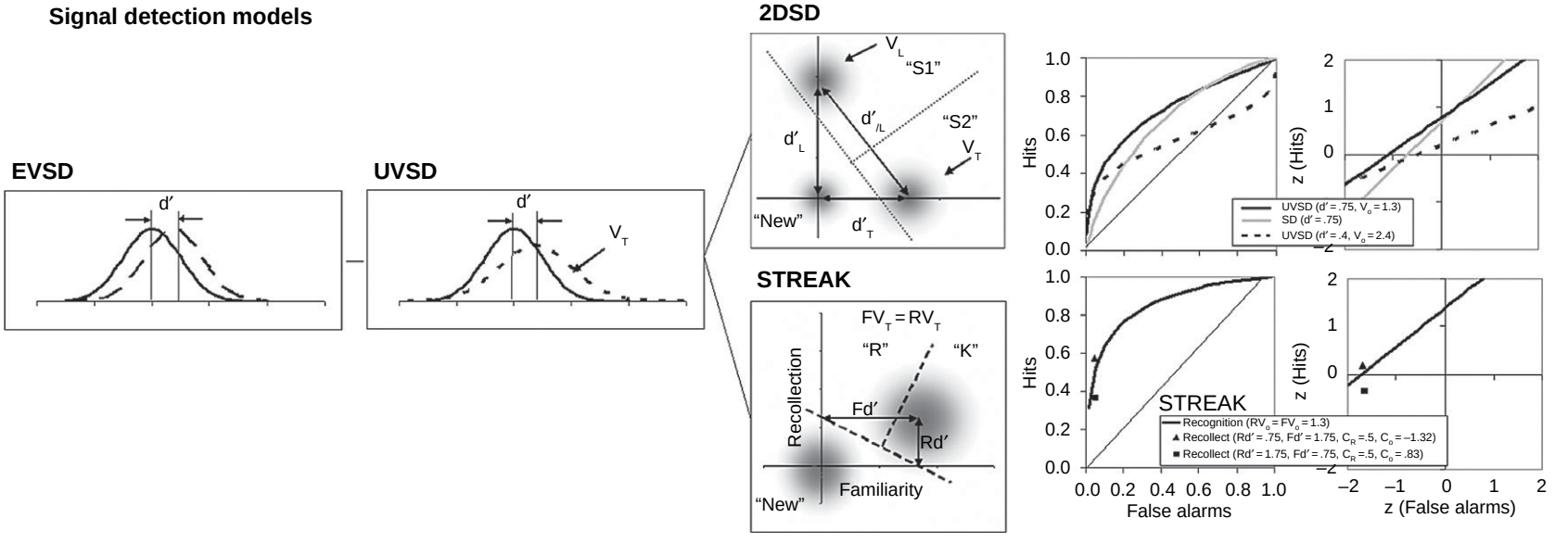

Figure 5 Strength distributions and predicted ROCs and zROCs for the signal detection models, including the equal-variance signal detection (EVSD), unequal-variance signal detection (UVSD), two-dimensional signal detection (2DSD), and sum-difference theory of remembering and knowing (STREAK) models. Predicted ROCs on the left are plotted in probability space and the ROCs on the right are plotted in z-space. Adapted from Yonelinas AP and Parks CM (2007) Receiving operator characteristics (ROCs) in recognition memory: A review. *Psychol. Bull.* 133: 800–832, with permission.

becomes more and more lax, changes will have larger effects on the proportion of new items that are recognized and decreasing effects on the proportion of old items that are recognized. Thus, the slope of the ROCs starts off very steep, but then starts to decrease and eventually levels out as the false alarm rate increases, leading to a curvilinear ROC.

The primary theoretical difference between the signal detection models and the threshold models is that the signal detection models assume that memory can never truly fail. That is, signal detection models assume that there is a memory signal that always provides some useful information. Therefore, signal detection models eschew the idea that memory is probabilistic (i.e., it succeeds or fails) and instead treat memory as deterministic, meaning that there is a memory signal for every item encountered – or essentially, that memory is always successful, even if it does not always return a strength value that leads to an accurate response.

2.23.4.1 Equal-Variance Signal Detection Model

The equal-variance signal detection (EVSD) model is the simplest signal detection model (see **Figure 5**), because it includes only one parameter – the accuracy measure d', the difference in average strength of the new and old item distributions. The model assumes that the variance of the targets' strengths is equal to that of the lures. Because the old and new distributions are assumed to have the same shape, the model generates a symmetrical ROC that has a slope of 1.0 in z-space. Thus, assessing the slope of the zROC provides a direct test of the equal-variance assumption.

Although the core assumptions underlying the EVSD model are the Gaussian and equal-variance assumptions, a number of auxiliary assumptions have sometimes been adopted. For example, many theories have assumed that the memory signal reflects a continuous scalar index of memory strength or familiarity (e.g., global memory models such as TODAM (Murdock, 1993) and SAM (Gillund and Shiffrin, 1984)). Alternatively, the memory signal could also reflect how many different aspects or features of the test item are remembered, or it may reflect the products of two or more separate memory processes such as recollection and familiarity (e.g., Johnson et al., 1993; Wixted and Stretch, 2004; Wixted, 2007). Another assumption, which is often adopted in order to account for subjective reports of remembering and knowing (Tulving, 1985), is that remember responses simply reflect stronger or more confident memories than know responses (e.g., Donaldson, 1996; Hirshman and Master, 1997; Dunn, 2004). Thus, when RK scores are plotted in ROC space they should fall along the same symmetrical ROC that is expected in item recognition, with the remember point (i.e., the proportion of correct remember responses vs. the proportion of incorrect remember responses) falling to the left of the recognition point (i.e., remember plus know responses).

2.23.4.2 Unequal-Variance Signal Detection Model

The unequal-variance signal detection model (UVSD) is probably the most common signal detection model of memory (see **Figure 5**), and it includes a distance measure between the means of the two distributions (similar to d'; see Wickens, 2002; Macmillan and Creelman, 2005, for details on accuracy measures for the unequal-variance model), as well as a second component: the variance of the old item distribution relative to the new item distribution (V_T). If the old item variance is greater than that of the new distribution, then the ROC will appear to be pushed up on the left side, as in **Figure 5**. If the old item variance is less than that of the new item distribution, the ROC will be pushed up on the right side (this is not illustrated, and it is rarely observed). Like the EVSD model, the UVSD model predicts curved ROCs that are linear when plotted in z-space. However, because the old item variance can vary, the model can produce asymmetrical ROCs (i.e., slopes in z-space greater or less than 1.0) – thus the variance parameter can also be thought of as a symmetry parameter, because it is the V_T parameter that makes an ROC symmetrical or asymmetrical. The inclusion of separate parameters for sensitivity and symmetry also suggests that the two memory components might be experimentally dissociable. That is, there may be variables that influence d' while leaving V_T unaffected, whereas other variables might influence V_T while leaving d' unaffected. However, the UVSD model does not indicate which experimental variables might produce such dissociations.

One property of the UVSD model that is often overlooked (although see Green and Swets, 1966; Decarlo, 2002) is that, if the variance of the old item distribution is greater than that of the new item distribution, the model can predict a curved ROC that drops below the chance diagonal (see **Figure 5**).

This will happen when the old item variance becomes large, and some portion of the old distribution falls farther to the left than the new distribution. Psychologically, this means that the encoding phase must have decreased, rather than increased, the memory strength of some of the studied items.

The UVSD model does not specify why the old and new variances differ, or why the old item variance almost always exceeds the new item variance (see the section titled 'Evaluation' that follows), but one common assumption is that the old distribution is more variable because of encoding variability (e.g., Hilford et al., 2002; Wixted, 2007). That is, because encoding will likely increase the strength of some items more than others, the old item distribution will be more variable than the new item distribution. Such an account predicts that the ROCs should be asymmetrical such that the z-slopes are less than 1, rather than being equal to or greater than 1. Although the encoding variability explanation seems intuitively logical and, therefore, a potentially good account of increased old-item variability, it is not technically consistent with the UVSD model. The encoding variability hypothesis describes differences in how much items increase in memory strength as a result of study, but, quite logically, does not allow for decreases. Thus, encoding variability *per se* does not lead to the expectation that the ROC should drop below the chance diagonal, as will happen whenever the old item variance exceeds the new item variance (although this is most evident when the slope gets very low).

As with the EVSD model, one can potentially explain RK reports by assuming that remember responses simply reflect high-confidence recognition responses (e.g., Donaldson, 1996; Hirshman and Master, 1997; Wixted and Stretch, 2004). The UVSD model accordingly predicts that the RK data should fall on the same function that is observed in recognition ROC studies. In contrast to the EVSD model, however, the recognition ROC can be asymmetrical.

2.23.4.3 Two-Dimensional Signal Detection Model

The two-dimensional signal detection model (2DSD) is an extension of the UVSD model that is aimed at explaining performance on source recognition tests (Hilford et al., 2002; Glanzer et al., 2004; for earlier development of multidimensional signal detection models see Tanner, 1956; Macmillan and Creeman, 1991; Ashby, 1992; Banks, 2000). In this model, there are two memory strength axes or dimensions (hence the name two-dimensional), one for each source. Therefore the distribution of strengths for items presented in Source A lies along the Source A dimension, and the strength distribution for items presented in Source B lies along the Source B dimension (see **Figure 5**). New items in the recognition test lie at the intersection of these two dimensions, and therefore there is a triangular relationship between the three distributions. The model assumes that studying items in one source will increase the items' strength along that source axis in the same way that study increases average strength in the EVSD and UVSD models (e.g., study of items in Source A will push the distribution up the Source A dimension). Note that the depiction of the model in **Figure 5** has transformed a model that exists in three dimensions (i.e., in the x, y, and z planes) into a two-dimensional illustration of the model viewed from the top. The 2DSD illustration **Figure 5** is similar to a topographical map because it is assumed that one is looking down at the model. There is an unseen axis (the y-axis) for the height of the distributions that would rise from the intersection of the two illustrated axes, and the circles actually represent three-dimensional Gaussian distributions (see Wickens, 2002; Macmillan and Creelman, 2005, for good introductions to multidimensional signal detection models). Although it is not illustrated, there is actually some overlap between the distributions lying along the two axes, as well as some overlap with the new item distribution.

The 2DSD model includes five free parameters – the strength and variance of each of the old item distributions relative to the new item distribution (with items from one source arbitrarily treated as targets and items from the other as the lures: target strength (d'_T), target variance (V_T), lure strength (d'_L), and lure variance (V_L)), as well as the distance between the two source distributions ($d'_{T/L}$). The target strength and lure strength refer to the distance between each source distribution and the new item distribution, thereby providing measures of item recognition for items from each source. The distance between the two source distributions is the measure of source recognition performance. Although the two source dimensions are presented as orthogonal in **Figure 5**, the angle is generally expected to be much less than 90° to account for the observation that people are more accurate in item than in source recognition (i.e., the distance between the two source distributions is typically shorter than between either

of those distributions and the new item distribution). In general, there are not enough data points available in ROC studies to allow the degree of this angle to be estimated, but it could in principle be allowed to vary as a means of measuring the relationship between source and item performance; however, this would require a novel source memory paradigm (e.g., Banks, 2000).

The 2DSD model assumes that people make item recognition decisions by setting a criterion that runs between the new item distribution and both of the old distributions (the diagonal line that runs from the upper left to the lower right in the 2DSD illustration in **Figure 5**). Source recognition decisions are made by setting another criterion that runs perpendicular to the item criterion and runs between the two old source distributions (the diagonal running from the upper right to the lower left).

In addition to the core assumptions of the UVSD model, the 2DSD model makes two further assumptions, one of which is simply that there are two dimensions of strength, one for each source. In addition, the model assumes that item and source judgments are based on the same underlying strength distributions and therefore predicts that performance on these tasks should be directly related. That is, manipulations that increase source recognition will also necessarily increase item recognition. Note, however, that the model does not predict exactly how closely item and source recognition will be related – that relationship will depend on the angle between the two source dimensions and the types of source information that the subject brings to bear when making the item discrimination.

The 2DSD model makes the same ROC predictions as the UVSD model. That is, because the model is based on Gaussian strength distributions, it predicts that ROCs (both item and source) should be curved in probability-space and linear in z-space. Because the model includes free parameters for the variance of the old item distributions, it can produce item recognition z-ROCs with slopes less than 1, and it can produce dissociations between ROC accuracy and asymmetry.

2.23.4.4 Sum-Difference Theory of Remembering and Knowing

The sum-difference theory of remembering and knowing (STREAK) is another two-dimensional extension of the UVSD model (see **Figure 5**; Rotello et al., 2004). The STREAK model was proposed to account for RK and item recognition ROC results. In a typical RK paradigm including remember, know, and new responses, an ROC can be constructed from two points: the remember hit and false alarm pair (which will be the lower left point on the ROC) and the remember + know hit and false alarm pair (also referred to as the 'recognition' point because it includes all recognized items). Adding in the 'new' responses would result in a point constrained to be (1,1). This is the same technique used to construct ROCs from confidence scales. The STREAK model assumes that one dimension of memory strength represents global familiarity and the other dimension represents the memory strength associated with recollection of specific details associated with an item. Every item, old or new, is assumed to have both recollection and familiarity strengths (indexed as Rd' and Fd', respectively), but the old items are expected to have higher strengths than the new items, on average. There are two distributions, corresponding to old and new items. New items lie at the intersection of the familiarity and recollection axes, and the old item distribution can occupy any space between the two axes (technically it can move beyond those axes as well, but that would indicate negative memory along one or both dimensions). Strength on each dimension is represented by the distance of the peak of the distribution from the axes. Thus, recollection strength is the distance of the peak of the distribution (the center of the circle in the STREAK illustration in **Figure 5**) from the recollection axis, and familiarity strength is the distance from the familiarity axis. The old item distribution is assumed to have greater variance than the new item distribution. In fact, in order for the model to be identifiable in standard RK experiments the new item variance is set at 80% of the old item variance (this approximates z-slopes of .80, the average slope found in item recognition). In confidence-based ROC studies, though, or studies in which both RK and confidence responses are collected, the old item variance is treated as a free parameter (V_T). Overall, then, the model has either two or three parameters, depending on whether V_T is allowed to vary or not.

STREAK assumes that people make item recognition decisions by setting a response criterion between the new and old item distributions that runs parallel to the line that would intersect the Rd' and Fd' values on their respective axes (this criterion is shown actually intersecting those points in **Figure 5**). RK decisions are made by selecting a second response criterion, which runs perpendicular

to the old/new criterion, that is used to determine if the item is more remembered or more familiar. Although not evident in the illustration of the model, the sum of the recollection and familiarity strength values dictates whether a person makes an old or new response (if recollection + familiarity > old/new criterion, 'old'), whereas the difference between the two strength values is used to make the RK response (if recollection − familiarity > RK criterion, 'remember'). Conceptually, STREAK treats standard item recognition in the same way as the UVSD model, but with 'memory strength' interpreted as the sum of recollection and familiarity strengths (see also Wixted, 2007, for a similar assumption in a UVSD model). In addition, the STREAK model assumes that recollection and familiarity lie along a single continuum and that people simply select a criterion between the two extremes in order to respond in RK experiments. This model differs markedly from the other signal detection models in that it explicitly assumes that two processes underlie recognition memory and produce the components that make up 'memory strength.'

Because the model is based on Gaussian strength distributions, it predicts curved item ROCs that are linear in z-space, like the UVSD model. If the V_T parameter is fixed at .80 (the new-to-old item variance ratio), it predicts asymmetrical ROCs with z-slopes of .80. However, if V_T is treated as a free parameter, then the item recognition process becomes identical to that of the UVSD model. The unique aspect of this model, however, is that because RK judgments are based on different decision rules than old/new judgments, the model can produce RK z-slopes that differ from the ROC z-slopes. That is, the model can produce a remember ROC point that can fall below, above, or along the regular item ROC, whereas the remember + know point has to fall exactly on the confidence ROC because it corresponds to all the items exceeding the old/new response criterion (see **Figure 5**). In this way, the RK-slope (i.e., the line joining the remember point to the recognition point to the right) can be greater, less than, or equal to that of the ROC slope.

2.23.4.5 Evaluation

The core assumption of all the signal detection models (i.e., the Gaussian assumption) is generally supported in tests of item recognition; that is, item ROCs have an inverted U shape in probability space and are approximately linear in z-space (e.g., **Figures 3** and **4**), a pattern that has been demonstrated repeatedly over the course of approximately 40 years. The EVSD model fails to account for the fact that z-slopes are less than 1 in item recognition, but all other signal detection models (which allow variance to differ between the targets and lures) account for this finding easily.

However, the results of relational recognition experiments present a challenge to all the signal detection models. ROCs in relational recognition tests can be either linear or curvilinear in probability space, but unlike item recognition, they are almost always U-shaped in z-space. For example, **Figure 6** presents associative ROCs for word pairs along with item ROCs for single words (Experiment 3 from Yonelinas, 1997). The item ROC is significantly concave in probability space and does not differ significantly from linear in z-space. Conversely, the

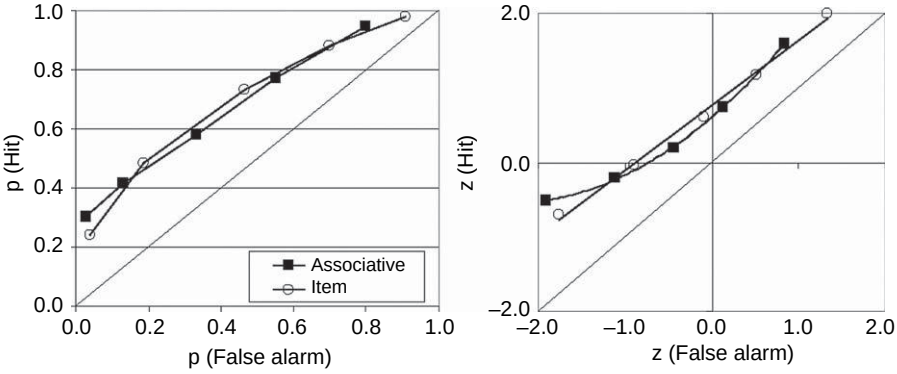

Figure 6 Item and associative recognition ROCs. The item ROC is concave in probability space and linear in z-space, whereas, the associative ROC is linear in probability space and convex in z-space. From Experiment 3 in Yonelinas AP (1997) Recognition memory ROCs for item and associative information: The contribution of recollection and familiarity. *Mem. Cognit.* 25(6): 747–763.

associative ROC is linear in probability space and convex in z-space. Similar findings have been reported in other associative recognition studies (e.g., Yonelinas et al., 1999; Kelley and Wixted, 2001; Healy et al., 2005), in tests of source recognition (Yonelinas, 1999a; Slotnick et al., 2000; Hilford et al., 2002; Decarlo, 2003; Glanzer et al., 2004; Slotnick and Dodson, 2005), and in plurality reversed recognition tests (e.g., Rotello et al., 2000; Arndt and Reder, 2002). In fact, the regularity of the U-shaped zROCs across relational studies is quite striking. In 52 out of 59 conditions taken from 17 studies of relational recognition the quadratic coefficient was positive (Parks and Yonelinas, 2007). (The quadratic coefficient is the term of a polynomial regression equation that quantifies the degree of U-shaped curvature in a function. Positive quadratic coefficients indicate that the function is U-shaped, negative quadratic coefficients indicate that it has an inverted U shape, and 0 indicates that there is no U-shaped curvature.) Thus, the vast majority of the relational recognition studies have resulted in U-shaped zROCs. U-shaped zROCs directly contradict the Gaussian assumption of the signal detection models, and therefore indicate that they are unable to account for these data.

Although relational recognition presents a serious challenge to the signal detection models, the models are able to account for other types of recognition data, though of course some models perform better than others. The worst is the EVSD model, which can only account for inverted-U-shaped ROCs and fails to account for any other of the common findings in the recognition ROC literature. The other models, however, have had some success in accounting for some aspects of RK studies. The UVSD and 2DSD models can make the auxiliary assumption that RK responses are just confidence responses split into two categories, with 'remember' reflecting high and 'know' reflecting low confidence. Thus, the models predict that the remember and remember + know points should fall along the same ROC produced by confidence ratings. In fact, studies that have directly compared RK and confidence responses have shown that RK scores and confidence judgments typically fall along the same ROC. For example, **Figure 7** shows remember, and remember + know responses plotted along with confidence-based ROCs from the same subjects (the top function is from Experiment 1 of Wixted and Stretch (2004), and the bottom function is from Experiment 1 of Yonelinas et al. (1996)). As can be seen in **Figure 7**, the RK points fall along the same functions that fit the confidence

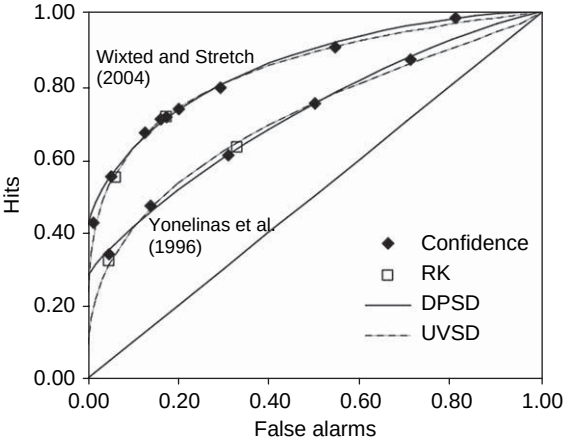

Figure 7 Confidence ROCs and RK ROCs from Wixted JT and Stretch V (2004) In defense of the signal detection interpretation of remember/know judgments. *Psychon. Bull. Rev.* 11: 616–641 and from Yonelinas AP, Dobbins I, Szymanski MD, Dhaliwal HS, and King L (1996) Signal-detection, threshold, and dual-process models of recognition memory: ROCs and conscious recollection. *Conscious. Cogn.* 5: 418–441. The figure illustrates that when subjects make RK and confidence responses to each test item, the RK ROC points fall along the same function as the confidence ROC. The figure presents the fits of the DPSD and UVSD models and indicates that both models fit the empirical data quite well.

ROCs. Wixted and Stretch (2004) reexamined several previous studies that had collected RK and confidence judgments and found that the z-slopes from the two procedures were similar in 15 out of 16 different experiments from six different published studies. Malmberg and Xu (2006) also found that RK and confidence ROCs were indistinguishable. Overall, direct comparisons of RK and ROC results indicate that, in a vast majority of cases, sensitivity (z-intercept) and asymmetry (z-slope) are similar for recognition confidence and RK judgments and, thus, generally support the predictions of the UVSD and 2DSD models. The convergence of RK and confidence ROCs is also consistent with the STREAK model, but because it predicts that the remember point can have virtually any relationship to the confidence ROC, this convergence is not particularly useful for evaluating STREAK.

Although the UVSD, 2DSD, and STREAK models can account for the convergence of RK and recognition ROCs, all but the STREAK model are challenged by another common RK finding. Specifically, RK and confidence ratings produce very similar ROCs when they are directly compared within experiments,

but in many other pure-RK experiments the z-slopes are much higher than would be expected from the item ROC literature. For example, Rotello et al. (2004) reviewed 373 published RK conditions and found that the average RK slope was close to 1.0, whereas item recognition slopes have an average around .80 (also see Dunn (2004) for a similar analysis), and in many cases the RK z-slopes were well above 1.0, something almost never seen in item recognition studies. Thus, the RK results are not always consistent with those from recognition ROC studies. Although the materials and test procedures may differ across the RK and ROC studies, thus complicating the comparison of the z-slopes, it is difficult to attribute the observed differences to this factor alone (see Wixted and Stretch, 2004).

To account for high RK z-slopes, Rotello et al. (2004) proposed the STREAK model, which can produce different slopes for confidence and RK studies, giving it an important advantage over the other signal detection models. However, a number of alternative measurement-artifact accounts have also been put forward to account for these results. For example, the RK z-slope would be artificially increased if subjects' criterion between remember and know responses varies over trials (Wixted and Stretch, 2004). Examining the z-slopes of the aggregate data (e.g., Rotello et al., 2004) rather than examining the subject-level z-slopes can also artificially increase the RK z-slope (Malmburg and Xu, 2006). Finally, Parks (2007) suggested that the remember responses might fall below the ROC if subjects adopt a strict definition of remembering such that only some aspects of the study event are treated as adequate to support a remember response. However, as of yet, none of these hypotheses have been extensively tested, and the reason for the high z-slopes remains somewhat of a mystery.

Other data that present some important challenges for the signal detection models are results from exclusion paradigms. In exclusion tests, subjects must reject or 'exclude' lures that are related in some way to the studied items or pairs. For example, on an associative test, subjects must reject rearranged pairs (e.g., 'magnet' – 'sheep,' after studying 'magnet' – 'phone' and 'corn' – 'sheep'); in conjunction tests they must reject compound words composed of previously studied words (e.g., 'blackbird,' after studying 'blackboard' and 'jailbird'); and in exclusion source tests, subjects must reject items from one source and accept only items from another source (e.g., accept only heard items as 'old' after studying both an auditory and a visual list). If unstudied items or pairs are included in the test list, then it is possible to plot the proportion of incorrectly accepted lures against the proportion of incorrectly accepted unstudied items or pairs, and this produces what we refer to as an 'exclusion ROC.' Like standard item ROCs, exclusion ROCs are curved downward, but they quickly approach the chance diagonal and in some cases pass below it. **Figure 8** presents representative exclusion ROCs from studies of source memory (Yonelinas, 1999a), word-pair recognition (Kelley and Wixted, 2001; Healy et al., 2005), and word-conjunction recognition (Lampinen et al., 2004). Each of the exclusion ROCs indicates that when subjects adopt a strict criterion they accept more lure items than new items (i.e., the ROC is above the chance diagonal), but as their criterion becomes more lax they are equally or more likely to accept a new item than a related lure item (i.e., the ROC approaches or goes below the chance diagonal). Curved, negative-going exclusion ROCs have been observed in several relational recognition experiments (Yonelinas, 1994; Kelley and Wixted, 2001; Lampinen et al., 2004; Healy et al., 2005), and the pattern appears fairly robust. Exclusion ROCs are linear in z-space, similar to item recognition, but their slopes range from .4 to .6 and, thus, appear to be considerably lower than those typically seen in item recognition tests.

The EVSD model cannot produce ROCs that cross the chance diagonal and, thus, fails once again, but the other signal detection models can account for the exclusion data. However, they do so by adopting rather questionable parameter values. For example, the UVSD model can often fit exclusion data well. However, in order to fit the conjunction data (Lampinen et al., 2004) in **Figure 8**, for example, the model parameters indicate that the study phase led to an average decrease in memory strength for the related lures ($d' = -1.20$), as well as a two- to threefold increase in the variance of the lures relative to the new items ($V_O = 2.71$). The fits of the UVSD model to the other exclusion ROCs are just as surprising. In the associative test (Kelley and Wixted, 2001), the model suggests that the study phase had virtually no effect on the average memory strength of the rearranged items (i.e., $d' = .096$), yet it almost doubled the variance of those items relative to the new pair distribution (i.e., $V_O = 1.83$), and a similar pattern was seen in the exclusion source test (i.e., $d' = 0.16$, $V_O = 1.95$; Yonelinas, 1994). These parameters indicate that the study phase increased the

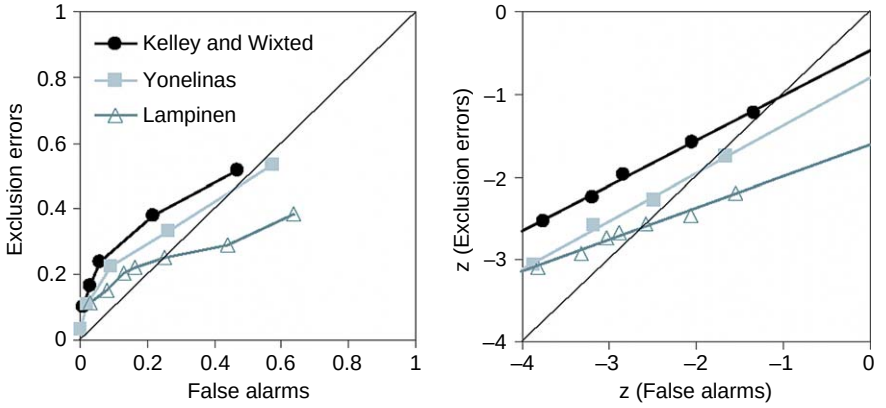

Figure 8 Exclusion ROCs (left), which plot the probability of incorrectly accepting a related lure against the probability of accepting a new item, for (1) word-pair recognition (strong item condition from Experiment 1 in Kelley R and Wixted JT (2001) On the nature of associative information in recognition memory. *J. Exp. Psychol. Learn. Mem. Cogn.* 27: 701–722); (2) source recognition (short list condition from Experiment 1 in Yonelinas AP (1994) Receiver-operating characteristics in recognition memory: Evidence for a dual-process model. *J. Exp. Psychol. Learn. Mem. Cogn.* 20: 1341–1354); and (3) conjunction recognition (the multiple presentation condition from Experiment 2 in Lampinen JM, Odegard TN, and Neuschatz JS (2004) Robust recollection rejection in the memory conjunction paradigm. *J. Exp. Psychol. Learn. Mem. Cogn.* 30: 332–342). The exclusion ROCs are concave and negative-going in probability space. The right panel depicts the same data in z-space and shows that the zROCs are linear.

strength of about half of the exclusion items and decreased the strength of the other half, yet did not alter the Gaussian shape of the distributions. These paradoxical conclusions suggest that the model is simply not appropriate for exclusion ROCs.

2.23.5 Hybrid Models

Various models have been proposed that combine the assumptions of signal detection theory and threshold theory. These models assume that a signal detection process is supplemented by either a recollection process or a probabilistic attention process. Generally, these models differ from the previous models in that they assume that both types of processes (a threshold process and a signal-detection process) contribute to recognition memory. Therefore, the following models all assume that some component of recognition memory is deterministic and always successful in some sense (like the signal detection models), whereas another component is probabilistic and therefore subject to failure (like the threshold models).

2.23.5.1 Dual-Process Signal Detection Model

The dual-process signal detection model (DPSD) (e.g., Yonelinas, 1994, 2001) integrates signal detection theory and threshold theory within a dual-process framework of recognition memory (e.g., Atkinson and Juola, 1974, Mandler, 1980; Jacoby, 1991). The DPSD model was the first hybrid model in the recognition memory literature, and thus it has been applied to the widest range of recognition paradigms so far. The model assumes that recognition memory judgments are based on a recollection process whereby qualitative information about the study event is retrieved (e.g., where or when an item was studied), or if recollection fails, recognition is based on a familiarity assessment process like that underlying the equal-variance signal detection model (see **Figure 9**). Thus, recollection and familiarity are assumed to be qualitatively different processes that yield different types of mnemonic evidence. All items are assumed to evoke a familiarity signal, but only some items will be recollected. As such, recollection is indexed as the probability that subjects recollect some aspect of the study event (R_T), whereas familiarity is indexed as the increase in familiarity related to the study phase (d'). It is assumed that subjects can recollect different types or amounts of information about a study event, but that recollection will sometimes fail and no qualitative information will be retrieved. Because it is subject to failure, recollection is described as a threshold process. Note that recollection is assumed to have a distribution of strength, but the model does not specify what kind of distribution it

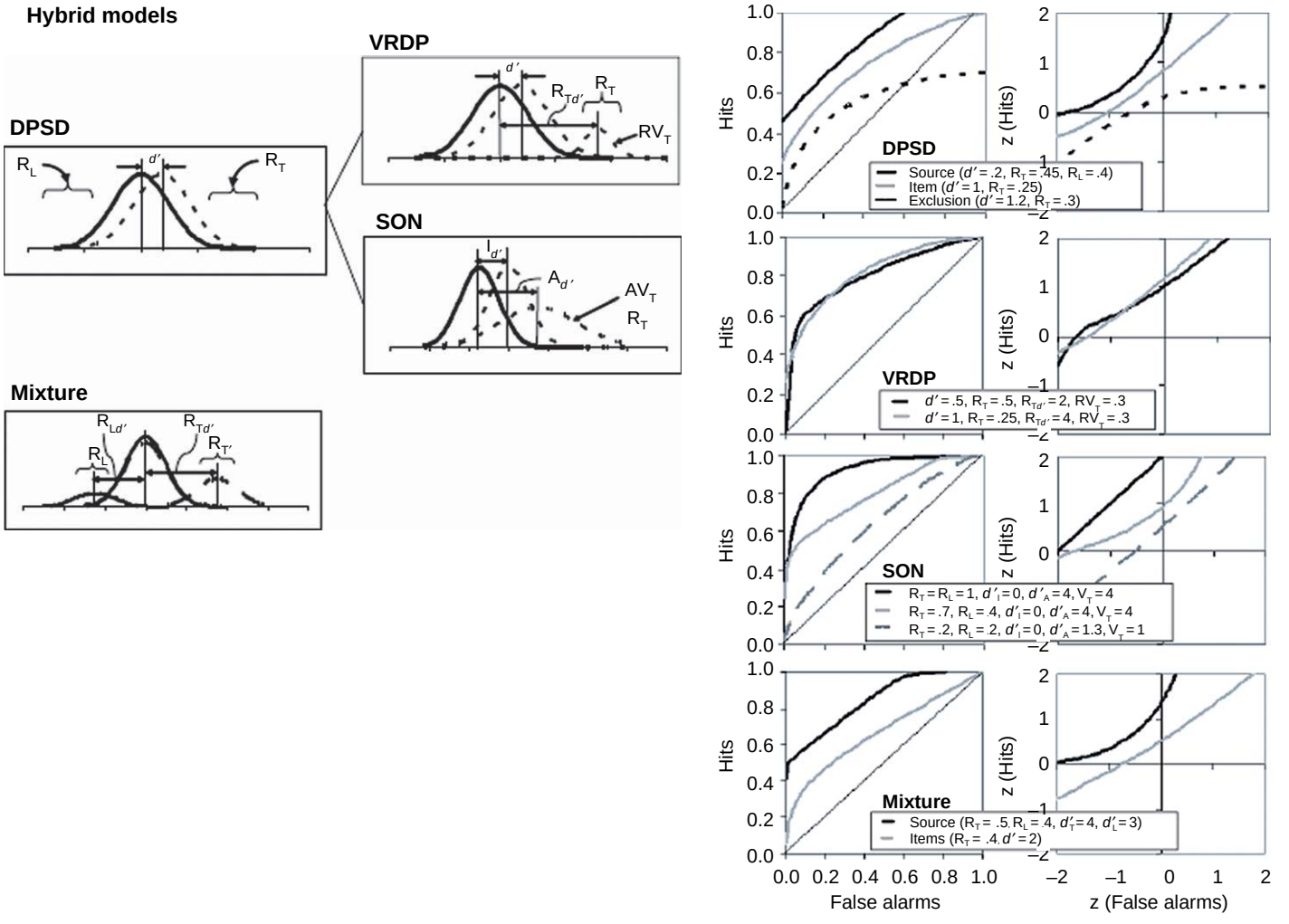

Figure 9 Strength distributions and predicted ROCs and zROCs for the hybrid memory models, including the dual-process signal detection (DPSD), variable-recollection dual-process (VRDP), some-or-none (SON), and mixture models.

is and thus measures recollection in the probability of success (there is an arrow labeled R_T pointing to an empty space in the depiction of DPSD in **Figure 9** because of this; i.e., the model doesn't specify what the recollection distribution looks like). Also, because recollection produces qualitative evidence of prior occurrence, it is assumed to lead to a relatively high-confidence recognition response. Familiarity-based responses are expected to be spread across the entire range of response confidence.

Unlike the signal detection models, which predict only curvilinear ROCs and linear zROCs, the DPSD model predicts different ROC/zROC shapes for different types of recognition depending on the relative contribution of recollection and familiarity. In tests of item recognition, the model predicts curved asymmetrical ROCs (see **Figure 9**). The familiarity component of the model leads to symmetrical curved ROCs like that of the signal detection model, but recollection increases the proportion of high-confidence recognition responses to old items and thus pushes the ROC up on the left side, making it asymmetrical. Although the predicted item ROCs are approximately linear in z-space, the threshold recollection process leads the zROCs to be slightly U shaped (Glanzer et al., 1999; Yonelinas, 1999b). However, the predicted curve for item recognition is typically so slight that it is not detectable (Yonelinas, 1999b). Overall, the two processes have different effects on ROC shape, which allows the model to predict experimental dissociations between sensitivity and symmetry. In general, both processes can increase overall performance, but recollection makes the ROC asymmetrical, whereas familiarity makes the ROC curved downward.

Because the shape of the ROC is determined by recollection and familiarity, and the functional nature of these processes has been reasonably well characterized (see Yonelinas, 2002), the DPSD model can be used to generate predictions about how different experimental variables will influence ROC shape. For example, because manipulations like deep versus shallow levels of processing and full versus divided attention increase recollection much more than familiarity, the model predicts that these manipulations should lead the z-slope of the ROCs to decrease as performance goes up. In contrast, manipulations that have comparable effects on both processes, such as study duration, should lead the z-slopes to be roughly constant as performance goes up. Additionally, because recollection is expected to be particularly disrupted in patient groups such as medial temporal lobe amnesics, the degree of asymmetry should differ for amnesics and controls, with much more symmetrical ROCs for amnesics than for controls. Further, if the hippocampus is critical for recollection, but not for familiarity (e.g., Eichenbaum et al., 1994; Aggleton and Brown, 1999; Yonelinas, 2002), an ROC analysis should indicate that individuals with selective hippocampal damage have a deficit in recollection, but not familiarity.

Because the model assumes that recollection and familiarity serve as two different bases for recognition responses, it allows recollection and familiarity to act in opposition to one another, as in exclusion tests (e.g., Jacoby, 1991), for which it predicts curved and negative-going ROCs (see **Figure 9**). For example, when subjects are instructed to accept items from one source as 'old' but to reject items from a different source as 'new,' familiarity for the to-be-excluded items will lead to a yes response and downward-curved ROCs. However, recollection, which is used to reject the excluded-source items, pushes the ROC downward. This happens because recollection decreases the number of exclusion errors (which are plotted on the y-axis instead of the hit rate in exclusion ROCs) by leading to many high-confidence rejections (i.e., many 'sure new' responses). Thus, while familiarity in the absence of recollection will result in a typical-looking ROC by leading to exclusion errors across the confidence range, recollection limits the number of errors that will be made, thereby forcing the right side of the ROC down. The resulting ROC is curved downward and crosses the negative diagonal as the familiarity response criterion is relaxed (Yonelinas, 1994; Yonelinas et al., 1995). In z-space the exclusion ROC is generally linear and will have a z-slope of less than 1.0, but with large recollection values the zROC starts to exhibit an inverted-U shape.

The DPSD model has also been applied to tests of relational recognition (see **Figure 9**; e.g., Yonelinas, 1997, 1999a). For relational recognition, an additional recollection parameter (R_L) is required to account for the probability of recollecting a lure item as a lure. For example, in source memory tests, the probability of recollecting items from the two different sources can be quite different; thus a separate recollection parameter is required for each source. One of the sources is arbitrarily referred to as the target source and the other as the lure source. Similarly, in associative memory tests subjects must discriminate between studied pairs (targets) and rearranged pairs (lures). Like the predicted item ROCs, familiarity

leads the relational ROCs to be curved, and recollection tends to make them more linear. However, because familiarity is usually expected to play a lesser role in relational than in item recognition, and because recollection contributes to the recognition of lures as well as targets, the ROCs should be more noticeably linear, and the zROCs more U-shaped.

It is sometimes assumed that subjects are aware of recollection and familiarity, and that they can report on their occurrence (e.g., Yonelinas and Jacoby, 1995; Yonelinas et al., 1996). Remember responses are assumed to provide an indirect index of recollection and thus should be associated with high-confidence recognition responses, whereas know reports provide an index of familiarity in the absence of recollection, and thus can be associated with high and low levels of confidence. If this is the case, then the RK responses should fall along the same function as the confidence ROC. That is, the remember ROC point should fall to the left of the remember + know responses, and both points should fall along the confidence ROC. The remember point should be close to the highest confidence ROC point, but will be shifted slightly to the right if there are also high-confidence responses based on familiarity. Because the RK and ROC points should fall along the same function, the z-slope of the RK and ROC results should be comparable.

Finally, the DPSD model has also been applied to neural monitoring methods, such as ERP and fMRI methods, as well as studies of medial temporal lobe amnesics. At the most basic level, the model predicts that there should be two distinct neural signals associated with recognition performance, one related to measures of recollection and another related to measures of familiarity. In line with recent neuroanatomical models (e.g., Aggleton and Brown, 1999), the model also assumes that the hippocampus preferentially supports recollection, whereas the surrounding neocortex, such as the perirhinal cortex, supports familiarity-based recognition. Medial temporal lobe amnesics, who often have more damage to the hippocampus than to surrounding neocortex, are therefore expected to have greater recollection than familiarity deficits. The recollection deficits are expected to lead to more symmetrical ROCs than those typically seen for healthy control subjects. Finally, if the damage is restricted to the hippocampus, the model predicts that familiarity should be preserved and only recollection will be impaired.

2.23.5.2 Variable-Recollection Dual-Process Model

The variable-recollection dual-process model (VRDP) is a modification of the DPSD model in which recollection is assumed to be a thresholded signal detection process (see **Figure 9**; Sherman et al., 2003; for related modifications of the DPSD model also see Macho, 2004; Healy et al., 2005). As in the DPSD model, familiarity is treated as an equal-variance signal detection process (d'), and recollection is a threshold process in the sense that only some of the studied items will be recollected (R_T). The critical modification is the explicit modeling of recollective strength. Specifically, recollected items produce a Gaussian distribution with a mean level of strength (Rd') and some variability around that mean (RV_T). Thus, items will be recognized if they are recollected and their recollection strength exceeds the response criterion, or if they are not recollected, but the familiarity strength exceeds the response criterion. Conceptually, the VRDP and the DPSD model are nearly identical. However, by allowing the variance of recollection to vary, the VRDP model allows for the possibility that recollection-based responses could receive lower confidence ratings than familiarity-based responses, contrary to the assumptions of the DPSD model. Psychologically, this would imply that recollection of contextual details sometimes produces less reliable evidence of prior occurrence than a feeling of familiarity. However, the VRDP model retains the assumption that recollection is the dominant process and, when successful, will dictate the final recognition response.

In general, the VRDP model makes the same predictions as the DPSD model. For example, the predicted item recognition ROCs are identical to the DPSD model, because if the recollection strength distribution falls above the high-confidence response criterion, then all the recollection responses lead to high-confidence responses, and the model collapses into the original DPSD model. However, if some portion of the recollection distribution falls below the high-confidence response criterion, then some of the recollected items can receive lower confidence responses. Thus, the model can produce ROCs that are slightly more curved than the DPSD model. In z-space, the ROCs are slightly U shaped across most of the range, but they can bend downward as the response criterion becomes strict (see **Figure 9**). The model is relatively new and has not been applied to many paradigms yet, and thus its predictions have

not yet been specified. However, our own calculations suggest that the model is flexible enough to predict a wide array of ROC results. Nonetheless, it will clearly be important for more theoretical and empirical work to be done to further flesh out the model's predictions.

2.23.5.3 Some-or-None Model

The some-or-none model (SON) is another modification of the DPSD model proposed by Kelley and Wixted (2001) to account for associative recognition (see **Figure 9**). The model assumes that memory judgments are based on assessments of associative memory strength and item memory strength, both of which are signal detection processes. Kelley and Wixted use the terms item memory and associative memory, but to be consistent with the other models we use the terms familiarity and recollection, respectively. Although both familiarity and recollection are signal detection processes, the retrieval of associative information is also probabilistic, meaning that only some proportion of the pairs will be recollected, and thus recollection is also a threshold process. Because the model assumes that recollection can fail, but that it varies in strength when successful, recollection is referred to as some-or-none. The item and associative distributions each require a strength parameter (d'_I and d'_A, for item and associative strength, respectively). The variance of the old item strength distribution is assumed to be 1.0 (equal to that of the new item distribution), whereas the variance of the old associative strength distribution is free to vary (V_A). The strength distributions for item and associative information are illustrated in **Figure 9**. The probability of retrieving associative information about a studied pair (R_T) is assumed to be greater than or equal to the probability of retrieving information about a rearranged pair (R_L).

Importantly, and in contrast to the other dual-process models, the SON model assumes that item and associative strength are combined and equally weighted when making associative recognition judgments. In fact, this is really the only difference between the SON and VRDP models. That is, although both models allow recollective strengths to fall below familiarity strengths, the VRDP model still assumes that recognition decisions are dominated by recollection such that successful recollection will dictate the final response. This is not the case for the SON model. In the SON model, an intact pair will be recognized if associative retrieval is successful (R_T) and the sum of the associative and individual item strengths exceeds the response criterion; or if associative retrieval fails but the item strength still exceeds the response criterion. In contrast, a rearranged pair will be correctly rejected if it is recollected as rearranged (R_L), and the item strength minus the associative strength is lower than the response criterion; or if recollection fails and the item strength is lower than the response criterion. Importantly, the resulting strength distributions for intact and rearranged pairs (which are not illustrated) form mixtures of two Gaussian distributions (item strength alone, and item plus or minus associative strength when recollection is successful); because the mixed distributions are usually not Gaussian, the model can produce nonlinear zROCs.

Kelley and Wixted (2001) argued that the variance of the associative distribution must be much greater than that of the item strength distribution in order to produce appropriate ROC results, but subsequent work with the model suggested that this was not a necessary assumption and indicated that the model could fit associative recognition without making this restrictive assumption (Macho, 2004; Healy et al., 2005). Our calculations support the latter claim.

In exploring the effects of the different parameter values on the predicted ROCs, we found that the model was capable of producing a wide variety of curved and linear ROCs at various different levels of performance (see **Figure 9**). Like threshold theory, the R_T and R_L parameters determine the apparent intercepts of the ROCs, and thus they control the degree of ROC asymmetry. That is, R_T determines the left y-intercept, and R_L determines the upper x-intercept. We refer to the intercepts as 'apparent' because the Gaussian distributions lead the ROCs to curve prior to actually intersecting the axes. In general, as associative strength increases, the predicted ROCs become more linear. This occurs because the farther apart the distributions are, the less Gaussian the mixed (item + associative) distributions will be. In contrast, as the variance of the associative strength decreases (i.e., approaches the variance of the item distribution), the ROCs become more curved. In z-space, the model predicts ROCs that have a slope less than 1.0 when R_T / R_L. The zROCs are approximately linear but become nonlinear as associative strength increases, generally exhibiting a U shape, but bending slightly downward at extreme criterion values. Importantly, when R_T and R_L approach 1.0, the predicted ROC will become symmetrical,

and as long as the associative strength is not too high, the zROC will be linear. That is, as recollection approaches 1.00, the mixed distribution upon which judgments are based becomes Gaussian.

2.23.5.4 Mixture Model

The mixture model is another extension of signal detection theory that has been proposed to account for item and source recognition (see **Figure 9**; DeCarlo, 2002, 2003; Hilford et al., 2002). In tests of item recognition, it is assumed that memory judgments are based on the assessment of item strength in a manner consistent with the signal detection model (e.g., it assumes equal-variance Gaussian memory strength distributions). However, an attentional process is also included such that only some proportion of the studied items will increase in memory strength (i.e., the attended items). Decarlo uses the term λ (lambda) to designate the probability that a target item is attended, but to be consistent with the other models we use the term R_T. In this way, the new items form a normal strength distribution, but the old items form a mixture (which is not illustrated) of two equal-variance normal strength distributions (which are illustrated), one overlapping with the new item distribution and the other shifted to the right by some constant (d'). The one overlapping with the new items are those items that were part of the study phase but that were unattended; those that increase in strength are the items that were attended. Thus, the primary difference between this model and the previous hybrids is that it treats recognition as a simple evaluation of strength or familiarity (i.e., as a single process), and assumes that the threshold component (attention) is involved only during the study phase.

One motivating factor behind the development of this model was to avoid a perceived problem with the UVSD model, which is that the model predicts ROCs that cross the chance diagonal. As discussed earlier, the increased variance of old items relative to new items means that the study phase may have led some items to decrease, rather than increase, in memory strength. That is, it places no restriction on how the old items change in memory strength in order to achieve that greater variance. The mixture model avoids this problem by only allowing the memory strength to be increased or to remain unaffected by the study event.

The same model is used in tests of source memory except that memory strength is assumed to reflect how strongly each item matches one of two sources (**Figure 9**). That is, studying items in one source (i.e., the arbitrarily chosen 'target' source) increases source strength and shifts the items to the right (R_T), whereas studying items in the other source (i.e., the lure source) decreases source strength and shifts the items to the left (R_L). As in the item recognition model, only items that are attended at study will be associated with a change in memory strength; thus the source model requires two strength parameters and two attention parameters. Both the item and source models can be extended by adding additional parameters to allow for different levels of attention, but the effects of such modifications have not been explored.

In tests of item recognition, the model predicts asymmetrical curved ROCs that are approximately linear in z-space, with slopes less than 1.0 (see **Figure 9**). The equal-variance Gaussian distributions underlying the model lead it to generate curved symmetrical ROCs, but because only some of the old items increase in strength, this effectively increases the variance of the old item distribution relative to the new item distribution, leading the ROC to be asymmetrical and to have a z-slope of less than 1. Although the ROCs are approximately linear in z-space, the predicted zROCs can have a slight U shape and can even exhibit a subtle downward trend at the extreme criterion values. The nonlinearity arises because of the probabilistic attention process that effectively divides the old items into two distributions (i.e., the attended and unattended items). When attention is very low or high, the mixed distribution (i.e., the mixture of the attended and unattended items, which is not illustrated) is effectively normal, because nearly all items fail to increase in strength (low attention), or nearly all items do increase (high attention). At intermediate values of attention, though, the two portions of the mixture distribution will be more removed from one another, depending on the average strength of the attended items, thereby leading the overall old item (or mixed) distribution to be non-Gaussian.

In tests of source memory, the predicted ROCs are similar to those predicted in item recognition, but because there are two, rather than one, probabilistic attention parameters influencing performance, the ROCs tend to be flatter in probability space and more noticeably U shaped in z-space (see **Figure 9**).

To determine how the model's parameters influence the shape of the item ROC, we explored different parameter values and found that the degree

of predicted nonlinearity in the zROC is not greatly affected by changes in attention when strength is held constant (and relatively low), except at the extremes. However, when attention is midrange, increases in strength result in more pronounced nonlinearity because the two portions of the mixture distribution move farther apart, making the resulting mixed distribution less normal in shape. In item recognition, increases in strength also produce more asymmetrical ROCs (i.e., the z-slope decreases). However, the attention parameter has a nonmonotonic relationship to z-slope. That is, intermediate levels of attention lead the old items to be distributed between the attended and unattended distributions, which increases old item variance and leads to z-slopes of less than 1.0. However, as attention either decreases toward 0 or increases toward 1.0, all the old items are forced into the lower portion (unattended) or the upper portion (attended) of the old item distribution, respectively, producing a symmetrical ROC (z-slope = 1.0).

Because the model assumes that one of the memory processes underlying the ROCs is an attentional encoding process, several general predictions can be made about the effects of different experimental manipulations on ROCs. For example, manipulations expected to influence attention at encoding (e.g., study duration, levels of processing, dividing attention, and word frequency – see Decarlo (2002, 2003) for a discussion of these variables) will not necessarily affect the degree of z-linearity, unless they result in large differences in attention and strength. However, increasing attention at study can lead to a decrease in slope (i.e., an increase of the variance of the old item distribution), then as attention increases further and goes toward 1.0 the pattern should reverse and could lead to an increase in slope (i.e., the variability of the mixed old item distribution decreases back toward that of the new item distribution). In contrast, experimental manipulations that do not affect attention during encoding (e.g., study-test delay or manipulations at the time of test) should only affect the ROCs by changing the strength parameter. Thus, these manipulations should lead the zROCs to become more nonlinear as strength increases (assuming a constant midrange attention parameter), and in the case of item recognition, the z-slope should decrease.

2.23.5.5 Evaluation

The hybrid models can account for item recognition as well as the relational recognition ROC data. The existing data do not clearly differentiate between the different hybrid models, although there is some suggestion that the models that incorporate recollection and familiarity process may fare best.

Because the hybrid models include a Gaussian signal detection process they can produce inverted U-shaped ROCs typically seen in item recognition and sometimes seen in relational recognition. And because they all incorporate a threshold process (recollection or attention), they can produce U-shaped zROCs found in relational recognition. Thus, the models are able to account for the shapes of the ROCs seen in both item and relational recognition studies, in both probability and z-space. Indeed, because the relative contributions of recollection and familiarity are expected to differ for the two types of tests, the hybrid models that incorporate those processes predict the different ROC shapes found in the two types of tests. The models also deal well with the variations in asymmetry found in item ROCs. For example, for the DPSD model, increases in recollection should be accompanied by increases in sensitivity and decreases in z-slope – a pattern found for manipulations such as levels of processing and divided attention (e.g., Yonelinas, 2001; Yonelinas et al., 1996). When both recollection and familiarity increase, overall sensitivity should increase too, but the degree of asymmetry should remain constant – a pattern seen with manipulations like repetition and study duration (see Glanzer et al., 1999, for a review). Because the SON and VRDP models are extensions of the DPSD model, it is likely that they too can account for these results. The mixture model can also account for these results, because it can accommodate changes in strength as well as changes in asymmetry.

The hybrid models also accurately predict the convergence found between RK and confidence ROCs when they are directly compared under the same conditions. For example, for the DPSD model, recollection is expected to lead to high-confidence responses, so the RK data should fall on the same function as the ROCs. A similar account can be provided by the VRDP model. Neither the SON nor the mixture model has been directly applied to RK results yet, but they might account for this convergence by assuming that remember responses simply reflect the strongest items.

The DPSD, VRDP, and SON models can all account for the curved and negative-going exclusion ROCs, as described earlier. Because each of these models includes separate recollection and familiarity processes that can work in opposition to each other,

the familiarity component of the model produces the curvilinear shape of the ROC, and the recollection component pushes the ROC toward the chance line as the criterion becomes more lax. The mixture model has not yet been applied to exclusion data, but our simulations suggest that it is not able to account for the exclusion ROCs in its current form. That is, the model predicts that the probability of accepting a new item should always be greater than the probability of accepting a lure item in an exclusion test, and therefore the entire exclusion ROC falls below the chance line, contradicting observed data.

However, none of these hybrid models can easily account for the high RK z-slopes that are sometimes reported (e.g., Rotello et al., 2004). The DPSD model predicts that the remember point should fall on the confidence ROC function, although it may be possible that the model could account for these high slopes by incorporating a false recollection parameter (Rotello et al., 2004). If successful, then a similar modification might allow the other models to account for these data as well, but this possibility remains to be explored. If the measurement artifact accounts of this finding can be ruled out then, these results will pose a serious problem for these models, and would suggest an approach like that underlying the STREAK model might be useful.

The DPSD and VRDP models both predict that distinct neural correlates of recollection and familiarity should be found and, therefore, match the neural findings from ERP and fMRI methods to date (Eichenbaum et al., 2007). That is, ERP studies have identified two dissociable correlates of accurate recognition decisions (see **Figure 10** for an example; for reviews see Friedman and Johnson, 2000; Mecklinger, 2000; Rugg and Yonelinas, 2003; Curran et al., 2006). The first is a frontocentral negativity that onsets about 400 ms after stimulus onset, and the second is a late positive component largest over the left parietal region that arises approximately 500 ms after stimulus onset. The slower component has been linked to recollection in the sense that it is associated with (1) remember compared to know responses, (2) accurate compared to inaccurate source recognition responses, (3) accurate compared to inaccurate judgments in plurality-reversed recognition, and (4) high-confidence old responses in item recognition. In contrast, the earlier effect has been linked with familiarity in the sense that it is associated with accurate recognition, but does not distinguish between (1) remembering and knowing, (2) accurate compared to inaccurate source

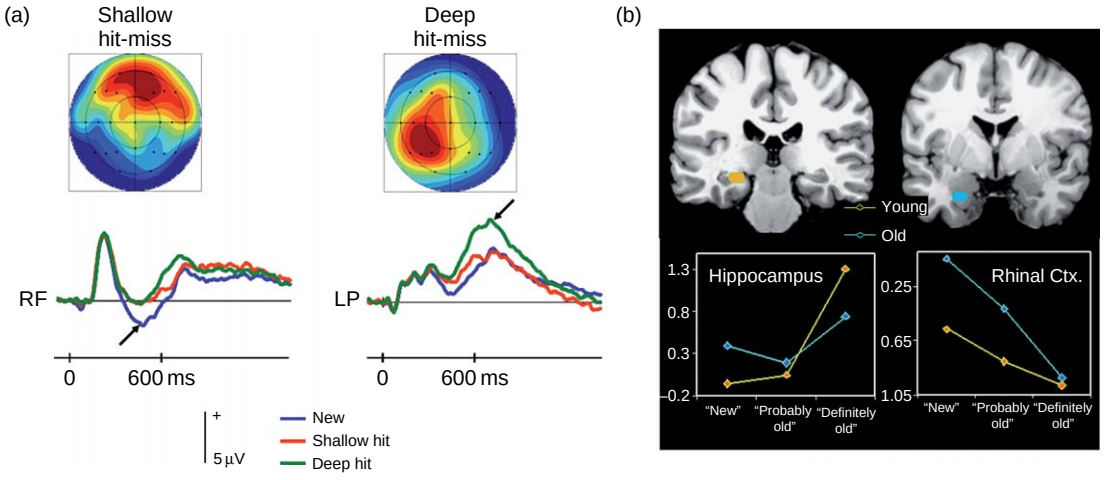

Figure 10 (a) ERPs illustrating an early N400 negativity (left panel) associated with familiarity and a late positive component (right panel) related to recollection. From Rugg MD, Allan K, and Birch CS (2000). Electrophysiological evidence for the modulation of retrieval orientation by depth of study processing. *J. Cogn. Neurosci.* 12(4): 664–678. In this study, the familiarity effect was evident for deeply and shallowly encoded items, whereas the recollection effect was most pronounced for the deeply encoded items. (b) Neural activity related to recognition confidence. From Daselaar SM, Fleck MS, Dobbins IG, Madden DJ, and Cabeza R (2006) Effects of healthy aging on hippocampal and rhinal memory functions: An event-related fMRI study. *Cereb. Cortex* 16(12):1771–1782. Hippocampal activity was associated with high-confidence old responses, but did not change across lower levels of confidence. In contrast, rhinal activity decreased monotonically as recognition confidence increased.

recognition, (3) accurate compared to inaccurate plurality recognition judgments, or (4) high versus low recognition confidence.

fMRI studies have revealed similar dissociations (for a review see Eichenbaum et al., 2007). For example, studies using RK, relational recognition, and ROC confidence methods have indicated that recollection is consistently associated with hippocampal activation and only rarely with perirhinal activation. In contrast, familiarity is consistently associated with perirhinal activation and only rarely related to changes in hippocampal activation (see **Figure 10** for an example).

The ERP and fMRI results present challenges to the mixture and SON models. First, the mixture model assumes that recognition decisions are based solely on an assessment of familiarity strength and therefore does not deal well with the finding of dissociations between neural signals during test. Because the SON model includes two components, it can deal with these dissociations, but it assumes that recognition responses are based on the summed and equally weighted familiarity and recollection information, such that items high in memory strength may reflect any combination of familiarity and recollective strengths. Thus, the relationship seen between measures of recollection, distinct neural signals, and high-confidence recognition responses is not readily explained by the model. Specifically, because the information from the two processes is combined to make a decision, it is not clear why the neural correlates of these processes would have such specific relationships to recognition confidence.

Results from studies of medial temporal lobe amnesia also seem to be in accord with the predictions of the DPSD and VRDP models. First, studies using RK, ROC, and confidence methods of measuring recollection and familiarity have shown that patients with damage to the hippocampus and surrounding cortex have deficits in recollection and a smaller, but consistent, deficit in familiarity (e.g., Cipolotti et al., 2006; Yonelinas et al., 1998, 2002). Second, patients with damage that is restricted to the hippocampus have relatively selective recollection deficits (Aggleton et al., 2005; Yonelinas et al., 2002; but see Manns et al., 2003; Yonelinas et al., 2004, for discussion). These differences have been demonstrated experimentally in rats – damage restricted to the hippocampus produced a selective recollection deficit (Fortin et al., 2004). And in accord with the DPSD model's third prediction, amnesics' ROCs are typically more symmetrical than those of controls (e.g., Yonelinas et al., 1998, 2002; Aggleton et al., 2005; Cipolotti et al., 2006; Wais et al., 2006), and this is true even when performance is equated between the groups (e.g., Yonelinas et al., 1998). The SON model may also be able to account for these data because it includes both recollection and familiarity, but it is less clear how the mixture model would deal with these findings, because it assumes that recognition is based on a single strength-assessment process.

Overall, the DPSD, VRDP, and SON models deal with the neural findings the most easily. The DPSD has been most extensively applied to these data and specifically predicts the dissociations of neural signals, the relationship between hippocampal activity and recollection measures, as well as the differences in ROC asymmetry found in amnesics and controls. The VRDP model, as an extension of the DPSD model, can also account for these data. The SON model has not been applied to neural data, but given its similarity to the DPSD model, should be capable of handling these patterns, though whether the parameters will provide psychologically reasonable accounts is still unknown. The mixture model is the only one to face immediate challenges by the neural data. Specifically, because it assumes a single process underlying recognition performance at test, it cannot easily account for the dissociations found in the ERP and fMRI literature or for the patterns of differential deficits in amnesia.

2.23.6 Alternative Theoretical Frameworks

The models just evaluated represent a broad range of different theoretical approaches to recognition, but the list is hardly exhaustive. The current analysis, however, is relevant to many more current models, because many of the predictions reviewed here parallel those of models that we did evaluate. In this section we discuss the implications of the current findings to a number of these alternatives.

A number of models have adopted the assumption underlying threshold theory, such as multinomial models (for a review see Batchelder and Riefer, 1990) that are often applied to source memory tests (e.g., Bayen and Murnane, 1996; Bayen et al., 1996; Belleza, 2003). Given the poor performance of the threshold models in item recognition, it follows that the multinomial models also fail to provide an acceptable account of item recognition. The story in source recognition is a little more complicated, where it

appears as though the threshold notion does work reasonably well for some source memory studies, in the sense that the ROCs can be close to linear. However, even if restricted solely to source decisions, multinomial models still seem inadequate because of the finding that source ROCs can often be curved. This indicates that the threshold notion is not entirely correct for relational recognition either. Although there may be ways of modifying the multinomial models to bring them more in line with the curved ROCs (e.g., adding more thresholds or considering different response strategies, Malmberg, 2002), it is clear that without modification those models do not provide a very good account of item or relational recognition performance.

The problems that arise for signal detection theory also have far-reaching implications, because so many current theoretical accounts of recognition memory have been built upon this framework. For example, the d' statistic used to measure memory sensitivity depends critically on the validity of the Gaussian and equal-variance assumptions. Although the Gaussian assumption appears to be approximately right in tests of item recognition, it is certainly not appropriate in relational recognition tests, and the equal-variance assumption is violated in nearly every item recognition experiment (i.e., ROC slopes are less than 1.0). However, even if one opts to use d' despite these problems, it is not a sufficient measure by itself – a measure of the second memory component (i.e., the variance ratio, recollection, or attention) is necessary to accurately describe performance as well. The observed ROCs are also problematic for various other models that are based on signal detection theory. For example, global memory models such as TODAM and SAM generally base recognition judgments on an assessment of a Gaussian memory strength signal, and as such they are not consistent with the U-shaped zROCs observed in relational recognition tasks. Moreover, the dissociation of sensitivity and asymmetry observed in item and relational recognition studies also presents problems for these models (for earlier discussions see Ratcliff et al., 1992; Clark and Gronlund, 1996). For example, models like SAM and Minerva 2 (Hintzman, 1984) predict that the ROCs should become more asymmetrical as performance increases, which is not consistent with what is seen with manipulations like study duration, which increase performance but not the slope. In contrast, TODAM predicts that the slope should remain relatively constant, which is not consistent with what is observed with manipulations like levels of processing, which affect both performance levels and slope. Note, however, that some of these models include recall mechanisms that might be used to supplement standard recognition in such a way as to produce non-Gaussian memory strength distributions, though it is not yet known whether such modifications would produce the observed pattern of ROC results. The SAC model of Reder et al. (2000) is another computational model (it starts at the level of representations), but one that incorporates familiarity and recollection processes. The model assumes that familiarity reflects the assessment of the activation of word nodes, whereas recollection reflects the assessment of activation of nodes that represent specific events. Both processes rely on assessments of activation in a manner consistent with signal-detection theory, so the model does not provide an account for the U-shaped zROCs, but whether it can be modified to do so is not yet clear (for discussion see Diana et al., 2006).

One computational model that appears to be consistent with the dual-process models considered is the complementary learning systems model (e.g., McClelland et al., 1995; O'Reilly and Rudy, 2001; Norman and O'Reilly, 2003). The model is based on the assumption that the hippocampus supports recollection by developing minimum overlapping representations of prior episodes, whereas the surrounding cortex gradually tunes populations of cortical units to respond strongly to different stimuli in such a way that it can discriminate between familiar and new items. A review of the model goes beyond the scope of the current paper (for a detailed discussion see Norman and O'Reilly, 2003), but the results from preliminary simulations are promising, because they indicate that the model can account for the differential importance of hippocampal versus the surrounding neocortex in recollection and familiarity and successfully predicts behavioral properties of recollection that other computational models have not (Elfman et al., unpublished data). For example, the hippocampus produces a threshold output such that it can produce linear ROCs, whereas the cortex produces curvilinear ROCs. It is not yet clear whether the model is able to account for the full body of results that have been discussed; however, models such as this one are particularly promising, because they aim to incorporate the behavioral and neuroanatomical knowledge about recollection and familiarity within the same theoretical framework.

2.23.7 Conclusions

In this chapter, we have examined several quantitative models of recognition memory, highlighting their assumptions and predictions, and finally focusing on how well those predictions have been supported by the data. The evidence strongly disconfirms the pure threshold and signal detection models. Pure threshold models fail outright in nearly every recognition domain. Although there are some sets of relational recognition data that threshold theories can adequately describe, they still fail to explain the wider range of findings of both linear and curvilinear ROCs. Pure signal detection models fare better than the pure threshold models, but they too face a fairly overwhelming challenge. They are unable to deal with curvilinear zROCs and therefore cannot account for the relational recognition results, including source, associative, and plurality-reversed recognition. However, they may still be relevant in particular tasks – for instance the UVSD model accounts for item recognition very well, and STREAK can account for nearly any RK pattern found. In our view, though, a model aimed at describing recognition performance should be aimed at more than a single task, especially given that there are several models which do so already.

The examination of ROC data from a wide range of recognition memory paradigms also indicates that single-component models of recognition memory are inadequate, and that there are at least two functionally and anatomically distinct component/processes involved in recognition. To account for ROC results, current models have incorporated several different theoretical divisions such as the distinctions between recollection/familiarity, item/associative information, attention/familiarity, and strength/variance. Although there is support for all of these distinctions, in general, only the hybrid models assuming the contribution of signal detection and threshold processes were successful at accounting for the existing literature (e.g., recollection/familiarity; attention/familiarity).

Although the existing results argue strongly against pure threshold and pure signal detection models and strongly in favor of the hybrid models, the current review does not provide definitive evidence for the superiority of one hybrid model over the others. And importantly, models that produce non-Gaussian distributions by means other than including both signal detection and threshold processes have yet to be seriously explored. Our hope, however, is that in examining the theoretical background and core assumptions of the existing models we have come to more clearly see which classes offer the most promise in explaining the data, as well as the important empirical and theoretical questions that need to be answered. In so doing, we hope that the next phase of ROC research will focus on testing competing predictions of these various hybrid models and lead to a deeper understanding of recognition memory.

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2.24 Memory Search: A Matter of Time

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2.24.1 Introduction

> In short, we make search in our memory for a forgotten idea, just as we rummage our house for a lost object. In both cases we visit what seems to us the probable neighborhood of that which we miss. We turn over the things under which, or within which, or alongside of which, it may possibly be; and if it lies near them, it soon comes to view. (William James, 1890: 290)

In most instances, memory retrieval occurs in a seemingly automatic and effortless fashion, as when we recognize an acquaintance or type in a password. These successful, and underappreciated, acts of retrieval suggest memory is a direct, immediate, content-addressable type of filing system. That is, we do not appear to engage in a search of memory in the literal sense of the term. There are occasions, though, when the desired information does not come easily to mind, and we then must engage in a more conscious and effortful interrogation of memory, as in William James' example above. Everyone has, from time to time, tried to remember where they parked their car in a busy lot, or what that funny joke was they heard last week. A salient feature of these types of retrieval attempts is that they seem to involve a search of memory that takes some appreciable amount of time.

Many researchers have measured response time (or reaction time or response latency; hereafter termed RT) in different memory tasks as a means to try to identify the nature of the underlying retrieval processes.

The measurement and interpretation of response time have a long history. For example, Donders (1868/1969) proposed a method to measure the

time it takes to complete a particular set of mental processes, and Jastrow (1890) argued that, by working backward from response time, one might be able to infer the particular processing structures that were used to perform the task. Luce (1986) and Welford (1980) discussed the broader use of response time to study cognitive processes and the detailed methods used in the analyses of response times.

In this chapter we review the analyses of RT for different tests of explicit memory as a way to examine and compare different characteristics of intentional memory search and retrieval processes. Measures of RT and response accuracy are sometimes viewed as complementary because they often show the same effects, suggesting that they are reflections of the same underlying processes. These measures, however, are not equivalent because some variables can have large effects on accuracy but have little or no effect on response latency (e.g., Rohrer and Wixted, 1994), and RT can be highly informative when accuracy is perfect (e.g., Mewhort and Johns, 2000). The problem of speed–accuracy tradeoffs can also lead to incorrect interpretations of RT if error rates across conditions are not considered (e.g., Pachella, 1974; Wickelgren, 1977). Thus, one should be cautious in considering only one of these two measures of performance. See Kahana and Loftus (1999) for a review and comparison of accuracy and RT in the study of memory. The measurement and analysis of RT include mean RT, the analysis of RT distributions, and speed–accuracy tradeoff functions. These measures will be considered in turn for the principal tasks that have been used to study different features of human memory – item recognition, associative recognition, cued recall, and free and serial recall.

2.24.2 Item Recognition

2.24.2.1 Item Recognition for Subspan Lists

The beauty of Helen of Troy is said to have launched a thousand ships. It might also be the case that Sternberg's (1966) classic study of memory search in short-term memory launched a thousand experiments. In Sternberg's procedure, participants view short lists of items (usually one to six) presented one at a time. Each list is followed by either an old or new test probe. Participants are instructed to respond whether or not the probe is a member of the preceding study set as quickly as possible while minimizing errors. In the varied-set version of this procedure there is one test following each list, and list length varies over trials. In the fixed-set variant, a given list is prememorized and followed by a series of test probes, and this process is repeated for lists of different lengths. Because the lists are less than the span of immediate memory, accuracy is very high, and RT is the primary dependent measure.

Sternberg found the same pattern of results in both the varied- and fixed-set versions of this task: RT increased in a linear fashion with the number of items in the memory set for both correct old and new decisions. This pattern of results has been replicated many times by many different researchers. An example of the linear RT set size function for the varied-set version of the Sternberg task is shown in the left panel of **Figure 1**. The linearity of the memory set function is not affected by considerable practice at the task (Kristofferson, 1972) and is found for a wide range of stimulus materials – letters, digits, faces, geometric shapes, colors, and words (cf. Sternberg, 1975), suggesting that this result reflects a basic property of the short-term memory search process.

Sternberg (1966, 1969) proposed that these results reflect a high-speed serial search process. That is, the probe item is compared to each item in the memory set one at a time. The time for each individual comparison is given by the slope of the function relating RT to set size, which is typically in the range of 35 to 40 ms per item. Such a search rate is indeed extremely fast – in the order of 25 items per second. The comparison process was also assumed to be exhaustive such that the probe is compared with every item in the memory set before a single yes or no match decision is made. This assumption is based on the finding that the slopes of the correct 'yes' and 'no' memory set functions are parallel. An exhaustive search process would seem inefficient compared to a self-terminating search process that would end when a positive match is found. Sternberg argued, though, that an exhaustive search is more efficient because it requires only one match decision following the entire comparison process as opposed to a decision after each individual comparison that would be required for a self-terminating serial search process.

The plausibility of Sternberg's exhaustive serial search process was later called into question, not on logical grounds but on empirical grounds. One problem concerned the characteristics of the underlying RT distributions. Schneider and Shiffrin (1977) noted that the variance increased more for positive than for negative responses at larger set sizes. An exhaustive search process predicts that, like mean RT, the

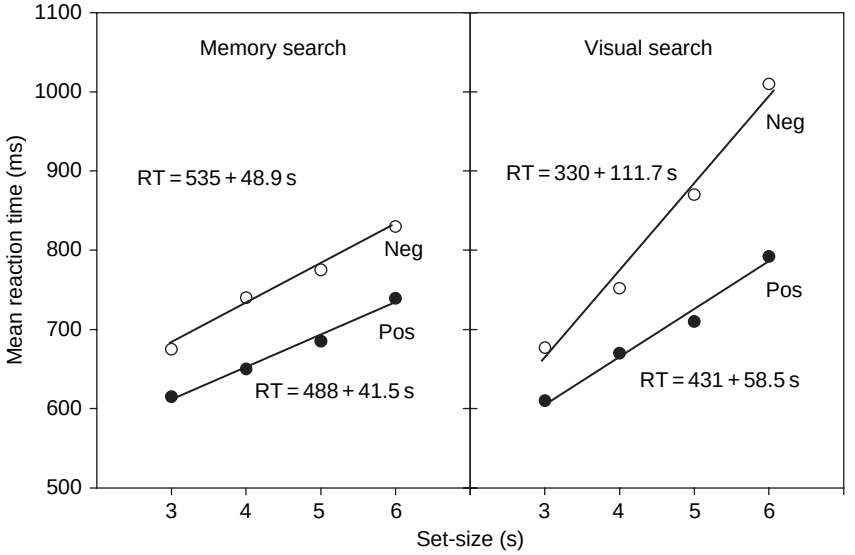

Figure 1 This figure shows mean response time (RT) for correct positive (solid circles) and correct negative (open circles) responses as a function of set size for Sternberg's (1966) short-term search task (left panel) and Neisser's (1963) visual search task (right panel). The data are from Hockley WE (1984) Analysis of response time distributions in the study of cognitive processes. *J. Exp. Psychol. Learn. Mem. Cogn.* 10: 598–615; used with permission.

variance of the RTs should be similar for old and new responses.

Hockley (1984; see also Hockley and Corballis, 1982) analyzed RT distributions in the Sternberg paradigm using the ex-Gaussian distribution to describe the shape and the changes of the RT distributions with memory set size. Ratcliff and Murdock (1976) showed that the ex-Gaussian distribution provides a very good description of observed RT distributions for a number of recognition memory phenomena. It has subsequently been used by investigators to characterize RT distributions in a variety of cognitive tasks (e.g., the Stroop effect; Heathcote et al., 1991).

The ex-Gaussian distribution is the convolution of normal and exponential distributions and is described by the following equation:

$$f(t) = \frac{e^{-(t-\mu)/\tau+\sigma^2/2\tau^2}}{\tau\sqrt{2\pi}} \int_{-\infty}^{(t-\mu)/\sigma-\sigma/\tau} e^{-\frac{y^2}{dy}}$$

where μ (mu) and σ (sigma) represent the mean and standard deviation, respectively, of the normal distribution component, and τ (tau) represents the parameter and mean of the exponential distribution component. Although the equation appears quite daunting, this distribution is much simpler conceptually. Imagine a normal distribution, and then extend or stretch out the right tail of this distribution (examples are shown in **Figure 2** and in **Figures 8 and 12** later in the chapter). In general terms, mu reflects the left or leading edge of the RT distribution, while tau reflects the elongated right tail, or the positive skew, of the distribution. Thus, mu and tau quantify two important properties of RT distributions, namely, the minimum or fastest RTs and the spread of the distribution determined by the slowest responses.

Hockley (1984) contrasted the nature of the RT distributions in the Sternberg memory search task with a visual search task. The visual search task was based on the search experiments reported by Neisser (1963) in which participants are first presented with a target letter followed by a vertically presented set of three to six letters. Participants determined whether or not the target item was contained in the search set. The RT set size functions for positive and negative responses are shown in the right panel of **Figure 1**. The results of the visual search task were consistent with a self-terminating serial search process where the visual search proceeds from the top to the bottom of the column of letters. Correct mean RT was a linear function of the size of the search set, the slope of the function for negatives was almost twice as steep as that for positives, and the serial position functions showed a recency gradient.

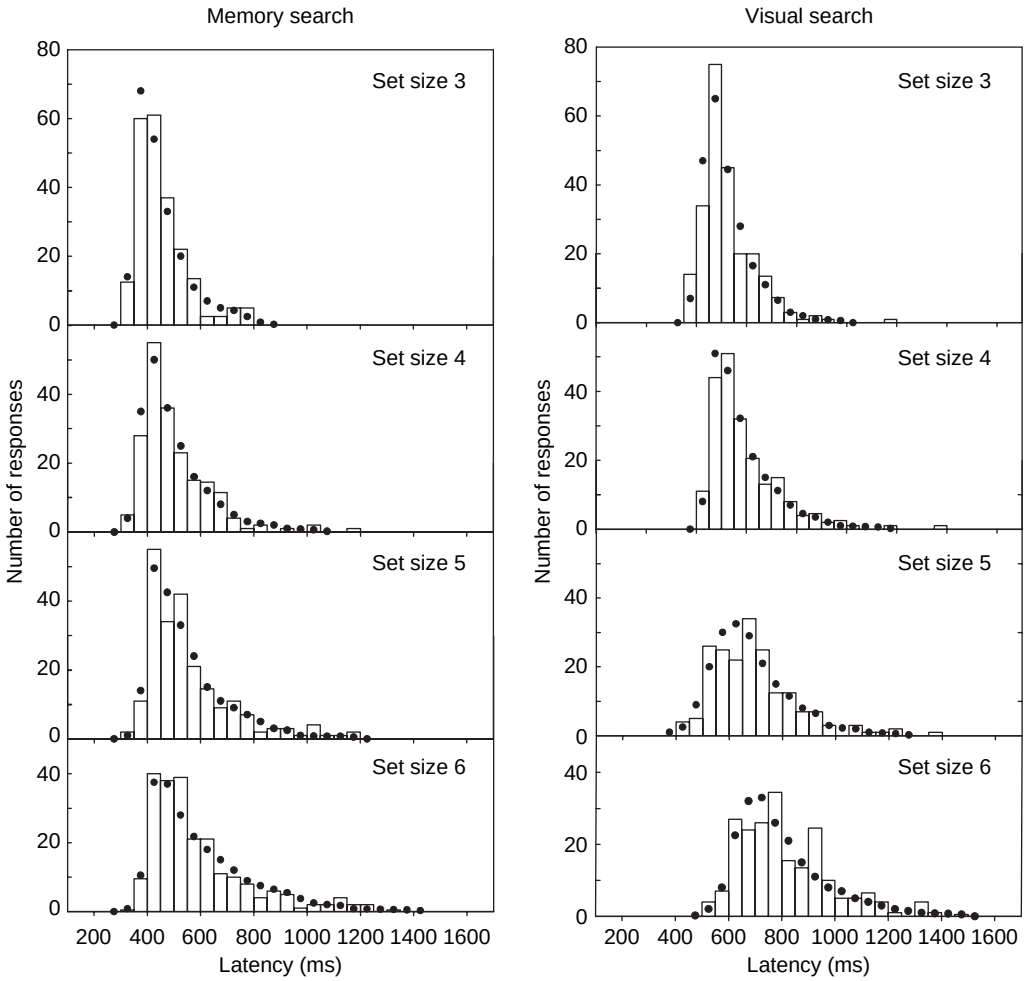

Figure 2 The bar graphs show the observed RT distributions for correct negative responses for one participant for the memory search task and the visual search task. The fits of the ex-Gaussian distribution are shown by the dots. Data are from Hockley WE (1984) Analysis of response time distributions in the study of cognitive processes. *J. Exp. Psychol. Learn. Mem. Cogn.* 10: 598–615; used with permission.

The analysis of the RT distributions for the visual search task were also consistent with a self-terminating serial search process, as the increase in mean RT was largely captured by increases in the parameter mu, which reflects changes in the leading edge of the latency distribution, or the fastest responses. In contrast, while the pattern of mean RTs in the memory search task replicated Sternberg's findings as shown in **Figure 1**, the increase in mean RT was largely seen in the tau parameter, which reflects the increasing skew of the RT distribution, or an increase in the slower responses.

The differences in the changes of the underlying distributions in the visual and memory tasks are illustrated in **Figure 2**. This figure shows the observed RT distributions and the fits of the convolution model for correct negative responses in each task for one of the six participants in the experiment. In the visual search task the entire distribution shifts to the right as set size increases. Thus the minimum as well as the maximum RTs increase with the number of items in the search set. In contrast, the minimum or fastest RTs change very little in the memory search task, a result that is inconsistent with a serial search process. These results for the Sternberg task have been replicated more recently by Ashby et al. (1993).

A second empirical problem for Sternberg's serial search hypothesis concerned the findings of serial position effects (e.g., Corballis, 1967; Burrows and Okada, 1971; Corballis et al., 1972; Ratcliff, 1978; Aube and Murdock, 1974; Monsell, 1978; Murdock and Franklin, 1984; McElree and Dosher, 1989).

Contrary to predictions of exhaustive scanning, mean RT for yes decisions was influenced by the position of the target item in the memory set. Generally, except for a small primacy effect, these studies showed that mean RT increased with decreasing recency of the positive test probe. Monsell (1978) demonstrated that when serial position is defined in terms of recency, or the number of items intervening between study and test (test lag), the serial position functions for memory set sizes of one to five were the same for all positions except the primacy item. The coexistence of both serial-position and set-size effects led to the suggestion that set-size effects arise due to the effects of serial position (Murdock, 1971, 1985; Monsell, 1978).

McElree and Dosher (1989) replicated Monsell's (1978) recency results using the response signal version of the speed–accuracy trade-off (SAT) procedure. This procedure provides a way to examine the time course of retrieval (Wickelgren and Corbett, 1977; Corbett and Wickelgren, 1978; Wickelgren et al., 1980; Dosher, 1981). In this paradigm retrieval is interrupted at different temporal intervals (typically between 0.1 and 3 s) after the probe is presented by having participants make a recognition decision as soon as the cue to respond is given. By examining performance over the different intervals one can assess the increase in accuracy as retrieval time increases until accuracy reaches an asymptote. SAT functions are characterized by three parameters: an intercept or starting point of the function, the rate of rise from chance accuracy to asymptotic or final level of accuracy, and the asymptotic level of accuracy. The intercept provides a measure of when information first becomes available, the rise parameter indexes the rate of accrual of information over time, and the asymptote reflects the maximum level of accuracy. **Figure 3** shows McElree and Dosher's SAT functions for different serial positions for memory set sizes of 3 and 5. These functions show that serial position primarily affects the asymptotic accuracy of recognition performance. The differences in the retrieval dynamics of the functions (the intercept and rate parameters) were restricted to differences between probes from the most recent serial position (the last study item before the test probe) and all other probes. Thus, with the exception of the last item, serial position influenced accuracy but not the speed of retrieval.

One criticism of the response signal procedure is that it cannot distinguish between variable all-or-none processing and continuous accumulation of information. That is, if all of the retrieved information

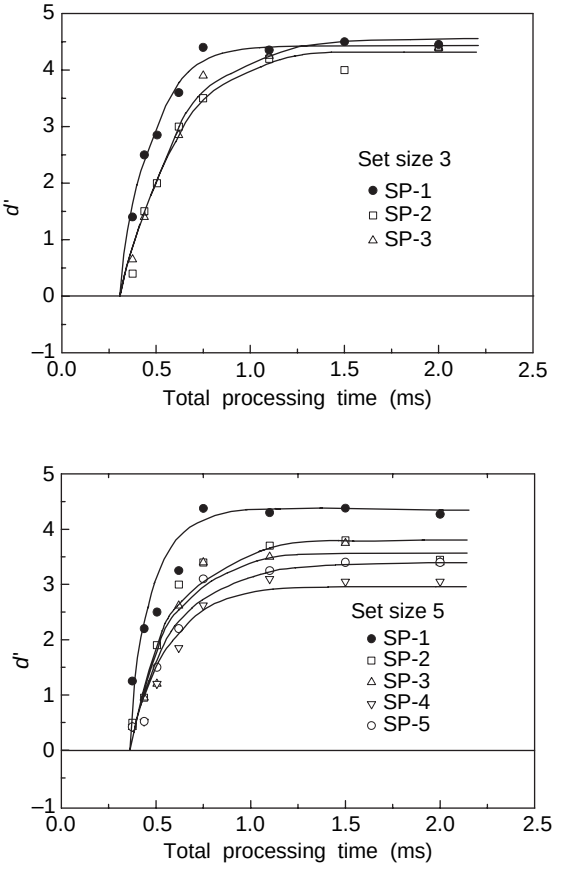

Figure 3 This figure shows mean d' values (a measure of discrimination or sensitivity from signal detection theory; cf. Macmillan and Creelman, 2005) as a function of total processing time for each serial position for set size 3 (top panel) and set size 5 (bottom panel). The solid lines represent the best-fitting exponential SAT functions. The results are from McElree B and Dosher BA (1989) Serial position and set size in short-term memory: The time course of recognition. *J. Exp. Psychol. Gen.* 118: 346–373; used with permission.

becomes available at one time and this time is variable, the SAT curves from the response signal procedure would still increase in a gradual fashion. Meyer et al. (1988) introduced a variant of the response signal procedure – speed–accuracy decomposition (SAD) – as a way to address this problem. In the SAD procedure regular (no signal) trials are randomly interspersed with signal trials, and the RT distributions are compared (see Ratcliff (1988) and Kahana and Loftus (1999) for a discussion of these SAT procedures). These results provide support for the view that information accumulates continuously.

A third problem for Sternberg's model was the finding of repetition effects (Burrows and Okada, 1971; Baddeley and Ecob, 1973). Responses to items

that were repeated in the memory set (e.g., 9 1 9 3) are faster than responses to nonrepeated items. Most damaging, perhaps, for Sternberg's serial search account were the findings of recency effects for negative probes. Both RT and accuracy suffer the more recently a negative probe on a current trial had been presented as a memory set item on a previous trial (e.g., Atkinson et al., 1974; Monsell, 1978; Hockley and Corballis, 1982; McElree and Dosher, 1989). This finding demonstrates that recognition decisions are influenced by the longer-term presentation history of the items outside the current short-term memory set, a finding that is not easily reconcilable with serial search models of immediate memory (Monsell, 1978; Ratcliff, 1978).

Van Zandt and Townsend (1993) evaluated the different classes of exhaustive and self-terminating search models in light of the results observed in the Sternberg paradigm and found that exhaustive processing models were not tenable. They concluded that self-terminating models provide the best description of rapid visual and memory search processes. A number of researchers have proposed versions of strength-based models as alternatives to Sternberg's original model (e.g., Wickelgren and Norman, 1966; Baddeley and Ecob, 1973; Murdock, 1985; McElree and Dosher, 1989). Ratcliff's (1978) diffusion model is one of the most developed and influential models illustrating this approach.

In Ratcliff's (1978) theory of memory retrieval the recognition test probe is compared to all of the items in the search set in parallel. Evidence is accumulated for each comparison based on the degree of relatedness between the probe and the memory item. This process is modeled by a continuous random walk process; positive evidence causes the random walk to approach an upper match boundary, while negative evidence drives the process downward toward a nonmatch boundary. A positive recognition decision is made when one of the comparison processes reaches the match boundary. If all the comparison processes terminate at the nonmatch boundary a negative decision is made. Ratcliff showed that this model can produce all of the findings observed in the Sternberg paradigm: linear and parallel RT set size functions, serial position effects, and the appropriate characteristics of the RT distributions and the SAT functions.

2.24.2.2 The Extralist Feature Effect

In Ratcliff's (1978) retrieval model, the latency of correct negative decisions depends on the slowest mismatch between the test probe and the items in memory that enter into the comparison process. Thus, negative decisions are treated as a default option that is reached when there is insufficient evidence (or strength or familiarity) to support a positive decision. Mewhort and Johns (2000; Johns and Mewhort, 2002, 2003) have recently challenged this notion. They proposed instead that correct rejections are based on information in the probe that contradicts the information represented in the study set.

Mewhort and Johns (2000) used stimuli that comprised a small number of features with finite values (e.g., a red star, a yellow triangle, etc.). Like Sternberg (1969), Mewhort and Johns used subspan study lists, and they also conditionalized the data on sure (or high confident) recognition responses to ensure that they were examining retrieval based on accurate encoding of the study items. The use of two-dimensional stimuli allowed the researchers to vary the similarity between the probe and the studied or target items. In the example given by Mewhort and Johns, a participant studies the following set of items: a blue cross, a red triangle, and a green circle. A yellow diamond would be a negative probe that does not share any features with the studied items (condition 0:0), a yellow cross or a red diamond would be a negative probe that shares one of its two features with the study set (condition 1:0), and a blue triangle or a green cross would be a negative probe that shares both of its features with the study set (condition 1:1). The similarity of the negative probe could also be varied in terms of the number of study items that share the same feature. For example, if two of the study items had the feature red, then a red star lure would share a repeated feature of the study set (condition 2:0).

Mewhort and Johns (2000; Johns and Mewhort, 2002, 2003) found that participants were fastest to correctly reject negative probes when they did not share a feature with the study set (the extralist feature effect) and became progressively slower as the number of shared features increased. That is, condition 0:0 was faster than condition 1:0, which in turn was faster than condition 1:1. In addition, the number of times a probe feature was studied did not affect RT when an extralist feature was present. That is, mean RT in condition 2:0 was similar to that of condition 1:0. This finding poses problems for familiarity-based accounts of recognition. Mewhort and Johns also showed that the extralist feature effect could be found using words as stimuli where the

manipulated features were the letters within the words, and also for prememorized lists that exceeded memory span.

As Mewhort and Johns argued, these results do not support the view that correct rejections are a default decision that occurs when insufficient evidence accrues to support a positive response. Rather, the extralist feature effect shows a clear role of contradiction in recognition decisions, at least when the information supporting contradiction is available, and also perhaps when the memory set is very well-defined.

2.24.2.3 Item Recognition for Supraspan Lists

Burrows and Okada (1975) measured RT for memorized lists of items that varied from 2 to 20 in length. RT was an increasing function of list length. Their results were best fit by bilinear functions with the break point occurring between lists of six and eight items, the traditional measure of memory span, and they argued that these results support the view that memory search processes are different above and below short-term memory span. Burrows and Okada noted, though, that a single continuous logarithmic function also provided a good description of the relationship between RT and list length. Similar results were found by Banks and Fariello (1974), who tested memory for lists of pictures of common scenes that varied from 2 to 24.

Hockley and Corballis (1982) replicated and extended Burrows and Okada's results. They also compared conditions in which negative probes were and were not repeated across lists in a session. Their results are presented in **Figure 4**. Again, mean RT increased as a function of memory set size, and a bilinear function with the break point at memory span provided a better fit than a single logarithmic function. When negatives were repeated, the slopes of each limb of the bilinear function and the rate constant of the logarithmic function were almost doubled. The fact that this manipulation had a similar effect on both subspan and supraspan list lengths suggests that these results reflect the operation of one retrieval process rather than retrieval from different memory systems.

Similar results have also been observed in the continuous recognition paradigm. In this procedure items are repeated in a long list of items, and participants make a recognition decision for each list presentation. Thus, an item is 'new' on its first appearance in the list and 'old' on its second presentation. Accuracy decreases and response latency increases as a function of the number of items, or test lag, between the first and second presentations of an item. Hockley (1984) found that a logarithmic function provided a good description of the increase in RT with test lag that varied from 1 to 24 intervening items. Moreover, he also found that both between-list and within-list stimulus manipulations (nouns versus nonnouns) influenced the slope of the logarithmic function with no appreciable effect on the intercept, whereas item repetition decreased the intercept with little effect on the slope.

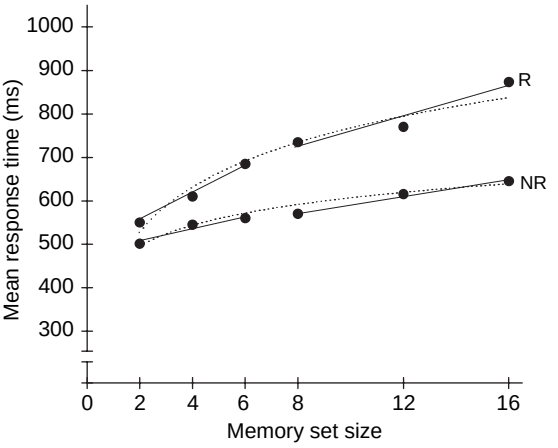

Figure 4 This figure shows mean response time (RT) as a function of memory set size (M) for lists with repeated negatives (R) and lists with nonrepeated negatives (NR). The dashed lines represent the best fitting logarithmic functions ($RT(R) = 415 + 159 \log_e M$; $RT(NR) = 445 + 81 \log_e M$), and the solid lines show each limb of the best-fitting bilinear functions with the breakpoint at the estimate of short-term memory span ($RT(R) = 468 + 38\,M$, and $RT(NR) = 471 + 20M$ for set sizes 2, 4, and 6; $RT(R) = 605 + 17M$, and $RT(NR) = 522 + 10M$ for set sizes 8, 12, and 16).

Findings from the response deadline SAT procedure further support a recency or strength-based view of recognition memory over both the subspan and supraspan range. Wickelgren et al. (1980) used a single-item probe recognition task to examine the temporal dynamics of retrieval for different serial positions of 16-item lists. They fit SAT retrieval functions for three subspan serial positions (16, 15, and 14, or the last three items of the list), and three supraspan sets of items (serial positions 13–11, 10–6, and 5–3). Asymptotic accuracy decreased monotonically with the decreasing recency of the test probe's serial position, indicating that memory strength

declines systematically over this range. Retrieval speed (as estimated by the intercept and rate parameters of the SAT functions), however, was constant across all serial positions exception for the last (most recent) item, which was processed 50% faster than the items from all of the other serial positions. Wickelgren et al. concluded that retrieval speed is constant except for the last item, which is in a privileged state of active awareness. (See McElree, 2001, for further evidence that the capacity of focal attention is limited to a very small number of representations, perhaps just one.)

2.24.2.4 Short-Term versus Long-Term Memory

Sternberg (1966) developed his subspan item recognition paradigm in order to study memory search processes in short-term or immediate memory. But is the distinction between short-term and long-term memory relevant to item recognition perfomance? When list length has been varied above and below memory span, both bilinear functions with the breakpoint at or near memory span, and continuous functions provide a good description of the increase in RT with list length or the lag between study and test (e.g., Banks and Fariello, 1974; Burrows and Okada, 1975; Hockley, 1984). Which function provides the more appropriate description of changes in RT with list length?

Three findings indicate that the continuous function provides the more meaningful description of recognition performance. First, the effects of repeating items from previous trials (e.g., Monsell, 1978; McElree and Dosher, 1989) show that recognition is not solely based on the current contents of short-term memory. Second, manipulations have similar effects on subspan and supraspan list lengths and have been shown to differentially affect the intercept and slope parameters of the continouous span functions (e.g., Hockley and Corballis, 1982; Hockley, 1984). Finally, results from the response-signal SAT procedure show that retrieval speed is constant below and above span except for the most recent item which appears to be in a privileged state of awareness (Wickelgren et al., 1980; McElree and Dosher, 1989; McElree, 2001). All of these results indicate that, with the possible exception of the most recent item, recognition proceeds in the same fashion above and below memory span.

2.24.2.5 Regularities of Item Recognition

Ratcliff and Murdock (1976) summarized a number of functional relationships between accuracy and response latency obtained in the study-test recognition paradigm. These relationships are shown in **Figure 5**. They provide a set of benchmarks that any model of recognition performance must be able to accommodate. In general, changes in mean RT mirror changes in accuracy. Accuracy increases while mean RT decreases with the confidence of the recognition decision. Accuracy and RT for high-confidence responses change in a complementary fashion as a function of output (test) and input (study) position and the number of study presentations of the items. Correct 'new' decisions (correct rejections) are almost as fast as correct 'old' decisions (hits). In addition, the changes in mean RT are seen to a greater extent in the tau parameter (the measure of skewness or variance) than in the mu parameter (minimum latencies) of the ex-Gaussian analysis of the RT distributions. As discussed previously, similar effects of recency, repetition, and list length effects, and changes in RT distributions and SAT functions have been observed in item recognition for subspan lists, indicating that these effects are basic characteristics of recognition memory.

Ratcliff (1978) showed that his diffusion model could not only fit the pattern of results found in the Sternberg paradigm, but also provide an impressive account of the accuracy and latency results obtained in the study-test paradigm and illustrated in **Figure 5**, as well as results from the prememorized list and continuous recognition procedures. Ratcliff's diffusion model is a formal theory of the retrieval and decision process, but it does not provide an account of how items are represented in memory or how they are compared. Nevertheless, the diffusion model can be incorporated into models that do make explicit assumptions concerning how items are represented and compared, such as Gillund and Shiffrin's (1984) search of associative memory (SAM) model and Hintzman's (1988) MINERVA 2 model.

A more recent regularity of recognition memory is the mirror effect (Glanzer and Adams, 1985, 1990). This effect refers to the finding that, when two classes of stimuli, A and B, are compared and class A is more accurately recognized than class B, the difference in accuracy is seen both in terms of a higher hit rate for class A old items and a lower false alarm rate for class A new items. If one thinks in terms of the underlying distributions representing the strength of the old and

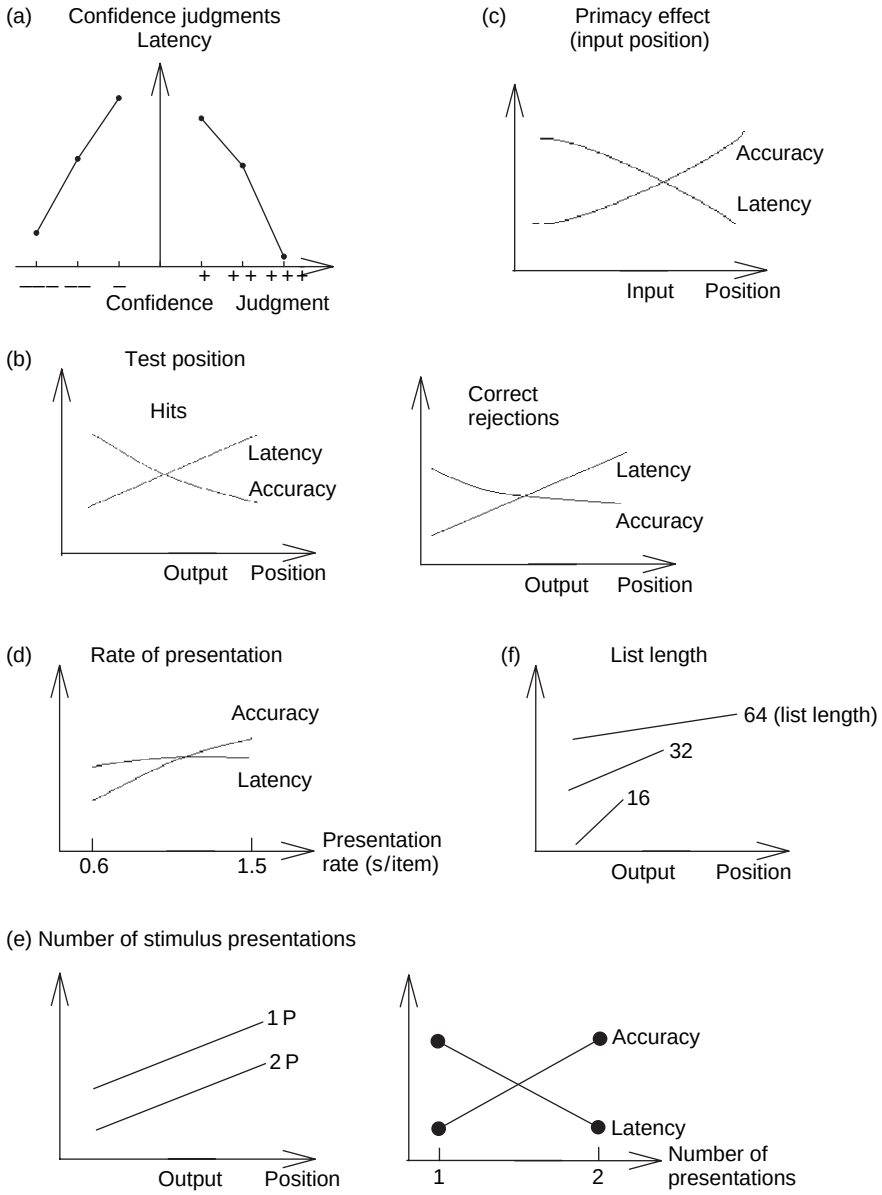

Figure 5 This figure summarizes the functional relationships of recognition decisions described by Ratcliff R and Murdock BB Jr. (1976) Retrieval processes in recognition memory. *Psychol. Rev.* 83: 190–214; used with permission. These relationships show that: (a) mean RT decreases as confidence increases; (b) accuracy and latency vary inversely with test (output) position, (c) study (input) position, (d) presentation rate at study, and (e) number of presentations at study; and (f) the increase in response latency as a function of output position decreases as list length increases. (Note that relationships (b) through (f) are for high-confidence responses only.)

new items of each class (as assumed in signal detection theory), then the order of the class A and B new item distributions mirror the order of the class A and B old item distributions. The mirror effect is also reflected in the pattern of mean RTs for the correct 'old' (hits) and 'new' (correct rejections) responses of each stimulus class (Hockley, 1994). The comparison of low- and high-frequency words, where low-frequency words are more accurately recognized than high-frequency words, is a prototypical example of the mirror effect in item recognition.

The mirror effect posed a challenge for simple strength-based models of recognition memory, because these models cannot easily account for why, for example, low-frequency items have a lower average strength than high-frequency items when they are new, but

have a higher average strength than high-frequency items after one study presentation. The mirror effect provided the impetus for a new generation of single-process models of recognition memory that could accommodate the mirror effect (e.g., Anderson and Milson, 1989; Shiffrin and Steyvers, 1997; McClelland and Chappell, 1998; Dennis and Humphreys, 2001; Murdock, 2003). The word-frequency mirror effect has also been taken as evidence in support of dual-process models of item recognition (Joordens and Hockley, 2000; Reder et al., 2000).

2.24.2.6 Dual-Process Views of Recognition

A debate that has received considerable attention has been waged between single-process theories of recognition decisions on the one hand (e.g., the global matching models of Murdock (1982), Gillund and Shiffrin (1984), and Hintzman (1988) and the more recent models of Shiffrin and Steyvers (1997), McClelland and Chappell (1998), and Dunn (2004)) and dual-process views on the other (e.g., Atkinson and Juola, 1973, 1974; Mandler, 1980; Jacoby, 1991; Yonelinas, 1994; Joordens and Hockley, 2000; Reder et al., 2000). Although single-process models differ in many interesting ways, they share a common assumption that recognition decisions are based on a single evidence dimension that has been variously characterized as memory or matching strength or familiarity. In contrast, the unifying assumption of dual-process theories is that recognition involves both familiarity and the recollection or retrieval of specific details of a prior experience (for recent reviews see Yonelinas, 2002; Diana et al., 2006).

No one questions that recollection can play a role in memorial decisions; indeed, a number of paradigms have been developed to examine the retrieval of specific details of the occurrence of past instances such as plurality discrimination (where participants must discriminate between old words such as *frog* and highly related distractors such as *frogs,* e.g., Hintzman and Curran, 1994; Rotello and Heit, 1999). Malmberg et al. (2004), for example, have proposed a version of Shiffrin and Steyvers' (1997) retrieving effectively from memory (REM) model that incorporates a recollection component for such cases. The controversial question is not about recollection *per se*, but rather whether recollection routinely plays an important role in the normal course of item recognition.

In the dual-process framework, it is generally assumed that familiarity-based processes occur in a rapid and automatic fashion. In contrast, recollection is a slower and more intentional retrieval process. Studies of the temporal dynamics of recognition decisions have provided evidence in support of this view. As discussed earlier, in the response-signal SAT procedure, participants must respond at different temporal deadlines during retrieval, allowing one to plot the growth of the accuracy of recognition decisions with increasing retrieval time. Studies using this procedure have shown that decisions that can be informed by familiarity alone, such as item recognition, can be made more accurately earlier than decisions that require retrieval of specific details such as source judgments (e.g., Hintzman and Caulton, 1997; Hintzman et al., 1998; McElree et al., 1999). These results are consistent with the view that familiarity is available very early after the presentation of the test probe, whereas recollection is a slower retrieval process. Another finding that indicates the early availability of familiarity is seen in false alarms to lures that are similar to studied items. These incorrect responses show an initial early increase and then a decrease with response lag (e.g., Dosher, 1984; Dosher and Rosedale, 1991; Hintzman and Curran, 1994), suggesting that the early responses are based only on familiarity. Somewhat later, item-specific information is retrieved that provides a basis for correctly rejecting similar lures.

Boldini et al. (2004) found a dissociation using the response-signal SAT procedure that they interpreted as further support for the dual-process view of recognition. Under incidental learning conditions, these investigators varied the level of processing of the items at study (pleasantness ratings that would promote deep processing versus maintenance rehearsal that supports only shallow encoding; cf. Craik and Lockhart, 1972) and whether or not the perceptual characteristics (modality) of the stimuli matched between study and test (auditory-visual vs. visual-visual presentations). Modality or perceptual match influenced recognition performance at the short response-signal delays (<300 ms), while level of processing affected accuracy at longer delays (>300 ms). Boldini et al. concluded that both a fast familiarity-based process and a slower recollection-based process contribute to recognition memory decisions.

A number of researchers have adopted Tulving's (1985) remember-know response procedure to distinguish between familiarity- and recollection-based recognition decisions. In this procedure, participants are instructed to classify their old decisions as either 'remember' if they recall any specific detail or details of the prior episode, or 'know' if the test item felt

familiar but no specific details of the previous experience were recollected. Researchers have demonstrated that a number of variables differentially affect these two types of responses (see Gardiner and Richardson-Klavehn, 2000, for a comprehensive review). For example, elaborative rehearsal (deep processing) affects remember but not know responses, whereas maintenance rehearsal (shallow processing) affects know responses without influencing responses classified as remember (Gardiner et al., 1994). Such dissociations have been taken as evidence for the separate contributions of familiarity and recollection to recognition decisions.

This subjective response procedure, however, is not without its critics. Donaldson (1996) and Hirshman and Master (1997) have argued that remember and know responses reflect differences in confidence (or strength of evidence) rather than distinguishing between decisions that are based on two different types of memorial information. Extending this view, Dunn (2004) developed a signal-detection type model in which remember and know responses are derived from two different decision criteria that bisect a single dimension of familiarity. In this model a new decision would be made if the familiarity associated with the test probe was below the lower of the two decision criteria, and an old 'know' response would be made if the familiarity value exceeded the lower criterion. If the familiarity value exceeded the higher of the two criteria, the old decision would then be classified as 'remember.' Dunn showed that such a model can, with appropriate placement of the two decision criteria, account for all of the dissociations between remember and know responses that are taken as support for the dual-process view. In reply, dual-process theorists have questioned whether participants are capable of adjusting their decision criteria in each experimental condition in the manner that Dunn assumed in his model (Diana et al., 2006).

The interpretation of the latency of remember and know responses is also controversial. Remember responses have typically been found to be faster than know responses (Dewhurst and Conway, 1994; Dewhurst et al., 1998, 2006; Henson et al., 1999; Hockley et al., 1999). Wixted and Stretch (2004) have shown that a single-dimension signal-detection model of remember/know responses predicts just this result. In contrast, this finding would appear to be in conflict with the results obtained with the response-signal SAT procedure that shows that familiarity processes are faster than recollection, and with the predictions of dual-process theory. Yonelinas (2002), however, suggests that the slower latency of know responses is an artifact of the remember/know instructions. These instructions specify that a know response should be made when there is no contextual information available to support a remember response. As a consequence, participants must assess recollection before making a know response. Remember responses, in contrast, can be made as soon as any contextual details are retrieved.

A different dual-process interpretation of the latency of remember and know responses has been offered by Henson et al. (1999) and Dewhurst et al. (2006; see also Gardiner et al., 1999; Konstantinou and Gardiner, 2005). Henson et al. suggested that know decisions take longer because it is more difficult to make recognition decisions in the absence of the recollection of contextual details. Dewhurst et al. showed that slower know responses are found both when participants make a single timed remember/know/new decision (one-step procedure) and when the untimed remember-know decision follows a timed old/new response (two-step procedure). Perhaps more interestingly, know responses were also slower when the remember-know decisions were made retrospectively. In this experiment, test items were presented once, and participants made old/new decisions. The test items were then presented a second time, and the participants were asked to indicate whether their previous old/new decisions had been based on familiarity or recollection. The mean RT of the old decisions that were subsequently identified as based on recollection (796 ms) was faster than the old decisions later classified as know (930 ms) or guess responses (1059 ms). Dewhurst et al. reasoned that these RTs reflect genuine differences in the speed of the recognition decision that is not influenced by the requirement to make a remember-know distinction. They concluded that the faster RTs for remember responses reflect the greater ease in making recognition decisions that are supported by the recollection of contextual information. Thus, in their view, recollection need not be a slow and effortful process but "can occur rapidly and automatically" (Dewhurst et al., 2006: 158).

2.24.2.7 Judgments of Event Frequency

It has been previously noted that different tasks, such as source or plurality judgments, have been used to examine memory for specific details of the prior presentation of events. Another illuminating task concerns memory for frequency, or the number of

separate occurrences of an event. Memory for frequency has been studied, in part, to try to determine how multiple occurrences of the event are encoded and represented in memory (for reviews see Howell, 1973; Hintzman, 1976, 1988). Our ability to remember the frequency of events is surprisingly accurate, even in the absence of any intention to do so, and it has been suggested that the encoding of frequency information represents an automatic process (Hasher and Zacks, 1979, 1984).

Logically, estimates of the frequency of prior events could be made in several different ways. Participants might, when asked, try to recollect and count each individual occurrence of an event. Or, participants could estimate the number of occurrences based on the cumulative strength or familiarity that is associated with the event. In either case, participants could also adjust or extrapolate their counts or derived estimates to compensate for failures of encoding or retrieval. These different possibilities form part of the multiple-strategy perspective of frequency estimation developed by Brown (1995, 1997, 2002). This framework provides an excellent example of the flexible manner in which we can interrogate and use our memory. Brown's basic distinction between enumeration-based and nonenumeration-based strategies of frequency estimation also provides a compelling example of a dual-process type of account of memory decisions.

Brown (1995) examined frequency judgments for words presented in either a variable or a consistent context. In each condition participants studied a list of pairs, each consisting of a category label and an exemplar. Different category labels were presented different numbers of times in the list, varying from 0 to 16. In the variable-context condition a different exemplar was presented with each category label (e.g., MAMMAL – dog, MAMMAL – tiger, MAMMAL – horse), whereas in the consistent-context condition the exemplar was always the same (e.g., CITY – London, CITY – London, CITY – London). At test, participants estimated the frequency of the different categories.

A major finding that indicated that subjects used an enumeration or counting strategy in the variable-context condition and a nonnumeration strategy in the consistent-context condition was the changes in the mean RTs of the frequency judgments. The mean frequency estimates and the mean RTs as a function of presentation frequency are shown in **Figure 6**. Mean RT increased sharply as a function of presentation frequency in the variable condition, consistent

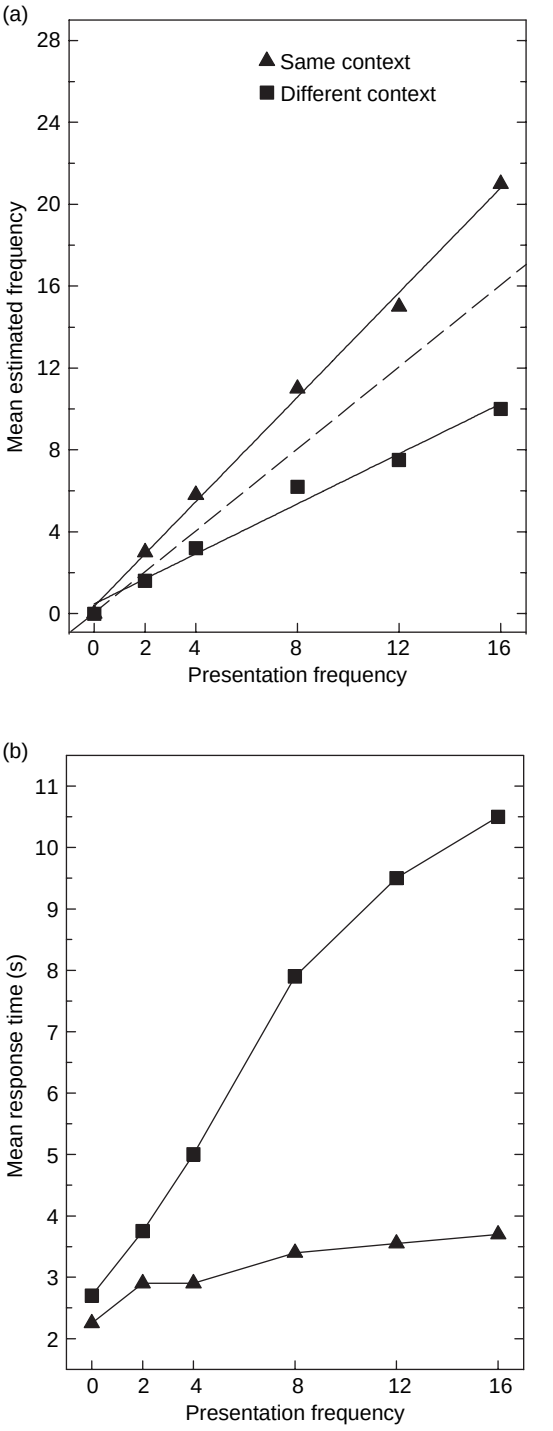

Figure 6 The figure shows the mean frequency estimates (a) and the mean response times (b) for the different-context and same-context conditions from Brown NR (1995) Estimation strategies and the judgment of event frequency. *J. Exp. Psychol. Learn. Mem. Cogn.* 21: 1539–1553; used with permission. In (a), the solid lines represent the best linear fit to the means, and the dashed line represents the actual presentation frequency.

with participants' retrieving and counting the different presentations of the target categories. The different contexts in this condition would support the search and retrieval of the individual occurrences of the categories. In contrast, the increase in mean RT with presentation frequency was far more modest in the same-condition context, indicative of participants' using a more global or inferential estimation strategy that did not involve searching and retrieving memories of the individual presentations.

Three additional findings supported the use of different estimation strategies in the same- and different-context conditions. Participants in the variable-context condition tended to underestimate actual frequency (consistent with failing to retrieve all instances of each event), while participants in the same-context condition tended to overestimate frequency (consistent with a strength-based estimation strategy). Moreover, information about the frequency range influenced the magnitude of participants' estimates in the same- but not in the variable-context condition. Finally, the participants' own verbal protocols indicated their use of the two different strategies.

The evidence indicates that there are two different bases for item recognition decisions and judgments of event frequency – a form of general or global familiarity and recall or recollection. In the next section we consider two tasks that involve memory for associations, or information that represents the relations between items. Each task is believed to involve a recall process, and each task is supported by retrieval cues presented at the time of test.

2.24.3 Associative Recognition and Cued Recall

2.24.3.1 Associative Recognition

In the standard associative recognition paradigm, participants study random pairs of items and then, at test, try to discriminate between intact or studied pairs and new pairings of rearranged study items. This procedure provides a relatively pure test of memory for the associations between unrelated items formed at study, because both intact and rearranged pairs consist of two old items that individually would be similar in their degree of familiarity. Since this task requires memory for specific associations formed at study, it is usually assumed that recall or recollection plays a dominant role in associative recognition decisions (e.g., Yonelinas, 1997; Hockley and Consoli, 1999; Cameron and Hockley, 2000; Rotello and Heit, 2000; Verde and Rotello, 2004).

Gronlund and Ratcliff (1989) compared the time course of the availability of item and associative information using the response-signal SAT procedure following the study of random word pairs. The response signal functions they obtained in their second experiment are shown in **Figure 7**.

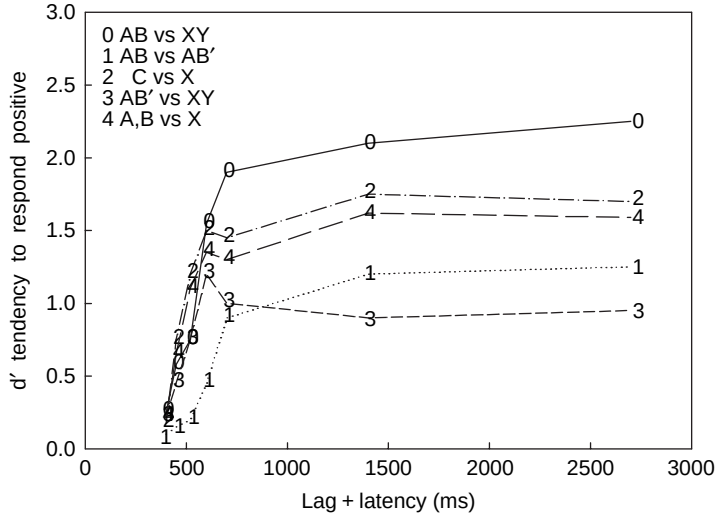

Figure 7 This figure shows averaged response signal functions from Gronlund SD and Ratcliff R (1989) Time course of item and associative information: Implications for global memory models. *J. Exp. Psychol. Learn. Mem. Cogn.* 15: 846–858; used with permission. (The d' tendency to respond positively is given as a function of the total processing time [lag + latency]. Function 0 is AB vs. XY; Function 1 is AB vs. AB′; Function 2 is C vs. X; Function 3 is AB′ vs. XY; and Function 4 is A,B vs. X.)

These functions represent the different types of discriminations that the participants were asked to make. Discriminating between two studied old words versus two new words is shown by Function 0, between intact studied pairs versus rearranged pairs by Function 1, between studied old single words versus new words by Function 2, between rearranged pairs versus pairs of new words by Function 3, and between old words studied in pairs versus new words by Function 4. Functions 0, 2, 3, and 4 represent discriminations that can all be based on item information, or memory for the individual words from the study list. These functions all show similar initial increases in accuracy with response time.

In contrast, the discrimination of intact versus rearranged pairs represented by Function 1 requires memory for the associations made at study. The familiarity of the individual items cannot aid in this discrimination. This function remains at chance at the earliest test lags, and discrimination is delayed relative to the other functions. Gronlund and Ratcliff (1989) also showed that these results are not due to the requirement that participants must read both words of the test pair to discriminate intact from rearranged pairs. Similar results were obtained when the first word of each test pair was presented 200 ms before the second word, and the response signal was measured from the onset of the second word.

Gronlund and Ratcliff (1989) considered two possibilities to explain why the availability of associative information is delayed relative to the availability of item information. One was that for associative recognition a compound cue must first be created. This cue would provide the means to derive a joint match of the two words to memory. Such a matching operation would provide a familiarity value in the same manner as is generally assumed for item recognition. The second possibility was that, whereas item recognition is based on familiarity, associative recognition is based on a slower recall process. In the second view, associative recognition would be similar in nature to cued recall.

2.24.3.2 Cued Recall

In the typical episodic memory cued-recall task, participants study random pairs of items, and at test, one item of each pair is presented as a cue to recall the associated member of the pair. We can also consider questions that require participants to retrieve a specific label, name, or fact in a cued-recall test of semantic memory.

In self-paced recall tasks the typical finding is that average error latency is longer than the latency of correct responses, and these two variables are interpreted differently. Correct latency is taken to be a measure of the amount of information about the item that is available in memory. Error latency, on the other hand, is interpreted as an index of the rememberer's willingness to continue searching memory for the item (e.g., Millward, 1964; MacLeod and Nelson, 1984).

In the cued-recall experiments carried out by MacLeod and Nelson (1984), participants studied number-noun pairs (e.g., 48-dollar) or noun-noun pairs (e.g, forest-elbow) and at test tried to recall the second item given the first item of the pair as a retrieval cue. Participants responded aloud, and a voice key was used to measure response time. The instructions emphasized accuracy with no mention that response time was being measured. Participants could take as much time as they needed, but they had to make a response on every trial even if it was a guess. Across their experiments MacLeod and Nelson manipulated retention interval (1, 3, or 5 weeks), levels of processing at study (classifying the nouns in terms of physical size or number of syllables), and study versus test trial repetitions. All of these manipulations had large and reliable effects on accuracy. For example, in Experiment 1 the mean probability of an error increased with retention interval: 0.54 (1 week), 0.75 (3 weeks), and 0.79 (5 weeks). The corresponding mean latencies (in seconds) for correct responses were 3.78, 6.90, and 7.11, and for incorrect responses were 18.12, 17.91, and 14.97. The results suggest a positive correlation between accuracy and correct RT, but the increase in mean RT was not statistically reliable (similar patterns and statistical outcomes were observed in all of the experiments). The results are, though, very clear in showing that mean RTs for incorrect responses do not vary with the manipulations that affected accuracy and are very much slower than correct responses, consistent with the view that correct and incorrect RTs are measuring different processes.

There is considerable evidence to support the conclusion that the latencies of errors are a measure of the rememberer's willingness to continue searching memory in the belief that the additional effort might prove successful. Thompson (1977, as described by MacLeod and Nelson, 1984) measured response times to respond to general-information questions. Then she asked her participants to make feeling-of-knowing judgments in terms of the likelihood of recognizing the correct answer for the questions that they could not recall. Finally, she gave participants a forced-choice recognition test

for the nonrecalled answers. The initial error latencies were reliably correlated with the feeling-of-knowing judgments, but not with the final recognition performance. Error latency thus appears to reflect what the rememberer believes to be in memory whether or not the belief is true.

Costerman et al. (1992) and Nelson et al. (1984) have also shown that retrieval latencies are longer for nonrecalled targets that are given higher feeling-of-knowing ratings. Participants will also spend more time attempting to retrieve the correct answer when they are in a 'tip-of-the-tongue' state (when they have a strong feeling that a particular answer is in memory and can be retrieved) compared to retrieval failures not accompanied by such a feeling (Schwartz, 2001). Finally, people will also spend longer searching memory and make more correct responses when the rewards for correct responses are high and the penalties for slowness are low, and will spend less time at retrieving with a resultant lower success rate when the penalties for slowness are high and the rewards for correct responses are lower (Barnes et al., 1999).

We have separately considered associative recognition and cued recall. Nobel and Shiffrin (2001) compared the retrieval dynamics of these two tasks with item recognition in order to determine the similarities and differences in the retrieval processes that are involved in each task.

2.24.3.3 A Comparison of Item versus Associative Recognition and Cued Recall

Nobel and Shiffrin (2001) used a voice key to measure RT for item recognition and cued recall following study lists of random word pairs. They found that both correct responses (hits vs. correct recalls) and incorrect responses (false alarms vs. intrusions) were much slower for cued recall compared to item recognition. These differences are clearly captured in the plots of the RT distributions fitted by the ex-Gaussian distribution and shown in **Figure 8**. The RT distributions for cued recall and item recognition are markedly different; the cued-recall distributions are shifted to the right, with a greater positive skewness and higher variance.

Nobel and Shiffrin also compared the retrieval dynamics of discrimination for item recognition (old vs. new single items), associative recognition (intact vs. rearranged study pairs), and paired recognition (intact pairs vs. pairs of new words) with cued recall using the response-signal SAT procedure. Their SAT functions for the three recognition tasks

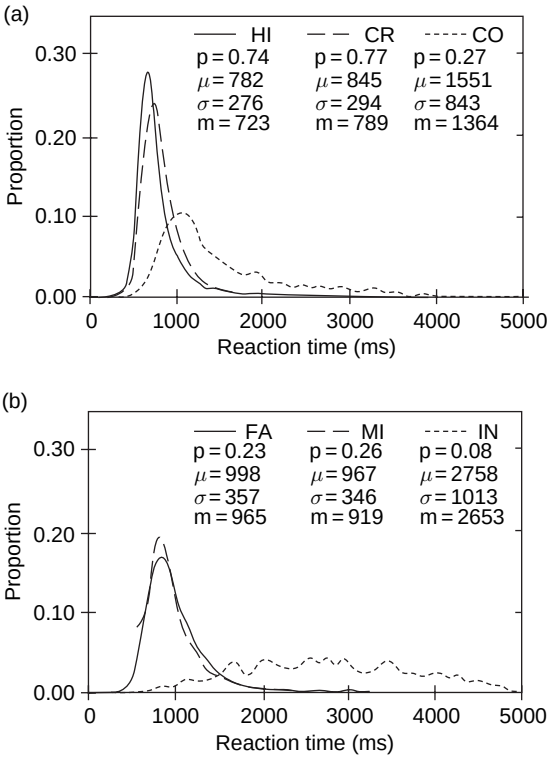

Figure 8 This figure shows the response time distributions for correct responses: (a) item recognition hits (HI) and correct rejections (CR), and correct cued recalls (CO), and for incorrect responses: (b) item recognition false alarms (FA) and misses (MI), and recall intrusions (IN). Observed by Nobel PA and Shiffrin RM (2001) Retrieval Processes in recognition and cued recall. *J. Exp. Psychol. Learn. Mem. Cogn.* 27: 384–413; used with permission. The proportion of each type of response (p), mean RT (μ), standard deviation (σ), and median RT (m) are shown in each panel.

are shown in the top portion of **Figure 9**, and the function for cued recall is shown in the bottom portion. These results show that, compared with item and paired recognition, the retrieval dynamics of associative recognition and cued recall are much slower. Moreover, the retrieval dynamics of associative recognition were statistically consistent with those of cued recall.

Based on their different temporal properties, Nobel and Shiffrin proposed that different retrieval processes give rise to associative and cued-recall performance on the one hand, and single-item and paired recognition on the other. In their view, item and paired recognition are based on a familiarity process that involves parallel access to recent episodic representations and leads to relatively fast 'old' or 'new' responses. In contrast, they argued that cued recall and associative recognition are carried out

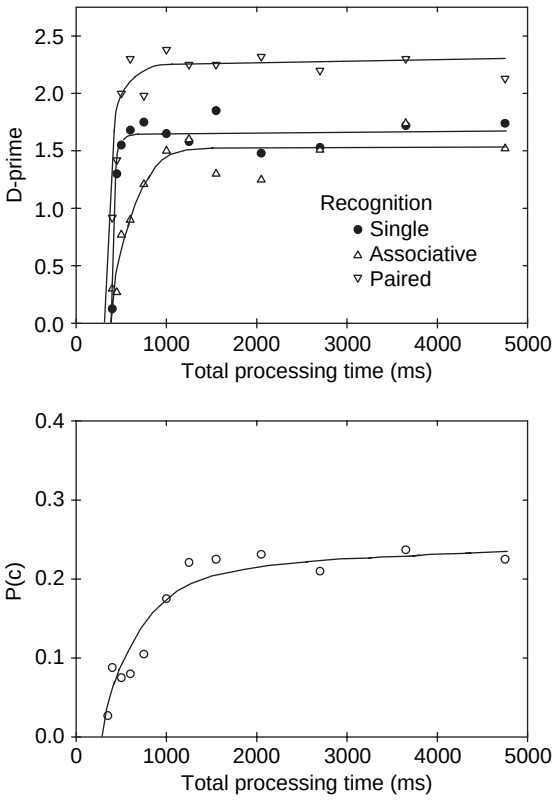

Figure 9 These functions show signal-to-response accuracy as a function of total processing time for single item, associative, and paired item recognition (top panel), and correct cued recall (bottom panel). The data are from Nobel PA and Shiffrin RM (2001) Retrieval processes in recognition and cued recall. *J. Exp. Psychol. Learn. Mem. Cogn.* 27: 384–413; used with permission.

through a memory search process that involves successive sampling and recovery until the relevant representation of the target is found, or the search is abandoned. Diller et al. (2001) provide a formal description of these processes and fits of this model to their results. The general search process that Nobel and Shiffrin suggested underlies cued recall and associative recognition has also been proposed to account for free recall performance.

2.24.4 Recall

2.24.4.1 Analyses of Interresponse Times in Free Recall

Free recall requires participants to output as many items from the study list as possible in any order. Thus, it is a sequential task that is spread out over time. While response times in recognition and cued-recall tasks are relatively fast and typically in the order of a few seconds, free recall occurs over many minutes and is characterized by much longer pauses between responses. For this reason an extended sequential search process is assumed in most models of free recall (e.g., Atkinson and Shiffrin, 1968; Shiffrin, 1970; Raaijmakers and Shiffrin, 1980; Metcalfe and Murdock, 1981; Rohrer and Wixted, 1994; Howard and Kahana, 1999).

Although the analysis of the temporal dynamics of free recall has a long history dating back to the seminal work of Bousfield and Sedgewick (1944), measures of response latency in studies of free recall have not been as common as in studies of recognition memory. Indeed, much of the research on free recall has used probability of recall as the dependent measure. This can be problematic, or even misleading, as Roediger and colleagues (e.g; Roediger and Thorpe, 1978; Roediger et al., 1982; see also Wixted and Rohrer, 1994) have pointed out. For example, in a typical free-recall experiment, performance is evaluated after a fixed retrieval period usually lasting only a few minutes. Few studies have examined recall over extended retrieval periods, yet recall can continue to increase even after 20 minutes (e.g., Roediger and Thorpe, 1978).

In the study of free recall, most of the analysis and modeling of response time has been based on interresponse times (IRTs), the time between consecutive retrievals (e.g., Bousfield et al., 1954; Murdock and Okada, 1970; Roediger et al., 1977; Roediger and Thorpe, 1978; Roediger and Tulving, 1979; Raaijmakers and Shiffrin, 1980; Gronlund and Shiffrin, 1986). The basic finding is that IRTs increase in a positively accelerated fashion with output position.

Bousfield and Sedgewick (1944) asked their participants to recall as many different items as possible from specific categories (e.g., quadruped mammals, U.S. cities) for a period of 18 min. Every 2 min the participants were also asked to draw a line under the last recalled item. Bousfield and Sedgewick then plotted the cumulative number of items recalled as a function of time. This analysis revealed that recall slowed continuously; the greatest number of recalled items occurred in the first interval, and fewer numbers of items were recalled in each successive interval. These latency distributions are well described by the cumulative exponential:

$$R(t) = N(1 - e^{-t/\tau})$$

where $R(t)$ equals the total number of items recalled by time t, N represents asymptotic recall (r recall after

infinite time), and τ represents the mean latency of the recalled items (Bousfield and Sedgwick, 1944; Indow and Togano, 1970; Roediger et al., 1977). An example of a cumulative recall latency distribution and its best-fitting cumulative exponential distribution is shown in **Figure 10**.

IRTs have also been shown to increase in a positively accelerated fashion with output position, and at any given output position, IRT is a good predictor of the number of items yet to be recalled (Raaijmakers and Shiffrin, 1980). Representative data from Murdock and Okada (1970) are illustrated in **Figure 11**. In this experiment, participants studied lists of 20 common words. Vocal responses were tape-recorded, and IRTs were measured as a function of output position. Each of the different curves in **Figure 11** represents a different total of words recalled that varied from four to nine. Rohrer and Wixted (1994) have shown that the increase in IRTs with output position is found for different list lengths and presentation rates of the study list and is thus a general feature of free recall.

Rohrer and Wixted (1994; see also Wixted and Rohrer, 1994) analyzed the characteristics of IRT distributions by fitting the observed latency distributions with the ex-Gaussian distribution that has been used to describe response time distributions for recognition decisions. **Figure 12** shows the recall latency distributions with the best-fitting ex-Gaussian distributions for different list lengths that varied from three to nine words. Longer study lists resulted in a decrease in the probability of recall and an increase in the latency of recall. The estimates of mu, representing the minimum response times, averaged about 1 s and did not vary significantly with list length. In contrast, the estimates of tau, reflecting the skewness of the distributions, increased reliably from approximately 3 to 7 s across list length. Thus, all of the increase in response time with list length was seen in the exponential component of the ex-Gaussian distribution. Rohrer and Wixted proposed that free recall is based on a relatively brief, normally distributed initiation stage (represented by mu) followed by an exponentially distributed search stage (represented by tau).

Bousfield and Sedgewick (1944) and McGill (1963) noted that the exponential increase of the cumulative latency distributions is consistent with a random search model of memory. In this model, individual items are randomly sampled from a search set, evaluated, and replaced. Thus, early in the process almost every sample from the search set will yield a new item to report. As the random search process continues, however, the probability of retrieving a previously sampled item increases until almost every iteration produces an already sampled item. Retrieval of each new item, therefore, becomes progressively slower. Rohrer and Wixted (1994; Wixted and Rohrer, 1994) also supported this basic account of free recall and considered both serial and

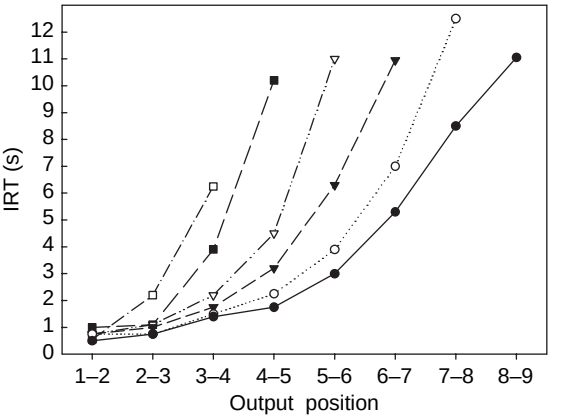

Figure 11 The increase in interresponse time (IRT) with output position. The six curves represent different total numbers of words recalled varying from four to nine. Data from Murdock BB Jr. and Okada R (1970) Interresponse times in single-trial free recall. *J. Exp. Psychol.* 86: 263–267; used with permission.

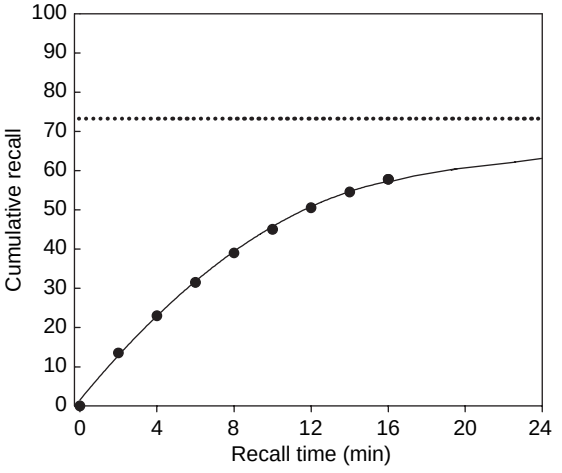

Figure 10 This figure shows a cumulative free recall latency distribution based on Wixted and Rohrer's (1994) plot of Bousfield and Sedgewick's (1944) results for the cumulative recall of pleasant activities over time. The solid curve represents the best-fitting exponential function and the dotted line indicates the asymptotic level of recall.

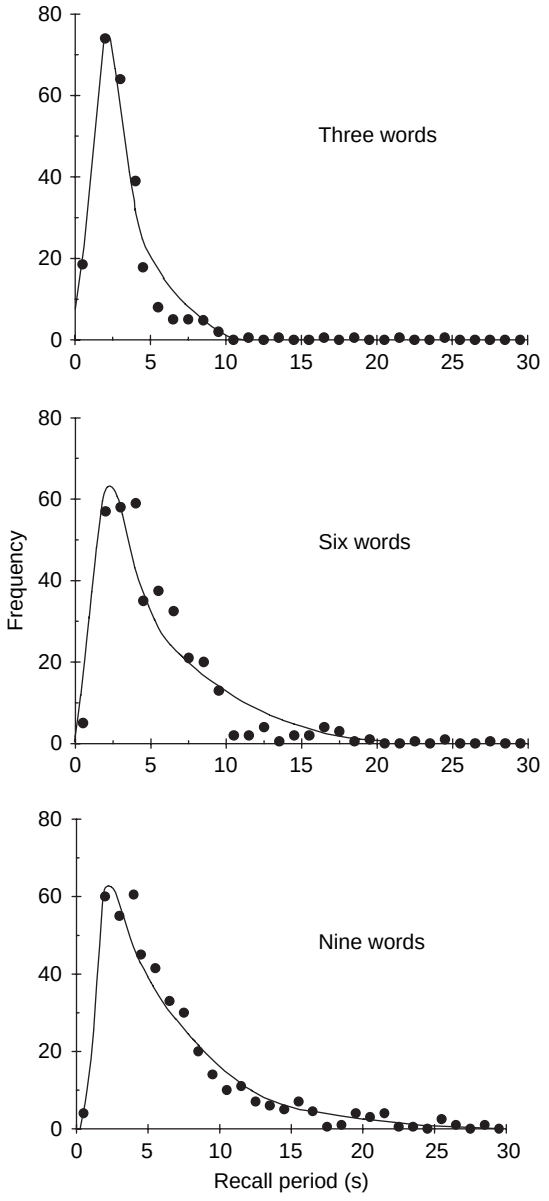

Figure 12 This figure shows recall latency distributions, grouped into 1-s bins, for three different study list lengths reported by Rohrer D and Wixted JT (1994) An analysis of latency and interresponse time in free recall. *Mem. Cogn.* 22: 511–524; used with permission. The fit of the ex-Gaussian distribution is also shown for each observed distribution.

parallel forms of the search process. They also noted that Raaijmakers and Shiffrin's (1980) search of associative memory model, which was developed as an extension of Shiffrin's (1970) random search model, shares many aspects in common with the random search account.

2.24.4.2 The Search of Associative Memory Model

The SAM model (Raaijmakers and Shiffrin, 1980, 1981; Shiffrin and Raaijmakers, 1992) is a sophisticated model of recall that has been successfully applied to a number of different phenomena. Although SAM has given way to the REM model (Shiffrin and Steyvers, 1997), the extension of REM to free recall is borrowed from SAM (Shiffrin, 2003). Very briefly, it is assumed in SAM that memories are represented as 'images' that vary in terms of their associative strengths to each other and the retrieval cue. The retrieval cue serves to activate the search set. In free recall the retrieval cue is the context of the study list. Retrieval consists of repeated sampling of the search set, and the retrieval probability of each item is a function of its associative strength. When an item is retrieved and output, it serves as the retrieval cue for the next sampling. This process continues until a stop rule is invoked based on the elapsed time without a successful retrieval. As Wixted and Rohrer (1994) note, if all images in the search set were activated to the same degree by the retrieval cue, and this remained the case during the entire recall period, then the search process would be equivalent to the random-search-with-replacement model.

A central notion in SAM is that items are associated to varying degrees. Kahana (1996) provided evidence for such associations between items presented sequentially in tests of free recall. Kahana reanalyzed data from a number of free recall studies in terms of the probability of recalling a given item as a function of its distance in the study list from the last item recalled (the conditional response probability). An example of such a function is shown in the left panel of **Figure 13**. Two aspects of these results are notable and are consistent across studies. Kahana termed these aspects of the results contiguity and asymmetry. Contiguity refers to the fact that items tend to be recalled after items that were studied in adjacent list positions. That is, item 8 is more likely to be recalled after item 7 is recalled than after item 5. Asymmetry refers to the finding that for items that were adjacent to each other in the study list and that were recalled after each other, forward transitions (recall item 7 then recall item 8) are about twice as likely as backward transitions (recall item 8 then item 7).

Kahana also examined conditional response latency functions, where IRTs between successively recalled items are plotted as a function of their

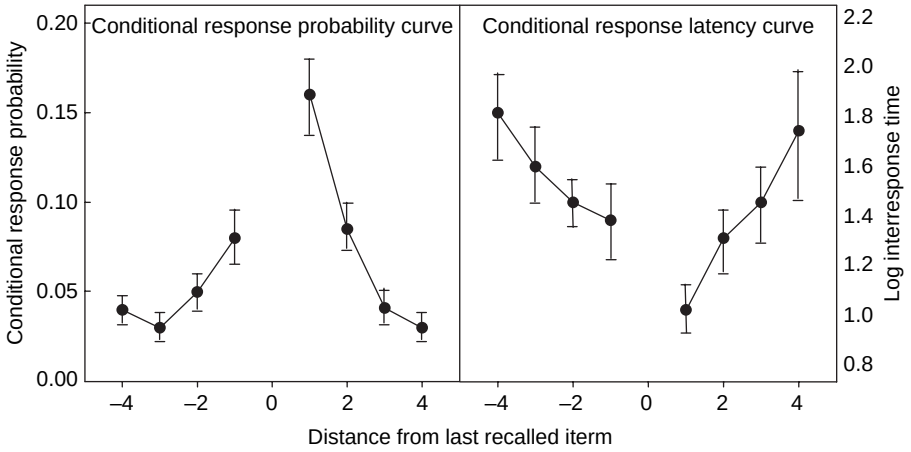

Figure 13 The conditional response probability (left panel) and conditional response latency (right panel) curves reported by Kahana MJ (1996) Associative retrieval processes in free recall. *Mem. Cogn.* 24: 103–109, based on Murdock and Okada's (1970) free recall results; used with permission. (The error bars reflect 95% confidence intervals for each mean.)

proximity in the study list. These IRTs are shown in the right panel of **Figure 13**, and it is clear that the IRT functions mimic the conditional response probability functions – mean IRT increases as the distance between the items' positions in the study list increases. These results indicate that participants studying random lists of words tend to associate the list items in a pair-wise fashion, and the conditional response probability and conditional response latency functions reflect the strength of these associations, as SAM would predict.

2.24.4.3 The Search Set

A critical aspect of any version of the random search-with-replacement model is defining the search set. In the SAM model it is assumed that the retrieval cue serves to activate the search set. Research has shown that certain types of retrieval cues are able to delineate or reduce the search set, whereas others do not.

In categorized free-recall tasks, participants study random lists of words drawn from different semantic categories such as precious stones and occupations and, at test, recall the semantically related items together (i.e., semantic clustering; Bousfield, 1953). Within-category IRTs increase with output position, and IRTs are longer when recall changes from one category to another (Pollio, 1964; Patterson et al., 1971; Graesser and Mandler, 1978; Wingfield et al., 1998). The pattern of IRTs is consistent with a random search and replacement model that operates at the level of the individual categories and with the SAM model given the assumption that the association between items is greater for within-category than between-category items.

Wixted and Rohrer (1994) discuss two studies of semantic memory that illustrate how some retrieval cues can serve to narrow the search set, while other types of cues cannot. Herrmann and Murray (1979) asked participants to recall items from large categories such as bodies of water, and smaller, nested categories such as lakes. Metlay et al. (1971) asked participants to recall items from different categories (e.g., names of U.S. presidents) that could be further subdivided (e.g., presidents' names that contain the letter *y*, $n = 5$, or the letter *s*, $n = 11$). Analyses of the cumulative latency functions indicated that participants could search lakes separately from the larger category of bodies of water, but could not restrict their search for presidents based on letters in their names. Presumably, lakes are stored as a separate category in semantic memory, and people can use this information to reduce the search set. Presidents' names, however, are not stored by their component letters, and the participants thus had to generate the entire set of 32 names and only report those names that conformed to the letter cue.

Roediger and Tulving (1979) provide two episodic memory examples of the failure of retrieval cues to restrict the search set. They presented participants with lists of 64 words in which there were eight words from eight different common semantic categories (e.g., vegetables). In addition, the words in each category began with the same set of eight letters. Participants were informed about the categorical nature of the lists, and the list was presented blocked by

category, with the category name presented before the category exemplars. The participants were not informed of the initial letters of the words in each category. There were three groups of subjects. A control group was instructed to recall all items from all categories. A second group was given four letters on their recall sheets and instructed to recall only the words that did not begin with those letters. The third group was given four category names and were told not to recall items from these categories, but only recall items from the other four categories which were not named. As one would expect based on the results of Metlay et al. (1971), participants could not restrict their search based on letter cues. Surprisingly, and in contrast to the results of Herrmann and Murray's (1979) nested category-cued experiment, participants could also not restrict their search based on the category cues. The mean cumulative recall functions for the critical words were the same in all three groups of Roediger and Tulving's experiment. It is not at all clear why participants would apparently 'waste time' in retrieving an excluded category or continue to search a category after retrieving and rejecting one exemplar from an excluded category.

2.24.4.4 Serial Recall

Serial recall requires participants to report list items in the order of their presentation. The emphasis on order information in this task has led to a focus on transposition errors that occur when an item is recalled in an incorrect list position. Transposition errors are categorized as anticipation errors when an item is recalled in an earlier serial position, and postponement errors when an item is recalled in a later list position.

Transposition errors can be measured in terms of the numeric difference between an item's study position and recalled position. Thus, an item recalled in its correct position would have a transposition value of zero. Anticipation errors have a negative transposition value, and postponement errors have a positive value. Transposition gradients can be measured by plotting the proportion of recalled items as a function of the transposition value. As summarized by Farrell and Lewandowsky (2004), these gradients reveal three regularities of serial recall. First, the gradients peak at a value of zero because most items are recalled in their correct position. Second, the probability of errors declines as the absolute transposition value increases; most errors occur near their correct position. Finally, the transposition gradients tend to be symmetrical. That is, the anticipation and postponement error gradients mirror each other.

In contrast to the long history of measuring response time in free recall, latency has only recently been used as a dependent measure of serial recall performance. Investigators have examined total output times (Dosher and Ma, 1998; Hulme et al., 1999) and correct IRTs for each serial position (e.g., Anderson and Matessa, 1997; Anderson et al., 1998; Cowan et al., 1998; Kahana and Jacobs, 2000; Mayberry et al., 2002; Oberauer, 2003; Farrell and Lewandowsky, 2004). In forward serial recall for subspan list lengths, mean IRTs typically show longer output times for recall of the first item and a relatively flat latency serial position curve for recall of the subsequent items. The delay in recalling the first item may reflect an initial preparatory stage (Farrell and Lewandowsky, 2004). Thus, when cumulative latency is plotted as a function of serial position, the increase in time is approximately linear (e.g., Dosher, 1999). Representative error transposition gradients and serial position curves for accuracy, latency, and cumulative latency reported by Farrell and Lewandowsky (2004) are shown in **Figure 14**.

IRT serial position functions for list lengths that exceed memory span also show the longer output time for the first item, but the remainder of the curve appears to show a more inverted-U shape with faster IRTs at the beginning and end of the list. **Figure 15** shows such functions obtained by Kahana and Jacobs (2000). In this experiment, participants learned lists of 11, 12, and 13 items over 12 experimental sessions. The latency serial position functions are similar for each list length and degree of learning (the first two sessions vs. the last six sessions of the experiment). For middle serial positions, IRTs vary in a nonmonotonic fashion, indicating consistencies in the participants' temporal groupings of the items. The averaged IRT functions, however, obscure individual variability of the groupings. Kahana and Jacobs examined these functions for each participant and reported that the individual IRT functions show that some participants grouped the lists in a consistent manner with longer IRTs for every second, third, or fourth item in the list. The IRT functions for other participants did not show such pronounced patterns, but still showed strong tendencies for longer IRTs in the middle and shorter IRTs at the beginning and end of the lists. Kahana and Jacobs suggested that these patterns reflect the participants' grouping of items into different-sized

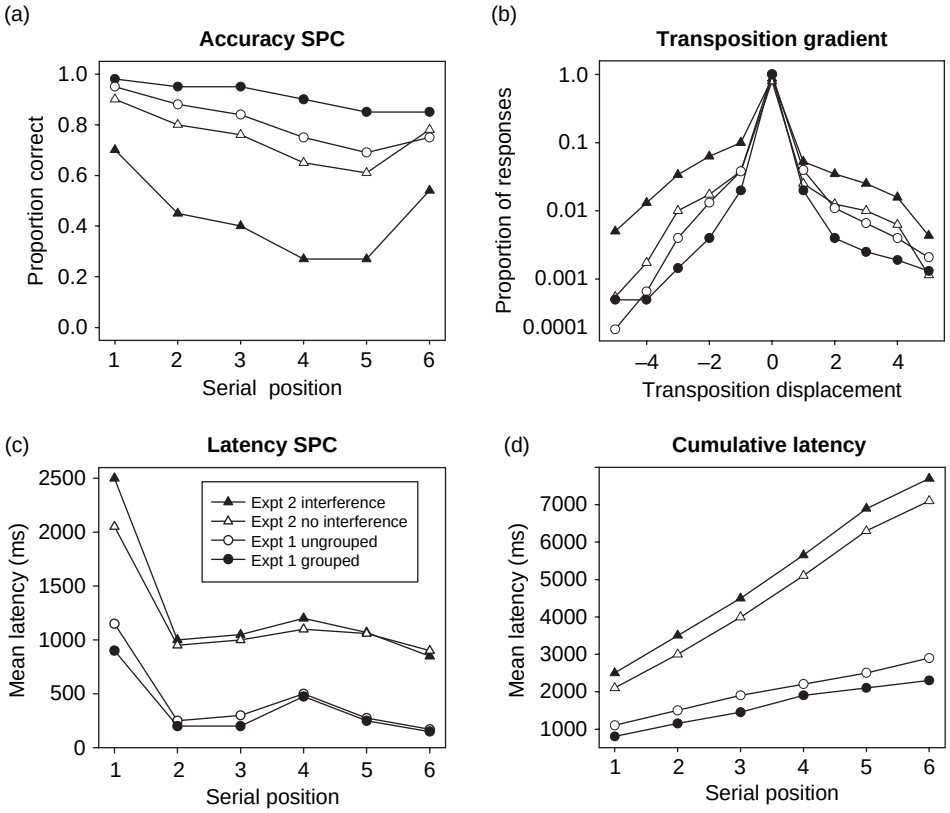

Figure 14 This figure shows the serial position curves (SPC) for (a) accuracy, (b) transposition error gradients, (c) latency serial position curves, and (d) cumulative latency serial position curves for Experiments 1 and 2 of the study of serial order recall from Farrell S and Lewandowsky S (2004) Modelling transposition latencies: Constraints for theories of serial order memory. *J. Mem. Lang.* 51: 115–135; used with permission.

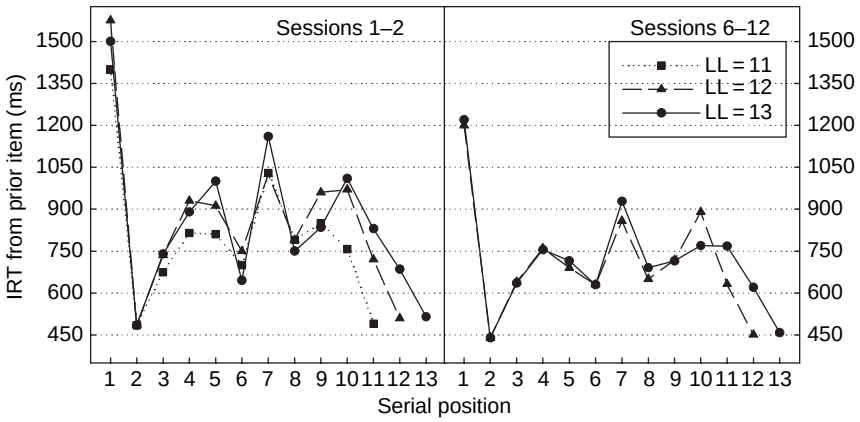

Figure 15 Average interresponse times (IRT) as a function of serial position for all perfect trials for each list length. From Kahana MJ and Jacobs J (2000) Interrersponse times in serial recall: Effects of intraserial repetition. *J. Exp. Psychol. Learn. Mem. Cogn.* 26: 1188–1197; used with permission. The left panel shows data from the lists from sessions 1–2, and the right panel shows the results for sessions 6–12.

chunks such that IRTs are longer for the first item of a group than the items within a group.

2.24.5 Conclusions and Controversies

2.24.5.1 Matching versus Searching

It is quite clear that there are both qualitative and quantitative differences between the temporal dynamics of item recognition on the one hand, and associative recognition, cued recall, free recall, and serial recall on the other. Item recognition responses are generally quite fast for both correct old and new decisions. In contrast, RT is much slower in associative recognition and recall-based tasks, and the latencies of correct and incorrect recall responses are measures of different processes.

It seems reasonable to conclude, therefore, as did Nobel and Shiffrin (2001), that item recognition is generally based on a strength-of-evidence variable, or familiarity, that is derived from a relatively fast matching process that is carried out in parallel, whereas associate recognition and recall are based on slower, sequential, search-based processes. Such a conclusion must be correct in the broader sense. Familiarity alone would usually provide a sufficient basis for item recognition decisions, but would not suffice for associative recognition or recall where more specific information must be retrieved. Such a sweeping conclusion, however, glosses over a number of important questions and details that remain to be resolved. This chapter concludes with brief considerations of some of these controversies.

2.24.5.2 Familiarity versus Recollection in Item Recognition

Most, if not all, researchers agree that recollection can play a role in recognition decisions, and most global matching models of recognition memory allow for decisions to be based on either matching strength or on recollection of details (see Hintzman and Curran, 1994, for a discussion of this point). The controversial question is whether recognition is largely based on familiarity, as single-process theorists advocate, or routinely involves recollection, as dual-process theorists have argued.

An argument in favor of the single-process view is that recognition decisions are typically much faster than decisions involving recall. If recollection involves a slower search process, it cannot be a general feature of recognition decisions. Nobel and Shiffrin (2001), however, point out that response latency would not be sufficient to distinguish between single-process and dual-process views of recognition if it were assumed that recollection involves a fast, truncated search process. An example of such a truncated search process would be stopping after the retrieval of the first sample in a successive sampling and recovery search process. Nobel and Shiffrin also noted that it might be almost impossible to distinguish between single- and dual-process views of recognition if there is a strong correlation between familiarity and the probability of retrieval success of such a truncated search process.

The notion of a truncated search process underlying recollection also provides an answer to the question of whether recollection-based recognition decisions are slow relative to familiarity-based decisions (e.g., Yonelinas, 2002) or the reverse (e.g., Dewhurst, et al., 2006). Recognition decisions that are based on any retrieved detail of the prior experience, and that participants characterize as a 'remember' rather than a 'know' old response, could be associated with very fast RTs, as Dewhurst et al. (2006) have proposed, when retrieval is based on a truncated search process. In contrast, recognition decisions that must be based on the retrieval of a specific detail or details of the prior episode, such as source discriminations or discriminations between targets and highly similar lures (e.g., 'frog' vs. 'frogs'; Hintzman and Curran, 1994), would be much slower decisions, because the search process could not be truncated or abbreviated. Thus, recollection might be fast or slow, depending on the task requirements.

2.24.5.3 Defining the Search Set

In models of recognition memory the search set is often defined in functional terms. For example, Ratcliff (1978) used a resonance metaphor to describe the memory set. In this metaphor, the test probe and item representations in memory are seen as tuning forks. The tuning fork representing the probe rings and evokes sympathetic vibrations from all items in memory that have tuning forks with similar frequencies. Thus the search set is defined by the degree to which items in memory are similar to the test probe. Search sets are also defined by degree of activation or match in several global matching models such as MINERVA 2 (Hintzman, 1988), SAM (Gillund and Shiffrin, 1984), and REM (Shiffrin and Steyvers, 1997). In distributed global matching models such

as TODAM (theory of distributed associative memory; Murdock, 1982) and CHARM (composite holographic associative recall model; Metcalfe, 1982), the functional memory set is the entire memory representational system. In all of these models the size of the search set is not a critical issue, because the potentially large number of items in the search set that are not highly activated, or are very dissimilar to the probe, do not greatly influence the decision system and thus do not affect performance.

The size of the functional search set may also not be a problem for dual-process models of recognition that assume that recollection is based on a truncated search process, and for models of associative recognition and cued recall. In these cases, the search process may be very focused because of the retrieval cues that are present at test.

The size of the search set is, though, a critical aspect of any version of the random search-with-replacement model of recall. In the SAM model it is assumed that retrieval cues serve to activate the relevant search set. These retrieval cues are the context and successively retrieved items, and they will activate images in memory that are associated with them. Thus, the functional search set comprises the memory images that have an association with a given set of cues. SAM has been successively applied to a broad range of recall phenomena, but its applications have largely been restricted to recall of a given list based on the associations formed between the list items. Thus the functional search set for most of the simulations of SAM has been restricted to the study list.

Sirotin et al. (2005) present an extension of the SAM model (eSAM) that adds a semantic memory store, a contextual drift mechanism, and a memory search mechanism that uses both episodic and semantic associations. These researchers show that eSAM is capable of simulating the effects of both preexperimental semantic knowledge and prior episodic information in an episodic free-recall task. These additions mean that the functional search set in eSAM is thus potentially very large. This model has not been extended to fitting IRTs, and the temporal characteristics of many of the effects that the model has been applied to have not been evaluated. Thus, the implications of the assumptions of this extended search model for the temporal dynamics of free recall are not known.

As previously discussed, research has shown that certain types of retrieval cues are able to delineate or reduce the search set, whereas others do not. For example, cues defining a semantic category are effective cues in recall from semantic memory (e.g., Hermann and Murray, 1979) and give rise to semantic clustering in recall of categorized lists (e.g., Graesser and Mandler, 1978). While participants can use such relevant cues to guide search and retrieval, it is not clear why the participants in Roediger and Tulving's (1979) episodic memory study could not use similar types of cues to omit categories and exemplars from the search process.

2.24.5.4 Contradiction and Knowing Not

A problem conceptually related to the issue of defining the relevant search set is the question of how we know what we do not know, or what Kolers and Palef (1976) termed 'knowing not.' Most models of recognition memory, such as Sternberg's serial search model of short-term memory, Ratcliff's diffusion model, and the family of global matching models, treat a negative response as a default decision that is reached when there is insufficient evidence to support a positive decision. In this view, the latency of correct rejections cannot take less time than the slowest positive responses. This has been shown to be generally the case in standard recognition studies involving lists of unrelated words.

Mewhort and Johns (2000; Johns and Mewhort, 2002, 2003) have challenged this view with their demonstration that participants were fastest to correctly reject negative probes when they did not share a feature with the study set (the extralist feature effect). Mewhort and Johns argued that negative decisions are not a default decision, but rather, are based on an assessment of contradictory evidence. Kolers and Palef (1976) also challenged the default interpretation of negative responses based on their demonstration that participants can make faster negative than positive responses when deciding whether or not they had visited different cities. Shanon (1974, as cited in Kolers and Palef, 1976) found that the latency of negative responses was faster when the participant also had not visited the country in which the city is located. That is, participants were faster to say that they had never visited Paris if they had also never been to France. Kolers and Palef took this as evidence that participants might have used a hierarchical search strategy. With such a strategy one might be able to quickly respond that they have not been to Paris if they have not been to Europe or have not been to France, but their response time would become progressively slower the more specific or fine grained the search

set must become. To borrow Mewhort and Johns' concept of contradiction, participants are faster to reject an item when the features of the probe (have you been to Toronto) contradict a large search set (North America) than progressively smaller search sets (Canada and Ontario). Thus, knowing not may involve the use of contradiction or be another manifestation of the extralist feature effect. Both effects may require that the appropriate search set be distinct or well defined, and that the relevant feature dimension or dimensions be highly salient.

2.24.5.5 Temporal Dynamics and Models of Memory

The analysis of RT provided the initial basis for search models of free recall and has provided a means to test and compare models of recognition and cued recall and, more recently, serial recall. Nevertheless, models of memory have not, for the most part, been directly concerned with accounting for RT. Gronlund and Ractliff (1989) pointed out that a general problem of the first generation of global matching models of item recognition was that they were essentially static and thus did not have mechanisms that are able to naturally predict the temporal dynamics of the search, retrieval, and decision processes. Diller et al. (2001) essentially echoed this comment when they noted that

> . . . although the use of single-step retrieval for recognition and sequential search for cued recall has rather obvious implications for RT predictions, the SAM and REM models have been restricted for the most part to accuracy predictions. (Diller et al., 2001: 414).

In a similar vein, Farrell and Lewandowsky (2004) commented that models of serial recall have neglected recall times because they have historically been concerned with measures of accuracy.

Gronlund and Ratcliff went further to suggest that temporal retrieval dynamics "provide a set of phenomena with which the next generation of theories must deal" (Gronlund and Ractliff, 1989: 857). This prediction has not been fulfilled because, I believe, we still do not fully understand the relationship between accuracy and RT. As we have seen, it is typically the case that accuracy and latency covary. That is, a number of variables such as confidence, list length, presentation rate, number of presentations, test, and study position influence accuracy and RT in complementary ways. It is therefore tempting to conclude that these two dependent variables are "two sides of the same coin" (to coin the phrase used by Kahana and Loftus, 1999). But there are also examples in which accuracy and RT are not highly correlated and therefore provide different measures of performance (e.g., MacLeod and Nelson, 1984; Rohrer and Wixted, 1994; Nobel and Shiffrin, 2001). As Kahana and Loftus (1999) argued, accuracy and RT can only be two sides of the same coin when the cognitive process of interest is a single operation that acts on a single type of information. Clearly, the processes underlying memory search and retrieval are much more complex, and thus accuracy and RT, although often highly correlated, cannot be simply two sides of the same coin.

Because it is not obvious how to marry the processes that produce changes in accuracy with those that give rise to the observed changes in RT, different approaches have been taken to model accuracy and RT. To use models of item recognition as an example, in Ratcliff's (1978) diffusion model the temporal dynamics are a property of the comparison process. In contrast, Hockley and Murdock (1987) proposed a dynamic model of the decision system which evaluates the outcome of the matching process over time to provide a means for Murdock's (1982) distributed associative model of memory, TODAM, to account for RT. Diller et al. (2001) adopted yet another approach in their assessment of retrieval completion (ARC-REM) model that they developed to enable REM to account for RT in recognition and recall. In this model it is assumed that the features of the probe are activated and become part of the comparison process gradually over time. The rememberer, given time, will wait until a sufficiently high proportion of probe features have become active and then interrupt the retrieval process to read out the current odds value and respond accordingly. This model has the ability to dissociate accuracy from RT.

Just as there are different ways to represent information in memory and different ways to search and retrieve this information, there are different ways to represent the temporal dynamics of the search, retrieval, and decision processes. I do not believe we will be able to solve one answer at a time. Rather, we must continue to explore different ways to represent the interplay between the encoding, representation, and retrieval of information on the one hand, and their associated temporal dynamics on the other, in order to eventually understand how they are related to one another.

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2.25 Mathematical Models of Human Memory

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2.25.1 Introduction

In this chapter, I will provide a brief introduction to formal models of memory. Although such approaches have become quite successful, it would be an overstatement to say that they enjoy a great popularity among mainstream experimental researchers interested in human memory processes. There are probably several reasons for this skepticism, but an important one seems to be that it is not always easy to see what a model adds compared to a verbal theory or explanation. In this chapter, I will discuss a number of the most important theoretical approaches, paying special attention to the issue of what these models can do that could not be done using only verbal theorizing.

Formal or mathematical models of memory can be broadly classified in terms of their scope and generality. At the simplest end, we have descriptive models that try to characterize lawful empirical regularities. Memory researchers, for example, have tried to characterize the form of the forgetting function, the function that relates memory performance (percent recalled or some other measure) to the retention interval, the time since the item was studied. Although several promising candidate functions have been proposed (most notably power and logarithmic functions; see Wixted and Ebbesen, 1991), the issue of which function best describes the forgetting curve has not been resolved. One reason is that many candidate functions capture the basic aspects of the forgetting curve, i.e., a curve that is characterized by a decreasing rate of decline (the older the trace, the less likely it is that it will be forgotten in the next unit of time). Another reason is that the comparison between different functions is complicated by the fact that some models are more versatile than others (can handle more different shapes, can mimic data generated by other models), which means that it is easier for such a model to fit any given set of data, although at the expense of its generalizability to new data (for a discussion of these issues, see Lee (2004) and Myung and Pitt (2002)). Hence, although such descriptive models may be useful for predictive purposes, a shortcoming of these models is that they are limited in scope, predicting only one type of relation. What is lacking in such models is an account of what causes the forgetting, making it difficult to devise experimental tests that would pit one model against the other. Similar issues arise in attempts to model the learning curve, the function that describes the increase in performance as a function of the number of learning or training trials.

At the next level, we have models that try to account for the basic learning and forgetting data in terms of what happens to individual memory traces. One issue that the descriptive models usually do not discuss is whether the proposed forgetting (or learning) function describes each and every memory trace or just the average of a large number of separate curves. This question was the main focus of a large number of studies conducted in the 1950s and 1960s. In a series of studies using the so-called RTT paradigm, in which one study or reinforcement trial (R) was followed by two test trials (T) without any additional study in between, it was shown that the probability of a correct response (success) on the second test trial given no success at the first test trial was nearly zero and much lower than the average probability of a success. This seemed to be

indicative of one-trial or all-or-none learning: The item was either completely learned on the study trial or not at all. This contradicted the standard assumption that learning was gradual. Such gradual learning functions were predicted by so-called linear operator models that assumed that the probability of a success on a given trial n was a simple linear function of the probability of success on the previous trial. Thus,

$$p_{n+1} = Q(p_n) = \alpha p_n + \beta \qquad [1]$$

where α and β are parameters that depend on the nature of the reinforcement given on trial n. The crucial assumption here was that this function described the behavior of each and every item independent of whether the response to that item had been correct on trial n.

To account for the results of the RTT paradigm, an alternative model was proposed in which the learning of an item was all-or-none: The item was either learned, always leading to a correct response, or not learned, in which case the probability of a success was at chance level. This model still predicts a gradual learning curve because such a curve represents the average of a number of items and subjects, each with a different moment at which learning takes place. The learning process in the all-or-none model may be represented by a simple Markov chain with two states, the conditioned or learned state (L) in which the probability correct is equal to 1, and the unconditioned state (U) in which the probability correct is at chance level (denoted by g). The following matrix gives the transition probabilities, the probabilities of going from state X (L or U) on trial n to state Y on trial $n+1$.

$$\begin{array}{l} \text{state on trial } n+1 \quad \text{P(Correct)} \\ \qquad\quad L \quad\quad U \\ L \begin{bmatrix} 1 & 0 \\ c & 1-c \end{bmatrix} \begin{bmatrix} 1 \\ g \end{bmatrix} \\ U \\ \text{state on trial } n \end{array} \qquad [2]$$

Strong support for the all-or-none model was obtained in an experiment by Bower (1961) in which subjects were presented lists of ten paired associate items consisting of a consonant pair and either the digit 1 or 2. This experiment was a breakthrough in the mathematical modeling of learning and memory because it did not just fit the learning curve but also a large number of other statistics (such as the distribution of the number of errors and of the trial of last error). The model fitted Bower's data remarkably well and this set a new standard for mathematical modelers.

One of the key predictions of the model was what became known as presolution stationarity: If the all-or-none assumption holds, the probability of responding correctly prior to learning (or prior to the last error) had to be constant:

$$P(e_{n+1}|e_n) = \text{constant for all } n \qquad [3]$$

Figure 1 shows the data from Bower's (1961) experiment and the predictions from the all-or-none and linear models. The data are in almost perfect agreement with the predictions of the all-or-none model and clearly inconsistent with those of the linear model. It may be shown that this presolution stationarity property is crucial for the all-or-none model in that the combination of this property together with the distribution of the trial of last error is a sufficient condition for the all-or-none model. That is, if both of these properties hold, the all-or-none model has to be the correct model. Since this property is strong evidence for the all-or-none model, it is understandable that proponents of gradual learning models tried to reconcile the finding with a model in which learning was more gradual. The argument that was used was based on the idea that the result might be explained if individual differences in the speed of learning were assumed. If items and/or subjects differ in their learning rate, errors on later trials might be

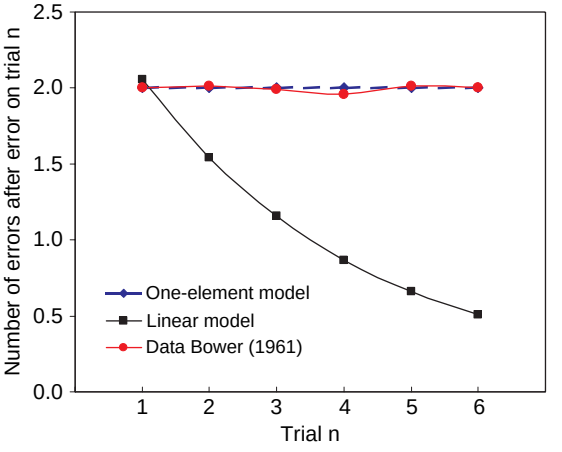

Figure 1 Number of errors following an error on trial n as a function of n. Data from Bower GH (1961) Application of a model to paired-associate learning. *Psychometrika* 26: 255–280; predictions for the all-or-none model and the linear model.

coming mainly from the more difficult items and from subjects with slower learning rates. However, in an ingenious analysis, Batchelder (1975) showed that this could not work. Batchelder analyzed the predictions of the linear operator model (eqn [1]) using a completely arbitrary distribution for the learning rate parameter and proved that it was impossible for the linear operator model to fit these results.

The success of the all-or-none model soon led to a series of related models that were based on the notion of discrete changes in the state of studied items. One issue that was investigated was whether this notion could account for transfer effects based on underlying conceptual categories. For example, suppose that several lists of paired associates are learned in succession where the stimulus items that belong to a particular conceptual category all have the same response. If learning is all-or-none, we might assume that a particular item will be learned in an all-or-none fashion as long as the conceptual relation is not yet discovered, but that once the relation has been discovered (which itself involves an all-or-none process) any new item belonging to the same category will start in the learned state rather than the unlearned state (i.e., no errors will be made on this item). Greeno and Scandura (1966), Batchelder (1970), and Polson (1972) showed that a relatively simple generalization of the all-or-none model gave a good account for the results of such experiments.

Although the all-or-none model was quite successful, the experiments that it was applied to were extremely simplified (simple stimuli, coupled with one of two possible responses). From the outset it was clear that the model would not hold for more complex experiments. However, perhaps the basic idea of the all-or-none model could be generalized in such a way that more complex learning tasks might be described as involving a series of stages, each stage being completed in an all-or-none manner. The most successful attempt at this type of generalization of the all-or-none model can be seen in the work of Greeno and associates (Greeno, 1968, 1974; James and Greeno, 1970; Humphreys and Greeno, 1970). Greeno did an extensive theoretical and empirical analysis of a two-stage learning model. As there are now two learning rate parameters, one for each stage, it becomes possible to look at the factors that affect each of these parameters and hence provide an interpretation for what the separate stages stand for. Contrary to the traditional two-stage theory of paired-associate learning (Underwood and Schulz, 1960), which maintained that the first stage involved a process of response learning and the second stage stimulus–response association, the results from the two-stage model proposed by Greeno were largely consistent with the idea that the first stage involved storage of the pair and the second stage learning to retrieve the pair.

Perhaps the most significant extension of the all-or-none model was proposed by Atkinson and Crothers (1964), who included the notion of a short-term memory state. The assumption here was that an item could move to a short-term state when it was studied but that it could move back to the unlearned state on subsequent trials when other items were being studied. Thus, such an item would show short-term forgetting: When tested immediately after having been studied, the response would be correct; however, when retested after several intervening trials, the probability of a correct response would be back at the baseline level (unless the item had moved to the learned state). The learning process in such models can be described using two transition matrices, one that applies when the target item is presented ($\mathbf{T_1}$) and one that applies when another item is presented ($\mathbf{T_2}$):

$$\mathbf{T}_1 = \begin{array}{c} \\ L \\ S \\ U \end{array} \begin{array}{c} \begin{array}{ccc} L & S & U \end{array} \\ \begin{bmatrix} 1 & 0 & 0 \\ d & 1-d & 0 \\ wc & w(1-c) & (1-w) \end{bmatrix} \end{array} \qquad [4a]$$

$$\mathbf{T}_2 = \begin{array}{c} \\ L \\ S \\ U \end{array} \begin{array}{c} \begin{array}{ccc} L & S & U \end{array} \\ \begin{bmatrix} 1 & 0 & 0 \\ (1-f)r & (1-f)(1-r) & f \\ 0 & 0 & 1 \end{bmatrix} \end{array} \qquad [4b]$$

where L is the state in which the item has been learned, S is the short-term memory state, and U is the state in which the item is not learned.

Several variants of such LS-models (Long-Short) were introduced, including ones that assumed that there could be additional storage (as well as forgetting) on intervening trials (note the parameter r in $\mathbf{T_2}$). This notion is of course related to the more general concepts of rehearsal and consolidation. The idea of storage on trials intervening between presentations might provide an explanation for the spacing effect, the finding that (in general) spaced study presentations are more beneficial for

later recall than massed presentations. Bjork (1966), Rumelhart (1967), and Young (1971) developed (increasingly complex) models to account for such spacing effects in paired-associate recall, leading to a model that became known as the General Forgetting Theory. However, these models never gained much popularity, perhaps because they were introduced at a time when the emphasis in the formal modeling of memory processes shifted to the next level following the 1968 publication of the Atkinson-Shiffrin model.

The theoretical framework that was proposed by Atkinson and Shiffrin (1968) made a distinction between structural properties of the memory system that were fixed and permanent, and control processes that operated on those structures. Control processes included such processes as rehearsal, coding, and retrieval strategies. The Atkinson-Shiffrin model assumed that information first enters a Short-Term Store (STS) and that the processing within STS determines storage in a permanent memory system, Long-Term Store (LTS). Information that is still present in STS at the time of testing will be readily available, but information that is no longer in STS will have to be retrieved from LTS. The probability of successful retrieval from LTS was a function of the strength of the LTS trace, which was itself determined by the nature of the processing in STS.

An important advancement of the Atkinson-Shiffrin theory was the model that was proposed for rehearsal processes in STS. It was assumed that at any time only a few items could be simultaneously in STS and that once STS was filled, any new item would have to replace one of the other items in STS. This idea led to the introduction of the concept of a rehearsal buffer as a simple model for rehearsal in STS, or rather a family of models since various alternatives were considered that differed in whether older items were more or less likely to be replaced by a new item. In these models, it was assumed that storage in LTS is directly related to the length of time that a particular item stays in the buffer. This storage assumption has often been misinterpreted as implying that the Atkinson-Shiffrin theory would assume that only time in STS determines how much information gets stored in LTS. However, Atkinson and Shiffrin proposed that rehearsal in STS is a control process and that the nature of the processing in STS will vary depending on the requirements of the task. In some tasks, the emphasis will be on simply maintaining the information in STS, but in other tasks the emphasis is on coding the information in LTS. This distinction between coding and rehearsal (or elaborative and maintenance rehearsal as it was later called) made it possible to accommodate levels-of-processing effects, i.e., the notion that the nature of the processing in STS determines the probability of later recall. Thus the standard textbook story that supposes that there is a fundamental difference between the Two-Store model and the levels-of-processing framework proposed by Craik and Lockhart (1972) is incorrect (see also Raaijmakers, 1993).

The major significance of the Atkinson-Shiffrin model was that it was not simply a model for one specific experimental task but a general framework within which models could be formulated for specific tasks. Thus, in addition to the short-term memory tasks investigated in the 1968 paper, the same general framework was applied to search and retrieval processes in long-term memory (see Shiffrin, 1968; Shiffrin and Atkinson, 1969), free recall (Shiffrin, 1970), and recognition memory (Atkinson and Juola, 1974). This was a major step forward compared to, for example, the General Forgetting Theory that did not allow a simple generalization to free recall or recognition paradigms. This type of approach in which a general framework is presented within which specific models are developed for specific tasks is a common characteristic of most current models of memory. In the next sections, I will discuss a number of such approaches with special attention given to the question how these models are able to provide novel explanations for experimental findings.

2.25.2 The ACT Model

The first model that we will discuss in more detail is the ACT theory developed by John Anderson. The ACT theory (Adaptive Control of Thought) has its roots in early theories of spreading activation (Collins and Loftus, 1975) and the work of Newell and Simon on cognitive architectures. ACT is not just a model for memory processes but aims to provide a general framework or architecture for all cognitive tasks (sometimes termed a Unified Theory for Cognition). Although ACT has undergone many changes since it was first presented in 1976, there are a number of aspects of the theory that have remained more or less the same over the years. First, ACT does not make a fundamental distinction between semantic and episodic memory. All knowledge facts and all experiences are stored in a single declarative memory system. Second, ACT makes a distinction between a working memory system, a declarative memory system, and a procedural memory

system. Declarative memory is modeled as a large set of interconnected nodes or chunks, while the procedural system has the form of a large set of production rules (rules of the form IF conditions A, B, and C are satisfied, THEN action Y is performed) that fire whenever their conditions are satisfied. Although one sometimes gets the impression that ACT is more a programming language in which various cognitive tasks may be modeled, the ACT framework has been used to develop detailed quantitative models for various memory tasks that do make specific and testable predictions.

In the original ACT model (Anderson, 1976), retrieval of a target item B from a cue item A was based on a notion of spreading activation in which a particular node was either active or inactive. The spreading of activation was controlled by the relative strength of the links from the cue to the nodes that were connected to the cue node. Once a node was activated, it would in turn start to activate other nodes associated with it (a threshold was assumed to prevent activation of all nodes). Since activation is all-or-none, response latency was determined by the time it took for activation to spread to the target node. However, using a primed lexical decision task, Ratcliff and McKoon (1981) showed that the semantic distance between the prime and the target does not affect the time at which the facilitation due to priming begins to have its effect, although it does affect the magnitude of the facilitation. Anderson (1983a,b) proposed a revised version of ACT, named ACT*, in which nodes were no longer activated in an all-or-none fashion. In ACT*, each node had a continuously varying activation value. The larger the activation value, the faster and the more likely it was that the trace would be retrieved.

Anderson (1981, 1983b) showed how this model could be used to explain a number of memory phenomena. In ACT*, performance is determined by the strength of the target trace relative to that of other traces associated with the retrieval cues used. On each presentation of an item, there is a probability that a trace will be formed and once formed, further presentations provide additional strength to the trace. The strength added to a trace was assumed to decay according to a power law. More specifically, the trace strength (S) for a trace that has been strengthened n times is equal to:

$$S = \sum_{i=1}^{n} t_i^{-b} \qquad [5]$$

where t_i is the time since the i-th strengthening and b is a decay parameter (between 0 and 1).

Anderson (1981, 1983b) showed that the ACT* model predicts a large number of standard findings from the memory literature. One intriguing result that came out of this analysis was that performance in recall tasks is a function of both the absolute and the relative strength of the target trace. In ACT*, the probability of recall is a function of both relative and absolute strength, but the latency is a function of the relative strength only. Anderson (1981) demonstrated that this implies that in a standard interference task there will be an interference effect on latency, even when the conditions are equated on percent correct. This result implies that it will not be possible to completely equate interference and control conditions at the end of second-list learning, as was implicitly assumed in many experiments on interference and forgetting (e.g., when both conditions learn to the same criterion). Basically, this prediction is due to the fact that if probability of recall is a function of both relative and absolute strength, it must be the case that in the condition in which it takes longer to reach a particular recall criterion, the absolute strength will be larger at the point where the criterion is reached. Hence, to get equal percent recall, this must be compensated for by a lower relative strength, hence a longer latency.

In a similar way, it can be shown that if the second list is again learned to a fixed criterion, performance on the second list may show proactive facilitation instead of interference, when it is tested after a delay in such a way that differences in relative strength are less important and performance is mostly determined by the absolute strength of the target trace. The latter may be experimentally accomplished by giving an unpaced test in which subjects are given ample time to produce the response. In such a test, differences in relative strength become less important since eventually the trace will be retrieved, although it may take a long time. Anderson (1983b) reports results that confirm this counterintuitive prediction. Mensink and Raaijmakers (1988) showed that these predictions hold not only for the ACT* model, but for all models in which performance is a function of both relative and absolute strength.

The latest version of ACT, called ACT-R (ACT-Rational), is based on a number of assumptions that are quite different from ACT*, yet the model shares enough features with the older models to justify using the same acronym. There are two important differences with ACT*. First, ACT-R no longer assumes a spreading activation conception of memory retrieval. Rather, it is assumed that activation of a memory trace or chunk is a

direct function of the association between the source elements (the retrieval cues) to that chunk and there is no spread of activation to other chunks from a chunk that is not itself a source of activation. Second, ACT-R is based on the assumption that the cognitive system is a rational system, i.e., the rules that govern the activation of information from memory are such that they optimize the fit to the environmental demands. This rational approach to cognition has been very influential (see also more recent models such as the REM model (Shiffrin and Steyvers, 1997) that will be discussed later in this chapter).

To appreciate this rational approach, it is helpful to consider some of the results discussed by Anderson and Schooler (1991). Anderson and Schooler showed that many of the functional relationships that we know from standard memory experiments (e.g., the typical learning and forgetting functions) can also be seen in the environment with material that has little to do with memory *per se*. For example, the probability that a particular word will appear in the headline of *The New York Times* or the probability that one will get an e-mail from a specific person obey the same functional relations as we know from memory research. If a particular word has appeared in the headline the probability that it will appear again after X days follows the same power law that we are familiar with when looking at standard retention functions. Thus, the basic idea of ACT-R is that the cognitive system has developed in such a way as to provide an optimal or rational response to the information demands of the environment: The probability that a particular item will be remembered at a particular time reflects the probability that it will be needed at that time.

This rational approach is reflected in the equations that ACT-R uses to describe the activation of a particular trace given that specific cues are present. In the ACT-R approach to memory (see Anderson et al., 1998) it is assumed that the activation of a chunk i depends both on its base-level activation (B_i, a function of its previous use) and on the activation that it receives from the elements currently in the focus of attention:

$$A_i = B_i + \sum_j W_j S_{ji} \qquad [6]$$

where S_{ji} is the strength of the association from element j to chunk i and W_j is the source activation (salience) of element j. If we interpret the base-level activation as similar to the prior odds of the chunk being needed and the second term as similar to the (log) likelihood of the trace given the available evidence (the cues), then the similarity of eqn [6] to Bayes' rule becomes evident. (According to this rule, the logarithm of the posterior odds is equal to the log prior odds plus the log likelihood ratio.) According to ACT-R,

$$S_{ji} = S + \ln(P(i|j)) \qquad [7]$$

where $P(i, j)$ is the probability that chunk i will be needed when element j is present or active. Note that since $P(i, j) \leq 1$ the logarithm of $P(i, j)$ will be ≤ 0 and hence S represents the maximum value that S_{ji} can obtain. For all practical purposes, these S_{ji} may be viewed as reflecting the associations between the cues j and the target trace. In ACT-R (see Anderson et al., 1998: 344), it is typically assumed that if there are m elements associated to the cue j, each will have a probability of $1/m$, hence:

$$S_{ji} = S + \ln(1/m) = S - \ln(m) \qquad [8]$$

Note that this equation assumes that for the associative activation S_{ji} it does not matter that a particular association may have become stronger in the course of the experiment: all that matters is the number of associative links from the cue to other elements or its fan. This seems a rather strong assumption, yet it does play an important role in ACT-R's handling of data from recognition experiments.

The first part of eqn [6], the base-level activation, reflects the activation that remains from previous presentations of the target trace or chunk. The activation of a chunk is subject to decay so that the longer ago the chunk was strengthened, the less the contribution of that activation to the current base-level activation. The equation for the base-level activation is thus given by:

$$B_i = \ln\left(\sum_{j=1}^{n} t_j^{-d}\right) + B \qquad [9]$$

In this equation, n is the number of times the chunk has been retrieved from memory, t_j indicates the length of time since the j-th presentation or rehearsal, and d and B are constants. It is evident that eqn [9] is closely related to eqn [5] that describes the activation in ACT*.

Finally, as in ACT*, it is assumed that the latency of a response is an exponentially decreasing function

of the activation level of the corresponding chunk. However, unlike ACT*, ACT-R does not simply look at the activation of the target trace but takes into account other traces or chunks that might be activated. It is assumed that the system will always retrieve the chunk with the highest activation (provided it is above the threshold). Due to the presence of noise in the system, the activation values will not have a fixed value but rather a probability distribution (a logistic distribution is assumed). The probability that a chunk with a mean activation value of A_i (and variance σ^2) is above a threshold τ is then equal to:

$$\Pr(i) = \frac{1}{1 + \exp[(A_i - \tau)/s]} \quad \text{where} \quad s = (\sigma\sqrt{3})/\pi \qquad [10]$$

If there are more chunks above threshold, the system will choose the one with the largest activation. The probability that the target chunk has the largest activation is given by an equation similar to the Luce choice rule:

$$P(\textit{choose } i) = \frac{\exp(A_i/t)}{\sum_j \exp(A_j/t)} \quad \text{where} \quad t = (\sigma\sqrt{6})/\pi \qquad [11]$$

Although ACT-R is much more than a model for memory, it does explain quite a number of findings from the memory literature. We will briefly discuss two such applications, the analysis of recognition memory proposed by Anderson et al. (1998) and the model for spacing effects developed by Pavlik and Anderson (2005).

Any ACT-R model begins with the specification of a number of production rules. In the recognition model, the basic production rules are the rules for Yes and No responses, which simply state that if a trace is found that corresponds to seeing the item in the list context, a Yes response will be made and another rule that applies when the first one fails and that generates a No response. Hence, contrary to most other current models for recognition, ACT-R is not based on a signal-detection-like approach but rather on the retrieval of a trace representing the item in the list context. Note that in such a model negative responses (No responses) are not based on a low familiarity value but on the fact that the rule for generating a positive response passes a waiting time threshold. Although such an approach may work well for explaining data observed on positive responses, there are some problems when negative responses are to be explained. First, this type of model has no simple solution to generate fast negative responses. Second, the model predicts that negative responses are not affected by various experimental factors (e.g., list length) unless one assumes that the waiting time threshold itself is a function of those factors (a solution that is hard to defend).

According to ACT-R, performance in a standard recognition task is determined by the activation of the chunk representing the tested item. According to eqn [6], this is a function of the base-level activation and the associative activation that it receives from the cues (the presented word and the list context). Hence,

$$A = \ln\left(\sum_{j=1}^{n} t_j^{-d}\right) + B + W_W S_W + W_L S_L \qquad [12]$$

where W_W is the weighting given to the word, S_W is the strength of the association from the word to the trace, W_L is the weight of the list context, and S_L is the strength of the context association. According to Anderson et al. (1998: 348), the first term may be approximated by:

$$\ln\left(\sum_{j=1}^{n} t_j^{-d}\right) = \ln\left(\frac{anT^{-d}}{1-d}\right) = C + \ln(n) - d\ln(T) \qquad [13]$$

where C captures the constant terms. Since $W_W S_W$ is also a constant and S_L is equal to $S - ln(L)$ according to eqn [8], the activation of eqn [12] may be written as:

$$A = B' + \ln(n) - d\ln(T) - W_L \ln(L) \qquad [14]$$

where B' combines all the constant effects, n equals the number of presentations/rehearsals, T is the time since presentation, L is the list length, d is the decay rate, and W_L is the attentional weighting of the list context. In their analyses, Anderson et al. (1998) set d and W_L equal to 0.5.

One interesting finding that this model predicts (and that would have been difficult to foresee without actually running the simulations) is the differential effect of list length and list strength in recognition. The list length effect refers to the effect of the number of other items on the list, while the list-strength effect refers to the effect of the strength of those other items (where strength might be manipulated by such factors as presentation time or additional presentations). In recall paradigms, both of these effects are present but in recognition tasks there is no effect of list strength (or a slightly reversed effect), although

there is a list-length effect. Shiffrin et al. (1990) showed that it is very difficult for many models to predict both the presence of an effect of the number of other items, yet no effect of the strength of those other items. ACT-R's recognition model, however, does explain this intricate pattern of results. The basic reason is that in ACT-R, strength manipulations affect the base-level activations whereas the length of the list mainly affects the associative activation (i.e., the fan effect; see eqn [13]). There are a few other factors that play a role (such as small differences in retention interval when presentation time or the length of the list is varied) but the main effects are due to these two factors. Hence, increases in strength affect the base-level activation for the tested item but do not affect the interfering effect of the other items on the list. Of course, one might question the assumption that strength manipulations do not affect the associative activation (as was the case in ACT*), but even so, the ACT-R analysis points to a possible solution to the puzzle of length and strength effects, a pattern of results that has proved difficult to accommodate in other models for recognition.

Pavlik and Anderson (2005) presented an application of ACT-R to account for spacing effects in paired associate recall tasks. They showed that their model could account for all of the standard findings in the spacing literature including a new experiment that they performed in which spacing was varied over much longer intervals than is normally the case in these experiments. In their experiment, there were two sessions separated by 1 or 7 days. During the first session, the subjects learned the English translations for a number of Japanese words. The pairs were presented four or eight times during the first session with interpresentation spacings of two, 14, or 98 trials. During the second session, they were given a number of test trials on the pairs learned during the first session. The data showed a crossover interaction such that the shorter spacings led to better performance at the end of the first session but worse performance at the start of the second session (see **Figure 2**).

In the application of ACT-R to this experiment, the associative activation will be constant and hence the analysis focuses on the base-level activation. Without any modifications, the ACT-R model does not predict such spacing effects (Pavlik and Anderson, 2005: 570), so some changes are necessary. The most likely candidate is the decay rate parameter d (see eqn [9]). In order to account for spacing effects, the decay rate has to be made

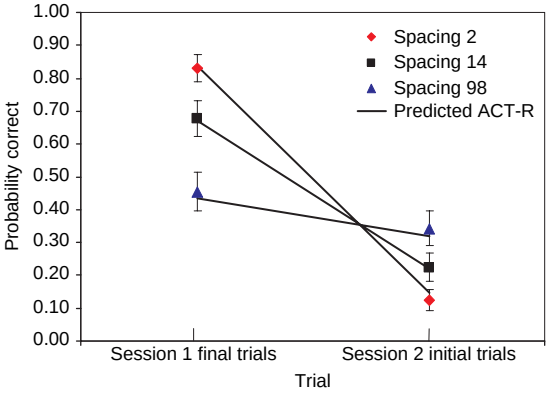

Figure 2 Probability of a correct response before and after the retention interval as a function of the spacing between the presentations during session 1. Observed data from Pavlik PI and Anderson JR (2005) Practice and forgetting effects on vocabulary memory: An activation-based model of the spacing effect. *Cogn. Sci.* 29: 559–586; predictions from the ACT-R model. Error bars correspond to two standard errors.

sensitive to the intervals between successive presentations. The formulation that Pavlik and Anderson (2005) used is based on the assumption that the decay rate for the contribution from the j-th presentation is a function of the activation at the time of the j-th presentation. Thus, eqn [9] is replaced by the following equation for the activation after n presentations:

$$B_n = \ln\left(\sum_{j=1}^{n} t_j^{-d_j}\right) \text{ with } d_j = ce^{B_{j-1}} + a \qquad [15]$$

If at the start of the j-th presentation the activation was high (i.e., the activation after j–1 presentations, B_{j-1}), d_j will be larger and thus the contribution from that trial at later tests will be lower due to the more rapid decay. Hence, the effect of long spacing intervals (characterized by low activation at the end of the retention interval) will be longer lasting and this more than compensates for their longer retention intervals, thus leading to a spacing effect.

These two examples illustrate the way in which task-specific models are constructed within the ACT-R framework. As mentioned before, ACT-R is an ambitious attempt to provide a unified theory of cognition. As such, restricting the evaluation to just its contribution as a memory model clearly does not do justice to the theory as a whole. However, even though the ACT-R has not been evaluated as extensively as some of the other memory models, the theory has already made a large number of contributions (see Anderson et al., 1998; Pavlik and Anderson,

2005). There have also been extensions of the framework to implicit memory effects, but these need to be investigated more thoroughly to determine whether they are indeed viable explanations of priming effects. A recent extension of the ACT-R framework is the identification of specific modules within ACT-R with specific regions in the brain. Anderson and colleagues (Anderson et al., 2003, 2004, 2005) have shown that the duration of those components can be mapped onto the BOLD response obtained in the associated brain regions (using the assumption that the duration but not the intensity of a specific component is reflected in the BOLD response). This of course opens up a whole new approach to the validation of the general ACT-R theory and also provides a much-needed theoretical framework for the interpretation of neuroimaging data. All in all, then, ACT-R represents an excellent example of the trend toward more general theories that has characterized recent research on mathematical models for memory processes.

2.25.3 The SAM and REM Models

2.25.3.1 The SAM Model and Related Models

The next model that we will discuss is the SAM model (Raaijmakers and Shiffrin, 1980, 1981b) and a number of related models that have been proposed in recent years. The SAM model (Search of Associative Memory) started out as a model for free recall (Raaijmakers, 1979). It was soon realized that the model could be generalized to paired-associate recall (Raaijmakers and Shiffrin, 1981a) and recognition (Gillund and Shiffrin, 1984). The model was subsequently extended to handle interference and forgetting (Mensink and Raaijmakers, 1988, 1989) and, more recently, spacing effects (Raaijmakers, 2003). Related models in which a semantic memory component was added have been proposed by other researchers, e.g., PIER2 (Nelson et al., 1998) and eSAM (Sirotin et al., 2005). In addition, Shiffrin and coworkers have developed a new model, REM, that is in many ways similar to SAM, but provides a solution to some problems relating to recognition memory, and that has also been extended to semantic and implicit memory paradigms (Shiffrin and Steyvers, 1997; Schooler et al., 2001; Wagenmakers et al., 2004).

The original SAM model was based on a search model proposed by Shiffrin (1970). It shared a number of characteristics with the Atkinson-Shiffrin theory such as the notion of a STS buffer as a model for rehearsal processes and the assumption that storage in LTS is a function of the nature and duration of rehearsal in STS. SAM assumes that when a specific event occurs (this could be anything but in most analyses it is simply the presentation of an item on a study list) various types of information are stored in the memory trace representing that event. Any type of information might be stored in the trace (the memory image, as it is usually called in SAM), but the model uses a classification in item, associative (interitem), and contextual information. Retrieval of information from LTS is a cue-dependent process, i.e., what is retrieved from LTS depends on the information that is present in STS at the time of the retrieval. In applications of SAM to typical memory paradigms such as free recall or recognition, the cues may be words from the studied list, category cues, and contextual cues.

Whether or not a specific memory trace is retrieved depends on the relations between the cues and the information stored in the trace. These relations are defined in a retrieval structure, a matrix that gives the associative strengths between possible cues and the stored memory image. A crucial assumption in SAM is that when several cues are used simultaneously (e.g., context and a retrieved item), the overall strength of the set of cues (Q_1, Q_2, etc.) to a specific trace is given by the product of the individual associative strengths:

$$A(i) = \prod_{j=1}^{m} S\left(Q_j, I_i\right) \qquad [16]$$

where $A(i)$ is the combined strength or activation of image I_i, and $S(Q_j, I_i)$ is the strength of association between cue Q_j and image I_i. The most important aspect of this eqn [16] is the assumption that individual cue strengths are combined multiplicatively into a single activation measure. This multiplicative feature focuses the search process on those memory traces that are strongly associated with all cues, the intersection of the sets of traces activated by each cue separately. An important aspect of SAM is that retrieval strategies are implemented in the choice of retrieval cues but once a specific set of retrieval cues is used, the retrieval process is automatic and completely determined by the relations between the retrieval cues and the information stored in memory.

The activations $A(i)$ determine both the probability of retrieval of a memory trace in recall tasks as well as the probability that an item will be recognized

as having been presented on the study list. It is assumed that in recall tasks the probability of being able to generate the answer depends on selecting or sampling the correct target trace and on the probability that enough relevant features from the stored trace are activated to enable the reconstruction or recovery of the answer. It is assumed that the system may sample several times before giving up, but if recovery fails once sampled, it will fail again if the same trace is sampled a second time using the same cues.

More specifically, the probability of sampling a trace is assumed to be proportional to the activation strength of the trace:

$$P_S(I_i) = \frac{A(i)}{\sum A(k)} \qquad [17]$$

The probability of recovery is assumed to be an exponential function of the summed strengths of the retrieval cues to the sampled image:

$$P_R(I_i) = 1 - \exp\left[-\sum_{j=1}^{m} S\left(Q_j, I_i\right)\right] \qquad [18]$$

Combining these assumptions, an equation can be derived that gives the probability of recall for a simple cued recall test in which the same set of cues is used for a maximum of *Lmax* retrieval attempts:

$$P_{recall}(I_i) = \left[1 - (1 - P_s(I_i))^{L_{\max}}\right] P_R(I_i) \qquad [19]$$

The above equations apply to cued recall. SAM was, however, initially developed as a model for free recall, which is more complicated since during the search process other list items may be retrieved and these may then be used as new retrieval cues. In SAM it was assumed that during the presentation of the list items, a few items may be simultaneously rehearsed and that storage of context, item, and interitem information was a function of this rehearsal process. That is, the amount of information that is stored for an item was assumed to be a function of the time that that item was rehearsed or the time that a specific pair was simultaneously rehearsed (in case of the interitem associations). For this part of the model, a buffer model similar to that of Atkinson and Shiffrin (1968) was used. At the time of testing, any items still in the buffer are first recalled (unless of course there are no items available anymore in the buffer) and then the process of retrieval from LTS itself starts. Initially, the search process is based solely on the context cues that are available but as soon as a list item is retrieved, that item is used as an additional cue. If this item+context search is not successful (i.e., if there are *Lmax* consecutive retrieval attempts that do not lead to new items being recalled) the system will revert back to using only the context cue. This process continues until no more new items can be recalled (within a reasonable time). For this latter aspect, a stopping criterion was used based on the total number of failed retrieval attempts (*Kmax*), but other stopping rules are also possible (although we have not seen a case where the nature of the stopping rule seems to matter). SAM also assumes that new information may be stored during the retrieval process. That is, if a new item is successfully retrieved, the associative connections between the probe cues and the sampled image are strengthened. Although conceptually simple, it turns out to be virtually impossible to derive analytical predictions for the model for free recall, hence all analyses have been done using Monte Carlo simulations.

Raaijmakers and Shiffrin (1980) reported a large number of such simulation results and showed that the SAM model gave an excellent account of many standard findings from the free recall literature. These included serial position curves, the effects of list length and presentation time, cumulative recall data, the phenomenon of hypermnesia, and many others. As an example, **Figure 3** gives the predictions from SAM and the observed data for the experiment of Roberts (1972) in which presentation time and list length were varied over a wide range.

Of particular interest was the prediction by SAM of the part-list cuing effect (extensively discussed in Raaijmakers and Shiffrin, 1981b). This effect refers to the finding that presenting a random sample from the list items as additional cues did not have the expected positive effect on the recall of the remaining list items as one would have expected based on the notion that subjects use interitem associations during recall. SAM's ability to generate the part-list cuing effect was rather surprising since it ran counter to the then standard interpretation of that effect in terms of inhibitory factors. Subsequent experiments (reported in Raaijmakers and Phaf, 1999) demonstrated the viability of SAM's account of the part-list cuing effect.

SAM assumes that recall and recognition involve the same basic process of activating information. However, when a specific item X is tested for recognition, the response is not based on the retrieval of information from just the trace corresponding to X (although there is no principled reason why it could not be) but on the overall activation of the memory system induced by the retrieval cues. The overall activation is used as the familiarity measure in the

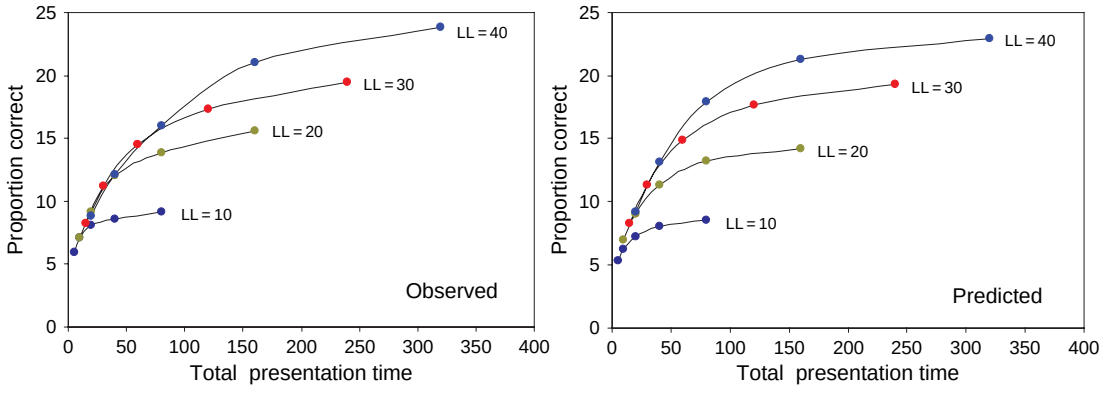

Figure 3 Observed (left panel, Roberts WA (1972) Free recall of word lists varying in length and rate of presentation: A test of total-time hypotheses. *J. Exp. Psychol.* 92: 365–372) and predicted (right panel) mean number of correct recalls in free recall as a function of presentation time and list length (LL). Predictions are based on the SAM model with parameter values as given in Raaijmakers JGW and Shiffrin RM (1980) SAM: A theory of probabilistic search of associative memory. In: Bower GH (ed.) *The Psychology of Learning and Motivation: Advances in Research and Theory*, vol. 14, pp. 207–262. New York: Academic Press.

standard signal detection model for recognition. This approach to recognition is termed a global familiarity model, in contrast to local familiarity models that are based on the familiarity or activation of the target trace. The global familiarity model is currently the most popular approach to modeling recognition and is used in a variety of models other than SAM (e.g., TODAM, MINERVA2, REM). One obvious advantage of the global familiarity approach is that it provides a simple way to deal with false alarms, the recognition of nonlist items (the distractor items), without having to make any additional assumptions. In the SAM recognition model developed by Gillund and Shiffrin (1984), the global familiarity measure is simply the overall activation in response to the retrieval cues used, i.e., $\sum A(k)$, with $A(k)$ as in eqn [16].

In the SAM model, the role of context cues in episodic memory retrieval is emphasized. Many experiments have shown that testing in a context that is different from the context at the time of encoding leads to a decrease in performance (especially in free recall tasks) compared to testing in the same context. This holds both for changes in the environmental context (e.g., Godden and Baddeley, 1975; Smith, 1979; Grant et al., 1998) and changes in the internal state or context (Eich et al., 1975; Eich, 1980). Mensink and Raaijmakers (1988, 1989) extended this notion to within-session changes in context. They assumed that within an experimental session there are gradual changes in context and that the context that gets stored in a trace is a selection from the currently available context elements. The model that they developed was adapted from Stimulus Sampling Theory (Estes, 1955) and assumed that there was a random fluctuation between a set of available or current context elements and a set of (temporarily) unavailable context elements. Mensink and Raaijmakers (1988) showed how such a notion of context fluctuation in combination with the SAM model for cued recall could account for many of the traditional results in the area of interference and forgetting. Using the same basic model, Raaijmakers (2003) showed that it could also account for standard spacing effects. A related analysis of contextual fluctuation processes as well as an application to free recall was developed by Howard and Kahana (1999; see also Kahana, 1996). Whereas in the original Raaijmakers and Shiffrin (1981b) analysis of free recall, a constant context was assumed during presentation and testing of a single list, Howard and Kahana (1999) made the reasonable assumption that context varies even within a single list and that upon retrieval of a specific trace not just the item information would be retrieved, but also the stored context information. They showed how such a model could account for a number of detailed aspects of recall processes.

Nelson et al. (1998) developed a model (PIER2) related to SAM that they showed could successfully explain a large number of findings on the effects of extralist cues on recall. In these experiments, a list of items is studied; at test, the subjects are given a cue and they are told that the cue item is meaningfully related to one of the list items. The basic idea of PIER2 is that during encoding of a list of words, explicit as well as implicit representations (traces) are formed. The implicit representation is an

automatic by-product of the comprehension process. Extralist cued recall may result from retrieving either the explicit or the implicit representation (or both). The PIER2 model focuses on the contribution to recall resulting from the implicit representation. It assumes that during encoding the study or target item as well as its associates are activated and that the activation strengths of both the target item and the associates are a function of their interconnectedness. At the time of testing, when the extralist cue is presented, a sampling function similar to that of SAM is used in which the probability of sampling the target item is proportional to the relative cue-to-target activation strength, relative to the strengths of the connections of the cue to its other associates and the strengths of the connections of the target to its other associates. Thus, the more unique the cue-to-target association (both at the cue side and at the target side) the higher the probability of sampling the (implicit) target representation. Using this sampling model, Nelson et al. (1998) showed that it successfully accounted for many results from previous experiments on extralist cuing.

Even though the SAM model has been quite successful in explaining a large variety of experimental results, the model in its original form fails to account for the list-strength effect (or rather the lack of it) and a number of other results in recognition (see Shiffrin et al., 1990). It soon became clear that in order to be able to explain these results, it would have to be assumed that the extent to which a trace is activated by an unrelated item cue should decrease as the number of features stored in that trace is increased (i.e., as the trace gets stronger). In SAM and most other models, it was assumed that the associative strength was a function of the number of overlapping elements, hence it should either stay the same or increase with the number of features stored.

A solution to this problem was found by adopting a so-called Bayesian or rational approach. In this type of approach (Shiffrin and Steyvers, 1997; McClelland and Chappell, 1998), it is assumed that the system, when confronted with an item that has to be accepted or rejected on a recognition test, makes an optimal decision based on the information that is stored in memory and knowledge of the rules that govern storage of information in memory. In the next section, we will discuss the REM model developed by Shiffrin and Steyvers (1997) as an example of this approach. A similar, independently developed model was presented by McClelland and Chappell (1998). Both of these models are based on the notion of differentiation, i.e., as an item gets stored better, it also becomes easier to differentiate from other items and will less likely be activated by cues representing other items. Although the models are quite similar in spirit (and would be considered equivalent on a purely verbal level), Criss and McClelland (2006) show that the two models are in fact not equivalent and will make different predictions for specific experiments (e.g., associative recognition). However, this analysis is beyond the scope of the present chapter.

2.25.3.2 The REM Model

As mentioned before, the REM model (Retrieving Effectively from Memory) is based on the assumption that the memory system behaves as an optimal decision-making system. On a simple recognition test, old and new items are presented and the subject has to decide whether the test item is old or new. REM assumes that the stored memory traces consist of samples of features from the studied items. Features may be stored correctly or incorrectly but as the study time increases, more features will be stored correctly. It is assumed that at test the system matches the features of the test item to each of the traces in memory. For a test item that was indeed on the list, there will of course be a relatively high number of matches and not many mismatches for the trace corresponding to that item. For all other traces (corresponding to the other items on the list) there will be more mismatches. For a distractor test item, all traces will have a relatively high number of mismatches and relatively few matches (since none of these traces corresponds to the test item). Hence, the number of matching and mismatching features gives information about whether the test item was on the list.

It is assumed that the system evaluates the evidence according to standard rules of probability theory and makes an optimal choice based on the available evidence. More specifically, the system chooses whichever response has the higher probability given the observed feature matches and mismatches in all the memory traces. Mathematically, the decision criterion is given by the posterior odds ratio, which according to Bayes' rule may be written as the product of the prior odds and the likelihood ratio:

$$\Phi = \frac{P(old|data)}{P(new|data)} = \frac{P(old)}{P(new)} \times \frac{P(data|old)}{P(data|new)} \qquad [20]$$

It can be shown that in REM, the likelihood ratio is given by the average likelihood ratio for the

individual list traces (assume L episodic images are compared to the test probe):

$$\Phi = \frac{1}{L}\sum_j \frac{P(D_j/old)}{P(D_j/new)} = \frac{1}{L}\sum_j \lambda_j \qquad [21]$$

Hence, an old response would be given if $\Phi > 1$. An interesting result from this analysis is that the decision rule turns out to be an example of the global familiarity approach to recognition memory. There are, however, two major differences between the REM and the SAM models for recognition. One is that in SAM the response criterion is basically arbitrary, whereas in REM there is a natural criterion corresponding to a likelihood of 1.0. The other difference is that in REM the activation value λ_j may be shown to be a function of both the number of matching and nonmatching features. For a simple version in which we simply count the number of matching and mismatching features, disregarding the exact value of the features (i.e., whether it is a very common or not so common value), it may be shown that the contribution to the overall likelihood for item j is given by:

$$\lambda_j = \left(\frac{\alpha}{\beta}\right)^{m_j}\left(\frac{1-\alpha}{1-\beta}\right)^{q_j} \qquad [22]$$

where α is the probability of a match given storage for the correct trace, β is the probability of a match given storage for an incorrect trace (α must obviously be larger than β), and m_j and q_j are the number of matches and mismatches, respectively, for trace j. Thus, the higher the number of matching features, the higher the likelihood, and the higher the number of mismatching features, the lower the likelihood. Earlier we mentioned the need to include information regarding the mismatching features in determining the activation of a trace in order to be able to account for list-strength effects. List-strength effects may be shown by comparing mixed lists composed of both strong and weak items, with pure lists consisting of only strong or only weak items. If there is a list-strength effect, the performance on the weak items in the pure weak list should be better than that on the weak items in the mixed list, and the performance on the strong items should be worse in the pure strong list compared to the mixed list. As shown in **Figure 4** (these results were obtained using a simulation program developed by David Huber), the REM model indeed predicts no decrease in recognition performance due to increasing strength of the other list items, although it does predict a decrease as a function of an increase in the number of other list items.

Equation [21] also suggests a similarity between REM and SAM in that the likelihood ratio for a particular trace in REM seems to play a similar role as the activation values in SAM. This suggests that it might be possible to generalize REM to recall paradigms by substituting the likelihood ratios for the activation values. This approach has the desirable feature that most, if not all, of the SAM recall predictions hold for REM as well. Diller et al. (2001) showed that this indeed produces a viable model for

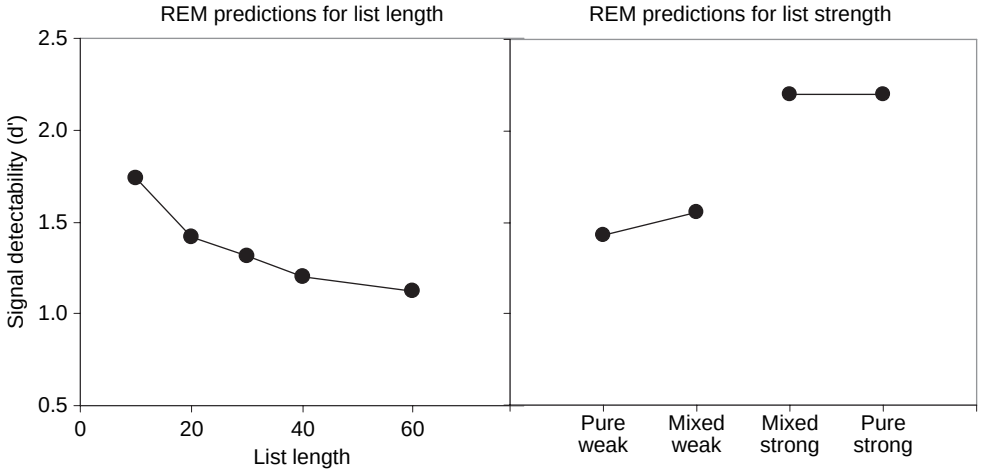

Figure 4 Predicted values for signal detectability (d′) as a function of list length (left panel) and list strength (right panel) according to the REM model (parameter values: g = 0.4, c = 0.7, u = 0.05; see Shiffrin RM and Steyvers M (1997) A model for recognition memory: REM: Retrieving effectively from memory. *Psychon. Bull. Rev.* 4: 145–166).

recall provided that one raises the likelihood ratios to a constant power. Thus, they defined the probability of sampling trace i as

$$P_S(I_i) = \frac{\lambda_i^{\gamma}}{\sum \lambda_k^{\gamma}} \quad [23]$$

Soon after the REM model for recognition was developed, it was realized that it might be fruitfully generalized to other domains, in particular semantic and implicit memory. In this more general version of REM, it is assumed that when an item is encountered, (a sample of) its features are stored in an episodic trace but also in a lexical/semantic system. Hence, the lexical/semantic trace accumulates information from all prior occurrences and is updated each time the item is presented (see Schooler et al., 2001).

Schooler et al. (2001) developed a REM-based model to account for priming effects in perceptual identification. The model gave a successful account of the results obtained by Ratcliff and McKoon (1997) in the forced-choice identification paradigm. In these experiments, a word (e.g., LIED) is briefly flashed and then masked. The subject is then presented with two alternatives (e.g., LIED and DIED) and has to choose which of these two was the word that was flashed. The critical result in this paradigm is that there is priming (i.e., an increase in the probability of choosing an item that was previously presented on a study list) but only when the two alternatives at the test are perceptually similar (LIED, DIED), but not when they are perceptually dissimilar (e.g., LIED, SOFA). Schooler et al. showed that this pattern of results can be explained in REM by the assumption that a small number of context features are added to the lexical/semantic trace of an item as a result of the prior presentation. These additional context features will obviously have a high probability of matching the later test context, hence will increase (although by a small amount) the number of matching features for the trace corresponding to the primed alternative. The crucial aspect in the REM explanation is that for similar alternatives the outcome of the feature match will often be the same, hence only a relatively small number of perceptual features will be relevant for the decision to choose one or the other alternative. As a result, the additional matches provided by the context features will have a larger effect when the alternatives are perceptually similar than when they are dissimilar.

To see this more clearly, **Figure 5** shows the distributions for the number of critical features for each of the choice alternatives that match the flashed item. Critical features are features that potentially can make a difference between the two alternatives. Since there are fewer critical features that differentiate similar alternatives, the number of matching critical features will also be lower. Assume that the foil item was presented on the prior study list and that this results in just one additional match due to context overlap between study and test. As shown in **Figure 5**, this additional match will have a clear effect for the similar alternatives: There is much more overlap between the distributions, and hence the probability that the target has more matches compared to the foil will decrease quite a bit. For the dissimilar alternatives, the added match due to context has only a small effect on the probability of choosing the target (the probability of a correct response). Hence, the effect of prior study will be much larger for the similar alternatives compared to the dissimilar ones.

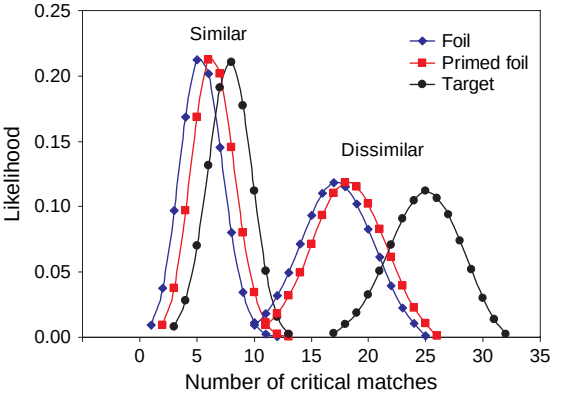

Figure 5 Predicted likelihood distribution for the number of critical matches for similar and dissimilar choice alternatives according to the REM model of Schooler LJ, Shiffrin RM, and Raaijmakers JGW (2001) A Bayesian model for implicit effects in perceptual identification. *Psychol. Rev.* 108: 257–272.

Wagenmakers et al. (2004) presented an application of REM to standard lexical decision tasks in which it was assumed that a lexical decision is based on the evaluation of the likelihood that the presented item corresponds to a word in the lexical system versus a nonword (just as a recognition decision is based on the evaluation that the test item corresponds to an item stored in the episodic system). There is a time-dependent encoding process such that as encoding time increases more and more probe features become available. The likelihood at time t is determined from the features available at

that time. The model was evaluated using signal-to-respond tasks and gave a good account for the effects of several major factors such as word frequency, repetition priming, and nonword lexicality.

Raaijmakers (2005) gives an outline of how the REM model may be extended to several other implicit and semantic memory paradigms such as associative priming, semantic categorization tasks, and associative repetition priming. A common feature of all of these applications is that the lexical/semantic system is assumed to be a much more flexible system than in many traditional accounts and that lexical/semantic traces do contain contextual features and hence are sensitive to recent episodes in which the item was presented.

2.25.4 Neural Network Approaches

All the approaches that I have discussed thus far do not make specific reference to how the processes that are postulated are actually implemented in the brain. The models in this section on the other hand take the analogy to neural processes in the brain as their starting point. It is assumed that information is distributed over sets of nodes in a neural network rather than as separate traces as in the models discussed thus far. Information is coded not in separate nodes or individual links but in the pattern of strengths over a large number of links or nodes. Hence, each individual node or link participates in the representation of many items or associations. Whenever a specific cue item is presented, the corresponding input nodes are activated and this activation is propagated through a network of links, leading to a specific pattern of activation at the output nodes and this pattern defines the output or the item retrieved from memory. The crucial property of these models (and the one that initially attracted the most attention) is that they provided a mechanistic account of the critical property that distinguishes human memory from other types of memory (such as hard disks), namely its associative character. That is, associative memory systems have the property that if the association *A-B* is stored, presentation of the cue *A* will automatically retrieve *B* without the need to know where *B* (or *A-B*) was stored. In models such as ACT and SAM, this property is assumed, but in neural network models, a computational account is given that generates the associative property, rather than assuming it.

To illustrate this, consider a very simple neural network model in which there is an input layer of neurons and an output layer of neurons and in which each input neuron is connected to each output neuron (e.g., Anderson et al., 1977). Items are represented by vectors, i.e., a series of activation values over the input or output neurons. In order to store the association *A-B*, the connections between the input *A* and the output *B* have to be modified in such a way that presenting *A* at the input side will produce *B* at the output side. This may be accomplished by modifying the connections between the *A* and *B* vectors in such a way that if the *i*-th value of *A* and the *j*-th value of *B* are both high, the connection is made stronger. More generally, if $\mathbf{f_i}$ is the feature vector for item *A* and $\mathbf{g_i}$ is the feature vector for item *B*, then the connections between the input nodes and the output nodes are increased by an amount equal to the product of the feature values. Using vector notation, this is equivalent to the assumption that the changes in the synaptic strengths are modified according to the matrix $\mathbf{M_i}$:

$$\mathbf{M_i} = \mathbf{f_i}\mathbf{g_i}' \quad [24]$$

Thus, if a list of such pairs is studied, the strengths are modified according to the matrix $\mathbf{M}$ with $\mathbf{M} = \sum \mathbf{M_i}$. Presenting an item as a cue to such a system amounts to postmultiplying the matrix $\mathbf{M}$ with the item vector. It is relatively easy to show that in the ideal case where all items vectors are uncorrelated and of unit length, such a model will show the associative property, i.e., on presentation of the item *A* ($\mathbf{f_i}$) the system will generate the associated item *B* ($\mathbf{g_i}$):

$$\mathbf{M}\mathbf{f_i} = \sum \mathbf{M_j}\mathbf{f_i} = \sum_{j \neq i} \left(\mathbf{g_j}\mathbf{f_j}'\right)\mathbf{f_i} + \left(\mathbf{g_i}\mathbf{f_i}'\right)\mathbf{f_i} = \mathbf{g_i} \quad [25]$$

The example given above is the simplest model of this kind and much more complicated models or networks have been proposed. All of these models, however, share the basic assumption that the associative information is encoded in the links or connections between the neurons. Item information is represented by the pattern or distribution of the activation values at the input and output layers. Note that the same nodes are used to represent all the items: The information is distributed over many nodes. Such models are therefore often called connectionist or distributed memory models. They may contain several layers of neurons with connections between successive layers (see Ackley et al., 1985; Rumelhart et al., 1986). Since the associative property that all of these models share may also be expressed as implying

that the model learns to predict the output vector given a specific input vector, it is not surprising that connectionist models have been developed not just to simulate human memory but also to compute any type of predictive relation between a specific input and specific output (i.e., associating a spoken output or phonemes based on the written input text, as in the NETtalk model; Sejnowski and Rosenberg, 1987). These more complex variants do not learn the associations in a single step (as in the simple model described earlier), but require several iterations in which the links between the nodes in the network are gradually changed. Basically what these models do is perform a kind of nonlinear regression using a least-squares fitting procedure to predict the output values given the input values.

Although these models have been quite successful in other domains, their success as a general framework for human memory is more limited. There are a number of features of these models that are problematic when they are used as models for episodic memory.

The most basic problem is known as catastrophic forgetting (McCloskey and Cohen, 1989; Ratcliff, 1990). This property is related to the fact that these models focus on extracting generalized rules from a series of exemplars rather than on storing individual items. The issue is that distributed memory models tend to forget all previously learned information on learning a new set of items. This is most clearly shown in the application of the back-propagation model to a retroactive interference experiment in which two lists are learned in succession (see McCloskey and Cohen, 1989). After learning the second list, humans will show some forgetting for the first list but the forgetting is far from complete. A typical back-propagation model, however, will show complete forgetting of the first list and in fact learning of the second list only starts after the first list has been completely unlearned. Such drastic forgetting is quite different from what is observed in experiments with humans, hence the name catastrophic forgetting. The basic reason for this incorrect prediction is that all the information is contained in the strengths or weights of the links in the network, and since these weights are freely adjusted during second-list learning to optimize second-list performance, there is nothing that prevents the complete forgetting of the first list information. Similar problems for recognition memory performance were demonstrated by Ratcliff (1990), who also showed that the model fails to predict a positive effect of amount of learning on the d′ measure for recognition.

It should be noted that these problems are not inherent to distributed memory models but seem to be limited to those connectionist models that assume that learning an item involves an optimization of the weights given to the links in order to tune the network to the information that it is currently being trained on. Murdock (1982, 1993), for example, developed a general framework for memory based on a distributed representation (TODAM, Theory of Distributed Associative Memory) in which item and associative information are added to a single memory vector (similar to the simple vector model described earlier) without any additional tuning. In TODAM, item information is simply added to the trace, while associative information (say the association A–B) is modeled by computing a vector that corresponds to the convolution of the vectors representing **A** and **B** (denoted as $\mathbf{A}^{*}\mathbf{B}$). Murdock showed that in such a model when A is presented as a cue, B (or at least a noisy version of the **B** vector) may be retrieved by computing the correlation of the **A** vector with the memory vector. TODAM does not suffer from the catastrophic forgetting problem presumably because a second list adds information (and hence noise) to the memory vector but does not destroy the information from the first list.

In order to prevent these problems in connectionist models, changes have to made to the basic structure of such models. One solution is to eliminate the strong version of the distributed memory assumption. For example, it might be assumed that there are a large number of nodes or connections and that learning a particular item or an association uses only a small proportion of these (e.g., so-called sparse distributed networks). Alternatively, it might be assumed that information concerning first-list learning continues to be stored in memory for a relatively long period after the learning of that list (a version of consolidation theory). In this way, the two lists become one list and a compromise is found between first- and second-list performance (see McClelland et al., 1995, for an ingenious version of this approach). Yet another approach is to relax the assumption that specific items are stored in a distributed manner, for example, competitive learning models using a winner-take-all principle in which retrieval results in a single unit are activated (retrieved) or a novelty-detection assumption that enables the system to allocate new items to units not already used to represent other items (e.g. Murre, 1992).

There are other problems that are not as easy to remediate in distributed memory models. For example, Shiffrin et al. (1990) showed that many network models have problems simultaneously predicting the presence of list-length effects and the absence of list-strength effects in recognition memory. Extra items harm performance by changing weights, but strengthening other items also changes the weights and should therefore cause similar harm. As yet, there is no clear solution for this problem within the framework of distributed memory models.

Despite these problems, neural network models continue to have a major influence on memory theories. These models have the advantage of a much closer link to neurobiological approaches and, more importantly, they still provide the only mechanistic explanation for the associative memory property. A nice example of a modern neural network model is the Complementary Learning Systems (CLS) approach proposed by McClelland et al. (1995) and further elaborated by O'Reilly and Rudy (2001) and Norman and O'Reilly (2003). The CLS approach is based on the realization that the memory system must combine two seemingly incompatible functions: Storage of episodic memories and integration of information to enable generalization. The first requires storage of specific, separated traces, whereas the second requires overlapping representations. The phenomenon of catastrophic forgetting shows that standard distributed representations are not a suitable model for episodic memory, although they do allow generalization. The solution in the CLS approach is to assume two separate but interactive systems: A rapidly changing system (assumed to be located in the hippocampal system) and a more slowly changing system (assumed to be cortical or neocortical). The hippocampal system is assumed to employ sparse compressed representations to minimize interference between traces, while the cortical system uses more standard distributed (overlapping) representations. It is assumed that there is a slow consolidation process that transfers information from the hippocampal to the cortical system. During recall, a cue will activate a corresponding pattern in the cortical system and if this pattern is sufficiently close to a stored hippocampal trace, the hippocampal system will settle on that trace, which then sends back activation to the cortical system, leading to the reinstatement of the original event pattern. Catastrophic interference in the neocortical system is avoided by a kind of consolidation process in which storage of new information is interleaved with renewed activation of older information.

McClelland et al. (1995) show how such a model may be used to explain a variety of findings from both human and animal experiments. For example, the fact that amnesic patients are unable to recall recent episodic experiences yet are able to recall older memories and do show implicit memory is attributed to a defect in the hippocampal system coupled with an intact cortical memory system. Norman and O'Reilly (2003) presented simulation results showing that the CLS model gives a good account of recognition memory. For example, the model may predict little or no list-strength effect in recognition if the recognition decision is mostly based on familiarity stemming from the cortical system (rather than on recall based on the hippocampal system). It is not clear, however, how the CLS model would handle both the absence of list-strength effects and the presence of list-length effects in recognition (see Norman and O'Reilly, 2003: 632).

However, even though these newer versions of connectionist modeling provide a solution for a number of the problems that plagued older connectionist models, there are several remaining issues. One is that it is not always clear which aspects of the model are responsible for a specific prediction. Although this is also a concern with other general modeling approaches, the issue is particularly relevant for these models. When a model successfully predicts a specific phenomenon, one also wants to know which aspects of the model are crucial for that prediction and which elements of the model (or the simulation) are incidental. For example, the model may employ a specific learning rule to optimize the weights or a specific equation for the decay of activation values. When one tries to understand why the model predicts the phenomenon, it is important to know whether it would still predict the phenomenon when a different learning rule or a different equation for decay (or perhaps no decay at all) is assumed. Thus, the ability to simulate a specific result does not yet mean that one has an explanation for that phenomenon (see also McCloskey, 1991; see O'Reilly and Farah, 1999, for a contrasting point of view). In many cases (for example, the prediction of the part-list cuing effect in SAM, see Raaijmakers and Phaf, 1999), a substantial amount of work is involved in figuring out why the model makes the prediction, but it is the additional work that ultimately leads to a model-independent explanation of the phenomenon. Such analyses are especially needed when it is difficult for other researchers to run the required model simulations.

2.25.5 Models for Serial Order Memory

In this section, I will discuss a number of models that have been proposed to account for memory for serial order information. Such models focus on explaining memory for item and order information in relatively short lists. For example, subjects might be presented with one or more lists of five items and then be given a test in which the items have to be recalled in the correct order, or they might be given the items at test (in a different order) and then asked to provide the correct order of presentation. The empirical evidence for (or against) these models is discussed by Healy and Bonk (*See* Chapter 2.05). We will restrict our discussion to the mathematical formulations that have been used.

A classic approach in this area is Estes' perturbation model (Estes, 1972). In this model, it was assumed that during study, items are associated or linked to their serial positions. However, during the retention interval, the item may shift (perturb) to a neighboring position. If one assumes that movements to an earlier or to a later position are equally likely, then the probability that an item occupies a particular position n at a given time t is given by the following difference equation:

$$P_{n,t} = (1-\theta)P_{n,t-1} + (\theta/2)P_{n-1,t-1} + (\theta/2)P_{n+1,t-1} \quad [26a]$$

For the endpoints we have a slightly different equation:

$$P_{1,t} = (1-\theta/2)P_{1,t-1} + (\theta/2)P_{2,t-1} \quad [26b]$$

for the first position and similarly for the final list position.

These relatively simple equations allow one to calculate the probability distribution for each item on the list. The model predicts better recall for items in the beginning and end positions than for items in the middle of the list since these items will have had less opportunity to perturb. Nairne (1992) obtained data for five-item lists at retention intervals of 30 s, 4 h, and 24 h and showed that the perturbation model gave a good quantitative account of the data. Note that in order to apply the model, one needs to estimate not just the perturbation parameter θ but also the number of cycles of perturbation (the number of times that eqn [26] is applied). It is easy to see that the model can also handle a number of other findings such as a higher accuracy if there are longer intervals between successive items (longer intervals will lead to less perturbation).

The perturbation model is an example of a bin model in which items are placed in or linked to serial positions rather than to one another. That is, a common view of serial order memory is that order memory is derived from item-to-item associations (the temporal order of a string such as ABCD is remembered through the pairwise associations A-B, B-C etc). What the perturbation model shows is that this type of view is not a necessary one and that an alternative view in which order information is not based on item-to-item associations but on memory for positional information can also give a good account of the data. However, a number of problems have been mentioned in the literature regarding such bin models, the most important one being that these models give no account of the recall of item information (cuing with a specific position automatically leads to recall of the linked item). In addition, it seems to be assumed that at test, the successive bins are always searched in the correct order (a rather strong assumption in the case of somewhat longer lists).

A prime example of a chaining model for serial order memory is the model proposed by Lewandowsky and Murdock (1989). Their model was based on the TODAM framework for memory, one of the distributed memory models discussed earlier. In this application of TODAM, it was assumed that recall starts by using a context cue to generate the first item, and then this item is used as a cue to generate the second item, and so on. A key problem for any type of chaining model is how to proceed if at a particular point no item is recalled. In TODAM, even though the retrieved vector may not enable the recall of a given item (the process of cleaning up the output vector via comparison to a lexicon may not succeed), the retrieved vector may still be used as a further cue.

Finally, Brown et al. (2000) developed a model for serial memory (termed OSCAR) that relies on contextual information to generate temporal information. In their model, context is represented as a series of oscillators that produce a dynamically changing state. The output from the oscillators forms a context vector. The model assumes that the overall context is made up of several such context vectors. During presentation of the list of items, each item vector is associated with the state of each context vector at the time of presentation. Thus, item 1 is associated to context vector 1 at time 1, context vector 2 at time 1, etc. Similarly, item 2 is associated to context vector 1 at time 2, context vector 2 at time 2, etc. All of the item–context associations for

each context vector are stored in an association matrix, similar to eqn [24]. At recall, the initial state of the context vectors is reinstated and these are then used to regenerate the context vectors at the following times. To recall the item that was presented at time *m*, context vector 1 at time *m* is multiplied with the memory matrix corresponding to context vector 1 (see eqn [25]), which produces an approximation to item *m*. Similarly, the context vector 2 is used in the same way, also leading to an approximation to item *m*, and so on for all context vectors. Finally, the item in a separately stored vocabulary of items that provides the best overall match to the various approximations of item *m* is then produced as the response. Thus, in this model, recall of a series of ordered items is based on the recall of gradually changing contexts that provide the temporal information for order memory. The OSCAR model is an example of a model for order recall that is based not on interitem associations but on the retrieval of temporal information that is specific to the time that a particular item was studied. The model provides a mechanism for how the system recalls the various contexts as well as the items that were presented. What is not clear, however, is how essential the specific formalization that Brown et al. (2000) used is for the predictions generated by OSCAR (e.g., which properties of the context vectors are essential, and are oscillators really required to enable the model to make these predictions).

2.25.6 Concluding Remarks

In the previous sections, I have presented an overview of several global frameworks for human memory. In this section, I return to the question raised in the introduction about what makes such models useful for understanding human memory processes.

Perhaps the most important advantage of having a formal model is that it makes it possible to prove that a specific argument or verbal explanation of a phenomenon is indeed valid (or the reverse: Show that it is not a valid argument). Many striking examples of such results may be found in the literature, for example:

- Batchelder's (1975) demonstration that the results from experiments on all-or-none learning could not be explained as being due to selection effects due to individual differences, as was thought by many proponents of theories in which learning was assumed to be more gradual.
- The demonstration by Hintzman and Ludlam (1980) that a purely exemplar-based classification model (MINERVA) could explain the finding that prototypical information seemed to be forgotten slower than the instances themselves. This finding had been generally interpreted as implying the existence of a prototype representation that was assumed to show a slower decay than the instance representations. The MINERVA model, however, did not contain any prototype representation and yet predicted the observed pattern of forgetting.
- The analysis of the part-list cuing paradigm using the SAM model (Raaijmakers and Shiffrin, 1981b) that showed that the lack of a positive cuing effect was entirely compatible with a model that was strongly based on the use of interitem associations. This analysis led to a new explanation for part-list cuing effects that we would not have thought of prior to running the analyses.

There are many such examples in the literature, and they do not necessarily have to be positive (in the sense of providing a new or alternative explanation). In some cases, computational analyses may show that a model fails to predict a finding that one would have intuitively thought that it should be able to predict. For example, the demonstration by McCloskey and Cohen (1989) of the catastrophic forgetting phenomenon shown by typical connectionist models had a big impact on the field. Similarly, Murdock and Lamon's (1988) demonstration that simple connectionist models failed to predict improved recognition performance with an increasing number of presentations was also initially met with disbelief.

What these examples show is that formal modeling may help to sharpen theoretical analyses by showing which results directly follow from a specific set of assumptions, which results cannot be predicted by the model, and which results may be predicted by the model but only under specific conditions (e.g., specific sets of parameter values). However, in order to be able to draw such conclusions, the modeler should not be content just to show that his or her model can predict the results of a particular set of experiments. This should be considered step one in the analyses and should be followed by additional analyses to determine the robustness of the prediction (does it vary in a qualitative sense when parameters are set to different values) as well as analyses to determine which aspects (assumptions) of the model are really crucial for the prediction. The latter aspect is often left out but is in my view the essence of the modeling approach: Models

should not be used as black boxes that in some mysterious way generate a specific pattern of data, but should preferably be used as analytical tools to assist the theoretical analysis of those data (what does it tell us about human memory processes?).

The latter point is related to the view that a model that is applied to a specific experimental paradigm is really a combination of (1) a set of core theoretical assumptions (the general theory), (2) a number of auxiliary assumptions related to the implementation of the model and specific computational aspects (e.g., an assumption that each trial adds the same amount of strength to a trace, or the specific learning rule used in a connectionist model), and (3) a set of task-specific assumptions (say a particular rehearsal strategy that is assumed or the rules that are used in generating an overt response based on the retrieved information). In this view, the ultimate goal of mathematical modeling is not simply fitting a set of data but to provide insight into the basic structure and processes in a particular domain. As such, there is no real difference with non-mathematical approaches. The basic advantage of the modeling approach is that it provides an analytical tool that can be used to experiment in a way that is not possible with verbally stated theories.

Viewed in this way, the progression of simple models that could only be applied to a single type of experiment to the more general approaches that we have discussed in this chapter is a major step toward a more coherent and comprehensive theory of learning and memory processes.

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2.26 Associative Retrieval Processes in Episodic Memory

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> [Y]ou are wrong to say that we cannot move about in Time. For instance, if I am recalling an incident very vividly I go back to the instant of its occurrence: I become absent-minded, as you say. I jump back for a moment.
>
> H. G. Wells, *The Time Machine*, 1898

In the above quote from Wells' classic science-fiction novel, the protagonist compares his actual travels through time to the mental time travel one experiences through the act of reminiscence. During our childhood, many of us have fantasized about actual time travel. If we could only return to a previously experienced episode of our lives and re-experience that episode in light of our new found knowledge, perhaps that knowledge would lead us to act differently, or simply to appreciate that previous experience in new and different ways.

Although true time travel remains beyond our reach, the act of remembering is a form of time travel that we can exercise at will. Our power to remember previously experienced events can put us back in the approximate mental context of that earlier episode and allow us to interpret that episode in light of our current knowledge. In so doing, we also alter our memory of the episode in permanent ways, such that each remembering brings back not only the original encoding context, but also some elements of the context of previous rememberings.

In 1972, Endel Tulving coined the term episodic memory to refer to the form of memory that allows us to associate the many different types of information constituting an event into a spatiotemporal context and to later use the content of the event to retrieve its context. Episodic memory places us in the memory, marking the memory's position on our personal, autobiographical, timeline. Retrieval of episodic memories constitutes a form of time travel in which we recover the encoding context of the previously experienced event. Other important forms of memory, such as perceptual priming and semantic memory, do not have this feature.

Episodic memory not only supports the vivid recollection of formative life events; it also enables us to remember where we parked our car in the morning, whether we took our medicine, and whom we met at a social engagement. Dramatic failures of these everyday aspects of episodic memory can result from damage to the medial temporal lobe of the brain (Spiers et al., 2001). More subtle impairments of episodic memory accompany the normal aging process (Salthouse, 1991; Kausler, 1994).

Ever since Ebbinghaus carried out his seminal studies in 1885, most laboratory studies of human memory have focused on episodic memory. In these experiments, lists of items[1] constitute sequences of mini-experiences presented in a controlled fashion. Subjects then attempt to recall or recognize the previously studied items under a variety of conditions designed to probe and challenge their memorial abilities.

2.26.1 Association and Context

Association has served as the core theoretical construct throughout the history of writings on memory. An association is not observed; rather, it is inferred from the tendency of one item to evoke another. Associations that come to mind quite naturally, like the association of king and queen or of bread and butter, relate to the meaning of the constituent items. This meaning develops through extensive experience, presumably involving the temporal co-occurrence of the items in many different situations. But associations can also be formed between nominally unrelated items in a single exposure. For example, when attending closely to a pair of items presented in temporal proximity (e.g., a name–face pair) we can quickly take hold of the association, at least temporarily. Sometimes, a salient new association may be encoded well enough after a single encounter that it can be recalled, or at least recognized, after a long delay.

The classic laboratory method for studying the encoding and retrieval of episodically formed associations is the paired-associate (or cued-recall) task. In this task, subjects study a list of randomly paired words, name–face pairs, or the like. Later, subjects are presented with one member of each studied pair as a cue to recall its mate. The paired-associate task has subjects explicitly learn associations among items. In the case of words, effective learning of the paired associates depends strongly on the formation of linguistic mediators, the use of imagery, or other strategies that involve elaboration of the meaning of the constituent items (for reviews, see, Paivio, 1971; Murdock, 1974; Crowder, 1976). One may ask whether strategies are strictly necessary for the formation of associations between contiguously presented items. We will return to this question at the end of the present chapter.

The idea of interitem association only takes us so far in thinking about episodic memory. To perform any episodic task one must have some means of distinguishing the current list from the rest of one's experience. For example, if we learn the association between the words *fountain* and *piano* in one setting, and then we later learn the association between *fountain* and *slipper* in another setting, how do we flexibly retrieve either *piano* or *slipper*, and how do we recall the setting in which the word was learned?

The idea that associations are learned not only among items, but also between items and their situational or temporal context was widely recognized in the first half of the twentieth century (Hollingsworth, 1928; Carr, 1931; McGeoch, 1932; Robinson, 1932). This idea formed the basis for Underwood's classic explanation of spontaneous recovery as described in his 1945 dissertation.

Despite its recognition among early memory scholars, the idea of context available at the time was too vague to find favor among the behavioristically oriented learning scholars who dominated in the post-war period (McGeoch and Irion, 1952). Whereas associations could be viewed as an experimentally determined increase in the probability of a stimulus evoking a response, context is not easily tied to experimental manipulations. To scholars of a strictly empirical orientation, the difficulty of controlling and manipulating context, especially internally generated context, greatly limited its utility as an explanatory construct. These scholars feared the admission of an ever-increasing array of hypothesized and unmeasurable mental constructs into the scientific vocabulary (e.g., Slamecka, 1987).

The notion of temporal context regained respectability in the memory literature after the appearance of Gordon Bower's temporal context model in 1972 (Bower, 1972; see also, Bower, 1967). The related notion of temporal coding processes was also emphasized by Tulving and Madigan (1970) in their influential review of the state of the field. According to Bower's model, contextual representations are composed of many features which fluctuate from moment

[1] Although Ebbinghaus used consonant-vowel-consonant (CVC) syllables as stimuli, most modern studies use words due to their relatively consistent interpretation and coding across participants.

to moment, slowly drifting through a multidimensional feature space. Whereas previous investigators had noted the importance of temporal coding (e.g., Yntema and Trask, 1963), Bower's model, which drew heavily on the classic stimulus-sampling theory developed by William K. Estes (1955), placed the ideas of temporal coding and internally generated context on a sound theoretical footing. The Bower–Estes model provided the basis for more recent computational models of temporal context and its central role in episodic memory (Mensink and Raaijmakers, 1988; Howard and Kahana, 2002).

2.26.2 Associative Processes in Free Recall

The cognitive revolution of the 1960s brought a shift away from the paired-associate and serial learning tasks which had served as the major experimental approach to the study of human verbal memory until that time. The more cognitively oriented researchers were especially drawn to free recall. In the free recall task, subjects study a sequence of individually presented items. At test, they are simply asked to recall all of the items they can remember in any order they wish.[2] There is no experimenter-imposed structure on the nature of the recall process. By analyzing the order in which subjects recall list items, one can gain considerable insights into the memory processes operating under these relatively unconstrained conditions. In contrast, the paired-associate task imposes a strong, experimenter-defined, organization on the to-be-learned materials: subjects are aware that they must link the paired items at study and that they will later be asked to recall a specific target item in response to a given cue.

The scientific literature on free recall has followed two distinct strands. One strand of research focused on how subjects learn a list over the course of successive study-test trials. In a classic study, Tulving (1962) demonstrated that over repeated trials in which the input sequence is randomized, the sequences of recalled items becomes increasingly consistent from trial to trial. In learning lists of random words, subjects appeared to create a kind of organization of the materials, with the level of recall tracking the degree of organization (see Sternberg and Tulving, 1977, for a review of measures of subjective organization). Earlier work by Bousfield and colleagues (Bousfield, 1953; Bousfield et al., 1954) had shown that when subjects studied lists that included strong semantic associates, their sequence of recalls was organized semantically, a phenomenon termed category clustering. Tulving's work showed that organization was a far more general phenomenon, seen even in lists whose items lacked any obvious categorical or semantic organization. Tulving's work on organization and memory spawned several decades of work aimed at understanding the role of organization in the learning process (see Tulving, 1983, for a review).

The second strand of research on free recall focused on how subjects recalled a list after a single study trial. In his classic analysis of the serial position curve in free recall, Murdock (1962) reported the relation between list position and recall probability. On an immediate recall test, subjects exhibited a striking recency effect, recalling the last few items more frequently than items from earlier list positions. These recency items were typically the first items recalled in the sequence of responses (Deese and Kaufman, 1957; Nilsson et al., 1975). Among the earlier (prerecency) items, subjects exhibited superior recall for the first three or four list items than for items from the middle of the list (the primacy effect).

Murdock varied both list length and presentation rate, and found that both manipulations produced a dissociation between the level of recall of recency and prerecency items. Specifically, he found that increasing list length or speeding the presentation rate resulted in lower recall of early and middle items, but did not affect recall of the more recent items. In addition to list length and study time (presentation rate), other variables that boost recall of prerecency items have little or no effect on recency items. For example, lists of similar words are better recalled than unrelated words (Craik and Levy, 1970), and lists of common words are better recalled than lists of rare words (Sumby, 1963; Raymond, 1969; Ward et al., 2003).[3] In both of these cases, however, the enhanced recall is not seen for the recency items. In contrast, the recency effect is significantly greater for auditorally than for visually presented lists, while modality of presentation has no effect on prerecency items (Murdock and

[2] In 1894, E. A. Kirkpatrick published the first study using the free-recall method. This was the same year that Mary Calkins introduced the paired-associate technique. Because of the unconstrained nature of the free-recall technique, Ebbinghaus (1911) found it to be crude and superficial. However, interest in free recall surged following a series of influential studies published between 1953 and 1962 by Weston Bousfield, James Deese, Ben Murdock, Leo Postman, and Endel Tulving.

[3] In item recognition, normative word frequency has the opposite effect, with rare words being better recognized than common words (MacLeod and Kampe, 1996).

Walker, 1969). Moreover, asking subjects to perform a brief unrelated distractor task at the end of the list (e.g., solving arithmetic problems for 15 s) greatly reduces the recency effect while having no adverse consequences on recall of prerecency items (Postman and Phillips, 1965; Glanzer and Cunitz, 1966). **Figure 1(a)** shows the effect of a brief distractor task on the serial position curve in free recall. These and other dissociations between recency and prerecency led many investigators to embrace the notion of distinct memory systems: a short-term store (STS) responsible for the recency effect, and a long-term store (LTS) responsible for the primacy effect and for the level of recall for prerecency items (Waugh and Norman, 1965; Atkinson and Shiffrin, 1968; Glanzer and Cunitz, 1966).

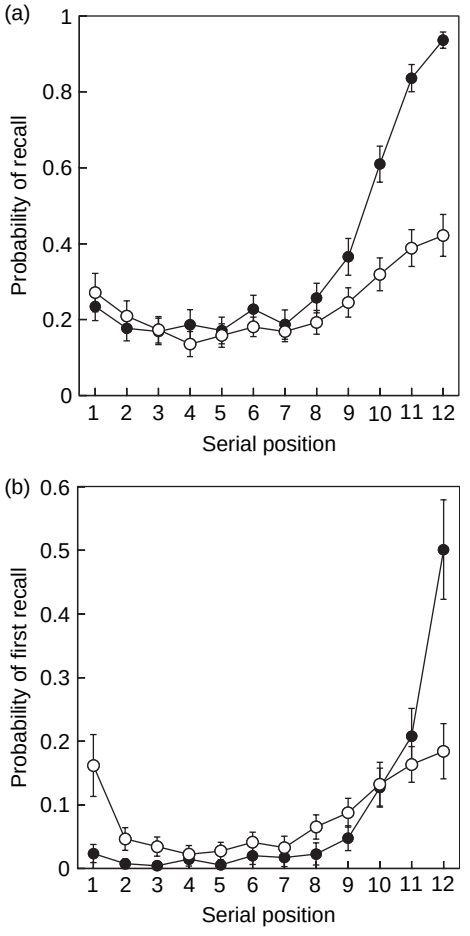

Figure 1 The recency effect in immediate and delayed free recall. After studying a list of 12 common words, subjects were either asked to recall items immediately (filled circles) or following a 15-s arithmetic distractor task (open circles). (a) Serial position curves. (b) Probability of first recall functions show the probability that the first recalled item was presented in a given serial position. These functions thus illustrate the relative tendency to begin recall with primacy or recency items. Data are from Howard MW and Kahana MJ (1999) Contextual variability and serial position effects in free recall. *J. Exp. Psychol. Learn. Mem. Cogn.* 25: 923–941 (Experiment 1). Error bars denote 95% confidence intervals.

2.26.2.1 Retrieval Dynamics in Free Recall

Although traditional serial position-based analyses fueled much of the theoretical debate concerning the memory processing underlying free recall (and for that matter serial recall), such analyses discard information about sequential dependencies in retrieval, information which is crucial for understanding the structure of episodic memory storage, and the process of episodic memory retrieval. By measuring the order in which list items are recalled, we can decompose the retrieval process into a measure of how subjects initiate recall and a measure of how they make transitions among successively recalled items.

As mentioned above, subjects typically initiate recall with one of the final list items. This tendency can be quantified by measuring the probability with which subjects initiate recall at each serial position. **Figure 1(b)**, which shows the probability of first recall as a function of serial position, reveals a strong tendency for subjects to initiate recall with one of the final list items (Hogan, 1975; Laming, 1999). In delayed free recall, this tendency is markedly diminished (Howard and Kahana, 1999). By studying subjects' subsequent recall transitions, one can see that temporally defined, interitem associations exert a strong influence on output order and inter-response times in free recall. These associations are inferred from participants' tendency to successively recall items from nearby list positions. As shown in **Figure 2(a)**, the probability of recalling a word from serial position $i+\text{lag}$ immediately following a word from serial position i is a sharply decreasing function of |lag|. Positive values of lag correspond to forward recall transitions; negative values of lag correspond to backward recall transitions.[4] In calculating the conditional response probability as a

[4] For example, if the list had contained the subsequence '*absence hollow pupil*' and a participant recalled *hollow* then *pupil*, the recall of *pupil* would have a lag of +1. If, instead, the participant recalled *hollow* then *absence*, the recall of *absence* would have a lag of −1. In this case, the participant is moving backward in the list. *Absence* followed by *pupil* would yield a lag of +2.

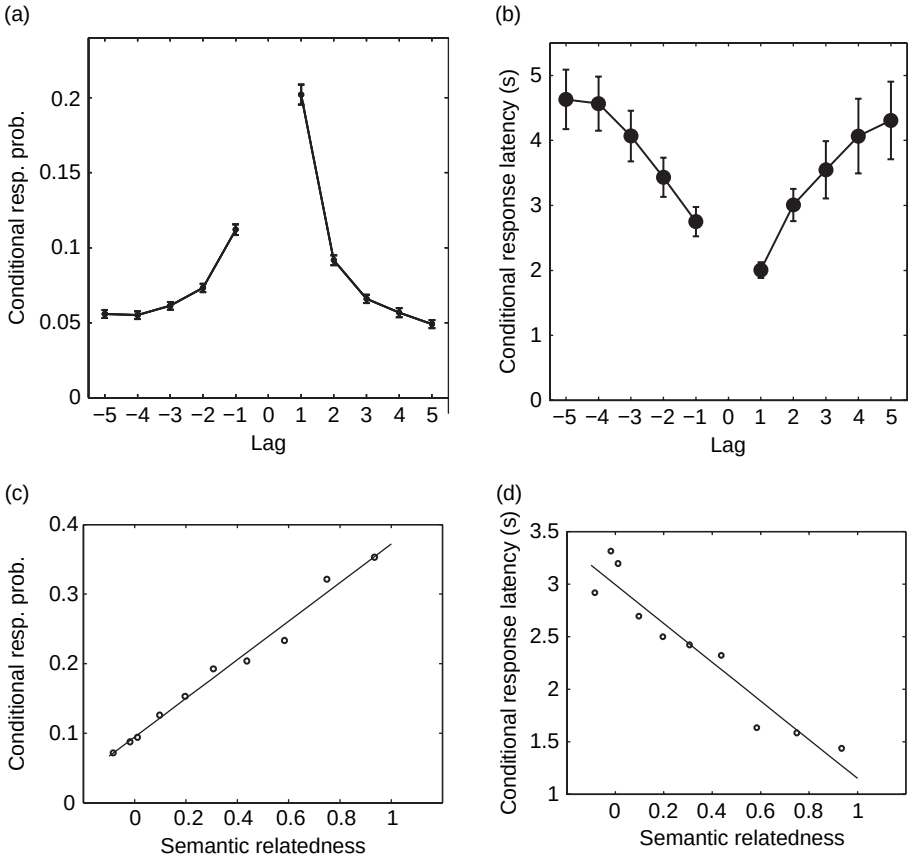

Figure 2 Associative processes in free recall: effects of temporal contiguity and semantic relatedness. (a) The conditional–response probability as a function of lag (or lag-CRP) shows the probability of recalling an item from serial position $i+\text{lag}$ immediately following an item from serial position i. This curve is based on data from 20 experimental conditions (Murdock BB (1962) The serial position effect of free recall. *J. Exp. Psychol.* 64: 482–488; Murdock BB and Okada R (1970) Interresponse times in single-trial free recall. *J. Verb. Learn. Verb. Behav.* 86: 263–267; Murdock BB and Metcalfe J (1978) Controlled rehearsal in single-trial free recall. *J. Verb. Learn. and Verb. Behav.* 17: 309–324; Roberts WA (1972) Free recall of word lists varying in length and rate of presentation: A test of total-time hypotheses. *J. Exp. Psychol.* 92: 365–372; Kahana MJ, Howard MW, Zaromb F, and Wingfield A (2002) Age dissociates recency and lag recency effects in free recall. *J. Exp. Psychol. Learn. Mem. Cogn.* 28: 530–540; Howard MW and Kahana MJ (1999) Contextual variability and serial position effects in free recall. *J. Exp. Psychol. Learn. Mem. Cogn.* 25: 923–941; Zaromb FM, Howard MW, Dolan ED, Sirotin YB, Tully M, Wingfield A, et al. (2006) Temporal associations and print-list intrutions in free recall. *J. Exp. Psychol. Learn. Mem. Cogn.* 32(4), 792–804; Kimball DR and Bjork RA (2002) Influences of intentional and unintentional forgetting on false memories. *J. Exp. Psychol. Gen.* 131: 116–130; Kahana MJ and Howard MW (2005) Spacing and lag effects in free recall of pure lists. *Psychon. Bull. Rev.* 12: 159–164; Kahana MJ, Dolan ED, Sauder CL, and Wingfield A (2005a) Intrusions in episodic recall: Age differences in editing of overt responses. *J. Gerontol. Psychol. Sci.* 60: 92–97). (b) The conditional-response latency as a function of lag (or lag-CRL) shows the mean inter-response time between successive recalls of items from serial positions i and $i+\text{lag}$ (Howard MW and Kahana MJ (1999) Contextual variability and serial position effects in free recall. *J. Exp. Psychol. Learn. Mem. Cogn.* 25: 923–941; Murdock BB and Okada R (1970) Interresponse times in single- trial free recall. *J. Verb. Learn. Verb. Behav.* 86: 263–267; Zaromb FM, Howard MW, Dolan ED, Sirotin YB, Tully M, Wingfield A, et al. (2006) Temporal associations and prior-list intrutions in free recall. *J. Exp. Psychol. Learn. Mem. Cogn.* 32(4): 792–804; Kahana MJ, and Howard MW (2005) Spacing and lag effects in free recall of pure lists. *Psychon. Bull. Rev.* 12: 159–164). Error bars represent 95% confidence intervals across experiments. (c) The conditional-response probability as a function of semantic relatedness (semantic-CRP) reveals that subjects are more likely to recall items that are semantically related to the just-recalled item. Semantic-relatedness was measured using the word-association space technique (Steyvers M, Shiffrin RM, and Nelson DL (2004) Word association spaces for predicting semantic similarity effects in episodic memory. In: Healy AF (ed.) *Cognitive Psychology and its Applications: Festschrift in Honor of Lyle Bourne, Walter Kintsch, and Thomas Landauer*. Washington, DC: American Psychological Association). (d) The conditional-response latency as a function of semantic relatedness (semantic-CRL) shows that subject transitions are made more quickly when they are to related items.

function of lag, or lag-CRP, we estimate the probability of a transition to a given lag by dividing the number of transitions to that lag by the number of opportunities to make a transition to that lag.

2.26.2.2 The Contiguity Effect

The analysis of retrieval transitions in free recall reveals a strong tendency for neighboring items to be recalled successively. We refer to this phenomenon, illustrating participants' reliance on temporal associations to guide recall, as the contiguity effect. As shown in **Figure 2(a)**, the contiguity effect exhibits a marked forward bias, with associations being stronger in the forward than in the backward direction. The basic form of the contiguity effect does not appear to depend on experimental manipulations. The lag-CRP functions are virtually identical across manipulations of presentation modality (visual vs. auditory), list length, and presentation rate (Kahana, 1996).

The contiguity effect also appears in the form of shorter inter-response times between recall of items from neighboring list positions. This can be seen in the conditional response latency (lag-CRL) function shown in **Figure 2(b)** (see Kahana and Loftus, 1999, for a further discussion of the accuracy–latency relation). The contiguity effect, as seen in both accuracy and latency data, may reflect a kind of mental time travel undertaken during memory search and retrieval. In recalling an item, the subject may 'travel back' to the time of its presentation, making it more likely that subsequent recalls will come from nearby serial positions.

2.26.2.3 The Semantic Proximity Effect

In free recall, participants do not rely solely on newly formed episodic associations; they also make use of their pre-existing semantic associations among list items. We can quantify subjects' use of semantic associations in free recall by computing the conditional probability of a recall transition as a function of an item's semantic relatedness to the just-recalled item (we term this function the semantic-CRP). This approach requires a measure of the semantic relatedness of arbitrary word pairs. To obtain such measures, we turn to computational models of semantic spaces. Landauer and Dumais (1997) developed latent semantic analysis (or LSA); this project involved the statistical analysis of a large text corpus, allowing them to derive a measure of word-relatedness from the tendency for words that share meaning to co-occur in paragraphs. Steyvers et al. (2004) developed a word association space (or WAS) based on the large University of South Florida word association database (Nelson et al., 2004). Both LSA and WAS provide measures of the semantic relatedness for a great many pairs of words in the English language. The measure is quantified as the cosine of the angle between the vectors representing the two words in a high-dimensional space. Completely unrelated words would have $\cos\theta \approx 0$, and strong associates would have $\cos\theta$ values between 0.4 and 1.0. For a more thorough treatment and discussion, see Howard et al. (2007).

The semantic-CRP shows that the stronger the semantic relation between two list words, the more likely it is that they would be successively recalled (**Figure 2(c)**). In addition, the stronger the semantic association between two successively recalled words, the shorter the inter-response time would be between the two words (**Figure 2(d)**). This analysis illustrates the powerful influence of semantic relatedness on recall of randomly chosen word lists. Even when lists lack any strong associates or any obvious categorical organization, recall transitions are driven by the relative semantic strengths among the stored items. Consistent with the findings of category clustering and subjective organization described above, the contiguity effect decreases, and the semantic-proximity effect increases, across learning trials in which the order of word presentation at study is randomized on each trial (Klein et al., 2005; Howard et al., 2007).

2.26.2.4 Normal Aging Affects Contiguity but Not Recency

It is well known that older adults perform more poorly on episodic memory tasks than their younger counterparts (Verhaeghen and Marcoen, 1993; Kausler, 1994). The age-related memory impairment is particularly marked in recall tasks that require subjects to use temporally defined associations, such as cued recall and free recall (Naveh-Benjamin, 2000; Wingfield and Kahana, 2002; Hoyer and Verhaeghen, 2006).

The analysis of retrieval transitions, as described above, can be used to directly assess subjects' reliance on temporal associations in free recall. Kahana et al. (2002) examined the difference between recency

and contiguity effects in younger and older adults. Half of the subjects in each age group were given an immediate free recall test; the other half were given a delayed free recall test. As expected, younger adults recalled more words on both immediate and delayed tests, and the distractor task attenuated the recency effect for subjects in both age groups. The critical finding was that older adults exhibited a significantly diminished contiguity effect, as seen in their lag-CRP functions (**Figure 3(b)**). In contrast, younger and older adults initiated recall in the same manner; their probability of first recall functions were virtually identical both in the immediate and in the delayed free-recall conditions (**Figure 3(a)**). Although older adults exhibited a markedly reduced contiguity effect, their semantic-proximity effect was unimpaired (unpublished observation). These findings suggest that the mnemonic deficit observed for older adults is largely restricted to the ability to form and/or utilize temporally defined associations. This is consistent with previous reports of age-related deficits in the formation and retrieval of episodic associations (e.g., Naveh-Benjamin, 2000).

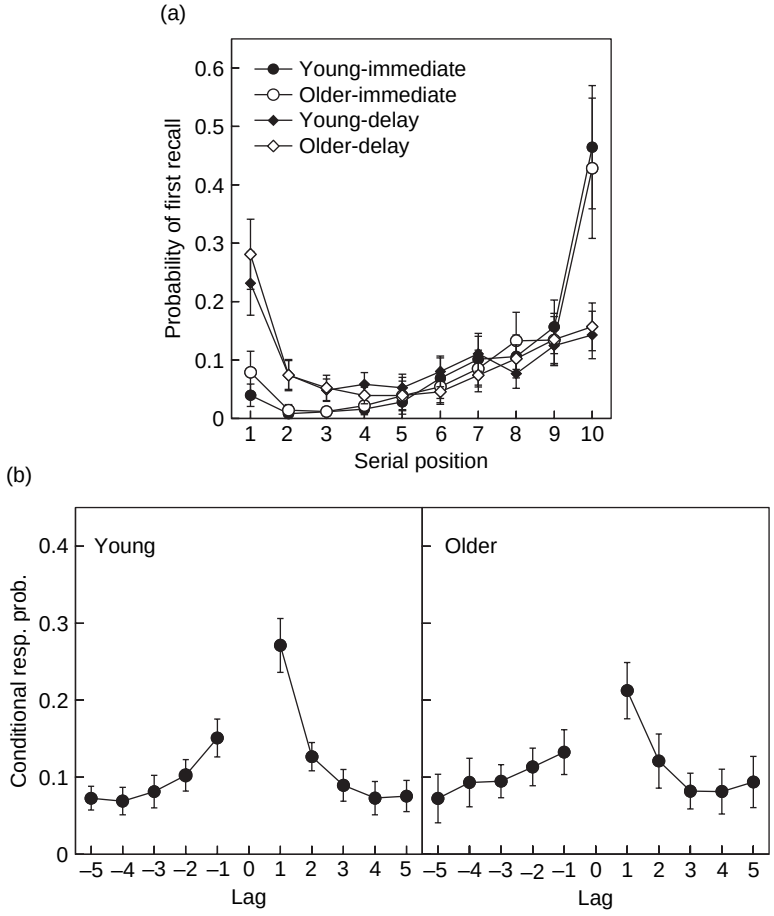

Figure 3 Selective effect of aging on associative processes in free recall. (a) Probability of first recall from immediate and delayed free recall for younger and older adults. Data taken from Kahana MJ, Howard MW, Zaromb F, and Wingfield A (2002) Age dissociates recency and lag recency effects in free recall. *J. Exp. Psychol. Learn. Mem. Cogn.,* 28: 530–540. Figure reprinted with permission from Howard MW, Addis KA, Jing B, and Kahana MJ (2007) Semantic structure and episodic memory. In: McNamara D and Dennis S (eds.), *LSA: A Road Towards Meaning.* Hillsdale, NJ: Laurence Erlbaum and Associates. (b) Conditional response probability (CRP) for younger and older adults from the delayed condition of Kahana MJ, Howard MW, Zaromb F, and Wingfield A (2002) Age dissociates recency and lag recency effects in free recall. *J. Exp. Psychol. Learn. Mem. Cogn., 28*, 530–540.

2.26.2.5 Long-Range Interitem Associations

Bjork and Whitten (1974) conducted an experiment which challenged the traditional STS-based account of recency effects in free recall. They were interested in seeing how well subjects could recall a list of word pairs under conditions designed to eliminate between-pair rehearsal. To eliminate between-pair rehearsal, they had subjects perform a difficult distractor task following the appearance of each pair, including the last one. Because the distractor was expected to displace any items in STS, Bjork and Whitten did not expect to find a recency effect. To their surprise, they found a strong recency effect, with the final few pairs being recalled better than pairs from the middle of the list. They called this the long-term recency effect. Their procedure, in which a distractor task is given following every item, including the last, is called continuous-distractor free recall. **Figure 4** illustrates the continuous-distractor free recall procedure alongside the more traditional immediate and delayed free recall procedures.

Condition	Recency
Immediate PEN CAR ROSE ··· BIND ***	Yes
Delayed PEN CAR ROSE ··· BIND [1+2=]***	No
Continuous distractor PEN [6+2=]CAR [3+7=]ROSE [1+1=] ··· BIND [2+5=]***	Yes

Figure 4 Illustration of immediate, delayed, and continuous-distractor paradigms. The row of asterisks indicates the start of the recall period.

The long-term recency effect has now been replicated many times using both single words and word pairs, and across delays ranging from tenths of seconds (Neath, 1993) to days (Glenberg et al., 1983). The magnitude of the long-term recency effect depends critically on both the duration of the distractor given after the last word (the retention interval) and on the duration of the distractor intervening between list words (the interpresentation interval). For a given retention interval, increasing the interpresentation interval results in more recency and better recall of the final item.

Kahana (1996) interpreted the contiguity effect as evidence for associations formed in STS. If associations are formed between items that are active together in STS (as postulated by Glanzer, 1972; Raaijmakers and Shiffrin, 1980), then this would predict the contiguity effect because nearby items spend more time together in STS than remote items. However, because a long interitem distractor should displace items in STS, the contiguity effect should be significantly attenuated in continuous-distractor free recall.

Howard and Kahana (1999) tested this hypothesis by measuring the contiguity effect in continuous-distractor free recall. **Figure 5(a)** illustrates the contiguity effect for interpresentation intervals ranging from 0 s (standard delayed free recall) to 16 s. As can be seen, the contiguity effect was relatively constant across this range of interpresentation intervals. This result is quantified in **Figure 5(b)** by fitting a power function ($P = a|\text{lag}|^{-b}$) to each participant's lag-CRP curve and using the b parameter as an estimate of the contiguity effect (the a parameter determines the overall scale of

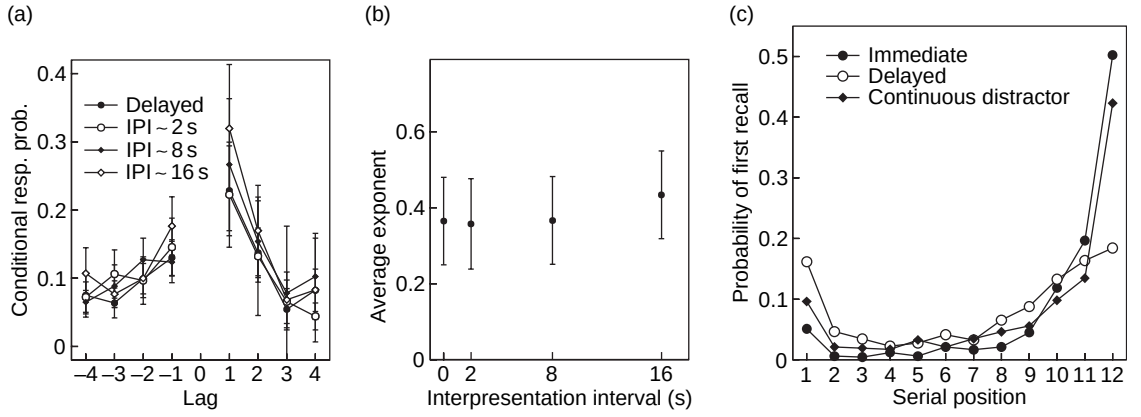

Figure 5 Long-range contiguity and recency effects. (a) Lag-CRP as a function of the length of the distractor task in continuous-distractor free recall. (b) To quantify the contiguity effect, power functions were fit to the lag-CRP curves for each participant in each condition. Error bars represent 95% confidence intervals. (c) The probability of first recall functions for immediate, delayed, and continuous-distractor free recall (Howard and Kahana, 1999).

the function). Insofar as the contiguity effect is insensitive to the absolute delay between list items, it exhibits an approximate time-scale invariance. Although 16 s of a distractor had virtually no impact on the contiguity effect, the same amount of distractor activity presented at the end of the list was sufficient to eliminate the end-of-list recency effect (**Figure 5(c)**).

As shown in **Figure 5**, the contiguity effect persists even when the study items are separated by 16 s of a demanding distractor task. However, recent work shows that the contiguity effect is evident on even longer time scales. Howard et al. (2008) presented subjects with a series of lists for free recall. At the conclusion of the session, subjects were given a surprise final free recall test in which they were instructed to remember as many words as possible from the 48 study lists in any order. Howard et al. (2008) measured the contiguity effect in this final free recall period both for transitions within a list as well as between lists. They found that transitions between nearby lists were more frequent than transitions between lists that were farther apart in the experiment. This contiguity effect extended about ten lists, or several hundred seconds, extending the range over which contiguity effects are observed in free recall by a factor of ten. Moreover, this paradigm offers several potential advantages over continuous-distractor free recall. In continuous distractor free-recall, subjects have an incentive to try and rehearse items across the distractor intervals. Because the subject is only asked to recall the most recent list in the Howard et al. (2008) study, and intrusions from prior lists are scored as errors, there is no strategic reason for subjects to rehearse across lists in anticipation of the surprise final free recall test. In continuous-distractor free recall, the consistency of associations across delay intervals was inferred from observing lag-CRP curves across conditions that differed in their IPI. It is conceivable that this was due in part to different strategies across experimental conditions. In contrast, in the Howard et al. (2008) study, both within-and across-list associations were observed simultaneously during the final free recall period.

2.26.2.6 Interim Summary

We have shown how both temporal contiguity and semantic relatedness strongly predict the order and timing of subjects' responses in the free-recall task. The contiguity effect (**Figure 2(a, b)**) illustrates how episodic associations are graded, exhibiting power-function decay with increasing lag. Recall of an item has a tendency to evoke not only adjacent list items, but other nearby items as well. In addition, episodic associations appear to be asymmetrical, favoring retrieval of items in the forward order.

Whereas the previous two characteristics of episodic association can be accommodated within the view that neighboring items become associated when they cooccupy a short-term buffer (or working memory system), analyses of episodic association in continuous-distractor free recall show that the contiguity effect persists across time scales. That is, using a distractor task to temporally segregate list items does not disrupt the associative mechanism. Moreover, contiguity can even be observed in recall transitions among items studied as part of different lists, separated by several minutes. The tendency for an item to evoke a nearby item thus depends on the relative spacing, not the absolute spacing, of the list items.

A critical question for memory theory is whether the contiguity effect is specific to free recall, or whether similar associative processes operate in other memory tasks. It is possible that some of the phenomena described in the preceding section are a consequence of specific strategies that subjects use in the free-recall paradigm. In particular, by allowing participants to recall items in any order, we may be observing participants' biases in favoring particular kinds of transitions (e.g., forward over backward, adjacent over remote) rather than revealing the underlying associative structure. This criticism is blunted by our finding that the lag-CRP and lag-CRL functions vary little across experiments that differ significantly in their methodologies, even including the introduction of a long interitem distractor (see **Figure 5**). Nonetheless, it is important to take a broader look at the question of associative processes in episodic memory. In the next section, we show how associative processes can be seen in the pattern of subjects' errors in free recall, serial recall, and cued recall. We then examine the question of associative processes in item recognition. The final section of this chapter discusses these empirical data in terms of the major theories of associative processes in episodic memory.

2.26.3 Memory Errors Reveal Associative Processes

The study of the errors made in a variety of memory tasks shows that even when the memory system goes awry and produces a response that is incorrect in the context of a given experiment, the processes

generating this error appear to be influenced by the same factors that guide correct responses. In this section, we consider how subjects' recall errors reveal characteristics of the associative processes operating in free recall, serial recall, probed recall, and cued recall tasks.

2.26.3.1 Prior-List Intrusions in Free Recall

It is well known that incorrect recalls (intrusions) often arise due to the semantic relations between studied and nonstudied items. For example, after studying a list of items that include the semantic associates of a critical word, participants often incorrectly recall that critical word even though it was not presented on the list (Deese, 1959; Roediger and McDermott, 1995; Roediger et al., 1998; Gallo and Roediger, 2002). Although semantic association is a major determinant of false recall, episodic memory processes also appear to play an important role. For example, in free recall of randomly arranged word lists, prior-list intrusions – incorrect recalls of words that were presented on an earlier list – are often more frequent than extralist intrusions – incorrect recalls of words that were not presented during the course of the experiment. This suggests that the recent study of an item increases the probability that it will be (incorrectly) recalled. Moreover, prior-list intrusions exhibit a strong recency effect, being most likely to come from the list immediately preceding the target list (Murdock, 1974; Zaromb et al., 2006); the number of prior-list intrusions coming from earlier lists decreases sharply (see **Figure 6(a)**).

In a recent study, Zaromb et al. (2006) asked whether contiguity-based associations would also tend to induce false recall. They conducted several free-recall experiments in which some items in a given list had also appeared on earlier lists. In all cases, participants were instructed to recall only the items from the most recently presented list. By creating lists that contained mixtures of novel items and items repeated from earlier lists, Zaromb et al. found that recalls of repeated items were more likely to be followed by prior-list intrusions than were recalls of novel items. This finding would emerge if temporal associations forged on prior lists compete with the associations formed in the current list, and if these older associations occasionally win in the competition. As further support for the role of contiguity-based associations, Zaromb et al. found that repetition-evoked prior-list intrusions came from the same prior lists as the repetitions themselves,

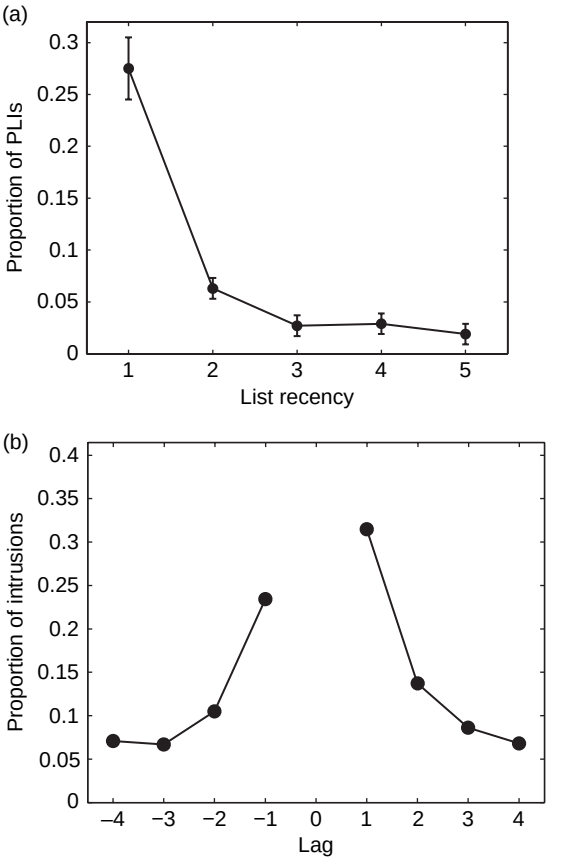

Figure 6 Effects of recency and contiguity on intrusions in free recall. (a) Prior-list intrusion (PLI) recency effect. Proportion of intrusions coming from one to five lists back. In calculating these PLI-recency functions for items originally presented one to five lists back, we excluded the first five trials from the analysis. That is because PLIs from five lists back could only occur on trials 6 and later. (b) Successive PLIs that came from the same original list tend also to come from neighboring positions in their original list. Thus, temporally defined associations influence PLIs in free recall (Zaromb et al., 2006).

and from positions near the repetitions in those lists. When subjects committed two same-list prior-list intrusions in succession, those intrusions tended to come from neighboring positions in their original list, exhibiting a temporal contiguity effect similar to that seen for correct recalls (see **Figure 6(b)**).

2.26.3.2 Intrusions in Serial and Probed Recall

We next consider the effect of contiguity on retrieval in serial-order memory. In a serial-recall task, participants are instructed to recall the list items in order of

presentation, rather than in any order as in free recall. In requiring ordered recall, the serial-recall task demands that subjects store information not only about which items were on the list, but also about their order. Thus, the serial-recall task exerts greater control over the manner of encoding and retrieval than does free recall.

Although subjects can only make one correct response in a given output position, they can commit many different types of errors. The orderly pattern of subjects' errors in serial recall can teach us a great deal about the underlying processes. For example, it is well known that when recalling an item in the wrong position this item tends to be misplaced near the correct (target) position (e.g., Lee and Estes, 1977). This finding has also been documented extensively in reordering tasks, where subjects are given all of the target items and asked to place them in their correct studied order (e.g., Nairne, 1990a, 1990b).

The traditional method for measuring error gradients is to plot the probability of an item studied in serial position i being recalled in position $i+\text{lag}$. This approach works especially well in reordering tasks where all the items are placed in some position. With longer lists, where only some of the items are recalled, it is especially important to correct for the availability of different lags, as we have done in our lag-CRP analysis of free recall. For these lag-CRP analyses, we compute the probability of recalling an item from position i in position $i+\text{lag}$ conditional on the possibility that an item could be placed in position $i+\text{lag}$ (for example, we make sure that the item from that position has not already been recalled). **Figure 7(a)** shows an analog of the lag-CRP derived from errors observed during serial recall (Kahana and Caplan, 2002). In addition to revealing the tendency for errors to come from nearby list positions, this curve shows a clear asymmetry effect, with errors in the forward direction being significantly more likely than errors in the backward direction.[5] Thus, the temporal gradient of errors in serial recall is strikingly similar to the temporal gradient of correct responses observed in free recall (see Klein et al., 2005, for a direct comparison of free recall and serial recall).

The analysis of errors in serial recall is complicated by the fact that each response depends on the sequence of prior responses (Giurintano, 1973). An alternative approach to measuring serial-order memory is to present subjects with a single item from a previously studied list and ask them to recall the item that preceded or followed the probe item (Murdock, 1968; Woodward and Murdock, 1968). Analysis of error gradients obtained in forward and backward probed recall provide an even cleaner test of the asymmetry effect observed in both free and serial recall. **Figure 7(b)** shows error gradients in a probed recall study reported by Kahana and Caplan (2002). The top panel shows that when subjects were given item i and asked to recall item $i+1$, responses tended to come from nearby positions, with a forward bias ($i+2$ is more likely than $i-1$). The bottom panel of **Figure 7(b)** shows that when subjects were probed in the backward direction (i.e., given item i and asked to recall item $i-1$), the same forward asymmetry was obtained (see also Raskin and Cook, 1937).

2.26.3.3 Intrusions in Paired-Associate Recall

The preceding section documented two characteristics of errors in serial recall and in probed recall of serial lists: (1) subjects' intrusions tend to be items studied near the position of the target item and (2) subjects' error gradients exhibit a forward asymmetry, with errors being more likely to be items following than items preceding the target item. The temporal gradient of retrieval transitions in free recall as seen in the lag-CRP, and the gradient of subjects' intralist intrusion errors in both serial and probed recall could reflect a common methodological aspect of these tasks. In both free and serial recall tasks, the to-be-learned items constitute an unbroken series such that storing and retrieving associations among neighboring items is useful for performing the task. An important exception to this is continuous-distractor free recall, in which list items are separated by a demanding distractor task. Nonetheless, even in continuous-distractor free recall, subjects may be motivated to make associations between neighboring items.

Paired associate memory provides an interesting contrast to both free and serial recall. In the standard paired-associate procedure, subjects are asked to learn a list of nonoverlapping pairs of words. Following this study phase, subjects are cued for recall of specific pairs (either in the forward or the backward order). Unlike free and serial recall, in which subjects must learn an entire list, subjects in the paired-associate task have no reason to learn associations other than those binding the items within

[5] As with the lag-CRP analysis of free recall, this analysis corrects for the number of available to-be-recalled items.

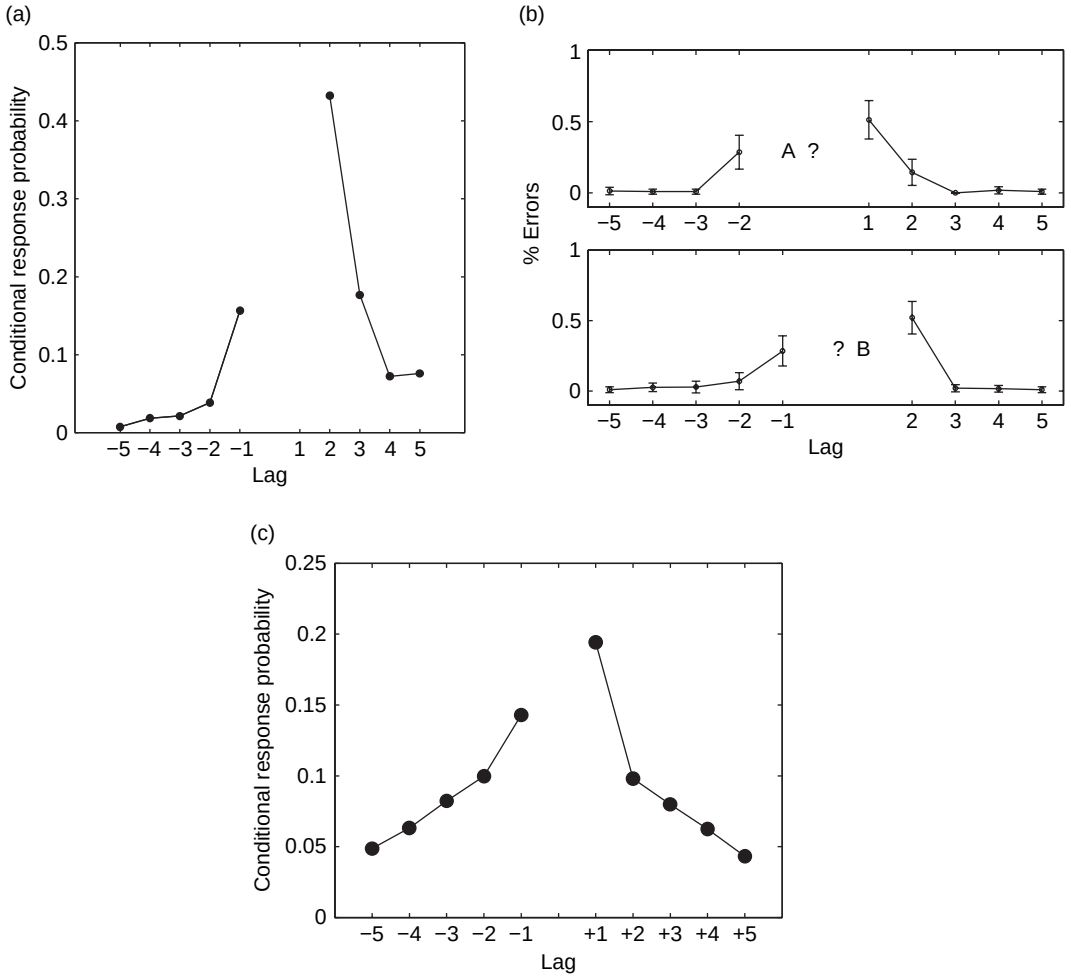

Figure 7 Intrusions reveal associative tendencies in serial-recall, probed-recall, and cued-recall tasks. (a) Lag-CRP analysis of errors in immediate serial recall. Correct responses (lag of +1) were excluded from this analysis. (b) Conditional error gradients in forward (top) and backward (bottom) probed recall; subjects are given item *i* as a cue for item $i+1$ (upper panel), or $i-1$ (lower panel), and they recall some other item $i+$ lag. Data are from Trial 1 of Kahana and Caplan's second experiment (Kahana MJ and Caplan JB (2002) Associative asymmetry in probed recall of serial lists. *Mem. Cognit.* 30: 841–849). (c) Following study of 12 randomly chosen noun-noun pairs, subjects were given a standard cued recall test. The probability of incorrectly recalling a word from pair-*j* in response to a cue word from pair-*i* decreased with increasing lag, measured in pairs. (Davis OC, Geller AS, Rizzuto DS, and Kahana MJ (2008) Temporal associative processes revealed by intrusions in paired-associate recall. *Psychon. Bull. Rev.* 15(1): 64–69).

each studied pair. Recall is strictly cued by the experimenter so there is no benefit to recalling any item other than the one being probed. Whereas associations in both free and serial recall have a strong forward bias, associations in paired-associate tasks are generally symmetric, with nearly identical recall rates for forward and backward probes (for reviews see Ekstrand, 1966; Kahana, 2002). This surprising result led Gestalt psychologists to propose an *associative symmetry hypothesis* (Köhler, 1947; Asch and Ebenholtz, 1962). According to this hypothesis, associations are learned by incorporating the representations of the constituent items into a new holistic representation. Formalized in computational models, this hypothesis implies that the strengths of forward and backward associations are approximately equal and highly correlated (Rizzuto and Kahana, 2001; Kahana, 2002; Caplan et al., 2006; Sommer et al., 2007).

In light of the distinct features of the paired-associate task, one may wonder whether subjects form temporal associations beyond those required to learn the pairings set forth in the experiment. Davis et al. (2008) addressed this question by examining subjects'

pattern of intralist intrusions in paired associate recall. In a cued recall task, there are a number of types of errors a subject could make. Intralist intrusions are incorrect responses where the subject recalls an item from a different pair than the cue came from. Davis et al. (unpublished data) hypothesized that if a common associative process underlies all recall tasks, intralist intrusions would be more likely to come from neighboring list pairs. Consistent with CRP analyses from other paradigms, Davis et al. conditionalized the probability of committing an intrusion from a given lag on the availability of the pair at that lag. Although intralist intrusions constituted only 5% of subjects' responses, these intrusions exhibited a strong tendency to come from neighboring pairs. This can be seen in **Figure 7(c)**, which shows that the conditional probability of an intralist intrusion decreased monotonically with the number of pairs (lag) separating the intrusion from the probed item. This effect was not limited to an increased tendency to commit intrusions from adjacent pairs; even when adjacent pairs were excluded, a regression analysis demonstrated that the across-pair contiguity effect was highly reliable.

Because the order of test was randomized with respect to the order of study, there was no reason for subjects to adopt a strategy of learning interpair associations. Indeed, such a strategy would have been counterproductive insofar as it would induce high levels of associative interference between pairs (Primoff, 1938). As such, these findings of associative tendencies in subjects' intralist intrusions suggest that these temporally defined associations arise from a basic and most likely obligatory memory process that causes items studied in nearby list positions to become associatively connected.

This spectrum of findings reveals that free recall is not alone in providing evidence for the centrality of contiguity effects in human memory. All of the major recall paradigms – free recall, serial recall, and paired-associates learning – show graded effects of temporal contiguity; in many cases these effects are revealed in the patterns of errors made by subjects. Taken together, these findings allow us to glimpse the workings of a general-purpose 'engine of association' that is tapped by all of these varied tasks. Furthermore, the observation of long-range contiguity, both in free recall and in subjects' intrusions in paired-associate recall, challenges the view that intentional encoding is necessary for the formation of contiguity-based associations.

2.26.4 Associative Processes in Item Recognition

Theories of item recognition and cued recall typically assume that these two tasks are based on distinct and possibly independent sources of information (Murdock, 1982; Gillund and Shiffrin, 1984; Kahana et al., 2005b) According to these theories, item recognition relies on item-specific information, whereas recall tasks rely on associative (or relational) information (Humphreys, 1978; Hunt and McDaniel, 1993). This view is supported by experimental dissociations between item recognition and free recall (e.g., the word frequency effect; Kinsbourne and George, 1974) and by the finding that words that are recallable often cannot be recognized, and vice versa (e.g., Tulving and Thompson, 1973; Tulving and Wiseman, 1975).

Despite these differences between recall and recognition, both tasks assess memory for an event encoded within a temporal context. Given the ubiquitous character of the contiguity effect across all of the major recall paradigms, it is natural to ask whether contiguity exerts some influence on retrieval in item recognition, at least under conditions where subjects' recognition judgments are accompanied by a feeling of recollection. More specifically, one might hypothesize that recognizing an item as having been previously studied would partially reinstate the item's encoding context, which in turn might facilitate subsequent recognition of neighboring items.

To test this hypothesis, Schwartz et al. (2005) manipulated the serial lag between successive memory probes in an item recognition study that used landscape photos as stimuli. The recognition test was a sequence of test probes that included the old items from the list intermingled with an equal number of new items that served as lures. Subjects pressed one of six keys in response to each probe, rating their confidence that it was seen before from 1 (sure new) to 6 (sure old). A recognition test might include the subsequence of test probes $(\ldots O_{23}, N, O_{12}, O_7, N, N, O_{39}, \ldots)$, where N denotes a new item and O_x denotes an old item from position x in the study list. The lag between two successive old items $(\ldots O_i, O_j \ldots)$ is just the distance, $j - i$, between the items on their initial presentation.

Suppose that recognition of a test item, O_i, brings forth the mental state – or temporal context – that prevailed when O_i was first encoded. Suppose further

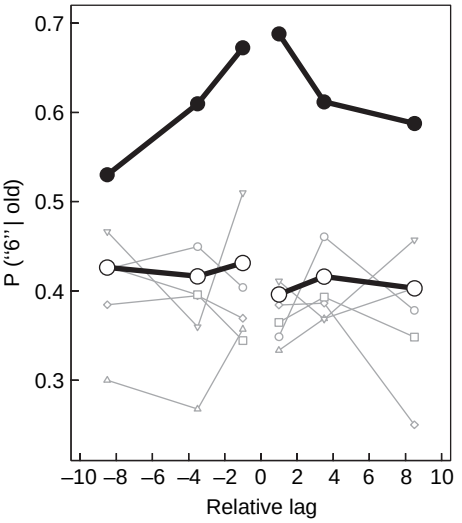

Figure 8 Contiguity effects in item recognition are specific to highest-confidence responses. Probability of a highest confidence (6) response to an old-item test probe as a joint function the relative lag of, and the response given to, the preceding old-item probe. Large filled circles represent 6 responses to the prior test probe. Open symbols represent one of the other five possible prior responses; downward-facing triangles, boxes, triangles, upward-facing diamonds, and circles represent responses 1–5 respectively. Large open circles collapse data over responses 1–5. Data are from Schwartz et al. (2005). Shadows of the past: Temporal retrieval effects in recognition memory. *Psychol. Sci.* 16: 898–904.

that this retrieved mental state contributes to the retrieval environment that determines subsequent recognition judgments. Then, if the very next test item is O_j, we would predict that memory for O_j should be enhanced when lag $= j - i$ is near zero.

The data in **Figure 8** show that when two old items are tested successively, memory for the second is better if it was initially presented in temporal proximity to the first. This tendency, however, was wholly attributable to cases in which the first item received a highest-confidence response. These highest-confidence old responses may be considered to reflect successful recollection of specific attributes of the encoding episode, whereas lower-confidence old responses are assumed to reflect the familiarity of an item whose attributes are not recollected (Yonelinas, 1999; Sherman et al., 2003). Schwartz et al. (2005)'s observation of contiguity effects in item recognition suggests that recollection of an item not only retrieves detailed information about the item tested, but also retrieves information about the item's neighbors.

We have now seen that the contiguity effect appears in all of the major episodic memory paradigms, including free recall, serial recall, probed recall, paired-associates, and even item recognition. The ubiquitous nature of this phenomenon implores us to search for an explanation in terms of fundamental principles of memory function. This search is the topic of the next section.

2.26.5 Theories of Episodic Association

Four major theories have been proposed to account for associative processes in episodic memory: (1) associative chaining, (2) associations formed in working memory (or buffer theory), (3) hierarchical associations (or chunking theory), and (4) contextual retrieval theory. In this section, we examine the implications of each of these four theories for the key empirical findings concerning contiguity-based associations in episodic memory.

Chaining theory, which originates in the writings of the associationists (e.g., Herbart, 1834) and in the early experimental work of Ebbinghaus, (1885/1913), assumes that when the memorial representations of two items become simultaneously active, or become active in rapid succession, the items' representations become associated in the sense that activation of one will evoke the other. A key feature of chaining is that associations are formed on the basis of temporal contiguity at study and that an item's representation is assumed to remain active only until the occurrence of the next item in the list.

Buffer models elaborate the basic chaining idea to include a mechanism that maintains an item's representations in the system past its actual presentation, allowing direct interitem associations to be created between items that are presented further apart in time (remote associations). Whereas classic chaining models assume that only two items are simultaneously active, buffer models allow for a larger number of items to be maintained in an active state and provide rules that determine when an item enters and leaves the active state (i.e., the buffer; Raaijmakers and Shiffrin, 1981).

Hierarchical associative models are based on the idea that multiple items can become unitized into a higher-order, conjunctive, representation which is distinct from any of the constituent items. These models have been particularly useful in describing the process of serial learning and serial recall (Johnson, 1972; Martin and Noreen, 1974; Lee and Estes, 1977; Murdock, 1995b, 1997). They assume

that associations between items are mediated by a higher-level (super-ordinate) representation.

Finally, contextual retrieval theory assumes that items are associated with a time-varying representation of spatiotemporal/situational context (Estes, 1955; Bower, 1972; Burgess and Hitch, 2005). Successively presented items are associated with this context representation, which then can be used as a cue to retrieve those item representations during the recall period. Importantly, associations arise when items retrieve their encoding context, which in turn cues neighboring items (Howard and Kahana, 2002).

Although we consider each of these major theories in turn, they are not mutually exclusive. In some cases, modern theories of episodic memory make use of more than one of the ideas presented above. For example, some modern buffer models also use a representation of temporal context to differentiate items on the current target list from items on previous lists (Mensink and Raaijmakers, 1988; Sirotin et al., 2005).

As we see it, any theory of associative memory retrieval needs to account for (at least) seven critical behavioral findings regarding temporal-associative processes. The first of these is the contiguity effect – the tendency for neighboring items to be recalled successively. The second critical finding is the asymmetry effect – the tendency for subjects to make transitions to items studied in subsequent list positions. This forward asymmetry is remarkably robust in free recall, being observed in every dataset that reports output order effects. The third critical finding is the long-range contiguity effect – the observation of contiguity effects in continuous-distraction free recall and in a final free-recall task. This finding illustrates how episodic associations are not limited to successively studied items, or even to items studied within a short time period. Rather, contiguity-based associations appear to span many intervening items. The fourth critical finding is that when items are repeated across lists, prior-list intrusions in free recall tend to come from serial positions close to the original presentation (Zaromb et al., 2006). This illustrates the tendency for associations formed on prior lists to influence memory for the current list. Fifth, the tendency for intrusions in serial-recall and probed-recall paradigms is to come from list positions close to the target item. This tendency also exhibits a forward asymmetry effect, where errors tend to be items from subsequent list positions. Sixth, the tendency is for intrusions in paired-associate paradigms to come from neighboring pairs. Although this effect exhibits some forward asymmetry, memory for the items within a pair is strikingly symmetric, with recall accuracy being nearly identical for forward and backward probes (Ekstrand, 1966; Kahana, 2002). Finally, the seventh critical finding is the observation of a contiguity effect in an item recognition task (though this effect appears to be limited to probe items that receive highest confidence old responses). In the sections below, we review the ability of the four major theories of episodic association to account for these findings.

In addition to the temporally defined associative processes reviewed above, a parallel set of findings concerns recency-sensitive processes in memory retrieval. Murdock (1974) summarizes the literature on primacy and recency effects in immediate recall and recognition tasks. Briefly, recency is the most prominent feature of the serial position curves obtained in free recall, paired-associate recall, probed recall, and item recognition. In serial recall, the primacy effect is more prominent than the recency effect. This is largely due to the fact that serial recall requires that subjects initiate recall at the start of the list. Although within-list recency effects in recall tasks are largely attenuated by an end-of-list distractor, recency returns in continuous-distractor free recall (Bjork and Whitten, 1974; Glenberg et al., 1980; Howard and Kahana, 1999). Recency is also observed over much longer time scales than the presentation of a single list, as evidenced by the observation that prior-list intrusions tend to come from recent lists (Murdock, 1974; Zaromb et al., 2006). Similarly, on a final free recall test, subjects are far more likely to recall items from recently studied lists (Craik, 1970; Tzeng, 1973; Glenberg et al., 1980; Howard et al., 2008). Thus, any theory of episodic memory must be able to accommodate recency across very long time scales. Whereas immediate recency effects have often been attributed to the operation of a short-term store, or buffer, longer-range recency effects are often attributed to a contextual coding process. A critical question is whether these recency effects have a common basis or whether they arise from distinct mechanisms (Greene and Crowder, 1984; Raaijmakers, 1993; Davelaar et al., 2005).

2.26.5.1 Chaining Theory

According to early conceptualizations of chaining theory, studying an item leads to the creation or strengthening of forward and backward connections

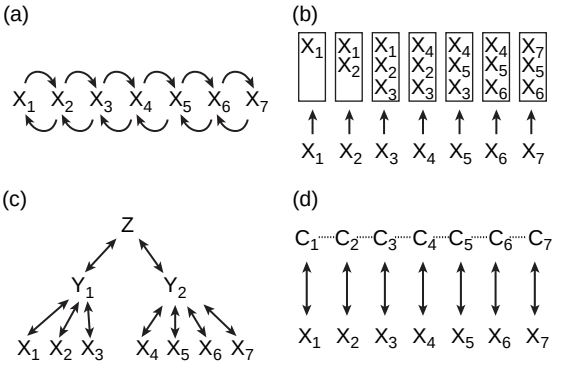

Figure 9 Illustration of the four types of memory models. (a) Chaining Theory. Each item is associated with its immediate neighbors. (b) Buffer Theory. Items are inserted into a fixed-capacity buffer and reside there until displaced. (c) Hierarchical Association Theory. Conjunctions of items are used to create higher-level representations, which are associated with the original items. (d) Contextual Retrieval Theory. A slowly changing context representation is associated with each of the items.

to the immediately preceding item, with associations being stronger in the forward direction (**Figure 9(a)**). As this classic version of chaining theory has often been associated with behaviorism and its rejection of mentalistic constructs, chaining has been a frequent source of ridicule at the hands of cognitively oriented theorists.

Modern chaining theories (e.g., Lewandowsky and Murdock, 1989; Chance and Kahana, 1997) improve on earlier conceptualizations in a number of critical ways. First, modern chaining theories represent each item as a collection of abstract features or attributes rather than as a single node. Second, associations are conceptualized as networks of connections between the processing units that represent the attribute values. These associative networks can be seen as representing a new entity rather than simply linking two preexisting knowledge structures. The associative retrieval process is thus able to recover a partial representation of an item and use that representation as a cue for subsequent recalls. In addition, the attribute representation of items provides a natural way of characterizing the similarities among item representations. By capturing the similarities among items, chaining models can simulate critical aspects of the behavioral data, such as the effect of semantic similarity on recall.

Lewandowsky and Murdock (1989) used the mathematical operations of convolution and correlation to simulate the chaining of associations among item representations in memory. This mathematical approach has also been used by Murdock and his colleagues to simulate data on free recall (Metcalfe and Murdock, 1981), paired associates, and item recognition (Murdock, 1982, 1992). Similar models have also been developed using Hebbian weight matrices to store associations (Humphreys et al., 1989; Rizzuto and Kahana, 2001; Kahana et al., 2005b).

Table 1 illustrates chaining theory's predictions regarding the seven critical findings reviewed above. It is not surprising that chaining theory predicts a contiguity effect in both immediate and delayed free recall (Kahana, 1996). Although most theories do not make explicit accounts of latency, it would be relatively straightforward to model the effect of contiguity on latency by using the strength of association to drive a diffusion model (e.g., Ratcliff, 1978).

Chaining theory is consistent with the idea that associations learned on earlier lists can induce subjects to commit intrusions when those earlier items are repeated in the target list. Further, when intrusions beget intrusions, chaining theory predicts that those intrusions should exhibit similar contiguity effects within the prior list that they came from (Zaromb et al., 2006). However, to accurately simulate the relatively modest interlist effects observed in the data, chaining theory must be augmented with a list context representation that is used to focus retrieval on the items in the target list (e.g., Sirotin et al., 2005).

Chaining theory can accommodate the forward asymmetry of the contiguity effect by differentially weighting the storage of forward and backward associations. This is not easily accomplished within the convolution-correlation formalism of Murdock and colleagues, but it can be easily implemented in a Hebbian matrix model (Pike, 1984; Kahana, 2002). Even so, employing differential weighting of forward and backward associations does little to explain the phenomenon.

The standard version of chaining theory assumes that associations are forged among neighboring items. One can extend the standard chaining model to produce the gradient of remote associations seen in the contiguity-effect in free recall by modeling the rehearsal process. When presented with an item for study, subjects often think about that item in relation to recently studied items. This rehearsal process will cause the functional order of study to differ from the nominal order of presentation (Brodie and Murdock, 1977; Tan and Ward, 2000), resulting in the remote

Table 1 The ability of four major theories of association to account for contiguity phenomena across memory tasks.

Theory	Contiguity	Asymmetry	Long-range contiguity	Prior-list intrusions	Probed recall intrusions	Across-pair intrusions	Contiguity in item recognition
Chain	✓	*	×	✓	✓	✓	*
Buffer	✓	*	×	✓	✓	✓	*
Vertical	✓	*	×	✓	✓	*	*
Context	✓	✓	✓	✓	✓	✓	✓

The ✓ symbol means that the model can account for the data without modification. The * symbol means that the model requires some modification from the standard version to account for this data-point (see text for elaboration of each case). The × symbol means that the model is unable to account for this data-point.

associations of the kind seen in **Figure 2(a)**. The standard approach to modeling rehearsal in free recall is to assume that rehearsal is controlled by a working memory buffer that actively maintains (and rehearses) a small number of items (e.g., Raaijmakers and Shiffrin, 1980). We discuss the predictions of these so-called buffer models in the next subsection.

The more serious challenge to chaining theory comes from the observation of preserved long-range contiguity effects in free recall. It is hard to envision how chaining models would explain the approximate time-scale invariance of the contiguity effect, as shown in **Figure 5(b)**. Nearest-neighbor chaining theory, even when augmented with a rehearsal buffer and a list-context representation, would predict a diminished contiguity effect when subjects perform a demanding distractor task following each study item. For chaining theory to explain the long-range contiguity effect in continuous-distractor free recall, one would have to assume that remote associations extend through distractor intervals and even across entire lists. To explain the gradient of intrusions observed in recall of paired-associates (**Figure 7(c)**), one would need to assume that remote associations automatically link items that were studied in nonadjacent pairs.

The finding of associative effects in item recognition is also not easily explained by chaining theory, as it would require associations to be automatically formed between items even when there is no task demand to do so. If chained associations were automatically formed between neighboring items, and if compound cueing operates at retrieval (e.g., McKoon and Ratcliff, 1992), then chaining theory should be able to predict the associative effects seen in **Figure 8**.

It would be misleading to imply that chaining theory should be evaluated solely on the basis of the select phenomena highlighted in **Table 1**. In the domain of serial recall, where chaining theories have been most thoroughly investigated, the basic chaining model offers strikingly counterfactual predictions concerning subjects' recall errors, particularly in lists that incorporate repetitions of identical or similar items (Ranschburg, 1902; Lashley, 1951; Crowder and Melton, 1965; Crowder, 1968; Henson et al., 1996; Henson, 1998; Kahana and Jacobs, 2000).

2.26.5.2 Working Memory Buffers and Dual Store Theory

Chaining theory makes the implicit assumption that the just-presented item is somehow maintained long enough to become associated with the current item. In essence, the just-presented item must be maintained in some type of working memory buffer. Dual-store memory models, such as the Atkinson–Shiffrin model and its more modern descendant, the SAM retrieval model, elevate the working memory buffer to a far more prominent role (Raaijmakers and Shiffrin, 1980; Sirotin et al., 2005). These models assume a working memory buffer that is capable of holding multiple items during list presentation. Any items residing in the buffer at the time of test may be recalled without a lengthy search process. Moreover, the rules that determine how items enter and leave the buffer can be designed to simulate the process of strategic rehearsal, thus enabling the models to account for aspects of free-recall data that are believed to depend on the pattern of rehearsals that occur during list presentation (Rundus, 1971; Brodie and Murdock, 1977; Tan and Ward, 2000; Laming, 2006). The critical assumption for our purposes is that items that are co-resident in the buffer become associated, and the size of the buffer determines the range of remote associations among items (see **Figure 9(b)**).

The SAM retrieval model, and its latest variant, eSAM, offers the most comprehensive model of free recall currently available (Raaijmakers and Shiffrin, 1980; Sirotin et al., 2005). The model's ability to explain a wide range of data, including findings concerning semantic organization effects, comes at the expense of a greater number of assumptions and mechanisms that are built into the model. For example, the eSAM model incorporates associations between items that share time in the buffer (essentially chaining) as well as associations between a time-varying list context signal and items. These associations reside in an episodic memory matrix that is distinct from a semantic memory matrix which is also used in retrieval. eSAM (and SAM) include a dynamical probabilistic recall process which keeps track of which items have already been recalled given a particular set of cues. Finally, a postretrieval recognition test is used to determine whether a retrieved item should be recalled or rejected due to its weak strength to the current list context.

It is important to note that buffer models such as those described by Davelaar et al. (2005) and Sirotin et al. (2005) have been shown to account for a very wide range of recall phenomena. For example, buffer models provide a natural explanation for the striking recency effect observed in immediate free recall and its marked attenuation following a brief interval of distracting activity. Because retrieval of items remaining in the buffer produces the recency effect in immediate recall tasks, buffer-based models can also neatly explain the numerous dissociations between recall of recency and prerecency items, as well as dissociations between immediate and continuous distractor free recall (Davelaar et al., 2005). Although they cannot easily account for long-range contiguity effects, buffer models still represent an important benchmark in the episodic memory literature.

2.26.5.3 Hierarchical Association Theory

Hierarchical models of association (e.g., Johnson, 1972; Lee and Estes, 1977; Murdock, 1995a, 1997; Anderson and Matessa, 1997; Anderson et al., 1998) attempt to explain how subjects unitize (or chunk) groups of items to create new conjunctive representations in memory. Whereas both chaining and buffer models define associations as directly linking neighboring items, hierarchical models assume that associations are mediated by a superordinate representation that provides access to two or more neighboring items. An item can be used to retrieve the superordinate representation (or chunk) which in turn can retrieve the other items associated with it. This kind of hierarchical associative structure is illustrated in **Figure 9(c)**.

Hierarchical theories of association have been largely motivated by the observation that practiced subjects tend to rhythmically group items during serial learning (e.g., Müller and Pilzecker, 1900). Because it is difficult to study subjects' grouping strategies in an unconstrained learning situation, researchers have devised methods to encourage specific grouping strategies whose consequences can be reliably measured. Such experimenter-imposed grouping is typically achieved by inserting pauses at regular intervals during list presentation.

There are four major consequences of experimenter-imposed grouping. First, consistent grouping leads to better serial recall, with the highest levels of recall observed for group sizes of three or four items (Wickelgren, 1967). Second, the grouping effect is largest for auditorally presented lists (Ryan, 1969). Third, grouping leads subjects to recall items in the correct within-group position but in the wrong group (Johnson, 1972; Brown et al., 2000). Fourth, subjects inter-response times during recall are longer at group boundaries (Maybery et al., 2002). These and related findings inspired the development of hierarchical associative models which have been applied with great success to data on serial recall (e.g., Estes, 1972; Lee and Estes, 1977; Murdock, 1993, 1997).

Hierarchical, or vertical, associations can be used to create representations that bridge time, which would help to explain some of the critical findings listed in **Table 1**. If the model is able to make a higher-level bridging representation associating successively presented items, then it can capture the contiguity effect. It is less clear whether a model like this can capture the asymmetry effect (Murdock, 1995b). Long-range contiguity effects pose a greater challenge, as they would require hierarchical representations to be robust to distraction, and to keep building up across lists. Hierarchical associations may be able to capture the contiguity effect in recognition, but this would require that the hierarchical representations are formed when there is no task demand to do so.

The preceding discussion refers to a type of hierarchical representation that bridges representations that are separated in time; however, another class of

hierarchical models forms higher-level representations that bridge various simultaneously active lower-level representations. In particular, the connectionist model of episodic memory introduced by McClelland et al. (1995), and further developed by Norman and O'Reilly (2003) posits that the hippocampus serves as the locus of a higher-level representation that represents the conjunction of all of the features activated in the various cortical areas that project to it. This hippocampally based episodic representation is associated with all of these lower-level features such that the later activation of a subset of those features allows the episodic representation to be retrieved; it then projects out to the cortical areas and reactivates the full set of originally active features.

2.26.5.4 Contextual Retrieval Theory

The effective use of memory depends on our ability to focus retrieval on those memories learned within a given spatiotemporal context (e.g., Carr, 1931; McGeoch, 1932). According to temporal-context models, the memory system associates each studied item with the contextual features present at the time of encoding. At the time of test, the current state of context is a good retrieval cue for recently studied memories (Bower, 1972; Howard and Kahana, 2002). Because retrieval results from a competition among activated memory traces, one observes recency both in immediate and in continuous-distractor free recall (Bjork and Whitten, 1974; Crowder, 1976; Howard and Kahana, 1999).

Howard and Kahana (2002) proposed an extension of the classic Estes-Bower context theory that was designed to explain the observation of long-range contiguity effects. According to their temporal context model (TCM), recall of an item results in a partial reinstatement of the context that was present when that item was studied. This retrieved context then serves as a retrieval cue for other items with a similar context at study, which are most likely to be items from nearby serial positions, thus yielding the contiguity effect.

TCM provides a natural explanation for the robust contiguity effects found in continuous-distractor free recall, as retrieval transitions are driven by the relative similarity between the temporal contexts of different list items. As long as a similar duration of distracting activity separates each item from its neighbors, TCM predicts that the transitions among neighboring list items will be largely independent of the absolute temporal separation of the items in the list.

According to TCM, context is a vector that changes gradually as a result of items being activated in semantic memory. TCM provides a formal mathematical model of how temporal context evolves as a consequence of item encoding and retrieval. It also describes an associative architecture, implemented as a neural network, that links both items to context and context to items.

A given state of temporal context will cue recall items via the context-to-item associative network. Consistent with Tulving's notion of encoding specificity (Tulving, 1983), the optimal cue for an item is the context in which it was encoded. Because context changes gradually, the state of context at the time of test will overlap most strongly with the contexts associated with recent items. This gives rise to the recency effect seen in all episodic memory tasks. Primacy is accommodated within TCM by assuming that early list items receive more rehearsals and/or increased attentional resources (Brodie and Murdock, 1977; Tan and Ward, 2000).

Just as contextual states can retrieve items in semantic memory, so too can items retrieve their associated contextual states. In TCM, it is this process of contextual reactivation that drives the evolution of the context vector itself. Contiguity effects arise because the retrieved contextual states overlap with the encoding context of nearby items. For a more complete treatment, the reader is referred to Howard and Kahana (2002) and Howard et al. (2006). For a discussion of a potential mapping between TCM and the structure and function of the medial temporal lobe, see Howard et al. (2005).

According to TCM, the forward-bias in the contiguity effect arises because recall of an item retrieves both the context stored during list presentation (which is similar to both the prior and subsequent list items) and the pre-experimental contextual states associated with the item. Because the pre-experimental contextual states associated with an item is added to the context vector at the time of the item's encoding, that part of the retrieved context is similar to the contextual states associated with subsequent list items but not prior list items. Thus, the context retrieved by an item includes a symmetric component (the contextual state associated during list presentation) and an asymmetrical component (the pre-experimental contextual states). The combination of these two components produces the forward asymmetry seen in the contiguity effect (**Figure 2(a)**).

Retrieved context is one way that contiguity effects could arise across wide-ranging time scales, such as those observed in continuous-distractor free recall, final free recall, and recall of paired-associates. Dennis and Humphreys (2001) suggested that temporal context may underlie recognition judgments as well. In this case, one might predict that high confidence yes responses reflect successful retrieval of context. The contiguity effect seen in item recognition (**Figure 8**) could arise if the retrieved contextual representation of an item combined with the subsequent test probe.

2.26.6 Conclusions and Open Questions

The evidence we have reviewed shows how retrieval of episodic memories is a cue-dependent process that reflects the temporal contiguity and the semantic relatedness of the cue and the target items. Analyses of retrieval transitions in free recall demonstrate that both temporal and semantic factors have a dramatic effect on retrieval. Although subjects may recall items in any order they wish, the recall of a given item is predictable on the basis of its semantic relatedness and temporal contiguity to the just recalled item.

The contiguity effect, as seen in **Figure 2(a)**, exhibits a strong forward asymmetry, with recall transitions being nearly twice as likely in the forward than in the backward direction. This tendency to make forward transitions contrasts with the overall tendency to begin recall at the end of the list (Kahana, 1996). Contiguity and asymmetry are ubiquitious in free recall. The basic lag-CRP and lag-CRL curves have the same form for lists of different lengths and presentation rates, for different presentation modalities, for different word frequencies, etc. Although reduced for older adults, the contiguity and asymmetry effects have the same basic form across age groups.

The contiguity effect is not limited to free recall; rather, it is a nearly universal characteristic of retrieval in episodic memory. Contiguity is seen in the pattern of correct recalls, inter-response times, and intrusions in free recall, and in the memory errors seen in probed recall, serial recall, and paired-associate recall. Even in item recognition, contiguity appears when subjects respond with high confidence.

One of the most striking and theoretically significant features of the contiguity effect is its persistence across time scales. In free recall, the contiguity effect is not reduced when list items are separated by 16 s of distractor activity. In recall of paired associates, contiguity appears in subjects' tendency to recall items from nearby pairs, thus demonstrating that contiguity does not depend on subjects intention to learn the association between neighboring items.

Four major theories have been proposed to explain episodic associations: Chaining theory, buffer theory, hierarchical association theory, and retrieved context theory. Whereas all of these theories can account for the basic contiguity effect, retrieved context theory offers the only adequate account of the long-range contiguity effect. Retrieved context theories, such as TCM, provide a basis for synthesizing the associative effects observed across all of the major episodic recall and recognition paradigms. In TCM, associative effects appear because retrieved context of a given item overlaps with the encoding context of nearby items. This approach constitutes a departure from traditional accounts of association, such as those assuming direct interitem associations (chaining or buffer theory) or those that assume hierarchial associative structures.

Although the presence of contiguity across time scales supports the contextual retrieval account of episodic association, it does not preclude the operation of other factors as suggested by the alternative theories. For example, it is possible to envision a hierarchical associative model or a buffer-based associative model that also includes a contextual retrieval mechanism.

Despite the enormous strides in our understanding of episodic association, a number of intriguing puzzles remain to be solved. One unsolved puzzle concerns the asymmetric nature of episodic associations. Although the forward asymmetry is a striking feature of associations in free recall, serial recall, and probed recall, the data do not reveal striking asymmetries in all episodic tasks. Moreover, recall of individual paired associates is almost perfectly symmetrical, with subjects exhibiting nearly identical rates of forward and backward recall, and with forward and backward recall being highly correlated at the level of individual pairs (Kahana, 2002).

Perhaps the most important of these puzzles is the question of how the rich structure of semantic associations in human memory could arise simply due to the repeated presentation of related items in temporal proximity. Computational models of semantic memory, such as LSA (Landauer and Dumais, 1997) and the topics model (Griffiths and Steyvers, 2002, 2003) provide some clues as to how such a reconciliation might be possible. LSA and the topics model

extract information about the temporal contexts in which words appear to estimate their meaning. Specifically, in these models, temporal context is defined as a passage of text. The hyperspace analog of language (HAL, Lund and Burgess, 1996) and BEAGLE (Jones and Mewhort, 2007) models define temporal context as a sliding window of a fixed number of words. This suggests the possibility of a unification of computational models of semantic memory and models of episodic memory based on contextual retrieval (Dennis and Humphreys, 2001; Howard and Kahana, 2002), in that each process may rely on the presence of a slowly-drifting source of contextual information.

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2.27 Episodic Memory: An Evolving Concept

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2.27.1 Introduction

Although the term episodic memory did not exist until about 35 years ago, it captures much of what philosophers, psychologists, and lay people have meant by memory or remembering. Episodic memory – or the recollection of events from one's personal past – is therefore one of the most fundamentally important concepts in the study of human memory. It is the capacity for episodic memory that enables one to recollect the multitude of details surrounding one's most cherished moments.

A challenge inherent in writing a review chapter on episodic memory is that it is not a static term; the essence of the term episodic memory has morphed and broadened considerably over the short time of the term's existence. It should be no surprise, then, that different empirical evidence has been brought to bear on the different meanings. A further twist is that a single person, Endel Tulving, both introduced the term (in 1972) and has modified its meaning many times in the years since. As a result, his theorizing and adaptation of the concept has spawned much of the relevant literature, and this chapter draws very heavily upon his work and emergent ideas.

We have chosen the following approach in organizing this chapter. We begin by attempting to identify a few of the historical landmarks or prominent features proposed in the conceptual development of episodic memory. We then choose two topics to consider in some depth. Specifically, we consider evidence supporting the proposition that episodic memory is a distinct memory system, different from other types of memory. We then consider research bearing on the suggestion that episodic memory may represent only one facet of a more general cognitive capacity that enables mental time travel into both the subjective past and future.

2.27.2 Historical Landmarks

2.27.2.1 A Taxonomic Distinction: Episodic and Semantic Memory

The concept of episodic memory was formally introduced in a seminal chapter by Tulving (1972), who

drew a distinction between memory for specific events (episodic memory) and memory for general knowledge and facts (semantic memory). For example, remembering that the word elephant had been present in a list of previously studied words, recounting the events surrounding the day of one's college graduation, or reminiscing about the most recent Christmas dinner with a family member would be considered instances of episodic memory. Knowing that elephants live in Africa, the name of the college one attended, and that a family gathering typically implies a special occasion would be classified as examples of semantic memory (*See* Chapter 2.28).

In 1972, Tulving explained that laboratory studies of human memory had long been concerned with episodic memory. That is, most experiments were of the same general design: Present events for study and then measure how well they are remembered at a later time. At this time, episodic memory was associated with a certain type of task: Those that required recall or recognition of a prior episode.

Although episodic and semantic memory are both declarative (i.e., may be articulated) and can be differentiated from memory that cannot be expressed in terms of representational information (i.e., procedural memory, or memory of how to perform a skill, see Squire, 1987), there exists a fundamental and straightforward distinction between episodic and semantic memory: Episodic memory involves remembering an episode from one's past that is specific to time and place, whereas semantic memory involves general knowledge that is not associated with specific episodes.

Tulving summarized his seminal 1972 chapter as having made "a case for the possible heuristic usefulness of a taxonomic distinction between episodic and semantic memory and two parallel and partially overlapping information processing systems" (Tulving, 1972: p. 401). At the time, the episodic/semantic distinction was offered as a proposal that the two types of memory may be separable. As will be seen, the concept of episodic memory quickly grew to denote more than its originally intended meaning. The taxonomic distinction between episodic and semantic memory, however, is a central feature of the original conceptualization that has stood the test of time. Indeed, this distinction has been adopted by the field and is in widespread use.

Before proceeding further, it is worth considering the similarities and differences between the term episodic memory and a few other, related terms. Autobiographical memory refers to personal memories of one's own life. These can be of two types: episodic or semantic. Consider the following examples: Remembering the first day of grammar school would rely upon episodic memory, whereas knowing the name of one's grammar school relies upon semantic memory. Both examples, however, represent autobiographical (self-related) memory. We should acknowledge, though, that researchers define autobiographical memory in different ways, so not all would agree with this classification scheme. Explicit memory is another term related to episodic memory. Explicit memory is a term often used as a heuristic for the type of memory used on an explicit test of memory; an explicit test is one in which a person is asked to willfully attempt to retrieve the past. Explicit memory can be contrasted with implicit memory, which is the unintentional manifestation of memory (e.g., if you were to read this chapter a second time, it would likely be read faster).

2.27.2.2 Subjective Awareness

The role of subjective awareness in memory has long been a topic of interest for the field (e.g., feeling-of-knowing judgments, tip-of-the-tongue states; for a historical review see Metcalfe, 2000). In 1983, Tulving published *Elements of Episodic Memory*, in which he explicitly applied such ideas to his own work. In that volume, Tulving proposed that memories for personal episodes are characterized by a strong feeling of re-experiencing the past. In contrast, Tulving argued that retrieval of general knowledge from semantic memory lacked this phenomenological quality. That is, although someone may know a fact (e.g., that St. Louis is the site of a famous arch) and is aware that he or she acquired knowledge of this fact in the past, one does so in a way that does not necessitate re-experiencing the instance in which the fact had been learned.

Tulving further argued that the feeling of re-experiencing a previously encountered event is the *sine qua non* of episodic memory. He outlined a general framework (General Abstract Processing System, or GAPS; **Figure 1**) by which to understand the act of remembering from episodic memory (Tulving, 1983). The GAPS framework was intended to highlight many issues associated with retrieval from episodic memory. We focus here on how this framework predicts the emergence of subjective awareness (or recollective experience, as it was referred to in 1983). As can be seen in **Figure 1**, an encoded event is converted into a latent memory trace (or engram;

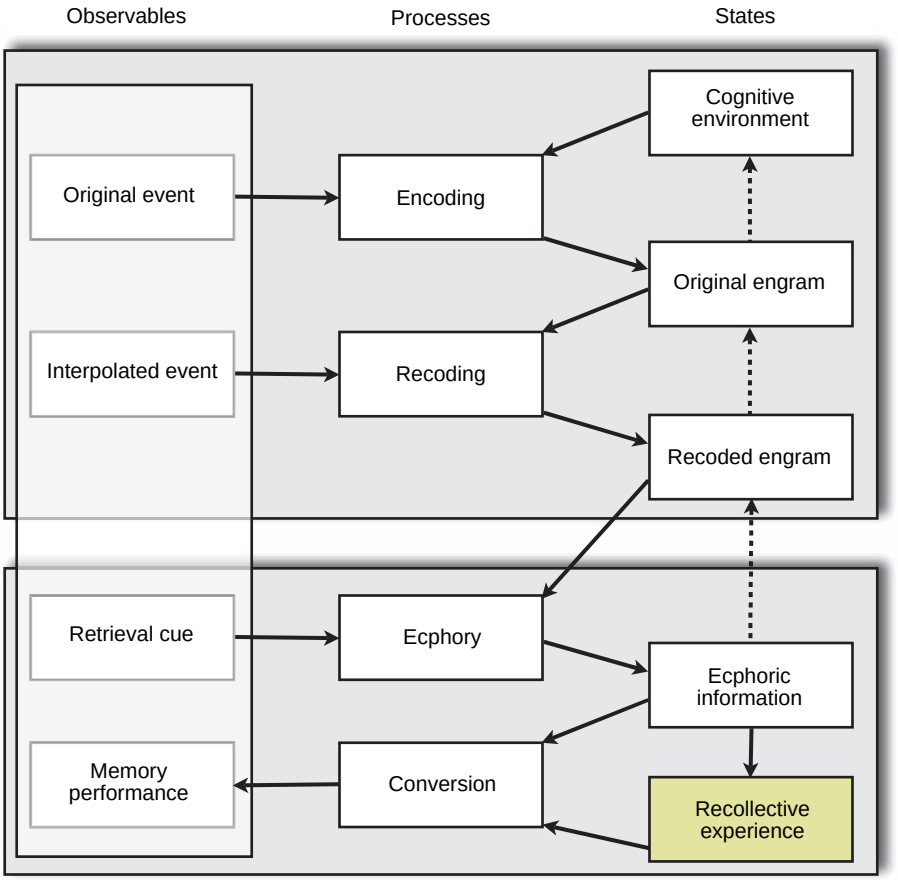

Figure 1 General Abstract Processing System: A conceptual framework for understanding retrieval from episodic memory. Adapted from Tulving E (1983) *Elements of Episodic Memory.* New York: Oxford University Press.

Semon, 1904). However, it is unlikely that the event will be remembered exactly as it had originally occurred. For instance, the latent engram related to that event is subject to recoding (e.g., by virtue of related interpolated events). The recoded engram then interacts with a retrieval cue to produce ecphory: The evocation of information from a latent engram into an active state (Semon, 1904; Tulving and Madigan, 1970; Tulving, 1976; Schacter et al., 1978). That is, the synergistic product of the memory trace and the retrieval cue determine the nature of what is remembered (ecphoric information; *See* Chapter 2.16; Tulving, 1982), which in turn determines recollective experience. Accordingly, the rememberer will become aware of the encoded event to the extent that ecphoric information is representative of the original episode. At this time, no data were presented that directly assessed a participant's recollective experience for the contents of his or her memory.

On the basis of the notable absence of phenomenological data from the majority of verbal learning experiments (but see Metcalfe, 2000, who discusses various exceptions), Tulving (1983) suggested that students of psychology had not yet begun the study of episodic memory. Of course, this claim directly contradicts his previous (Tulving, 1972) assertion, which he declared in 1983 to have been "not very well thought out" (Tulving, 1983: p. 9). Prior research had assumed a correlation between a learner's behavioral response and subjective awareness. That is, if a learner was able to recall or recognize having previously encountered a given stimulus item (e.g., a word from a previously presented list) it was assumed that he or she mentally re-experienced the original event. It is now well-established that there is no direct correlation between behavior on a memory test and the cognitive processes underlying that behavior (Schacter, 1987; Tulving, 1989a; Jacoby, 1991; Roediger and McDermott, 1993; Toth, 2000; *See*

Chapter 2.33). Tulving (2002b) reflected on this issue by pointing out that episodic memory is concerned with what happened where and when. Typical verbal learning experiments assessed the what aspect but left when and where unqueried.

With this problem in mind, Tulving (1985b) devised a research paradigm designed to illustrate that a learner in a memory experiment does not necessarily remember the instance in which he or she experienced an event that he or she knows occurred in the past. This procedure was a starting point for exploring the nature of subjective awareness.

2.27.2.3 The Remember/Know Paradigm

The remember/know paradigm was introduced as a tool for investigating a learner's subjective awareness of a prior study episode (Tulving, 1985b), although current procedures have been modified somewhat from the original implementation (see Rajaram, 1993). For the most part, a remember/know experiment takes the form of the typical laboratory memory experiment. Learners study a set of stimulus materials at time one (e.g., a list of words) and take a memory test on those materials at time two. The innovation that Tulving introduced was to ask learners at the time of the memory test whether they actually remembered the exact prior occurrence of a given study item (e.g., the word ocean), or whether they just knew that the item had been presented, but could not remember the precise instance of its original presentation (Tulving, 1985b; Gardiner, 1988; Rajaram, 1993; Gardiner and Richardson-Klavehn, 2000; *See* Chapter 2.17).

Tulving (1985b) showed that learners could easily make these mental distinctions and that both remember and know responses were present during tasks that previously had been thought to tap episodic memory (i.e., recognition, cued recall, and even free recall). This important finding suggested that learners had two routes by which to recover the contents of a past study episode. Remembering was identified as the hallmark of episodic memory and was further associated with a unique mental state called autonoetic (self-knowing) awareness, implying a feeling of personally re-experiencing the past. Knowing was associated with semantic memory and noetic (knowing) awareness, a mental state lacking the feeling of personally re-experiencing the past. Further, memory tasks were found to vary in the degree to which they relied upon remembering, with free recall demonstrating the greatest level of remember responses (i.e., the greatest reliance on episodic memory). Hence, an important conclusion here is that no memory test is a pure measure of episodic memory, and tests designed to assess episodic memory differ in the degree to which they rely on the construct, with none achieving a pure assessment of episodic memory.

It is interesting to note that the subjective (autonoetic) awareness that Tulving had identified as a central component of episodic memory was similar to what pioneers of memory research had in mind when discussing remembering. For instance, William James (1890) wrote of remembering as, "a direct feeling; its object is suffused with a warmth and intimacy to which no object of mere conception ever attains" (James, 1890: p. 239). Hermann Ebbinghaus, (1885) adopted a generally understood conceptualization of memory that had been put forth by John Locke, defining remembering as the emergence of a sought after mental image that is "immediately recognized as something formerly experienced" (Ebbinghaus, 1885: p. 1). According to Locke, memory was the power of the mind "to revive perceptions, which it has once had, with this additional perception annexed to them, that it has had them before" (Locke, 1975: p. 150).

2.27.2.4 Retrieval Mode

Aside from the subjective awareness (or lack thereof) thought to accompany memory retrieval, Tulving (1983) outlined various other features by which he distinguished episodic from semantic memory (see also Tulving, 2005). At the time, the listing of differences was meant as a starting point for discussion, rather then any acknowledgment of hard-set facts. Importantly, the features on which episodic and semantic memory were hypothesized to differ were divided into three categories, each separately focusing on the information handled by episodic and semantic memory, their operations, and their applications. The main point of these subcategories was to emphasize that the distinction between episodic and semantic memory was more than just a difference in the type of information under consideration.

For instance, Tulving (1983) made a distinction regarding the manner in which access is gained to episodic and semantic knowledge. According to Tulving, access to information from episodic memory is deliberate and requires conscious effort. Conversely, semantic knowledge may be accessed in a relatively automatic fashion. For instance, stimuli in the environment are immediately interpreted on the basis of

semantic knowledge. When reading a novel, the meanings of words come to mind with relative ease. However, it is only when one is in a particular state of mind that is focused on their personal past that the same stimulus may remind one of a particular episode. For example, single words have been shown to act as effective cues for the retrieval of personal autobiographical memories (Crovitz and Schiffman, 1974; Robinson, 1976); this is only the case, however, when participants are specifically instructed to use those words as retrieval cues. This state in which one focuses attention on their past and uses incoming information as cues for past experiences is referred to as retrieval mode (Tulving, 1983; Lepage et al., 2000). A potential exception to this rule involves spontaneous conscious recollection, wherein personal memories suddenly come to mind. One common example is the evocation of an emotional memory (e.g., one's first kiss) by a particular piece of music (see Berntsen, 1996, 1998). Similar examples have been offered in the prospective memory literature (McDaniel and Einstein, 2000; Einstein et al., 2005).

Tulving (1983) argued that retrieval mode constituted a necessary condition for retrieval from episodic memory but admitted, "we know next to nothing" about it (Tulving, 1983: p. 169). In terms of the behavioral literature on the topic, the same statement holds true today. Although subsequent research on the topic has illuminated the nature in which the presence/absence of retrieval mode may be manipulated in the context of a memory experiment (e.g., retrieval intentionality criterion, Schacter et al., 1989), we have not learned much more about the state itself.

Recent advances in neuroimaging techniques (see section titled "Functional neuroimaging") have revived interest in the study of retrieval mode. For example, Lepage et al. (2000) suggested that brain regions showing similar patterns of brain activity during either successful or failed attempts of episodic retrieval (relative to a control task that does not engage episodic retrieval processes) can be taken as neuroanatomical correlates of retrieval mode. Reviewing the relevant literature, Lepage et al. identified six frontal lobe regions (mostly right lateralized) that appear to become active whenever participants attempt to retrieve past information, regardless of whether they are successful or not. Thus, the underlying nature of retrieval mode has not yet been delineated, but neuroimaging techniques may prove useful in approaching this issue.

2.27.2.5 Subjective Awareness, Self, and Time

As we have mentioned, the concept of episodic memory has been considerably refined over the years. According to Tulving's most recent conceptualization, episodic memory is a recently evolved, late-developing, and early-deteriorating past-oriented memory system, more vulnerable than other memory systems to neuronal dysfunction, and probably unique to humans. It makes possible mental time travel through subjective time, from the present to the past, thus allowing one to re-experience, through autonoetic awareness, one's own previous experiences (Tulving, 2002b: p. 5).

Thus far we have highlighted subjective (autonoetic) awareness as the defining feature of retrieval from episodic memory. Equally important are concepts of self and subjective time (Tulving, 2002a,b). That is, episodic memory requires the capacity to represent a psychologically coherent self that persists through subjective time, whose past experiences are recognized as belonging to the present self (self-contiguity; Klein, 2001). Klein (2001; see also Klein et al., 2004) argues that a breakdown of self-contiguity disrupts the ability to represent past and present mental states as being aspects of the same personal identity, thus leaving an individual incapable of identifying a current mental state as one that was previously experienced. Klein (2001) reviews compelling evidence to support this claim. For example, individuals with schizophrenia – a population characterized by impairments in self-contiguity – have profound deficits in episodic memory (McKenna et al., 1994).

2.27.2.6 The Episodic Memory System

As can be seen by the 2002 definition (quoted in the previous section), episodic memory grew to encompass much more than the type of memory that allowed one to recall or recognize prior events. It became a hypothetical neurocognitive memory system that is characterized, relative to other memory systems, by its unique function and properties (Tulving, 1984, 1985a; Sherry and Schacter, 1987; Schacter and Tulving, 1994). Of course, this basic idea was foreshadowed somewhat by the earlier description (even in the 1972 description regarding partially overlapping processing systems), but the earlier emphasis had been on the basic taxonomic

distinction and not on the much more bold claim that it is a memory system.

What exactly is a memory system, and what might the criteria be for establishing one? These questions have spurred a great deal of controversy, much of which appeared in the context of the emerging literature on implicit memory in the late 1980s and early 1990s (Tulving, 1985a; Sherry and Schacter, 1987; Roediger et al., 1990, 1999; Schacter and Tulving, 1994; Buckner, 2007). Some theorists were concerned that the lack of stringent criteria would lead to a proliferation of putative memory systems, many of which were probably not well justified. We wish to sidestep that general debate here; our view is that although the criteria for establishing a memory system are not well-specified (and are often not met even when specified), there is nonetheless strong evidence that episodic memory represents a fundamentally different kind of memory than semantic memory and that the hypothesis that it is indeed a distinct memory system is certainly viable. Here we choose to focus on what was meant by this claim that episodic memory should be considered a memory system and review some of the evidence bearing on the claim.

First, the episodic memory system enables its owner to process (i.e., encode, store, and retrieve) personally experienced episodes. In this way, it allows one to accomplish a feat not possible without the system. Secondly, episodic memory can be differentiated from semantic memory on a variety of dimensions (Tulving, 1972, 1983). We have already addressed one of these dimensions at length, namely the conscious awareness that characterizes episodic (autonoetic awareness) relative to semantic (noetic awareness) memory. Hence, episodic memory has a set of properties that differentiate it from other systems.

It is important to note that the episodic memory system is hypothesized to be related to and have evolved from phylogenetically earlier systems, including semantic memory (Tulving, 1985b, 1995). That is, the ability to consciously re-experience a specific event from the past may have grown out of a more general ability to use the past in an informative fashion, albeit one lacking a sense of subjectively reliving the event (see **Figure 2**). The episodic memory system "depends upon but goes beyond the capabilities of the semantic system. It could not operate in the absence of the semantic system" (Tulving, 1989b: p. 362). Of course, the evolutionary relation between episodic memory and semantic memory is not subject to laboratory investigation. As will be seen, a similar relation appears to exist in the course of ontogenetic development, though, whereby episodic memory emerges in the presence of fully functioning semantic memory.

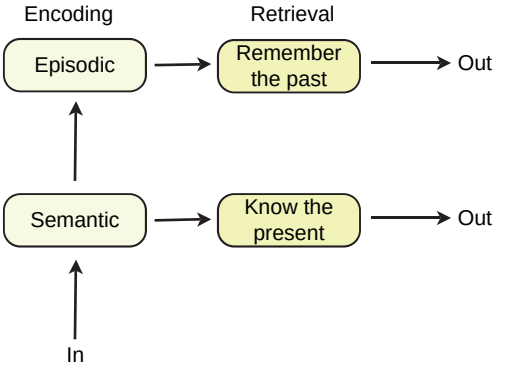

Figure 2 Sketch of the relations between semantic and episodic memory. Information can be encoded into semantic memory independent of episodic memory but must be encoded into episodic memory through semantic memory. Encoded and stored information is potentially available for retrieval from one of the two systems or from both of them. Adapted from Tulving and Markowitsch (1998).

In the following section, we present evidence from neuropsychology, functional brain imaging, and developmental psychology consistent with the idea that episodic memory may in fact represent a viable neurocognitive system or is at least functionally dissociable from semantic memory.

2.27.3 Converging Evidence for the Episodic Memory System

The idea that episodic memory might represent a distinct memory system emerged largely out of the behavioral psychological literature, where it was shown that a particular independent variable might affect performance on one measure or set of measures (e.g., measures thought to draw largely upon episodic memory) but not affect performance (or affect performance in the opposite direction) on different measures, thought to draw largely on semantic memory. For example, level of processing during encoding affects the likelihood of later remembering but not knowing (when the remember/know paradigm is used; see Yonelinas, 2002, for review). Perhaps the most compelling evidence for the idea comes from brain-based studies, particularly neuropsychological

studies. Here it can be shown that some patients lose the ability to use episodic memory while retaining other classes of memory, including semantic memory. Following, we review some of this evidence.

2.27.3.1 Neuropsychology

Neuropsychological observations of brain-damaged individuals have contributed a great deal to our understanding of the organization of human memory in the brain. Perhaps the most famous contribution is that of Scoville and Milner (1957), who reported the case of patient HM. HM incurred dense amnesia following a bilateral resection of the medial temporal lobes. Since then, a great deal of converging evidence from neuropsychological observations of human patients, neurological experimentation using animal subjects, and more recent advances in functional brain imaging techniques has corroborated Scoville and Milner's original observation: The medial temporal lobes play an important role for memory (for an early reference, see Bekhterev, 1900).

Of particular interest, Scoville and Milner (1957) classified the impairment observed in patient HM as one of declarative memory. That is, no distinction was made between episodic and semantic memory. Of course, this is not surprising given that the distinction was not introduced to the neuropsychological community for another 30 years (Tulving, 1985b; although see Nielsen, 1958, for a foreshadowing of the distinction). Another potential reason the distinction was not made is because it was not readily apparent. HM's surgical resection encompassed large portions of the medial temporal lobes, including, but not limited to, the hippocampal formation. It has recently been considered that hippocampal damage is particularly associated with deficits of episodic memory, whereas semantic memory problems arise as a result of adjacent cortical damage (Mishkin et al., 1997; Aggleton and Brown, 1999). Accordingly, both episodic and semantic memory may have been damaged in patient HM.

Vargha-Khadem and her colleagues have recently reported on a set of three amnesic patients, each of whom sustained bilateral pathology restricted to the hippocampus following an anoxic episode in early life (ranging from birth to 9 years; Vargha-Khadem et al., 1997). Unlike most amnesic patients, their ability to acquire knowledge remains intact. As a result, all three patients have been able to progress through the educational system with little trouble. However, all three are severely impaired in their ability to recall events, even those that occurred minutes previously. These cases represent a clear dissociation between episodic and semantic memory function in the presence of brain damage restricted to the hippocampus.

Although dissociations between episodic and semantic memory are rarely clear-cut, there do exist many case reports in which one is relatively more impaired than the other. Most such cases have reported greater deficits of episodic memory relative to semantic memory (e.g., Cermak and O'Connor, 1983; Calabrese et al., 1996; Kitchener et al., 1998; Levine et al., 1998; Viskontas et al., 2000), although the reverse pattern also occurs (e.g., Grossi et al., 1988; De Renzi et al., 1997; Yasuda et al., 1997; Markowitsch et al., 1999). The reversed pattern (i.e., greater impairment of semantic than episodic memory) is not well accommodated by the idea that episodic memory requires semantic memory to operate.

It is important to note that these case studies are characterized by various etiological factors and resulting patterns of brain impairment that are not restricted to the medial temporal lobes. In general, there is good reason to believe that the operations of various memory systems (including episodic and semantic) depend upon highly distributed and interacting regions of the brain (Mesulam, 1990; Nyberg et al., 2000). For instance, although the role of hippocampus is well established, deficits of episodic memory are also highly correlated with frontal lobe pathology (e.g., Ackerley and Benton, 1947; Freeman and Watts, 1950; Stuss and Benson, 1986; Wheeler et al., 1997).

As an example of relative impairment of episodic memory, consider patient ML (Levine et al., 1998). Following a severe closed-head injury, patient ML became amnesic for pretraumatic events. Although ML retained the capacity to recount many autobiographical facts, he was unable to re-experience any specific event associated with them. For instance, ML could recount the name of a high school teacher perfectly well but was unable to recollect any experience associated with that individual. In brief, the episodic component of patient ML's autobiographical memory was missing. His pathology was restricted to right ventral frontal lobe, including the unicinate fasciculus, a band of fibers connecting frontal and temporal cortices. Patient ML is one of many brain-damaged patients who have lost much of their episodic and semantic memory, with no accompanying anterograde (posttrauma) amnesia.

That is, these patients are able to learn new information. With respect to these patients' retrograde (pretrauma) memory problems, semantic memory typically recovers, while episodic memory remains largely impaired.

As an example of disproportionate impairment of semantic memory, consider the report by Grossi et al. (1988) of a student who lost her ability to reproduce factual knowledge that she had learned prior to her injury. For instance, she was unable to recount various facts learned in school, although she could remember specific meetings with instructors. Summarizing over many such observations, Kapur (1999) concluded that, "loss of factual, semantic memories is readily dissociable from loss of memory for personally experienced events" (p. 819).

Perhaps the most well-documented example of a dissociation between episodic and semantic memory is a patient known as KC, who has been investigated by Tulving (1985b) and his colleagues at the University of Toronto. At the age of 30, patient KC sustained damage to several regions of his brain (including the medial temporal lobes) following a closed-head injury from a motorcycle accident (Rosenbaum et al., 2000, 2005). As with many amnesic patients, neuropsychological testing revealed that KC had retained many of his cognitive capacities. For instance, his intelligence and language faculties remain largely unaffected; he can read and write; he is able to focus and pay close attention to a conversation; he is capable of performing a wide variety of mental tasks, including visual imagery; and his short-term memory is normal.

KC also knows many details about his personal past. Among other things, he knows the names of many of the schools that he attended, the address of his childhood home, the make and color of his former car, and the location of his family's summer home. That is, KC's semantic knowledge of information acquired prior to the brain trauma remains largely intact. Nonetheless, KC cannot remember a single personal episode associated with this knowledge. For instance, although he can readily describe the process of changing a flat tire, he cannot remember ever having performed this task. In fact, KC cannot remember a single episode from his lifetime. This lack of episodic memory extends to highly emotional events; KC has no recollection regarding the untimely death of his brother or a bar fight that left him with a broken arm.

Given the diffuse nature of KC's brain pathology, it remains unclear what the precise cause of the clear dissociation between episodic and semantic memory might be, although strong arguments can be made regarding damage to regions of KC's medial temporal lobes (e.g., Vargha-Khadem et al., 1997; Klein et al., 2002) and frontal cortex (see Wheeler et al., 1997). Regardless, the story of patient KC is a remarkable one and suggests that there may emerge a biological dissociation between episodic and semantic memory.

As a whole, these studies show that various forms of deficits can be found with respect to episodic and semantic memory. Note, however, that there has not yet been successful resolution of how the current concept of episodic memory could accommodate finding a properly functioning episodic memory system occurring in a person with semantic memory deficits. Nonetheless, the more general finding that episodic and semantic memory can be dissociated not just as a function of independent variables but also in neuropsychological patients is consistent with the idea that episodic memory should be considered a memory system.

2.27.3.2 Functional Neuroimaging

There now exist seemingly countless neuroimaging studies of episodic memory. Here we identify a few general patterns that indicate a brain-based dissociation between episodic and semantic memory. We have found it necessary to be brief, and we suggest that the interested reader seek some of the in-depth reviews that detail the wealth of studies that have shaped our understanding of episodic memory and how it is represented in the brain.

Traditional psychological studies and (especially) lesion studies do not allow the easy separation of retrieval from storage. In neuroimaging studies, however, retrieval effects can arguably be better isolated. Here we focus primarily on retrieval from episodic memory for a couple reasons. First, the encoding of information into episodic memory seems to rely largely upon retrieval of information from semantic memory (Tulving et al., 1994; see also Prince et al., 2007). Storage is a phase not well studied with the methods under consideration here. Finally, retrieval has been argued to be the foundation for understanding memory; indeed, Roediger (2000) entitled a chapter "Why retrieval is the key process in understanding human memory."

Functional neuroimaging techniques, such as positron emission tomography (PET) and functional magnetic resonance imaging (fMRI), allow neuroscientists to examine the healthy human brain at work.

When participants engage in a given cognitive task, PET or fMRI provide information about the level of cerebral blood flow (PET) or blood oxygenation level (fMRI) localized in the brain regions recruited for the task. Such metabolic changes correlate highly with underlying neuronal activity and thus provide important insights into the brain structures that might underlie specific cognitive tasks.

One challenge in conducting brain-imaging research lies in experimental design. In the typical design, metabolic changes associated with two cognitive tasks are contrasted with one another in hopes of isolating the neural correlates of the cognitive process of interest. Researchers attempt to contrast a pair (or in some cases a set) of tasks that are highly similar to one another but that vary on one key dimension. Note that such a contrast highlights differences between tasks but (in the absence of a third, low-level baseline task) is unable to address areas of common activation.

For instance, in order to identify the neural correlates associated with retrieval from episodic memory, studies have contrasted a task that draws upon episodic memory with a second retrieval task that does not involve the reinstatement of specific spatial-temporal details (e.g., retrieval of general knowledge, which draws upon semantic memory). Although one may be certain that one task reasonably depends more on episodic memory and the other more on semantic memory, neither task is a direct window into the type of memory it is designed to reflect; confidence is gained, however, when results replicate across studies and tasks. This approach makes testable the assumption made by Tulving that retrieval from episodic memory relies upon semantic memory but adds to it certain other processes or brain regions. It is therefore possible to see whether episodic memory seems to rely upon the same brain regions as semantic memory with the addition of others.

With the neuropsychological studies just reviewed in mind, one could make some predictions with respect to how episodic and semantic memory might differ. Relative to some lower-level baseline task, semantic and episodic memory would be expected to reveal very similar activity. To the extent that episodic memory indeed builds upon semantic memory, any differences seen would be expected to be in the direction of greater activity for episodic than semantic memory. Specifically, retrieval from episodic memory would be expected to rely more upon hippocampus (and potentially surrounding structures) than would semantic memory.

In general, neuroimaging studies of episodic memory do not line up perfectly with the neuropsychological studies, and the precise reasons behind this situation are still unclear (Buckner and Tulving, 1995). One way in which the data are consistent with the theory is that in general, activation for retrieval from semantic and episodic memory tasks is very similar, with many (but certainly not all) differences tending to go in the direction of episodic retrieval. One puzzling finding is that the hippocampus is not reliably seen as particularly active during retrieval from episodic memory, especially as typically studied, with verbal materials (Fletcher et al., 1997; Schacter and Wagner, 1999). However, neuroimaging studies of episodic memory using autobiographical memories as the content of retrieval, rather than word lists learned in the laboratory, do overlap nicely with lesion studies (e.g., hippocampal activity is commonly reported in neuroimaging studies of autobiographical memory retrieval). Thus, questions regarding the differences obtained using differing methodologies may ultimately need to focus on the tasks being used in conjunction with the method of inquiry.

Direct comparisons of tasks designed to rely on episodic and semantic memory have not been reported as often as one might think (but for some examples see Shallice et al., 1994; Fletcher et al., 1995; Nyberg et al., 1996; McDermott et al., 1999a,b). Those who have done so show that regions within frontal cortex are more active for episodic than semantic memory. In the early 1990s (when the literature was based largely on PET methodology), retrieval-related activation in frontal cortex was almost always right-lateralized in or near Brodmann Area (BA) 10 (for a review see Buckner, 1996); more recent studies using fMRI tend to show bilateral or left-lateralized activity here. Following this relatively unanticipated finding, much work has been devoted to attempting to identify the processing underlying these prefrontal regions involved in episodic retrieval. Some hypotheses regarding the processes include retrieval mode (the mental set of attempting to retrieve the past, LePage et al., 2000), retrieval success (McDermott et al., 2000), postretrieval processing (a set of processes following the initial recovery of information in the retrieval phase; see Rugg and Wilding, 2000), or the amount of retrieval effort extended (Schacter et al., 1996). Different regions certainly contribute to different processes, but it is not yet clear which regions are contributing which processes (or even if the correct processes have

been identified). A precise understanding of the situation awaits further work.

Another somewhat surprising finding is the role parietal cortex appears to play in episodic memory. Contrasts of episodic memory tasks with semantic memory tasks tend to activate regions within bilateral inferior parietal cortex (within BA 40) and within medial parietal cortex (precuneus and posterior cingulate/retrosplenial cortex, e.g., McDermott et al., 1999b), and contrasts of episodic retrieval with other comparison tasks have elicited similar findings, which have led to recent attempts to identify the role of parietal cortex in memory (Shannon and Buckner, 2004; Wagner et al., 2005). Although the possible importance of parietal cortex in episodic retrieval was at the time unanticipated from the lesion literature, a closer look at the lesion literature shows that lesions on medial parietal structures can indeed produce what has been called retrosplenial amnesia (Valenstein et al., 1987).

Of historical importance is an early generalization in functional imaging studies of human memory, which suggested an apparent asymmetry between episodic encoding and retrieval processes: Hemispheric Encoding/Retrieval Asymmetry (HERA; Tulving et al., 1994). In general, episodic encoding was thought to be more strongly associated with left frontal lobe activity (than right), whereas episodic retrieval was more strongly associated with right frontal lobe activity (than left). Because episodic encoding is believed to involve a high degree of semantic elaboration of incoming information, semantic retrieval has also been associated with left frontal lobe activity. As reviewed above, most researchers would probably argue that the more profitable approach is to attempt the ascription of processes to specific cortical regions (rather than making broad generalizations to larger regions of cortex, e.g., the role of the right frontal lobe). Nonetheless, the HERA idea was influential in the late 1990s and served as a guiding framework for a number of studies.

In this short review, we have necessarily omitted many relevant issues from consideration. Among those are fMRI studies of remembering and knowing (e.g., Henson et al., 1999; Eldridge et al., 2000; Wheeler and Buckner, 2004) and studies from the tradition of autobiographical memory (see Maguire, 2001 for review). Further, event-related potential (ERP) studies anticipated the importance of parietal cortex in retrieval (Rugg and Allan, 2000) and some of the differences seen in remembering and knowing.

To summarize, initial contrasts of episodic and semantic memory were expected to elucidate the role of the hippocampus in episodic memory. Although some studies showed such activation, many did not. Attention then turned to the role of frontal cortex in remembering (with an accompanying new look at the neuropsychological literature). Most recently, the role of parietal cortex has become of great interest. The questions being asked are essentially of the flavor of which regions contribute which processes. In our view, this approach is the best one to take at this point (see, too, Roediger et al., 1999). Neuroimaging studies have not well adjudicated the question of whether episodic memory is a memory system but have clarified thinking with respect to how (in process terms) episodic and semantic memory differ and what the neural substrates of those different processes might be. Note that this review has focused on studies that are somewhat relevant to the question of whether episodic memory can be thought of as a memory system dissociable from semantic memory; other related issues (e.g., a comparison between remembering and knowing or between successful and unsuccessful retrieval attempts) have not been addressed, as we see them as less critical to the question under consideration here (although they address fundamentally important issues in the topic of remembering).

2.27.3.3 Development of Episodic Memory: The Magic Number 4 ± 1

Episodic memory is a late-developing memory system that emerges in the context of an already existing ability to draw upon the past in an informative fashion. Beginning at an early age, children are able to acquire vast amounts of knowledge from their surroundings. For instance, within the first few years of life, a child will have learned and retained the meanings of thousands of words and detailed knowledge pertaining to the identities of various objects in their environment. This early accumulation and utilization of knowledge is best characterized in terms of semantic memory. That is, although children know about many things that they have learned in the past, the capacity to reliably remember specific events does not emerge until approximately 4 years of age.

As with various other developmental milestones, episodic memory emerges in a gradual manner. Specifically, although most 3 year olds have great difficulty with tasks that are believed to require episodic memory, there do appear glimpses that this capacity is beginning to manifest itself. For instance, by the age of

3 years, many children are capable of reporting the content of an event that they had previously witnessed in the laboratory (Howe and Courage, 1993; Bauer et al., 1995; Bauer and Werenka, 1995). However, the descriptions are typically vague, and it is difficult to know whether these children remember the precise episodes they describe, or whether they just know about them.

Johnson and Wellman (1980) have presented data suggesting that the ability to discriminate between the mental states of remembering and knowing does not emerge until the age of 5 years. In their study, few 4 year olds, some 5 year olds, and most first-grade children demonstrated an understanding of the distinction. This finding is consistent with the claim that children under the age of 4 years are likely relying upon semantic memory when reporting on events from their past.

A great deal has been learned about the emergence of episodic memory through the use of source memory tests (Johnson and Raye, 1981; Johnson et al., 1993). Not only do such tests require the participant to remember the content of a prior study episode, but the participant must also remember the context (e.g., when, where, etc.) in which that content was learned. Source memory tasks are believed to be good tests of episodic memory in that a correct response requires the reinstatement of specific spatial–temporal aspects of the originally encoded event. Studies that have adapted the source memory paradigm for use with children are consistent in their findings: The capacity for episodic memory appears to emerge around the age of 4 years.

In a particularly clear demonstration, Gopnik and Graf (1988) had 3-, 4-, and 5-year-old children learn the contents of a drawer under one of three conditions. The children were told about the contents of the drawer, were allowed to see the contents of the drawer for themselves, or were given hints so they could infer the contents of the drawer. During a later test, the researchers were interested in the children's ability to answer two questions: What was in the drawer, and how do you know? With regard to the first question, retention of the contents of the drawer was comparable across all age groups. All children knew what they had seen. This was not the case when the children were required to discriminate the source of their knowledge. Although the 5-year-old children made few mistakes in describing the manner in which they had learned about the contents of the drawer, the 3 year olds performed at chance levels (see also Wimmer et al., 1988; O'Neill and Gopnik, 1991). That is, only the 5-year-old children remembered the circumstances under which they had seen the contents.

This basic finding has been replicated many times (e.g., Lindsay et al., 1991; Taylor et al., 1994; see Wheeler, 2000b; Drummery and Newcombe, 2002, for a review). In general, 3 year olds show initial signs of a developing episodic memory system, but for the most part they have great difficulty when they are required to report specific details of past occurrences. By the age of 5 years, most children appear to possess fully functioning episodic memory, although this capacity is likely to continue to develop thereafter (for related discussion, see Nelson, 1984; Gopnik and Slaughter, 1991; Flavell, 1993; Howe et al., 1994; Perner and Ruffman, 1995; Wheeler et al., 1997; Wheeler, 2000a,b; Tulving, 2005; Piolino et al., 2007). With respect to the purposes of our present discussion, children of all ages (except those younger than 8 months; Wheeler, 2000b) possess intact semantic memory, the context in which episodic memory develops.

2.27.4 Episodic Memory and Mental Time Travel

Finally, we consider the most recent conceptual development regarding episodic memory, namely, its relation to mental time travel. The idea, initially delineated by Tulving (1985a), is roughly that humans (and perhaps only humans) possess the ability to mentally represent their personal past and future (see also Suddendorf and Corballis, 1997; Tulving, 2002a). That is, just as we can vividly recollect our personal past, we can also, with a seemingly equal level of vividness and efficacy, mentally represent personal future scenarios (episodic future thought).

Beginning with the pioneering work of Hermann Ebbinghaus (see also Nipher, 1876), students of psychology and neuroscience have expended more than 100 years of thought and careful experimentation toward an understanding of human memory. However, there has been surprisingly little inquiry into episodic future thought. According to Tulving and his colleagues, both capacities represent an important component of autonoetic consciousness, which is the ability to "both mentally represent and become aware of subjective experiences in the past, present, and future" (Wheeler et al., 1997: p. 331).

Next, we review evidence suggesting that the capacity for episodic future thought (Atance and

O'Neill, 2001) is intricately related to the ability to vividly recollect one's past. Specifically, it has been argued that impairments to both capacities co-occur following brain damage (Tulving, 1985; Klein et al., 2002), that both share similar neural networks (Okuda et al., 2003; Addis et al., 2007; Szpunar et al., 2007), and that both appear rather late in ontogenetic development (Busby and Suddendorf, 2005).

2.27.4.1 Neuropsychology

For an example of selective damage, consider again patient KC. Along with a selective deficit of episodic memory, KC is unable to project himself mentally into the future. When asked to do either, he states that his mind is "blank"; when asked to compare the kinds of blankness in the two situations, he says it is the "same kind of blankness" (Tulving, 1985: p. 4).

A similar profile is exhibited by patient DB, studied by Klein and colleagues (Klein et al., 2002); DB experienced an anoxic episode following cardiac arrest and can no longer recollect his past, nor can he project himself into the future. Interestingly, Klein et al. revealed that DB was able to think about the past and future in a nonpersonal (semantic) manner. That is, while DB could not report any of what he had personally experienced in the past or any of what he might experience in the future, he could report general facts related to the past, along with what might generally occur in the future (e.g., concerns about global warming).

Hassabis et al. (2007) replicated and extended these findings in a more systematic fashion. In that study, the authors presented a set of five amnesic patients with brain damage localized to the hippocampal formation. Each of these patients is densely amnesic for personal episodes but retains intact semantic memory. To test whether the profound deficit of episodic memory was accompanied by a deficit in episodic future thought, the authors tested the patients' ability to form mental images of novel future experiences. Specifically, the patients were presented with a series of 10 cues and asked to imagine themselves in the context of either novel (e.g., castle) or familiar (e.g., possible event over next weekend) settings. Relative to those of control subjects, the patients' images were "fragmentary and lacking in coherence" (Hassabis et al., 2007: p. 1728).

The aforementioned case studies represent only a few of many reports about amnesic patients. Most other investigations into the phenomenon of amnesia have, for the most part, focused on the memory problems inherent in such patients. For instance, many others have been interested in investigating the relative effects of brain damage on episodic versus semantic memory (Kapur, 1999; Wheeler and McMillan, 2001). Thus, it remains uncertain whether comparable impairments in backward- and forward-going aspects of mental time travel are common in all such patients.

Nevertheless, there do exist prior reports describing amnesic patients as living in the permanent present (Barbizet, 1970; see also Lidz, 1942), and cases similar to the ones mentioned above have been reported (Stuss, 1991; Dalla Barba et al., 1997; Levine et al., 1998). In addition, there exist extensive reviews of case study reports on patients with frontal lobe damage (e.g., Luria and Homskya, 1964; Luria, 1969). One common characterization of these patients is that they seem to be detached from the past and unconcerned about matters related to their personal future (Ackerley and Benton, 1947; see also Freeman and Watts, 1950; Ingvar, 1985; Fuster, 1989; Wheeler et al., 1997; Wheeler, 2000a).

2.27.4.2 Functional Neuroimaging

The psychological study of episodic future thought has been attempted only sporadically (D'Argembeau and Van der Linden, 2004, 2006; Szpunar and McDermott, in press), and the search for its neural substrates has begun only very recently. Note that we draw an important distinction between episodic future thought and more general thoughts of the future, such as planning, which has received extensive attention in the literature and is thought to rely heavily on regions within frontal cortex (Stuss and Benson, 1986; Shallice, 1988; Fuster, 1989). The set of procedures under examination here – comprising episodic future thought – are arguably a necessary precursor to planning; without the ability to envision oneself spending a weekend with friends on the ski slopes, for example, it is unlikely that one would plan the weekend.

Consider a recent PET study by Okuda et al. (2003). Participants were asked to speak aloud for 1 min about their near future (the next few days), far future (next few years), near past (recent few days), and far past (last few years). Activity during these states was compared to each other and to a fifth, baseline state, which involved talking about the meaning of various words. Two regions in anteromedial frontal cortex and medial temporal cortex were more active for the future conditions than the past conditions; other regions (in nearby medial frontal

and medial temporal cortex) exhibited the opposite effects (more activity for past conditions relative to future). The authors suggested that remembering the past and planning for the future likely share common neural correlates and that it may be necessary for past experiences to be reactivated in order to facilitate an effective plan for future events (see too Burgess et al., 2000). Their data suggest that specific regions within frontal and medial temporal cortex might be suited for these functions. Although quite interesting, these data are of questionable relevance to the topic under consideration because in speaking about the future, the participants in this study tended not to focus upon specific future episodes but instead spoke about intentions, conjectures, and schedules. In contrast, these aspects were not much present when speaking about the past (i.e., the past tended to focus on specific episodes). In the other two studies to be considered, participants were asked to focus on specific episodes (either episodes that might take place in the future or ones that indeed took place in the past).

Szpunar et al. (2007) used fMRI to identify brain regions that might be important for representing oneself in time and then to examine those regions to see whether or not they are similarly engaged by past and future thought. In order to accomplish this goal, participants were asked to perform a set of three tasks. In all of these tasks, participants viewed a series of event cues (e.g., birthday party) and were asked to envision a specific scenario in response to the cues. In one task, the instructions were to recollect a personal memory of that kind of event (e.g., a specific previous birthday party). The second task instructed subjects to use the cue to think of a specific future scenario involving the cue. Activity common to both tasks (i.e., a conjunction of the past and future tasks) was contrasted with a third task that involved many of the processes common to past and future thought (e.g., mental construction of lifelike scenarios) but that lacked a sense of representing oneself in time. Specifically, the control task required participants to use the cue as a starting point for imagining former U.S. President Bill Clinton in a specific scenario. Bill Clinton was chosen because pretesting showed that he is easy to visualize in a variety of situations.

As can be seen in **Figure 3**, several regions in the brain's posterior cortex were similarly engaged during personal past and future thought, but not during the control task. These regions were located in the occipital cortex, the posterior cingulate cortex, and the medial temporal lobes. Previous research had shown that these regions are consistently engaged during tasks such as autobiographical memory (Svoboda et al., 2006) and mental navigation of familiar routes (Ghaem et al., 1997; Mellet et al., 2000;

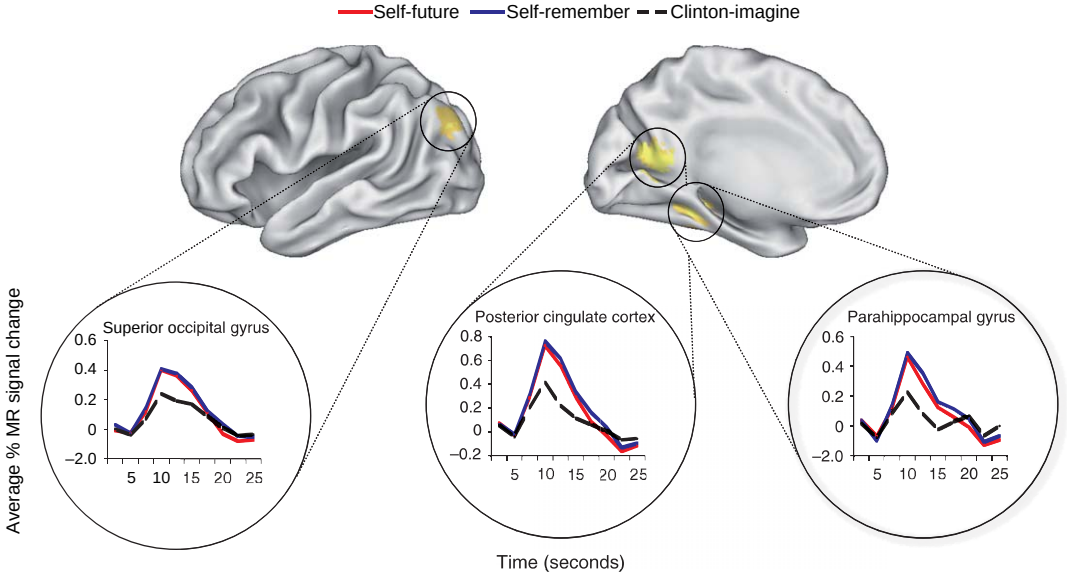

Figure 3 Percent signal change for brain regions exhibiting indistinguishable patterns of activity across time while participants envisioned their personal future and recollected the past. Imagining a familiar individual in similar scenarios resulted in a pattern of activity different from both the past and future tasks. Regions appear within superior occipital gyrus, posterior cingulate cortex, and parahippocampal gyrus. Data from Szpunar, Watson, and McDermott (2007).

Rosenbaum et al., 2004), which encourage participants to recount previously experienced settings (Aminoff et al., 2007). Szpunar et al. hypothesized that asking participants to envision a personal future scenario likely required similar processes. That is, in order to effectively generate a plausible image of the future, participants reactivate contextual associations from posterior cortical regions (cf., Bar and Aminoff, 2003; Okuda et al., 2003; Bar, 2004). Postexperiment questionnaires indicated that participants did tend to imagine future scenarios in the context of familiar settings and people.

A similar pattern of fMRI data has been presented by Addis et al. (2007), who parsed episodic future thought and remembering into two separate phases: construction and elaboration. That is, subjects were given cues (e.g., car) and asked to envision themselves in the future or to remember a past event. Once the event was in mind, they were to press a button and to then keep thinking about the event for the remaining time of the 20 s. They then rated the level of detail, the emotional intensity, and the perspective (first person or third person) before moving to the next trial. Of most interest to the present discussion is the construction phase (in part because the activity during the elaboration phase could not be separated from the activity during the three subsequent rating phases). Relative to baseline tasks that involved sentence generation and imagery, constructing the past and future episodes led to equivalent activity in a set of posterior cortical regions similar to those reported by Szpunar et al. (2007).

In light of such findings, Schacter and Addis (2007a) have proposed what they call the constructive episodic simulation hypothesis. They argue that one important function of retaining personal memories is the ability to sample their contents in mentally constructing (predicting) novel future scenarios (see also Szpunar and McDermott, in press). That past and future thought are so closely related provides insight into why certain populations who lack access to specific personal details of their past (e.g., brain damaged amnesic patients) are also unable to imagine specific personal future scenarios.

Finally, it should be noted that although this is a very recently emerging topic of interest, we anticipate that the above-mentioned studies will act as a catalyst for future research. Several early concept papers and reviews on the topic have also been put forth (Buckner and Carroll, 2007; Miller, 2007; Schacter and Addis, 2007a; Szpunar and McDermott, 2007). There is a recent but clear trend in thinking about episodic memory to include episodic future thought.

2.27.4.3 Development of Episodic Future Thought

A small but growing line of research suggests that the ability to project oneself into the future emerges in concert with the ability to vividly recollect the past. For instance, Busby and Suddendorf (2005) have shown that it is not until about the age of 5 years that children are able to accurately report what they will or will not do in the future (i.e., tomorrow), as well as what they have or have not done in the past (i.e., yesterday). Many of these studies have focused on requiring children to predict future states (e.g., Suddendorf and Busby, 2005) and have revealed both that the emergence of this capacity is not based simply on semantic knowledge related to the future event (Atance and Meltzoff, 2005) and that it is not dependent on language (Atance and O'Neill, 2005).

2.27.5 Is Episodic Memory Uniquely Human?

Perhaps the most intensely debated topic regarding episodic memory is whether this capacity, and mental time travel more generally, is uniquely human. There is no dispute that nonhuman animals possess memory. For example, consider a dog that buries a bone in the backyard and retrieves it the following day. How does the dog accomplish this task? Perhaps the animal mentally travels back in time, as we might. Alternatively, the animal may simply know that the backyard is somewhere where things are buried and may be able to make use of salient cues to locate the object it desires. Or the animal may know exactly where the bone is without remembering the episode in which it was placed there. We suspect most dog owners would suggest that the animal surely remembers where it had buried the bone and would likely be willing to offer many other examples to support the claim. But is this what happens?

As it turns out, this is a very difficult question to answer. If we assume that subjective (autonoetic) awareness is the central component of episodic memory, then we are not able to get very far. Much of the evidence for the concept of autonoetic awareness comes by way of verbal reports regarding the subjective state experienced during the act of remembering the past (e.g., remembering vs. knowing). Because we

cannot directly ask a nonhuman animal to describe its mental state, the prospect of identifying autonoetic awareness in other species is dim (Clayton et al., 2005). This state of affairs has led some to argue that there should be other means by which to investigate episodic memory in nonhuman animals.

Clayton et al. (2003) suggest that one alternative is to characterize episodic memory in terms of the spatial–temporal information that is encoded about an earlier event (what, where, and when) and the nature by which this information is represented (i.e., as an integrated whole) and utilized. The authors argue that animal studies must consider these behavioral criteria if they are to demonstrate convincing evidence of episodic memory in nonhuman animals. Clayton et al. further review prior attempts using primates, rats, and other animals that fall short of meeting these criteria.

Clayton, Dickinson, and their colleagues have presented several impressive demonstrations of an integrative memory capacity in the western scrub jay (e.g., Clayton and Dickinson, 1998, 1999). In their studies, the scrub jays are given the opportunity to cache both preferable but perishable (e.g., wax worms) and nonpreferable but less perishable (e.g., nuts) foodstuffs (see **Figure 4**). Given that the scrub jays' preferred snack will perish sooner, the birds must remember not only what they stored and where they stored it, but also when the foodstuff had been stored. Although the scrub jays will prefer to search for their favored treat, there is little point if that snack is no longer edible. It appears that the scrub jays are able to integrate these aspects of the original caching episode and search accordingly. That is, the scrub jays are able to appropriately adjust recovery attempts of the differentially perishable caches depending on how long ago they had stored the food items.

Figure 4 A western scrub-jay caching wax worms.

Even such convincing evidence of an integrated spatial–temporal memory of the past leaves open questions regarding the mental life of this species of bird. As a result, Clayton et al. (2003; Clayton and Dickinson, 1998) refer to this capacity as episodic-like memory, while others question whether this feat represents episodic memory or some other mechanism that may be driven by specific learning algorithms (Suddendorf and Busby, 2003; Suddendorf, 2006; see also Tulving, 2005).

Tulving (2005) has suggested that although mental states cannot be reported by other species, they may in fact be inferred, particularly in the context of mental time travel into the future (e.g., Emery and Clayton, 2001; Dally et al., 2006). Specifically, Tulving argues that comparative studies of episodic memory per se may be futile, in that demonstrations of episodic-like memory in other species may be explained away by simpler mechanisms that need not evoke episodic memory in its true sense (involving autonoetic consciousness). However, it may be possible to construct a situation in which an animal's future-directed behavior may not be attributed to other, simpler means.

Achieving such a situation, however, is no simple matter. A great deal of evidence suggests that even our nearest primitive relatives are incapable of truly future-oriented behavior (for reviews see Roberts, 2002; Suddendorf and Busby, 2003). According to the Bishof-Kohler hypothesis, an animal's foresight is necessarily restricted because it cannot anticipate future needs (for a more in-depth discussion see Suddendorf and Corballis, 1997). For instance, although chimpanzees display preparatory behaviors for future food consumption, it is unclear whether such behaviors indicate foresight beyond the near future (e.g., Boesch and Boesch, 1984; Byrne, 1995). Based on a review of the relevant literature, Roberts (2002) also concluded that higher-order primates appear to be "stuck in time."

Future studies will require clever experimental designs that will allow researchers to examine whether a particular species is able to plan for the future in a manner that is not instigated or maintained by its present motivational state, and in the absence of any immediate benefits associated with a future-directed action (see Mulcahy and Call, 2006; Raby et al., 2007). As it stands, the capacity to mentally represent the personal past and future has only been convincingly demonstrated with human beings (usually over the age of 4 years). Although future research will provide us with a better understanding

as to how unique this capacity is to humans, it will likely remain that this capacity holds a special status for humankind (Suddendorf and Corballis, 1997; Tulving, 2002a).

2.27.6 Concluding Remarks

As with all concepts of scientific inquiry, episodic memory is an evolving one that is largely shaped through the intricate relationship between data, theory, and available methods of inquiry. The concept of episodic memory started out as a taxonomic distinction that might possess some heuristic usefulness for future research. It has now expanded to encompass a dissociable system of the human brain that enables its owner to accomplish a feat (i.e., becoming autonoetically aware of episodes from one's past) that could not otherwise be possible. Currently, episodic memory represents a concept of great interest to many fields (e.g., clinical psychology, comparative psychology, developmental psychology, experimental psychology, functional brain imaging, neuropsychology, and psychopharmacology). There is little doubt that the continuing accumulation of data from these various areas of research, together with their unique methods of inquiry and furthering technological advancements, will ensure that researchers on the topic will continue to ask new and exciting questions.

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2.28 Semantic Memory

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Semantic memory entails the enormous storehouse of knowledge that all humans have available. To begin with, simply consider the information stored about the words of one's native language. Each of us has approximately 50,000 words stored in our mental dictionary. With each entry, we also have many different dimensions available. For example, with the word 'dog' we have stored information about how to spell it, how to pronounce it, its grammatical category, and the fact that the object the word refers to typically has four legs, is furry, is a common pet, and likes to chase cats (sometimes cars, squirrels, and other rodents), along with additional sensory information about how it feels when petted, the sound produced when it barks, the visual appearance of different types of dogs, emotional responses from past experiences, and much, much more. Of course, our knowledge about words is only the tip of the iceberg of the knowledge we have available. For example, people (both private and public) are a particularly rich source of knowledge. Consider how easy it is to quickly and efficiently retrieve detailed characteristics about John F. Kennedy, Marilyn Monroe, Bill Clinton, a sibling, parent, child, and so on. Indeed, our semantic, encyclopedic knowledge about the world appears limitless.

One concern reflected by the examples above is that semantic memory seems to be all inclusive. In this light, it is useful to contrast it with other forms of memory, and this is precisely what Tulving (1972) did in his classic paper distinguishing semantic and episodic memory. According to Tulving, semantic memory "is a mental thesaurus, organized knowledge a person possesses about words and other verbal symbols, their meaning and referents, about relations among them, and about rules, formulas, and algorithms for the manipulation of these symbols, concepts and relations" (1972, p. 386). In contrast, episodic memory refers to a person's memory for specific events that were personally experienced and remembered. So, the memory for the experience of having breakfast yesterday (e.g., where one was seated, how one felt, the taste of the food, who one was with) would fall under the umbrella of episodic memory, but the fact that eggs, cereal, and toast are typical breakfast foods reflects semantic knowledge. However, as we shall see, there is some controversy regarding where episodic memory ends and semantic memory begins. Indeed, we would argue that semantic memory penetrates all forms of memory, even sensory and working memory (Sperling, 1960; Tulving and Pearlstone, 1966; Baddeley, 2000), because tasks that are assumed to

tap into these other types of memory often are influenced by semantic memory.

So, what is indeed unique about semantic memory, and how has this area of research contributed to our understanding of learning and memory in general? One issue that researchers in this area have seriously tackled is the nature of representation, which touches on issues that have long plagued the philosophy of knowledge or epistemology. Specifically, what does it mean to know something? What does it mean to represent the meaning of a word, such as DOG? Is it simply some central tendency of past experiences with DOGS that one has been exposed to (i.e., a prototype DOG), or is there a limited list of primitive semantic features that humans use to capture the meaning of DOG, along with many other concepts and objects? Is the knowledge stored in an abstracted, amodal form that is accessible via different routes or systems, or is all knowledge grounded in specific modalities? For example, the meaning of DOG might be represented by traces laid down by the perceptual motor systems that were engaged when we have interacted with DOGs in the past.

In this chapter, we attempt to provide an overview of the major areas of research addressing the nature of semantic memory, emphasizing the major themes that have historically been at the center of research. Clearly, given the space limitations, the goal here is to introduce the reader to these issues and provide references to more detailed reviews. The vast majority of this work emphasizes behavioral approaches to the study of semantics, but we also touch upon contributions from neuropsychology, neuroimaging, and computational linguistics that have been quite informative recently. We focus on the following major historical developments: (1) the nature of the representation, (2) conceptual development and learning, (3) insights from and limitations of semantic priming studies, (4) interplay between semantic and episodic memory tasks, and (5) cognitive neuroscience constraints afforded by comparisons of different patient populations and recent evidence from neuroimaging studies. For further discussion of this latter area, the interested reader should see Chapter 2.29.

2.28.1 Nature of the Representation

Although the question of how one represents knowledge has been around since the time of Aristotle, it is clear that cognitive scientists are still actively pursuing this issue. One approach to representation is that we abstract from experience a prototypical meaning of a concept, and these ideal representations are interconnected to other related representations within a rich network of semantic knowledge. This is the network approach. Another approach is that there is a set of primitive features that we use to define the meaning of words. The meanings of different words and concepts reflect different combinations of these primitive features. This is a feature-based approach. Historically, the distinction between these two approaches has been central to research addressing the nature of semantic memory.

2.28.2 Network Approaches

One of the first landmark studies of knowledge representation came from computer science and was based on the important dissertation of A. M. Quillian. Quillian (1968) developed a model of knowledge representation called the Teachable Language Comprehender. A goal of this model was to formulate a working program that allowed efficient access to an enormous amount of information while minimizing redundancy of information in the network. Quillian adopted a hierarchically organized network, a portion of which is displayed in **Figure 1**. As shown, there are two important aspects to the network: nodes and pathways. The nodes in this network are intended to directly represent a concept in semantic memory, so for example, the word BIRD has a node that represents BIRDNESS. These nodes are interconnected in this network via labeled pathways, which are either 'isa' directional pathways or property pathways. Specifically, one can verify that BIRDS are indeed ANIMALS by finding an isa pathway between BIRDS and ANIMALS. Likewise, one could verify that 'A ROBIN BREATHES' by finding the isa pathway between robin and bird, and between bird and animal, and then accessing the property pathway leading to BREATHES from ANIMALS. In this sense, the model was quite economical, because most properties were stored only at the highest level in the network in which most of the lower exemplars included that property. For example, BREATHES would only be stored at the ANIMAL level, and not at the BIRD or CANARY level, thereby minimizing redundancy (and memory storage) in the network. Quillian also recognized that some features may not apply to all exemplars below that level in the network (e.g., ostriches are birds, and birds fly), so in these cases, one needed to include a

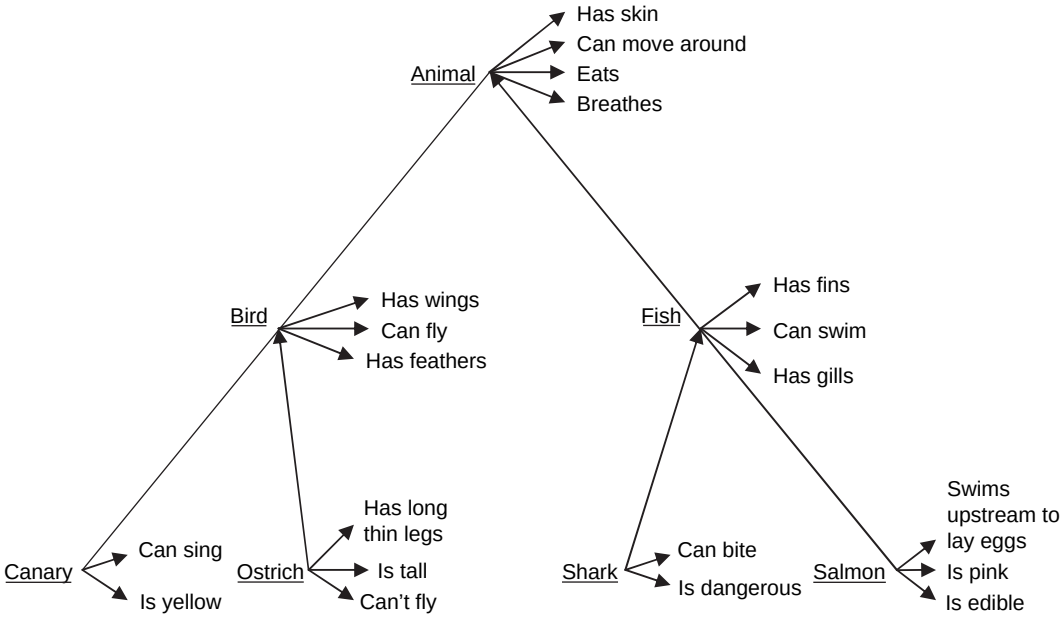

Figure 1 Hierarchically arranged network. Taken from Collins A and Quillian MR (1969) Retrieval time from semantic memory. *J. Verb. Learn. Verb. Behav.* 8: 240–247.

special property for these concepts (such as CAN'T FLY attached to OSTRICHES).

The economy of the network displayed in Figure 1 does not come without some cost. Specifically, why would one search so deeply in a network to verify a property of a given concept, that is, why would one have to go all the way to the ANIMAL concept to verify that 'CANARIES BREATHE'? It seems more plausible that we would have the property BREATHES directly stored with the CANARY node. Of course, Quillian was not initially interested in how well his network might capture performance in humans, because his goal was to develop a computer model that would be able to verify a multitude of questions about natural categories, within the constraints of precious computer memory available at the time.

Fortunately for cognitive psychologists, Quillian began a collaborative effort with A. Collins to test whether the network model developed by Quillian could indeed predict human performance on a sentence verification task, that is, the speed to verify such sentences as 'A CANARY IS A BIRD'. Remarkably, the Collins and Quillian (1969) study provided evidence that appeared to be highly supportive of the hierarchically organized network structure that Quillian independently developed in artificial intelligence. Specifically, human performance was nicely predicted by how many 'isa' and 'property' pathways one needed to traverse to verify a sentence. The notion is that there was a spreading activation retrieval mechanism that spread across links within the network, and the more links traversed the slower the retrieval time. So, the original evidence appeared to support the counterintuitive prediction that subjects indeed needed to go through the 'CANARY IS A BIRD' link and then the 'BIRD IS AN ANIMAL' link to verify that 'CANARIES BREATHE', because this is where BREATHES is located in the network.

The power of network theory to economically represent the relations among a large amount of information and the confirmation of the counterintuitive predictions via the sentence verification studies by Collins and Quillian (1969) clearly encouraged researchers to investigate the potential of these networks. However, it soon became clear that the initial hierarchically arranged network structure had some limitations. For example, the model encountered some difficulties handling the systematic differences in false reaction times, that is, the finding that correct 'false' responses to 'BUTTERFLIES ARE BIRDS' are slower than responses to 'SPIDERS ARE BIRDS.' Importantly, there was also clear evidence of typicality effects within categories. Specifically, categories have graded structure, that is, some examples of BIRD, such as ROBINS, appear to be better examples than other BIRDS, such as OSTRICHES.

There were numerous attempts to preserve the basic network structure of Collins and Quillian (1969), and indeed, some general models of cognitive performance still include aspects of such network structure. Collins and Loftus (1975) took a major step forward when they developed a network that was not forced into a hierarchical framework. This is displayed in **Figure 2**. As shown, these networks are basically unstructured, with pathways between concepts that are related and the strength of the relationship being reflected by the length of the pathways. Collins and Loftus further proposed that the links between nodes could be dependent on semantic similarity (e.g., items from the same category, such as DOG and CAT, would be linked), or the links could emerge from lexical level factors, such as cooccurrence in the language. Thus, DOG and CAT would be linked because these two items often occur in similar contexts. Because the strength of spreading activation is a function of the distance the activation traversed, typicality effects can be nicely captured in this framework by the length of the pathways. Of course, one might be concerned that such networks are not sufficiently constrained by independent evidence (i.e., if one is slow the pathway must be long). Nevertheless, such networks have been implemented to capture knowledge representation in both semantic and episodic domains (see Anderson, 2000).

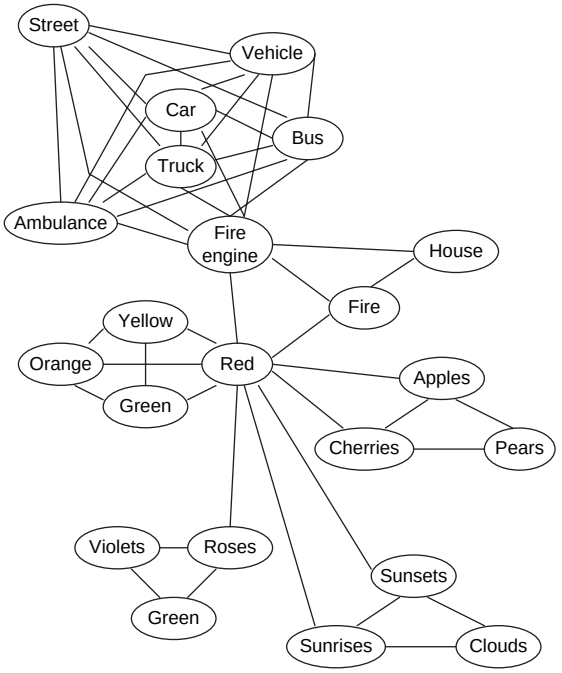

Figure 2 Semantic network. From Collins AM and Loftus EF (1975) A spreading-activation theory of semantic processing. *Psychol. Rev.* 82: 407–428.

More recently, there has been a resurgent interest in a type of network theory. Interestingly, these developments are again driven from fields outside of psychology such as physics (see Albert and Barabasi, 2002) and biology (Jeong et al., 2000). This approach is very principled in nature in that it uses large existing databases to establish the connections across nodes within a network and then uses graph analytic approaches to provide quantitative estimates that capture the nature of the networks. In this light, researchers are not arbitrarily constructing the networks but are allowing the known relations among items within the network to specify the structure of the network. This approach has been used to quantify such diverse networks as the power grid of the Western United States and the neural network of the worm, *C. elegans* (Watts and Strogatz, 1998). Once one has the network established for a given domain (i.e., providing connections between nodes), one can then quantify various characteristics of the network, such as the number of nodes, the number of pathways, the average number of pathways from a node, and the average distance between two nodes. Moreover, there are more sophisticated measures available such as the clustering coefficient, which reflects the probability that two neighbors of a randomly selected node will be neighbors of each other. In this sense, these parameters quantify the characteristics of the targeted network. For example, when looking at such parameters, Watts and Strogatz (1998) found that naturally occurring networks have a substantially higher clustering coefficient and relatively short average distances between nodes compared with randomly generated networks that have the same number of nodes and average connectivity between nodes. This general characteristic of networks is called 'small world' structure. These high clustering coefficients may reflect 'hubs' of connectivity and allow one to access vast amounts of information by retrieving information along the hubs. In popular parlance, such hubs may allow one to capture the six degrees of separation between any two individuals that Milgram (1967) proposed and that has been popularized by the game "six degrees of separation with Kevin Bacon".

What do worms, power grids, and parlor games have to do with semantic memory? Steyvers and Tenenbaum (2005) used three large databases reflecting the meaning of words to construct networks of semantic memory. These included free-association

norms (Nelson et al., 1998), WordNet (Miller, 1990), and Roget's Thesaurus (1911). For example, if subjects are likely to produce a word in response to another word in the Nelson et al. free-association norms, then a connection between the two nodes was established in the network. Interestingly, Steyvers and Tenenbaum found that these semantic networks exhibited the same small world structure as other naturally occurring networks; specifically, high-clustering coefficients and a relatively small average path distance between two nodes. As shown in **Figure 3**, if one moves along the hub of highly interconnected nodes, an enormous amount of information becomes readily available via traversing a small number of links.

Of course, it is not a coincidence that naturally occurring networks have small world structure. The seductive conclusion here is that knowledge representation has some systematic similarities across domains. Indeed, Steyvers and Tenenbaum (2005) and others have suggested that such structure reflects central principles in development and representation of knowledge. Specifically, Steyvers and Tenenbaum argue that as the network grows, new nodes are predisposed to attach to existing nodes in a probabilistic manner. It is indeed quite rare that a new meaning of a word is acquired without it being some variation of a preexisting meaning (see Carey, 1978). Hence, across time, nodes that are added to the network will be preferentially attached to existing nodes. This will give rise to a high degree of local clustering, which is a signature of small world network structure. We return to the issue of how concepts develop in a later section.

It is noteworthy that Steyvers and Tenenbaum (2005) have also provided empirical support from their network analyses. For example, they have found that word frequency, or the degree to which a word is encountered in language, and age of acquisition, defined as the average age at which a child learns a given word, effects in naming and lexical decision performance naturally fall from this perspective. Naming and lexical decision are two of the most commonly used word recognition tasks used in research investigating the nature and structure of semantic memory. In naming (or speeded pronunciation), a participant is asked to read a presented stimulus aloud as quickly as possible, whereas in lexical decision, he or she is asked to indicate whether a letter string is a real word or a pseudoword (i.e., a string of letters that does not correspond to the spelling of a real word). In both tasks, the primary dependent measure is response latency. The general assumption is that the speed required to access the pronunciation of a word or to recognize a string of letters reflects processes involved in accessing stored

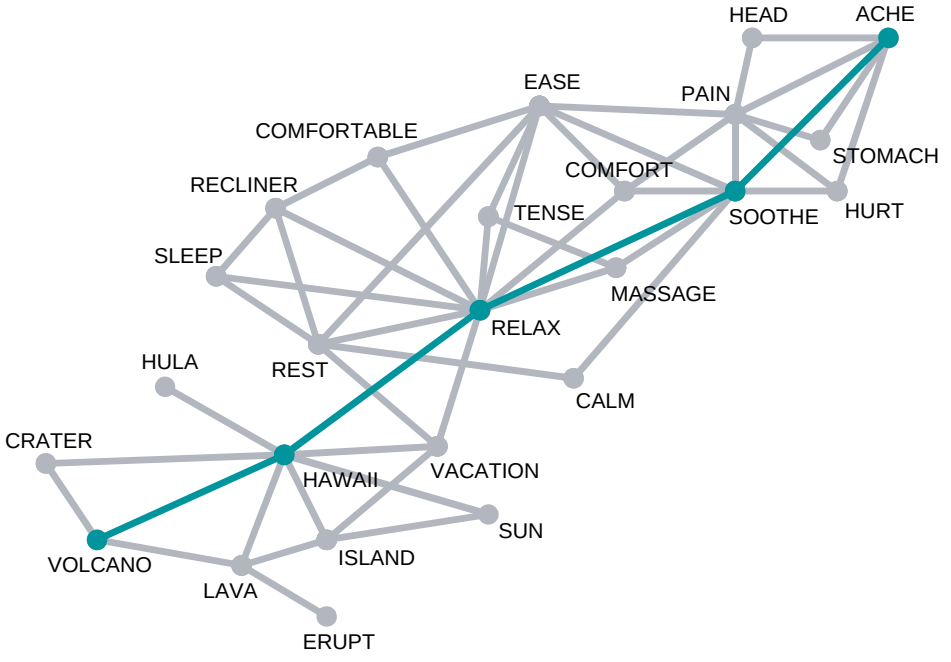

Figure 3 Segment of small world semantic network. Courtesy of Marc Steyvers.

knowledge about that word. Interestingly, Steyvers and Tenenbaum found a reliable negative correlation between number of connections to a node (semantic centrality) in these networks and response latency, precisely as one might predict, after correlated variables such as word frequency and age of acquisition have been partialed out (also see Balota et al., 2004). Clearly, further work is needed to empirically confirm the utility of these descriptions of semantic structure and the mechanisms by which such networks develop over time. However, the recent graph analytic procedures have taken a significant step toward capturing semantic memory within an empirically verified network.

2.28.3 Feature Analytic Approaches

An alternative to concepts being embedded within a rich network structure is an approach wherein meaning is represented as a set of primitive features that are used in various combinations to represent different concepts. Of course, this issue (distributed representation of knowledge, by way of features, vs. a localist representation, via a node to concept relationship) is central to attempts to represent and quantify learning and memory in general. We now turn to a review of the feature-based approaches in semantic memory.

The original Collins and Quillian (1969) research generated a great deal of attention, and soon researchers realized that categories reflected more graded structures than was originally assumed. Specifically, some members of categories are good members (ROBIN for BIRD), whereas other members appear to be relatively poor members (VULTURE for BIRD) but are still definitely members of the category (see Battig and Montague, 1969; Rosch, 1973). In addition, there was a clear influence of goodness of an exemplar on response latencies in the sentence verification task described above. Specifically, good exemplars were faster to verify than poor exemplars, referred to as the typicality effect. The Collins and Quillian hierarchical network model did not have any obvious way of accommodating such degrees of category membership.

Smith et al. (1974) took a quite different approach to accommodate the results from the sentence verification task. They rejected the strong assumptions of network theory and proposed a model that emphasized the notion of critical semantic features in representing the meaning of a word. So, for example, the word BIRD might be represented as animal, two legged, has wings, sings, is small, flies, and so on. There is no hierarchical organization within this model, but concepts reflect lists of critical features. They also distinguished between two classes of features, defining features and characteristic features. Defining features are the necessary features that an exemplar must have to be a member of a category. So, for example, all birds must eat, move, lay eggs, and so forth. On the other hand, characteristic features are features that most, but not all, exemplars have, such as small, flies, sings.

The second important aspect of the Smith et al. (1974) perspective is the emphasis on the decision processes engaged in the classic sentence verification task (see Atkinson and Juola, 1974; Balota and Chumbley, 1984, for similar decision models applied to short-term memory search and lexical decision, respectively). In verifying a sentence such as 'A ROBIN IS A BIRD,' subjects first access all (both defining and characteristic) features associated with ROBIN and all features associated with BIRD. If there is a high degree of overlap in the features, that is, above some criterion, the subject can make a fast 'yes' response. This would be the case in 'A ROBIN IS A BIRD,' since both defining and characteristic features provide a high degree of overlap. On the other hand, some exemplars of a given category may overlap less in characteristic features such as in 'AN OSTRICH IS A BIRD'. Although ostriches are clearly birds, they are not small and do not fly, which are characteristic features of birds. Hence, in such cases, the subject needs to engage in an additional analytic checking process in which only the defining features are compared. This additional check process takes time and so slows response latencies. Hence, the model can naturally capture the typicality effects mentioned above, that is, robins are better exemplars of birds than ostriches, because robins can be verified based on global overlap in features, whereas ostriches must engage the second, more analytic comparison of only the defining features, thereby slowing response latency.

In addition to accounting for typicality effects, the feature analytic model also captured interesting differences in latencies to respond 'no' in the sentence verification task. Specifically, subjects are relatively fast to reject 'A CARP IS A BIRD' compared with 'A BUTTERFLY IS A BIRD.' Carps do not have many overlapping features with birds, and so the subject can quickly reject this item, that is, there is virtually no overlap in features. However, both butterflies and

birds typically have wings, are small, and fly. Hence, the subject must engage the additional check of the defining features for 'BUTTERFLY IS A BIRD,' which ultimately leads to slower response latencies, compared with the sentence 'A CARP IS A BIRD.'

Although there were clear successes of the Smith et al. (1974) feature analytic approach, there were also some problems. For example, the model was criticized for the strong distinction between characteristic and defining features. In fact, McCloskey and Glucksberg (1979) provided a single process random walk model that accommodated many of the same results of the original Smith et al. model without postulating a distinction between characteristic and defining features. According to the random walk framework, individuals sample information across time that supports either a yes or no decision. If the features from the subject and predicate match, then movement toward the yes criterion takes place; if the features do not match, then movement toward the no criterion takes place. This model simply assumed that the likelihood of sampling matching feature information for the subject and predicate is greater for typical members than nontypical members, and therefore the response criterion is reached more quickly for typical than nontypical members, thus producing the influence on response latencies. The distinction between single- and dual-process models is a central issue that pervades much of cognitive science.

A second concern about the Smith et al. (1974) model is that they did not directly measure features but, rather, inferred overlap in features based on multidimensional scaling techniques, in which an independent group of subjects simply rated the similarity of words used in the sentence verification experiment. In this way, one could look at the similarity of the words along an N-dimensional space. Interestingly, Osgood et al. (1957) used a similar procedure to tackle the meaning of words in their classic work on the semantic differential. Osgood et al. found that when subjects rated the similarity across words, and these similarity ratings were submitted to multidimensional scaling procedures, there were three major factors that emerged: Evaluative (good–bad), potency (strong–weak), and activity (active–passive). Although clearly this work is provocative, such similarity ratings do not provide a direct measure of the features available for a concept. So, if there are indeed primitive features, it seems necessary to attempt to more directly quantify these features.

McRae and colleagues have been recently attempting to provide such constraints on feature analytic models (McRae et al., 1997; Cree et al., 1999; Cree and McRae, 2003; McRae, 2004; McRae et al., 2005). The goal here is to develop a feature-based computational model implemented in an attractor network capable of capturing the statistical regularities present in semantic domains. The general notion underlying attractor network models is that knowledge is distributed across units (which might be thought of as features) and that the network settles into a steady pattern of activity that reflects the representation of a concept. The conceptual representations that form the basis of semantic knowledge in the model are derived from feature norming data. To collect norms, groups of participants are asked to list features for a number of concepts (e.g., for DOG, participants might list BARKS, FURRY, CHASES CATS, etc.). McRae and colleagues propose that when participants are asked to list features of various basic-level category exemplars (e.g., DOG and APPLE are basic-level concepts from the superordinate category of MAMMALS and FRUIT, respectively), the resulting lists of features reflect the explicit knowledge people have of these concepts. Importantly, McRae does not claim that the nature of the representation consists of a feature list; rather, he argues, the features are derived from repeated multisensory interactions with exemplars of the concept, and in a feature listing task, subjects temporarily create an abstraction for the purpose of listing features that can be verbally described. Currently, feature norming data are available for 541 concrete objects, representing a wide variety of basic-level concepts. Importantly, the model can account for many empirical observations in semantic tasks, as discussed below.

The major assumption implemented by McRae and colleagues' model is that semantic knowledge, as represented by feature lists, involves the statistical averaging of feature correlations among members of similar categories. Features are correlated if they co-occur in basic-level concepts. For example, HAS FUR is highly correlated with HAS FOUR LEGS, as these two features cooccur in numerous exemplars of the mammal category. However, HAS FUR and HAS WINGS have a low (almost nonexistent) correlation, as these two features do not co-occur frequently. The argument is that individuals are highly sensitive to the regularity of the correlations, which are tapped by semantic tasks. As demonstrated by McRae et al. (1999), the strength of the feature

correlations predicted feature verification latencies in both human subjects and model simulations, with stronger correlations yielding faster response latencies than weaker correlations when the concept name was presented before the feature name (e.g., DOG-FUR). In addition, the correlation strength interacted with stimulus onset asynchrony (the time between the onset of the concept name and the onset of the feature name, SOA). Specifically, the effect of feature correlations was larger at shorter SOAs, with only high correlations predicting response latencies. However, at longer SOAs, even weakly correlated features influenced response times, indicating that, as more time was allowed for the effects of correlated features to emerge, even the more weakly correlated feature-concept pairs benefited from the shared representation. In another series of studies, McRae et al. (1997) reported that the strength of feature correlations predicted priming for exemplars from the living things domain but not for exemplars from nonliving things domains, for which priming was instead predicted by individual features. This finding is consistent with evidence that, compared to living things, nonliving things tend to have a lower degree of correlated features (also see section 2.28.8.1).

Several interesting extensions of McRae and colleagues' work on the role of features in organizing semantic knowledge have been recently reported. Pexman et al. (2002) examined the role of the number of features associated with a concept and found that items with more features were responded to faster in both naming and lexical decision tasks after a number of other variables known to influence visual word recognition latencies had been factored out. Pexman et al. (2003) reported similar results in a semantic categorization task and in a reading task. These findings were interpreted as supporting the distributed nature of semantic representations in which features are assumed to reflect access to conceptual knowledge, and this information quickly comes on line in isolated word recognition tasks.

In a related vein, there is recent evidence from the categorization literature that categories with richer dimensionalities (i.e., more features and more correlations among features) are easier to learn than categories with fewer dimensions (Hoffman and Murphy, 2006). Thus, rather than resulting in combinatorial explosions that make learning impossible, rich categories with many features lend themselves well to learning – a finding that is nicely mirrored in how people, even very young children, quickly and reliably learn to recognize and classify objects in the world. Indeed, it seems that learning to categorize complex objects, which might be quite similar in terms of features, is something most individuals can do reliably and easily. One concern that arises when one examines the richness of the stimuli in the environment, is the potentially infinite number of features that are available to identify a given concept. In fact, critics of feature-based models have argued that the number of possible feature combinations would result in combinatorial explosion, as knowing even a few features of a category could easily result in an enormous number of ways in which the features could be correlated and integrated (see Murphy, 2002, for a discussion). However, as McRae (2004) notes, two points are relevant in addressing this issue. The first is that the feature correlations tend to influence performance largely in implicit tasks – thus reducing the necessity of explaining how an individual can explicitly use the vast amount of information available. The second point is that the feature vectors that underlie semantic representations are generally sparse. In other words, the absence of a specific feature is uninformative, so, for example, knowing that a dog does not have feathers is relatively uninformative. Thus, although feature-based models might not fully capture the richness of the knowledge that individuals have about concepts, they have been useful in advancing research in the field of semantics.

2.28.4 Concept Learning and Categorization

Since semantic memory deals with the nature of representation of meaning, and categories are central to meaning, it is important to at least touch on the area of categorization and how concepts develop. In their classic book, Bruner et al. (1956) emphasized the importance of categorization in organizing what appears to be a limitless database that drives complex human learning and thought. Categorization has been viewed as a fundamental aspect of learning and indeed has been observed early in childhood (Gelman and Markman, 1986) and in other species such as pigeons (Herrnstein et al., 1976). Ross (*See* Chapter 2.29) provides a much more focused discussion of this topic.

One intriguing question that arises when one considers the content of semantic memory concerns the grain size and structure of the representations. In other words, is there a level at which objects in the

real world are more or less easy to learn and categorize? One possibility is that the world is initially perceived as a continuum in which there are not separate 'things.' Through repeated interactions with verbal labels or other forms of learning, an individual learns how to discriminate separate objects (e.g., Leach, 1964). This approach places the burden on an extensive and demanding learning process. An alternative approach is that the human cognitive system is ideally suited to detect and recognize objects at a specific grain or level. The assumption that the system is biased toward recognizing specific patterns implies that the process of learning the appropriate verbal labels that refer to specific items in the environment is significantly easier. This problem – how very young children learn that when their mother points to a dog and says 'dog' the referent of the phonological pattern in question refers to an entire object, and not to furry things, things of a certain color, or loosely attached dog parts – has been extensively discussed by Quine (1960).

In an elegant series of experiments, Rosch and colleagues (e.g., Rosch et al., 1976) provided empirical evidence that there is indeed a specific level at which categories of objects are represented that contains the most useful amount of information. For example, identifying a given object as a DOG implies that one recognizes that the specific exemplar is a dog, although it may differ from other dogs one has encountered. Simply knowing something is a dog allows one to draw upon a pool of stored knowledge and experiences to infer appropriate behaviors and interactions with the categorized object. However, knowing the object is an animal is not as informative, given the wide variability among animals. For example, interactions with an elephant are likely to be quite different from those one might have with a spider. Conversely, classifying the exemplar as a Collie or as a German Shepherd does not add a significant amount of inferential power for most purposes.

Rosch et al. (1976) argued that at the basic level, categories are highly informative and can be reliably and easily discriminated from other categories. Exemplars of basic-level categories (e.g., DOGS, BIRDS, CARS, etc.) have many attributes in common, tend to be similar in shape and in how one interacts with them, and allow easy extraction of a prototype or summary representation. The prototype can be accessed and serves as a benchmark against which novel exemplars can be compared: Those that are highly similar to the prototype will be quickly and easily classified as members of the category. Exemplars that differ from the prototype will be recognized as less typical members of a category (e.g., penguins are quite different from many other birds). Hence, typicality effects fall quite nicely from this perspective.

Historically, there has again been some tension between abstract prototype representation and more feature-based approaches. Consider the classic work by Posner and Keele (1968). Although cautious in their interpretation, these researchers reported evidence suggesting that prototypes (in this case a central tendency of dot patterns) were naturally abstracted from stored distortions of that prototype, even though the prototype was never presented for study. They also found that variability across a sufficient number of distortions was critical for abstracting the prototype. These results would appear to support the notion that there is a natural tendency to abstract some representation that is a central tendency of exemplars that share some common elements. So, 'dogness' may be abstracted from the examples of dogs that one encounters. This could suggest that there is indeed a unified representation for dogness.

An alternative approach is that there is nothing unique about these central tendencies but, rather, such representations reflect the similarity of the episodically stored representations in memory. This is a particularly important observation because it suggests that there is a blending of different types of memories, that is, categorical information is simply decontextualized episodic memories. Consider, for example, the classic MINERVA model developed by Hintzman (1986, 1988). In this computational model, each episodic experience lays down a unique trace in memory, which is reflected by a vector of theoretical features. There is no special status of category representations or hierarchical structure. Rather, categorization occurs during retrieval when a probe (the test item) is presented to the system, and the feature vector in the probe stimulus is correlated with all the episodically stored traces. The familiarity of a test probe is a reflection of the strength of the correlations among elements in memory. Because the schema overlaps more with multiple stored representations, that is, it is the central tendency, it will produce a relatively high familiarity signal or strength in a cued recall situation. The importance of the Hintzman approach is that there is no need to directly store central tendencies, as they naturally arise out of the correlation among similar stored traces in the feature vectors. Moreover, as Hintzman argues, there is no need to propose a

qualitative distinction between episodic and semantic memories, because both rely on the same memory system, that is, a vast storehouse of individual feature-based episodic traces.

The notion that categories are a reflection of similarity structure across memory traces and can be generated during retrieval clearly has some appeal. Indeed, Barsalou (1985) demonstrated the importance of ad hoc categories that seem to be easily generated from traces that do not inherently have natural category structure; for example, what do photographs, money, children, and pets have in common? On the surface, these items do not appear to be similar – they do not belong to the same taxonomic category, nor do they share many features. However, when given the category label "things to take out of the house in the case of a fire," these items seem to fit quite naturally together because our knowledge base can be easily searched for items that are in the house and are important to us. As Medin (1989) has argued, similarity depends on the theoretical frame that a participant uses to guide a search of memory structures. There appears to be an unlimited number of ways in which similarity can be defined, and hence similarity discovered. For example, lichen and squirrels are similar if one is interested in specifying things in a forest. This brings us to the remarkable context dependency of meaning, and the possibility that meaning is not defined by the stimulus *per se* but is a larger unit involving both the stimulus and the surrounding context. The word DOG in the context of thinking about house pets compared with the word DOG in the context of guard dogs or drug-sniffing dogs probably access quite different interpretations, one in which the focus is on companionship, furriness, and wagging tails, the other in which the more threatening aspects of dogness, such as sharp teeth, are accessed. One might argue that the context activates the relevant set of features, but even this is difficult until one has sufficient constraint on what those features actually are.

2.28.5 Grounding Semantics

In part because of the difficulties in defining the critical features used to represent meaning and potential problems with the tractability of prototypes of meaning, several researchers attempted to take novel approaches to the nature of the representations. There are two general approaches that we review in this section. First, because of the increase in computational power, there has been an increased reliance on analyses of large-scale databases to extract similarities across the contexts of words used in various situations. This perspective has some similarity to the exemplar-based approach proposed by Hintzman (1986, 1988) and others described earlier. In this sense, meaning is grounded in the context in which words and objects appear. The second approach is to consider the perceptual motor constraints afforded by humans to help ground semantics, that is, the embodied cognition approach. We review each of these in turn.

2.28.5.1 Grounding Semantics in Analyses of Large-Scale Databases

This approach attempts to directly tackle the poverty of the stimulus problem when considering the knowledge that humans have acquired. Indeed, since the days of Plato, philosophers (and more recently psychologists and linguists) have attempted to resolve the paradox of how humans can acquire so much information based on so little input. Specifically, how is it that children learn so much about the referents of words, when to use them, what their syntactic class is, what the relations among referents are, and so on, without explicit instruction? Some have argued (e.g., Pinker, 1994) that the poverty of the stimulus is indeed the reason one needs to build in genetically predisposed language acquisition devices. However, recent approaches to this issue (e.g., Latent Semantic Analysis, or LSA, Landauer and Dumais, 1997; Hyperspace Analogue to Language, or HAL, Burgess and Lund, 1997) have suggested that the stimulus input is not so impoverished as originally assumed. One simply needs more powerful statistical tools to uncover the underlying meaning and the appropriate database.

In an attempt to better understand how rich the stimulus is when embedded in context, Landauer and Dumais (1997) analyzed large corpora of text that included over 4.6 million words taken from an English encyclopedia, a work intended for young students. This encyclopedia included about 30 000 paragraphs reflecting distinct topics. From this, the authors constructed a data matrix that basically included the 60 000 words across the 30 000 paragraphs. Each cell within the matrix reflected the frequency that a given word appeared in a given paragraph. The data matrix was then submitted to a singular value decomposition, which has strong similarities to factor analysis to reduce the data matrix to

a limited set of dimensions. Essentially, singular value decomposition extracts a parsimonious representation of the intercorrelations of variables, but, unlike factor analysis, it can be used with matrices of arbitrary shape in which rows and columns represent the words and the contexts in which the words appear. In this case, the authors reduced the matrix to 300 dimensions. These dimensions reflect the intercorrelations that arise across the words from the different texts. So, in some sense the 300 dimensions of a given word will provide information about the similarity to all other words along these 300 dimensions, that is, the degree to which words co-occur in different contexts. The exciting aspect from this data reduction technique is that by using similarity estimates, the model actually performs quite well in capturing the performance of children acquiring language and adults' performance on tests based on introductory textbooks. In this way, the meaning of a word is being captured by all the past experiences with the word, the contexts with which that word (neighbors) occurs, the contexts that the neighbors occur in, and so on.

The remarkable success of LSA, and other similar approaches such as HAL (Burgess and Lund, 1997), provides a possible answer to the poverty of the stimulus problem, that is, when considering the context, the stimulus is indeed very rich. In the past, we simply have not been able to analyze it appropriately. Moreover, the model nicely captures the apparent contextual specificity of meaning in that meaning is defined by all the contexts that words have appeared in and hence will also be constantly changing ever so slightly across subsequent encounters. Finally, the model is indeed quite important because it does not rely on a strong distinction between semantic and episodic memory since it simply reflects past accumulated exposure to language. In this sense, it has some similarity to the Hintzman (1986) model described above.

2.28.5.2 Grounding Semantics in Perceptual Motor Systems

An alternative approach that has been receiving considerable recent attention is that meaning can be grounded in perceptual-motor systems (e.g., Barsalou, 1999). Briefly, this perspective is part of the embodied cognition approach that posits that the cognitive system of any organism is constrained by the body in which it is embedded (Wilson, 2002). Thus, cognition (in this case meaning) is not viewed as being separable from perceptual, motor, and proprioceptive systems; rather, it is through the interactions of these systems with the environment that cognition emerges. Furthermore, the type of representations that an organism will develop depends on the structure of the organism itself and how it exists in the world. This approach has its roots in Gibson's (1979) ecological psychology, as it is assumed that structures in the environment afford different interactions to different organisms. It is through repeated interactions with the world that concepts and knowledge emerge. Importantly, the very nature of this knowledge retains its connections to the manner in which it was acquired: Rather than assuming that semantic memory consists of amodal, abstract representations, proponents of embodied approaches argue that representations are grounded in the same systems that permitted their acquisition in the first place (Barsalou et al., 2003).

According to the modality-specific approaches to knowledge, a given concept is stored in adjacent memory systems rather than being abstracted. For example, in Barsolou's (1999) account, knowledge is stored in perceptual symbol systems that emerge through repeated experience interacting with an object or an event. Briefly, Barsalou assumed that when a percept is encountered, selective attention focuses on context-relevant aspects of the percept and allows modal representations to be stored in memory. Repeated interactions with similar events or members of the same category result in the formation of a complex, multimodal representation, and a simulator emerges from these common representations. Simulators are the basic unit of the conceptual system and consist of a frame (which is somewhat similar to a schema), the purpose of which is to integrate the perceptual representations. Simulators provide continuity in the system. Importantly, the representations that are stored include not only modal, perceptual information (e.g., sounds, images, physical characteristics) but also emotional responses, introspective states, and proprioceptive information. Retrieving an exemplar or remembering an event is accomplished by engaging in top-down processing and activating the targeted simulator. Importantly, a given simulator can yield multiple simulations, depending on the organism's goal, the context, and the relevant task demands. For example, different simulations for DOG are possible, such that a different pattern of activity will occur if the warm and furry aspect of dog is relevant or whether the aspect of being a guard or police dog is relevant. Of course, this nicely captures the context sensitivity of meaning. Barsalou (1999) argues that perceptual

symbol systems are as powerful and flexible as amodal models, as they are able to implement a complete conceptual system (see also Glenberg and Robertson, 2000).

Evidence in support of modal approaches to semantics can be found in both behavioral and cognitive neuroscience studies. We briefly review some of this evidence here, although a full review of the neuroscience literature is beyond the scope of this chapter (*See* Chapter 3.07 for further discussion of this area). For example, there is evidence from lesion studies that damage to the pathways supporting a specific modality results in impaired performance in categorization and conceptual tasks that rely on that same modality. Specifically, damage to visual pathways generally results in greater impairment in the domain of living things, which tend to rely heavily on visual processes for recognition. Conversely, damage to motor pathways tends to impair knowledge of artifacts and tools, as the primary mode of interaction with these items is through manipulation (see Martin, 2005). Consistent with the lesion data, neuroimaging studies indicate that different regions of the cortex become active when people process different categories. Regions adjacent to primary visual areas become active when categories such as animals are processed (even if the presentation of the stimulus itself is not in the visual modality), whereas regions close to motor areas become active when categories such as tools are processed. These findings have been interpreted as consistent with the hypothesis that people run perceptual-motor simulations when processing conceptual information (Barsalou, 2003).

Pecher et al. (2003) reported evidence from a property verification task indicating that participants were faster in verifying properties in a given modality (e.g., BLENDER-loud) after verifying a different property for a different concept in the same modality (e.g., LEAVES-rustling) than when a modality switch was required (e.g., CRANBERRIES-tart). Pecher et al. argued that the switch cost observed was consistent with the hypothesis that participants ran perceptual simulations to verify the properties (in this case sounds) rather than accessing an amodal semantic system. In a subsequent study, Pecher et al. (2004) observed that when the same concept in a property verification task was paired with two properties from different modalities, errors and latencies increased when verifying the second property. Pecher et al. interpreted this finding as indicating that recent experiences with a concept influence the simulation of the concept. Importantly, researchers have argued that such results are not simply a result of associative strength (i.e., priming) nor of participants engaging in intentional imagery instructions (Barsalou, 2003; Solomon and Barsalou, 2004).

Although the results summarized above are compelling and are supportive of the hypothesis that sensory-motor simulations underlie many semantic tasks, the majority of these studies have examined tasks such as property verification and property generation. The question thus arises of whether the results are somehow an artifact of the task demands, and specifically whether these results reflect the structure of the semantic memory system or whether subjects are explicitly retrieving information as they notice the relations embedded within the experimental context. Glenberg and Kaschak (2002) extended the evidence for embodiment effects to a novel series of tasks that do not appear as susceptible to task demand effects. In these experiments, participants read a brief sentence and judged whether the sentence made sense or not. The critical sentences contained statements that implied motion either toward the participant (e.g., "Nancy gave you the book") or away from the participant (e.g., "You gave the book to Nancy"). Participants responded by moving their hand toward themselves or away from themselves. Glenberg and Kaschak found what they called the action-sentence compatibility effect: When the required response was consistent with the movement implied in the sentence, participants were faster than when the implied motion and the actual physical response were inconsistent. These data appear most consistent with the view that when processing language, people relate the meaning of the linguistic stimulus to action patterns.

2.28.6 Measuring Semantic Representations and Processes: Insights from Semantic Priming Studies

As described above, there have been many empirical tools that have been used to provide insights into the nature of semantic memory. For example, as noted earlier, some of the early work by Osgood et al. (1957) attempted to provide leverage on fundamental aspects of meaning via untimed ratings of large sets of words and multidimensional scaling techniques. With the advent of interest in response latencies, researchers turned to sentence verification tasks that dominated much of the early work in the 1970s

and 1980s. Although this work has clearly been influential, the explicit demands of such tasks (e.g., explicitly asking subjects to verify the meaningfulness of subject-predicate relations) led some researchers in search of alternative ways to measure both structure and retrieval processes from semantic memory. There was accumulating interest in automatic processes (LaBerge and Samuels, 1974; Posner and Snyder, 1975) that presumably captured the modular architecture of the human processing system (Fodor, 1983), and there was an emphasis on indirect measures of structure and process. Hence, researchers turned to semantic priming paradigms.

Meyer and Schvaneveldt (1971) are typically regarded as reporting the first semantic priming study. In this study, subjects were asked to make lexical decisions (word-nonword decisions) to pairs of stimuli. The subjects' task was to respond yes only if both strings were words. The interesting finding here was that subjects were faster to respond yes when the words were semantically related (DOCTOR NURSE), compared with when they were unrelated (BREAD NURSE). This pattern was quite intriguing because subjects did not need to access the semantic relation between the two words to make the word/nonword decisions. Hence, this may reflect a relatively pure measure of the underlying structure and retrieval processes, uncontaminated by explicit task demands. Moreover, the development of this paradigm was quite important because researchers thought it may tap the spreading activation processes that was so central to theoretical developments at the time.

The research on semantic priming took a significant leap forward with the dissertation work of Neely (1977), who used a framework developed by Posner and Snyder (1975) to decouple the attentional strategic use of the prime-target relations from a more automatic component. In this study, subjects only made lexical decisions to the target, and subjects were given explicit instructions of how to use the prime information. For example, in one condition, subjects were told that when they received the prime BODY, they should think of building parts (Shift condition), whereas in a different condition, subjects were told that when they received the category prime BIRD, they should think of birds (Nonshift condition). Neely varied the time available to process the prime before the target was presented by using SOAs ranging from 250 to 2000 ms. The important finding here is that the instructions of what to expect had no influence on the priming effects at the short SOA (i.e., priming occurred if the prime and target had a semantic relationship, independent of expectancies), but they did have a large effect at the long SOA, when subjects had time to engage an attentional mechanism (i.e., the priming effects were totally dependent on what subjects were told to expect, independent of the preexisting relationship). Hence, Neely argued that the short SOA data reflected pure automatic measures of the semantic structure and retrieval processes and could be used as a paradigm to exploit the nature of such semantic representations.

A full review of the rich semantic priming literature is clearly beyond the scope of the present chapter (see Neely, 1991; Lucas, 2000; Hutchison, 2003, for excellent discussions of the methodological and theoretical frameworks). However, it is useful to highlight a few issues that have been particularly relevant to the current discussion. First, there is some controversy regarding the types of prime-target relations that produce priming effects. For example, returning to the initial observation by Meyer and Schvaneveldt (1971), one might ask if DOCTOR and NURSE are related because they share some primitive semantic features or are simply related because they are likely to co-occur in the same contexts in the language. Of course, this distinction reflects back on core assumptions regarding the nature of semantic information, since models like LSA might capture priming between DOCTOR and NURSE, simply because the two words are likely to cooccur in common contexts. Researchers have attempted to address this by selecting items that vary on only one dimension (see, e.g., Fischler, 1977; Lupker, 1984; Thompson-Schill et al., 1998). Here, semantics is most typically defined by category membership (e.g., DOG and CAT are both semantically related and associatively related, whereas MOUSE and CHEESE are only associatively related). Hines et al. (1986), De Mornay Davies (1998), and Thompson-Schill et al. (1998) have all argued that priming is caused by semantic feature overlap because of results indicating priming only for words that shared semantic overlap versus those did not, when associative strength was controlled. However, Hutchison (2003) has recently argued that the studies that have provided evidence for pure semantic effects (i.e., while equating for associative strength), actually have not adequately controlled for associative strength based on the Nelson et al. (1998) free-association norms. Clearly, equating items on one dimension (associative strength or semantic overlap) while manipulating

the other dimension is more difficult than initially assumed. In this light, it is interesting to note that two recent review papers have come to different conclusions regarding the role of semantics in semantic priming based on such item selection studies. Lucas (2000) argued that there was clear evidence of pure semantic effects, as opposed to associative effects, whereas Hutchison (2003) was relatively more skeptical about the conclusions from the available literature.

Balota and Paul (1996) took a different approach to the meaning versus associative influence in priming via a study of multiple primes, instantiating the conditions displayed in **Table 1**. As one can see, the primes were either both related, first related, second related, or both unrelated to the targets, and the targets could either be homographic words with distinct meanings (e.g., ORGAN) or a nonhomographic words (e.g., STRIPES). As one can see, the primes were related to the targets at both the semantic and associative level for the nonhomographs (e.g., LION and STRIPES are both related to TIGER at the associative and semantic level), but for the homographs the primes were related to the targets at only the associative level (e.g., PIANO and KIDNEY are only related to ORGAN at the associative level, since KIDNEY and PIANO are different meanings of ORGAN). Thus, one could compare priming effects in conditions in which primes converged on the same meaning of the target (nonhomographs) and priming effects where the primes diverged on different meanings (homographs). The results from four experiments indicated that the primes produced clear additive effects, that is, priming effects from the single related prime conditions nicely summated to predict the priming effects from the double related prime conditions for both homographs and nonhomographs, suggesting that the effects were most likely a result of associative level information. Only when subjects directed attention to the meaning of the word, via speeded semantic decisions, was there any evidence of the predicted difference between the two conditions. Hence, these results seem to be supportive of the notion that standard semantic priming effects are likely to be the result of associative-level connections instead of meaning-based semantic information. Of course, the interesting theoretical question is how much of our semantic knowledge typically used is caused by overlap in the contexts in which items are stored as opposed to abstracted rich semantic representations.

Table 1 Prime-target conditions from the Balota and Paul (1996) multiprime study

Nonhomographs			
Condition	*Prime 1*	*Prime 2*	*Target*
Related–related	LION	TIGER	STRIPES
Unrelated–related	FUEL	TIGER	STRIPES
Related–unrelated	LION	SHUTTER	STRIPES
Unrelated–unrelated	FUEL	SHUTTER	STRIPES
Homographs			
Condition	*Prime 1*	*Prime 2*	*Target*
Related–related	KIDNEY	PIANO	ORGAN
Unrelated–related	WAGON	PIANO	ORGAN
Related–unrelated	KIDNEY	SODA	ORGAN
Unrelated–unrelated	WAGON	SODA	ORGAN

Balota DA and Paul ST (1996) Summation of activation: Evidence from multiple primes that converge and diverge within semantic memory. *J. Exp. Psychol. Learn. Mem. Cogn.* 22: 827–845.

Hutchison (2003) notes two further findings that would appear to be supportive of associative influences underlying semantic priming effects. First, one can find evidence of episodic priming in lexical decision and speeded word naming tasks. In these studies, subjects study unrelated words such as (CITY-GRASS) and are later presented prime-target pairs in a standard lexical decision study. The interesting finding here is that one can obtain priming effects in such studies, compared to an unrelated/unstudied pair of words (see McKoon and Ratcliff, 1979). Thus, the semantic priming effects obtained in word recognition tasks can also be produced via purely associative information that develops within a single study exposure. However, it should be noted here that there is some question regarding the locus of such priming effects and that one needs to be especially cautious in making inferences from the episodic priming paradigm and the role of task-specific strategic operations (see, e.g., Neely and Durgunoglu, 1985; Durgunoglu and Neely, 1987; Spieler and Balota, 1996; Pecher and Raajmakers, 1999; Faust et al., 2001).

The second pattern of results that Hutchison (2003) notes as being critical to the associative account of semantic priming effects is mediated priming. In these situations, the prime (LION) is related to the target (STRIPES) only through a non-presented mediator (TIGER). So, the question is whether one can obtain priming from LION to STRIPES, even though these two words appear to be semantically unrelated. Although de Groot (1983) failed to obtain mediated priming effects in the

lexical decision task, Balota and Lorch (1986) argued that this may have resulted from the task-specific characteristics of this task. Hence, Balota and Lorch used a speeded pronunciation task and found clear evidence of mediated priming. Evidence for mediated priming has also now been found in versions of the lexical decision task designed to minimize task-specific operations (e.g., McNamara and Altarriba, 1988; McKoon and Ratcliff, 1992; Sayette et al., 1996; Livesay and Burgess, 1998). Of course, it is unclear what semantic features overlap between LION and STRIPES, and so these results would appear to be more consistent with an associative network model, in which there is a relationship between LION and TIGER and between TIGER and STRIPES, along with a spreading activation retrieval mechanism (see McKoon and Ratcliff, 1992; Chwilla and Kolk, 2002, for alternative accounts of the retrieval mechanism).

In sum, although the semantic priming paradigm has been critical in measuring retrieval mechanisms from memory, the argument that these effects reflect amodal semantic representations that are distinct from associative information has some difficulty accommodating the results from multiprime studies, episodic priming studies, and mediated priming studies. As noted earlier, there are available models of semantic memory (e.g., Burgess and Lund, 1997, HAL; Landauer and Dumais, 1997, LSA) and categorization (e.g., Hintzman, 1996, MINERVA) that would strongly support the associative contributions to performance in such tasks and, indeed, question the strong distinction between semantic and episodic memory systems. Hence, this perspective predicts a strong interplay between the systems. We now turn to a brief discussion of the evidence that directly addresses such an interplay.

2.28.7 The Interplay Between Semantics and Episodic Memory

Memory researchers have long understood the influence of preexisting meaning on learning and memory performance (see Crowder, 1976, for a review). Indeed, in his original memory manifesto, Ebbinghaus (1885) was quite worried about this influence and so purposefully stripped away meaning from the to-be-learned materials by presenting meaningless trigrams (KOL) for acquisition. Of course, semantics has penetrated episodic memory research in measures of category clustering (see Bousfield, 1953; Cofer et al., 1966; Bruce and Fagan, 1970), retrieval-induced inhibition (see Anderson et al., 1994), and release from proactive interference (see Wickens, 1973), among many other paradigms. Indeed, the interplay between preexisting knowledge and recall performance was the centerpiece of the classic work by Bartlett (1932). Researchers realized that even consonant–vowel–consonant trigrams were not meaningless (see Hoffman et al., 1987). At this level, one might even question what it would mean to episodically store in memory totally meaningless information.

One place where researchers have attempted to look at the interplay between semantic and episodic structures is within the episodic priming paradigm described earlier. In these studies, participants receive pairs of unrelated words for study and then are later given prime-target pairs that have either been paired together or not during the earlier acquisition phase. For example, Neely and Durgunoglu (1985) investigated the influence of studying previous pairs of words and word–nonword combinations on both lexical decision performance and episodic recognition performance (also see Durgunoglu and Neely, 1987). Although there were clear differences between the tasks in the pattern of priming effects (suggesting dissociable effects across the two systems), there were also some intriguing similarities. For example, there was evidence of inhibition at a short prime-target SOA (150 ms) in both the episodic recognition task and the lexical decision task from semantically related primes that were in the initial studied list but were not paired with the target. It appeared as if this additional semantic association had to be suppressed in order for subjects to make both the episodic recognition decision and the lexical decision. The finding that this effect occurred at the short SOA also suggests that it may have been outside the attentional control of the participant.

The power of preexisting semantic representations on episodic tasks has recently taken a substantial leap forward with the publication of an important paper by Roediger and McDermott (1995), which revisited an earlier paper published by Deese (1959). This has now become know as the DRM (after Deese, Roediger, and McDermott) paradigm. The procedure typically used in such studies involves presenting a list of 10–15 words for study (REST, AWAKE, DREAM, PILLOW, BED, etc.) that are highly related to a critical nonpresented item (SLEEP). The powerful memory illusion here is that subjects are just as likely to recall (or recognize) the critical nonpresented item (SLEEP) as

items that were actually presented. Moreover, when given remember/know judgments (Tulving, 1985), participants often give the critical nonpresented item remember judgments that presumably tapped detailed episodic recollective experience. It is as if the strong preexisting semantic memory structure is so powerful that it overwhelms the episodic study experience.

It should not be surprising that many of the same issues that have played out in the semantic memory research have also played out in the false memory research. Indeed, one model in this area is the activation monitoring (AM) framework (e.g., Roediger et al., 2001a), which suggests that subjects sometimes confuse the activation that is produced by spreading activation that converges on the critical nonpresented item (much akin to the Collins and Loftus, 1975) with the activation resulting from the study event. This framework attempts to keep separate the episodic and semantic systems but also shows how such systems can interact. In contrast, Arndt and Hirshman (1998) have used the Hintzman (1986) MINERVA framework to accommodate the DRM effect by relying on the similarity of the vectors of the individually stored words and the critical nonpresented items. As noted above, the MINERVA framework does not make a strong distinction between episodic and semantic systems. Moreover, the MINERVA model is more a feature-based model, whereas the AM framework *a priori* would appear more akin to a prototype model, but no strong claims have been made along this dimension. A further distinction between the AM framework and the MINERVA approach concerns the relative contributions of backward associative strength (BAS, or the probability that a list item will elicit the target, or critical lure, on a free-association task) and forward associative strength (FAS, or the probability the critical lure will elicit a list item in such a task). According to AM accounts, the critical variable is expected to be BAS, as the activation flows from the list items to the critical lure. However, according to MINERVA, FAS should be more important, as the similarity between the probe (i.e., the critical lure) and the stored episodes (i.e., the list items) should be a more powerful determinant of memory performance. Results from a multiple regression analysis reported by Roediger et al. (2001b) indicated that, in the DRM paradigm, BAS was the better predictor, thus supporting the AM framework. (We thank Roddy Roediger for pointing this out.)

The question of the nature of the representation (i.e., associative vs semantic) underlying these powerful memory illusions has also been studied. For example, Hutchison and Balota (2005) recently utilized the summation paradigm developed by Balota and Paul (1996), described earlier, to examine whether the DRM effect reflects meaning-based semantic information or could also be accommodated by primarily assuming an associative level information. Hence, in this study, subjects studied lists of words that were related to one meaning or related to two different meanings of a critical nonpresented homograph (e.g., the season meaning of FALL or the accident meaning of FALL). In addition, there were standard DRM lists that only included words that were related to the same meaning of a critical nonpresented word (e.g., such as SLEEP). Consistent with the Balota and Paul results, the results from both recall and recognition tests indicated that there was no difference in the pattern of false memory for study lists that converged on the same meaning (standard DRM lists) of the critical nonpresented items and lists that diverged on different meanings (homograph lists) of the critical nonpresented items. However, when subjects were required to explicitly make gist-based responses and directly access the meaning of the list, that is, is this word related to the studied list, there was clear (and expected) difference between homograph and nonhomograph lists. Hutchison and Balota argued that although rich networks develop through strategic use of meaning during encoding and retrieval, the activation processes resulting from the studied information seem to primarily reflect implicit associative information and do not demand rich meaning-based analysis.

There is little doubt that what we store in memory is a reflection of the knowledge base that we already have in memory, which molds the engram. Hence, as noted earlier, semantic memories may be episodic memories that have lost the contextual information across time because of repeated exposures. It is unlikely that a 50-year-old remembers the details of hearing the Rolling Stones' "Satisfaction" for the first time, but it is likely that, soon after that original experience, one would indeed have vivid episodic details, such as where one was, who one was with, and so on. Although this unitary memory system approach clearly has some value (e.g., McKoon et al., 1986), it is also the case that there is some powerful evidence from cognitive neuroscience that supports a stronger distinction.

2.28.8 Representation and Distinctions: Evidence from Neuropsychology

Evidence for the distinction of multiple memory systems has come from studies of patients with localized lesions that produce strong dissociations in behavior. For example, the classic case of HM (see Scoville and Milner, 1957) indicated that damage to the hippocampus resulted in impairment of the storage of new episodic memories, whereas semantic knowledge appeared to be relatively intact (but see MacKay et al., 1998). Hence, one might be overly concerned about the controversy from the behavioral studies regarding the distinct nature of semantic and episodic memory systems. However, there are additional neuropsychological cases that are indeed quite informative about the actual nature of semantic representations.

2.28.8.1 Category-Specific Deficits

There have now been numerous cases of individuals who have a specific lesion to the brain and appear to have localized category-specific deficits. For example, there have been individuals who have difficulty identifying items from natural categories (e.g., animals, birds, fruits, etc.) but have a relatively preserved ability to identify items from artificial categories (e.g., clothing, tools, furniture). At first glance, such results would appear to suggest that certain categories are represented in distinct neural tissue that have or have not been disrupted by the lesion. Such a pattern may also be consistent with a localized representation of meaning instead of a distributed feature-based representation in which all concepts share vectors of the same set of primitive features.

Unfortunately, however, the interpretation of impaired performance on natural categories and intact performance on artificial categories has been controversial. For example, such deficits could occur at various stages in the information flow from discriminating visually similar items (e.g., Riddoch and Humphreys, 1987) to problems retrieving the appropriate name of an object (e.g., Hart et al., 1985). Such accounts do not rely on the meaning of the categories but suggest that such deficits may reflect correlated dimensions (e.g., difficulty of the visual discrimination) that differ between natural and artificial categories. In this light, it is particularly important that there have been cases that have shown the opposite pattern. For example, Sacchett and Humphreys (1992) reported an intriguing case that shows disruption of the performance on artificial categories and body parts but relatively preserved performance on natural categories. They argued that one possible reason for this pattern is that this individual had a deficit in representing functional features, which are more relevant to artifactual representations and body parts than natural categories such as fruits and vegetables. Whatever the ultimate explanation of these category-specific deficits, this work has been informative in providing a better understanding of how members within categories may differ on distinct dimensions.

In a similar vein, one hypothesis that has been suggested to explain domain differences in category-specific deficits is the sensory/functional hypothesis (Warrington and McCarthy, 1987; Farah and McClelland, 1991; Caramazza and Shelton, 1998). According to this proposal, natural categories such as animals depend heavily on perceptual information (especially on visual discriminations) for identification and discrimination. Conversely, functional information is more important for recognition of artifacts, such as tools. Thus, damage to regions of sensory cortex is expected to result in selective impairment of natural kinds, whereas damage to regions in or adjacent to motor cortex would result in impairment in artifacts. Although compelling, this view is not endorsed by all researchers. Caramazza and colleagues, in particular, have argued that the sensory/functional hypothesis fails to account for some of the patterns of deficits observed and some of the finer-grain distinctions. In particular, it is difficult for this model to account for the selective sparing or impairment of fruits and vegetables, body parts, and musical instruments that have been reported (see Capitani et al., 2003, for a recent review). Thus, the question of whether and how the type of knowledge that is most critical for supporting the representation of a particular domain is involved in category-specific deficits remains open.

To address this controversy, Cree and McRae (2003) extended the sensory/functional hypothesis to include a broader range of types of knowledge. They developed a brain region taxonomy that included nine different forms of knowledge, including sensory/perceptual in all modalities (vision, taste, audition, etc.), functional, and encyclopedic. Encyclopedic features included information about items such as LIVES IN AFRICA for ELEPHANT – in other words,

information that likely was learned and not experienced directly. Cree and McRae then developed a nine-dimensional representation for the 541 concepts for which they had norming data and estimated the salience of each type of knowledge for each object and each category. In a series of cluster analyses, Cree and McRae found that the knowledge types nicely predicted the tripartite distinction between living things, artifacts, and fruits and vegetables reported in several neuropsychological case studies. In addition, Cree and McRae examined several distributional statistics, including the number of distinguishing and distinctive features and similarity to obtain a measure of confusability (i.e., the extent to which a given concept might be confused with another concept from the same category). The categories they examined did appear to be differentially sensitive to these measures, and the implemented model reflected patterns of impairment observed in patients. They concluded that knowledge type does underlie the organization of conceptual representations and that selective impairment in a particular brain region involved in maintaining such knowledge can result in the observed patterns of impairment in patients with category-specific deficits. Although many questions remain, it is clear that evidence from individuals with category-specific deficits has provided considerable insight into both the nature of category representation and the underlying neural representations.

2.28.8.2 Semantic Dementia

The most common form of dementing illness is dementia of the Alzheimer type (DAT). However, there is also a relatively rare and distinct dementia, referred to as semantic dementia (SD), which overlaps with DAT in features such as insidious onset and gradual deterioration of comprehension and word-finding ability. SD is a variant of frontal temporal dementia and typically involves one or both of the anterior portions of the temporal lobes. The consensus criteria for SD (Hodges et al., 1992) include impairment in semantic memory causing anomia, deficits in both spoken and written word comprehension, a reading pattern consistent with surface dyslexia (i.e., impairment in reading exception words such as PINT but preserved reading of regular words and nonwords that follow standard spelling to sound rules, such as NUST), impoverished knowledge about objects and/or people with relative sparing of phonological and syntactic components of speech output, and perceptual and nonverbal problem solving skills. These individuals are often quite fluent, but their speech is relatively limited in conveying meaning. They are particularly poor at picture naming and understanding the relations among objects. For example, the Pyramids and Palm Trees test developed by Howard and Patterson (1992) involves selecting which of two items (e.g., a palm tree or a fir tree) is most similar to a third item (e.g., a pyramid). Individuals with SD are particularly poor at this task and so would appear to have a breakdown in the representations of the knowledge structures.

An interesting dissociation has been made between SD individuals and DAT individuals. In particular, Simons et al. (2002) recently found a double dissociation, wherein individuals with SD produced poorer picture naming than individuals with DAT; however, individuals with SD produced better performance than individuals with DAT on a later episodic recognition test of these very same pictures (also see Gold et al., 2005). Clearly, the selective impairment across these two groups of participants is consistent with distinct types of information driving these tasks. Of course, one must be cautious about the implications even from this study, because it is unlikely that either task is a process-pure measure of episodic and semantic memory (see Jacoby, 1991), but clearly these results are very intriguing.

Recently, Rogers et al. (2004) proposed a model of semantic memory that maintains strong connections to modality-specific systems in terms of both inputs and outputs and has been particularly useful in accommodating the deficits observed in SD. This model has some interesting parallels to Barsalou's (1999) proposal, in that it assumes that semantic memory is grounded in perception and action networks. In addition, like the model proposed by McRae et al. (1997), Rogers et al. suggest that the system is sensitive to statistical regularities, and these regularities are what underlie the development of semantics. The particular contribution of Rogers et al.'s model, however, is that although semantic representations are grounded in perception–action modality-specific systems, the statistical learning mechanism allows the emergence of abstract semantic representations. Importantly, inputs to semantics are mediated by perceptual representations that are modality specific, and as a result, the content of semantic memory relies on the same neural tissue that supports encoding. However, different from Barsalou and colleagues' account, Rogers et al. do

suggest that there is a domain-general, abstracted representation that emerges from cross-modal mappings. Thus, although the system relies on perceptual inputs, the abstract representations can capture cross-modality similarities and structures to give rise to semantic memory.

Rogers et al. (2004) implemented a simple version of their model using a parallel distributed-processing approach in which visual features provided the perceptual input and are allowed to interact in training with verbal descriptors through a mediating semantic level. Importantly, the semantic representations emerge through the course of training as the network learns the mappings between units at the visual and verbal levels. The units the model was trained on consisted of verbal and visual features generated in separate norming sessions. Once training was complete, several simulations were reported in which the model was progressively damaged in a way that was thought to mimic varying levels of impairment observed in individuals with SD. Overall, the model nicely captured the patterns of performance of the patients. Specifically, one pattern often observed in SD is a tendency to overregularize conceptual knowledge. For example, individuals might refer to all exemplars of a category using the superordinate label or a single label that is high in frequency (e.g., calling a DOG an ANIMAL or a ZEBRA a HORSE). This is possibly a result of the progressive failure in retrieving idiosyncratic information that serves to distinguish exemplars, such that only the central tendency (e.g., a prototype or most typical exemplar) remains accessible. Thus, less common items might take on the attributes of higher-frequency exemplars. The model displayed similar patterns of generalization as the SD individuals, a finding explained in terms of changes in attractor dynamics that resulted in the relative sparing of features and attributes shared by many exemplars but a loss of more distinctive features. This model provides an interesting account of semantic memory and the deficits observed in individuals with SD, one in which both perceptually based information and abstracted representations interact to give rise to knowledge of the world.

2.28.9 Neuroimaging

Investigations into the nature of semantic memory have benefited from recent advances in technology that allow investigators to examine online processing of information in the human brain. For example, positron emission technology (PET) and functional magnetic resonance imaging (fMRI) allow one to measure correlates of neural activity *in vivo* as individuals are engaged in semantic tasks (see Logothetis and Wandell, 2004). Although a full review of the substantial contributions of neuroimaging data to the questions pertaining to semantics is beyond the scope of this chapter (*See* Chapter 3.07 for a review), we briefly examine some of the major findings that have helped constrain recent theorizing about the nature and locus of semantic representations. Two major brain regions have been identified through neuroimaging studies: left prefrontal cortex (LPC) and areas within the temporal lobes, particularly in the left hemisphere.

The first study to report neuroimaging data relevant to semantic memory was conducted by Petersen et al. (1988), who used PET techniques to localize activation patterns specific to semantic tasks. Subjects were asked to generate action verbs upon presentation of a concrete object noun, and activity during this task was compared with the activity occurring during silent reading of the words. Petersen et al. reported significant patterns of activity in LPC, a finding that has since been replicated and extended to other types of attributes. Martin et al. (1995) extended this work to show that the specific attribute to be retrieved yielded different patterns of activation. Specifically, the locus of activation involved in attribute retrieval tends to be in close proximity to the neural regions that are involved in perception of the specific attributes. Thus, retrieval of visual information, such as color, tends to activate regions adjacent to the regions involved in color perception, whereas retrieval of functional information results in activation of areas adjacent to motor cortex. These findings mesh nicely with the perceptual/motor notions of representation in semantic memory reviewed above (e.g., Barsalou, 1999; Rogers et al., 2004). In addition, Roskies et al. (2001) reported that not only were regions in lateral inferior prefrontal cortex (LIPC) preferentially active during tasks that required semantic processing, but specific regions were also sensitive to task difficulty. Thus, it appears that frontal regions are involved both in the active retrieval from semantic memory and in processing specific semantic information.

Many researchers have suggested, however, that although frontal regions are involved in semantic retrieval, the storage of semantic information is primarily in the temporal regions (see Hodges et al., 1992). Indeed, another area that has been implicated in semantic processing is in the ventral region of the

temporal lobes, centered on the fusiform gyrus, and especially in the left hemisphere. This area shows significant activation during word reading and object naming tasks, indicating it is not sensitive to the stimulus form but to the semantic content therein (see Martin, 2005, for a review). Furthermore, within this area, different subregions become more or less activated when subjects view faces, houses, and chairs (e.g., Chao et al., 1999), suggesting that different domains rely on different regions of neural tissue. This, of course, could be viewed as consistent with the category-specific deficits reviewed above. However, as noted by Martin and Chao (2001), although peak activation levels in response to objects from different domains reflect a certain degree of localization, the predominant finding is a pattern of broadly distributed activation throughout the ventral temporal and occipital regions, which is consistent with the idea that representations are distributed over large cortical regions.

Recently, Wheatley et al. (2005) reported data from a semantic priming study using fMRI that also converges on the notion of perceptual motor representations of meaning. Subjects silently read related, unrelated, or identical word pairs at a 250-ms SOA while being scanned. The related pairs consisted of category members that were not strongly associatively related (e.g., DOG-GOAT, but see the discussion above regarding the difficulty of selecting such items). Given the relatively fast SOA and that no overt response was required, Wheatley et al. argued that any evidence for priming should be a reflection of automatic processes. Consistent with other evidence that indicates there are reliable neural correlates of behavioral priming that were evidenced by reduced hemodynamic activity (Wiggs and Martin, 1998; Mummery et al., 1999; Rissman et al., 2003; Maccotta and Buckner, 2004), Wheatley et al. found decreased activity for identity pairs and a slightly smaller, but still significant, decrease for related pairs relative to the unrelated pairs condition. Importantly, Wheatley et al. were able to compare patterns of activation as a function of domain. Consistent with proposals by Barsalou (1999), they found that objects from animate objects yielded more activity in regions adjacent to sensory cortex, whereas manipulable artifacts resulted in greater activity in regions adjacent to motor cortex. These findings were taken as evidence that conceptual information about objects is stored, at least in part, in neural regions that are involved in perception and action.

Although the Wheatley et al. (2005) study used a task that was likely to minimize strategic processing, one question that remains to be addressed is whether the automatic and strategic processes involved in semantic priming tasks (see earlier discussion) can also be dissociated in neural tissue. In a recent study, Gold et al. (2006) reported that several of the brain regions previously implicated in processing during semantic tasks are differentially sensitive to the automatic and strategic processes involved in lexical decision tasks. In three experiments, Gold et al. manipulated prime target relatedness, SOA, and whether primes and targets were orthographically or semantically related. Long and short SOAs were intermixed in scanning runs to assess the relative contributions of strategic and automatic processes (see Neely, 1991). A comparison of orthographic and semantic priming conditions was included to determine whether any areas were particularly sensitive to the two sources of priming or whether priming effects are more general mechanisms. The results clearly indicated that different regions responded selectively to different conditions. Specifically, midfusiform gyrus was more sensitive to automatic than strategic priming, but only for semantically related primes, as this region did not show reduced activity for orthographic primes. Four regions were more sensitive to strategic than automatic priming, two in left anterior prefrontal cortex and bilateral anterior cingulate. Even more intriguing, the two regions in LIPC were further dissociated: The anterior region showed strategic semantic facilitation, as evidenced by decreased activity, relative to a neutral baseline, whereas the posterior region showed strategic semantic inhibition, or increased activity, relative to the neutral baseline. In addition, the medial temporal gyrus showed decreased activation concurrently with the anterior LIPC, supporting previous claims that these regions show greater activation in tasks that are more demanding of strategic processes but reduced activation when the strategic processes are less demanding (Wagner et al., 2000; Gold et al., 2005). In sum, it appears that the behavioral dissociations between automatic and strategic processes in priming tasks are also found in the neuroimaging data. The complexity of the patterns of activation involved in semantic tasks appears to indicate that the retrieval and storage of semantic information is indeed a distributed phenomenon that requires the coordination of a wide array of neural tissue.

2.28.10 Development and Bilingualism

Although we have attempted to provide a review of the major issues addressed in semantic memory research, there are clearly other important areas that we have not considered in detail because of length limitations. For example, there is a very rich area of developmental research addressing the acquisition of meaning in children (see Bloom, 2000, for a comprehensive review), along with work that attempts to capture the nature of semantic memory in older adulthood (see, e.g., Balota and Duchek, 1989). Of course, we touched upon these issues earlier when discussing how the small world networks of Steyvers and Tenenbaum (2005) develop over time, along with the work by Rosch (1975) on the development of categorization. Given that meaning is extracted from interactions with the environment, the developmental literature is particularly important to understand how additional years of experience mold the semantic system, especially in very early life. There are many interesting connections of this work to topics covered earlier in this chapter. For example, regarding the influence of preexisting structures on false memory, it is noteworthy that young children (5-year-olds) are more likely to produce phonological than semantic false memories, whereas older children (around 11 years and older) are more likely to produce the opposite pattern (see Dewhurst and Robinson, 2004). Possibly, this is a natural consequence of the development of a rich semantic network in early childhood that lags behind a more restricted phonological system.

Another very active area of research involves the nature of semantic representations in bilinguals (see Francis, 1999, 2005, for excellent reviews). For example, researchers have attempted to determine whether there is a common semantic substrate that is amodal, with each language having specific lexical level representations (e.g., phonology, orthography, syntax, etc.) that map onto this system. This contrasts with the view that each language engages distinct semantic level representations. Although there is still some controversy, the experimental results seem more consistent with the assumption that the semantic level is shared across languages, at least for skilled bilinguals. Evidence in support of this claim comes from a diverse range of tasks. For example, in a mixed language list, memory for the language of input is generally worse than memory for the concepts (e.g., Dalrymple-Alford and Aamiry, 1969). In addition, one finds robust semantic priming effects by translation equivalents (words in different languages with the same meaning, e.g., DOG in English and HUND in German), which is consistent with at least a partially shared semantic representation (e.g., de Groot and Nas, 1991; Gollan et al., 1997).

2.28.11 Closing Comments

The nature of how humans develop, represent, and efficiently retrieve information from their vast repository of knowledge has for centuries perplexed investigators of the mind. Although there is clearly considerable work to be done, recent advances in analyses of large-scale databases, new theoretical perspectives from embodied cognition and small world networks, and new technological developments allowing researchers to measure, *in vivo*, brain activity, are making considerable progress toward understanding this fundamental aspect of cognition.

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2.29 Concept and Category Learning in Humans

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2.29.1 Introduction

Concepts and categories are crucial for intelligent thought and action. A child needs to learn to tell toys from tools and which types of dogs can be petted. A student needs to learn to distinguish the principle underlying a math problem, so that relevant principle knowledge can be applied. Researchers need to be able to decide what type of person has asked them to collaborate, to understand the concept of confounding, and to be able to communicate new ideas to others. The focus of this chapter is understanding the learning of categories and concepts. This learning is critical not only because little of this knowledge is

innate but also because the learning of concepts and categories is a large part of acquiring knowledge.

We use concept to refer to a mental representation, an idea, that picks out a class of entities. A category is the class or set of entities that is referred to by the concept. One has a concept of dog, and then there is the category of dogs (both real and imagined). Different things in the category can all be treated similarly, with respect to some purpose. Cars are members of a category because they have much in common in terms of appearance and use. However, one might want to make finer distinctions, such as separating cars that can carry much stuff for moving from sports cars; at other times, such as in compiling assets, one might want to think of an expensive sports car as part of the same class as a boat and house. When possible, we use categories to refer to the learning or use of the members and concepts to refer to the knowledge of categories. Despite this distinction, they are often used interchangeably because it is often true that one is dealing with both together.

We also need to address briefly two important issues: Origins and types. First, although some researchers argue for the independent existence of concepts in the world (separate from the organisms that perceive them), we, as psychologists, believe it is more useful to think of them as arising jointly from the fit between the world and the organism. Although it is true that we do not just have any concepts, it is also true that different organisms have different concepts, and even among humans, the concepts we have often are a function of human activity. Second, although much of the work has focused on object categories, we clearly have many other categories besides objects, people, situations, problems, scenes, and so on. We try to include a variety of types in our discussion (also see Medin et al., 2000).

In this chapter, we provide an overview of the work on concept and category learning, with a focus on experimental work and modeling. We begin by considering the functions of concepts. We then address a large body of research that has examined conceptual structure for classification, with particular emphasis on prototype and exemplar approaches and models. We argue that this work has missed some important aspects of both how categories are learned and the importance of structure and prior knowledge. A goal of this chapter is to integrate the work on concepts and categories into the areas of cognition in which they are so crucial. All cognitive activity relies on concepts in some way, so an understanding of how they are learned is likely to have an impact on much other research. We provide an integration in three ways. One, we examine the learning of concepts and categories from a goal-oriented view, asking how the ways in which they might be learned influence the representation available for other cognitive activities. Two, we consider more complex concepts, as would be needed to account for results in most areas in which concepts are used. Three, we address this integration more directly by considering two very different areas of cognition, problem solving and language.

2.29.1.1 Functions of Concepts and Categories

Concepts and categories are fundamental building blocks of cognition. Murphy (2002) calls them the mental glue in that they link our past experiences (with toys, dogs, mathematical problems, collaborators) to our current ones. They are a part of all cognitive, and many noncognitive, theories. We mention here a few functions.

2.29.1.1.1 Classification

Classification is the determination that something is a member of a particular category: A carrot, an extrovert, a permutations problem, an instance of insurgency. This action allows one to access knowledge about the category that can be used for a variety of other functions.

2.29.1.1.2 Prediction and inference

When an object is classified as a carrot, you can predict how it will taste, how crunchy it will be. You can use knowledge of the category to infer how it was grown and how similar it is to a beet. Prediction is often considered a key function of categories (e.g., Anderson, 1991) in that it allows for selection of plans and actions.

2.29.1.1.3 Understanding and explanation

People need to know not just what, but why. We can explain aberrant behavior if we know that the person was grieving or drunk. This characterization might change our future actions with the person. If we start watching a TV program part-way through and cannot understand what is happening, being told it involves a love triangle may help us to make sense of the events.

2.29.1.1.4 Communication

We are social animals, and much of our activity is geared toward interacting with others. Concepts provide a kind of social glue as well, as they facilitate communication and allow us to learn new concepts by indirect experience.

Concepts and categories underlie much of mental life, and their learning is complex. We must learn not just to classify items: Knowing something is a carrot, a permutations problem, or an extrovert does not help unless we know enough about carrots, permutations, or extroverts to accomplish our goals (e.g., how to prepare the carrot, solve the problem, quiet the extrovert). Thus, as we learn to tell what category an item is in, we also have to learn much else about the concept to allow prediction, explanation, and communication. The corresponding distinction between knowledge that allows one to classify and knowledge that allows one to perform other conceptual functions is a central one for this chapter, as well as for understanding concepts and categories in other areas of cognition.

2.29.2 Conceptual Structure

We turn now to the structure of concepts. How are categories mentally represented to allow these various functions to be accomplished? This question is essential for any examination of concept and category learning, because it provides a clear target for what must be learned. We can only provide a brief overview, but fuller descriptions can be read in Medin (1989) and Murphy (2002).

2.29.2.1 Views and Models

What determines which items go together in a category and which items are in different categories? A common intuition is that it depends upon similarity – more similar items are in the same category. One can think of items as consisting of a set of features. Similarity is defined as the overlap of features (such as Tversky, 1977) or, if one prefers spatial metaphors, as closeness in some multidimensional space (e.g., Shepard, 1962a,b). This idea of similarity underlies many views of conceptual structure, three of which are summarized in **Table 1**. We briefly present these views and associated formal models with a focus on classification learning.

2.29.2.1.1 Classical view

The classical view of concepts takes a strict view of similarity: All items in a category must have a specific set of features. If an item has those features, it is in the category; if it does not, it is not. One can think of this as a definitional view of categories: The features are singly necessary and jointly sufficient for category membership. A triangle is any closed two-dimensional figure that has three straight sides. Any item that has all of those features is a triangle, and any item that does not have all of those features is not a triangle. In addition, the view includes the rule-based idea, in which items are classified as being in a category if they meet some rule, such as red, or red and large (e.g., Bruner et al., 1956). This view has a long history (see Murphy, 2002), and it matches many intuitions about category members sharing some common characteristics. However, because the classical view assumes all members possess the same set of common features, it does not explain why some category members are more typical than others (e.g., robins vs. penguins) or why it has proven so difficult for people to come up with a set of defining features for most categories (Wittgenstein, 1953, has a famous example of trying to define the category games). There are no current formal models that rely solely on a classical view.

2.29.2.1.2 Prototype view

The prototype view (or probabilistic view) keeps the attractive assumption that there is some underlying common set of features for category members but relaxes the requirement that every member have all the features. Instead, it assumes there is a probabilistic matching process: Members of the category have more features, perhaps weighted by importance, in common with the prototype of this category than with

Table 1 Similarity-based views of conceptual structure and their classification decisions

Classical	Unitary description: Definitional, rule-based	Classification: Category member if and only if all features are true of an item
Prototype	Unitary description (prototype): Probabilistic	Classification: Category member if more (weighted) features are true of the prototype than of other prototypes
Exemplar	No unitary description: Disjunctive representation	Classification: Category member if more similar to (weighted) category members than to members of other categories

prototypes of other categories (and perhaps some minimum match level is required). An early presentation is available in Rosch and Mervis (1975), with a more recent presentation in Hampton (2006).

This type of representation has major implications for how to think about categories. First, some members may have more of the prototype features than others, such as a robin having more bird prototype features than a penguin. People tend to judge robins as better examples of birds than are penguins and are faster to classify robins as birds than they are to classify penguins as birds. Second, this view suggests that the category boundaries, which were strict in the classical view, may be fuzzy, with some cases that are far from the prototype and maybe even almost as close to another prototype. For example, whales have many fish-like properties, and bats have many bird-like properties. Both are viewed as poor examples of the mammal category and are slow to be verified as members of that category. Overall, this view leads to a set of category members that tend to have a family resemblance – no defining features, but some features will be possessed by many members and some features by a few members (similar to an extended family). Rosch and Mervis (1975) argue that this type of family resemblance characterizes many natural categories. See the top half of **Figure 1** for a simple example.

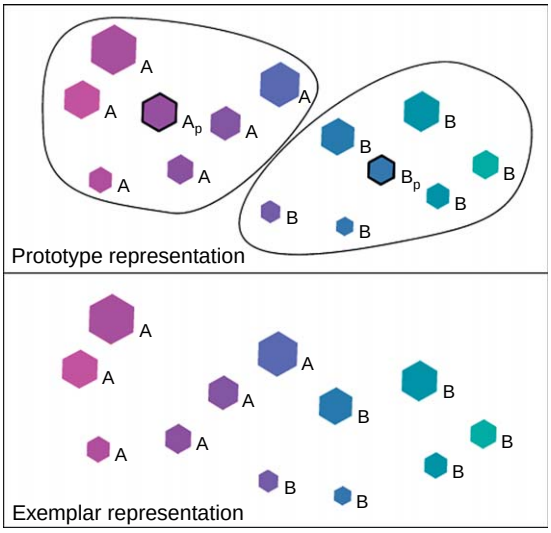

Figure 1 Sample prototype and exemplar representations. Members of Category A tend to be large and pink, whereas members of Category B tend to be small and turquoise. The prototype representation in the top panel also includes a prototype item with the bold outline and P subscript, which was not presented but represents the center of the presented items. (Although the prototypes here can be characterized as instances, more generally prototypes are summary representations.) The exemplar representations in the bottom panel include all individual category members, but do not group them or include a center.

Smith and Minda (1998, 2000; Minda and Smith, 2001) proposed a model of prototype-based classification that matches an item to the various prototype representations and picks the most similar (see also Hampton, 1993). Using the spatial distance idea of similarity, the prototype models (a) determine the distance from the test item to each prototype, (b) compute the similarity from the distance, and (c) choose the category prototype as a function of the similarity. There are several specific choices to make as to how to formalize these ideas. For the reader interested in formal models, we provide a simple one with additive similarity and just two categories in **Table 2**.

The learning of the category is the building of this summary representation. Exactly how the prototype is learned is not usually specified. One possibility, for simple cases, is to assume a simple associationist-like mechanism, with frequently occurring values being more reinforced. The point is that the earlier experiences are used to build the summary representation,

Table 2 Sample prototype model calculations (assuming *N* dimensions, city-block[a] metric, and two categories, A and B)

To calculate	*Formula*	*Comment*
(a) Distance of test item, *t*, to prototype, *P*	$d_{tP} = \sum_{k=1}^{N} w_k \lvert t_k - P_k \rvert$	w_k Weights each dimension *k* by attention (or importance)[b]
(b) Similarity of test item, *t*, to prototype, *P*	$S_{tP} = 1 - d_{tP}$	Additive similarity [c]
(c) Probability of choosing Category *A*	$P(A\lvert t) = \frac{S_{tP,A}}{S_{tP,A} + S_{tP,B}}$	Luce Choice rule over prototypes' similarities to the test item

[a]City-block metric is common when the dimensions' distances seem to be evaluated separately and then added. It is as if one is walking in a city and can turn only at the corners, rather than as the crow flies (Euclidean). It is used here as an illustration of one distance metric.
[b]The difference between the values of the test item and prototype on each dimension is summed, weighted by the attention given to that dimension. The sum of the weights is constrained to be equal to 1 ($\Sigma\, w_k = 1$) as a limited-capacity system (total amount of attention is limited).
[c]Similarity is assumed to be a linearly decreasing function of psychological distance.

which is then slightly revised with new experiences (in the same way one can keep various statistics of a sample as each new item is added).

2.29.2.1.3 Exemplar view

The idea of a summary representation is attractive, and we clearly know a lot about categories such as birds. However, an alternative view is that the knowledge we use in making category judgments such as classification is not this summary representation but the set of category members: The conceptual structure is a collection of the mental representations of category members. When an item is presented, the person matches the representation of the item to the various previous items' representations and uses some subset (perhaps even just one item) selected by their similarity to the current item's representation. Thus, to classify a hawk as a bird, one would retrieve some earlier representations of hawks or similar birds and note their bird category; to decide what it ate, one would again use these more specific representations.

We know this lack of a summary representation seems strange at first thought, but often a new item will remind one of some old item from which one can make a classification or prediction ("is this person like my friend Bill in other ways?"). The exemplar view is an extension of this idea to allow the use of specific instances to classify the current item. Imagine you see a new ostrich-like animal with some unusual bird characteristics. If you relied on the bird prototype, you might not classify it as a bird. However, if you used similar birds, then you would likely classify it as a bird, as well as predict it probably does not fly or eat worms.

This exemplar view accounts for the prototype-like effects mentioned earlier. Some category members, such as robins, will be viewed as more typical than others, such as penguins, because a robin will be similar to many birds (not just the large number of robins, but also sparrows, thrushes, etc.), whereas a penguin will be similar mainly to the few penguins one has seen. The uncertain cases will be exemplars that are not very similar to any items in a category and may even be a little bit similar to atypical items in another category (see **Figure 1**, lower panel).

There are a number of formal exemplar models, with the best known being the Context Model (Medin and Schaffer, 1978), which was extended in a number of ways by the Generalized Context Model (GCM, Nosofsky, 1986). Like the prototype model, exemplar models also use the similarity of the test item to each category, but because there is no summary representation, the similarity of the test item to the category is just the sum of the similarity of the test item to each of the items in the category. The other main difference from prototype models is that multiplicative, rather than additive, similarity is assumed. Multiplicative similarity means that the similarity is not a linear function of the psychological distance, but instead, very close items (ones that are similar to the test item on many dimensions) matter much more. More specifically, exemplar models (a′) compare the similarity of the test item to all items, (b′) compute the similarity of the test item to each category by summing the test item's similarity to each of the category members, and then (c′) choose the category as a function of similarity. The equations for one such model are presented in **Table 3** for those readers with an interest in formal models. Most exemplar models capture only classification performance, but ALCOVE (Kruschke, 1992) extends the GCM to allow some

Table 3 Sample exemplar model calculations (assuming *N* dimensions, city-block[a] metric, and two categories, A and B)

To calculate	*Formula*	*Comment*
(a′) Distance of test item, *t*, to exemplar, X_j	$d_{tj} = \sum_{k=1}^{N} w_k \lvert t_k - X_{jk} \rvert$	w_k Weights each dimension *k* by attention (or importance)[b]
(b′) Similarity of test item, *t*, to exemplar, X_j	$S_{tj} = \exp(-c \cdot d_{tj})$	Multiplicative similarity,[c] c indicates sharpness of generalizations
(c′) Probability of choosing Category *A*	$P(A\lvert t) = \frac{\sum_{i \in A} S_{ti}}{\sum_{i \in A} S_{ti} + \sum_{i \in B} S_{ti}}$	Luce Choice rule over exemplars' similarities to the test item

[a]City-block metric is common when the dimensions' distances seem to be evaluated separately and then added. It is as if one is walking in a city and can turn only at the corners, rather than as the crow flies (Euclidean). It is used here just as an illustration of one distance metric.
[b]The difference between the values of the test item and prototype on each dimension is summed, weighted by the attention given to that dimension. The sum of the weights is constrained to be equal to 1 ($\Sigma\, w_k = 1$) as a limited-capacity system (total amount of attention is limited).
[c]Multiplicative similarity is not a linear function of psychological distance. Rather, close items (small distance) have much greater effect than in a linear function. The exponential function is commonly used and has a quick drop-off with distance. (Note that the exponential of the sum of distances across each dimension is equal to multiplying the exponential of each dimensional distance, since $\exp[a+b] = \exp[a] \cdot \exp[b]$.)

learning. In addition, there is no learning of summary representations, because only individual items need to be stored.

2.29.2.2 Evaluations of Prototype and Exemplar Models

There have been many (many) comparisons of prototype and exemplar models (for a review, see Murphy, 2002). The general result is that exemplar models do as well or better than prototype models in most cases (though see Smith and Minda, 1998, 2001). Our view, summarizing over many results, is that the advantage is largely a result of two factors: similarity calculation and selectivity (Ross and Makin, 1999). First, the exemplar models' multiplicative similarity (compared to the prototype models' usual assumption of additive similarity) means that the model does not combine features independently but, rather, is sensitive to the relational information among the features (e.g., Medin and Schaffer, 1978). The combination of features is being used beyond their separate contribution to determining classification. Thus, if one encountered small birds that sang and large birds that squawked, a prototype representation would not be sensitive to that particular relational (cooccurrence) information, whereas an exemplar model would. Although it is not a usual assumption, prototype models might also incorporate multiplicative similarity (e.g., Smith and Minda, 1998); this helps the fit, but it does not mimic the predictions of the exemplar model. (Multiplicative similarity is a nonlinear function, so calculating multiplicative similarity on the mean (prototype) is not the same as the mean of multiplicative similarities on the individual instances.) In fact, exemplar models implicitly keep all the statistical information (e.g., frequency, variability, cooccurrence) by keeping all the exemplars. One might argue that a prototype model could also keep various statistical information around to make it equivalent to such exemplar models (e.g., Barsalou, 1990), but no one has proposed such a formal model. Second, because exemplar models have no summary representation, the same knowledge is not used for all the different decisions. Thus, even unusual items can be classified by similarity to earlier unusual similar members. The ability to use different knowledge for different decisions means that the exemplar model can classify unusual items without compromising its ability to easily classify more typical items. This flexibility is important in allowing the exemplar model to fit a variety of classification data.

The exemplar model fits the data well for many classification experiments but has difficulties with other aspects of category-related judgments. One major issue is that it has no place for these summary representations that we all find attractive in thinking about concepts. In particular, to answer the question as to why these items are all members of the same category, the exemplar view is left with the unsatisfying answer that "they all have the same category label." That may be fine for arbitrary experimenter-defined categories in the laboratory but seems woefully inadequate for permutation problems, extroverts, and love triangles. (Note that the classical view can point to a definition and the prototype view to similarity to some common summary representation.)

2.29.2.3 Combined Models

It seems likely that people are not restricted to a single means of representation and that we might combine advantages of prototype and exemplar models, or at least general and more specific knowledge. We consider two types of proposals. First, one could take a prototype model and an exemplar model and simply combine them with some means of determining whether a decision would be based on the prototypes or exemplars. Smith and Minda (1998) show that a combined model can provide a better account of the data, with the prototype being more influential earlier, when each item to be classified has been presented only a few times, and the exemplar model controlling responses more as the same items are presented often. They do not specify a control mechanism, but perhaps the model that has greater confidence in its choice might determine the classification.

Second, there have been models that build upon simple models with a more integrated approach. Interestingly, rules, which can be viewed as simple classical models, are making a comeback: They appear to work better as part of the answer rather than the sole answer. ATRIUM (Erickson and Kruschke, 1998) combines simple rules with an exemplar model, ALCOVE (Kruschke, 1992). The authors provide experimental evidence showing that both types of knowledge can influence a task and then address how the two types of knowledge might be integrated to provide an account of the data. All inputs are examined by both the rule and exemplar modules, with the response a weighted function

determined by how much attention is given to each. The model learns to shift attention between the modules as a function of which module is better at classifying particular inputs. (Also see the RULEX model by Nosofsky et al., 1994.)

A very different combined model, COVIS, has been proposed by Ashby and colleagues (Ashby and Ennis, 2006, present an overview). Human category learning is assumed to be mediated by a number of functionally distinct neurobiological systems, and the goal is to elucidate these systems and their behavioral consequences. An explicit system is important for rule-based tasks – those tasks with a focus on a single dimension for which people might generate and test hypotheses. Another, procedural-based, system deals with information-integration tasks that require combining information from multiple dimensions. This model combines rule- and procedural-based knowledge to account for a variety of behavioral results and data from neuropsychological patients.

2.29.2.4 Evaluation of Work on Conceptual Structure

2.29.2.4.1 Successes

The separation of different views of conceptual structure has generated much research. The prototype view has greatly extended our understanding of natural categories. The formal modeling on the exemplar approach has shown that exemplar representations coupled with multiplicative similarity are able to account for a wide variety of classification results. The more recent prototype modeling work shows that some findings that seemed problematic for prototype models may not be, though the exemplar model still seems to have an edge on overall accounts of the results. Although almost all of the exemplar work has focused on learning artificial categories, some recent work suggests the exemplar models may fit some real-world categories as well (reviewed in Storms, 2004).

2.29.2.4.2 Limitations

We label this part of the evaluation 'limitations' because the difficulties are not failures but restrictions. The simple point, to be elaborated in the next section, is that the field has examined only a small part of the picture for conceptual structure and learning. Thus, although the prototype and exemplar approaches and models have been explored extensively, especially in the laboratory, our understanding of concept and category learning may be quite limited.

First, almost all the work on adult category learning until a decade ago focused on classification learning, how people learn to assign items to specified categories. We learn concepts and categories in many ways – such as by interactions, inferences, problem solving, instruction – yet these have received little attention in research on category learning. Not only is much of the laboratory work limited to classification learning, it has rarely varied from a small range of particulars (here is an incomplete list): two categories, small number of features, small number of values per feature, small number of items per category, and divorced from any prior knowledge. Given all the possible ways that even the classification paradigm might be done, these seem very limiting. Of course, it is possible that all the ways of learning and all the possible ways of changing the classification paradigm will not matter in terms of our understanding of category learning, but the evidence suggests just the opposite. As elaborated in the next section, it appears that many of these changes lead to important differences in what is learned.

Second, the items being learned have been limited. In addition to the ways mentioned, almost all have been objects (or descriptions of objects), with little examination of problems, people, situations, scenes, and so on. In addition, the items in most experiments have generally consisted of features only, with no relational structure beyond cooccurrences. None of the main classification theories developed in the exemplar-prototype debates allow relations in their item representations. Given that real-world categories have much relational structure, as well as much prior knowledge, it is unclear how well these findings will relate to more complex cases.

2.29.3 Beyond Classification and Featural Representations

In this section, we consider some recent work that begins to address these limitations. First, research has extended concept and category learning from a focus on classification to consider other means of category learning. Second, we consider two formal models that were designed to examine category learning beyond classification, the Rational Model and SUSTAIN. Third, we review some work that has gone beyond representations of features to ask how more complex categories might be learned, including the influence of prior knowledge. Finally, we consider how far this

new research has gone in providing a resolution, or at least a partial resolution, to these limitations.

2.29.3.1 Category Learning Beyond Classification

As we outlined at the beginning, concepts and categories have many functions, of which classification is just one. Classification is an important one: By determining what category an item is in, one has access to much relevant knowledge about that item. However, the near-exclusive focus on classification learning in laboratory experiments is problematic for two reasons. First, if we learn categories in multiple ways, it seems prudent to examine more than classification learning to get a full understanding of category learning. Second, classification learning has an important difference from most other conceptual functions. In classification learning, the goal is to determine what category the item is in. This requires figuring out what distinguishes the competing categories. However, most other functions, such as prediction, understanding, or communication, require using what you know about a particular category, with the other categories often not mattering at all. That is, classification requires distinguishing between categories, but most other functions require within-category knowledge. For example, Chin-Parker and Ross (2004) found that classification learning led to learning only about diagnostic features (those that are predictive of category membership). People learned nothing about the other features that were not predictive of a category, even though they occurred 80% of the time in both categories. This result is exactly as predicted by classification theories, such as the exemplar models mentioned earlier (e.g., Nosofsky, 1986). However, if one is predicting what a new animal eats, it requires knowing more than what type of animal it is; one also needs to know what food is eaten by animals of that type. If one is solving a math problem, knowing the type of problem is helpful only if it allows access to relevant information about how to solve problems of that type.

A number of laboratory tasks have been examined over the last 10 years that extend our understanding of category learning by examining types of learning other than classification. These tasks emphasize how categories allow us to accomplish the goals we have: Predict, solve problems, explain. We focus on three tasks here: Inference learning, in which the classification is provided; category use, in which the learner uses the category to learn some other task; and an unsupervised learning, in which no category information is provided. We give a rough outline of their procedural differences from classification learning in **Table 4** and a rough outline of the processing in **Figure 2**.

First, one can learn about categories by inferring features of category members. Inference learning is a

Table 4 Simplified procedures for various category-learning laboratory tasks

Classification	An item is presented Subject responds with one experimenter-defined category label Feedback given on classification Next item is presented
Inference	An item (one feature missing) is presented, along with category label Subject responds with value of missing feature Feedback given on inference Next item is presented
Category use	An item is presented Subject responds with one experimenter-defined category label Feedback given on classification Subject uses category and item to do some task (inference, problem solve) Feedback given on second task Next item is presented
Unsupervised[a] (using Minda and Ross, 2004, to be specific on procedure)	An item is presented Subject uses item to do some category-related task (prediction) Feedback given on task Next item is presented

[a]Note: no mention made of category, but category is useful for prediction.

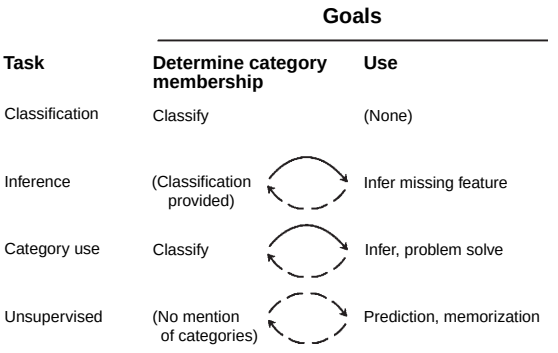

Figure 2 Schematic to illustrate the relation among the different laboratory tasks used to study category learning.

paradigm that can be compared to classification directly: Rather than presenting *N* features and asking what category the item is in, the label is given along with *N*−1 features, and the person's task is to supply the missing feature (Yamauchi and Markman, 1998). Suppose there is an item in Category A that has three features; we can characterize this item as {A a1 a2 a3}. In a classification trial, the learner would be provided with {? a1 a2 a3} and have to provide the label A, whereas in an inference trial, the learner would get {A a1 a2 ?} and have to provide the missing feature, a3. The procedure is presented in **Table 4**. This task matches a common means of interacting with categories in which one knows the category and parts of the item but has to infer a missing feature: Given this dog, what will it eat? Or given this type of situation, what will happen next? This task appears to be very similar to classification learning but leads to rather different learning. In inference learning, people learn prototypical features that are not diagnostic (Chin-Parker and Ross, 2004) and features that vary in their exact presentation (Yamauchi and Markman, 2000). Focusing within a category leads people to learn what the category is like, not just what distinguishes it from another category (Yamauchi and Markman, 1998; see Markman and Ross, 2003, for a review; Johansen and Kruschke, 2005, for counterarguments). However, if one knows what a category is like (e.g., dogs, permutations), one can often tell if an item is a member of the category as well. In addition, inference learning, by focusing on what the category members are like, may also help people to understand the underlying coherence of relational categories (Erickson et al., 2005).

Second, other work has considered classification as part of a larger goal-related task and asked how category knowledge might be influenced. The rationale is that classification is not usually the goal; usually we want to do something with the classification. We classify an object as a pencil and use it to write, a person as a psychopath and stay away, a math question as a permutations problem and apply the appropriate formula. Ross (1997, 1999, 2000) has conducted research with a category use paradigm in which one not only classifies the item but also then uses the item to perform some goal-oriented task, such as make an inference about the item or solve the problem (see **Table 4** and **Figure 2**). In these studies, the uses of the category (e.g., inference or problem solving) influence the category representation, even for later classification. Performance on later classification tests is not a function just of the diagnosticity of the features but also of their importance for the use. Those features involved in the inference, for example, are viewed as more central to the category than equally predictive features that are not involved in the use. To be more concrete, Ross (1997) had people learn to classify patients (sets of symptoms) into two diseases and then choose the treatment to give that patient (which depended on both the disease and the symptoms). Two symptoms were equally and perfectly predictive of the disease, but only one was also predictive of the treatment. After learning, people were given a single symptom and asked which disease they would think a patient had if this was all they knew of the symptoms. Although both symptoms were perfectly predictive of the disease, learners were much more likely to correctly classify the one that was also predictive of the treatment. Even when we explicitly classify items, we continue to learn about the category from other nonclassification uses, and this knowledge influences a variety of later category-related judgments, including classification. Related results have been found with problem solving (Ross, 1997, 1999), and even with young children (Hayes and Younger, 2004; Ross, et al., 2005).

The goals for which categories are used during learning can have additional consequences that may influence the representation of real-world categories. Brooks et al. (2007) argue that tasks in which classifications are used for a different primary task may lead to a very different learning of the classification knowledge than classification alone. In a clever set of experiments, they show that the other task diverts attention away from analyzing the category structure for classification, such that learners believe the category has defining features when it does not. When we divert our resources to using the category, the

knowledge learned from the classification may be a less central part of our category representation.

Third, we can look at cases in which people are not even told there are categories. If we have a helpful teacher/parent, we may get feedback on the category membership (classification) or labeled items (inference), but in many cases, such as much of our informal learning, we may not. In unsupervised learning tasks, the categories are not provided to the learner (e.g., Ahn and Medin, 1992; Heit, 1992; Wattenmaker, 1992; Clapper and Bower, 1994; Billman and Knutson, 1996; Kaplan and Murphy, 1999; Clapper and Bower, 2002; Love, 2003). Here, the particular interactions people have with the items may affect later formation of categories (e.g., Lassaline and Murphy, 1996). Many of these unsupervised tasks focus on the items and the categories, often through observation, memorization, or sorting. When there is a more goal-oriented learning task, such as predicting a critical feature or solving a problem, any learning about the categories or items is incidental to attaining the goal. Minda and Ross (2004) found in a prediction task (where category membership was crucial to the prediction) that the unsupervised learning led to paying more attention to a wider set of features than did classification learning.

In sum, although classification learning has been the dominant paradigm for studying category learning, including other tasks may provide a more complete picture of category learning. Classification is a critical function of categories, but it is critical because it provides access to knowledge about the item that can be used to infer, predict, understand, or explain. Classification learning promotes learning what distinguishes the categories, whereas these other functions of categories tend to promote learning what each category is like.

2.29.3.2 Formal Models That Do More Than Classify: Rational Model and SUSTAIN

Most category-learning models have focused on classification learning, but a few have considered other category functions. We consider two prominent models, J. R. Anderson's (1990, 1991) Rational Model of categorization and SUSTAIN (Love et al., 2004), with an emphasis on learning mechanisms and extensions to multiple functions of categories.

2.29.3.2.1 The Rational Model of categorization

The Rational Model is a component of Anderson's (1990, 1991) rational analysis of cognition. The central claim of the model is that categories serve as a basis for prediction. Classification is a specific type of prediction in which the task is to predict the category label. The model stores clusters of exemplars with similar properties. The primary goal of the model is to develop clusters that optimize the accuracy of a variety of category-based predictions.

Predictions in the model are based on conditional probabilities. The probability that a new exemplar with a set of properties F has property j (on dimension i) is:

$$P_i(j|F) = \sum_k P(k|F)P_i(j|k), \qquad [1]$$

where $P(k|F)$ is the probability that an item belongs to cluster k given that it has properties F, and $P_i(j|k)$ is the probability that an item in cluster k has property j on dimension i. The idea behind equation 1 is that predicting a missing property involves the summing of probabilities across multiple clusters, with the influence of each cluster weighted by its probability, given the new item. The sum of these values across all clusters is the probability that the new item has property j.

Clusters are learned that optimize the accuracy of predictions. The first exemplar forms its own cluster, and each added exemplar is either placed into an existing cluster or a new one that contains only itself, whichever maximizes within-cluster similarities. Thus, a new item that is dissimilar to the earlier items is more likely to begin a new cluster. The probability that a new item will be placed into cluster k depends on (1) how similar the item and cluster features are and (2) the size, or base rate, of the cluster, with the item more likely to be placed into a cluster that represents more items.

Two aspects distinguish this model from the classification models described earlier. First, clusters differ from prototype and exemplar representations in that the goal of clustering is more abstract: to capture statistical structures in the environment suitable for making predictions. Interestingly, clusters sometimes mimic each of the other approaches (cf. Nosofsky, 1991), suggesting that aspects of both prototype and exemplar representations are useful.

Second, the model predicts features as well as category labels, so it can be used for nonclassification tasks, such as inference learning (see Yamauchi and Markman, 1998). In addition, it provides an explanation for how people might induce a missing feature of an exemplar when the category is unknown. Suppose

that you hear an animal rustling behind a bush, and you think it is probably a dog but possibly a raccoon. What is the probability that the animal barks? In terms of equation 1, dog and raccoon are different values of *k*. Murphy and Ross (1994, 2005; Malt et al., 1995; Ross and Murphy, 1996) tested this hypothesis in a large number of studies and usually found evidence against it. Their work shows that people instead tend to base predictions on the most likely category only (dog in the previous example). Although the Rational Model was not supported, it provided a strong alternative view and has led to a consideration of when single categories and multiple categories might be used for predictions.

2.29.3.2.2 SUSTAIN

SUSTAIN (Supervised and Unsupervised STratified Adaptive Incremental Network; Love et al., 2004) is a network model of category learning that shows great flexibility compared with prototype and exemplar models for two main reasons. First, like the Rational Model, it seeks to build clusters that capture regularities or structure in the environment. Second, unlike the Rational Model, this search for structure is guided by the goals of the learner. This goal-oriented learning allows the model to account for a wide variety of category learning results beyond classification.

Categories in SUSTAIN are represented by clusters. Unlike the Rational Model, these clusters are not collections of exemplars but, rather, summary representations of encountered exemplars. The clusters formed are influenced by the goals of the learner, such as increased attention to the feature (including category label) being predicted and the features most relevant for this prediction. The details of the model are complex, so we outline the main steps of performance and learning here, then turn to a simple example.

The performance, such as prediction of a feature, relies upon the clusters. The item is compared to each cluster, and the most similar cluster determines the prediction. The summary representation of each cluster includes a distribution of expectations for each dimension (e.g., how likely the different values are to occur). The item's features are compared to these various distributions, with selective attention occurring through the tuning of receptive fields on each dimension (akin to visual receptive fields that are, for example, sensitive to a small range of orientations at particular locations). The activation of the cluster increases with the similarity of the item's features to the summary representation (weighted by the selective attention weights). The different clusters can be thought of as trying to explain the input for the particular goal, with lateral inhibition among the clusters leading to a winning cluster that determines the output. Thus, unlike the Rational Model that sums over all the clusters (see equation 1), only the most likely cluster is used to determine the prediction (consistent with the Murphy and Ross, 1994, results).

SUSTAIN is biased toward simple category representations (i.e., few clusters) but does develop more elaborate clustering schemes for complex stimulus sets. Learning involves updating old clusters and developing new ones. In supervised learning, if the correct prediction is made, SUSTAIN will compare the output of the winning cluster to the target response and make small adjustments in receptive field tunings and summary representation values in the direction of the target values, if needed. These changes will lead to a repetition of the item producing a cluster output closer to the target values. If an incorrect prediction is made, a new cluster is created that is centered around the item. (In unsupervised learning, a new cluster is formed if the current item is not sufficiently similar to any existing cluster.)

This explanation is a bit abstract, so we illustrate with a simple example. Imagine there is an object that can be described by three binary features: shading (filled = 0, empty = 1), color (blue = 0, red = 1), and shape (circle = 0, triangle = 1). Thus, we can represent an (empty red circle) as (1, 1, 0). Suppose this was the first item presented, then Cluster 1 (CL1a) would simply be (1, 1, 0) as seen in **Figure 3**. Now suppose the second item was (empty red triangle), item (1, 1, 1), and it was similar enough to be put in the same cluster. The updated Cluster 1 (CL1b) would be adjusted to be (1, 1, 0.5). The last value does not represent a triangular circle but, rather, an increased probability that a new item represented by that cluster will be a triangle. If the third stimulus (empty blue circle), (1, 0, 0), is not sufficiently similar to CL1b (unsupervised learning) or does not predict the correct response (supervised learning), the model recruits a new cluster CL2a to represent that item, as shown in the figure.

Recall that SUSTAIN prefers simple cluster sets when possible. To demonstrate, if the stimuli in **Figure 3** were divided by shape into two categories, it is likely that SUSTAIN would develop two clusters, each at the center of the front and back face of the cube, (0.5, 0.5, 0) and (0.5, 0.5, 1). These would indicate a high probability of circle//triangle for the front/back cluster and intermediate probabilities for other dimensions. This is a simple clustering solution, because it strongly emphasizes just one feature.

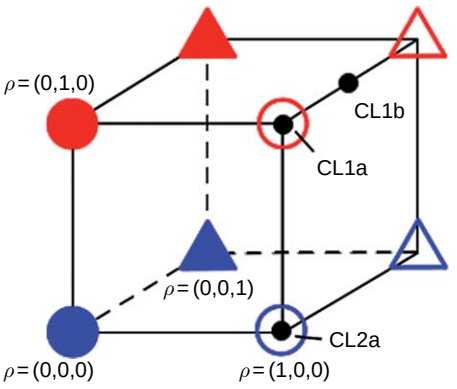

Figure 3 Binary-valued stimuli to illustrate working of SUSTAIN. Coordinates are shading (0 = filled, 1 = empty), color (0 = blue, 1 = red), and shape (0 = circle, 1 = triangle). Black dots represent clusters CL1a, CL1b, and CL2a following the presentation of items (1, 1, 0), (1, 1, 1), and (1, 0, 0), respectively.

Like the Rational Model, the clusters in SUSTAIN adapt to their learning environment, but they are also sensitive to the goals of the learner (e.g., Barsalou, 1985; Solomon et al., 1999; Medin, et al., 2006). In supervised learning, new clusters are created in response to an incorrect decision, allowing the model to adjust to specific learning criteria. This differs from clustering in the Rational Model, where new clusters are created in response to exemplars that are generally dissimilar to existing clusters.

SUSTAIN's ability to adapt to different category functions is also seen in comparing classification and inference learning. Using a family resemblance structure (where all items are similar to a prototype for the category), Love et al. (2000) found that inference learning often led to a single cluster (i.e., close to the prototype), whereas classification tended to create several clusters per category, indicating the use of simple rules and memorization when classifying. (See also Love and Gureckis, 2005, for a related application to a real-world difference in goals.)

In summary, SUSTAIN focuses on the relationship between learning goals and category structure. In a sense, SUSTAIN generalizes the contributions of the Rational Model by proposing that, in addition to capturing the structure of the environment, categories also capture a learner's goal-specified relationship with the environment.

2.29.3.3 Beyond Featural Representations

Much of the current work on category learning is limited not just in how the categories are learned but also in what is learned. The categories learned in these studies are different from real-world categories in two important ways. First, the category structure is feature-based with only very simple relations among the features. Most, if not all, real-world categories have much more complex structures, and relations are essential. Second, the materials are usually devised to make as little contact with any world knowledge as possible, to allow an unconfounded examination of category learning. However, the learning of most (and perhaps all) real-world categories is influenced by our world knowledge: By minimizing the influence, we may be ignoring a major influence in the learning. We discuss each of these ideas with a brief examination of relevant models.

2.29.3.3.1 Relational information

Most of the categories studied in experimental settings with adults consist of a small number of features (usually 2–5), with some of the features predictive of each category. These simple structures, cleverly designed, are often sufficient to provide tests of particular aspects of current formal models. The underlying (implicit) assumption is either that real-world categories have such structures (which they do not) or that the learning principles derived from studying such simple structures will apply to learning more complex structures. This latter possibility remains feasible, though we present evidence throughout this section suggesting that there are many important differences. The main point we wish to make here is that the category structures that have typically been examined are a very small subset of the possible structures and do not have much to do with real-world category structures (see Murphy, 2005).

Let's take a simple example of relational structure, within-category correlations. We can classify an item as a bike using features such as handlebars, tires, and such. Bicycle handlebars include ones that are either dropped or straight, and bicycle tires include ones that are knobby or slick. However, these are not independent features: Dropped handlebars and slick tires usually go together (racing bikes), as do straight handlebars and knobby tires (mountain bikes). These within-category correlations are common in many real-world categories: They do not add to the category predictiveness of the features, but they are important as signals to additional category structure, such as subcategories. When within-category correlations have been examined in laboratory studies of classification learning, they do not appear to be

learned easily, if at all (Chin-Parker and Ross, 2002). This difficulty is generally consistent with classification learning models, because the within-category correlations do not improve the classification predictiveness. Despite this unanimity of classification models and laboratory classification learning data, people do learn within-category correlations in the world. They even learn it in the laboratory when given inference learning (Chin-Parker and Ross, 2002) or when prior knowledge promotes the correlation (Murphy and Wisniewski, 1989).

The point is that as one moves to more complex relations among features, we know little about category learning. Of course, the structure of real-world categories is far more complex than a single within-category correlation: Imagine the structure for carrots or permutations. The dominant classification models are all feature based and do not allow for complex relational information (though see Pazzani, 1991). The problem is that adding relational information greatly complicates models and brings a host of additional issues that need to be addressed (e.g., Hummel and Holyoak, 2003).

2.29.3.3.2 Knowledge

What people know affects what they learn: Cricket fans gain much more from watching a cricket match than do those of us who know nothing about the game. Not surprisingly, the effect of knowledge is also true in category learning: Knowledge can have large effects on the ease of learning as well as what is learned (see Murphy, 2002: chapter 6, for a review). Continuing the example on within-category correlations, knowledge has a large effect. Murphy and Wisniewski (1989) had people learn categories containing within-category correlations. For example, their materials included correlated features that were conceptually related (for a clothing category: worn in winter, made of heavy material) or not (blue, machine washable). They found that in the absence of knowledge relating the features, classification learners did not learn these within-category correlations. However, in the condition in which knowledge related the features, classification learners did show sensitivity to the correlations. Ahn and her colleagues argue that there are so many possible correlations in the world that people cannot notice all of them, so people use their knowledge to notice correlations that are meaningful to them (e.g., Ahn et al., 2002; though see McRae, 2004).

We mention briefly three other effects of knowledge on learning. First, the learning of new categories that are consistent with prior knowledge is greatly accelerated compared with learning unrelated or inconsistent categories. For example, Wattenmaker et al. (1986) showed that categories consisting of items whose features related to a theme such as honesty (e.g., returned the wallet he had found in the park) were faster to learn than categories whose items had unrelated features. Second, even learning to classify items into categories consistent with knowledge but for which a prior concept is unlikely to be available (e.g., arctic vehicles) is faster than learning to classify into unrelated categories (e.g., Murphy and Allopenna, 1994). Third, the learning influence of prior knowledge does not restrict learning to only those features related to the prior knowledge, but generally, those are learned more quickly than the unrelated features of the items (e.g., Kaplan and Murphy, 2000).

How does knowledge influence category learning? Wisniewski and Medin (1994) suggest three important possibilities (also see Heit and Bott, 2000). First, knowledge might weight or select features of the item. Second, knowledge might allow one to infer additional, relevant features. Third, although these first two possibilities view knowledge as independent of the learning process, knowledge and learning may be more tightly coupled or interactive. Learning may influence the activated knowledge, which may then influence later learning. As one example, learners might begin to change how they interpret some features as they see how the features relate to their goal. The main point of this work is that knowledge and concept/category learning cannot be thought of separately: Our knowledge of particular concepts is intimately intertwined with other knowledge, and we use that other knowledge both to help learn new information about concepts and that this learning in turn may influence our other knowledge.

Despite this large influence of knowledge on category learning, most category learning research has examined cases in which knowledge influences are minimized (Murphy, 2005). If there are interactions between the influences of knowledge and the learning, some (unknown) part of what we learn from examining category learning in the absence of knowledge may not be applicable to the cases in which knowledge is used. In addition, there may be learning processes with knowledge that are not required in the knowledge-free classification learning experiments.

There has been some progress in considering how to account for the influence of prior knowledge on category learning in a more general way, including

Heit's Baywatch model (Bayesian and empirical learning model, e.g., Heit and Bott, 2000) and KRES (Knowledge-Resonance model; Rehder and Murphy, 2003). Space precludes much description of these complex connectionist models, but both models add nodes to represent prior knowledge in addition to the usual ones to represent the features of the items. The presented item activates its features but also activates prior knowledge that it is related to, providing an additional source of activation for the category decision. The models account for a wide variety of data. For example, KRES predicts that the learning of categories consistent with prior knowledge is accelerated compared with unrelated categories, and even features unrelated to the prior knowledge are learned. In addition, this model incorporates the interactive view between knowledge and learning by the learning influencing the connection weights between features and between features and prior knowledge.

These models of prior knowledge do not include relational representations. Relational models are beginning to be developed (e.g., Hummel and Ross, 2006; Kemp et al., 2006; Tomlinson and Love, 2006) but need also to address the pervasive influences of prior knowledge.

2.29.3.4 Directions for Providing Integration

We have considered some limitations of the current category learning work both in terms of the learning tasks and in terms of the featural representations. Different means of learning about categories provide a variety of knowledge about the category not just for classification but also to support all the category-based cognitive activities. In addition, the knowledge about a category has to be intimately related to our other conceptual knowledge to be useful. What does this suggest about concept and category learning?

A main lesson that we have taken from a consideration of these various limitations is that the study of concepts and category learning needs to be integrated into other areas of cognition. Conceptual knowledge needs to support many cognitive activities, not just classification, and examining these category-based activities across a variety of domains will both point out places in which we need to further our understanding of category learning and help to make the work on category learning more relevant to other areas of cognition. Much of our learning depends on our goals, so considering more than category learning is an important part of ensuring this integration.

Murphy and Medin (1985), in a seminal paper, proposed that the study of conceptual structure could not rely on similarity to explain why objects might cohere, or go together, in a category. Although similarity might be a useful heuristic in some cases, it is too unconstrained to provide an explanation of category coherence. They proposed that the coherence of the category depended on its fit to people's prior knowledge – the naive theories people have. This proposal changes the idea that the category members are similar to that they have some similar underlying rationale. Their internal structure is defined not just by features but also by relations connecting features. In addition, their external relations are critical: They must relate somewhat consistently with other knowledge the person has. This view is often called the theory view to make clear that it views category coherence as depending upon people's theories, not simple similarity.

This proposal has had a major influence on how conceptual structure is thought about and investigated. It was instrumental in leading to much of the work on how knowledge influences category learning. We mention two interesting illustrations. First, Wisniewski and Medin (1994) gave subjects a set of children's drawings of people and asked them to provide a rule for each category; they were told either that one group of drawings was from creative children and the other from noncreative children or, for other subjects, that one group was from city children and the other from farm children. The rules generated were very different, picking up on aspects of the drawings that were consistent with some ideas of those types of people. For example, one feature was seen as a pocket, indicating detail, when it was from the drawings by creative children, but was seen as a purse when it was from drawings of city children. Second, Ahn and Kim (2005; Kim and Ahn, 2002) have examined clinical psychologists' understanding of various psychodiagnostic categories (such as depression or anorexia). Although the training they receive emphasizes a prototype representation (classification is often in terms of some criterial number of features being present), the clinical psychologists often apply their causal theories of the disorders to help diagnose and determine treatments. Thus, this theory view has led to a wealth of interesting research relating prior knowledge to concept learning. The main shortcoming of this view is the

lack of specific details on how knowledge influences learning.

There are likely to be many cases in the learning of complex categories in which the constraints imposed by theories are not sufficient even for classification. For example, in learning to classify members of many real-world categories, there may be hundreds of potential features that could be important for determining category membership, so what determines which features people use? Knowledge may reduce the number of likely features and relations but still leave too large a number to consider. One possibility is that the importance of the features and relations for the overall goal of the task may provide a heuristic as to which ones are important for classification. For example, one might not be able to tell how to classify a particular math problem, but as one gets experience solving problems of that type, those aspects of the problem critical for solution may provide a good clue as to how to classify future problems. The comparison to other category members with respect to these useful features may also help to lead to a deeper understanding of why the problem is solved in this way.

It is important to clarify this suggestion and make clear how it relates to the general integration goal of this chapter. Concepts and categories support a variety of functions. The usefulness of this knowledge across the different functions provides constraints that one cannot get from a single function. In addition, the changes to the representations as one both uses and gets feedback on one function provides knowledge that can be used for other functions. For example, learning to classify complex items, with many features and relations, is a very difficult task if one relies only on feedback from the classification (which may be why classification learning experiments typically use few features and values). However, if these same categories are used for inferences or problem solving, that use provides suggestions as to what features and relations one might consider. Similarly, background knowledge can be used to help focus on relevant features and relations or even to learn new features that are important for later classifications (e.g., Wisniewski and Medin, 1994). The apparent difficulty of learning complex concepts is partly a result of thinking about it as some isolable process that relies only on classification and feedback on the classification. People have many sources of information from both the various interactions with the items and their prior knowledge to help in learning new concepts and categories.

2.29.4 Integrating Concepts and Categories into Cognition

We have been arguing throughout this chapter that it is important to think about concept and category, learning more broadly to integrate it into the many cognitive activities in which they play such a critical role. In this section, we illustrate this possibility by examining concepts and categories in two very different areas, problem solving and language.

2.29.4.1 Problem Solving

Categories play a critical role in human problem solving. Being able to correctly classify a problem as a permutations problem allows you to recall and apply the appropriate formula. In this section, we describe how category knowledge can influence various aspects of the problem-solving process, how problem categories change with experience, and how problem solving can affect the category representation.

Most models of problem solving consist of some version of the following five stages: (1) problem identification and creating a mental representation of the initial problem state and goal; (2) identifying and selecting a set of operators, procedures, or strategies to make progress toward that goal; (3) applying those operators and generating a solution; (4) assessing whether the solution satisfies the goal; and (5) storing the solution with other knowledge about the problem/category (Newell and Simon, 1972; Bransford and Stein, 1993; Pretz et al., 2003).

Categories impact all aspects of this process and are especially critical in the early stages (**Figure 4**). The process of problem identification is a classification that determines whether or not the current problem is like other problems encountered in the past. After the problem is classified, the problem solver can then recall and apply a set of procedures, strategies, or rules to solve the problem, such as recalling the appropriate formula for a permutations problem. Category knowledge may also be helpful in later stages of problem solving, such as evaluating whether or not a potential solution satisfies the known constraints of the problem type.

The problem goal is also important. Since category knowledge is used in the service of accomplishing some particular task, knowing how the goal relates to the problem features is a critical part of understanding the problem and identifying the appropriate solution procedures to solve it. As an illustration,

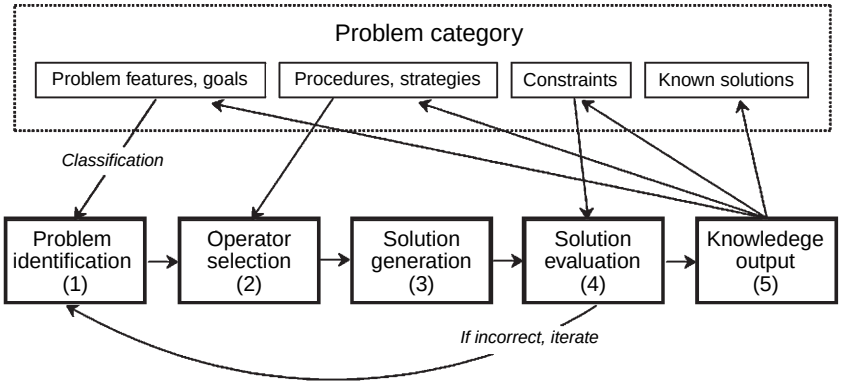

Figure 4 Five stages of problem solving (lower boxes) and relation to knowledge of problem categories (upper box). Arrows connecting boxes in the lower portion of the figure represent the general order of processing. Arrows connecting boxes between the upper and lower portions represent interactions between problem solving and category knowledge.

imagine that, as you are ready to drive to work, you notice the lights were left on, and the car does not start. If you classify the situation as a dead car battery, you can search your relevant category knowledge, perhaps recalling that one solution is to use jumper cables and ask your next-door neighbor for a jump. However, if your primary goal was to get to work on time, you might access other relevant knowledge such as calling a friend for a ride. For categories to be helpful for problem solving, they must go beyond simple diagnostic features and include information about the problem or goal procedures or strategies to accomplish that goal, and knowledge to evaluate the solution.

2.29.4.1.1 Differences between expert and novice category representations

Much research has shown that experts categorize problems with the principle or structural features of the domain, whereas novices rely on using the surface features (Larkin et al., 1980; Bedard and Chi, 1992). Chi et al. (1981) found that when physics experts were asked to sort a set of problems into those that could be solved similarly, they sorted on the basis of the underlying physics principles, such as Newton's Second Law, whereas novices sorted on the basis of the surface characteristics, such as problems with inclined planes. The physics experts had learned to associate the deep principles with the problem features and could take advantage of this knowledge for problem categorization.

The finding that experts use the deep principles of the domain to classify and reason about novel problems has been shown in many other domains including chess (Chase and Simon, 1973; Gobet and Simon, 2000), computer programming (Adelson, 1981), electronics (Egan and Schwartz, 1979), and mathematics (Silver, 1979, 1981), among others. How does one go from a novice category representation to that of an expert? One suggestion from the problem-solving literature is that much of the learning comes as a by-product of the problem-solving activity itself (Ross and Kennedy, 1990; Cummins, 1992). There are multiple possibilities for how this can occur, including adding new knowledge to the category, modifying previous knowledge by weighting particular category features, adding constraints to the category to further specify the category boundary, deleting inappropriate knowledge, or acquiring new, more specific categories. These possibilities suggest not only that categories are a critical aspect of human problem solving but that category representations can be adapted and changed through problem solving.

2.29.4.1.2 Problem solving and category learning

Much experimental work shows that problem solving can affect category learning (Ross, 1996, 1997, 1999). For example, Ross (1996, Experiment 2a) conducted a category use experiment (similar to the ones mentioned in the section 'Beyond classification and featural representations') in which students learned to classify equations into two categories and then solve them. The solutions of the equations differed, and the question was whether this would affect the participants' category representations and influence their future classifications. **Table 5** shows a sample of the materials. Every problem had an x and a y variable, and half of the participants solved for variable x and the other half for variable y. The equations were

Table 5 Sample materials from Ross (1996)

Phase of experiment	*Equation*	*Solution*	*Solve for* x	*Solve for* y
Study				
Type 1	$9 + \frac{sx}{t} = \frac{5qy + b}{f}$		SMD	MSD
Type 2	$sp + \frac{ry}{2} = \frac{g + 9x}{b}$		MSD	SMD
Test				
	$k + \frac{9z}{a} = \frac{s + r}{t}$	SMD	Type 1	Type 2
	$s + \frac{2}{n} = \frac{9 + mz}{p}$	MSD	Type 2	Type 1

SMD, subtract, multiply, divide; MSD, multiply, subtract, divide. At test all subjects solved for *z*. The ''solve for *x*'' and ''solve for *y*'' labels refer to the study conditions.
From Ross BH (1996) Category representations and the effects of interacting with instances. *J. Exp. Psychol. Learn. Mem. Cogn.* 22: 1249–1265. Copyright 1996 by the American Psychological Association. Reprinted with permission of the author.

created so that those who solved for variable *x* would use a subtract-multiply-divide (SMD) solution procedure on type 1 problems and a multiply-subtract-divide (MSD) solution procedure on type 2 problems, whereas those who solved for variable *y* would use the opposite procedures on the two problem types. At test, they classified novel test problems and solved for a new variable *z*. Tests requiring a SMD solution procedure tended to be classified as type 1 problems for those participants who solved for *x* and as type 2 problems for those who solved for *y* (and vice-versa for test problems that required the MSD solution procedure). Although the two groups classified the same problems into the same categories during learning, they classified the test items into opposite categories because of how they interacted with the items. The way in which problems are solved can influence which categories people have.

Beyond traditional problem solving, Medin and colleagues show strong influences of extended interactions on how tree experts classify and reason (Medin et al., 1997; Lynch et al., 2000; Proffitt et al., 2000). Taxonomists and maintenance workers sorted a set of trees on the basis of morphological features, but each weighted the importance of those features differently, whereas the landscapers sorted more on the basis of utilitarian features, such as providing shade or ornamental quality (Medin et al., 1997). The experts' category representations were influenced by how they interacted with items in the category. Proffitt et al. (2000) found that all of the groups used ecological-causal domain knowledge in addition to general taxonomic knowledge to make inductions. These results are consistent with the laboratory results showing that experience interacting in a domain influences a person's category representation and subsequent reasoning from those categories.

The role of concepts and categories in problem solving is pervasive. For categories to be useful for problem solving, they require more than simple featural representations, including information about the goals, solution procedures, and constraints of the problem. Problem categories change with experience: There are general shifts from surface feature representations to structural (relational) representations, as well as influences of the particular uses.

2.29.4.2 Language

Categories are also critical in language use. We focus on one area within language performance (i.e., the encoding of syntactic number) to explore different kinds of categories, the processes that operate on them, and the functions such categories serve in language performance.

In general, the types of categorical structures necessary to support language processing seem to depart considerably from those focused on in category learning research. For example, during language production, transforming a message that is full of meaning into an utterance that is full of sound (Bock and Miller, 1991) may involve (at least) coordinating systems of categories corresponding to syntactic structure (e.g., the syntax of phrases or sentences), grammatical functions and thematic roles (e.g., subject, object, agent, patient), word types (e.g., noun, verb), word-specific grammatical information (e.g., grammatical gender and number), word meanings, morphophonology, and prosodic structure. Particular to syntactic number, the production of a

lexical singular (e.g., cat/argument/dustbuster) or a lexical plural (e.g., cats/arguments/dustbusters) is thought to be rooted in a kind of categorization involving the apprehension of the referent of a noun as a single thing or more than one thing (Eberhard et al., 2005). Some categorizations are not simple: A bowl full of "fresh or dried food that is usually made from flour, eggs, and water formed into a variety of shapes" may be conceived of as a mass or as comprising individual units and so the speaker may elect to call it pasta or noodles, respectively. This categorization determines the appropriate lexical number characteristics of the noun.

Consistent with our emphasis on category use, assigning lexical number (singular or plural) is not an end in and of itself – lexical number characteristics serve the function of communicating to a listener the numerosity of referents, and they are critical in the computation of grammatical agreement (such as between a subject and a verb; Eberhard et al., 2005; but see Vigliocco et al., 1996). Grammatical agreement in turn serves important communicative functions such as linking pronouns to their referents and helping listeners syntactically bind subjects to their predicates when syntactic ambiguity arises. For example, subject–verb agreement helps disambiguate who has rabies in "The *dog* chasing the *men* that *has* rabies."

Beyond categorizing a referent as one thing or more than one thing, singular nouns in many languages divide into count nouns and mass nouns. The count/mass distinction is thought by some to reflect distinct modes of construal relevant to individuation and allows an interesting examination of concepts in language use.

Count nouns like animal(s), argument(s), and noodle(s) must have a determiner in the singular form (*Animal is fierce), are regularly pluralized, and take the quantifiers many and few. Mass nouns such as wildlife, evidence, and pasta do not need to take a determiner in the singular (Wildlife is flourishing), are not regularly pluralized, and take the quantifiers much or little. What are the psychological implications of this distinction? The cognitive individuation hypothesis proposes that count nouns denote individuated entities and mass nouns denote nonindividuated entities (Mufwene, 1984; Wierzbicka, 1988; Jackendoff, 1991; Bloom, 1994; Bloom and Kelemen, 1995; Bloom, 1996; Wisniewski et al., 1996; Wisniewski et al., 2003; Middleton et al., 2004). The class of individuated entities includes common objects such as cats, blenders, and airplanes but also includes things bounded spatially (even to an absence of matter, e.g., a hole, Giralt and Bloom, 2000) or temporally (events, such as a footrace or a party; Bloom, 1990). Individuation can apply to entities linked by common fate or goal (e.g., a gang, a flock) or common purpose (a bikini may be conceived as an individual because the two pieces perform one function; Bloom, 1996), as well as from a variety of other factors (see Goldmeier, 1972; Jackendoff, 1991; Soja et al., 1991; Bloom, 1994).

2.29.4.2.1 Categorization and cognitive individuation

The process of individuation is not just a categorization based on the physical features on an entity. Cognitive individuation involves active construal of an entity, which can be flexibly applied and has important consequences. Specifically, if a person individuates an entity, that person predicates that features of the entity must hold specific functional relationships to each other (Wisniewski et al., 2003; Middleton et al., 2004). For example, if one individuates a configuration of wood as a table, one is comprehending how the configuration of four upright pieces of wood and a horizontal wooden plane go together to support the important function of supporting stuff. This construal does not allow pieces to be randomly removed or rearranged. In contrast, if one categorizes the table as a nonindividuated entity, one might focus on the material rather than the configuration. If so, one might predicate the important property of 'is flammable,' which does not depend on the configuration.

Evidence that individuation is a flexible process in which different outcomes (e.g., individuation vs. nonindividuation) can arise given the same stimulus was reported by Middleton et al. (2004; Experiment 4). One group of participants viewed a bounded pile of coarse decorative sugar in a box (a novel stimulus) and chose to refer to it with count or mass syntax. A second group viewed the stimulus, followed by a mode of singular interaction where they repeatedly took an individual grain and placed it through one of several holes in a rectangular piece of cardboard. Participants in this second group were more likely to refer to the stimulus with count syntax than the control group. This demonstrates that individuation is not directly tied to the features of a stimulus. Rather in this case, how a person interacted with the entity was related to its individuation status, and this in turn was reflected in the syntax they used. This point introduces the functionality of the count/mass distinction: Using count or mass syntax provides a means to communicate distinct construals of

an entity in terms of individuation. This may be particularly useful when the mode of construal as an individual or nonindividuated entity is atypical for a referent. Consider 'too much woman' as in "[S]he's [Jennifer Lopez] too much woman for that piece of snore [Ben Affleck]." Using mass syntax with what is typically a count noun (i.e., woman) allows the speaker to communicate construal of womanly attributes as lying on a continuum, with Jennnifer Lopez falling on the high end (at least, too high for Ben Affleck). Attributes of other common objects can be construed as lying on a continuum, as communicated in "[M]any border collies are destroyed because they proved to be too much dog for their owners," where 'dogness' may be some value along a continuum composed of activity level, obedience, ferocity, and so on. (These examples are extracted from American Web sites.)

Language may not just reflect concepts, it may influence the representation of concepts. The boundaries of basic categories may not be invulnerable to the effects of language (e.g., Boroditsky, 2001; Gordon, 2004). As one example, Imai and Gentner (1997; see also Imai, 1995) showed that Japanese- and English-speaking children differentially weighted the importance of a similar substance and similar configuration when choosing which item was the same as an example. This issue of how language may lead to differences in categorical structure is potentially very important inasmuch as we learn a large proportion of our concepts through communicating by direct instruction or implicitly through conversation (see Markman and Makin, 1998).

2.29.5 Conclusions

In this chapter, we have presented an overview of research on concept and category learning, but we have done so from a particular perspective. Although classification models and experiments have dominated the laboratory work in this area, some recent work has questioned both the focus on classification and its use of simple featural-based items. This work has promoted a broader examination of concept and category learning in three ways. First, a variety of category-learning paradigms are being investigated, along with models that can perform other category functions besides classification. Second, the complexity of the material being learned has increased to include relational categories, prior knowledge, and nonobject categories. New models are also being proposed to begin to account for these complexities. Third, this perspective encourages a consideration of how concept and category learning may be viewed in other areas of cognition. These advances should provide a richer, broader view in the future, so we can better understand the learning of concepts and categories and their crucial role for intelligent thought and action.

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2.30 Language Learning

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2.30.1 Introduction

2.30.1.1 What Is Language, and What Makes It a Unique Learning Problem?

Language presents an unparalleled problem for any account of human learning. As adults we have little insight into, or memory of, the learning task we faced during infancy and early childhood. Adult language processing is normally so efficient that we cannot introspect the cognitive or neural processes that accompany such prosaic language-uses as making small talk or listening to a story – processes that include attention-modulation, classification, retrieval, inference, and cognitive control.

By examining language development in infants and children we may gain insight into the challenges, progress, and process of this singularly important and universal learning task. The overall topic is extremely complex, and a thorough treatment would include detailed consideration of phenomena

including multilingualism, second-language learning, language loss, aging, developmental disabilities, genetics, and animal learning. Due to space limitations, however, and the intricacies of each of these topics, this overview will focus on the paradigmatic phenomenon of first-language learning by healthy infants and children.

To begin, we must address the thorny question of what makes language a unique modality for social information, and why language acquisition might be a singularly difficult problem for the cognitive and social sciences.

2.30.1.2 Why Is Language Hard to Learn?

Human languages (numbering between 3,000 and 8,000, depending on definitions and some unknowns) share some basic features. The world's languages differ in many regards: the set of sound-distinctions that change the meaning of an utterance (or movements that change the meaning of a sign); how meaning-elements can be altered and combined to express complex meanings; what specific meanings are encoded by words (and by derived words, phrases, or idioms); and how various speakers and listeners in a community may use language for different purposes in different situations. Given the stunning variability of the world's languages, effort has been made to identify linguistic universals. The broadest universals are: hierarchical structure (e.g., rules for combining, changing, and deleting/reducing phonemes (speech sounds), morphemes (smallest meaning units), words, and phrases); arbitrariness of form; modifiability of forms (by assimilation, simplification, or metaphor); and combinatorial complexity. Other universals are more specific. For example, despite phonological variability across languages, there is a common ordering by which, for example, vowels are accrued. As another example, all languages can somehow refer differently to *self* versus *other*. Other universals are more like parameters that take one of several 'values,' and these must be learned by children presumably from culture-specific input (as opposed, in the case of 'true' universals, to learning from some universal experiences).

Despite these universals, the profound differences between languages make it hard to specify children's ability to learn language. Children must be prepared to learn language with phonological tone variations (e.g., Mandarin dialects; Yoruba) or percussive 'click' or air-ingestive noises (Sindhi, Xhoso, Zulu), or languages like Hawaiian with very limited phonology. In terms of morphology (i.e., patterns of variation in word structure), children must be prepared to learn languages with limited verb morphology but extensive use of auxiliary verbs, like English, or languages with extensive verb inflections and vowel harmony (i.e., where the root vowel changes the verb's inflections), like Hungarian. Specific examples abound: children learning English must make some verbs reflexive by adding '[possessive pronoun]-self,' whereas children learning Hebrew must learn to affix /hit-/ to most verbs – unless the first sound is a fricative (e.g., /s-/), in which case there is a complex switching of phonemes in the root verb and inflected affix. Mohawk uses a morphological inflection /-atat-/ to indicate reflexive action, but also has a 'semireflexive' morpheme to indicate relatively high involvement or self-generation of an activity. Many more examples can be found in syntax: for example English-speaking children learn that roles (subject, object) are cued by word order; Italian-speaking children must learn to use other cues (e.g., animacy). In semantics, English-speaking toddlers must learn that 'diaper' and 'underwear' refer to things with similar shapes that cover the same body parts but different material and contexts of use, whereas 'hat' and 'gloves' differ in shape and body parts but share material and contexts of use; and 'clothes' refers to all of these but is a mass noun (which usually refer to uncountable things like liquids). Finally, in pragmatics Spanish-speaking children must learn different second-person pronouns for adults and peers; Japanese children must learn different honorifics for men and for women; English-speaking children may say 'you' in all cases.

Thousands of between-language differences like these highlight the complexity of the learning problem faced by children and the difficulty of specifying what children might be prepared to learn *a priori*. Yet even within a language the learning problems are daunting. For example, the regular English past tense inflection is an affix /-ed/ after the main verb. But in fact the phonological form can be /-d/ (e.g., 'bugged'), /-t/ ('marked'), /-ed/ ('blasted'), or /-id/ ('melted'). Moreover, different speakers or dialects use different variants of the same ending, *and* the phoneme before the ending changes the sound of the ending. Thus, in spoken language there is much more variability of form than in writing. Also, there are many irregular past-tense forms: 'run'/'ran' or 'swim'/'swum' (medial vowel change); 'is'/'was' (initial consonant-vowel change); 'go'/'went' (different word), etc. Ignoring the reasons for these

differences, from the learner's position most of these are at best only loosely predictable. They must be memorized as exceptions, or inferred from subtle patterns (e.g., verbs ending in /-ing/ ['bring'; 'sing'] have a medial vowel change (/-i-/ to /-u-/).

Children sometimes learn these exceptions early: for example 3-year-olds are sensitive to a constraint on pluralizing the head of a compound noun, which sounds grammatical for irregulars ('mouse-eater' to 'mice-eater') but not regular nouns (e.g., 'rat-eater' to *'rats-eater') (Gordon, 1985). No one teaches children this explicitly, but by age 3 children have begun to learn not only the obvious rules but less-obvious conditional regularities like these. This illustrates children's preparedness to acquire a massive hierarchical system of probabilistic heuristics and exceptions for allowable forms of words, phrases, and sentences. There is much evidence that 2- and 3-year-olds are learning to treat language as an abstract, modifiable, combinatorial system of conventional forms, transformations, and uses (Gordon, 1985; Clark, 1997; Tomasello, 1999; Bates et al., 2003). Yet 3-year-olds still have much to learn. This is a critical point: it is often assumed that children are astoundingly good language-learners despite the intrinsic difficulty of the task. Certainly the task seems difficult, but compared with what? Vision? Motor skills? Such questions are difficult to answer because they require an information-theoretic comparison of different learning problems, and making any such comparison in an even-handed way would be difficult or impossible. Similarly, it is nearly meaningless to claim that children learn language 'quickly.' Compared with what? Learning calculus? Learning to drive? Any such comparison is so problematic that the absurd difficulty of the question becomes obvious. Children require a good five years of steady, ample language input (for hours every day), with massive social and physical support, to achieve fluency. The cost of failure is exclusion from social interaction and information. Stated like this, it becomes hard to defend any assumptions about the specialization of language-learning processes.

To move from fuzzy assumptions toward a clearer understanding of the language learning problem and how children solve it, the following sections summarize what infants learn in the first year, what toddlers learn in the second and third years, what preschoolers learn in the third and fourth years, and what older children continue to learn thereafter. A critical issue throughout is how these changes differ from language to language. It is critical because we want a valid characterization of the universal capacity for language learning. First, though, we address two factors that are part and parcel of that capacity: the ecological context of first-language learning and the relation of language learning to the human genome.

2.30.1.3 The Context of Language-Learning 'in the Wild'

Language is used differently in different communities, and this is part of the learning problem for infants. Infants and toddlers are not consciously reflecting on language as a learning 'problem' akin to some monumental homework assignment. Their motives are to affiliate with caregivers, maximize hedonic states and minimize unpleasant ones, predict what other people will do, and join in positive social exchanges whenever a felicitous opportunity arises. Language is an integral part of the events that fit these motives: it is present in all sorts of social events from birth and even before (DeCasper and Fifer, 1980). The point is often forgotten: infants are not *trying* to learn language. They are trying to satisfy dynamic endogenous needs and modulate their affective states. This requires action, reaction, and learning about dynamic social environments. Language is a diffuse category of information that variably (but not randomly) occurs concurrently with social events. Sometimes language information is correlated with ongoing social events and variables, as during one-on-one baby-talk. Other times it is independent of the infant's experience, as when a caregiver chats with another adult while feeding the infant. Thus, an infant's language input is *sometimes* tailored to her ongoing experience. Sometimes it is not.

Thus, to understand how infants and children can acquire any language we must consider in part the range of language-uses in infants' social environments. For example adults modify their speech when speaking to infants, and infants prefer to hear infant-directed speech (Cooper and Aslin, 1990; Pegg et al., 1992). It therefore seems infant-directed speech should facilitate infants' language learning, and in fact it can facilitate adults' learning (Golinkoff and Alioto, 1995). However, there are language communities where adults do not address babies. Still, infants in these communities seem to acquire language at about the same rate as infants who regularly hear infant-directed speech (Lieven, 1994), though they might experience some delays in productive competence (Brown, 1998). Thus infant-directed speech is

not a 'hard' prerequisite of learning, although it might accelerate *some* aspects of language acquisition.

The points are that (1) all cultures do not communicate with their infants in similar ways; (2) it is not obvious how different cultural practices influence language learning: we must test these empirically (e.g., Bornstein et al., 1998). Other examples in the following sections show that our intuitions of 'what matters' in language learning often lack empirical support.

2.30.1.4 How to Think About Genetic Factors in Language Learning

There is no doubt that language is a species-specific capacity. Some universal language features are not acquired by any other species, however smart their members are in other regards. Specifically, hierarchical structure, modifiability/extensibility of forms, and combinatorial complexity are all absent or profoundly limited in our nearest evolutionary neighbors, the great apes (Terrace et al., 1979; Deacon, 1997). What, then, allows learning in nearly every child in every human community?

Explanations from genetic causes have limited power to explain language outcomes (Braine, 1992; Elman et al., 1996; Karmiloff-Smith, 1998). Certainly language learning requires genetic coregulation of brain development that leads, in a protracted and dynamic cascade of multifactorial changes, to particular information processing phenotypes. Yet that claim is very different from a stronger claim, that some specific evolutionary adaptation(s) were propagated in our ancestors *because they coded for* specialized, species-specific (and adaptive) language phenotypes. That is possible but entirely hypothetical. Nevertheless, strong claims for *specialized* genetic bases of language have been made, buttressed by reference to the well-publicized discovery of a family with many members who have severe language deficits (Gopnik, 1997). Affected family members shared a point mutation (i.e., single-amino-acid substitution) of a gene in chromosome 7 (Lai et al., 2001). The gene, dubbed FOXP2, induces RNA transcription to affect the expression of an indeterminate number of other genes, including some that code for proteins that affect neural structures (e.g., calcium channels). The downstream effects of FOXP2 mutations have been hotly debated. Vargha-Khadem et al. (1998) found differences in several brain regions including Broca's area (left inferior-frontal cortex), which is nonexclusively involved in language production, and caudate nucleus (in the basal ganglia), which is involved in motor coordination and which communicates with frontal cortex. Both changes might explain the profound speech deficits in affected family members. However, those deficits are hardly isolated: affected family members also show generalized motor coordination deficits. Given that language production is an incredibly elaborate feat of motor coordination, one would expect *general* motor problems to manifest as speech deficits. Another finding is that most affected members show mild verbal *and* nonverbal mental retardation. This is not surprising because a transcription factor could have widespread effects on neural development.

Comparative studies further complicate the FOXP2 story. Multiple species – mice, for example – have versions of FOXP2. Mice FOXP2 differs from human FOXP2 by three base changes (i.e., amino acid) (Enard et al., 2002). However, two of these are unique to humans and hypothesized to have evolved in the last 200 000 years. Thus, although FOXP2 interacts with brain development in complex ways to produce many cognitive and behavioral effects, it is possible that recent mutations lead to new hominid neural and cognitive phenotypes that 'tipped the scale' to permit, among other cognitive capacities, language. It is also possible that the correlation is spurious.

In sum, although genes must be related to language learning, researchers have only recently started asking more sophisticated questions about the relations: what role do FOXP2 and other genes play in emergent cascades of neural and neurochemical processes during brain development? How are the neural networks that develop for association learning, perceptual-motor learning, and social-information processing altered by the coactivation of mutated gene forms? Such questions are central to our eventual understanding of language abilities and their expression as developmental products. However, current answers to these questions are almost pure speculation.

2.30.1.5 Are There Critical Periods for Learning?

There is a popular idea that language fluency can be attained only during a limited window of age, after which brain plasticity becomes reduced and fluency is difficult or impossible to achieve (Lenneberg, 1967). This is consistent with evidence of partial reduction in plasticity with age (Stiles, 2000) and with computational models wherein early input patterns have a greater effect on learned network weights than later input patterns (i.e., 'starting small'; Elman, 1993; Smith et al., 2001).

Most evidence for a critical period in language learning comes from studies relating L2 (second-language) competency to age-of-acquisition, controlling for years of exposure. (Critical periods in phoneme discrimination are discussed in the section titled 'Classification problem: speech sounds in the infant's sound-scape.') Johnson and Newport (1989), for example, tested adult Korean and Chinese immigrants on English syntactic distinctions. They found a linear decline in competence with increasing age of acquisition, from 8 to 39 years, but no difference in starting ages ranging from 3 to 7 years. This suggests a gradual, continuous decline from about 7 years to adulthood in the capacity to master syntactic details of a new language.

Subsequent studies have shown that it is difficult to predict what syntactic competencies will be compromised by missing early experience. Mayberry and Lock (2003) compared adult English-speakers to adults who learned English in late childhood after learning (1) a signed language, or (2) a non-English spoken language, or (3) no language (as profoundly deaf infants in speaking family). Non-language learners were impaired in processing all sentence types, including simple ones. Early signers or non-English speakers were compromised only in processing complex or noncanonical sentences, especially dative alternations ("The father gave a boy a dog") and relative clauses ("The boy who is chasing the girl is happy"), both of which can be considered generally difficult English syntactic structures. However, performance on complex sentences did not differ between the latter groups, suggesting that modality of first language has little effect on what forms are easier or harder to learn in L2.

Despite such converging evidence for critical period effects in articulation and syntax, the exact nature and cause remain controversial. Many studies do not document or factor out the learning conditions of immigrants of different ages, but these conditions are quite important: child immigrants are often immersed in school, whereas adults might spend time with other L1-speaking adults and receive far less L2 input (Stevens, 1999). In fact, some researchers argue there is little or no compelling evidence of critical periods for language (Birdsong, 1992; Flege, 1999). For example, a study of U.S. census data from a large sample of Spanish- and Chinese-speaking adults found that educational attainment (in U.S. schools) accounted for more variance in self-reported English fluency (26% and 42%, respectively) than age of arrival (6% and 9%, respectively) (Hakuta et al., 2003). Notably, the modest (<10% of variance) schooling-independent effect of age showed no inflection during a particular age range: the function was nearly linear, indicating no discrete cutoff in learning capacity associated with, e.g., puberty. However, Stevens (1999) also used census data and found subtle nonlinearities when regressing the probability of immigrants responding that they speak English 'very well' or 'well' against age of immigration, with the greatest change between 1 and 7 years of age. However, because census methods have limited sensitivity and validity, and because behavioral evidence shows no age range during which L2 learning rapidly declines (Johnson and Newport, 1989), we tentatively conclude that there is no narrow period of development (i.e., 1–3 years) during which language learning becomes crystallized or limited. Future research could tackle intriguing questions, such as why some adults learn L2 and achieve complete fluency, but others learn L2 during adolescence and never approach fluency.

2.30.1.6 Summary

The past two decades have shed considerable light on some *general* questions about the human capacity to learn language. From comparative studies we have learned that, although some nonhuman animals can learn and use up to a few hundred abstract symbols and respond correctly to short, simple, concrete sentences (Savage-Rumbaugh et al., 1993), there is no evidence they can flexibly and productively use symbols for a wide range of meanings (e.g., abstract/nonphysical concepts) or truly flexible syntactic structures, not to mention morphology. Nor do nonhuman animals show prosaic language uses like word play, commenting on absent referents, metaphor, humor, or nonliteral speech.

It seems clear that there is no precipitous critical period for learning, although there is some evidence for a gradual decline in the probability of mastering subtle phonological and syntactic distinctions of a new language, over starting ages ranging from about age 7 years to adulthood.

Finally, recent studies of language change provide fascinating insight into children's role in language evolution (i.e., creolization): specifically in systematizing language structures (Senghas and Coppola, 2001; Senghas et al., 2004). For example, creole-signing children spontaneously create syntactic distinctions that mirror distinctions in natural languages (e.g., manner vs. path of motion), whereas those children

may conflate manner and path of motion in their nonlinguistic gestures (indicating that the distinction is not *obligatory* but specially formalized in the new language). Other studies indicate that the SVO (subject-verb-object) word order canonical in English, for example, is not a 'natural' order: new signers may create an SOV sign order (Sandler et al., 2005).

Keeping in mind these concerns and conclusions about the learning environment and the genome in first-language learning, we now describe critical findings about how children acquire fluency. The results are organized by age divisions that are roughly defined by changing age-related learning tasks and social contexts. Despite this organization, much of language learning will be ongoing and continuous rather than stage-like. In all that follows, it can be assumed that a developmentally constant demand is to understand what other people are trying to communicate, and master enough abstract language forms to interpret others' meanings and to produce one's own messages such that one's intentions and perspectives can be inferred by others. It should also be assumed, despite some organization into distinct sections, that children do not learn separate aspects of language (syntax vs. morphology vs. pragmatics), rather that these theory-laden and historical distinctions are typically interrelated in human language processing data (Bates et al., 2003).

2.30.2 What Is Learned in the First Year

2.30.2.1 Classification Problem: Speech Sounds in the Infant's Soundscape

Languages do not use all the same speech sounds. Adult speakers of language X cannot always pronounce, or even discriminate, some phonemes of language Y. For example English voiced bilabial consonants form two phonemes, [b] and [p], that differ only in voice onset time (VOT, or the time between onset of vocal cord vibrations and air release). By contrast, in Thai the same spectrum is divided into three phonemes. English-speaking adults perceive the VOT spectrum as two discrete categories, with a high-entropy region around the /b/–/p/ distinction, but do not perceive a third category in the region of the added Thai contrast. How, if adult speakers cannot even perceive all phonemes, do infants learn whatever complement of speech-sound distinctions is relevant in their language?

During the third trimester of gestation the fetal auditory system is sufficiently developed to begin learning some abstract properties of speech sounds produced by the mother. Although the amniotic sac filters the acoustic content of speech, enough invariants are retained in this filtered signal that, after birth, neonates prefer the sound of their mother's voice (DeCasper and Fifer, 1980). Neonates also perceive some phonetic distinctions such as the /b/–/p/ VOT contrast (Eimas et al., 1971). This suggests that the extensive and well-demonstrated plasticity of auditory cortex (Ohl and Scheich, 2005), which begins prenatally, responds in humans to acoustic invariants of speech.

During the first few months infants become sensitive to differences between phonemes (consonants and vowels), including differences in place of articulation and VOT (Trehub, 1973; Eimas, 1974). Phoneme perception develops such that by 9–12 months infants are sensitive to native contrasts but less sensitive to nonnative contrasts (Werker and Tees, 1999). Werker and Tees (1984) found a decline from 6 to 12 months in English-learning infants' discrimination of a Hindi /Ta/–/ta/ contrast and a Nthlakampx /k'i/–/q'i/ contrast (defined by place of articulation). These distinctions (unlike, e.g., /ba/–/da/) are also subtle for nonnative adults (Werker and Tees, 1999), but can be learned with practice (McClelland et al., 2002). This suggests a sensitive period in phonological development. Phonological processing difficulties for L2 distinctions might, in some cases, lead to larger difficulties with speech processing that resemble L1 language delays (Tallal, 2004).

Despite evidence for a sensitive period in phonological development during the first year, adaptation of the auditory system to language-specific input begins well before 9–12 months. Within their first few days infants discriminate native (French) from foreign (Russian) speech (Mehler and Cristophe, 1994), though discrimination depends partly on how phonologically different the languages are (Nazzi et al., 1998). Whatever neurological changes accompany 9- to 12-month-olds' loss of sensitivity to nonnative contrasts, it is not the case that younger infants are insensitive to native speech features.

2.30.2.1.1 What categories are infants prepared to learn? Insights from signed languages

To gain insight into what is distinctive about learning to perceive speech, we can consider how infants

learning signed languages acquire the basic linguistic units, that is, motor forms including hand shapes, manual motions, and other body motions (e.g., facial gestures). In what ways, if any, has brain development evolved to favor processing and learning of speech sounds over other modalities?

It is not clear that language learning is at all specialized for speech. Petitto and Marentette (1991) argued that deaf children learning signed languages begin manual 'babbling' by 10 months or earlier. The emergence of a production distinction between signing and gesturing suggests prior perceptual analysis of hand morphology of signs. How young can infants perceive differences in hand shape that carry meaning differences in a signed language? Schley (1991) found 3-month-olds could discriminate at least one hand-shape difference. Though this is not conclusive it suggests there is no great delay in perceptual learning of language-relevant forms in nonspeech modalities. Further, Baker et al. (2006) suggest a critical period in acquiring hand-shape phonology: hearing infants at 4 months classified same- from different-shape tokens (from ASL); 14-month-olds did not. The timing of this loss of sensitivity is roughly similar to loss of nonnative speech sound sensitivity (Werker and Tees, 1999) and is further supported by evidence that older infants learning spoken language lose the tendency to interpret novel gestures as symbolic (Namy and Waxman, 1998).

2.30.2.2 Beyond Phonology: Finding the Words

When do infants begin to perceive larger units – specifically, combinations of speech sounds that we hear as words and phrases? This has been a major topic of research in the past decade. For example Jusczyk et al. (1993) found that infants around 7 months discriminate (and prefer) the stress pattern of their native language (e.g., strong-weak in English, e.g., 'mother'; 'bottle'). This preference could help infants parse words in the speech stream; a critical ability because there are no clear acoustic markers of the boundaries of words. How else might infants learn to separate words and inflections in the ongoing speech stream?

Another source of word-boundary information is the likelihood that two phonemes will occur in sequence within some word in a given language. Consider the phrase 'pretty baby,' which has a word boundary between /-y/ and /b-/ but, for all the infant knows, might be three words (e.g., 'pritt ebay bee'). However, the probability of the phoneme sequence 'eeb' in English is much less than the probability of 'tee' or 'bay,' so the former parsing is more likely. Infants can learn such differences in transitional probabilities within minutes, simply by listening to an artificial language with controlled transitional probabilities (Saffran et al., 1996). Thus, before their first birthday infants encode cues to the structure of words. These learning abilities are not specific to word-learning nor to humans: infants can learn analogous transitional probabilities in musical motifs (Saffran et al., 1999) or sequences of visual shapes (Kirkham et al., 2002). Also, tamarin monkeys can learn transitional probabilities in speech phonemes (Hauser et al., 2001). Thus, however important the phoneme-sequence-learning capacity is, it is not sufficient for human speech processing. Also, infants might learn words spoken in isolation faster than embedded words (Brent and Siskind, 2001), suggesting that word segmentation is, despite sequence-learning abilities, resource demanding and/or error prone.

2.30.2.3 First Words: Content and Conditions of Learning

2.30.2.3.1 What do infants know about words?

Deciphering the speech stream involves more than segmenting individual words: children need to associate certain sequences of phonemes with contexts of use or kinds of referents. How do infants learn word meanings? Infants by 4 months attend more to the sound of their own name than another name with the same stress pattern (Mandel et al., 1995). By 7 months such preferences extend to high-frequency words (e.g., 'cup'; Jusczyk and Aslin, 1995). By 11 months infants represent the phonological details of familiar words (Swingley, 2005). How readily do infants learn such representations? Eight-month-olds, after hearing a word several times, discriminated it from other words as long as two weeks later (Jusczyk and Hohne, 1997).

It seems infants can learn and remember sounds of specific words several months before they start using them productively. However, increased attention to familiar patterns is not the same thing as symbolic understanding. When do infants learn to associate words with object types, people, events, or properties? At 8 months infants show a slight tendency to associate an object that was recently paired several

times with a novel word, but only if the speaker moved the object in synchrony with saying the word (Gogate and Bahrick, 1998, 2001). The importance of intermodal synchrony underscores the fragility of infants' word-referent associative learning. By 11–14 months, infants are sometimes above chance at attending to an object previously paired with a novel word 6 to 9 times (Woodward et al., 1994). However, it is unclear how much (or little) input is needed for various referents or situations, and whether infants learn anything beyond a weak intermodal association (Shafer and Plunkett, 1998). In other words, we still do not know when and how infants learn words as abstract symbols.

In interpreting all this literature a caveat is in order: much older preschoolers are sometimes insensitive to gross word-form violations (Barton, 1980), suggesting that phonological/lexical knowledge may remain immature long after infancy. The confusing range of sensitivity and insensitivity shown in various studies of infants and preschoolers (e.g., Fisher et al., 2004) demands more sophisticated models than currently exist. One issue is that infants are very sensitive to contextual factors (Naigles, 2002), so the exact conditions of input *and* of testing must be meticulously detailed and compared in order to make sense of different studies of infants' word-form knowledge.

2.30.2.4 Beyond Words: Learning Phrase Structure and Lexical-Syntactic Categories

Infants show some awareness of other linguistic patterns in the first year. Fernald and Mazzie (1991) showed that infants are sensitive to prosodic (i.e., melodic) contours of infant-directed utterances that correspond with different messages or meanings (e.g., approval vs. prohibition). Interestingly, prosodic patterns show some consistency across languages (Fernald et al., 1989; Grieser and Kuhl, 1998), suggesting that many societies come to exploit prosodic distinctions that are salient to infants, as a way to draw attention to distinct messages before infants can comprehend specific words or phrases.

Prosodic information might also help infants learn syntactic distinctions. Adults detect phrase and clause boundaries based on speech cues (intonation, stress, pauses, word duration), even when listening to a foreign language (Pilon, 1981). Although these cues are sometimes unpredictable or misleading, they may be more predictable in infant-directed speech (Stern et al., 1983). Several studies (e.g., Hirsh-Pasek et al., 1987; Jusczyk, 1997) indicate that 8- to 10-month-olds expect pauses at syntax-relevant clause and phrase boundaries. This preference is not specific to spoken language; infants also prefer pauses at phrase boundaries in classical music (Krumhansl and Jusczyk, 1990). Also, although prosodic structure could highlight syntactic structure in languages with strong word-order cues (e.g., English) it might be less useful in languages where syntax is carried by inflections (e.g., Hungarian; Icelandic). Still, English-learning infants as young as 2 months can use prosodic clause cues to represent two-word sequences, at least briefly (Mandel et al., 1996).

Infants in the first year might distinguish between kinds of words that correspond to different syntactic categories. Shi and Werker (2003) found 6-month-olds discriminate so-called content ('open-class') words (e.g., 'chair,' 'hide') from grammatical ('closed-class') words ('the,' 'you'), and prefer the former, even in a foreign language. No common phonological cue differentiates these word classes across languages, but some combination of cues is probabilistically available in any language (Morgan et al., 1996). The implication is that languages evolve a lexical 'division of labor *and* form,' so content words have more distinctive phonology than syntactic units. This might contribute to a developmental shift in the kinds of words infants learn as they populate their lexicon and acquire syntax (see the section titled 'New math: populating the lexicon').

It is not just that infants associate more interesting-sounding words with open-class units; they also learn sequences of words. Gómez and Gerken (2000) found that 12-month-olds developed expectations for order and repetition dependencies in small sets of artificial CVC words (e.g., 'pel' can start a sentence *or* follow 'vot'). After training, infants heard novel 'sentences' that were 'grammatical' or 'agrammatical' and listened longer to agrammatical sentences. Thus infants are sensitive to the same types of transitional probabilities between words that Saffran et al. (1996) showed for phonemes. This might support syntax learning.

This finding (see also Marcus et al., 1999) does not show that 12-month-olds have learned syntax, but that they are minimally sensitive to more- versus less-likely orderings of syllables or lexemes, given well-controlled input. Yet syntax involves more than order, and more than just CVC syllables. It involves a number of abstract categories or form classes, systematically related in various ways to other categories, under a system of complex

principles and probabilities for changing and combining units. Currently we only know that infants discriminate (1) familiar from less-familiar orderings of syllables; (2) acoustic and prosodic cues that correlate with phrase and clause boundaries; and (3) phonological cues that differentiate broad syntactic categories (e.g., content vs. grammatical words). It remains unknown how this learning contributes to later syntactic knowledge in the next several years.

2.30.2.5 First Uses: Reasons to Learn Language

Recall that, although language researchers describe infants as trying to solve a taxonomy of massively complicated mapping problems, that description is imposed upon the infant whose goals are to stay regulated, reduce uncertainty, and maximize hedonic states. Caregivers who help infants meet these goals sometimes emit streams of vocal noises (or gestures). Why should infants learn these? One reason must be that infants are motivated to affiliate with people, and interested in what people say. Infants must pick and choose information in rich environments. Some human features, such as faces (Fantz, 1963), voices (DeCasper and Fifer, 1980), and hands (Deák et al., 2006) tend to attract infants' attention. If caregivers also talk about their actions while infants are watching them, it can give infants good input for learning words. Hart and Risley (1995) showed that language input – amount and variability of speech – predicts infants' language skills into preschool. Similarly, Tamis-LeMonda et al. (2001) found maternal responsiveness (i.e., reacting quickly and appropriately to the infant's signals) at 9 and 13 months predicted language outcomes including age at first words and acquisition of 50 words. Notably, mothers' responses to infants' vocalizations and play prompts (e.g., acting on a toy while commenting) were the best predictors of language outcomes.

2.30.2.6 Using Social Inferences to Bootstrap Learning

A major shift in our understanding of child language was sparked by evidence that early language is interwoven with intentionality (i.e., awareness of other people's mental states and emotions). Although this awareness becomes more precise and explicit through childhood, its first measurable signs emerge around 9–18 months of age.

Much research has focused on attention-sharing, periods when two or more individuals shift attention to a common focus. Such episodes facilitate communication, because the topic of conversation can be highlighted by extra-linguistic behavior (i.e., if interlocutors comment on whatever has their attention, and both are focused on the same thing, they will tend to share topic). Research and theory of the development of attention-sharing skills in infants, and its relation to language development, is reviewed by Baldwin and Moses (2001), Deák and Triesch (2006), and Tomasello (1999). In short, infants sometimes follow an adult's gaze or pointing gesture by 12 months of age, though the ability improves from 9 to 18 months (Butterworth and Jarrett, 1991; Deák et al., 2000; Brooks and Meltzoff, 2002). Infants might be either more likely to do so, or do so for longer, if the parent verbally encourages them to follow (Flom and Pick, 2003). Thus, parents' speech acts initially have an attention-modulating function for infants.

Does sharing attention conversely facilitate language development? There is no evidence of this in the first year, but early attention-sharing skills seem to support rather sophisticated inferences in the second year (see the section titled 'Inferring the meaning behind the words').

2.30.3 What Is Learned in the Second Year

During the second year toddlers' language will advance in several critical ways. Some burgeoning sensitivities of infants become active. Research points to advances in three major areas: lexical knowledge, pragmatics, and syntax. These areas are tightly related, but because research often treats them separately, the following section treats them (artificially) as separate.

2.30.3.1 New Math: Populating the Lexicon

There has been controversy about what kinds of words toddlers first understand. The first 50 words typically include many generic object labels ('bottle'), proper names ('Lara,' 'mommy'), words for actions or modifiers ('up,' 'more'), and social routine words ('bye bye') (Nelson, 1973). One debate is whether first words are highly context restricted or under extended and, therefore, limited in abstraction. Snyder et al. (1981) found about half of 13-month-olds' first 50 words were in fact contextually restricted; yet some

should be by definition (e.g., 'bye-bye,' 'peek-a-boo'). Huttenlocher and Smiley (1987) found infants rarely use labels in contextually idiosyncratic ways. Toddlers know so much less than adults about the referent categories of words that their semantic representations must be limited or distorted. Yet one study hints at fairly rapid corrections. Woodward et al. (1994) found 18-month-olds better than 13-month-olds at extending a novel word to a new exemplar like the training object. Thus, toddlers quickly learn to generalize generic words from first (idiosyncratic) referents to abstract classes and thereby reduce contextually restricted uses.

Once toddlers extend words taxonomically, they must still adjust the boundaries of the referent category. Toddlers sometimes overextend words (e.g., use 'ball' for all spheres; Rescorla, 1980) or underextend them (e.g., excluding penguins from 'bird'). Yet such errors do not indicate an inability to map words onto sensible categories. Most overextensions, for example, are based on spurious perceptual or functional similarities (Clark, 1973; Nelson, 1979). Also, there is no evidence that toddlers *typically* over- or underextend words. Many overextensions have a pragmatic basis and do not reflect systemic conceptual confusion (Thompson and Chapman, 1977). That is, when a 1-year-old calls a stranger 'daddy' she is probably not questioning her own legitimacy, but noticing some similarities between a novel referent (strange man) and a familiar one (daddy). Given the child's many lexical gaps, such remote similarities might constitute the only basis for choosing a rarified 'known' word to indicate the referent.

As children receive input they will modify the boundaries of word-meanings using factors such as typicality (White, 1982; Wales et al., 1983). However, we do not know which input factors alter these boundaries, or how.

Despite these early challenges, toddlers make rapid progress in populating their lexicons. One story is that after children learn 50–75 words their rate of word learning accelerates: the 'naming explosion.' This suggests that, after learning some symbolic mappings, toddlers achieve insight about the abstract meanings of words. We do not, in fact, know what higher-order realizations or inferences, if any, facilitate 1-year-olds' word-learning ability. Here, however, are some relevant facts.

First, 1-year-olds tend to interpret others' actions as symbolic. These include gestures as well as words (Namy and Waxman, 1998; Childers and Tomasello, 2002, 2003), so the acceleration is not strictly based on some insight about word-like sound strings. Second, many infants accelerate in word learning around 50–75 words, but others do not (Fenson et al., 1994). Thus, individual infants differ in word-learning trajectory, for reasons that remain unclear despite decades of attempted explanations (e.g., Nelson, 1979).

One hypothesized explanation is that an acceleration in word learning is related to new classification skills (Gopnik and Meltzoff, 1992). Evidence is suggestive but inconclusive. Another idea is that as children learn more words they develop more robust connections among the word representations in neural networks. As the neural representation patterns (i.e., vectors) evoked by particular words become more stable and better defined, this stability can make it easier to learn new word-referent associations (Plunkett et al., 1992; Gasser and Smith, 1998). For example, as children learn words they learn how certain word types (e.g., object labels) are associated with certain referent features (e.g., shape and material), and this can guide inferences about new word meanings (Smith et al., 2003). Thus, increasing semantic knowledge supports new word learning. This is an important principle of word learning throughout childhood and adolescence (Anglin, 1993; Deák, 2000b).

The acceleration in 1-year-olds' word learning is not uniform across kinds of words. An important finding (Fenson et al., 1994; Bates and Goodman, 1999) is that nouns dominate infants' first 50–100 words; however, relational words (i.e., verbs and adjectives) are thereafter learned relatively faster, and become a relatively larger proportion of new vocabulary. Another shift occurs after toddlers know about 300–500 words; learning of grammatical words and morphemes then accelerates. An exciting finding is that this pattern holds (in broad strokes at least) across at least a few Indo-European languages including Italian (Caselli et al., 1999; Devescovi et al., 2005), which differs from English in syntax. There are language-specific differences in vocabulary growth trends, but the relation between vocabulary growth and acceleration of relational words (first) and grammatical words (second) appears robust.

2.30.3.2 Inferring the Meaning Behind the Words

In the second and third years the attentiveness that even younger infants show toward other people,

especially in propensity to share attention and monitor others' emotions, becomes more sophisticated and interwoven with language. For example toddlers can use nonlinguistic social cues to reduce uncertainty of a speaker's referential meaning. Baldwin (1995) found 18-month-olds map a novel word onto whatever the speaker was attending to, not what the infant was attending to, even if they were attending to different things. Toddlers do even more sophisticated tracking and encoding of social cues accompanying others' speech acts. Akhtar et al. (1996) had 2-year-olds and two adults looking at objects in boxes. All participants looked at three objects, and then one adult left the room. The remaining adult then examined the fourth object, and the absent adult then returned, looked in the box and said, "... I see a gazzer in there!" Toddlers tended to associate 'gazzer' with the fourth object, though the returning adult never had picked it up, and the adult who picked it up had never said the word. From this it seems toddlers can infer the most plausible referent of a particular speaker's comment or label. Although this finding has invited competing explanations, converging evidence (Diesendruck et al., 2004) shows that 2-year-olds do in fact use social information (e.g., who was present when some referent was the focus of attention, the speaker's emotion while examining an object or performing an action, etc.) to associate words with referents. Toddlers also modify their own communicative behaviors to take into account an interlocutor's social knowledge (O'Neill, 1996), suggesting that they use information related to other people's mental states or knowledge in order to use and learn language effectively.

We cannot tell how reliably and accurately toddlers use social information to guide inferences about speakers' meanings. All studies are done in simplified, controlled 'best-case' environments, whereas the complex, messy world of everyday social interactions might be too variable to help toddlers make inferences. There are, however, two reasons to believe they can. First, young children with autism typically have profound deficits in joint attention and social inference skills and typically very delayed language skills in childhood and adulthood (Loveland and Landry, 1986; Mundy et al., 1990). Thus, infants who do not make use of social information have impaired language development (this is just correlational, but consistent with the hypothesis above). Second, there is naturalistic evidence that parents constrain the social context of their spontaneous communications with toddlers in somewhat predictable ways (Ninio and Snow, 1996; Pan et al., 1996). Thus, the messiness and unpredictability of everyday interactions is partly limited by parents.

2.30.3.3 Combinatorial Explosion: Putting Words Together

The robust relation between vocabulary growth and acquisition of relational and syntactic words (or morphemes) extends to toddlers' syntactic competence (Bates and Goodman, 1999; Devescovi et al., 2005). Apparently toddlers need a 'critical mass' of words for objects, relations, events and states before they can assemble these units productively. Besides this regularity, how does early syntactic expression and comprehension develop in the second year?

Much work has focused on toddlers' two-word utterances. Early combinations are produced with regularity about the same time as the 50–75 word threshold, or 18–24 months. In four children studied by Bloom et al. (1975) an MLU (mean length of utterances, in morphemes) of 1.5 or better (e.g., about half of utterances having two words) was achieved around 22–24 months. Toddlers' first 2-word productions are described as 'telegraphic' because they lack grammatical words and inflections. Nonetheless, they express a variety of relations including action ('Kathryn jumps'), locative action ('tape on there'), locative state ('I sitting'), static state ('Caroline sick'), recurrence ('more milk'), possession ('Mommy sock') and others (e.g., negation) (Bloom et al., 1975). Some types of relations (e.g., action) are systematically verbalized before others (e.g., locative state), even across languages (Braine, 1976). It is unclear whether this is due to conceptual, syntactic, or motivation factors. However, 1-year-olds show some sensitivity to input in the relational meanings they learn. Choi et al. (1999) found differences in Korean and English toddlers' acquisition of spatial predicates such that Korean toddlers are more attentive to spatial relations (e.g., tight- vs. loose-fitting containment) with distinct words in Korean.

A key issue concerns the early emergence of syntactic categories in two-word utterances (Bloom et al., 1975). Such utterances are usually syntactically (and semantically) ambiguous: does 'Mommy sock' denote possession, action (e.g., putting-on), spatial contiguity, or something else? Syntax might help us disambiguate these alternatives, but are there incipient syntactic categories in toddlers' first combinations? Bloom et al. examined subjects' ordering of morphemes and substitutions (e.g., saying

'her jumps' and 'Kathryn jumps'). Such pronominal constructions suggest an intermediate step toward abstract categories like 'subject'. Two of four children were extensive pronoun users, suggesting proto-syntactic classes, but the individual differences makes interpretation difficult (see also MacWhinney, 1978, for evidence on early diversity of morphosyntactic development). Valian (1986) later showed, however, young 2-year-olds' productions of several form classes (noun, determiner, adjective, preposition, noun phrase, and prepositional phrase) to be well differentiated. Also, two-word speakers understand fully formed sentences better than telegraphic ones (Shipley et al., 1969). Thus, 2-year-olds know more about the correct syntax of individual words than it seems from the combinations they produce, and even 1-year-olds might have some rudimentary expectations (e.g., associating the first noun in a sentence with an actor; Hirsh-Pasek and Golinkoff, 1996). A critical question is how infants and toddlers acquire this knowledge. This has been controversial (Braine, 1976; Maratsos and Chalkley, 1980; Bates and MacWhinney, 1982; Pinker, 1984; Tomasello, 1992), and an adequate treatment is impossible due to space limitations. Nevertheless we will provide a historical synopsis.

Maratsos and Chalkley (1980) proposed that toddlers register long-term patterns of co-occurrence in use (and non-use) of words in particular patterns or contexts, in order to eventually learn syntactic frames. This theory, a precursor of connectionist models and early alternative to a Chomskian learning acquisition device (a mythical organ by which language input is assimilated to an innate syntax), offered a plausible means of incremental input-dependent learning. This type of account and its limits are insightfully critiqued by Maratsos (1998).

For a flavor of the history of this sort of 'nativist versus empiricist' debate, consider the controversy over children's acquisition of transformations over rules-with-exceptions. The test case is English past-tense verb forms, with a regular /t/ or /d/ suffix, but various exceptions including vowel change ('come'/'came'), consonant change ('make'/'made'), word change ('go'/'went'), or no change ('cut'/'cut'). Such messiness is hardly unique to English past-tense: English plural nouns have the same property, as do, for example, German gender categories and many other syntactic forms in many languages. The question is how children can acquire diverse forms for the same type of transformation. A relevant finding is that toddlers sometimes overregularize, producing forms like 'goed,' 'runned,' or 'breaked' (not 'went,' 'ran,' or 'broke'). Notably, such forms are often not the earliest produced; toddlers sometimes produce 'went,' then 'goed' for a while, then ultimately the correct irregular (Cazden, 1968). This right-wrong-right progression intrigues linguists because it suggests a progression from individual word-forms to a syntactic rule to rule-with-exceptions. Marcus et al. (1992) found that past-tense overregularizations are infrequent but variable across time and child, and the right-wrong-right pattern is an idealization with high variability. Also, individual overregularization rates correlate with the frequency of irregulars in the child's lexicon and linguistic environment.

How can we explain the variability of these errors across time and child of these errors? Marcus et al. (1992) argued that exceptions must become strong enough as memory traces to be retrieved before the rule is applied. This idea is only partly explanatory, but it leaves open the possibility of fleshing out the account by testing simulations of learning in artificial neural networks (ANNs). Despite early (and often spurious) objections to this approach, it is clear that many complex patterns, including overregularizations, can be modeled by ANNs (Plunkett, 1992; Hadley et al., 1998; Morris et al., 2000; Lewis and Elman, 2001). For example, a syntactic distinction considered by Chomskian theorists to be unlearnable (under 'Poverty of the Stimulus' arguments; see Pullem and Scholz, 2002, for critique) was shown by Lewis and Elman (2001) to be learned by a fairly simple ANN taking training input from natural speech samples.

Toddlers' syntactic knowledge can also be tested in experimental paradigms. For example Akhtar and Tomasello (1997) show that 3-year-olds, but not 2-year-olds, readily induce, from just a few instances, whether a novel word is transitive or intransitive. Although 2-year-olds learned that novel words referred to actions, they did not appropriately generalize their transitive or intransitive status. (Naigles, 2002, offers another interpretation.) Moreover, toddlers will accept and interpret agrammatical uses of familiar verbs (*"The zebra goes the lion") in ways that suggest fluid phrase/frame structure representations (Naigles et al., 1992). In short, although toddlers are starting to learn the syntactic properties of different words and phrases, their specific knowledge is variable, ephemeral, and unorganized by abstract distinctions such as transitive/intransitive.

2.30.4 What Is Learned in the Third and Fourth Years

2.30.4.1 Acquiring Semantic Relations

As children's vocabulary grows beyond a certain size they must work out a variety of semantic relations, such as inclusion, overlap, and exclusion. For example, are all pets animals? Could any puppy be an herbivore? Deák and Maratsos (1998) showed that 3-year-olds readily produce different labels for an item, and these respect the same semantic relations that adults recognize: if asked about a dog puppet, "Is it a cat?" children reply, "No, it's a dog!". If asked "Is it a doll?" they reply "No, it's a puppet!". The near-errorless pattern of rejections and same-category substitutions suggests that 3-year-olds – and perhaps 2-year-olds (Clark and Svaib, 1997) – represent semantic relations. As early as children know enough words to begin filling in semantic frameworks, they can constrain inferences and naming decisions.

What about adding new words to semantic frameworks? Even 2-year-olds try to make reasonable interpretations of novel words with respect to other words they know, how the word was used, and properties of the referent (Waxman and Senghas, 1992). For example preschoolers can use contrast to interpret new words (Au and Glusman, 1990): if they hear something described "not the red one, but the chromium one," they infer that 'chromium' names an unfamiliar color. Contrary to some claims (Markman and Wachtel, 1992), 2- to 5-year-olds do not by default assume that each word refers to a mutually exclusive category (Waxman and Senghas, 1992; Mervis et al., 1994; Savage and Au, 1996; Deák and Maratsos, 1998; Deák et al., 2001). However, under circumstances like high working-memory load, preschoolers may adopt a temporary mutual exclusivity approach (Liittschwager and Markman, 1994), possibly to simplify the learning task (Deák, 2000a; Deák and Wagner, 2003).

How do preschoolers eventually learn appropriate semantic relations? First, speakers sometimes couch words in meaningful information, like statements of contrast (Au and Glusman, 1990; Callanan, 1990); however, such information is not always enough (Deák and Wagner, 2003) and is more useful to older children (Smith, 1979). Second, syntactic context is sometimes helpful (Naigles, 1990), though for many words in many languages it is a very weak cue. Third, children sometimes analogize from familiar morphological (Anglin, 1993) and semantic (Johnson et al., 1997) relations, but the limits on such analogizing are not known. In short, we usually do not know how preschoolers situate a new word in an existing semantic framework.

2.30.4.2 New Uses of Language

Preschool children's language skills develop in the service of social knowledge and interaction. Different language communities value different linguistic skills (Heath, 1983), and 3- and 4-year-olds are improving at using language for different purposes (i.e., genres such as narrative, conversation, or teasing), in different contexts (e.g., home vs. school; mealtime vs. circle time) and with different interlocutors (e.g., siblings, peers, parents) (Dunn and Shatz, 1989; Dunn, 1996; Slomkowski and Dunn, 1996; Pan and Snow, 1999). Navigating these different contexts requires very flexible linguistic skills, and although preschoolers are not yet fully fluent, the preschool years bring great advances in the ability to use language appropriately in different situations.

2.30.5 What is Learned in Later Childhood

2.30.5.1 Learning the Nuances

A cursory survey of the child language literature indicates that children show basic fluency by 4 years of age and mastery of basic morphological and syntactic structures by about 5 years.

What remains to develop is the ability to apply basic linguistic knowledge in contexts that are more challenging or complex, or that require integration of linguistic and paralinguistic (and nonlinguistic) information within and between utterances. For example, Campbell and Bowe (1983) told children stories with a low-frequency homonym (e.g., during a car trip a "hare ran across the road"). Children were shown to interpret 'hare' in its dominant meaning (i.e., 'hair'), though this interpretation was nonsensical. Although children have difficulty learning homonyms (Doherty, 2004), and answering ambiguous questions (Waterman et al., 2000), this particular error involves integrating information across utterances in order to interpret (i.e., represent meaning of) a statement. Similarly, 6-year-olds have trouble flexibly attending to paralanguage and semantic content to interpret mixed messages (e.g., "My mommy gave me a treat" said in a sad voice); they tend to rigidly attend only on the most salient kind of information (Morton et al., 2003). This might explain older

children's difficulty understanding jokes, irony, and sarcasm. In general, as children get older they can make more precise and context-appropriate inferences about a speaker's meaning, while maintaining syntactic, semantic, and pragmatic coherence over longer passages of conversation or narrative. This expansion in the 'scope' of linguistic performance is seen in semantic, syntactic, and discourse processing.

2.30.5.1.1 Learning the nuances of relational semantics

Children sometimes have difficulty inferring the extensions of semantic relations, especially when novel words are involved. Class inclusion relations (inclusion, overlap, and exclusion) are among the simplest between categories (though not the only ones; e.g., Lakoff, 1987). Thus, the assertion "some fish are eaten" requires representing some overlap between two classes (fish and food).

Recall that by 3 years children can use familiar words in semantically appropriate ways (Deák and Maratsos, 1998). By 4–5 years they can infer the relation of a novel word to familiar ones based on class inclusion statements. For example if told "A *pug* is a dog" (where *pug* is novel), kindergartners usually infer that a pug must be an animal, but do not infer that a pug is a dog if told "A pug is an animal" (Smith, 1979). Still, the use of semantic information improves with age. Deák and Wagner (2003) attempted to teach children several novel words and the relations between them using class-inclusion statements. Four- and 5-year-olds learned few relations, whereas 6- and 7-year-olds learned most. It is unknown why older children are better at using direct input to learn semantic relations. Perhaps they sometimes analogize from familiar semantic relations (Johnson et al., 1997).

2.30.5.1.2 Learning complex online syntactic judgments

Another synthetic linguistic skill is interpreting syntactic relations in the 'real time' of conversation. Adults quickly and reliably determine when a sentence is irreparably ungrammatical, as from an agreement error. However, adults can also withhold judgments in the face of an ambiguous sentence until all 'legal' interpretations of syntactic structure have been checked. For example in an auxiliary omission error such as "Mrs. Brown working at the library called home to say she would be late," adults can withhold judgment until the end of the sentence (Blackwell et al., 1996). Children, by contrast, prematurely try to resolve syntactic ambiguities before parsing is complete.

For instance, Trueswell et al. (1999; Hurewitz et al., 2000) demonstrated that 5-year-olds prematurely resolve a noun-modifier clause (e.g., "Put the frog on the napkin in the bowl") as destination-marking prepositional phrase. That is, they interpret "on the napkin" as a destination marker, placing an isolated toy frog onto an empty napkin instead of putting a frog already on a napkin into a bowl. The error unfolds as children listen to the sentence, as shown by eye-movement analysis: whereas adults shift gaze to the frog on the napkin, 5-year-olds look at the incorrect (second) frog early and do not show awareness of the ambiguity of the modifier. Interestingly, 5-year-olds can in other contexts correctly produce the same syntactic structure. Thus even when children can produce complex syntactic structures, they may make on-line parsing errors.

Children's syntactic judgments also become faster from 6 to 10 years. Children in this age range are in general slower than adults at detecting violations of agreement or word order, and are relatively slower to notice violations early in a sentence rather than late in the sentence (Kail, 2004). Moreover, semantic incongruity within a sentence seems to distract 6-year-olds and keep them from noticing syntax errors (Windsor, 1999). Such findings suggest limitations of working memory or processing efficiency. Grammaticality judgments require holding several sentence constituents in memory, and increased processing speed and efficiency from 2 to 10 years (Kail, 1991), as well as increased verbal working memory capacity (Gathercole et al., 1992), should make syntactic processing faster and more reliable.

2.30.5.1.3 Learning the nuances: reference, pragmatics, and implicature

Syntactic judgments and constructions fundamentally involve pragmatic factors (Bates and MacWhinney, 1982). As children gain fluency, and adults expect them to maintain good discourse cohesion, they must master a wide variety of devices for maintaining good discourse cohesion: topic–introducing-and-shifting (e.g., "There was this guy. He. . ."), topic-continuing (e.g., "yeah, and. . ."), perspective-shifting (e.g., "No, *he* didn't do it, *she* did!"), etc. As these examples show, pronouns and generic descriptions are important elements of discourse (Karmiloff-Smith, 1979). Adults, for example, find it jarring to

continue to use unique individuation within a narrative:

> Chris and Heidi went to a new restaurant. The waitress asked Chris and Heidi if Chris and Heidi wanted drinks. "No," said Heidi. Heidi had already had some wine.

Preschoolers can use pronouns for coherent reference; for example they use simple cues (e.g., gender) to pick out a pronoun referent (Blakemore, 1990). Five-year-olds who lag behind in this ability show other narrative comprehension deficits (Cain and Oakhill, 1999; Yuill and Oakhill, 1991). By 7 years, however, children select and substitute pronouns in more pragmatically appropriate ways (Lloyd et al., 1995; Hickman and Hendricks, 1999). Specifically, the ability to use an interlocutor's knowledge to select unambiguous referential terms develops from 4 to 7 years (Ackerman, 1993), during the same period when they improve at drawing inferences about a speaker's meaning based on nonliteral semantic and discourse implications (e.g., Özçalıskan, 2004).

2.30.5.2 From Fluency to Flexibility and Meta-Language

2.30.5.2.1 Cognitive flexibility in child language

Each of the linguistic achievements of late childhood involves greater precision of interpretation or production. This precision requires representing different perspectives (Clark, 1997), which in turn requires representational flexibility (Deák, 2003). Flexibility involves processes including shifting attention, generating/selecting new representations, suppressing prior cues and associations, etc. Deák (2000b; 2003) found that children's flexibility in using cues to infer novel word meanings develops from 3 to 6 years, and individual differences in flexibility predict vocabulary, but are unrelated to children's ability to inhibit lexical associations. One interpretation is that word-learning flexibility is independent of some related cognitive control processes, but nevertheless predicts word-learning efficacy. A significant question is whether the same kind of cue-using flexibility is used by children to make complex syntactic and discourse interpretation. There is as yet no evidence addressing this question.

Cognitive flexibility encompasses children's growing ability to formulate and select appropriate but nonobvious representations of a referent or sentence in light of contextual information. For instance, interpreting /har/ as a synonym for rabbit, not hair (Campbell and Bowe, 1983), requires flexibility and selectivity in retrieving alternate word meanings. Such sorts of cognitive control are prominent in mature language abilities.

Some claims about the development of cognitive flexibility have focused on limitations on cognitive resources such as working memory and inhibitory processes (Diamond, 1998). Evidence for these claims is mixed at best (Deák and Narasimham, 2003; Zelazo et al., 2003), but there is so little research on effects of working memory and inhibition on flexible representations during language processing that the matter is unresolved.

Another idea is that cognitive flexibility, including flexible language processing, rests on children's developing ability to coordinate multiple response-contingencies in their response selection (Zelazo et al., 2003). For example, Zelazo et al. (2003) claim 3-year-olds cannot use a two-level hierarchy of verbal rules to guide classification responses. Three-year-olds readily sort cards by either of two rules (color or shape), for example, but when asked to switch from one to the other they continue to follow the first rule (Zelazo et al., 1996). Is the problem their inability to handle the complexity of a hierarchy of rules? It seems 3-year-olds use quite complex linguistic contingencies to formulate or interpret syntactic utterances, at least in ideal circumstances (e.g., Bates and MacWhinney, 1982; Slobin, 1982), so it is difficult to assimilate natural language performance into Zelazo et al.'s (2003) theory. However, there is some evidence that children who do not flexibly respond to changing rules can benefit from semantic and pragmatic support (Munakata and Yerys, 2001; Kirkham et al., 2003). Also, studies of feedback suggest that children's errors are based on misunderstanding the rules or failing to notice rule-switch cues (Bohlmann and Fenson, 2005), consistent with the argument that cue comprehension is a critical factor in children's linguistic flexibility (Deák, 2003). Although it remains unclear how late-developing language skills intersect with the development of cognitive control, the two are not strongly correlated in individual 4- and 7-year-olds (Brophy et al., 2002), suggesting some dissociation.

2.30.5.2.2 Becoming an expert language user

As children's language skills become consolidated, they become faster and more accurate, especially when processing or producing more complex and

novel utterances. This is hardly surprising but it raises key issues. One is that there are no neurobiological accounts of later language development. This is surprising given recent findings that fairly brief language training interventions can measurably change children's neural activity during language processing. This indicates prolonged neural plasticity (Shaywitz et al., 2004).

Another issue is that the large literature on expertise acquisition (Feltovich et al., 2006) is disconnected from the literature on later language development – which might be considered a nearly universal type of human expertise. The result of this disconnect is an odd conceptual separation of similar phenomena. For example, children acquire expertise in phonology such that lexical representations are organized in phonological similarity neighborhoods (Luce and Pisoni, 1998), which have characteristic perceptual expertise effects (Vitevitch, 1997). Five- to 7-year-olds have fewer similarity neighborhoods than adults (Charles-Luce and Luce, 1990), but these become refined with phonological and vocabulary development. Another near-universal form of human expertise, face processing (Gauthier et al., 1999), is acquired in ways that reveal plasticity and input-driven effects. It is likely that in the next decade language expertise, like face processing and other examples of childhood expertise, will no longer be viewed in outmoded nativist terms, but as a complex, emergent product of input-expectant learning.

2.30.5.2.3 Knowledge about language

Older children develop metalinguistics, or the ability to reflect on language (Gombert, 1992). Metalinguistic awareness focuses on dissociation of representations, as between a word as an action and as a symbol of whatever it represents. For example children might have trouble judging which is a longer *word:* 'mosquito' or 'cow,' because they conflate the words with their referents. Metalinguistic development might facilitate discourse facility (e.g., Morton et al., 2003): to the extent that specific lexical and syntactic acts underspecify a speaker's meaning, the ability to reflect on speech acts *per se* can help children understand nonliteral language (e.g., irony, sarcasm, or figurative language; Levorato and Cacciari, 2002).

Young children's metalinguistic knowledge has been tested in synonym and homonym usage. Doherty et al. (2004) found preschoolers' ability to identify homonym word pairs (baseball bat vs. flying bat) improved from 3 to 4 years of age and predicted understanding of false beliefs (i.e., inferring that another person can have an incorrect belief) even when vocabulary development was controlled. This suggests that metalinguistic knowledge develops in conjunction with other meta-representational skills.

How does metalinguistic knowledge develop? Older preschoolers show a tenuous association of printed word to referential meaning (Bialystok, 1997). However, this seems to improve with bilingual experience, possibly because bilingual children have more experience dealing with the abstract nature of linguistic representations as they switch codes to talk with different people (Bialystok et al., 2000). However, this argument is tentative, as there is so little research on the development of metalinguistic knowledge.

2.30.6 Conclusions

Three critical positions have been alluded to above, and these are central to the ongoing study of child language learning.

First, as advances in neuroscience fundamentally change our understanding of human cognition, they challenge persistent myths and assumptions about language. Basic findings about the developing bases of language in the brain, including the plasticity of language development (Bates et al., 2003), render ideas like Chomsky's 'language acquisition device' quaint. The growing sophistication of computational simulations of language learning support neurally plausible accounts of language development. However, because methods for measuring neural function and change in infants and children are so limited, much remains to be discovered.

Second, despite extensive use of terms like 'syntax,' 'semantic,' 'morphology,' and 'discourse,' these are conveniences based on historical convention in linguistics. Though there do seem to be some aspects of nearly pure syntactic knowledge, for example (Maratsos, 1998), more typical are complex interrelations among aspects of linguistic knowledge (e.g., Hay and Baayen, 2005). For example, there are no *a priori* neural dissociations between syntax and semantics (Bates et al., 2002). The interrelatedness of linguistic knowledge can be shown in children as well as adults. An intriguing question is how neural and psychological specialization of various aspects of language emerge during development.

Finally, research on different populations, including infants and children with various developmental

disorders, and adults with neurological and sensory deficits, and a wide range of languages, will be necessary to understand typical language development. Studies of communicative learning in nonhuman species, and of nonlinguistic learning in humans (e.g., Childers and Tomasello, 2003) are also necessary. Despite the challenges of synthesizing such a vast range of research, the history of child language research clearly shows that a myopic focus on competent, healthy, educated English speakers leads to mistaken assumptions about the nature of language and language learning.

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2.31 Transfer and Expertise: The Search for Identical Elements

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2.31.1 Introduction

Imagine you are traveling to London from the United States for the first time in your life. Having arrived at London's Heathrow Airport, you rent a car and take to the road. What is going to happen? The answer to this question is central to the issues treated in this chapter. In contrast to the United States, cars in England are driven on the left, rather than on the right side of the road. In addition, the driver's seat is on the right side of the car, and the stick shift is to the left of the driver. Thus, although the skills of driving a car in the United States and England share many common features, they are also quite different. One might ask, then, how well a person will be able to drive in England, assuming that the person has driven a car before in the United States. These questions address the issue of 'transfer.'

Transfer is always considered relative to a baseline condition. For example, one might ask how an experienced American driver compares to an experienced native English driver when driving in London. In all likelihood, the American driver will perform less well than the English driver, thus demonstrating less than perfect transfer from the American situation to the English situation. Alternatively, however, one might also ask how an experienced American driver compares to a novice English driver. In that case, the American driver will probably outperform the English driver, thus demonstrating 'positive' transfer from the American to the English situation.

According to a well-known definition by Ferguson, 'transfer' refers to

> any effects resulting from repetition, in the ability to perform a specified task, either the same task under different conditions or a different task. (Ferguson, 1954: 99)

Transfer is thus concerned with any effects that performing one task (original task) has on the subsequent

performance of the same task under different circumstances or of a different task (transfer task).

Ferguson's definition of transfer makes it obvious that transfer is a very broad concept that relates to a large number of meaningful research questions. For example, does knowledge of Latin facilitate or impair the learning of mathematics? Or does listening to music by Mozart improve or not improve spatial reasoning? To get a better handle on the large variety of different questions and situations that transfer relates to, Perkins and Salomon (1992) suggested six dimensions that can be used to structure the domain of transfer: (1) positive versus negative transfer, (2) general versus specific transfer, (3) near versus far transfer, (4) vertical versus horizontal transfer, (5) literal versus figural transfer, and (6) low-road versus high-road transfer.

For example, transfer might be positive (facilitating) or negative (detrimental), and it might be general or specific. Transfer is positive if performing one task improves subsequent performance on a second task; transfer is negative when performing an original task leads to subsequent worse performance on the transfer task.

Transfer is said to be specific if only particular aspects of, rather than general attitudes toward, the original task affect the acquisition of a new task. In contrast, transfer is said to be general (or nonspecific) if it results from warm-up and learning-to-learn effects. Warm-up effects refer to the attainment of various sensory, postural, and attitudinal adjustments that are needed to tackle a task (e.g., adjusting eyes and head for proper viewing of items displayed on a computer screen, adapting to pacing conditions, etc.). These adjustments are usually made relatively early during task practice and, once made, need not be made again. Learning-to-learn refers to the attainment of general task procedures which, once activated, may then be applied to a subsequently encountered task situation. Examples include skill in detecting stimuli on a computer screen and discovering procedures that ease a mental calculation task. As will become evident later, in current information-processing theories the distinction between general and specific transfer is increasingly difficult to maintain. Different types of transfer (i.e., positive and negative transfer, or general and specific transfer, or near and far transfer) may well occur together, as when certain aspects of the original task facilitate the acquisition of the new task while other aspects hinder it.

As a psychological topic, 'transfer' has both a long and a controversial history. Theoretical discussions of transfer can be traced back to at least the early writings of Greek philosophers, such as Aristotle and Plato. At the time, transfer was an important concept because it helped to explain why a mind that was born blank (i.e., blank slate) was nevertheless capable of acquiring knowledge from the beginnings of its existence (c.f., the later writings of British Empiricists, such as Locke and Reid).

More recently, transfer has become a highly controversial topic for at least two different reasons: empirical and theoretical. Empirically, so-called 'far' transfer, that is transfer across conceptually different domains, has been notoriously difficult to obtain. Consequently, some theorists (e.g., Detterman, 1993) have even argued that transfer, at least transfer that has any practical implications, does not exist (but see, e.g., Halpern, 1998; Barnett and Ceci, 2002).

Theoretically, different conceptualizations of how the human 'mind' works have tended to foster radically different and incompatible views on the mechanisms underlying transfer that have been heatedly debated over the course of the past 100 years. To understand this point, it is necessary to realize that transfer is a higher-order phenomenon. That is, to explain transfer one need not only describe the mechanisms by which transfer is achieved but also the mechanisms underlying performance and learning in the two tasks between which transfer occurs. In other words, the theoretical explanation of how original and transfer tasks are learned and are performed constrains the theoretically possible explanations of how transfer is achieved.

2.31.2 Goals and Structure of the Chapter

In many contemporary texts on transfer, the main focus is on summarizing empirical findings that elucidate the conditions that lead or do not lead to different types of transfer. Thus, for example, it may be argued that transfer is affected by two classes of variables that relate to (1) the encoding of the original task, and (2) the retrieval of the transfer task. Variables affecting transfer via the encoding of the original task are, among many others, (1) the degree of learning of the original task, (2) the differential use of learning strategies, (3) the number and variability of shown examples, and (4) abstract training. Variables that affect transfer by increasing or

decreasing the likelihood that a transfer task is retrieved include (5) the similarity of surface and structural components of the original and transfer tasks, (6) the similarity of processing in the two tasks, as well as (6) the provision of hints (for an excellent recent summary of the variables that have empirically been shown to affect transfer, see, for instance, Kimball and Holyoak, 2000).

In contrast to this empirical focus, the focus of the present chapter is primarily theoretical. That is, our main focus is on discussing some of the most important theoretical conceptions of transfer that have been offered in the past 100 years. Empirical data are described only to the extent that they further understanding of the theoretical conceptions. There are two reasons for why we focus on theories rather than on empirical findings. First, there already exists a number of excellent recent summaries of empirical research on transfer (e.g., Kimball and Holyoak, 2000), and little is gained by providing yet another summary chapter. Second, we strongly believe that the results of empirical research on transfer can only be understood in the context of a firm and clear theory, and that a deep and thorough understanding of transfer is more likely to result from a discussion of theories than from a summary of empirical research findings.

The chapter has thus two goals. Our first and main goal is to present an overview of important theories of transfer that have been developed in the past 100 years. By comparing older with more recent theories, it will become very obvious in which way and why our understanding of transfer has changed, and where we are currently heading.

Our second goal is to discuss the relation between transfer and expertise, that is, the question of whether or not expertise in any given domain modulates transfer. Although perhaps surprising at first glance, this specific question is a natural consequence of our statement that transfer can only be understood in the context of performance and learning theories. If indeed an understanding of how tasks are performed and are learned in the first place necessarily precedes the formulation of a transfer theory, then it is at least not unreasonable to suspect that transfer might be qualitatively different at the beginning and advanced stages of learning.

We begin with a brief overview of the two most important theories on transfer that existed at the beginning of the twentieth century: the Doctrine of Formal Discipline and the Theory of Identical Elements. Second, we discuss, in some detail, transfer theories that are based on the notion of 'identical elements.' Not surprisingly, we show that what has been termed 'identical elements' varies widely across theories. Intertwined with the description of the transfer theories, we discuss the relation between transfer and expertise. Next, we return to the original idea of the Doctrine of Formal Discipline and argue that derivates of this idea are still heatedly debated in present-day psychology. Finally, we briefly summarize the current state of affairs and offer some conclusions.

2.31.3 Transfer Theories at the Beginning of the Twentieth Century

One of the first theories of transfer that not only was taken seriously by philosophers of mind but was widely applied to school settings as well has come to be known as the Doctrine of Formal Discipline (Locke, 1693). The Doctrine of Formal Discipline was based on a meta-theory of the human mind according to which the mind is divided into a number of general faculties, such as attention, reasoning, and memory (faculty psychology). Each faculty can be likened to a muscle that can be trained in a variety of ways. Improved muscle strength (e.g., improved reasoning ability) then benefits all mental tasks that require the specific muscle or faculty. Thus, studying mathematical problems, for example, not only improves mathematical ability but also philosophical thinking – at least to the extent that the two subject areas require the same reasoning faculty. Studying subjects such as Latin and geometry, therefore, is of pedagogical value because it serves to 'discipline' the mind.

The theory was literally uncontested for many years; Thorndike and Woodworth (1901a,b,c) were the first to provide strong empirical evidence against the Doctrine of Formal Discipline. In one of their experimental situations, for instance, they had participants estimate the areas of different-sized shapes and found little evidence of transfer of estimation from one shape to another. The mind, they concluded (Thorndike and Woodworth, 1901a: 248) is "a machine for making particular reactions to particular situations," and "spread of practice occurs only where identical elements are concerned in the influencing and influenced function" (Thorndike and Woodworth, 1901a: 249). With their Theory of Identical Elements, Thorndike and Woodworth proposed one of the most durable theories of transfer,

which laid the groundwork for most of the empirical and theoretical work that was to follow later.

Although the Theory of Identical Elements rapidly emerged as the most widely accepted principle describing transfer in the early years of the century and was believed to account for most of the available transfer data (e.g., Hunter, 1929; McGeoch, 1942), it was by no means without its critics. Orata (1928), for instance, criticized the Theory of Identical Elements for its inability to explain findings reported by Judd (1908) and proposed a mental model theory of transfer instead (for more recent elaborations on this topic see, for instance, Kieras and Bovair, 1984). In his study, Judd had two groups of primary schoolchildren throw darts at an underwater target. Only one of the groups had the principle of refraction explained to them. The groups performed equally well at the start when the target was submerged 30.5 cm. However, when the target depth was changed to 10 cm, the group that knew the principle of refraction performed better. Furthermore, there was no transfer from 30.5 to 10 cm in the group that did not have the principle of refraction explained. Although the actual group differences obtained were rather small, the pattern of this finding was later replicated by Hendrickson and Schroeder (1941).

At least part of the reason why Thorndike and Woodworth's Theory of Identical Elements has been so tremendously successful was that the theory was, on the one hand, general enough to cover many different task situations while, on the other hand, not too general to still be meaningful. The theory was really more a general framework than a specific model. What it was lacking (very obviously) was a clear definition of what 'identical elements' were, or even more basic, what the elements were in the two tasks or two skills between which transfer was to occur.

In the next section, we summarize historical and recent ideas on what 'identical elements' are. Most of the ideas have come out of the verbal learning and the information-processing traditions, which will be addressed in turn.

2.31.3.1 Verbal Learning Tradition

The domain of verbal learning has been one of the most carefully and thoroughly studied areas in modern psychology and, consequently, has generated a vast amount of theoretical and empirical information. For illustrative purposes, we begin our discussion in the area of serial verbal learning.

The objective in serial verbal learning is to learn a list of serially arranged words such that the list can be reproduced (in correct order) at a later point in time. The method of serial verbal learning was introduced by Ebbinghaus (1885) who arranged artificial verbal materials (nonsense syllables) into serial lists and read them to the beat of a metronome until they seemed just on the verge of being learned; then, he would look away from the sheet on which the list was printed, and would try to recite the list. Ebbinghaus's measure of learning was the amount of time it took him to learn the entire list; his measure of transfer was the percentage of time saved in relearning the same or similar lists.

2.31.3.2 Serial Learning Analysis

Ebbinghaus was not only the first to introduce the empirical method of serial verbal learning, but also the first to construct a theory for what is actually learned in serial verbal learning (Ebbinghaus, 1885). His theory was based on the ideas of British Associationists such as David Hume (1739/2000) and John Locke (1693). According to British Associationists, learning is viewed as the acquisition of connections, or associations, between the stimulus (S) and response (R) units that are indigenous to a given task. The basic requirements for the acquisition of associations are that the to-be-related S unit and R unit occur contiguously, that their contiguous occurrence be repeated, and that the evocation of the R unit be followed by some reinforcing event.

The basic principle of serial learning, according to Ebbinghaus, is that every item in a list of serially presented verbal items becomes associated with every other item, subject to two qualifications: First, the strength of an association between two list items varies inversely with their degree of remoteness, that is, with how far apart they are in the series. Second, forward associations, for any particular degree of remoteness, are stronger than backward associations. From these qualifications it appears that the basic mechanism that permits learning of a serial ordering is the formation of associations linking adjacent items in the forward direction. This chaining hypothesis was hardly considered hypothetical at all; it was almost self-evidently true to researchers between the time of Ebbinghaus and the late 1950s.

Given that Ebbinghaus's ideas of serial verbal learning were almost uncritically accepted until at

least the late 1950s, it is clear that most theories of transfer of verbal learning during this period had to be, and in fact were, based upon the concept of forward associations. If what was learned in serial learning were primarily the forward associations, then these associations had to be the identical elements, the basic components proposed by Thorndike and Woodworth that were to be transferred to a new task. Early evidence for this claim came from Ebbinghaus's own research with derived lists.

A derived list is one that contains the same items as a list learned earlier, but in an order that is altered in some prescribed, meaningful manner. These orders can be arranged such that associations of varying degrees of remoteness, forward or backward, are presumed to transfer from the original list to the learning of the second, derived list. A forward first-order derived list, for instance, is one formed by skipping one item in constructing the new list; thus the original list A-B-C-D-E-. . .-K yields the forward first-order list A-C-E-. . .-K. Similarly, second-order, third-order, etc., lists can be formed by skipping two items, three items, etc.

The derived list method offered Ebbinghaus a potent means for testing the validity of his theory. Specifically, he constructed a number of transfer lists having first-, second-, third-, and seventh-order degrees of forward remoteness. For each of these derived lists, he had learned the original list 24 h earlier. The results of the experiment were expressed as savings – how much more rapidly it was possible to learn the derived lists than the corresponding original lists – and confirmed the predictions of Ebbinghaus's theory: all conditions with regularly derived lists resulted in more savings than a random-order control condition. Furthermore, the closer the remote associations being transferred, the greater the savings.

Ebbinghaus's original derived list experiments were later harshly criticized on methodological grounds. The most serious concern was an objection raised by Slamecka (1964) to the procedure of skipping a regular number of items when deriving the second lists. Slamecka noted that with a regularity of this sort, participants might discover the rule for derivation and use the first list to mediate acquisition of the second, derived, list. In one of his own experiments, Slamecka (1964) compared the Ebbinghaus procedure with one in which the average degree of remoteness on a derived list was fixed, but the number of items skipped was variable. With this new procedure, Slamecka did not find positive transfer from the first to derived lists.

As a result of these and other criticisms, theorists became increasingly convinced that the serial methodology was not the method of choice for empirical work on transfer of learning and needed to be replaced with paired-associate learning. Although a stimulus-response analysis of learning was almost universally accepted at that time, with each item in the list serving both as a stimulus for the next item and as a response for the previous item, the stimuli and responses could not be manipulated independently of one another in an experiment. With paired-associate learning, however, stimulus and response similarity could easily be manipulated independently. Paired-associate learning, therefore, quickly became the method of choice for scientists working on theories of transfer.

2.31.3.3 Paired-Associate Analysis

2.31.3.3.1 One-component models

In paired-associate learning experiments, participants are asked to learn two paired-associate lists, one after the other. Of interest is the relation between the two lists in terms of which learning can be applied from the first to the second list. Using an alphabetic code, one may represent various transfer situations by a double pair where the first pair, A-B, denotes the first list, and the second pair denotes the second list. For example, in the A-B, A-D paradigm, the second list has the same stimulus units, A, as the first list, but different response units, D; that is, one must learn to make different responses on the second list in the presence of familiar stimuli. In the A-B, C-B paradigm, in contrast, one must make familiar responses in the presence of new stimulus units. The A-B, C-D paradigm is often considered to be a baseline, or control, paradigm because there is no deliberate similarity relation between the two lists, and duration of practice is typically identical for the two conditions.

Most of the original work on transfer of paired-associate learning, culminating in Osgood's (1949) transfer surface, described in the paragraphs that follow, was based upon the same theoretical framework that Ebbinghaus's model of serial verbal learning was based upon, namely, classical S-R theory. Consequently, transfer was explained in terms of the similarities of the S-to-R associations acquired in the context of Task 1 and Task 2. According to the logic of classical S-R theory, transfer between the tasks can be experimentally manipulated by varying

(1) the similarity between the S units of Task 1 and Task 2, (2) the similarity between the R units of Task 1 and Task 2, and (3) both the similarities between the S units and the R units in Task 1 and Task 2 simultaneously and independently.

To illustrate, let us consider the situation in which stimulus (S) similarity is varied (from A to A′ to A″; the prime indicates the decreasing similarity) and responses (R) are kept identical. In this case, associative strength from a given S-R pair of Task 1 is said to generalize to its Task 2 counterpart that maintains the same R unit. The extent of the generalization is dependent upon the degree of similarity between the S unit of Task 1 and the S unit of Task 2 and decreases monotonically with decreasing similarity. The underlying mechanism of transfer is said to be stimulus generalization.

Classical transfer theory predicts optimal positive transfer for the A-B, A-B paradigm, in which both stimulus and response are kept identical in Task 1 and Task 2; high positive transfer for the A-B, A′-B paradigm, in which the stimulus unit of Task 2 is closely related to the stimulus unit of Task 1; less positive transfer for the A-B, A″-B paradigm; and no transfer for the A-B, C-B paradigm. Transfer research has provided substantial support for most of these predictions. Positive transfer has been the usual outcome for the A-B, A′-B paradigm (for instance, Dallett, 1962), and the amount of positive transfer appears to decrease with decreasing intertask similarity between the S units (Dallett, 1962; Brown et al., 1966).

Most of the existing data on paired-associate transfer were summarized by Osgood (1949) in his transfer and retroaction surface (the term 'retroaction' refers to retroactive inhibition, the effect a second task can have in impairing retention of a first task). This three-dimensional graph, depicted in **Figure 1**, shows how much transfer there is to be expected between two tasks as a function of how similar they are in terms of their stimulus and response units. Assuming a constant first list, A-B, the various points on the graph show how much transfer is to be expected with different second lists. For example, if the second list is identical to the first, the A-B, A-B paradigm, there will be high positive transfer. If the stimulus units remain the same but require entirely new responses, as is true in the A-B, A-D paradigm, negative transfer results.

Several studies (e.g., Dallett, 1962; Wimer, 1963) have examined a fair sample of all the possible relations within the transfer surface. As one might expect,

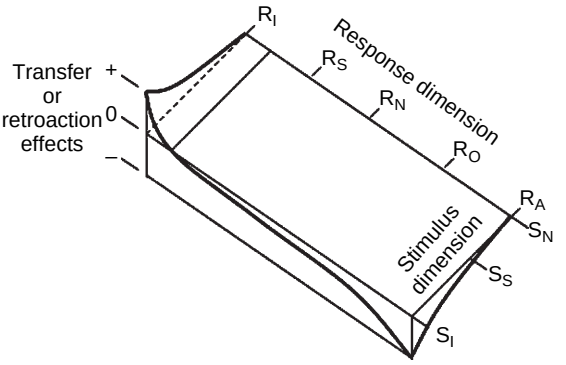

Figure 1 Osgood's transfer and retroaction surface. Note that the medial plane represents transfer effects of zero magnitude. Response relations are distributed along the length of solid and stimulus relations along its width. Adapted from Osgood CE (1949) The similarity paradox in human learning: A resolution. *Psychol. Rev.* 56: 132–143, with permission from Elsevier.

these experiments were rather large and complicated. One of them (Wimer, 1963), for instance, combined five degrees of stimulus similarity between tasks with five conditions of response similarity. In general, the broad outcomes of these studies are in agreement with each other and with the predictions of the Osgood surface.

Although classical S-R theory has been quite successful in predicting transfer for situations in which the similarity of S units and/or R units was manipulated between tasks, it has been much less successful in predicting transfer in a situation in which the S units of Task 1 are unrelated to the S units of Task 2 while the R units are still identical (A-B, C-B paradigm). Classical S-R theory predicts no transfer in this case. However, Twedt and Underwood (1959) found a trend toward negative transfer, and other investigators have found significant amounts of negative transfer (e.g., Kausler and Kanotti, 1963).

Furthermore, reasonably good success in explaining the direction and magnitude of transfer has been shown only for the paradigms the theory is equipped to handle. Excluded from consideration, for instance, are paradigms that fall outside these boundaries, such as the A-B, B-C paradigm, for which slight negative transfer has been repeatedly found (e.g., Murdock, 1958; Goulet and Barclay, 1965). Thus, in order to explain all possible transfer conditions, rather than only a few, classical S-R theory had to be expanded into what have been labeled multi-component theories.

2.31.3.3.2 *Multi-component models*

In multi-component models, it is assumed that the learning of paired-associate lists, and in general of any material, is a result not only of forward associative learning but also of backward associative learning, response learning, and stimulus learning (Martin, 1965; Kausler, 1966). Consequently, transfer may not only affect the acquisition of Task 2 forward associations but also any other of the proposed components. It should be clear that the addition of components, although not altering the basic logic of transfer, considerably complicates the analysis of transfer in paired-associate learning.

The direction and amount of overall transfer are now the combined result of the separate component effects. If negative transfer occurs for one component and zero transfer for all other components, then the overall transfer effect would necessarily be negative. However, if a second component contributes positive transfer, then the negative effect of the first component is diminished. Thus, predictions concerning overall transfer are based on the consideration of the specific effects of the separate components, with the amount of overall transfer simply being the algebraic sum of the amounts of transfer contributed by the separate components.

It should also become clear that the multi-component view of transfer is not compatible with Osgood's (1949) summary of transfer phenomena. Osgood's analysis is limited to situations in which learning consists only of acquiring the forward associative component. That is, Osgood's original transfer surface does not take into account familiarity with stimuli and/or responses. (Without going into details, we just briefly note that, in response to these problems, Martin (1965) called for a modification of Osgood's original transfer surface and proposed, instead, three different surfaces, one for each of three different components of transfer, namely, response learning, forward associations, and backward associations.)

2.31.3.4 Transfer and Expertise: Effects of First-Task Practice on Transfer of Paired-Associate Learning

In verbal learning theories of transfer, at least two different questions address the relation between transfer and expertise. First, one might wonder whether or not, in general, expertise facilitates or hinders transfer. That is, are experts 'better' or 'worse' at transfer both within and outside of their domain of expertise? To some extent, the answer to this question is trivial. Assuming that experts possess more knowledge in their domain than do nonexperts, they have, in terms of verbal learning theory, more stimulus representations, more response representations, and more associations at their disposal to transfer to a different task. Thus, they should be at an advantage in any situation that is similar to the situation they are expert at.

The question regarding potential benefits and costs of expertise, however, inevitably leads to a much more specific and theoretically much more compelling and important second possible question concerning the relation between transfer and expertise. In behaviorist single-component and multi-component views of transfer, this question boils down to how familiarity with or practice on the first task affects the content of transfer. That is, does expertise modulate what is transferred between two tasks? In multi-component views, this question can be much more complex and more difficult to disentangle than in the classical one-component approach. In classical S-R theory, to become more familiar with a task simply means that the forward associations grow stronger. Thus, familiarity with the first task enhances the effects of transfer, negative or positive, by increasing the strengths of S-to-R forward associations.

In multi-component approaches, however, the effects of familiarity can be selective; that is, the effects of familiarity can strengthen some components while not affecting others and vice versa. This added possibility makes research on first-task practice an elegant tool for testing the validity of multi-component views. Thus, prepracticing the stimuli but not the responses, for instance, will tend to make the stimuli more readily available for retrieval, but will not – or only minimally – affect the strength of the associations and the availability of responses. That is, by way of experimentally inducing familiarity with some components but not with others, the multi-component view of learning can be experimentally tested. Consequently, most of the studies in which practice on the first task was varied were primarily interested in proving their version of a multi-component learning theory, rather than in addressing the issue of whether the basic components of transfer change with practice on the first task. Nevertheless, these studies have provided some interesting insights and are briefly discussed.

Experimental studies on the effects of first-task practice on the acquisition of a second task can be classified in the following way: (1) studies in which

only the response units of Task 2 received practice in Task 1 (basically an A-B, C-B paradigm), and (2) studies in which only the stimulus terms of the second task were practiced in Task 1 (basically an A-B, A-C paradigm).

For example, as Martin (1965) points out, multicomponent views explain transfer in the A-B, C-B paradigm as depending largely on two factors: negative transfer due to backward interference and positive transfer due to response availability. Thus, with a low degree of practice, the strength of B-to-A (backward) associations carried over as the source of interference for the B-to-C associations should be relatively slight. On the other hand, even with a low degree of practice, response learning of the B units should be fairly complete by the end of Task 1 practice. Assuming the B units are of low meaningfulness (to avoid further complications), the positive transfer from response learning is likely to outweigh the slight negative transfer from associative learning, thereby yielding slight overall positive transfer.

With a high degree of Task 1 practice, however, the strength accrued for B-to-A associations should be sufficient to assure strong interference with the B-to-C associations. The negative transfer from backward associative learning should now outweigh the positive transfer from response learning (which is unlikely to increase beyond the amount manifested with a low degree of Task 1 practice), leading to overall negative transfer.

This balance of forces was nicely illustrated in a study by Jung (1962), in which he showed that with a low degree of Task 1 practice there was a trend toward positive transfer, while with a high degree of Task 1 practice the trend shifted to negative transfer. Similar results were obtained in a study by Schulz and Martin (1964) in which familiarity with Task 1 response units was achieved not via amount of practice but via degree of response meaningfulness.

It appears then that within the framework of multi-component views of transfer, the effects of Task 1 practice on the performance of Task 2 can be readily explained by introducing a strengthening mechanism: With practice, stimuli and responses become more easily available and associations become stronger. Importantly, the components of transfer do not appear to change with practice at all. Instead, what changes with practice is simply the strength or availability of transfer components, but not the nature of transfer.

2.31.4 Information-Processing Theories

Most of the theoretical positions we review next (Johnson; Kieras, Polson, and Bovair; Singley and Anderson), although differing on the specifics, share a common assumption: The acquisition of a task consists of separate learning stages that result in a multilevel hierarchy of organization. The basic idea conveyed by this hierarchy, and the one that fundamentally sets these new theories apart from earlier stimulus-response views, is that higher-level units are not simply associations of lower-level units, but are functionally distinct from lower-level units and can be accessed and used independently.

Two groups of nested-hierarchy theories differ on how this hierarchical organization comes about and in how it is used. The 'nonassociative recoding' position (Crowder, 1976), first articulated by researchers like Jensen, Bower (e.g., Trabasso and Bower, 1968), and Johnson (1969), relies primarily on information-processing machinery; the 'multilevel-associative' positions of Hebb (1961) and Estes (1972) stay within the associations-through-temporal-contiguity framework, but escape the limitations of the approach.

In the following, we concentrate on the 'nonassociative recoding' positions simply because their development has led directly to the modern conceptions of transfer. Starting with Johnson's classic coding theory, we then turn to the more recent approaches taken by Kieras, Polson, and Bovair, and by Singley and Anderson.

2.31.4.1 Johnson's Coding Theory

Although originally developed to explain recall of sentences, Johnson's coding theory (Johnson, 1969, 1970, 1972, 1978) presents a rather elaborate model of how the process of grouping lower-level knowledge units into higher-level units is established during learning and how higher-order units are used in task performance. Coding theory is based upon the concept of recoding that was originally developed by Miller (1956). Miller proposed that when participants are presented with items that are to be remembered, they can increase their recall by recoding subsets of items into higher-order units.

Johnson calls the memorial representation of a collection of items that are grouped together a 'code.' The specifics of coding theory can be summarized in the following five points. First, the main

theoretical property of codes is that they are unitary. They are single memory devices that can represent a number of individual items (i.e., memorial representation of a group of items). Because codes are unitary, they are recovered from memory in an all-or-none fashion; that is, once a code is recovered, all the information the code represents is recovered.

Second, codes are distinct from the information they represent. This distinction between code and content is analogous to the one often made between a category and the items from the category.

Third, an important implication of this distinction is that codes may be viewed as if they were opaque containers. That is, if the code and the represented information are distinct, then the recall of codes might be largely independent of the recall of the information represented by the code. For example, a participant might feel confident that she can remember a free-recall list because she remembers that it contained instances of birds, trees, and flowers. However, at the time of recall, she might discover that when she attempts to decode the category labels into their appropriate instances she is unable to remember the exact items that appeared in the list.

Fourth, codes can represent either individual items or other codes. Hierarchical coding occurs when the codes at one level of organization are recoded into higher-level codes. Note that any given set of items can be recoded into very different organizations. The sequence SBJFQLZ, for instance, could be organized by including SBJ in one chunk, FQLZ in another, and then recoding the codes for those two chunks into one higher-order code representing the entire sequence. Alternatively, the sequence could be organized into codes for three lower-order chunks like SB, JFQ, and LZ, and then the codes for these three chunks could be recoded into a code representing the entire sequence. For any given set of items, there exist many different organizations that can be imposed.

Finally, to allow for correct sequential retrieval of codes and items within codes, Johnson assumes that codes at any given level in the hierarchy and items within codes are distinguished by order tags.

The implications of Johnson's coding theory for an understanding of transfer should be fairly obvious. If learning a task is equivalent to learning a hierarchy of codes, rather than to learning a series of forward or backward associations, then codes, rather than associations, should be the identical elements, the components of transfer. Furthermore, what changes with practice and expertise is not simply the strength or availability of components, as in S-R, associative views, but the nature of the components themselves. With practice, lower-level codes will be recoded into functionally distinct higher-level codes.

Bower and Winzenz (1969) directly compared the S-R associative view of transfer with the code-based view in a study in which they repeatedly gave participants the same series of letter sequences. The Bower and Winzenz study was basically a replication of experiments by Hebb (1961) and Melton (1963) that had shown that the recall of a letter sequence was improved if it was repeated later in the session. Bower and Winzenz were able to show that this improvement from repetition occurred only if a sequence was grouped in the same way on both occasions. For example, if the series ABCDEFG was presented once with rhythmic grouping AB′CDE′FG and then later again with the grouping changed to ABC′DEF′G, then there was no improvement. However, if the same rhythmic breakdown was used on both occasions, then there was a gain from the first to the second trial.

Bower and Springston (1970) tested the same idea with prior experience from semantic, as opposed to episodic, memory. In their study, participants were asked to recall 12-letter strings that were broken into groups by pauses within the string. In some cases, the strings were broken by pauses so as to coincide with familiar acronyms, e.g., TV-IBM-TWA-USSR; but in other cases, the pauses did not coincide, e.g., TVI-BMTW-AU-SSR. Bower and Springston found that the facilitation occasioned by familiar acronyms was heavily dependent on whether the presented grouping corresponded with the acronym boundaries or not.

Note that in the Bower and Winzenz and Bower and Springston studies, the materials to be transferred from Task 1 to Task 2 were absolutely identical. That is, the effects reported could only be due to the differences in the organization of the materials. These studies, then, seem to demonstrate that codes, as defined by rhythmic grouping, rather than forward or backward associations, are transferred from one task to another. In a whole series of similar studies, Johnson and associates explored the role of codes in transfer of learning more systematically.

For instance, Johnson (1969, 1970) asked participants to learn a sequence of grouped letter triplets, such as SBJ′FQL′ZNG. Following the first-list learning, they were asked to learn another list that was identical to the first except that one letter in each of two groups was changed (e.g., SBJ′FQL′ZNG

changed to ′SXJ′FQL′TNG). After learning the second list, participants were asked to recall the first list.

The results of these experiments showed that the loss for the unchanged letters from a group with a change was about 50% relative to a rest control condition (no second list), whereas the loss of the unchanged letters in groups without changes was about 10% or less. These findings are consistent with Johnson's coding theory and do not seem to be readily explainable by an S-R–based associative view of transfer.

Note that the coding view also predicts that the amount of transfer is not affected by which particular letter in a group is changed and also that the number of letters changed in a group should have no relation to the amount of transfer. That is, if a middle letter from a group is changed, transfer is no different than if the first or the last letter is changed. Furthermore, whether one, two, or three letters in a group are changed should not affect transfer, either. Both of these predictions have been confirmed experimentally (Johnson, 1970).

These and similar experiments provide rather strong evidence for the view that codes, rather than associations, are the basic elements of transfer. Unfortunately, the effects of practice on transfer have never been experimentally explored. With practice, the theory assumes, functionally distinct higher-order codes are formed from lower-order codes. Therefore, the components of transfer are predicted to change. One reasonable assumption to make would be, for instance, that what is transferred from one task to another are only the (reusable) codes at the highest possible level of hierarchy, an interesting and also experimentally testable prediction.

The appraisal of Johnson's coding theory depends on what criteria one decides to apply. Johnson's accomplishments seem very impressive if one looks at those aspects of the theory that relate to the explanation and prediction of transfer phenomena. The experimental effects associated with the theory are both clear-cut and nonintuitive. However, Estes (1972), for instance, has complained with complete justification that, despite Johnson's (1978) later elaborations, the theory is primarily a metatheory. That is, it leaves many of the most fundamental processes – sequential retrieval, coding mechanisms, decoding mechanisms – underspecified.

One could argue that at least one of the reasons why the mechanisms of coding theory are relatively unexplored may have been the fact that information-processing machinery only later, with the introduction of production-system architectures, has developed the tools for a full-fledged process analysis. Next, we describe two recent information-processing approaches to transfer that, although different in their theoretical conceptualizations, are both implemented in a production-system architecture. Therefore, it will be useful to briefly describe the general properties of a production system.

2.31.4.2 An Overview of Production-System Models

Production systems have become an almost generic theoretical formalism in cognitive psychology. Numerous production-system models of cognitive processes have been developed. Examples include problem-solving models (Newell and Simon, 1972; Karat, 1983), models of text comprehension (Kieras, 1982), and models of learning (Anderson, 1982).

In general, production systems represent knowledge as a collection of rules. Processing mechanisms interpret these rules, thereby generating a sequence of cognitive operations and actions. In its most fundamental form, a production system consists of two data structures, a long-term declarative memory of facts and a production memory, that are connected through a processing cycle. The production memory can be described as a collection of IF-THEN condition-action rules (productions) of the form

IF (condition) THEN (action)

The processing cycle works as a three-stage recognize-act cycle. It matches the IF parts of one or more production rules (from production memory) with the contents of some active part of the declarative memory (often referred to as short-term memory or working memory), decides which of the matched rules to fire (conflict resolution), and executes the THEN part of the selected rules, resulting in either the activation, modification, or execution of an existing memory structure.

Note that the condition part of a production rule can contain information at very different levels of abstraction. For example, it might contain current goals or subgoals or information about the external task environment.

Production systems are usually hierarchically organized and goal-driven. That is, a sequence of production rules is organized such that the system moves from satisfying one subgoal to satisfying

another subgoal to satisfying a general goal and so on. This sequential, goal-driven behavior is modeled by having the production rule that just fired delete the subgoal accomplished and add a new subgoal to working memory. The sequential behavior of the model is, thus, controlled by manipulating the pattern of subgoals.

Next, we turn to two information-processing approaches that make direct use of the production-system formalism in their formulations of transfer theories. Both of these theories assume that production rules, rather than associations or codes, are the identical elements of transfer. Beyond this commonality, however, the two theories differ fundamentally.

2.31.4.3 Kieras, Polson, and Bovair's Theory of Transfer

Perhaps the simplest and most straightforward approach that any theory implemented in a production-system environment could take with regard to explaining transfer is the one taken by Kieras, Polson, and Bovair (e.g., Polson and Kieras, 1984, 1985; Kieras and Polson, 1985; Kieras and Bovair, 1986; Polson, 1987). Very much unlike the theories described so far, Kieras, Polson, and Bovair's approach to understanding transfer is not based upon a learning theory. Instead, these researchers, in essence, construct production systems for each of the two tasks involved in transfer, the original and the transfer task, and then examine them for the amount of production overlap by simply counting the number of productions that appear in both systems. The more productions the two tasks have in common, the greater the amount of transfer. Thus, according to this view, production rules are the identical elements of transfer that were postulated by Thorndike and Woodworth.

Production rules are transferred from one task to another if they are either identical or generalizable. They are identical if they have the same conditions and the same actions; they are generalizable when a condition can be derived from other conditions by replacing a constant with a variable. In fact, Kieras and Bovair (1986), for instance, note that in all their analyses of transfer, generalizations and identical rules were transferred equally quickly and were learned much faster than new production rules.

Note that transfer, in Kieras, Polson, and Bovair's view, is simply a matter of reusing old production rules in a new context. The model is simple and straightforward because it is based upon an essentially static representation of knowledge. All that is needed to predict amount and direction of transfer from one task to another is to construct two production systems that model task performances in the two tasks and to count the number of identical and generalizable production rules. Kieras, Polson, and Bovair take this approach even further by keeping the production systems they generate as simple as possible. As Kieras and Polson state, their production system is the

> . . . most elementary form of production system in that there are no conflict resolution rules, and very simple kinds of pattern matching are used in evaluating conditions. (Polson and Kieras, 1985: 207)

Likewise, they make no attempt to represent fundamental cognitive processes, such as memory retrieval or reading comprehension, and assume that each production takes the same amount of time to execute.

Note that, despite its simplicity, the identical-production-rules view of transfer has a clear advantage over classical associative theories of transfer: Productions are abstract entities that can be used to describe human behavior at different levels of abstractions. In associative models, stimuli and responses describe mostly overt behavior. Production rules, in contrast, can represent higher-order and lower-order goals as well as externally observable behavior or rule-based reasoning, etc. That is, they can be used to represent many different entities at various levels of generality. Thus, production rules are extremely flexible with regard to what they can represent.

Kieras, Polson, and Bovair have experimentally tested their model of transfer in various studies and in two different domains, namely, text editing (e.g., Polson and Kieras, 1985; Polson, 1987; Polson et al., 1987) and operating a simple device (e.g., Kieras and Bovair, 1986). The results of these studies demonstrate very clearly that production rules can, in fact, provide a very useful characterization of what is transferred from one task to another.

Kieras, Polson, and Bovair's approach to understanding transfer is best described as a cognitive engineering approach (Gray and Orasanu, 1987). As a general theory of transfer, however, the belief that the amount of transfer can be determined by simply counting productions can be criticized for at least two different reasons. First, production rules might differ with regard to how important they are in a particular system. Foss and DeRidder (1987) argue that productions that are higher up in the goal structure of a particular task might be more important for transfer than productions at a very 'late' level in the goal

structure. Thus, a change in the syntax between old and new systems may result in a substantial number of differences in the production rules that describe the behavior of the user, but if all these changes are 'late' in the goal structure, then they might not have the same impact on transfer as an equal number of changes in production rules that affect subgoals 'higher' in the goal hierarchy.

The second criticism that can be leveled against counting identical production rules is the one mentioned already, namely, the fact that this theory – because it is not closely tied to a learning theory – completely ignores the level of skill at which the original task is performed.

Both of these criticisms have been addressed in a theory of transfer that was formulated by Singley and Anderson (1985, 1989). These authors propose essentially an extension to Kieras, Polson, and Bovair's view of transfer that is heavily dependent upon Anderson's theory of learning (Anderson, 1982, 1983, 1986, 1987).

2.31.4.4 Singley and Anderson's Theory of Transfer

Like Kieras, Polson, and Bovair, Singley and Anderson (1985, 1989) propose that production rules are the common elements that are transferred from one task to another. Unlike Kieras, Polson, and Bovair, however, and very much in line with the theories discussed earlier, their view is based upon a powerful learning mechanism, implemented in Anderson's Adaptive Control of Thoughts (ACT*) theory of human cognition. In this learning mechanism,

> . . . knowledge comes in declarative form, is used by weak methods to generate problem solutions, and as a by-product, new productions are formed. The key step is the knowledge compilation process, which produces the domain-specific skill. (Singley and Anderson, 1989: 50)

This statement is central to ACT*'s account of learning. The theory breaks down the acquisition of skill into two major stages: a declarative stage, where a declarative representation of the skill is interpreted by general, nonspecific, problem-solving productions (such as analogy, means-ends analysis, pure forward search, etc.), and a procedural stage, where the skill is directly embodied in domain-specific productions. The transition from the declarative to the procedural stage is achieved by the process of knowledge compilation. Knowledge compilation consists of two separate mechanisms: the composition mechanism collapses sequences of general productions into a smaller number of highly specific productions (larger IF parts), and the proceduralization mechanism deposits domain knowledge from long-term memory directly into productions.

The initial use of weak methods and knowledge compilation are the main factors that influence the acquisition of domain-specific productions. However, two additional factors influence their execution, namely, strength accrual and working memory limitations.

The strength of a production determines how rapidly it applies. Production rules accumulate strength as they successfully apply; that is, as they are practiced and learned. Just as learning has an impact on production strength, it has an impact on working memory limitations; that is, working memory increases with practice. Because in ACT*, the only way errors can occur is through the loss of information in working memory (leading to the wrong production firing or the correct production firing but producing the wrong result), an increase in working memory will tend to decrease the likelihood of errors occurring.

Singley and Anderson's theory of transfer differs from Kieras, Polson, and Bovair's in two critical points. First, Singley and Anderson assign weights to the production rules shared by the two tasks, reflecting the frequency of their use. Production rules that are 'higher up' in the goal hierarchy will tend to be used more frequently than the more specialized productions at the lower end of the goal hierarchy and will therefore be assigned larger weights. Consequently, they will figure more prominently in transfer predictions than will productions at the lower end.

Second, Singley and Anderson assume that the components of transfer – production rules – change qualitatively with practice. According to the ACT* theory of learning, all productions arise initially from declarative encodings. With practice, the declarative component, however, will eventually become very small, and transfer will be predictable solely on the basis of procedural knowledge.

Singley and Anderson's theory of transfer makes four important predictions. First, during early stages of learning, transfer is a function of declarative and procedural knowledge. With practice, the impact of the declarative component will rapidly decrease, and

transfer will be a function solely of procedural knowledge.

Second, transfer of procedural knowledge is captured by the identical-production-rules theory. That is, the amount of transfer between two tasks depends directly on the number of shared productions in the two tasks.

Third, if the basic components of transfer are productions, then transfer has to be specific to the use of knowledge. This prediction is derived from the condition-action asymmetry of production rules. In particular, the conditions of a rule imply the actions but not vice versa. This property implies the use specificity of knowledge (that is, no transfer) in certain situations where two sets of production rules are based upon the same abstract knowledge but have been dedicated to different uses.

And finally, the theory predicts that negative transfer is minimal. If indeed transfer is determined by the number of shared productions (and that number can never be smaller than zero), then it follows that transfer can be negative only in cases where earlier learned conditions still apply in a new task but now lead to either wrong or nonoptimal actions.

In general, it can be said that the experimental evidence for all four predictions is strong. For instance, Singley and Anderson (1985, 1989) studied transfer among two line editors (ED and EDT) and a screen editor (EMACS). Twenty-four expert typists with no prior text-editing experience were taught a minimum core set of commands for each editor. In the line editors, the core set included commands for printing, deleting, inserting, and replacing lines, and substituting strings within lines. In the screen editor, the core set included commands for moving the cursor forward, backward, and up and down, and deleting characters, words, and strings.

Participants performed the above editing operations for 3 h each day and a total of 6 days. The EMACS group spent all 6 days on EMACS. All other groups spent the last 2 days using EMACS. The ED-EDT and EDT-ED groups spent 2 days on one line editor and the next 2 days on the other. The control group spent 2 days typing at the terminal.

Singley and Anderson split their analyses into two different parts, macroanalyses and microanalyses. Transfer data from the macroanalyses showed near total transfer between the two line editors, moderate transfer from line editors to screen editor, and slight transfer from typing-only to screen editor. The magnitude of predicted transfer (on the basis of relatively simple sets of production systems for the three editors) correlated to .98 with the magnitude of the observed transfer, representing an almost perfect linear relationship between the production overlap predictions and empirical measures of transfer. From these results, Singley and Anderson (1989) concluded that the massive amount of transfer between the two line editors was due to almost identical production systems at both the higher level and the lower level of goal hierarchies, whereas the moderate transfer from line editors to screen editor was primarily mediated by identical higher-order goal structures.

Microanalyses revealed that these general results could vary quite considerably for different subtasks of editing. Singley and Anderson split each unit task into various components of text-editing performance. Their results revealed (1) that a complex task like text editing can be decomposed into different component parts, (2) that each of these components seems to be learned and transferred separately and in accordance with the identical-production-rules theory, and (3) that various components of the task decompose rather cleanly into planning and execution phases, with transfer of planning being much more pronounced than transfer of execution, which can essentially be neglected. In general, this study strongly supported Singley and Anderson's (1989) claim that production rules are the basic components of transfer.

To test the prediction that transfer is confined to the same use of knowledge and does not occur between different uses of the same knowledge, McKendree and Anderson (1987) compared participants' ability in List Processing Language (LISP) evaluation skills. They gave twenty participants four consecutive days of practice in evaluating combinations of four basic LISP functions. Two functions combined items into a list (INSERT and LIST), and the other two extracted items from a list (CAR and CDR – assigned the more mnemonic names of FIRST and REST). Participants practiced 150 trials on each of the 4 days.

After the first and the last session, participants were given a transfer task that required them to generate functions similar to those they had just practiced evaluating. All of the transfer problems involved basic functions or pairs of functions that the participants had seen immediately before in the evaluation task. Yet, while error rates on the evaluation task decreased dramatically from Day 1 to Day 4 (35% to 15%), the transfer task showed little improvement (29.3% to 26.6%). Despite becoming significantly better at evaluating functions, participants on Day 4 were not any better at generating functions than they were on Day 1.

Singley and Anderson's (1985, 1989) theory is easily the best articulated, clearly specified, and most complete theory of transfer to date. By making full use of Anderson's ACT* learning mechanism, the theory is capable of generating interesting and counterintuitive predictions that have already been, and continue to be, subjected to rather thorough and extensive experimental tests.

2.31.5 Comparison of Theories of Transfer

2.31.5.1 The Content of Transfer

Older S-R-based views of what is learned when a task is acquired and what is transferred from one task to another differ fundamentally from more recent information-processing views. Classical S-R theory and its various descendants all have in common that the most important components of learning are assumed to be associations, whether forward or backward, between externally observable stimuli and responses. In contrast, more recent information-processing theories view learning as the acquisition of general memory structures that are capable of representing observable as well as nonobservable behavior (goals, operators, methods, etc.).

The transition from classical associative theories of transfer to the identical-production-rules view has not been one of degree but has been quite dramatic: Production rules are abstract entities that can be used to describe human behavior at different levels of abstractions. They represent higher-order and lower-order goals as well as externally observable behavior or rule-based reasoning, etc. Using production rules as the basic units of transfer is an entirely different ballgame than relying on associations, stimuli, and responses.

2.31.5.2 Effects of First-Task Practice/ Expertise on Transfer

A second important difference between the classical S-R view of transfer and more recent information-processing approaches relates to the effects of expertise on transfer. Classical S-R theory and its various descendants have clearly and unequivocally defended the continuous nature of transfer, characterized by strengthening of associations and increased accessibility of stimuli and responses as a result of increasing expertise. In sharp contrast, the information-processing theories of transfer discussed earlier (with the exception of the static approach taken by Kieras, Polson, and Bovair) have assumed that, because learning is a discontinuous process, transfer is discontinuous also. Thus, the elements of transfer change qualitatively with increasing experience and familiarity on the first task.

However, the assumption that different knowledge representations are transferred to a novel task when the first task has been practiced extensively versus when it has not been practiced extensively has so far not been directly and experimentally demonstrated. In fact, when Singley and Anderson, for instance, modeled text-editing performance in three different text editors, one of their simplifying assumptions was that "the transfer task has the same organization before and after a subject's exposure to the training task" (Singley and Anderson, 1989: 251). This particular aspect shared by all modern theories of transfer, then, clearly is in need of experimental validation.

2.31.6 The Doctrine of Formal Discipline Revisited

Virtually all theories of transfer reviewed in this chapter are based upon Thorndike and Woodworth's Theory of Identical Elements (Thorndike and Woodworth, 1901a,b,c); that is, all assume that performance on a second task is (primarily) facilitated by reusing previously acquired specific memory representations. One might conclude then that transfer is primarily specific rather than general. Note, however, that the nature of what specific elements and general methods are has changed so substantially over the past century, as discussed, that it has become virtually impossible to differentiate between general and specific transfer. In fact, by including cognitive constructs as among the common elements that can be transferred, the more recent production system–based approaches have basically eliminated the old distinction between specific and general transfer. The entire task, including aspects of the stimulus and response as well as more general cognitive aspects, can now be described and analyzed under a unified system.

In the last section of the chapter, we briefly discuss some findings that have recently been offered in support of the notion of general transfer. The question we are mainly interested in answering is whether

or not these recent findings do indeed lend new credibility to the old Doctrine of Formal Discipline or can alternatively be explained in terms of production system–based transfer theories, as our previous discussion suggests.

One particular recent line of research maintains that practice on a task not only leads to domain-specific task knowledge but to general reasoning skill as well. For example, Lehman et al. (1988; Lehman and Nisbett, 1990) compared the effects of graduate training in psychology, chemistry, medicine, and law on methodological and statistical reasoning. The authors' results revealed positive transfer of medicine and psychology graduate training to the ability to solve reasoning problems in everyday-life situations. In addition, graduate training in law positively affected the ability to solve conditional reasoning problems whereas graduate training in chemistry did not affect any of these abilities.

Thus, the Lehman et al. findings appear to support the assumption of general transfer. In accordance with the Doctrine of Formal Discipline, the authors concluded from their findings that

> . . . rules about assessing causality, rules for generalization, rules for determining argument validity, and rules for assessing the probativeness of evidence . . . (Lehman et al., 1988: 441)

can be taught to improve general thinking skill (see also, Fong et al., 1986; VanderStoep and Shaugnessy, 1997).

More recent experiments (Shraagen, 1993; Schunn and Anderson, 1999) lend further support to the findings of Lehman and colleagues. Schunn and Anderson compared domain experts (cognitive science researchers), task experts (social and developmental psychologists), and domain and task novices (undergraduates) regarding their ability to design memory experiments. Results showed that domain and task experts differed in terms of their domain-specific knowledge and procedures, whereas experts and novices differed in terms of general skills such as the quality of hypothesis generation, data evaluation, developing experimental designs, or drawing conclusions. In addition, the authors ruled out that these general skills were related to IQ. On the basis of the SAT, they divided the novices in their study into those with high abilities and those with low abilities. The performances of the two groups did not differ from each other, but, overall, the two groups were less able to apply general rules than were the two expert groups. The authors concluded that expertise leads to general skills that can be transferred to tasks in unfamiliar domains. The quality of reasoning within a specific domain, however, depends on domain-specific knowledge and processes.

In a subsequent study, Harrison and Schunn (2004) tested scientific reasoning abilities in the domains of psychology and biology of near-experts (graduate students; biologists and psychologists) and novices (undergraduates of both subjects). The authors used a bidirectional transfer paradigm; that is, all participants received problems from biology and psychology. Harrison and Schunn's results again showed that general skills that were applicable in both domains developed with increasing expertise; that is, near-experts were better able to transfer the general skills to unfamiliar domains than were novices.

Again, these findings appear to be consistent with the Doctrine of Formal Discipline. Training in scientific reasoning leads to skill that supports reasoning in other scientific domains or in everyday-life situations. Although the transfer effects were small and transfer was far from complete, the findings suggest that, beyond domain-specific knowledge, experience in one domain also leads to general skill that can facilitate problem solving in other domains. In accordance with findings by Ceci and colleagues (Ceci and Liker, 1986; Ceci and Ruiz, 1992, 1993), this effect of expertise is not related to IQ.

However, it can be shown that the findings described are consistent with and can be explained by production system–based transfer theories. Schunn and Anderson (1999), for instance, argue that participants acquire general search heuristics or production systems when they practice tasks in a domain as well as knowledge (declarative facts in ACT-R) that is used by the search heuristics. Some of the general heuristics that are implemented in production rules can be applied in other than the specific domains in which they have been acquired. This is the case if the new domain provides knowledge that the productions can use. That is, transfer is possible when previously acquired production rules are applicable to knowledge structures in the new domain – in other words, when the new and the old domain share identical features.

Furthermore, the production rule–based transfer theories assume that expertise leads to the strengthening of specific production rules and to the weakening of other rules (e.g., Anderson and Lebière, 1998). This, in turn, might lead to specific preferences in terms of

how to approach a given problem. Domain-specific expertise thus shapes how one interprets a given situation and this influence might increase with increasing expertise (e.g., Bransford and Schwartz, 1999; Chen and Klahr, 1999; Harrison and Schunn, 2004; Lehman and Nisbett, 1990). Interestingly, this assumption would predict that expertise in one domain might change how one approaches problems in a new, unfamiliar domain. This effect is what Schwartz et al. (2005) might have had in mind when they proposed to expand the definition of transfer to include 'preparation for future learning.'

The domain-general effect of expertise resembles well-known findings in the field of insight problem solving (e.g., Luchins, 1942; Maier, 1931; Wertheimer, 1959) or of perceptual learning (e.g., Gibson and Gibson, 1955; Goldstone, 1998). For example, in insight problem solving, participants' previously acquired knowledge can prevent their generating the 'correct' mental representation needed to solve the problem at hand (e.g., Knoblich and Oellinger, 2006).

Overall, the recent findings suggest that some general transfer exists. However, rather than supporting the assumption that just about **any** mental activity at Time 1 improves just about **any** subsequent mental activity at Time 2, as was suggested by the Doctrine of Formal Discipline, these findings can be explained in terms of production rule–based transfer theories.

2.31.7 Summary and Concluding Remarks

Our main goal in this chapter has been to describe and compare important theories of transfer that have been offered in the psychological literature in the past 100 years. It should be fairly obvious that the nature of transfer theories has changed dramatically from the early days of the Doctrine of Formal Discipline and the Theory of Identical Elements to behaviorist conceptions of transfer and to present-day production system–based conceptions. Currently, the production rule as the unit of transfer provides a quasi-standard way of describing what the identical elements of Thorndike and Woodworth are. As has been argued, the transition from the earlier associationist to a production system–based theory of transfer has not been gradual but has been fairly dramatic. Along with this transition, the earlier distinction between specific and general transfer has lost much of its appeal and meaning. In today's theories, production rules can represent every conceivable piece of information, and whether it refers to observable or unobservable behavior makes little difference.

Being able to specify Woodworth and Thorndike's identical elements is one thing; however, being able to describe and understand the actual process of transfer is a completely different thing. Note that, as the currently dominating theories of transfer, such as Singley and Anderson's, are formulated, they do not capture the actual process of transfer. It is simply not very likely that when they are confronted with a transfer situation, participants create memorial representations of two tasks and then match them against each other. This scenario might help to predict the amount of transfer between two tasks; it does not capture the process of transfer. It seems much more realistic to assume, for instance, that a second task is not represented in isolation in memory when learned, but instead is represented in terms of its deviations from the original task.

In this regard, Polson et al. (1987) describe an interesting process model of transfer in terms of a repair theory. They propose that transfer between overlapping complex skills is largely a repair process where the representation of the first skill is 'edited' in order to reflect the new demands of the transfer task. This repair process is composed of three steps: determining which elements of the old skill need to be repaired, that is, which elements are no longer valid in the transfer task; determining what the new, replacement elements should be; and making the repair.

Polson et al. point out that, in transfer environments that offer both instruction and feedback, the first two steps in the repair process are greatly simplified. However, in situations where the transition between tasks is poorly defined and must be discerned by the participants themselves, interference may be introduced. Indeed, the classic Einstellung phenomenon (Luchins, 1942) can be described as a transfer situation in which the changing demands of the transfer task have not been pointed out explicitly to participants.

Another dramatic demonstration of this effect in a different setting is the classic part-whole negative transfer effect in verbal learning (Tulving, 1966; Sternberg and Bower, 1974). The basic phenomenon is that participants experience negative transfer when learning a second list of words after learning an initial list that is either a subset (part-whole transfer) or superset (whole-part transfer) of the transfer list if

they are uninformed about the relation between the first and second word lists. However, if participants are explicitly informed that the transfer list either is a part of or contains the training-list words, the negative transfer turns to strong positive transfer, which is what one would have originally predicted on the basis of an identical elements approach.

Polson et al.'s repair theory has not been extensively tested and can only be regarded as a very rough first approximation of the actual process of transfer. However, it appears to be a rather safe bet to assume that research on the process of transfer will trade places with research on the basic elements of transfer in the very near future and will become one of the most important experimental battlegrounds for theories of transfer.

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2.32 Implicit Learning

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2.32.1 Introduction

All of us have learned much without parental supervision and outside of any form of planned academic instruction, and more generally without any intentional attempts to acquire information about the surrounding world. Countless examples could be found in domains as diverse as first-language acquisition, category elaboration, sensitivity to musical structure, acquisition of knowledge about the physical world, and various social skills. All of these domains have several features in common. In particular, they are commonly described as governed by complex abstract rules by scientists, whether they would be linguists, musicologists, physicists, or sociologists. Also, learning in those situations mainly proceeds through the learner's exposure to a structured environment, without negative evidence (i.e., without direct information about what would contradict the rules underlying the domain).

Despite the pervasiveness of these forms of learning in real-world settings, it is worth stressing that they have been virtually ignored by experimental psychology for decades. At the beginning of the cognitive era, the study of learning was essentially devoted to classical and operant conditioning on the one hand, and to the formation of concepts or problem solving processes on the other. The above phenomena seem hardly reducible to simple conditioning effects in regards to their complexity, and research on concept learning and problem solving does not provide *a priori* a better account, primarily due to the fact that the hypothesis testing strategies essential in these research domains do not seem applicable in situations where negative evidence is lacking. This empirical and conceptual vacuum opened the door to the upsurge of the nativist perspective, which characterized the cognitive approach from its outset.

This chapter presents a stream of research that is primarily aimed at exploring the forms of learning illustrated in the examples above through laboratory situations involving arbitrary materials (for overviews, see Berry and Dienes, 1993; Berry, 1997; Cleeremans et al., 1998; French and Cleeremans, 2002; Jimenez, 2003; Perruchet and Pacton, 2006; Reber, 1993; Seger, 1994; Shanks, 2005; Stadler and Frensch, 1998). This field of research evolved essentially from the end of the 1980s, although its roots are in the pioneering studies of Arthur Reber, who coined the term 'implicit learning' (IL) about 40 years ago (Reber, 1967). The implications of the results issued from IL research for the nativist/empiricist debate will be addressed in the final discussion, after having examined what is learned in this context, how 'implicit' is implicit learning, and the relations of laboratory research with real-world situations of learning.

2.32.2 Rules, Instance-Based Processing, or Sensitivity to Statistical Regularities?

2.32.2.1 Learning Rules

A large part of the literature on IL exploits the artificial grammar learning paradigm, initially proposed by Reber (1967). Participants first study a set of letter strings generated from a finite-state grammar that defines legal letters and permissible transitions between them (**Figure 1**). Typical instructions do not mention the existence of a grammar and are framed so as to discourage participants from engaging in explicit, intentional analysis of the material. Participants are then subsequently informed about the rule-governed nature of the strings and asked to categorize new grammatical and nongrammatical letter strings. Participants are typically able to perform this task with better-than-chance accuracy, while remaining unable to articulate the rules used to generate the material. This empirical outcome has been unambiguously confirmed by a vast number of subsequent experimental studies involving many variants of the situation.

Reber's (1967) original proposal was that participants have internalized the constraints embodied by

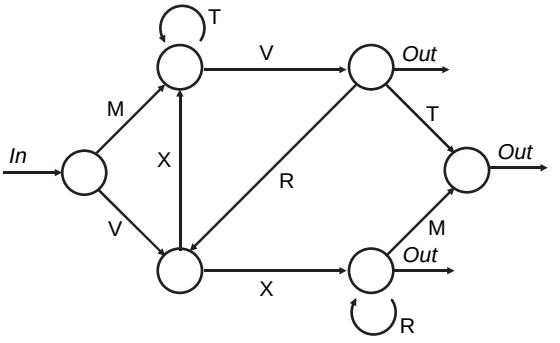

Figure 1 The artificial grammar used by Reber and Allen (1978), Dulany et al. (1984), and Perruchet and Pacteau (1990) among others. For example, MTTV and VXVRXVT are grammatical, whereas MXVT is not grammatical.

the generation of rules during training. Rule abstraction is assumed to occur during the study phase, when participants are exposed to a sample of letter strings generated from the grammar. During the test phase, participants are assumed to use the acquired knowledge, stored in an abstract format, to judge the grammaticality of new items. Other illustrations of this reasoning can be found in many subsequent studies. Let us consider those by Lewicki et al. (1988) and McGeorge and Burton (1990).

In the Lewicki et al. (1988) paradigm, participants were asked to perform a four-choice reaction-time task, with the targets appearing in one of four quadrants on a computer screen. They were simply asked to track the targets on the numeric keypad of the computer as fast as possible. The sequence looked like a long and continuous series of randomly located targets. However, this sequence was organized on the basis of subtle, nonsalient rules. Indeed, unbeknown to participants, the sequence was divided into a succession of 'logical' blocks of five trials each. In each block, the first two target locations were random, while the last three were determined by rules. The participants were unable to verbalize the nature of the manipulation and, in particular, they had no explicit knowledge of the subdivision into logical blocks of five trials, which was a precondition that had to be satisfied if they were to grasp the other rules. However, performance on the final trials of each block, the locations of which were predictable from the rules, improved at a faster rate and was better overall than performance on the first, random, trials. Lewicki et al. (1988) accounted for these results by postulating that the structuring rules were discovered by a powerful, multipurpose unconscious algorithm abstractor.

In the McGeorge and Burton (1990) study, which initiated a stream of research on the so-called 'invariant learning,' participants were asked to perform an arithmetic task on a set of four-digit numbers. Unbeknown to them, each four-digit number contained one '3' digit (the 'invariant'). In a subsequent forced-choice recognition test, participants were shown 10 pairs of four-digit numbers. They were told that one of the numbers in each pair was seen during the study phase, and that they had to find it. In fact, all the numbers were new, but half of them contained one '3' as in the study strings, while no '3' occurred in the other half. Participants choose above chance the numbers containing a '3,' although they were unable to report anything pertinent to the invariant digit. The authors inferred that participants had learned the critical rule unconsciously.

These results, and most of the others in the early IL literature, have been shown to be empirically robust in subsequent studies. However, two other interpretations have been proposed. Their common intuition is that people do not abstract the rules of the domain, but instead learn about the product of the rules.

2.32.2.2 The Instance-Based or Episodic Account

The first historical alternative to the abstractionist position in the field of artificial grammar learning is the so-called instance-based or memory-based model proposed by Brooks (Brooks, 1978; Vokey and Brooks, 1992). In Brooks' model, subjects who are shown grammatical strings during the study phase store the strings in memory, without any form of condensation or summary representation. During the test phase, they judge for grammaticality of test strings as a function of their similarity to specific stored strings. The instance-based model works because, if no special care is taken to generate the material, grammatical test items tend to look globally more similar to study items than ungrammatical test items.

Vokey and Brooks (1992) made independent the usually confounded factors of specific similarity and grammaticality, in order to assess the size of the effect of each factor on grammaticality judgments. Test items were classified as similar when they differed by only one letter from one study item, and dissimilar when they differed by two or more letters from any study items. The authors obtained a reliable effect of specific similarity on grammaticality judgments (see also McAndrews and Moscovitch, 1985). As expected, similar items were more often classified as grammatical than dissimilar items when their grammatical status (i.e., their consistency with the grammar) was kept constant. However, the grammaticality factor also had a significant, and usually additive, effect. Similar evidence was collected by Cock et al. (1994) in invariant learning. The authors demonstrated that similarity to instances in the study phase was even a more important factor than apparent knowledge of the invariant feature in the McGeorge and Burton (1990) paradigm.

To account for the fact that the similarity to a specific training item fails to account for all the

variance in performance, Vokey and Brooks (1992; Brooks and Vokey, 1991) suggested that the similarity may also be computed with the whole set of study items instead of a single one (see also Pothos and Bailey, 2000, for another measure of similarity). The currently prevalent interpretation keeps the idea of some kind of pooling or summation over multiple episodes, but privileges a formulation in terms of statistical regularities.

2.32.2.3 The Sensitivity to Statistical Regularities

While the instance-based model considers whole episodes (e.g., VXVRXVT in artificial grammar learning), this alternative account considers elementary components (e.g., the individual letters). The consequences are considerable. Indeed, large episodes are idiosyncratic, and hence they generate distinctive and independent memory traces. By contrast, elementary components occur iteratively under the same or other combinations, and hence it makes sense to describe the to-be-learned stimuli using statistical concepts, such as frequency, probability, or contingency. For this reason, this approach is designated here as the statistical account, even though it is most commonly referred to as the 'fragmentary' approach in the conventional IL literature. It is worth noting that the term 'statistics' does not necessary entail that learners perform statistical computations, an issue that will be addressed later (see the section titled 'Statistical computations and chunk formation').

The general principle of this account is straightforward: Organizing rules generates statistical regularities in the world, and people adjust their behavior to those regularities. Understanding how this account works in artificial grammar learning is easy. Looking at the grammar shown in **Figure 1**, it appears that that some associations between letters are possible (e.g., MV), and other impossible (e.g., MX), and that among the legal associations, some are more frequent than others (e.g., RX presumably occurs more often than RM). If participants learn something about the frequency distribution of the pairs of letters (bigrams) that compose the study strings, they should perform subsequent grammaticality judgments better than chance. Perruchet and Pacteau (1990) tested this hypothesis. They reasoned that, if subjects learn only bigram information when faced with the whole strings, the direct presentation of the bigrams, which precludes the use of any high-level rules, should not change the final performance. The prediction was confirmed; the performance of participants who had learned using the complete grammatical strings (as usual) and those who were trained using the bigrams from which these strings were composed were statistically indistinguishable. Other experiments from the same study and other studies (Dienes et al., 1991; Gomez and Schvaneveldt, 1994) confirmed the importance of bigrams knowledge, although they showed that participants also learn other piecemeal information, such as the location of permissible bigrams and the first and last letters of the strings.

The question is now: Does this interpretation work in general? It could be argued indeed that artificial grammar learning is especially well-fitted to a statistical interpretation, because the rules can be easily translated in terms of statistical regularities. To address this question, let us consider how the Lewicki et al. (1988) study presented above can be reinterpreted. Recall that a precondition to grasp the complex second-order dependency rules structuring the sequence was a parsing of the whole sequence into logical blocks of five trials, and that participants were fully unconscious of doing so. However, Perruchet et al. (1990) demonstrated that participants could learn the task without ever performing the segmentation of the sequence into logical blocks. Instead, they could become sensitive to the relative frequency of small units, comprising two or three successive locations. Some of the possible sequences of two or three locations were more frequent than others, because the rules determining the last three trials within each five-trial block prohibited certain transitions from occurring. In particular, an examination of the rules shows that they never generated back-and-forth movements. As a consequence, the back-and-forth transitions were less frequent *on the whole sequence* than the other possible movements. The crucial point is that these less frequent events, which presumably elicit longer reaction times, were exclusively located on the random trials. This stems not from an unfortunate bias in randomization, but from a logical principle: The rules determined both the relative frequency of certain events within the entire sequence and the selective occurrence of these events in specific trials (for an alternative interpretation based on connectionist modeling, see Cleeremans and Jimenez, 1998).

A similar reanalysis was performed by Wright and Burton (1995) on the McGeorge and Burton (1990) invariant task. Wright and Burton observed that a by-product of the invariant rule was to modify the probability of occurrence of observing a digit repetition in the strings. More precisely, the strings that contain one '3' include, on the mean, a smaller proportion of repeated digits than the strings in which no '3' occurs, all simply because the chances of generating repeated digits are lesser over three than over four successive drawings. The authors showed that at least a part of the participants' above-chance performance during the test was due to the fact that they tended to reject the items containing repetitions, rather than the items violating the invariant rule.

What is new in these examples (for a similar illustration, see the reinterpretation of Kushner et al. (1991) by Perruchet (1994b)) with regard to the artificial grammar-learning situation is the fact that the link between the generating rules and the distributional statistics of simple and salient events is far from obvious. The fact that rules may have remote consequences, the learning of which having effects similar to the learning of the rules themselves, may obviously be thought of as a drawback in the experimental designs, without any implication out of the laboratory studies. However, it may also be thought of as a quite fundamental outcome, essential to understand the power of the statistical approach in the natural situation of learning, as the section titled 'Discussion: about nativism and empiricism' will emphasize.

2.32.2.4 Rules versus Statistics: A Crucial Test

How can rule-based and statistical interpretations be discriminated? When the rules of a domain generate a set of events so restricted that all the possible events can be exhaustively experienced by a subject, it may be impossible to discriminate between the two types of interpretations. However, this case is largely deprived of interest. Indeed, the power of the rules is that they make people able to adapt to new situations from previous exposure to a subset of the events the rules can generate. Here is the hint for a crucial test.

For a first example, let us consider an argument for rules put forth by Reber and Lewis (1977) in the context of artificial grammar learning. In a given experiment, participants are exposed to a subset of the virtual full set of strings generated by the grammar, and this subset cannot be perfectly representative of the full set for all aspects. For instance, the frequency distribution of the observed bigrams has a high probability of departing to some extent from the frequency of the bigrams composing the full set of strings. Reber and Lewis argued that if participants abstract the rules of the grammar, they should be sensitive to the bigram frequency of the virtual full set of strings, and not the frequency of occurrence of the bigrams composing the strings actually displayed in the study phase. They provided empirical data supporting this hypothesis, and Reber (1989) construed these data as one of the main supports for his contention that studying grammatical letter strings gives access to the abstract structure of the grammar. The logic of the argument is indeed sound, but unfortunately, the supporting data turned out to be due to various methodological drawbacks inherent to the Reber and Lewis procedure (Perruchet et al., 1992). In fact, participants are sensitive to the frequency distribution of the bigrams they actually perceived.

In the preceding example, the possibility of discriminating interpretations based on rules and statistics stems from the fact that the subset of items to which participants are exposed are not representative of the whole set of items due to sampling biases. The same logic may be implemented in a more systematic way, by training participants with a given material and testing them with different material. The following section examines the findings obtained in these so-called 'transfer' situations, which have been heavily used in IL research.

2.32.2.5 The Phenomenon of Transfer: The Data

In the standard paradigm of transfer in artificial grammar learning, the letters forming the study items are changed in a consistent way for the test of grammaticality (e.g., M is always replaced by C, X by P, etc.). Reber (1969) and several subsequent studies (e.g., Mathews et al., 1989; Dienes and Altmann, 1997; Manza and Reber, 1997; Shanks et al., 1997; Whittlesea and Wright, 1997) have shown that participants still outperform chance level under these conditions. The principle underlying the 'changed letter procedure' has been extended to other surface changes. For instance, the training items and the test items may be, respectively, auditory items and visual items (Manza and Reber, 1997), color and color names, sounds and letters (Dienes and Altmann, 1997), or vice versa. Successful transfer was observed in each case.

The phenomenon of transfer has also been observed in invariant learning. McGeorge and Burton (1990) found that the selection of number strings containing the invariant digit persisted when study strings were presented as digits (e.g., 1234) and test strings as their word equivalents (e.g., one two three four; see Bright and Burton (1998) for similar examples of transfer in another invariant learning task).

Transfer has even been observed in infants. In Marcus et al. (1999), 7-month-old infants were exposed to a simplified, artificial language during a training phase. Then they were presented with a few test items, which were all composed of new syllables. For instance, in one experiment, infants heard 16 three-word sentences such as *gatiti, linana,* or *tanana,* during the study phase. All of these sentences were constructed on the basis of an ABB grammar. The infants were then presented with 12 other three-word sentences, such as *wofefe* and *wofewo.* The crucial point is that, although all of the test items were composed of new syllables, only half of the items were constructed from the grammar with which the infants had been familiarized. In the selected example, the grammatical item was *wofefe. Wofewo* introduces a structural novelty in that it is generated from a concurrent ABA grammar. The infants tended to listen more to the sentences generated by the ABA grammar, thus indicating their sensitivity to the structural novelty. In another experiment, infants were shown to be able to discriminate sentences generated by an AAB grammar. Similar studies using more complex material have been performed with 11-month-old infants (Gomez and Gerken, 1999).

2.32.2.6 The Phenomenon of Transfer: The Interpretations

2.32.2.6.1 Rules?

Marcus et al. concluded that infants have the capacity to represent algebraic-like rules and, in addition, "have the ability to extract those rules rapidly from small amounts of input and to generalize those rules to novel instances" (Marcus et al., 1999, p. 79). Demonstrations of transfer in more complex situations in adults have elicited similar comments. For instance, Reber, talking about performance in the changed letter procedure in artificial grammar learning studies, claimed that

> . . . the abstractive perspective is the only model of mental representation that can deal with the existence of transfer of knowledge across stimulus domains. (Reber, 1993: 121)

A rule-based interpretation may have difficulty accounting for the entire pattern of data, however. First, the traditional emphasis on positive results must not overshadow the fact that transfer failure has frequently been reported in the literature on IL. In the conclusion of their review on transfer in the most current IL paradigms, Berry and Dienes pointed out that

> . . . the knowledge underlying performance on numerous tasks . . . often fails to transfer to different tasks involving conceptually irrelevant perceptual changes. (Berry and Dienes, 1993: 180)

This empirical finding leads the authors to propose that limited transfer to related tasks is one of the important key features of performance in IL tasks. Moreover, in experiments where positive evidence of transfer is reported, performance levels on transfer situations are generally lower than performance levels on the original training situation. This so-called transfer decrement phenomenon raises a problem for a rule-based standpoint. In an authoritative discussion on the use of abstract rules, Smith et al. (1992) posit as the first of their eight criteria for rule use that "Performance on rule-governed items is as accurate with unfamiliar as with familiar material" (Smith et al., 1992, p. 7; see also Anderson, 1994, p. 35; Shanks, 1995, Ch. 5; Whittlesea and Dorken, 1997, p.66). Manza and Reber (1997) acknowledge this implication of their own abstractionist view. Clearly, this prediction of rule-based accounts has scarce experimental support at best.

However, observing that rule-based interpretation of transfer is, after all, not so well-fitted as might expected has limited interest until better interpretations are put forward. Are there alternatives?

2.32.2.6.2 Explicit inferences during the test?

In the standard situations of artificial grammar learning, most people are able to learn the abstract rules of the grammar when they are instructed to search for rules (Turner and Fischler, 1993) or when they are given incidental instructions which guide them toward the deep structure of the material (Wittlesea and Dorken, 1993). A first alternative possibility to account for transfer in IL studies is that transfer is due to the involvement of explicit reasoning, despite the instructions given to participants.

This account finds support in the examination of the tasks in which transfer routinely succeeds and tasks in which transfer fails. As noted by Newell and Bright (2002), the tasks that trigger transfer are those in which participants are instructed to use knowledge that they have acquired during training, such as artificial grammar learning tasks and invariant learning tasks. These instructions inevitably shift subjects to a rule-discovery mental set. The tasks in which subjects are not explicitly engaged to rely on what they saw in study phase, such as serial reaction-time (SRT) tasks and control interactive tasks, are far less prone to transfer. In SRT tasks, for instance, a target stimulus appears on successive trials at one of a few possible positions on the computer screen. Participants are simply asked to react to the appearance of the target by pressing a key that spatially matches the location of the target on a keyboard. Typically, the same sequence of trials is repeated throughout the session. Participants exhibit a decrease in reaction times with regard to a control condition, without ever being informed about the presence of a repeated structure. In this case, transfer to dissimilar surface feature typically fails (Stadler, 1989; Willingham et al., 1989).

The role of explicit reasoning in changed-letter transfer in artificial grammar learning is further suggested by the fact that transfer is performed better when the training session involves intentional (i.e., rule searching) rather than incidental instructions (Mathews et al., 1989). Whittlesea and Dorken (1993) failed to obtain changed-letter transfer in subjects whose attention was not focused on the structure of the situation. In the same vein, Gomez (1997) showed that changed-letter transfer occurred only in subjects who had sufficient explicit knowledge of the rules.

Although these studies suggest that transfer performance partly depends on the involvement of conscious and deliberate processes, it is difficult to account for all the positive results in those terms. To evoke only one counterargument, the observation of transfer in infants (Gomez and Gerken, 1999; Markus et al., 1999) can hardly be explained by the recourse to intentional rule-breaking strategies. Is it possible to account for transfer in IL without any recourse to rules?

2.32.2.6.3 Disentangling rules and abstraction

There is no doubt that the evidence of transfer is indicative of abstraction. However, the view that abstraction is indicative of rule formation and rule use has been heavily challenged. As cogently argued by Redington and Chater (2002), "surface-independence and rule-based knowledge are orthogonal concepts."

To begin with a simple case, let us consider Manza and Reber's (1997) results, showing a transfer between auditory and visual modalities in the artificial grammar learning area. These authors interpret their findings as providing support for their abstractionist, rule-based view. However, the phenomenon can be easily explained otherwise. Any sequence – such as VXMTR – presented orally will be immediately recognized when displayed visually, irrespective of whether this sequence is generated by a grammar or not. This is because the perceptual primitives, namely the letters V, X, and so on, are processed to an abstract level that makes them partially independent of their sensory format. The differences between the two explanations is worth stressing. In the former case, a rule-governed pattern is assumed to be extracted from the auditory stimuli before being applied to the visual stimuli. In the latter case, matching is directly performed at the levels of the perceptual primitives. The same comment can be applied to many other studies. For example, the transfer between colors and the name of colors (Dienes and Altmann, 1997) and the transfer between digits and their word equivalents (McGeorge and Burton, 1990) can also be accounted for by the natural mapping between the primitives involved in the experiment.

At first glance, the above explanation does not apply to all transfer results. As a case in point, it does not seem to work for the studies by Marcus et al. (1999) in which transfer is observed between, say, *gatiti* and *wofefe*, because there is no natural mapping between *ga* and *wo*, or *ti* and *fe*. Reinterpretation of the Marcus et al. data is possible along the same line, however, if one assumes that the perceptual primitives can be relational in nature. The relation that needs to be coded is the relation 'same-different,' or, in other words, the only ability that infants need to possess is that of coding the repetition of an event. Indeed, as pointed out by McClelland and Plaut (1999), *gatiti*, *wofefe*, and more generally all the ABB items, can be coded as different-same, whereas none of the other items can be coded using the same schema.

As surprising as this conclusion may be, the demonstrations of transfer stemming from the more complex situations of artificial grammar learning in adults imply the coding of no more complex relations than event repetitions (e.g., Tunney and Altmann,

1999; Gomez et al., 2000; for an overview, see Perruchet and Vinter, 2002, Section 6). Lotz and Kinder (2006) confirmed and extended this conclusion. They showed that the sources of information used in transfer tasks in artificial grammar learning studies concern the local repetition between adjacent elements, as well as the repetition of nonadjacent elements in the whole items.

Overall, this analysis demonstrates that transfer, such as observed in IL settings, is in no way indicative of rule knowledge. It is fairly compatible with a statistical approach, provided one acknowledges the possibility that statistical processes operate not only on surface features (such as forms or colors), but also on more abstract properties and on simple relational features, such as the repetition of events. The idea that transfer does not imply rule abstraction has also gained support from the possibility of accounting for transfer performance within a connectionist framework (Altman and Dienes, 1999; McClelland and Plaut, 1999; Seidenberg and Elman, 1999; Christiansen and Curtin, 1999). Note also that transfer has been claimed to be compatible with an interpretation focusing on instance-based processing, thanks to the notion of abstract analogy (Brooks and Vokey, 1991), although Lotz and Kinder (2006) failed to find an empirical support for this account.

2.32.2.7 A Provisional Conclusion

There is evidence that the analogy with specific items may account for a specific part of variance in performance. The Vokey and Brooks (1992) demonstration, presented in the section titled 'The instance-based or episodic account,' has been challenged (Knowlton and Squire, 1994; Perruchet, 1994a), but additional evidence has been provided since then (Higham, 1997). The interest of the instance-based model resides in its highlighting the fact that behavior may be implicitly affected by individual episodes rather than simply by large amounts of training. However, there is a consensus on the idea that this account cannot be thought of as exclusive. It seems inevitable to jointly consider the pooled influence of a series of events to account for the whole pattern of data.

The two main views accounting for the influence of multiple past events are based on rules and statistics, respectively, but there is no symmetry between the two accounts. Indeed, no one disputes the existence of statistical learning. This consensus comes from the human ability to learn in the countless situations in which regularities cannot be described by a set of rules, as the concept has been defined above. As a consequence, the only possible question is: Do we need rules, in addition to statistical learning, to account for implicit learning in rule-governed situations?

Here is the end of the consensus. On the one hand, many authors respond "no." Their position is based on the fact that the sensitivity to statistical regularities is able to account for performance in most of the experimental situations that were initially devised to provide an existence proof for rule learning, including transfer settings. In addition, when a direct test has been performed to contrast the predictions of the two models, predictions of the statistical account have been unambiguously confirmed (see also Perruchet (1994b) on the situation devised by Kushner et al. (1991) and Channon et al. (2002) on the biconditional grammar). On the other hand, other authors (e.g., Knowlton and Squire, 1996) argue that empirical evidence requires a dual process account, mixing statistical learning and rule knowledge. Their position stems from experimental studies in which learning persists in test conditions where the simplest regularities – those that are presumably captured by statistical learning – have been made uninformative (e.g., Knowlton and Squire, 1996; Meulemans and Van Der Linden, 1997; but see Kinder and Assmann, 2000). This kind of evidence is not fully compelling, however, because it is not possible to ascertain that all the possible sources of statistical knowledge have been taken into account (see for instance the reanalysis of Meulemans and Van der Linden (1997) by Johnstone and Shanks (1999)). A more principled demonstration, in which some specified content of knowledge would fail to be approximated by statistical learning, would provide a far stronger argument.

The remaining of this chapter focuses on statistical learning. This does not mean that the possibility of implicit rule learning can be considered as definitely ruled out. This presumably will never be the case, because proof of nonexistence is beyond the scope of any empirical investigation. Needless to say, this approach does not mean either that rule learning does not exit at all; there is clear evidence that humans are able to infer and use abstract rules when conscious thought is involved. The very existence of science should provide a sufficient proof for the skeptic.

The implications of focusing on statistical learning are that the questions and their experimental approach will be considered irrespectively of

whether the to-be-learned materials can be described in terms of rules or not. Note that this focus is in keeping with the recent literature on IL, which typically includes a number of situations that are not governed by rules such as SRT tasks with repeated sequences and word segmentation (e.g., Saffran et al., 1997), as well as other situations, such as contextual cuing (Chun and Jiang, 2003), that are not described in this chapter due to space limitations.

2.32.3 Learning about Statistical Regularities

Before examining the question of what processes underlie behavioral tuning to statistical regularities, one needs to identify the kinds of regularities to which humans are sensitive.

2.32.3.1 What Is Learnable?

2.32.3.1.1 Frequency, transitional probability, contingency

For many people, claiming that behavior is sensitive to statistical regularities amounts to saying that behavior is sensitive to the absolute or the relative frequency of events. For instance, participants in an artificial grammar experiment may have learned that MT occurred n times, or that a proportion p of the displayed bigrams were MT. Considering only frequency provides limited information, however. It may be interesting to know the probability for 'M' to be followed by 'T,' a measure called conditional or transitional probability. To assess whether 'M' is actually predictive of 'T,' this probability must be compared to the probability that another letter precedes 'T'. The difference between these two conditional probabilities is called DeltaP (Shanks, 1995). In addition, it may be worth considering the reverse relations, namely the probability for 'T' to be preceded by 'M.' This 'backward' transitional probability may be quite different from the standard, forward transitional probability. The normative definition of contingency in statistics (such as measured, for instance, by a χ^2 or a Pearson correlation) requires a consideration of the bidirectional relations. When data are dichotomized, for instance, Pearson correlation is the geometrical mean of the forward and backward DeltaP (for a more detailed presentation, see Perruchet and Peereman, 2004).

The focus on frequency in early studies on IL does not mean that human behavior is only sensitive to this variable. Indeed, all the measures of association are generally correlated, so evidence collected to support one specific measure is equivocal if no special care is taken for controlling the other measures. Aslin et al. (1998) demonstrated that participants were sensitive to the transitional probability in word-segmentation studies. These results have been replicated in visual tasks (e.g., Fiser and Aslin, 2001), so that most recent studies on statistical learning take for granted that the statistics to which people are sensitive are transitional probabilities. This conclusion could be premature, given the correlations between the different measures, and the paucity of studies including different measures. In fact, the literature on conditioning has long suggested that even animals such as rats or pigeons are sensitive to DeltaP (Rescorla, 1967). Perruchet and Peereman (2004) compared several measures of associations, and they found that participants were more sensitive to the bidirectional contingency than to simpler measures of associations (although in a specific context). A conservative conclusion could be that people are sensitive to more sophisticated measures of associations than co-occurrence frequency, and further study is needed for assessing more precisely which statistic is the more relevant in each context.

2.32.3.1.2 Adjacent and nonadjacent dependencies

A dimension orthogonal to the previous one concerns the distance between the to-be-related events. The early studies endorsing a statistical approach in the IL domain focused on adjacent elements (typically the bigrams of letters). The importance of adjacent relations, however, does not mean that it is impossible to learn more complex information. A number of studies in SRT tasks have investigated how reactions times to the event n improved due to the information brought out by the events n-1, n-2, n-3 (known as first-order, second-order, and third-order dependency rules, respectively), and so on. Second-order dependencies can be learned quite easily and are now used as a default in most SRT studies. Third-order dependency rules can also be learned, although less clearly (Remillard and Clark, 2001). However, higher-order dependency rules are seemingly much harder, or impossible to learn. For instance, even after 60 000 practice trials, Cleeremans and McClelland (1991) obtained no evidence for an effect of the event four steps away from the current trial.

In the situations discussed so far, the relations between distant events are not considered

independently from the intervening events. By contrast, in the AXC structures investigated in several recent studies, a relation exists between A and C irrespective of the intervening event X, which is statistically independent from both A and C. Examining whether learning those nonadjacent relationships is possible was prompted by the fact that these relations are frequent in high-level domains such as language and music. The studies investigating the possibility of learning nonadjacent dependencies between syllables or words (Gomez, 2002; Newport and Aslin, 2004; Perruchet et al., 2004; Onnis et al., 2005), musical sounds (Creel et al., 2004; Kuhn and Dienes, 2005), digits (Pacton and Perruchet, in press), and visual shapes (Turk-Browne et al., 2005) report positive results. However, most of them conclude that learning nonadjacent dependencies presupposes more restrictive conditions than those required for learning the relations between contiguous events. Gomez (2002) showed that the degree to which the A_C relationships were learned depended on the variability of the middle element (X). For Newport, Aslin, and collaborators (e.g. Newport and Aslin, 2004), the crucial factor is the similarity between A and C. Learning seems also much easier in a situation where the successive AXC units are perceptually distinct (e.g., Gomez, 2002), than in situations where they are embedded in a continuous sequence (e.g., Perruchet et al., 2004). By contrast, Pacton and Perruchet (in press) provided support for a view in which nonadjacent dependencies can be learned as well as adjacent dependencies insofar as the relevant events are actively processed by participants to meet the task demands.

2.32.3.1.3 Processing multiple cues concurrently

Up to now, we have examined how the learner exploits one source of information, for instance, event repetition. However, taken in isolation, a source of information often has a limited value in real-world settings. The system efficiency would be considerably extended if various sources could be exploited in parallel. The number of studies exploring this issue is still tiny, but they provide converging evidence for a positive assessment, as well in artificial grammar learning (e.g., Kinder and Assmann, 2000; Conway and Christiansen, 2006) as in SRT tasks (e.g., Hunt and Aslin, 2001). Studies on word segmentation have also demonstrated the possibility of combining statistical and prosodic cues (e.g., Thiessen and Saffran, 2003). The concurrent exploitation of various information sources can be simulated by connectionist networks (e.g., Christiansen et al., 1998), a feature that strengthens the plausibility of such a possibility in humans.

2.32.3.1.4 Does learning depend on materials?

An impressive amount of data suggests that statistical learning mechanisms are domain general. For instance, although most studies in artificial grammar learning involve consonant letters, a large variety of other stimuli have been used occasionally, such as geometric forms, colors, and sounds differing by their timbre or their pitch, without noticeable difference. Conway and Christiansen (2005) directly compared touch, vision, and audition, and found many commonalities, although sequential learning in the auditory modality seemed easier than with the other two senses. Likewise, data on word segmentation have been successfully replicated with tones instead of syllables (e.g., Saffran et al., 1999; Saffran, 2002).

However, the fact that IL processes have a high level of generality across and within sensory modalities does not mean that they apply equally well to any stimuli, as if statistical learning mechanisms were blind to the nature of processed material. The well-known difficulty of publishing null results certainly accounts for a part of the apparent universality of IL mechanisms. A closer look shows, for instance, that learning may depend on aspects of the material that could seem *a priori* irrelevant. For instance, there is overwhelming evidence of rapid learning in standard SRT tasks, in which a target appears in successive trials at one of a few discrete locations. Chambaron et al. (2006) explored a similar situation, except that participants had to track a target that moved along a continuous dimension. They fail to obtain evidence of learning in several experiments, hence showing that, in spite of a close parallel between continuous tracking tasks and SRT tasks, taking benefit from the repetition of a segment in continuous tracking task appears to be considerably more difficult than taking benefit from the repetition of a sequence in SRT tasks. Moreover, recent research on statistical learning has shown that learning was highly dependent on low-level perceptual constraints. For instance, for a given statistical structure, the acoustic properties of the artificial speechflow have been shown to be determinant for learning to segment the speechflow into words (e.g., Onnis et al., 2005). Shukla et al. (2007) provides evidence that possible world-like sequences, namely chunks of three syllables with

high transition probabilities, are not recognized as words if they straddle two prosodic constituents.

The efficiency of learning may also depend on high-level expectancies about the structure of the material. For instance, Pothos (2005) used an artificial grammar learning task in which the consonant letters were replaced by the name of cities. The sequences of cities were presented as the routes a salesman has to travel. In one group of participants, the training sequences matched the intuitive expectation that the salesman follows routes that link nearby cities, while in a second group, they conflicted with this intuition. Learning, as assessed by the comparison with an adequate control group, occurred only in the first group, as if a conflict between the knowledge acquired by processing the statistical structure of the stimuli and the intuitive expectations about stimulus structure prevented learning from occurring in the second group.

2.32.3.1.5 About the learners

Any discussion about learnability is meaningless without considering the characteristics of the learners. Most of the studies reported above have been performed on healthy adult participants.

Regarding first the effect of age, recent research on statistical learning has shown the surprising learning abilities of infants. These abilities have been initially revealed with auditory artificial languages (e.g., Saffran et al., 1996), but they are in no way limited to language-like stimuli. They concern sounds or combinations of visual features as well (e.g., Fiser and Aslin, 2001, 2002). Several studies have also investigated IL in children. They suggest that there is no noticeable evolution from 4- or 5-year-old children to young adults (e.g., Meulemans et al., 1998; Vinter and Detable, 2003; Karatekin et al., 2006). Other studies suggest that IL does not decline with healthy aging (e.g., Cherry and Stadler, 1995; Negash et al., 2003). Furthermore, a number of studies have reported impressive IL abilities in children (e.g., Detable and Vinter, 2004) and young adults (Atwell et al., 2003) with mental retardation, and in patients with psychiatric (e.g., Schwartz et al., 2003) and neurological disorders, including amnesia (e.g., Meulemans and Van der Linden, 2003; Shanks et al., 2006), Alzheimer's disease (Eldridge et al., 2002), Parkinson's disease (e.g., Smith and McDowall, 2006), closed-head injury (e.g., Vakil et al., 2002), and Williams Syndrome (Don et al., 2003). Statistical learning abilities have been shown in animals such as nonhuman primates (e.g., Hauser et al., 2001) or even rats (Toro and Trobalon, 2005).

These data have crucial implications for a number of fundamental and applied issues. They do not mean, however, that everyone shares equivalent abilities whenever statistical learning is concerned. In fact, comparative studies often select situations the difficulty of which is *a priori* well-suited for the full span of the investigated population. When the level of difficulty is increased, a difference often emerges. For instance, a deficit of performance has been observed in complex IL tasks in elderly people (e.g., Howard et al., 2004) and in amnesic patients (Curran, 1997; Channon et al., 2002). This dependency of IL with regard to learner's general competencies whenever the learning settings become complex enough is confirmed by studies on adult healthy people. For instance, Dienes and Longuet-Higgins (2004) observed that only participants experienced with atonal music were able to learn artificial regularities following the structures of serialist music.

2.32.3.2 Statistical Computations and Chunk Formation

2.32.3.2.1 Computing statistics?

Observing that performances in IL tasks conforms to statistical regularities may lead us to infer that learners compute statistics. Certainly, the idea that learners unconsciously compute statistics using the same algorithms as a statistician would use is somewhat implausible. However, the possibility of approximating the outcome of analytical computations through connectionist networks (e.g., Redington and Chater, 1998) offers a much more appealing alternative. Learning is performed by the progressive tuning of the connection weights between units within multilayer networks. Although different types of networks have been used (see Dienes, 1992), the Simple Recurrent Networks (SRN), initially proposed by Elman (1990), have been the most widely applied to IL. SRNs are typically trained to predict the next element of sequences presented one element at a time. Cleeremans and McClelland (1991) have shown that an SRN was able to simulate the performance of human learners in an SRT task in which the successive locations of the target were generated by a finite-state grammar, and the ability of SRNs to successfully account for performance in various IL paradigms has been confirmed since then in a number of studies (e.g., Kinder and Assmann, 2000). Certainly, due to the impressive

ability of connectionist networks to simulate learner's performance, the idea that learners actually perform statistical computations is taken for granted by a number of authors. This idea is not compelling however. Inferring statistical computation from statistical sensitivity may amount to repeating the same error as the early researchers in IL, who inferred rule abstraction from the behavioral sensitivity to rules. An alternative interpretation emerges from the observation that IL generally leads to the formation of chunks.

2.32.3.2.2 The formation of chunks

The fact that learning leads to the formation of chunks is largely consensual. This is obviously the case in recent studies on word segmentation and object formation, in which performance is directly assessed through chunk formation. But this is also true in most of the other situations of IL, in which chunks are not the explicit end-product of learning. A number of studies have shown that participants learn small chunks of two or three elements in artificial grammar learning settings. Chunking the material, far from being a degraded procedure, is a highly efficient mode of coding. Indeed, dealing with small units facilitates transfer and generalization. This happens because, given the structure of finite-state grammars, new items are formed by recombining old components. However, this is true only if the chunks respect the statistical structure of the material. To put the matter simply, assuming five events (A, B, C, D, and E), forming the chunks 'AB' and 'CDE' is beneficial only if A and B on the one hand, and C, D, and E on the other hand, form cohesive structures. If 'AB' is frequently followed by C, and 'DE' frequently occurs in other contexts, then this mode of chunking would be ill suited (Perruchet et al., 2002).

The formation of chunks forming cohesive structures can be easily accounted for by the idea that learners compute statistics. For instance, for Saffran and Wilson (2003), verbal chunks are inferred from statistical computations and then serve as the stuff for further statistical computations. Fiser and Aslin (2005) also consider that the visual input is chunked into components according to the statistical coherence of their components. To use the five-event example, AB and CDE would emerge as from some kind of cluster or factorial analyses once the correlational structure of the events has been computed.

2.32.3.2.3 Are statistical computations a necessary prerequisite?

Chunks consistent with the statistical structure of the material can also emerge without prior statistical computations. Simple memory mechanisms could be sufficient. To begin with, let us consider the ubiquitous phenomenon of forgetting. Because frequently repeated events tends to be forgotten to a lesser extent than less frequent events, forgetting leads us to be sensitive to event frequency, without any statistical 'computation.' Several models of IL (Servan-Schreiber and Anderson, 1990; Knowlton and Squire, 1996; Perruchet and Vinter, 1998a) rest on this intuition. Again, in the example, the chunks 'AB' and 'CDE' would emerge from the fact that A and B on the one hand, and C, D, and E on the other hand, occur more often together than any other combinations of events. In Perruchet and Vinter's Parser model, those chunks emerge simply because other associations of events (such as ABC), if they occur, are forgotten due to their relative rarity. The difference with the statistical account is that, instead of being inferred from the results of statistical computation, chunk formation is the primary mechanism, and the cohesive chunks are those that are selected among a number of other ones due to well-known laws of associative memory, primarily forgetting.

Chunking is often thought of as exclusively sensitive to the raw frequency. This would indeed be the case if the strength of memory traces only depended on the repetitions of events. However, it has long been known that forgetting is due in large part to the interference generated by the prior or subsequent events that are related in some way to the target event. Now, and this is the crucial point, taking into account the effect of interference in chunk formation amounts to considering other measures of association than the raw frequency of co-occurrences. For instance, implementing forward interference is sufficient to make chunk strength sensitive to transitional probabilities (Perruchet and Pacton, 2006, Box 3). Moreover, Perruchet and Peereman (2004) have shown that the Perruchet and Vinter's (1998a) Parser model, thanks to the role ascribed to interference in chunk formation, was even sensitive to contingency, that is, to a measure of association more comprehensive than conditional probabilities.

To recap, the current debate is between those who argue that statistical computations are performed first, with the chunks inferred on the basis of their results, and those who argue that the chunks are formed from the outset, with the sensitivity to

statistical regularities being a by-product of the selection of those chunks as a consequence of ubiquitous memory laws. Note that the two interpretations are equally consistent with associative learning principles. One advantage of the second option is its parsimony. Indeed, no additional computational devices have to be imagined to extract chunks from distributional information. In addition, the chunk model can be easily unified with the instance-based model, because both are grounded on standard memory laws. A unified view could find an integrative framework in the so-called processing account of IL. This account borrows, from research in memory, the idea that memory traces are no more than the by-product of the processing operations engaged during study, and that retrieval depends on the overlap between the processing undertaken during the study and the test phase (e.g., Roediger et al., 1989). Support for this view in IL studies stems from the demonstration of the encoding specificity effects in research into artificial grammar. For instance, Whittlesea and Dorken (1993) show that performances in the test phase are better if the processing involved during the test (pronouncing or spelling the letter strings) matches the processing involved during the study phase. Although the processing account is historically associated with the instance-based models of IL (e.g., Neal and Hesketh, 1997), its grounding principles could be applied to chunk-based models as well.

These considerations, however, cannot be considered as compelling. At this time, the available experimental studies intended to tease apart the predictions from statistical and chunk-based models have produced equivocal results (e.g., Boucher and Dienes, 2003). Clearly, the outcome of the debate is pending further empirical investigations.

2.32.4 How Implicit Is 'Implicit Learning'?

What defines implicitness in IL is far from being agreed upon. A distinction is made, in the following sections, between what occurs during the training phase and the test phase of an IL session. The study-test distinction has limited interest, insofar as in most real-world situations, and in several laboratory situations (such as SRT tasks) as well, any event both influences subsequent events and is itself influenced by the prior ones, hence serving the two functions simultaneously. However, this distinction provides a convenient means to tease apart different issues.

2.32.4.1 Implicitness during the Training Phase

2.32.4.1.1 Incidental and intentional learning

A feature which is a part of virtually all definitions of IL is the incidental nature of the acquisition process. IL proceeds without people's intention to learn. This characteristic is sometimes the only one to be retained, thus conflating the notion of IL and incidental learning (e.g., Stadler and French, 1994). The SRT tasks are often considered as those that offer the best guarantee of the incidental nature of learning, because this task is endowed with its own internal purpose, and it leaves quite limited time for thinking about the task structure. In most of the other IL tasks, instructions distract participants from thinking about the overall material structure, by focusing participants' attention on individual items. For instance, in artificial grammar learning, participants are generally asked for the rote learning of individual letter strings. In invariant learning, participants are asked to perform some arithmetic computation on each digit string. In other tasks, such as the word-segmentation task, participants are simply asked to listen to the artificial language, without specific demands.

2.32.4.1.2 Is attention necessary?

A question of major interest is whether performance improvement depends on the amount of attention paid to the study material during the familiarization phase. The main strategy consists in adding a concurrent secondary task during the training session, then observing whether performance improvement is equivalent to that observed in a standard procedure.

A few early studies claimed that the addition of a secondary task had no effect, or even could *facilitate* learning in very complex experimental settings. This leads to contrast the concepts of 'selective learning' and 'unselective learning' (e.g., Berry and Broadbent, 1988), with the latter being assumed to occur when the situation was too complex to be solved by attention-based mechanisms. The original results were not replicated, however (e.g., Green and Shanks, 1993), and to the best of our knowledge, the notion of unselective learning, as initially discussed in the studies conducted by Broadbent and colleagues, is no longer advocated.

The idea of two forms of learning, differing according to whether attention is required or not, has also been proposed in another context, but with the opposite stance. The hypothesis was that

attention is required for learning complex sequences in SRT tasks, while nonattentional learning is efficient for the simplest forms of sequential dependencies (Cohen et al., 1990). However, observing learning under dual-tasks conditions does not imply the existence of a nonattentional form of learning, because the secondary task might not deplete the attentional resources completely (Stadler, 1995). Closing their survey on the role of attention in implicit sequence learning, Hsiao and Reber concluded:

> We view sequence learning as occurring in background of the residual attention after the cost of the tone-counting task [commonly used as a secondary task in this context] and the key-pressing task. If there is still sufficient attention available to the encoding of the sequence, learning will be successful; otherwise, failure will result. (Hsiao and Reber, 1998: 487)

Regarding artificial grammars, Reber (e.g., 1993) has also acknowledged that attention to the study material is necessary for learning to occur. In support of this claim, Dienes et al. (1991) have shown that the accuracy of grammaticality judgments was lowered when subjects had to perform a concurrent random number generation task during the familiarization phase.

Note also that other studies have shown that, without at least minimal attentional involvement, even simple covariations or regularities turn out to be impossible to learn (Jimenez and Mendez, 1999; Hoffmann and Sebald, 2005; Pacton and Perruchet, in press; Rowland and Shanks, 2006b). The conclusion according to which improved performance in IL situation requires attention has been recently supported by studies on statistical learning using continuous speech flow (Toro et al., 2005) or visual displays (Baker et al., 2005; Turk-Browne et al., 2005). This conclusion comes as no surprise, because the major role played by selective attention in acquisition processes is an old and robust empirical finding (for another approach that emphasizes the role of attention, see Frensch et al., 1994).

2.32.4.2 Implicitness during the Test Phase

2.32.4.2.1 The lack of conscious knowledge about the study material

Is it possible to improve his/her performance without being conscious of what has been learned? A considerable amount of studies have addressed this question by exploring participants' explicit knowledge through postexperimental tests. Overall, a number of studies report that participants are aware of the knowledge they have acquired. However, other studies report that participants fail in the test of explicit knowledge. The question is: Are those negative results reliable? A number of potential drawbacks have been raised.

2.32.4.2.2 The Shanks and St. John information criterion

The first problem is linked to the fact that exploring whether knowledge is consciously represented primarily requires that the knowledge relevant for performing the task has been correctly identified. In an influential synthesis of the literature, Shanks and St. John (1994) coined this requirement as the 'information criterion.' The information criterion stipulates that the information the experimenter is looking for in the awareness test needs to match the information responsible for the performance change.

Although the cogency of this criterion may seem obvious, it must be realized that it entails that any conclusion about implicitness entirely depends on the response given to the 'what is learned' question raised in the prior sections. Any error in the hypothesized content of knowledge, far from being a "slightly embarrassing methodological glitch" (Reber, 1993, note p. 44, 114-115), has dramatic consequences on the inference that one may drawn about the implicit/explicit status of the acquired knowledge. For instance, Reber and Allen correctly pointed out that:

> . . . clearly a considerable proportion of subjects' articulated knowledge can be characterized as an awareness of permissible and nonpermissible letter pairs. (Reber and Allen, 1978: 210).

However, the authors did not realize that this form of knowledge was sufficient to account for performance. Instead, they attributed performance improvement to rule knowledge, which they concluded to be the result of unconscious abstraction. A large part of the earlier claims for the lack of conscious knowledge about the study material seemingly stems from this problem, also known as the problem of the 'correlated hypotheses' after the seminal studies by Dulany (1961) and Dulany et al. (1984).

2.32.4.2.3 The Shanks and St. John sensitivity criterion

According to Shanks and St. John, a second criterion is that the test of explicit knowledge is sensitive to all

of the relevant conscious knowledge. A test of free recall, such as used in the early studies on IL, is notoriously insensitive. For instance, participants may not report some knowledge they have about the material structure, because they have a conservative response criterion that makes them respond only when their knowledge is held with high confidence, or simply because they think this knowledge is irrelevant or trivial. For this reason, most studies now involve a test of recognition, in which participants have to discriminate items belonging to the training materials from new items. However, performing no better than chance in a recognition test is not necessarily a proof that participants lack any explicit knowledge about the task. For instance, Reed and Johnson (1994) used a recognition test after an SRT task and observed that recognition scores were at chance. In an attempted replication involving the same procedure, Shanks and Johnstone (1999) found instead very high levels of recognition. The only difference between the two studies was that participants in Shanks and Johnstone were rewarded by an extra sum of money for each correct decision. Performing the recognition test is somewhat tedious, and presumably participants in the Reed and Johnson study were not motivated enough to make the effort required to perform the task correctly.

2.32.4.2.4 The problems of forgetting

In the standard procedure, the explicit tests are postponed after the task of IL, thus raising the problem of the retention of the knowledge exploited during the implicit test. For instance, Destrebecqz and Cleeremans (2001) reported chance-level recognition in an SRT paradigm (at least for a group of participants). Notably, the test of recognition was administered after participants had performed another task, in which they had to generate sequences under various instructions (see below). Shanks et al. (2003) attempted to reproduce Destrebecqz and Cleeremans' dissociation between RT measures and recognition scores, but in conditions in which the two kinds of measures were taken concurrently. In three experiments, they failed to replicate the Destrebecqz and Cleeremans' dissociation and obtained instead clear evidence of recognition. Note that the problems of forgetting are made especially important due to the fact that a recognition test necessarily includes the exposure to new sequences (generally half of the test items). Because these new sequences are highly similar to old sequences, they are prone to generate interference for the subsequent test trials.

2.32.4.2.5 The problem of the reliability of measures

The scores in implicit and explicit tasks are often found to be correlated. For instance, in SRT tasks, Perruchet and Amorim (1992) reported that Pearson correlations over the sequence trials between RT and recognition scores ranged, in three experiments, from .63 to .98. However, some authors (e.g., Willingham et al., 1993) have argued that evidence for unconscious knowledge was given by the fact that learning could be still observed when the analysis was restricted to the subgroup of items (or the subgroup of participants) for which no evidence of explicit knowledge was gathered. This argument is questionable, however. As discussed in Perruchet and Amorim (1992), the method, in effect, dichotomizes the scores on the implicit measure on the one hand, and on the explicit measure on the other, to assign the items or the participants to a fourfold contingency table. Then inference for dissociation is drawn from the observation that some items or some participants fall into the discordant cells of the contingency table, or in other words, that the correlation is not perfect. The problem with this method is that a prerequisite for obtaining a perfect correlation is perfect reliability of measures. This condition is highly unrealistic for psychological measures, especially for the scores on implicit tests (Meier and Perrig, 2000; Buchner and Brandt, 2003). Shanks and Perruchet (2002) have developed this reasoning into a quantitative model, which assumes that the sources of error plaguing implicit and explicit measure are independent. Although the model involved a single underlying memory variable, it turned out to be able to generate a dissociation between RTs and recognition in SRT tasks that mimics fairly well the dissociation the authors reported themselves (despite the temporal synchrony of measures).

2.32.4.2.6 An intractable issue?

To sum up, the current evidence for the lack of conscious knowledge about the study material is weak at best. There is currently no identified condition allowing one to obtain a reproducible dissociation. Most of the experiments reporting above-chance performance in implicit measures and chance-level performance in explicit tests have been replicated in more stringent conditions, and it turns out that, as a rule, the dissociation no longer appears when appropriate controls are made.

These data do not allow clear conclusions. On the one hand, the preceding discussion makes it clear that

it is impossible to conclude to the existence of learning without any conscious counterpart. But, on the other hand, it should be also unwarranted to infer from the current findings that conscious awareness of the material structure is necessary for performance improvement. The first reason is a logical one, which has been met with regard to rule abstraction, namely, it is not possible to prove that something does not exist. There is yet another reason, linked to the fact that no task is process-pure, as has been well documented in the literature on implicit memory. This is especially true for the most sensitive tests, such as recognition. Jacoby (1983), and many others since, have argued that the relative fluency of perception, which relies on implicit process, may be used as a cue for discriminating old from new items in a recognition task, thus making a variable contribution to recognition judgments over and beyond a directed memory search factor. This entails that above-chance recognition after an IL task does not provide a compelling evidence for explicit knowledge. To date, it is not clear how further studies could solve this conundrum. Some authors (e.g., Higham et al., 2000) have suggested that those problems are intractable and should prompt researchers to give up any attempts to demonstrate learning without concurrent consciousness.

2.32.4.2.7 The subjective measures

The measures discussed so far are often called 'objective,' because it is the experimenter that judges the level of awareness of participants from their performance in specific tests. Another way of defining implicitness starts from the consideration of the phenomenal state of the participants such as it may be directly expressed. Two such 'subjective' measures of implicitness have been proposed in the literature, the guessing criterion and the zero-correlation criterion (Dienes et al., 1995). In both cases, participants are submitted to a test of explicit knowledge such as a recognition test, and they have to rate how confident they are about each decision. To check whether the guessing criterion is filled, the scores on the recognition test are restricted to those of the decisions that are accompanied by a subjective experience of guessing. If participants achieve above-chance discrimination while they report to be guessing, the guessing criterion is met. The zero-correlation criterion rests on the idea that, if knowledge is implicit, participants must not be more confident when they are correct than when they are incorrect. As a consequence, if participants have no introspection into the bases of their decisions, the correlation between confidence and accuracy should be null.

Can performances on standard IL tasks be called implicit according to these criteria? The literature again does not provide a clear response, with some studies reporting positive results and others negative results. In fact, the general picture appears similar to that observed with objective measures, with initially positive findings being not replicated when more sensitive measures are used. For instance, Dienes and Altman (1997) reported a zero correlation between confidence and accuracy in an artificial grammar learning task involving a transfer paradigm. Notably, participants had to assess their confidence on a continuous scale ranging from 50 to 100, where 50 was a complete guess and 100 was absolutely sure. Using the same scale, Tunney and Shanks (2003b) replicated this result. However, based on a study by Kunimoto et al. (2001), Tunney and Shanks reasoned that a binary confidence judgment could be more sensitive, maybe because participants might find it easier to express subjective states on a binary than on a continuous scale. When participants had to express their confidence on a binary scale, they were found to be systematically more confident in their correct decision than in their incorrect decision in several independent experiments. To conclude, irrespective of the *a priori* validity that one decides to ascribe to subjective measure of implicitness, it appears that there is to date no identified procedure that fulfills subjective criteria in a reproducible way.

2.32.4.2.8 The lack of control

One possible meaning of 'implicitness' is that of 'automaticity.' One of the key features usually attributed to automatic behavior is that it is irrepressible, irrespective of people's intentions to do so. Although recent literature on automaticity has questioned the possibility that any learned behavior - even reading, which is often construed as prototypical of automaticity – could actually be outside of control (e.g., Tzelgov et al., 1992), the question of whether the expression of knowledge in IL tasks shares this property deserves to be raised. Such a demonstration was provided by Destrebecqz and Cleeremans (2001) in an SRT task. In an application of Jacoby's process dissociation procedure (Jacoby, 1991) to this task, the authors asked participants to generate a sequence under two successive conditions during the test phase of an otherwise standard SRT procedure. In the first condition, they were told to generate the sequences they were previously exposed to, and if

they fail to remember them, to generate sequences as they come to their minds (the inclusion instructions). Then participants had to produce a sequence of keypresses that did not overlap with the training sequence (the exclusion instructions). Crucially, participants - at least a subgroup of participants trained without any interval between the response to a target and the appearance of the next target - were influenced by the training sequences despite their intention to prevent this from happening. They performed in the same ways irrespective of the instructions, and under exclusion instructions, they generated the training sequence more than would be expected from an appropriate baseline.

These findings, however, have proven to be difficult to replicate. In the same conditions, Wilkinson and Shanks (2004) found that participants were more influenced by the training sequence under inclusion than under exclusion instructions (see also Destrebecqz and Cleeremans, 2003). In three experiments, Wilkinson and Shanks also failed to replicate the results according to which parts of the training sequence were generated more often under exclusion instructions than in the baseline, even after more extensive training than used by Destrebecqz and Cleeremans (2001) - although they did not get any negative difference either, as it could be expected if participants were able to withdraw the parts of the training sequence from influencing their production. Overall, these and others results (see for instance Dienes et al., 1995; Tunney and Shanks, 2003a) offer only quite limited evidence for the conclusion that knowledge gained in IL settings lies outside of intentional control.

2.32.4.2.9 *The lack of intentional exploitation of acquired knowledge*

The fact that participants are seemingly able to withdraw the influence of prior training when they are asked to do so does not mean that, under standard conditions, this influence is intentionally mediated. The lack of intentional exploitation of stored knowledge seems to be a hallmark of the real-world examples given at the outset of this chapter. Presumably nobody has the intuition of applying strategically a core of learned knowledge when speaking his maternal language, hearing music, or conforming to physical or social rules. Is this intuition confirmed in experimental studies?

The question is made difficult by the fact that, in most cases, influences expected from the intentional exploitation of conscious knowledge about the relevant aspects of the situation would have the very same effects as those induced by unconscious processes. As a consequence, it has been suggested that performance in IL tests can be accounted for by the use of explicit knowledge about various aspects of the experimental situation (Dulany et al., 1984; Shanks and St. John, 1994). It is certainly impossible to rule out this contention in general. However, it should be unwarranted to generalize it to all IL tasks. Indeed, there are cases in which the conscious exploitation of explicit knowledge does not coincide with the expected results of unconscious processing. One example is provided by the grammar learning studies involving preference judgments. Indeed, there should be *a priori* no reason for the knowledge about the material to be used to guide a preference judgment. However, participants consistently prefer grammatical items (e.g., Manza et al., 1998).

Vinter and Perruchet (2000) proposed a new task of IL that was especially devised to eliminate the potential influence of intentional control. When adults are asked to draw a closed geometrical figure such as a circle, their production exhibits a striking regularity. If they begin the circle in the lower half, they tend to rotate clockwise, and if they begin the circle in the upper half, they tend to rotate counter clockwise. In Vinter and Perruchet experiments, participants were guided to draw geometrical figures in such a way that this natural covariation was inverted. This training induced important and long-lasting modifications of subsequent free drawings. The point of interest is that, even if participants had become aware of the inverted covariation between the starting point and the rotation direction they experienced during the training session, they should have no reason to modify their usual mode of drawing as they did. This study provides clear evidence for an adaptive mode in which subjects' behavior becomes sensitive to the structural features of an experienced situation, without the adaptation being due to the intentional exploitation of subjects' explicit knowledge about these features.

2.32.4.3 Processing Fluency and Conscious Experience

Let us now reverse the direction of the potential relation between learning mechanisms and conscious thought, in order to examine the level of dependency of conscious thought with regard to IL.

An influential model of how training in IL settings leads to a change in performance posits that training

induces a modification in the subjective experience of the learner. More specifically, the underlying idea is that training improves the fluency of perceptual processing for the studied materials. This account was initially proposed by Servan-Schreiber and Anderson (1990) in the context of their chunking theory. In fact, however, the improved processing fluency can also be attributed to other forms of knowledge, such as rules or memory for specific instances. Thus a fluency theory has been advocated as well by those researchers who maintain a role for rule-based processing (Zizak and Reber, 2004) and by those who consider that statistical computations are sufficient (e.g., Conway and Christiansen, 2005).

Let us examine how this account works in artificial grammar learning paradigms. The assumption is that, after training with a sample of grammatical strings of letters, new grammatical strings are processed fluently, or more precisely, more fluently than expected (Whittlesea and Williams, 2000), hence generating a feeling of familiarity leading itself to endorse the strings as grammatical. The two steps of this hypothesis have received experimental support. The fact that exposure to the training strings improved processing fluency has been shown by Buchner (1994). The test strings were displayed in such a way that they emerged progressively from noise, a procedure known as a perceptual clarification procedure. It turned out that grammatical strings were identified about 200 ms faster than ungrammatical ones. The fact that improved fluency in turn influences grammaticality judgments has been demonstrated using a method well documented in the literature on implicit memory, which consists in artificially enhancing the fluency of processing of selected items. During the test phase of an otherwise standard artificial grammar learning experiments, Kinder et al. (2003) exposed participants to test strings that did not differ in their grammatical status (they were all grammatical). The test strings were displayed in a perceptual clarification procedure as in Buchner (1994), except that some strings were clarified slightly faster than the others. Participants judged the former more often grammatical than the latter.

The fluency account suggests that IL modifies the subjective experience of the learner. However, the induced modifications appear to be quite minor, insofar as they are prompted by a gain of some fractions of second in processing speed. The frequent rapprochement of the concepts of IL and priming (e.g., Cleeremans et al., 1998; Conway and Christiansen, 2006; Kinder et al., 2003) is consonant with the idea that the training-induced modifications are relatively inconsequential. It is also possible to consider that the changes in the conscious experience of the learner are much more striking. For instance, in artificial grammar learning, participants normally learn to perceive the grammatical strings as a sequence of chunks the content of which is consonant with the structure of the grammar (e.g., the sequences of letters composing a recursive loop have high chance of being perceived as chunks, see Servan-Schreiber and Anderson, 1990; Perruchet et al., 2002). Likewise, in word-segmentation studies, the speechflow, which is initially perceived as an unorganized set of syllables, turns out to be perceived as a sequence of units, which match the words composing the language. More generally, an essential function of IL could be that of making the conscious perception and representation of the world isomorphic with world's deep structure. Because this change in subjective experience can be construed as a simple by-product of the attentional processing of the incoming information, Perruchet and Vinter (2002) have suggested the concept of 'self-organizing consciousness' to express the idea that IL shapes new conscious percepts and representations in a way which make them increasingly adaptive (see also Perruchet, 2005; Perruchet et al., 1997).

Neither the fluency account nor Perruchet and Vinter's (2002) self-organizing consciousness model is aimed to account for all behavioral changes observed in IL settings. For instance, although the fluency account is relatively consensual (partly due to the fact that it is mute with regard to the nature of knowledge inducing fluency), this account explains only a part of the performances observed in IL settings. Even in the artificial grammar learning paradigm, which is *a priori* a well-suited field of application, relative processing fluency does not seem to be able to account for the whole pattern of grammaticality judgments (Buchner, 1994; see also Zizak and Reber, 2004, p.23). However, these models point to the possibility of considering IL and consciousness not in terms of dissociation or independence, but rather in terms of dynamic interplay.

2.32.4.4 Summary and Discussion

To sum up, research of the last few decades has shown that it is surprisingly difficult to specify in what sense IL is implicit. The notion of unselective, nonattentional learning has vanished in light of

studies demonstrating that learning requires at least some forms of attentional processing of the incoming information. Likewise, there are quite limited supports to claim that while they perform the implicit test participants (1) have no conscious knowledge about the study material, (2) have the subjective experience of guessing, or (3) have no control over the expression of their knowledge. Of course, it is possible to include one or the other of these features within a definition of IL, and some authors did so (for a sample of definitions, see Frensch, 1998). However, endorsing this kind of definition leads to the somewhat paradoxical consequence of giving to a research domain the objective of checking whether this domain actually exists. To date, there is no specified paradigm in which one or the other of these criteria can be asserted in a consensual and reproducible way.

A feature that can be retained with higher confidence is the lack of intentional exploitation of stored knowledge. This does not mean, obviously, that this condition is fulfilled in each and every study, but rather that the existence of the phenomenon can be reasonably asserted on the basis of reproducible evidence. Accepting the role of unconscious influences, however, does not lead us to conceive IL and conscious experiences as divorced one from each other. There is indeed extnsive evidence that these unconscious influences primarily affect the conscious experience of the learner.

2.32.5 Implicit Learning in Real-World Settings

2.32.5.1 Exploiting the Properties of Real-World Situations

Although most of research on IL uses artificial, laboratory situations, natural situations have been used on occasion to shed light on specific issues. In this case, only the test phase is carried out in well-controlled experimental conditions, while implicit training is assumed to have occurred previously in natural settings. For instance, Pacton et al. (2001) exploited the very extended time scale on which real-world learning takes place to examine whether transfer decrement (see the section titled 'The phenomenon of transfer: the interpretations') is a transitory or an enduring phenomenon. The issue is important, because it can be argued that the transfer decrement commonly observed in laboratory settings, which is one of the arguments used against a rule-based view, is simply due to the fact that training is not extensive enough to allow the full development of rule abstraction. Pacton et al. explored the development of the sensitivity to certain orthographic regularities not explicitly taught at school. They showed that the decrement in performance due to transfer persisted without any trend toward fading over the 5 years of experience with printed language that they examined, hence strengthening the claim that IL is not mediated by rule knowledge.

2.32.5.2 Exploiting our Knowledge about Implicit Learning

The knowledge gained in laboratory studies is aimed at improving our understanding of world-sized issues. Explicit loans from the IL literature have been made occasionally in a number of domains, including child development (Perruchet and Vinter, 1998b), second-language acquisition (e.g., Ellis, 1994; Robinson, 2002), spelling acquisition (Kemp and Bryant, 2003; Pacton et al., 2005; Pacton and Deacon, in press), and the development of gustatory preferences (Brunstrom, 2004). To various degrees, the concepts and the methodology of laboratory studies have inspired researchers to progress in the understanding of these domains. In regard of the potential relevance of IL mechanisms in these and other domains, much more could be made in this direction, however. The only domain in which a sizeable amount of literature has emerged concerns the relationships between IL and natural language acquisition (e.g., Gomez and Gerken, 2000). This rapprochement is partly due to the fact that research on language has evolved on its own toward methods – the use of artificial languages – and concepts – notably around the notion of statistical learning – that are also at the heart of IL research.

The practical applications of IL, for instance for educative purposes or the reeducation of neurological patients, appear to be still sparser. Some methods have evolved that exploit principles which can be *a posteriori* related to IL principles, such as using conditions as similar as possible to natural learning to teach second language (after Krashen, 1981) or reading (for a review, see Graham, 2000). An extensive literature also concern the use of errorless learning for reeducative purposes in a neuropsychological perspective (see review in Fillingham et al., 2003). But most of these attempts have been conducted without considering the possible contribution of IL research (for a recent exception, see Saetrevik et al.,

2006). The explanations for this relative paucity are certainly manifold. One of them may be that learning in real-world situations most often involve some mixture of implicit (or incidental) and explicit (or intentional) learning. Now, the interactions between these forms of learning have not been at focus in the literature on IL, because, except in a few studies (e.g., Matthews et al., 1989), the objective has been to isolate implicit processes to examine them in their maximum state of purity. Further studies are needed to assess how, for instance, the learning of rules in explicit conditions may be combined with implicit statistical learning.

2.32.6 Discussion: About Nativism and Empiricism

Let us return to a question raised at the outset of this chapter, which stemmed from the lack of consideration during the behaviorist era of issues such as first-language acquisition, category elaboration, sensitivity to musical structure, acquisition of knowledge about the physical world, and various social skills. It was pointed out that this situation opened the door to the upsurge of a nativist perspective. Where do the studies reported in this chapter leave us?

At first glance, the mechanisms of IL, as they are revealed in laboratory studies, appear as definitely underpowered. The picture given by recent research stands far from the idea of the extraordinarily powerful processes that were imagined once, for instance by Lewicki et al., when they contended that "our nonconscious operating processing algorithms can do instantly and without external help" the same job as our conscious thinking achieves in relying on "notes (with flowchart or lists of if-then statements) or computer" (Lewicki et al., 1992, p.798). In fact, IL processes are probably unable to bring out to genuine rule knowledge, and the possibility of transfer are limited. In addition, the involvement of these processes seems to be dependent on selective attention. As pointed out above, the experimental study of learning around the 1960s was essentially devoted to classical and operant conditioning on the one hand and to the formation of concepts or problem-solving processes on the other hand. To make a long story short, IL mechanisms seem to be much nearer to the former than to the latter.

To be sure, experimental studies show that participants generally perform above chance in complex experimental settings. However, above-chance performance is generally attributable to the learning of some indirect, correlated aspects, which can be easily captured by elementary mechanisms. Everything happens as if IL often captured only nonessential aspects of the task. In experimental contexts, these correlated features are often considered as potential drawbacks, which need to be eliminated to reach the deep substance of the training material. For instance, studies in artificial grammar learning are often designed in such a way that bigram distribution becomes noninformative, studies in invariant learning often are controlled in such a way that the repetition of digits brings out no information about the invariant, and so on.

On the face of it, these data seem to provide fuel for a perspective in which the role of learning is minimized with regard to innate abilities. This is indeed the case if one considers that the knowledge base underlying the mastery of language and of the other high-level abilities alluded to above should be of the same form as the knowledge base that the scientist - for instance the linguist - acquires from an analytic investigation into his or her domain, that is, a formal, rule-based set of principles. This form of knowledge seems indeed to be definitely out of reach of IL processes.

However, a quite different perspective is possible. The general idea consists in assuming that learning in real-world setting proceeds as in the laboratory, that is, through the capture of correlated, apparently secondary aspects that can be grasped by elementary associative processes and that allow a good approximation of the behavior that would result from the knowledge of the formal structure of the domain. In order to be viable, such a perspective requires that the objective analysis of specific domains provides evidence for such correlated features. Quite interestingly, recent research on language has revealed a number of such features. The best-documented example is certainly the past-tense formation in English, in which it has been shown that regular and irregular verbs differ according to the distribution of their phonological and semantic features. Connectionist simulations have shown that exploiting those correlated cues leads to a very good approximation of the performance that would result from the formal knowledge of the *-ed* suffix rule, along with the knowledge of the exceptions (e.g., McClelland and Patterson, 2002). To consider another illustration, it has been shown that simple co-occurrence statistics (e.g., Redington et al., 1998) as well as phonological cues (e.g., Monaghan et al.,

2005) turn out to be highly informative about grammatical categories. These and other studies suggest that, as far as language is concerned, abstract classes and categories are often associated with simple statistical properties that make them tractable by general-purpose statistical learning mechanisms.

If further studies on language corpuses confirm and extend this kind of findings, and if the same kind of analysis proves to be successful in other high-level domains of competence, then IL mechanisms would appear extraordinarily powerful to promote behavioral adaptation. Indeed, those mechanisms are remarkably well-suited to exploit a massive amount of correlated cues. This approach appears to provide the first viable alternative to the nativist perspective that is still prevalent in the cognitive approach starting from Chomsky. The development of a full-blown empiricist alternative depends obviously upon further investigations on human learning processes, but also on the development of a nonconventional mode of description of the world humans are faced with.

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2.33 Implicit Memory and Priming

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2.33.1 Introduction

Priming refers to an improvement or change in the identification, production, or classification of a stimulus as a result of a prior encounter with the same or a related stimulus (Tulving and Schacter, 1990). Cognitive and neuropsychological evidence indicates that priming reflects the operation of implicit or nonconscious processes that can be dissociated from those that support explicit or conscious recollection of past experiences. More recently, neuroimaging studies have revealed that priming is often accompanied by decreased activity in a variety of brain regions (for review, see Schacter and Buckner, 1998; Wiggs and Martin, 1998; Henson, 2003), although conditions exist in which priming-related increases are also observed (e.g., Schacter et al., 1995; Henson et al., 2000; Fiebach et al., 2005). Various terms have been used to describe these neural changes, including adaptation, mnemonic filtering, repetition suppression, and repetition enhancement. These terms often refer to subtly distinct, though related, phenomena, and in some cases belie a theoretical bias as to the nature of such neural changes. Thus, throughout the present review, the term neural priming will be used to refer to changes in neural activity associated with the processing of a stimulus that result from a previous encounter with the same or a related stimulus.

When considering the link between behavioral and neural priming, it is important to acknowledge that functional neuroimaging relies on a number of underlying assumptions. First, changes in information processing result in changes in neural activity within brain regions subserving these processing operations. A second assumption underlying positron emission tomography (PET) and functional magnetic resonance imaging (fMRI) is that these changes in neural activity are accompanied by changes in blood flow, such that the energy expenditure that accompanies increased neuronal processing elicits the delivery of metabolites and removal of by-products to and from active regions, respectively. It is these local vascular changes that are measured: PET measures changes in cerebral blood flow and oxygen or glucose utilization, while fMRI measures the ratio of oxygenated to deoxygenated hemoglobin (i.e., the blood-oxygen-level dependent, or BOLD, signal). Related techniques such as event-related potentials (ERP) and magnetoencephalography (MEG), by contrast, measure the electrophysiological responses of neural populations more directly, although at a cost of decreased spatial resolution. While this chapter will focus on fMRI and to a lesser extent PET

studies of priming, ERP and MEG studies will be discussed when they are of special interest to the discussion of a particular topic.

Neuroimaging studies have provided new means of addressing cognitive theories that have traditionally been evaluated through behavioral studies. The primary goal of the present chapter is to examine how neuroimaging evidence has informed, influenced, and reshaped cognitive theories about the nature of priming. We focus on five research areas where such interaction has occurred: influences of explicit versus implicit memory, top-down attentional effects, specificity of priming, the nature of priming-related activation increases, and correlations between brain activity and behavior.

2.33.2 Influences of Explicit Versus Implicit Memory

Priming is typically defined as a nonconscious or implicit form of memory. This characterization is supported by numerous observations of spared priming in amnesic patients with severe disorders of explicit memory. However, starting with the earliest cognitive studies of priming in healthy volunteers, researchers have been concerned with the possibility that subjects may use some type of explicit retrieval to perform a nominally implicit task. This concern has led to the development of various cognitive procedures for estimating and removing the influences of explicit retrieval (e.g., Schacter et al., 1989; Jacoby, 1991). Two forms of such explicit 'contamination' have received attention in cognitive studies: (1) subjects realize that their memory is being tested, and intentionally retrieve study list words while performing a priming task to augment performance; (2) subjects follow task instructions, and therefore do not engage in intentional retrieval, but nonetheless unintentionally recollect that they had studied target items on the previous study list. With respect to the latter type of contamination, it has been noted that explicit memory often takes the form of unintentional or involuntary recollections of previous experiences in which there is no deliberate, effortful attempt to think back to the past; one is spontaneously 'reminded' of a past event that is accompanied by conscious recollection (e.g., Schacter, 1987; Schacter et al., 1989; Richardson-Klavehn et al., 1994; Richardson-Klavehn and Gardiner, 1998; Bernsten and Hall, 2004). We now consider findings from neuroimaging studies that provide insights into the nature of and relation between implicit and explicit influences on priming.

The explicit contamination issue arose in the first neuroimaging study of priming (Squire et al., 1992). In this experiment, subjects semantically encoded a list of familiar words prior to PET scanning and were then scanned during a stem completion task in which they provided the first word that came to mind in response to visual three-letter word stems. During one scan, subjects could complete stems with study list words (priming), and during another, they could complete stems only with new words that had not been presented on the study list (baseline). In a separate scan, subjects were provided with three-letter stems of study-list words, and were asked to think back to the study list (explicit cued recall).

Priming was associated with decreased activity in the right extrastriate occipital cortex compared with baseline, but there was also increased activity in the right hippocampal formation during priming compared with the baseline condition. In light of previous results from amnesic patients indicating that normal stem-completion priming can occur even when the hippocampal formation is damaged, it seemed likely that the observed activation of the hippocampal region reflects one of the two previously mentioned forms of 'contamination': subjects intentionally retrieved words from the study list or, alternatively, they provided the first word that comes to mind and involuntarily recollected its prior occurrence.

Schacter et al. (1996) attempted to reduce or eliminate explicit influences by using a nonsemantic study task (counting the number of t-junctions in each of target words), which in previous behavioral studies had supported robust stem-completion priming together with poor explicit memory for the target items (e.g., Graf and Mandler, 1984; Bowers and Schacter, 1990). Consistent with the idea that the priming-related hippocampal activation previously observed by Squire et al. reflects contamination from explicit memory that is not essential to observing priming, following the t-junction encoding task there was no evidence of priming-related increases in the vicinity of the hippocampal formation during stem completion performance relative to the baseline task, but there were priming-related decreases in bilateral extrastriate occipital cortex and several other regions.

Using PET, Rugg et al. (1997) found greater left hippocampal activity after deep encoding than after shallow encoding during both intentional (old/new

recognition) and unintentional (animate/inanimate decision) retrieval tasks. They also observed greater right anterior prefrontal activity during intentional retrieval than during unintentional retrieval after both deep and shallow encoding. These results suggest that increases in hippocampal activity during explicit retrieval, unaccompanied by corresponding increases in anterior prefrontal activity, reflect the presence of involuntary explicit memory.

A more recent event-related fMRI study by Schott et al. (2005) extends the findings of these early studies. During the study phase, subjects made nonsemantic encoding judgments in which they counted the number of syllables in each word. During the test phase, Schott et al. used a stem completion task and directly compared performance during intentional retrieval (i.e., try to remember a word from the list beginning with these three letters) and incidental retrieval (i.e., complete the stem with the first word that comes to mind). Importantly, they used a behavioral procedure developed by Richardson-Klavehn and Gardiner (1996, 1998) in which participants indicate whether or not they remember that the item they produced on the completion task had appeared earlier during the study task. This procedure could be applied to both the incidental and intentional tests, because on the intentional test subjects were told to complete stems even when they could not recall a study-list item. In the scanner, subjects used a button press to indicate whether they had covertly completed a stem; between these test trials, they provided their completions orally and indicated whether or not they remembered having seen the item during the study task. Stems completed with study-list words that were judged as nonstudied were classified as primed items, whereas stems completed with study-list words judged as studied were classified as remembered items. Both primed and remembered items were compared with baseline items that subjects judged correctly as nonstudied.

Similar to previous studies, Schott et al. (2005) documented activation reductions for primed items compared with baseline items in a number of regions, including extrastriate visual cortex. However, because the primed items in this study were, by definition, ones that subjects did not consciously remember having encountered previously, these data show more convincingly than earlier studies that priming-related activation decreases can reflect strictly nonconscious or implicit memory. Moreover, the authors also reported that their findings concerning priming-related reductions during the incidental tests were largely replicated during the intentional test. Thus, the results support the idea that priming effects can occur during both intentional and unintentional retrieval. Several other regions, including the right prefrontal cortex, showed greater activity during the intentional than the incidental task. In contrast to prior studies, the hippocampus showed greater activity during baseline than during priming, which the authors attributed to novelty encoding. Overall, these neuroimaging results support earlier behavioral distinctions between strategic controlled retrieval (i.e., intentional vs. incidental) and conscious recollection of the occurrence of previously studied items and show clearly that priming-accompanied activation reductions can occur without conscious recollection.

While the foregoing studies attempted to distinguish implicit and explicit aspects of priming by focusing on retrieval, other studies have done so by examining brain activity during encoding. Schott et al. (2006) examined subsequent memory effects, where neural activity during encoding is sorted according to whether items are subsequently remembered or forgotten (e.g., Brewer et al., 1998; Wagner et al., 1998). This study reported fMRI data from the encoding phase of the aforementioned stem completion experiment reported by Schott et al. (2005), where participants counted the number of syllables in each word. Consistent with results from earlier subsequent memory studies that examined explicit retrieval, Schott et al. found greater activation during encoding for subsequently remembered than for forgotten items in left inferior prefrontal cortex and bilateral medial temporal lobe. By contrast, encoding activity in these areas was not associated with subsequently primed items. Instead, subsequent priming was associated with activation decreases during encoding in bilateral extrastriate cortex, left fusiform gyrus, and bilateral inferior frontal gyrus. These regions were distinct from those that showed priming-related decreases during the stem completion test. Schott et al. suggest that their data indicate that priming, in contrast to explicit memory, is associated with sharpening of perceptual representations during encoding, an idea that is consistent with previous theories emphasizing the differential role of a perceptual representation system in priming and explicit memory (Schacter, 1990, 1994; Tulving and Schacter, 1990).

While the combined results from Schott et al.'s (2005, 2006) encoding and retrieval phases highlight

clear differences between priming and explicit memory, a related study by Turk-Browne et al. (2006), also using a subsequent memory paradigm, uncovered conditions under which the two forms of memory are associated with one another. Subjects made indoor/outdoor decisions about a series of novel scenes. Each scene was repeated once, at lags ranging from 2 to 11 items. Fifteen minutes after presentation of the final scene, subjects were given a surprise old/new recognition test. Turk-Browne et al. focused on a region of interest in the parahippocampal place area (PPA) that responds maximally to visual scenes (e.g., Epstein and Kanwisher, 1998). The critical outcome was that repeated scenes produced behavioral priming and reduced activation in the PPA, but only for those scenes that were subsequently remembered. Forgotten items did not produce either behavioral or neural priming. A whole-brain analysis revealed similar effects – neural priming for remembered items only – in bilateral PPA as well as in left inferior temporal gyrus and bilateral angular gyrus. However, forgotten items were associated with neural priming in the anterior cingulate.

Given the general trend that behavioral and neural priming both depended on subsequent explicit memory, Turk-Browne et al. suggested that their data reveal a link between implicit and explicit memory that involves some aspect of shared encoding processes – most likely that selective attention during encoding is required for both subsequent priming and explicit memory.

The neuroimaging evidence considered thus far reveals some conditions under which priming can occur independently of explicit memory and others where dependence exists. An experiment by Wagner et al. (2000) showed that priming can sometimes hinder explicit memory. They made use of the well-known spacing or lag effect, where reencoding an item after a short lag following its initial presentation typically produces lower levels of subsequent explicit memory than reencoding an item after a long lag (though in both cases, explicit memory is higher than with no repetition). Using an incidental encoding task (abstract/concrete judgment) and old/new recognition task, Wagner et al. documented greater explicit memory following a long- than a short-lag condition, consistent with previous behavioral findings. By contrast, they showed greater behavioral priming, indexed by reduced reaction time, and greater neural priming, indexed by reduced activity in the left inferior frontal lobe, following a short lag than a long lag. Moreover, there was a negative correlation between the magnitude of neural priming in the left inferior frontal region and the level of subsequent explicit memory. Thus, the short-lag condition that maximized priming also reduced explicit memory. Although the exact mechanism underlying the effect is still not known, Wagner et al. suggested that priming may impair new episodic encoding and later explicit memory by reducing encoding variability, that is, encoding different attributes of repeated items on different trials. To the extent that encoding variability normally enhances subsequent memory by providing multiple retrieval routes to an item (e.g., Martin, 1968), priming might reduce explicit memory because it biases encoding toward sampling the same item features on multiple trials. Whatever the ultimate explanation, these results highlight the role of a previously unsuspected interaction between priming and explicit memory in producing a well-known behavioral effect.

2.33.3 Top-Down Attentional Effects on Priming

Priming is often considered to be an automatic process (e.g., Jacoby and Dallas, 1981; Tulving and Schacter, 1990; Wiggs and Martin, 1998). However, recent neuroimaging evidence has revealed that, to some extent, behavioral and neural priming may be affected by top-down cognitive processes such as attention or task orientation.

2.33.3.1 Priming: Automatic/Independent of Attention?

Early evidence supported the notion that perceptual priming effects occur independent of manipulations of attention (for review see Mulligan and Hartman, 1996). However, subsequent findings from behavioral studies began to reveal that some perceptual priming effects do depend to some degree on attention at study (e.g., Mulligan and Hornstein, 2000).

In a seminal review that linked behavioral priming with the phenomenon of repetition suppression, Wiggs and Martin (1998) stated that this process "happens automatically in the cortex" and "is an intrinsic property of cortical neurons," and that "perceptual priming is impervious to ... attentional manipulations" (Wiggs and Martin, 1998: 231). Indeed, there is some compelling evidence from studies with monkeys

to suggest that repetition-related neural priming can occur independent of attention (e.g., Miller et al., 1991; Miller and Desimone, 1993; Vogels et al., 1995), but these findings do not speak directly to neural priming in humans. Some neuroimaging evidence shows that conditions exist under which both behavioral and neural priming are unaffected by manipulations of attention. A PET study by Badgaiyan et al. (2001) investigated the effects of an attentional manipulation during the study phase of a cross-modal priming task. Target words were aurally presented among distracter words at study under either full attention or under a divided-attention task. At test, visual word stems were presented in separate blocks for both target word types. Behavioral priming (faster reaction times) and neural priming (reduced regional cerebral blood flow in superior temporal gyrus) were of similar magnitude for words presented under full and divided attention conditions (see also Voss and Paller, 2006).

An fMRI study that we reviewed earlier (Schott et al., 2005) further demonstrated that changing the nature of the task to be performed during the test phase did not affect the level of behavioral or neural priming. Following shallow encoding of words at study, word stems were presented in separate blocks of either an implicit or an explicit memory task at test. Although the explicit task elicited a higher rate of explicit recollection of previously studied words, there were no differences in behavioral priming effects between the two conditions – i.e., subjects produced an equivalent number of previously studied words when cued with word stems in both test conditions. Moreover, an equivalent degree of neural priming was documented in left fusiform, bilateral frontal, and occipital brain regions in both implicit and explicit conditions. Thus, this experiment demonstrated that changing the task orientation at test had no effect on behavioral or neural priming.

Hasson et al. (2006) demonstrated comparable neural priming in some brain regions despite a change of task orientation across separate sessions (i.e., separate experiments with different tasks). In the first of two experiments, subjects listened to spoken sentences, some grammatically sensible, some nonsensible, and decided whether each sentence was sensible or not. In the second experiment, subjects passively listened to spoken sensible sentences only, making no judgments or responses. A direct contrast between the two tasks indicated that neural priming in temporal regions was equivalent across conditions. However, neural priming was also observed in inferior frontal regions, but only in the active condition in which subjects made sensible/nonsensible judgments. This finding suggests that attentional manipulations have variable effects on different brain regions.

The foregoing studies have demonstrated that behavioral and/or neural priming can occur independent of shifts in attentional demands or task orientation at study (Badgaiyan et al., 2001; Voss and Paller, 2006), at test (Schott et al., 2005), or between different tasks (Hasson et al., 2006). However, consistent with the latter finding by Hasson et al. of concurrent attenuation of priming in prefrontal regions associated with changing task demands, these null results do not rule out the possibility that under different task conditions, and in different brain regions, top-down attentional effects may play an important role in priming. We consider now (and also later in the chapter) recent evidence that supports this claim.

2.33.3.2 Priming: Modulated by Attention

Henson et al. (2002) reported one of the first neuroimaging studies to demonstrate that neural priming is modulated by top-down cognitive factors. Subjects viewed pictures of famous and nonfamous faces, each presented twice at random intervals within one of two separate, consecutive task sessions. During the implicit task session, subjects performed a continuous famous/nonfamous face discrimination task; during the explicit task session, subjects performed a continuous new/old face recognition task. Neural priming was observed in a face-responsive region in the right fusiform gyrus for repeated famous faces only, consistent with previous findings (Henson et al., 2000), as well as for both famous and nonfamous faces in a left inferior occipital region. Neural priming in these regions occurred only in the implicit task. As stimuli were identical across the different task conditions, the modulation of neural priming was attributed to top-down effects of task orientation.

Although there were effects of attention on neural priming, behavioral priming seemed to be unaffected by top-down factors. Rather, behavioral priming, as indexed by reduced reaction time to respond to repeated presentations of famous faces relative to initial presentations, was equivalent in the implicit and explicit tasks. This result implies a dissociation between behavioral priming and neural priming observed in these brain regions. Further, attentional modulation varied only between sessions, i.e., the

same task was performed on each stimulus during the initial and repeated presentations, leaving open the question of whether attentional factors exert an influence at study, at test, or on both occasions.

A subsequent fMRI study tested the hypothesis that attentional factors, specifically at study, have an impact on neural priming (Eger et al., 2004). During fMRI scanning, subjects performed a task at study in which two objects were simultaneously presented, one to the left and one to the right of a central fixation point. Importantly, subjects were cued to attend to either the left or right of center by a visual cue presented onscreen 100 ms prior to presentation of the 'prime' stimuli. A single 'probe' stimulus was subsequently presented in the center of the screen that matched the previously attended stimulus, matched the previously unattended stimulus, was the mirror image of one of these two stimuli, or was novel. Analyses of repetition-related behavioral facilitation (faster reaction times) and neural response reductions (fMRI BOLD signal decreases in fusiform and lateral occipital regions) revealed that behavioral and neural priming occurred only for probes that matched (or mirrored) the attended prime. Conversely, no behavioral or neural priming was documented when the probe stimulus matched (or mirrored) the unattended prime. Thus, this study showed that modulation of spatial attention affects behavioral as well as neural priming in object selective perceptual processing regions, and that these top-down attentional effects exert an influence specifically at the time of study.

In a face-repetition priming study, Ishai et al. (2004) reported that neural priming occurred only for repeated faces that were task relevant. Subjects were presented with a target face and then were shown a series of faces, including three repetitions of the target face, three repetitions of a nontarget face, and seven distracter faces. Participants were required to push a button each time the target face appeared, and thus were required to attend to all faces, although only the target face was task relevant. Significant neural priming (reduced BOLD response for the third relative to the first repetition) was observed in face-responsive regions, including inferior occipital gyri, lateral fusiform gyri, superior temporal sulci, and amygdala, but only for the target face repetitions; no neural priming was associated with repetition of nontarget faces.

Yi and colleagues (Yi and Chun, 2005; Yi et al., 2006) used overlapping scene and face images to also demonstrate that task-relevant attention has an effect even for simultaneously viewed stimuli. In one experiment, participants were presented with overlapping face and scene images and instructed to attend only to the face or the scene on a given trial (Yi et al., 2006). Neural priming in a face-responsive fusiform region was documented only for repeated faces that were attended, and not for scenes or unattended faces. Similarly, neural priming in a scene-responsive parahippocampal region occurred only for repeated scenes that were attended, and not for faces or unattended scenes. Surprisingly, even after sixteen repetitions of a stimulus every 2 s within a block, no trace of neural priming was observed for unattended stimuli in these respective regions (Yi et al., 2006).

Thus, while a number of neuroimaging studies have shown that both behavioral and neural priming can remain constant across study and test manipulations of attention or between different tasks with common stimuli, several studies reviewed here indicate that top-down effects of attention can have an impact on behavioral and/or neural priming, both at the time of study (Henson et al., 2002) and at test (Ishai et al., 2004), and have been shown to involve both spatial attention (Eger et al., 2004) and task-relevant selective attention (Ishai et al., 2004; Yi and Chun, 2005; Yi et al., 2006). To reconcile these ostensibly incongruent conclusions requires a more detailed consideration of the nature of subtle differences in various manipulations of attention, and importantly, of the particular brain regions involved.

Accordingly, recent studies (e.g., Hassan et al., 2006) have begun to dissociate various brain regions that are differentially sensitive to various attentional manipulations. In a study by Vuilleumier et al. (2005), participants viewed overlapping objects drawn in two different colors at study and were instructed to attend only to objects of a specified color. At test, these objects were presented singly among novel real and nonsense objects, and subjects indicated whether each object was a real or nonsense object. Behavioral priming was documented both for previously attended and ignored objects, with a relative boost in performance for objects that were attended. However, different brain regions showed differential sensitivity to the effects of attention on neural priming. A group of regions that comprised right posterior fusiform, lateral occipital, and left inferior frontal regions demonstrated neural priming only for attended objects presented in the original view. By contrast, bilateral anterior fusiform regions were insensitive to changes of viewpoint (original vs.

mirrored), but showed neural priming for unattended objects in addition to more robust neural priming for attended objects. Finally, neural priming in the striate cortex was view specific and more robust for attended than ignored objects.

In keeping with the latter findings, O'Kane et al. (2005) reported a similar dissociation between brain regions differentially sensitive to manipulation of top-down processes. Subjects were presented with words at study and performed a judgment of either size, shape, or composition in separate task blocks. At test, subjects performed a size judgment for all studied words presented among novel words. Behavioral facilitation, as measured by faster reaction times for size judgments at test, was observed for repeated relative to novel words, with an additional benefit when the judgment was the same at study and test (size/size) relative to when the judgment was switched (shape/size or composition/size). Neural priming in left parahippocampal cortex tracked the behavioral trend, showing reduced BOLD responses for repeated relative to novel words, with an additional trend toward increased priming when the task was the same across repetition. In left perirhinal cortex, however, neural priming occurred for repeated words only when the judgment was the same at study and test. The finding that perirhinal cortex is sensitive to semantic but not perceptual repetition provides evidence that this region is involved in conceptual processing.

Considered together, the neuroimaging studies reviewed here suggest that behavioral and neural priming are indeed modulated by top-down cognitive factors of attention or task orientation, but that this modulation exerts differential effects across different brain regions depending on the nature of the task. Neural priming within a given brain region may occur only to the extent that the processing of a stimulus reengages this region in a qualitatively similar manner across repetitions.

2.33.3.3 Neural Mechanisms of Top-Down Attentional Modulation

Although the effects of attention on priming have now been well documented, little is known about the neural mechanisms that underlie these top-down effects. Efforts to understand these mechanisms have been at the forefront of recently emerging neuroimaging research.

Increased attention at the time of study has been suggested as an important factor in priming. Turk-Browne et al. (2006), as previously reviewed in this chapter, reported that neural priming occurred only for repeated scenes that were later remembered, but not for those scenes that were later forgotten. They found that tonic activation, a general measure of regional neural activity, was elevated for scenes that were later remembered and that also elicited neural priming upon repeated presentation. While previous evidence indicates that increased attention results in increased neural firing rates within process-relevant brain regions, a recent fMRI study suggests that attention may also increase selectivity of the neural population representing an attended stimulus (Murray and Wojciulik, 2004).

Other neuroimaging approaches, including MEG and EEG, have been used to further characterize the nature of attentional modulations of neural priming as well. Evidence supporting the hypothesis that attention serves to increase specificity of perceptual representations was reported by Duzel and colleagues (2005) in a study using MEG. By investigating neural activity at study, they compared words that showed subsequent behavioral priming (faster reaction times) to those that did not show subsequent priming. They reported relatively decreased amplitude, but increased phase alignment, of beta and gamma oscillations for words that showed later priming, indicating increased specificity of the neural response for these words at the time of study. Further, they reported increased coordination of activity between perceptual and higher brain regions for words that showed subsequent priming, as measured by increased interareal phase synchrony of alpha oscillations. Importantly, this increased synchrony between perceptual and higher brain regions was detected immediately prior to the initial presentation of the subsequently primed stimuli, indicating an anticipatory effect. These results suggest that top-down processes, through anticipatory coordination with perceptual brain regions, increase specificity of perceptual representations at study. Such a process may also be necessary at test for successful priming. Gruber et al. (2006) reported that 'sharpening' of the neural response in cell assemblies (as measured by suppression of induced gamma band responses in ERPs) occurred for repeated visual stimuli only when the task was the same at both study and test, but not when the task was switched.

Therefore, through a combination of various neuroimaging techniques, researchers have begun to characterize the neural mechanisms that underlie attentional modulation of priming. These

mechanisms may constitute a link between the cognitive functions that are accessible to our conscious awareness and under our volitional control and the unconscious systems that facilitate fluency of mental processing.

2.33.4 Specificity of Priming

Priming effects vary in their specificity, that is, the degree to which priming is disrupted by changes between the encoding and test phases of an experiment. When study/test changes along a particular dimension produce a reduction in priming, the inference is that the observed priming effect is based to some extent on retention of the specific information that was changed; when level of priming is unaffected by a study/test change, the inference is that priming reflects the influence of an abstract representation, at least with respect to the changed attribute. Questions concerning the specificity of priming have been prominent since the early days of priming research in cognitive psychology, when evidence emerged that some priming effects are reduced when study/test sensory modality is changed (e.g., Jacoby and Dallas, 1981; Clarke and Morton, 1983) and can also exhibit within-modality perceptual specificity, shown by the effects of changing typeface or case for visual words (e.g., Roediger and Blaxton, 1987; Graf and Ryan, 1990), or speaker's voice for auditory words (e.g., Schacter and Church, 1992). Considerable theoretical debate has focused on the key issue raised by studies of specificity effects, namely whether priming reflects the influence of nonspecific, abstract preexisting representations or specific representations that reflect perceptual details of an encoding episode (for review and discussion of cognitive studies, see Roediger, 1990; Schacter, 1990, 1994; Roediger and McDermott, 1993; Tenpenny, 1995; Bowers, 2000).

Considering the early cognitive research together with more recent neuropsychological and neuroimaging studies, Schacter et al. (2004) recently proposed a distinction among three types of specificity effects: stimulus, associative, and response. Stimulus specificity occurs when priming is reduced by changing physical properties of a stimulus between study and test; associative specificity occurs when priming is reduced because associations between target items are changed between study and test; and response specificity occurs when priming is reduced because subjects make different responses to the same stimulus item at study and test. We will review here evidence from neuroimaging studies concerning each of the three types of priming specificity and consider how the imaging data bear on the kinds of theoretical questions that have been of interest to cognitive psychologists.

2.33.4.1 Stimulus Specificity

Most neuroimaging research has focused on stimulus specificity, which is observed by changing physical features of a stimulus between study and test. As mentioned earlier, cognitive studies have shown that priming effects are sometimes modality specific, that is, reduced when study and test sensory modalities are different compared with when they are the same. Such effects are most commonly observed on tasks such as word or object identification, stem completion, or fragment completion, which require perceptual or data-driven processing (Roediger and Blaxton, 1987). Amnesic patients have shown a normal modality-specific effect in stem completion priming (e.g., Carlesimo, 1994; Graf et al., 1985), suggesting that this effect is not dependent on the medial temporal lobe structures that are typically damaged in amnesics.

Early neuroimaging studies of within-modality visual priming that compared brain activity during primed and unprimed stem completion showed that priming is associated with decreased activity in various posterior and prefrontal cortical regions, but the decreases were observed most consistently in the right occipitotemporal extrastriate cortex (e.g., Squire et al., 1992; Buckner et al., 1995; Schacter et al., 1996). These and related findings raised the possibility that priming-related reductions in extrastriate activity are based on a modality-specific visual representation, perhaps reflecting tuning or sharpening of primed visual word representations (Wiggs and Martin, 1998). Consistent with this possibility, Schacter et al. (1999) directly compared within-modality visual priming to a cross-modality priming condition in which subjects heard words before receiving a visual stem completion task. They found priming-related reductions in extrastriate activity during within- but not cross-modality priming. Surprisingly, however, other neuroimaging studies of within-modality auditory stem completion priming also revealed priming-related activity reductions near the extrastriate region that was previously implicated in visual priming (Badgaiyan et al., 1999; Buckner et al., 2000; Carlesimo et al., 2004). These results remain poorly understood, but it has been

suggested that one part of the extrastriate region (V3A, within BA 19) is involved in multimodal functions, perhaps converting perceptual information from one modality to another (Badgaiyan et al., 1999).

Although the results of imaging studies comparing within- and cross-modality priming are not entirely conclusive, studies of within-modality changes in physical properties of target stimuli have provided clear evidence for stimulus-specific neural priming, which in turn implicates perceptual brain mechanisms in the observed priming effects. Studies focusing on early visual areas have provided one source of such evidence. Grill-Spector et al. (1999) found that activation reductions in early visual areas such as posterior lateral occipital complex (LOC) exhibit a high degree of stimulus specificity for changes in viewpoint, illumination, size, and position. By contrast, later and more anterior aspects of LOC exhibit greater invariance across changes in size and position relative to illumination and viewpoint. Evidence from a study by Vuilleumier et al. (2005) considered in the previous section likewise indicates a high degree of stimulus specificity in early visual areas, as indicated by viewpoint-specific neural priming in these regions.

Later visual regions can also show stimulus-specific neural priming, but several studies indicate that this specificity effect is lateralized. In a study by Koutstaal et al. (2001), subjects judged whether pictures of common objects were larger than a 13-inch-square box, and later made the same judgments for identical objects, different exemplars of objects with the same name, and new objects. Behavioral priming, indicated by faster response times, occurred for both identical objects and different exemplars, with significantly greater priming for identical objects. Reductions in activation were also greater for same than for different exemplars in the bilateral middle occipital, parahippocampal, and fusiform cortices. These stimulus-specific activation reductions for object priming were greater in the right than in the left fusiform cortex. Simons et al. (2003) replicated these results and further demonstrated that left fusiform cortex shows more neural priming for different exemplars compared with novel items relative to right fusiform cortex, indicating more nonspecific neural priming in the left fusiform. Also, left but not right fusiform neural priming was influenced by a lexical-semantic manipulation (objects were accompanied by presentation of their names or by nonsense syllables), consistent with a lateralized effect in which right fusiform is modulated by specific physical features of target stimuli and left fusiform is influenced more strongly by semantic features. In a related study by Vuilleumier et al. (2002), subjects decided whether pictorial images depicted real or nonsense objects, and subsequently repeated stimuli were identical, differed in size or viewpoint, or were different exemplars with the same name. Neural priming in the right fusiform cortex was sensitive to changes in both exemplar and viewpoint.

A similar pattern has also been reported for orientation-specific object priming by Vuillemer et al. (2005) in the overlapping shape paradigm described earlier, and Eger et al. (2005) reported a stimulus-specific laterality effect using faces. In the latter experiment, subjects made male/female judgments about famous or unfamiliar faces that were preceded by the identical face, a different view of the same face, or an entirely different face. Behavioral priming, indexed by decreased response times, was greater for same than different viewpoints for both famous and unfamiliar faces. Collapsed across famous and unfamiliar faces, neural priming was more viewpoint dependent in right fusiform gyrus than in left fusiform gyrus. In addition, for famous faces, priming was more nonspecific in anterior than more posterior fusiform cortex. Similarly, Vuillemer et al. (2005) report some evidence for greater stimulus-specific neural priming in posterior compared with anterior fusiform gyrus. Other studies indicate that later perceptual regions can exhibit largely nonspecific priming, both for visual stimuli such as scenes (Blondin and Lepage, 2005) and auditory words (Orfanidou et al., 2006; see also Badgaiyan et al., 2001). However, evidence provided by Bunzeck et al. (2005) suggests that effects in later perceptual regions are characterized by category specificity. In their study, subjects made male/female judgments about faces and indoor/outdoor judgments about scenes. Subjects responded more quickly to repeated faces and scenes compared with initial presentations, thus demonstrating behavioral priming. Face-responsive regions in fusiform and related areas showed selective activation reductions for repeated faces, whereas place-responsive regions in parahippocampal cortex showed decreases for repeated scenes.

By contrast, regions of inferior frontal gyrus and left inferior temporal cortex appear to respond invariantly to an item's perceptual features and are instead sensitive to its abstract or conceptual properties – even when the degree of perceptual overlap between initial and subsequent presentations of a stimulus is minimal to nonexistent. Neural priming has been observed in

these regions during reading of mirror-reversed words initially presented in a normal orientation (Ryan and Schnyer, 2006) and also when silently reading semantically related word pairs, but not for pairs that are semantically unrelated (Wheatley et al., 2005). Consistent with this observation, neural priming in these regions is independent of stimulus modality (Buckner et al., 2000) and has even been observed when the modality differs between the first and second presentations of a stimulus (e.g., visual to auditory; Badgaiyan et al., 2001; Carlesimo et al., 2003).

Overall, then, the foregoing studies reveal a fairly consistent pattern in which neural priming in early visual regions exhibits strong stimulus specificity, whereas in later visual regions, right-lateralized stimulus specificity is consistently observed (for a similar pattern in a study of subliminal word priming, see Dehaene et al., 2001). These effects dovetail nicely with previous behavioral studies using divided-visual-field techniques that indicate that visually specific priming effects occur to a greater extent in the left visual field (right hemisphere) than in the right visual field (left hemisphere) (e.g., Marsolek et al., 1992, 1996).

The overall pattern of results from neuroimaging studies of stimulus specificity suggests that, consistent with a number of earlier cognitive theories (e.g., Roediger, 1990; Schacter, 1990, 1994; Tulving and Schacter, 1990), perceptual brain mechanisms do indeed play a role in certain kinds of priming effects.

2.33.4.2 Associative Specificity

Research concerning the cognitive neuroscience of associative specificity began with studies examining whether amnesic patients can show priming of newly acquired associations between unrelated words. For example, amnesic patients and controls studied pairs of unrelated words (such as window–reason or officer–garden) and then completed stems paired with study list words (window–rea___) or different unrelated words from the study list (officer–rea___). Mildly amnesic patients and control subjects showed more priming when stems were presented with the same words from the study task than with different words, indicating that specific information about the association between the two words had been acquired and influenced priming, but severely amnesic patients failed to show associative priming (Graf and Schacter, 1985; Schacter and Graf, 1986). A number of neuropsychological studies have since examined associative specificity in amnesics with mixed results (for review, see Schacter et al., 2004), and it has been suggested that medial temporal lobe (MTL) structures play a role in such effects. Some relevant evidence has been provided by a PET study that used a blocked design version of the associative stem completion task (Badgaiyan et al., 2002). Badgaiyan et al. found that, as in previous behavioral studies, priming was greater when stems were paired with the same words as during the study task than when they were paired with different words. The same pairing condition produced greater activation in the right MTL than did the different pairing condition, suggesting that associative specificity on the stem completion task may indeed be associated with aspects of explicit memory. Given the paucity of imaging evidence concerning associative specificity, additional studies will be needed before any strong conclusions can be reached.

2.33.4.3 Response Specificity

While numerous behavioral studies had explored stimulus specificity and associative specificity prior to the advent of neuroimaging studies, the situation is quite different when considering response specificity, where changing the response or decision made by the subject about a particular item influences the magnitude of priming (note that we use the terms 'response specificity' and 'decision specificity' interchangeably, since behavioral data indicate that the effect is likely not occurring at the level of a motor response; see Schnyer et al., in press). Recent interest in response specificity has developed primarily as a result of findings from neuroimaging research. Dobbins et al. (2004) used an object decision priming task that had been used in studies considered earlier (Koutstaal et al., 2001; Simons et al., 2003), but modified the task so that responses either remained the same or changed across repeated trials. In the first scanning phase, pictures of common objects were either shown once or repeated three times, and subjects indicated whether each stimulus was bigger than a shoebox (using a 'yes' or 'no' response). Next, the cue was inverted so that subjects now indicated whether each item was 'smaller than a shoebox'; they made this judgment about new items and a subset of those that had been shown earlier. Finally, the cue was restored to 'bigger than a shoebox,' and subjects were tested on new items and the remaining items from the initial phase.

If priming-related reductions in neural activity that are typically produced by this task represent facilitated size processing, attributable to 'tuning' of relevant aspects of neural representations, then cue reversal should have little effect on priming (though it could disrupt overall task performance by affecting both new and primed items). According to the neural tuning account, the same representations of object size should be accessed whether the question focuses on 'bigger' or 'smaller' than a shoebox. By contrast, if subjects perform this task by rapidly recovering prior responses, and this response learning mechanism bypasses the need to recover size representations, then the cue reversal should disrupt priming-related reductions. When the cue is changed, subjects would have to abandon the learned responses and instead reengage the target objects in a controlled manner in order to recover size information.

During the first scanning phase, standard priming-related activation reductions were observed in both anterior and posterior regions previously linked with priming: left prefrontal, fusiform, and extrastriate regions. But when the cue was reversed, these reductions were eliminated in the left fusiform cortex and disrupted in prefrontal cortex; there was a parallel effect on behavioral response times. When the cue was restored to the original format, priming-related reductions returned (again there was a parallel effect on behavioral response times), suggesting that the reductions depended on the ability of subjects to use prior responses during trials. Accordingly, the effect was seen most clearly for items repeated three times before cue reversal.

Although this evidence establishes the existence of response-specific neural and behavioral priming, there must be limitations on the effect, since a variety of priming effects occur when participants make different responses during study and test. For instance, priming effects on the stem completion task, where subjects respond with the first word that comes to mind when cued with a three-letter word beginning, are typically observed after semantic or perceptual encoding tasks that require a different response (see earlier discussion on top-down attentional influences). Nonetheless, the existence of response specificity challenges the view that all activation reductions during priming are attributable to tuning or sharpening of perceptual representations, since such effects should survive a response change. Moreover, these findings also appear to pose problems for theories that explain behavioral priming effects on object decision and related tasks in terms of changes in perceptual representation systems that are thought to underlie object representation (e.g., Schacter, 1990, 1994; Tulving and Schacter, 1990), since these views make no provisions for response specificity effects. By contrast, the transfer appropriate processing view (e.g., Roediger et al., 1989, 1999) inherently accommodates such effects. According to this perspective, priming effects are maximized when the same processing operations are performed at study and at test. Although this view has emphasized the role of overlapping perceptual operations at study and at test to explain priming effects on tasks such as object decision, to the extent that the subject's decision or response is an integral part of encoding operations, it makes sense that reinstating such operations at test would maximize priming effects.

However, there is one further feature of the experimental paradigm that Dobbins et al. (2004) used to produce response specificity that complicates any simple interpretation. Priming in cognitive studies is usually based on a single study exposure to a target item, but neuroimaging studies of priming have typically used several study exposures in order to maximize the signal strength. As noted earlier, Dobbins et al. found that response specificity effects were most robust for items presented three times during the initial phase of the experiment (high-primed items), compared with items presented just once (low-primed items).

A more recent neuropsychological investigation of response specificity in amnesic patients highlights the potential theoretical importance of this issue (Schnyer et al., 2006). Schnyer et al. compared amnesics and controls on a variant of the object decision task used by Dobbins et al. (2004). Objects were presented either once (low primed) or thrice (high primed), and then responses either remained the same ('bigger than a shoebox?') or were switched ('smaller than a shoebox?'). Consistent with Dobbins et al. (2004), controls showed greater response specificity for high-primed objects compared with low-primed objects. Amnesic patients showed no evidence of response specificity, demonstrating normal priming for low-primed items and impaired priming for high-primed items. That is, healthy controls showed greater priming for high- than for low-primed objects in the same response condition, but amnesics failed to show this additional decrease in response latencies.

These results raise the possibility that different mechanisms are involved in priming for objects presented once versus those presented multiple times. Perhaps single-exposure priming effects on the object

decision task depend primarily on perceptual systems that operate independently of the MTL and thus are preserved in amnesic patients. In neuroimaging experiments, such effects might reflect tuning or sharpening of perceptual systems, independent of the specific responses or decisions that subjects make regarding the object. But for items presented several times, subjects may learn to associate the object with a particular response, perhaps requiring participation of medial temporal and prefrontal regions. These considerations also suggest that response or decision specificity in the object decision paradigm used by Dobbins et al. (2004) is better described in terms of stimulus-response or stimulus-decision specificity – that is, the formation of a new link between a particular stimulus and the response or decision. This idea is supported by recent behavioral data showing that response-specific priming occurs only for the exact object that was studied, and not for a different exemplar with the same name (Schnyer et al., in press). In any event, the overall pattern of results suggests that a single-process model is unlikely to explain all aspects of these neural or behavioral priming effects, a point to which we return later in the chapter.

2.33.5 Priming-Related Increases in Neural Activation

Our review so far has focused on behavioral facilitation and corresponding repetition-related reductions of neural activity associated with priming. However, under some conditions, priming has been associated with decrements in stimulus processing, such as slower responses to previously ignored stimuli relative to novel stimuli (i.e., the 'negative priming' effect – a term coined by Tipper, 1985) and poorer episodic encoding for highly primed items (Wagner, et al., 2000). Further, while repetition-related increases in neural activity have long been associated with explicit memory processes, neural increases associated with priming have also been documented, although less frequently. Neuroimaging studies have begun to investigate the nature of such neural increases and the conditions that elicit them. This research suggests a link between performance decrements and increased neural responses associated with priming and provides new evidence that speaks to competing cognitive theories of implicit memory.

2.33.5.1 Negative Priming

Negative priming (NP) occurs when a stimulus is initially ignored, and subsequent processing of the stimulus is impaired relative to that of novel stimuli. An early example of identity NP was demonstrated by Tipper (1985); overlapping drawings of objects drawn in two different colors were presented, and subjects were instructed to attend to and identify objects of only one specified color. At test, identification of previously presented objects that were ignored was significantly slower than identification of novel objects. The NP effect has since been documented across a diverse range of experimental tasks and stimuli (for review, see Fox, 1995; May et al., 1995). Efforts to characterize the nature of this processing have sparked a number of theoretical debates within the cognitive psychology literature. One of these debates has centered on the cause of NP (e.g., whether it relies on processes during encoding or later retrieval), while another has focused on determining the level of processing that ignored items undergo in order to elicit NP (e.g., perceptual vs. semantic processing).

Competing accounts of the cause of NP are offered by two theories. The selective inhibition model (Houghton and Tipper, 1994) proposes that representations of ignored stimuli are initially activated but are immediately inhibited thereafter by selective attention. Thus, upon subsequent presentation of a previously ignored stimulus, this inhibition must be overcome, resulting in slowed processing relative to novel stimuli. The episodic retrieval model (Neill and Valdes, 1992; Neill et al., 1992) proposes that ignored stimuli are fully encoded into an episodic representation, as are attended stimuli. Upon repeated presentation of a stimulus, episodic information from the initial presentation can provide a 'shortcut' to the previous response associated with that stimulus. Whereas this would facilitate processing of previously attended stimuli that were associated with a particular response, it is detrimental to processing of ignored stimuli with which no response was associated at study. Behavioral experiments have failed to produce unambiguous support for either of these models (Fox, 1995; May et al., 1995; Egner and Hirsch, 2005).

Neuroimaging can provide a useful way to test these theories, because they predict the involvement of different brain regions supporting either inhibitory or episodic processes. Egner and Hirsch (2005) reported data from an fMRI experiment using

a color-naming Stroop task that provide support for the episodic retrieval model. A region in the right dorsolateral prefrontal cortex (DLPFC) demonstrated increased activation for probe trials that were subject to NP relative to probe trials that had not been primed. The authors noted that this right DLPFC region has been associated with processes related to episodic retrieval (for review, see Stevens and Grady, 2007). Importantly, across individual subjects, activity in right DLPFC was positively correlated with response times during NP trials, but not nonprimed trials. These data support the theory that ignored stimuli, rather than being actively inhibited, are fully encoded at study, and that episodic retrieval at test contributes to the NP effect.

Another recent fMRI study investigated the level at which ignored stimuli are processed (i.e., perceptual vs. semantic/abstract) (Zubicaray et al., 2006). The authors reasoned that, if ignored stimuli elicit automatic activation of semantic representations at study, then brain regions that have been implicated in the storage and/or processing of these representations, such as the anterior temporal cortex (for review, see McClelland and Rogers, 2003) should be active during study of ignored stimuli. Overlapping drawings of different-colored objects elicited NP (slower reaction time for object identification at test) for previously ignored objects relative to novel objects. Analysis of fMRI data from the study session revealed a positive relationship between the magnitude of BOLD activity in the left anterolateral temporal cortex, including the temporal pole, and the magnitude of the subsequent NP effect. In agreement with Egner and Hirsch (2005), these data suggest that ignored stimuli are actively processed at study, and further indicate that this processing occurs at the level of abstract/semantic representations in higher conceptual brain regions.

2.33.5.2 Familiar Versus Unfamiliar Stimuli

There has been a long-standing debate in the cognitive psychology literature concerning priming of familiar versus unfamiliar stimuli (for review, see Tenpenny, 1995). According to modification/abstractionist theories (Morton, 1969; Bruce and Valentine, 1985), preexisting representations are required in order for priming to occur; these abstract representations are modified in some way upon presentation of familiar stimuli. According to acquisition/episodic theories (Jacoby, 1983; Roediger and Blaxton, 1987; Schacter et al., 1990), priming does not rely on a preexisting representation; rather, both familiar and unfamiliar stimuli can leave some form of a trace that can facilitate subsequent priming (although there may be limits; see Schacter et al., 1990; Schacter and Cooper, 1995). Neuroimaging studies have produced data relevant to this debate.

In a PET study, Schacter et al. (1995) reported behavioral priming for repeated unfamiliar objects, as shown by increased accuracy of possible/impossible judgments for structurally possible three-dimensional objects. However, in contrast to the more common finding of concomitant reduction in neural activity associated with behavioral priming reviewed earlier in the chapter, the authors reported increased activation in a left inferior fusiform region that was associated with priming of the possible objects.

In a more recent event-related fMRI study, Henson et al. (2000) reported data from four experiments using familiar and unfamiliar faces and symbols that directly tested the hypothesis that repetition-related neural priming entails reduced neural activity for familiar stimuli, but increased neural activity for unfamiliar stimuli. Behavioral priming (faster reaction times for familiarity judgments) was documented for repetition of both familiar and unfamiliar faces and symbols (although priming was greater for familiar than for unfamiliar stimuli). However, in a right fusiform region, repetition resulted in decreased activation for familiar faces and symbols, but increased activation for unfamiliar faces and symbols.

Henson et al. (2000) offered an account of their findings in terms of both modification and acquisition: while priming of familiar stimuli involves modification of preexisting representations, resulting in repetition suppression, priming also occurs for unfamiliar stimuli as a new representation is formed, resulting in repetition enhancement (for a generalized theory, see Henson, 2003). This suggestion is supported by evidence from a study by Fiebach et al. (2005), who concluded that neural decreases accompanying repeated words, in contrast to neural increases accompanying repeated pseudowords, reflect the sharpening of familiar object representations and the formation of novel representations for unfamiliar objects, respectively. Further, data from a previously reviewed study by Ishai et al. (2004) support this hypothesis as well; for unfamiliar faces, neural activation increased for the first repetition, but decreased in a linear trend thereafter, possibly reflecting the initial acquisition of an unfamiliar face representation, followed by subsequent modification of this newly

formed representation. Henson et al. (2000) further hypothesized that the repetition enhancement effect for unfamiliar stimuli would only occur in "higher visual areas, such as the fusiform cortex, where the additional processes such as recognition occur" (Henson et al., 2000: 1272). However, in a recent study using event-related fMRI, Slotnick and Schacter (2004) reported increased activation in early visual processing regions (BA 17/18) for repeated, relative to novel, unfamiliar abstract shapes. This finding suggests that earlier perceptual regions may also demonstrate activation attributable to processes involved in acquisition of new representations of unfamiliar stimuli.

2.33.5.3 Sensitivity Versus Bias

In number of studies by Schacter and colleagues (Schacter et al., 1990, 1991a; Cooper et al., 1992; Schacter and Cooper, 1993) participants studied line drawings of structurally possible and impossible objects and then made possible/impossible judgments at test to repeated presentations of the objects. Behavioral priming is measured as increased accuracy (and/or faster reaction time) for identifying an object as possible or impossible upon repeated presentations; significant priming is consistently observed for possible, but not impossible, objects. As mentioned earlier, a PET study of priming on the possible/impossible decision task revealed that increased activation in a left inferior/fusiform region was associated with priming of possible objects only (Schacter et al., 1995).

Schacter and Cooper proposed that such priming depends on the structural description system (SDS), a subsystem of the more general perceptual representation system (Tulving and Schacter, 1990). The proposal of an SDS was based on evidence of dissociations between priming (for possible, but not impossible, objects) and explicit tests of memory, across study-to-test object transformations (Cooper, et al., 1992; Schacter et al., 1993b), manipulations at encoding (Schacter and Cooper, 1993; Schacter et al., 1990), and in studies with elderly populations and amnesic patients (Schacter et al., 1991b, 1992, 1993b; and for review, see Soldan et al., 2006). In this view, priming of repeated objects reflects increased sensitivity (i.e., accuracy) on the part of the SDS, which is only capable of representing structurally possible objects.

An alternative theory is the bias account of priming in the possible/impossible object-decision task proposed by Ratcliff and McKoon (McKoon and Ratcliff, 1995, 2001; Ratcliff and McKoon, 1995, 1996, 1997, 2000). In this view, an encounter with an object, regardless of whether it is structurally possible or impossible, results in a subsequent bias to classify that object as 'possible,' leading to increased accuracy (i.e., positive priming) for repeated possible objects but decreased accuracy (i.e., negative priming) for impossible objects. However, this account also posits that explicit processes play a role in object-decisions, such that explicit memory of the study episode cues subjects as to whether the object is possible or impossible. It is argued, then, that this combination of bias and episodic information leads to robust positive priming for possible objects. By contrast, for impossible objects, the two factors cancel each other out, resulting in zero priming. Ratcliff and McKoon (1995) reported data from seven experiments that supported their hypothesis (for criticism of their conclusions, see Schacter and Cooper, 1995; for response, see McKoon and Ratcliff, 1995). Other bias accounts of object-decision priming have been proposed as well, such as the structure-extraction bias (Williams and Tarr, 1997).

Behavioral studies relevant to this debate continue to emerge, supporting either the sensitivity account of priming (e.g., Zeelenberg et al., 2002) or the bias account (e.g., Thapar and Rouder, 2001), but behavioral investigations alone have been inconclusive (Soldan et al., 2006). However, neuroimaging studies have recently produced evidence that speaks to the ongoing debate.

In a recent event-related fMRI study (Habeck et al., 2006), subjects performed a continuous possible/impossible object-decision task on structurally possible and impossible objects repeated four times each. Although the behavioral results did not correspond to sensitivity or bias models, or to previous findings (priming, as measured by faster reactions times, was documented for both possible and impossible objects), neural priming was documented for possible objects only. A multivariate analysis of the fMRI data revealed a pattern of brain regions in which activation covaried in a linear fashion (areas showing both repetition suppression and repetition enhancement) with repetition of possible objects only. No such pattern was observed for repetition of impossible objects. Further, there was a correlation between behavioral (faster reaction times) and neural priming for possible objects only.

Similarly, a recent ERP study by Soldan et al. (2006) reported data from two possible/impossible object-decision priming experiments using unfamiliar

objects that provide compelling evidence that the visual system differentially encodes globally possible versus globally impossible structures. In the first experiment, subjects made structural decisions (right/left orientation-decision task) about possible and impossible objects at study. In the second experiment, a functional decision (tool/support function-decision task) was performed at study. The behavioral results of the experiments were inconclusive with respect to sensitivity versus bias theories. However, the ERP data clearly failed to support bias theories, which hold that possible and impossible objects are processed similarly in the visual processing system. Rather, two early ERP components (the N1 and N2 responses) showed repetition enhancement for possible objects, but no neural effect for repetition of impossible objects, in both the structural and functional encoding experiments. Moreover, the magnitude of repetition enhancement in the N1 ERP component was correlated with behavioral priming for possible objects. These data support the theory that priming is supported by an SDS that encodes structurally possible objects only.

2.33.6 Correlations between Behavioral and Neural Priming

While neuroimaging studies have provided considerable evidence bearing on the neural correlates of priming, caution is warranted when interpreting the causal nature of such effects. Although a number of studies have documented the close overlap between neuronal activity and BOLD activity in the primate (Logothetis et al., 2001; Shmuel et al., 2006; for a human analogue see Mukamel et al., 2005), it is critical to determine whether functional neuroimaging data reflect the neural underpinnings of cognitive processes or index spurious activations that are epiphenomenal to the process of interest.

Initial studies used methodologies where blocks during which participants viewed repeated items were contrasted with blocks during which participants viewed novel items (e.g., Squire et al., 1992; Raichle et al., 1994; Buckner et al., 1995; Schacter et al., 1996; Wagner et al., 1997). The introduction of event-related fMRI (Dale and Buckner, 1997) later allowed researchers to intermix old and new items and delineate activity associated with individual trial-types, providing evidence that the neural priming that accompanies repeated items is not simply due to a blunting of attention or vigilance that may permeate extended periods of cognitive processing (e.g., Buckner et al., 1998). Together, studies of this sort have consistently documented the co-occurrence of behavioral priming and neural priming in a subset of the brain regions that are engaged during task performance with novel material (see **Figure 1**).

In order to establish a link between neural priming and behavioral priming, neuroimaging studies have attempted to demonstrate a relationship between the magnitude of both effects. That is, if neural priming is indeed related to behavioral priming, then the two should not only co-occur but should be directly correlated. A number of studies have reported a positive correlation between the magnitudes of behavioral priming and neural priming in frontal regions during tasks of a semantic or conceptual nature. Maccotta and Buckner (2004) showed that behavioral priming for repeated words in a living/nonliving classification task was significantly correlated with the magnitude of neural priming in regions of the left inferior frontal gyrus and pre-supplementary motor areas. Using the same task, Lustig and Buckner (2004) documented significant correlations between behavioral and neural priming in the left inferior frontal gyrus for young adults, healthy older adults, and patients with Alzheimer's disease (also see Golby et al., 2005). A similar pattern has been documented in the auditory domain: Orfanidou et al. (2006) found that the degree of auditory word priming on a lexical decision task was predicted by the extent of neural priming in left inferior frontal gyrus and supplementary motor areas. Others have found that the correlation between behavioral priming and prefrontal neural priming can be category specific. Using a classification task, Bunzeck et al. (2006) provided evidence that the correlations between neural and behavioral priming were specific for scenes in left inferior prefrontal cortex, but for faces in left middle frontal gyrus.

Consistent with the foregoing findings, in the aforementioned study by Dobbins et al. (2004), multiple regression analysis revealed that left prefrontal activity predicted the disruptive effects of response switching on behavioral priming for individual subjects: greater initial reductions in prefrontal activity were associated with greater subsequent disruptions of behavioral response times when the response was changed. To the extent that activation reductions in prefrontal cortex indicate less reliance on controlled processing and greater reliance on automatic processing, these data suggest that performance disruptions attributable to response switching reflect a need to

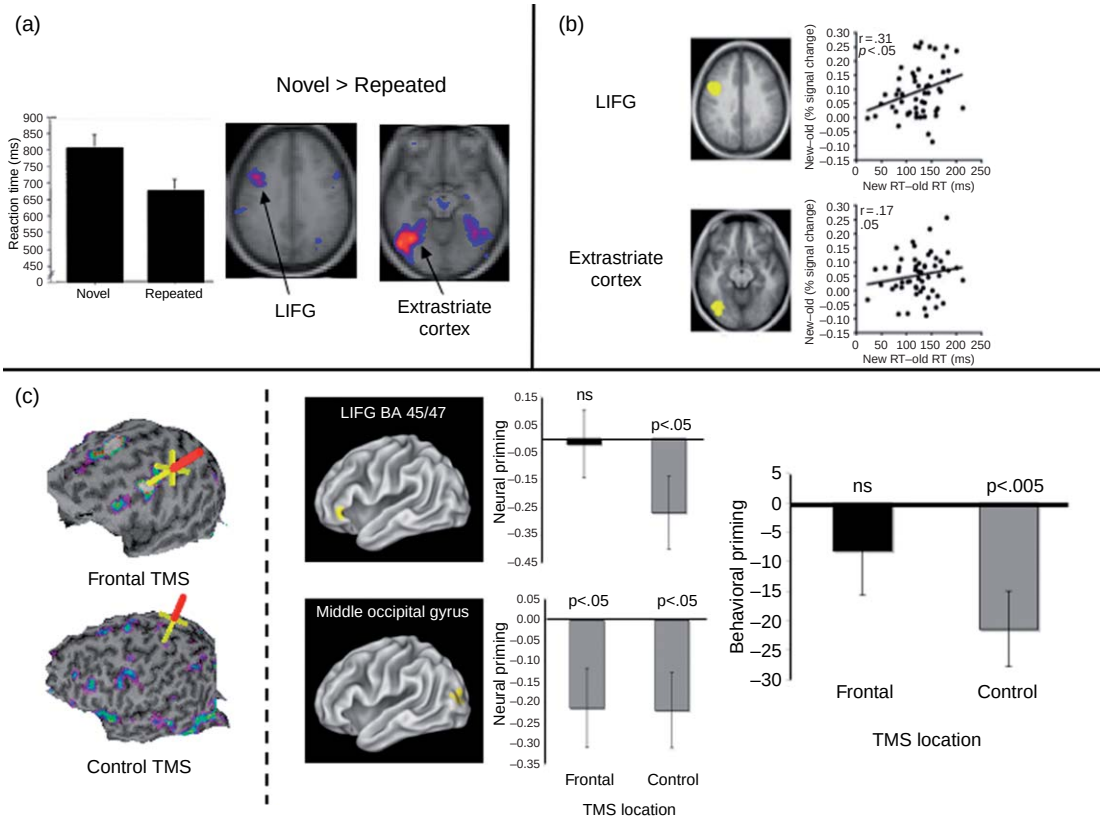

Figure 1 Correlations between behavioral and neural priming. (a) Semantic classification of visual objects using event-related fMRI reveals that the decrease in response time (behavioral priming) that accompanies classification of repeated items co-occurs with decreased activity (neural priming) in regions of the left inferior frontal gyrus (LIFG) and extrastriate cortex. (b) During semantic classification of words, the magnitude of behavioral priming is directly correlated with the magnitude of neural priming in the LIFG, but not the extrastriate cortex. (c) Transcranial magnetic stimulation (TMS) applied to a region of the LIFG (but not of a control location) during semantic classification of visual objects disrupts subsequent behavioral priming and the neural priming in LIFG during fMRI scanning. Neural priming in the middle occipital gyrus is unaffected by frontal or control TMS. Adapted from (a) Buckner RL, Goodman J, Burock M, et al. (1998) Functional-anatomic correlates of object priming in humans revealed by rapid presentation event-related fMRI. *Neuron* 20: 285–296, with permission from Elsevier; (b) Maccotta L and Buckner RL (2004) Evidence for neural effects of repetition that directly correlate with behavioral priming. *J. Cogn. Neurosci.* 16: 1625–1632, with permission from MIT Press; (c) Wig GS, Grafton ST, Demos KE, and Kelley WM (2005) Reductions in neural activity underlie behavioral components of repetition priming. *Nat. Neurosci.* 8: 1228–1233, with permission from the authors.

reengage slower controlled processes in order to make object decisions. This idea is consistent with the further finding that reductions in fusiform activity did not predict behavioral costs of switching cues, suggesting that these reductions may be incidental to behavioral priming during conceptual tasks.

Other evidence indicates that behavioral priming can correlate with neural priming in regions outside the prefrontal cortex as well. Bergerbest et al. (2004) found that behavioral priming for environmental sound stimuli correlated with neural priming in right inferior prefrontal cortex and also in two secondary auditory regions: bilateral superior temporal sulci and right superior temporal gyrus. Using a stem completion task, Carlesimo et al. (2003) found that the magnitude of behavioral cross-modality priming (auditory-to-visual) was correlated with the extent of activation reduction at the junction of the left fusiform and inferior temporal gyrus.

Turk-Browne et al.'s (2006) study of the relation between priming and subsequent memory effects, (where, as discussed earlier, neural activity during encoding is sorted according to whether items are subsequently remembered or forgotten) provided a different perspective on the correlation issue. Repeated scenes produced behavioral and neural

priming, but only for those scenes that were subsequently remembered. For these scenes only, there was also a correlation between the magnitude of behavioral and neural priming in the fusiform gyrus; this relationship approached significance in right inferior prefrontal cortex. As discussed earlier, the finding that the degree of behavioral and neural priming depended on subsequent memory points toward a link between implicit and explicit memory, perhaps involving shared attentional processes.

Together, these studies provide evidence for a relationship between behavioral priming and neural priming (also see Zago et al., 2005; Habeck et al., 2006). Correlations between the two variables generalize across paradigms (e.g., semantic classification, stem-completion) and are restricted to regions thought to mediate the cognitive operations engaged during the task. Although these correlations have been consistently reported with respect to neural priming in frontal cortices and to a lesser extent temporal cortex, few studies thus far have provided evidence for a correlation between behavioral priming and neural priming in earlier perceptual cortices – even though neural priming in the latter regions frequently accompanies item repetition.

The relationship between behavioral priming and neural priming in early visual regions was explicitly explored by Sayres and Grill-Spector (2006). Participants were scanned using fMRI in an adaptation paradigm during a semantic classification task on objects. Repetition of objects was accompanied by reductions in activity in regions of the LOC and posterior fusiform gyrus. However, in contrast to the correlations that have been observed between neural and behavioral priming in frontal and temporal regions, neural priming in earlier visual regions was unrelated to the facilitation in response time that accompanied repeated classification, thus providing more evidence that these two phenomena may be less tightly associated in these regions.

Although these correlations suggest that neural priming effects in prefrontal and temporal regions may support behavioral priming on a number of tasks, they do not allow conclusions regarding a causal role. It is possible that neural priming in these regions is necessary for behavioral priming. Alternatively, neural priming in other areas of the brain (e.g., regions of perceptual cortex) may subserve behavioral priming, and the neural priming observed in prefrontal and temporal cortex may simply reflect a feedforward propagation of the changes occurring in these other regions. In order to establish a causal relationship between behavioral priming and neural priming in frontal and temporal cortex, one would have to provide evidence of a disruption of behavioral and neural priming in these regions, accompanied by intact neural priming in perceptual cortices.

Wig et al. (2005) provided such evidence by combining fMRI with transcranial magnetic stimulation (TMS). TMS allows for noninvasive disruption of underlying cortical activity to a circumscribed region, thus inducing a reversible temporary virtual lesion (Pascual-Leone et al., 2000). In the study by Wig and colleagues, for each participant, regions of the left prefrontal cortex (along the inferior frontal gyrus) that demonstrated neural priming were first identified during semantic classification (living/nonliving) of repeated objects using fMRI. Each participant was then brought back for a TMS session where they classified a new set of objects using the same task. Short trains of TMS were applied to the previously identified prefrontal region during classification of half of these objects; classification of the remaining half of objects was accompanied by TMS applied to a control region (left motor cortex). Immediately following the TMS session, subjects were rescanned with fMRI while performing the semantic classification task on objects that were previously accompanied by prefrontal stimulation, objects previously accompanied by control-site stimulation, and novel objects. Results revealed that classification of objects that had been previously accompanied by left frontal TMS failed to demonstrate subsequent behavioral priming and neural priming in the left inferior frontal gyrus and lateral temporal cortex. By contrast, neural priming in early visual regions remained intact. Critically, these effects were not due to generalized cortical disruption that accompanied TMS; control-site stimulation had no disruptive effects on either behavioral or neural markers of priming. Consistent with this finding, Thiel et al. (2005) provided evidence for a disruptive effect of left-frontal TMS on behavioral priming during a lexical decision task. Together, these results provide evidence that behavioral and neural markers of priming in frontal and temporal regions are causally related, not just correlated.

In summary, correlations between behavioral and neural priming are observed consistently in prefrontal, and to some extent temporal, regions on priming tasks that include a conceptual component, such as semantic classification and stem completion. Although studies using such tasks have failed to

demonstrate a relationship between behavioral priming and neural priming in perceptual regions, behavioral demonstrations of perceptual priming are well documented (e.g., Tulving and Schacter, 1990; Schacter et al., 1993a). A key hypothesis to be evaluated in future investigations is that neural priming in perceptual cortices subserves perceptual priming. Establishing a causal relationship between the two necessitates careful consideration of the behavioral tasks used to demonstrate such effects. Further, it is likely that the behavioral advantage for repeated processing of an item is mediated by multiple processes and components of priming – both conceptual and perceptual – that contribute in an aggregate fashion to facilitate task performance (e.g., Roediger et al., 1999). Neuroimaging research can be helpful in attempting to tease apart the components of such effects and link them with the activity of specific brain regions.

2.33.7 Summary and Conclusions

Our review demonstrates that neuroimaging research has shed new light on cognitive theories of priming that were originally formulated and investigated through behavioral approaches within the field of cognitive psychology. The contributions of this research include advances with respect to long-standing theoretical debates about the nature of priming, as well as new lines of investigation not previously addressed by cognitive studies.

As alluded to earlier, evidence across several domains of neuroimaging research on priming is inconsistent with a single process account of the phenomenon, and instead supports the idea that multiple processes are involved in different types of behavioral priming and corresponding neural priming. Schacter et al. (2007) recently proposed a multiple-component view of priming, as depicted in **Figure 2**.

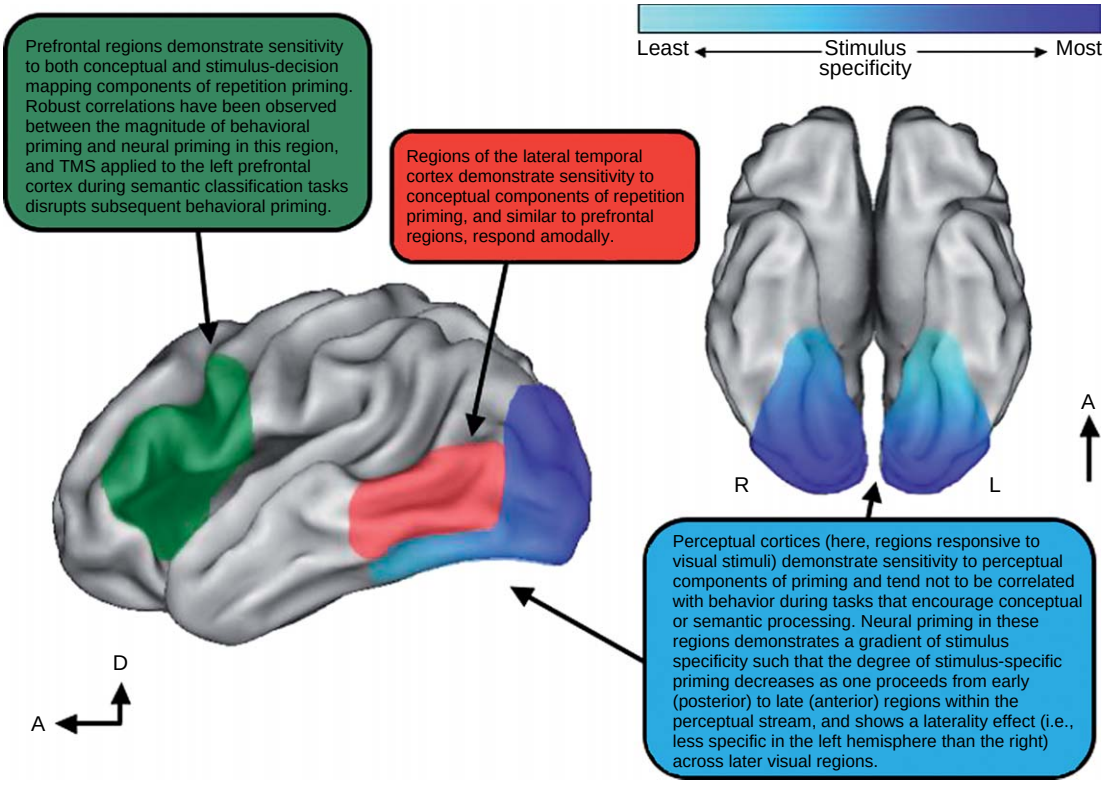

Figure 2 Schematic of proposed components of priming. Figure depicts partially inflated lateral view of the left hemisphere and ventral view of the left and right hemispheres. Lateral view is tilted in the dorsal-ventral plane to expose the ventral surface ('A' denotes anterior direction, 'D' denotes dorsal direction, 'L' and 'R' denote left and right hemispheres, respectively). Color-coding of anatomical regions is meant to serve as a heuristic for the proposed components. The color gradient within the ventral visual stream (blue) is meant to represent approximately the gradient of stimulus specificity that has been observed within these regions. TMS, transcranial magnetic stimulation. Adapted from Schacter DL, Wig GS, and Stevens WD (2007) Reductions in cortical activity during priming. *Curr. Opin. Neurobiol*. 17: 171–176, with permission from Elsevier.

This view suggests that there are at least two distinct mechanisms involved in neural priming. One corresponds roughly to what Wiggs and Martin (1998) called *sharpening* or *tuning,* which occurs when exposure to a stimulus results in a sharper, more precise neural representation of that stimulus (*See* Chapter 3.12; see also Grill-Spector et al. (2006) for more detailed consideration of sharpening and related ideas). Such tuning effects are likely to predominate in posterior regions that code for the perceptual representations of items, and perhaps in anterior regions that underlie conceptual properties of these items. Tuning effects, however, are unable to account for response-specific priming effects (e.g., Dobbins et al., 2004) and appear to be less correlated with behavioral priming observed during tasks that are semantic or conceptual in nature. The second proposed mechanism primarily reflects changes in prefrontal cortex that drive behavioral priming effects in a top-down manner, as initially controlled processes become more automatic (Logan, 1990; Dobbins et al., 2004).

While the view proposed by Schacter et al. (2007) suggests two possible components of priming, this is a preliminary model that needs to be extended, elaborated, and related more fully to distinctions among types of priming (e.g., perceptual, conceptual, associative) that have been long discussed in the cognitive literature. Traditional theories of priming laid the groundwork for understanding these components, and neuroimaging research will likely play a crucial role in resolving the questions that remain, in suggesting new lines of inquiry not previously conceived of, and in expanding our understanding of the nature of priming and implicit memory more generally.

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2.34 Motor Learning and Memory

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2.34.1 Introduction and Definition of the Field

Most of us have marveled at the extreme levels of movement skill shown by champion athletes, musicians and dancers, or artisans. Certainly, we almost take for granted our capabilities to drive a car, type on our computer keyboard, or play golf, as the many skills we seem to possess make life productive at work and enjoyable at play. But the capability for production of actions is far more important and fundamental than this. A moment's reflection will reveal that the capability to move our limbs is an essential aspect of life for, without movement capabilities, we could not feed ourselves, locomote to find food or shelter, or, for that matter, even reproduce or survive after birth. Viewed in this way, the capability to move purposefully and efficiently is a critical aspect of life itself, and its study requires little justification.

The study of movement capabilities is very broad, spanning many levels of analysis and touching many

different fields or departments within the typical university. Researchers frequently study these phenomena by focusing on such varied levels of analysis as the biochemical changes with muscle contractions or brain activity, the neural underpinnings of actions, the biomechanics of skilled activities, the learning of new actions, the changes in movement with age, and even the role of movements for societies (migrations or wars). For our purposes here, we narrow the focus to areas of study that are closely related to experimental psychology where, as with other areas within psychology, the concern is with observable behavior in individuals (mainly humans) and for the understanding of how this behavior is organized and produced. This focus has, in the past few decades, been called various names such as kinesiology, motor neuroscience, human performance, or motor control. This field is defined by the scientific study of movements by humans and animals, with attempts to understand the actions' characteristics and fundamental underpinnings and ultimately organizing the many variables' experimental effects into testable theory.

A part of the field of motor control has, since the earliest days of experimental psychology, been the focus on how skills are learned with practice and experience. The study of learning in general has always been a core focus for experimental psychology and, as we discuss later, the study of skill learning has undergone many changes in popularity over the years as well. The label motor learning is the one most often associated with the field directed at understanding the acquisition of skill (or movement capability) with practice or experience. This chapter focuses on this area directly.

2.34.2 Motor Learning: Acquisition of Procedural Knowledge

It is useful to make an important distinction about the kind of knowledge that is the product of motor learning, as it helps to set the field of motor learning apart from the larger field of human learning as it is studied in experimental psychology. Roughly speaking, knowledge can be thought of in two ways (or perhaps, as two ends of a continuum). Declarative knowledge is the capability to know that, as in knowing that the first American president was George Washington, or that $2 + 2 = 4$. A distinctly different kind of knowledge is procedural knowledge, which is the capability to know how to. . ., as in knowing how to ride a bicycle, how to type on a keyboard, or how to use a machine tool.

We suggest that, for the much of research efforts concerning human and animal learning in experimental psychology, the laboratory subject is acquiring declarative knowledge. For example, in verbal learning, the participant already knows how to say the proper answer or press the correct response key; what is learned is the selection of an existing behavior out of a large number of existing behaviors. In animal learning, the animal may learn to press the bar in the cage after some stimulus information is presented; learning how to press the bar is not of much interest. Also, learning to run a maze seems to be, at first glance, a highly motor activity; but running and learning to turn corners are not what is learned in the experiment – rather when and which way to turn are the critical issues.

For motor learning, on the other hand, the problem for the learner is how to produce a particular action, e.g., a dislocate-shoot-handstand on the still rings; the choice of which action to do is not the issue, as it is clear that this particular action is the goal. Now, the problem for the learner is to develop a way to control the musculature and limbs so that the designated action can be performed. Thus, motor learning does not seem to involve learning which previously acquired action to make, but rather it involves how to produce a given action. This is emphasized in motor-learning experiments, where tasks for which subjects have not had any previous experience are typically used (see Schmidt and Lee, 2005: Chapter 2).

We can probably think of counter-examples for animal learning. One might be the circus bear who has learned to ride a bicycle, or the dog who rides a surfboard, behaviors that are almost certainly not genetically acquired and must have been learned. If these kinds of behaviors were studied in animal-learning laboratories, we would be forced to call the product of this practice motor learning. But tasks like this are rarely studied in the laboratory, where the preferred method seems to be to use already acquired actions (e.g., bar presses) so that what is learned is when, or under what conditions, to press the bar – declarative knowledge. Even with these possible exceptions, the declarative/procedural distinction seems to be the chief factor that separates the field of motor learning from many other forms of learning examined in the laboratory. How procedural knowledge is acquired with practice is the defining feature of motor-learning research.

2.34.3 Brief Historical Background of Motor Learning Research

Historians owe a debt of gratitude to the work of McGeoch (1927, 1929, 1931), Irion (McGeoch and Irion, 1952; Irion, 1966), and Adams (1987), who provided detailed reviews of motor learning research during the past century. Like those reviews, the present chapter does not detail closely related areas of research such as the neurophysiology of motor control, or human factors and ergonomics research. Rather, we focus on studies of motor behavior, and how the permanent capability to achieve a goal-oriented action improves as the direct result of practice – the study of motor learning.

Early experimental investigations of motor learning were concerned with real-world skills, and the problems associated with their acquisition, retention, and transfer. For example, Bryan and Harter (1897, 1899) studied telegraphy (sending and receiving Morse code), documenting the changes that occur with improvements in skill (see also Taylor, 1911/1967). The acquisition (Book, 1908/1925) and retention (Hill et al., 1913; Hill, 1934, 1957) of typewriting skills represented another focus of study of learning real skills.

The initial stage or period of skill acquisition provided important information about the motor learning process, which was unavailable to the experimenter if the participant had already acquired some skill in the task to be learned. So, similar to the use of nonsense syllables as the unit of study in verbal memory experiments, psychologists created new tasks to examine how learning proceeded in the absence of previously acquired skill. Very simple tasks such as blindfolded line-drawing (Thorndike, 1927; Elwell and Grindley, 1938) and more complex tasks such as mirror tracing (Snoddy, 1926) and pursuit tracking (Koerth, 1922) were frequently used motor learning tasks.

The use of novel motor tasks also facilitated the study of various practice variables, as these variables were believed to have their most significant impact during the early stage(s) of skill acquisition. During the first half of the twentieth century, the most commonly studied motor learning variables included the effects of practice distribution (Bourne and Archer, 1956) and knowledge of results, or KR (Thorndike, 1927; MacPherson et al., 1948, 1949; Dees and Grindley, 1951). Like all behavioral science disciplines, certain topics in motor learning have gone through periods of intense scrutiny, during which considerable research was conducted and their results archived. Much of this work was driven by theories that, at the time, generated a great deal of interest. A good example in early motor learning research was the study of distribution of practice. A large volume of research was produced during the 1940s and 1950s concerning the effects of rest period durations between work periods, almost always using continuous tasks (such as pursuit tracking or mirror tracing). Much of this work was conducted with the purpose of testing the tenets of Hull's (1943) theory of habit strength and drive reduction. Practice distribution using continuous motor skills was viewed as a good behavioral vehicle to study Hull's theory in humans. So, when interest in Hull's theory waned, so too did experiments on practice distribution in motor learning (Adams, 1987; for a review of this work see Lee and Genovese, 1988). The study of KR suffered a similar fate. Augmented feedback, such as KR, was important to theorists who studied conditioning as a means to shape behavior.

The cognitive psychology revolution in the 1950s and 1960s ended most of the motor learning research undertaken in psychology departments. The new hot topics were memory and attention. By the 1970s, research output in the study of learning, such as paired associate learning and motor skills learning, was significantly reduced. Disinterest in motor-learning research continued in psychology departments through the end of the century and largely remains so today (Rosenbaum, 2005). However, motor learning research has become revitalized since the 1970s due mainly to four key factors.

2.34.4 Four Factors Contributing to the Modern Era of Motor Learning Research

2.34.4.1 Technology

By the early 1980s, researchers had generally become dissatisfied with simple measures of performance, such as reaction time or unidirectional error scores. Everyday actions involve complex movements, usually requiring the coordination of multiple degrees of freedom. The rapid advancements in computers and digital technology in the latter part of the century gave rise to more sophisticated methods to examine complex movements and the ability to record and analyze much more data than had previously been possible. Digital recording devices allowed the

simultaneous recording of multiple actions and computers enabled the power and flexibility to put them to work. The elegance of motor control could now be studied in detail, and relatively cheaply and easily so.

2.34.4.2 Relevance to Other Disciplines

A second factor that gave rise to the rebirth of motor learning research was the expansion of interest to other disciplines. Motor learning had always been relevant to physical educators who studied sport skills, and this interest continued to grow as technology facilitated the examination of specific sport skills in experiments (Williams and Hodges, 2004). Motor learning also grew as an area of research interest to scientists in other disciplines. Specialists in human factors and ergonomics found motor learning to be a fruitful area for understanding the learning process in occupation-related settings (e.g., Agruss et al., 2004). Various health-related disciplines discovered important relationships between motor learning and factors of direct relevance to them, such as stroke rehabilitation in physical therapy (Boyd and Winstein, 2003; Krakauer, 2006), voice rehabilitation (Verdolini and Lee, 2004; Yiu et al., 2005), and the acquisition of chiropractic (Descarreaux et al., 2006), dental (Wierinck et al., 2006), and surgical skills (Dubrowski et al., 2005; Brydges et al., 2007).

2.34.4.3 Two Important Papers

Although interest in motor skills research among psychologists waned, the role of psychological and cognitive processes as key components of the motor learning process became a motivating factor for renewed interest, and this interest can be localized to the publication of two important papers. The papers were important because they reported findings that were counterintuitive to popular thinking at the time. Shea and Morgan (1979) reported the findings of two groups of subjects who practiced three versions of a task that required subjects to learn patterns of arm movements and to perform them as rapidly as possible. A blocked group practiced the patterns in a drill-type order, which minimized the interference that practice of one task could exert on another task; all trials on one pattern were completed before trials began on a new pattern. Performance of this blocked group was very good: They achieved an asymptote level of reaction time and movement time

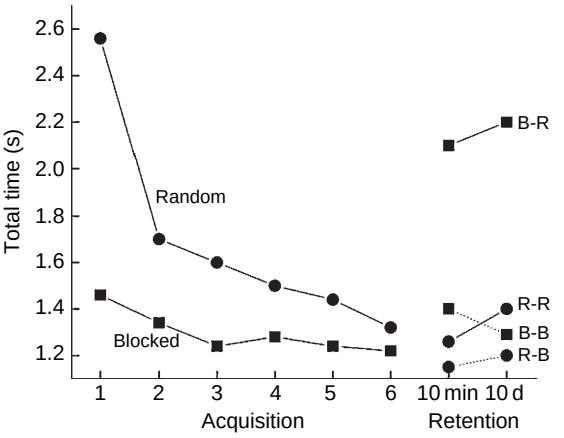

Figure 1 Blocked practice produces better performance during the acquisition trials than random practice. However, the random group performs better than the blocked group in immediate (10-min) and delayed (10-day) retention tests, regardless of how the retention tests are ordered (e.g., R–B = performance of the random group in blocked-ordered retention tests). Data from Shea JB and Morgan RL (1979) Contextual interference effects on the acquisition, retention, and transfer of a motor skill. *J. Exp. Psychol. Hum. Learn. Mem.* 5: 179–187.

very quickly (presented as total time in **Figure 1**). In contrast, performance of the random group, which never received practice trials on the same pattern more than twice in a row, was poor, never reached asymptote, and generally was worse than the blocked group throughout acquisition. These findings were consistent with well-known principles of the effects on interference on performance: Less interference produced better performance and vice versa.

The counterintuitive nature of Shea and Morgan's findings were revealed in retention and transfer tests conducted after delays of 10 min and 10 days. In these tests (conducted in both random and blocked orders), performance of the random group was superior to that of the blocked group on the blocked-ordered retention tests (compare the R-B and B-B symbols in **Figure 1**). And the effects of random practice were far superior in the randomly ordered retention tests (compare the R-R and B-R symbols in **Figure 1**). Apparently, the very same interference effects that resulted in performance deficits in acquisition also resulted in learning benefits as revealed in the retention tests. This finding was called the contextual interference effect (cf. Battig, 1979; Battig and Shea, 1980) and remains a topic of continued interest today (see the section titled 'Contextual interference').

The other important paper, published around the same time, was a review of the extant literature regarding the effects of KR in motor learning (Salmoni et al., 1984). The message delivered in this review paper was consistent in many respects to the message of the Shea and Morgan (1979) paper. Specifically, if one were to make an assessment about the efficacy of learning variables on the basis of performance during an acquisition session (i.e., when the manipulations were ongoing), then a very different conclusion would emerge compared to an assessment that was based on performance in a retention or transfer test (i.e., when the conditions of performance are equated). In their review of the KR literature, Salmoni et al. reported several instances in which the effects of a KR variable in acquisition were reversed in a retention or transfer test. Similar to the Shea and Morgan findings, these conclusions highlighted the important distinction between performance and learning and caused researchers to reassess their thinking about the factors and theoretical issues underlying the learning of motor skills.

We discuss these research areas in detail later. However, it is interesting to note that much of the interest in motor learning for researchers in other disciplines grew directly out of the counterintuitive nature of the findings reported in the new studies of contextual interference and KR effects. For some, it was the relative ineffectiveness of practice regimes in these specific, applied research areas that motivated this interest in motor learning research (e.g., Dubrowski et al., 2005).

2.34.4.4 Theory

The last factor that has spirited the growth of motor learning research concerns the product of skill acquisition – memory for motor skill – which is the domain of motor control. Perhaps this factor, more than any other, has resulted in the growth of motor learning from a theoretical perspective. Movement is the result of neural mechanisms that interact to result in action. At any one time, the true capability for motor skill is a concatenation of the current state of these interactions in memory, which is the direct result of practice. Two formalized theories of motor control, published in the 1970s, provided a strong rationale regarding how motor control is represented in memory. We begin by reviewing these theories and how they influenced motor learning research.

2.34.5 Motor Control: The Memory (Product) of Motor Learning

Over the years, there have been many separate statements or suggestions concerning what is learned in motor learning, that is, what is the product of practice? Two of these more formalized attempts are described next.

2.34.5.1 Adams's Theory

In the late 1960s, Jack Adams emphasized that learning could be conceptualized in terms of feedback-based, closed-loop processes, both for verbal learning (Adams and Bray, 1970) and motor learning (Adams, 1971). His motor-learning theory was based on the large body of empirical literature that used slow, self-paced, linear-positioning tasks, with the numerous practice variables that had been studied in this way. For these skills, the learner's task was to move a lever (or other manipulandum) to a particular goal location, and practice with KR was typically used.

For Adams, practice created what he called the perceptual trace – a memory structure that was the product of learning. After each attempt at positioning the lever, the subject receives KR about his or her error, which provides a basis for a more correct action on the next trial, and so on. Thus, for Adams, the function of KR was to guide the action toward the target over trials. Experience at the target location generated movement-produced (chiefly kinesthetic) feedback about that position, which was stored as a perceptual trace. With repeated trials increasingly near the target position, the most frequently experienced perceptual trace came to represent the sensory qualities of being at the target location. The process of reinforcing an increasingly narrow range of perceptual traces near the correct trace is illustrated in the three panels in **Figure 2**. Once this perceptual trace was sufficiently strong, on a subsequent attempt the subject would move to a position such that the difference (error) between his or her actual feedback at the moment and the acquired perceptual trace was minimal, using closed-loop processes. Thus, variations of KR that produced a faster approach to the target, or more accuracy at the target, were thought to be beneficial for learning because they made the perceptual trace more distinctive or reliable. This process also improved the subject's capability to detect his or her own errors (the difference between concurrent sensory feedback and the perceptual

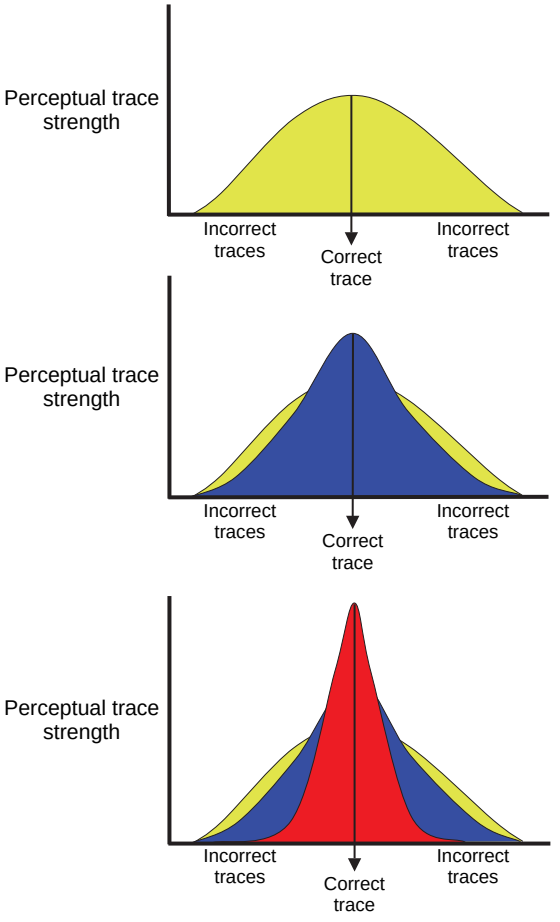

Figure 2 A conceptualization of Adams's (1971) theory. As repetitions accumulate, the representation strength of the correct perceptual trace becomes more fine-tuned in memory. With many repetitions, the correct perceptual trace becomes the dominant modal representation.

trace). In this manner, a strong perceptual trace would facilitate continued learning in the absence of external KR from the experimenter.

Adams's theory had a powerful effect on the field, generating considerable empirical activity in providing tests of the various predictions. As a result of this activity, research identified a number of shortcomings in the theory.

2.34.5.1.1 No open-loop processes

Adams intended his theory to be an account of slow, linear positioning tasks, and he made no attempt to explain the control of rapid actions. For rapid tasks, such as throwing, striking, or other brief movements of the limbs, evidence has mounted showing that feedback acts too slowly for it to be the basis for movement control. Alternatively, centrally generated, open-loop processes were proposed that were presumably responsible for producing such fast actions without requiring movement-produced feedback (Henry and Rogers, 1960; Keele, 1968). These open-loop processes have been described under the general heading of motor programs, about which we will provide additional discussion later. But, in short, the inability for Adams's theory to account for rapid actions was taken as a limitation.

2.34.5.1.2 Novelty

One logical problem with the theory was the inability to account for novel actions: Actions that the subject had never produced previously. If the basis for the perceptual trace was experience with the correct action, then how could the person ever make a variation of a skill that he or she had never practiced before, such as positioning a weighted limb in a new location? Perceptual traces, in this view, were limited to the actions that had actually been performed or practiced previously. A further implication is that, in order to perform the many varieties of actions that we do, we must have a very large number of perceptual traces in memory. This naturally brought up the concern regarding how the central nervous system could store and retrieve all of the traces necessary to allow us to move with the ease and efficiency that we can.

2.34.5.1.3 Generality

Another concern was the fact that skills seemed to demonstrate a particular kind of generality. That is, when we throw, we can throw in a very large number of ways, many of which have not been experienced before. It is as if the skill of throwing, for example, could be generalized to a wide number of similar but nonidentical throwing actions. Adams's theory did not provide a way for this kind of generalization to occur.

2.34.5.2 Schema Theory

Schema theory, proposed by Schmidt (1975), grew out of the several drawbacks to Adams's theory. In particular, the concern was to provide an account of learning in rapid as well as slower actions, to provide a way for novel actions to be performed, and to account for the generality that seemed to be shown in so-called classes of actions (such as throwing). As with Adams's theory, the concern was for discrete actions only, as other kinds of processes seem to operate in continuous tasks (e.g., swimming, steering a car).

2.34.5.2.1 Open-loop processes

Schmidt proposed that discrete actions such as overarm throwing are really a collection of actions that fall into the same class. Each member of the class is different in nearly countless ways (e.g., speed, trajectory, object size, throwing distance), but each member of the class had certain features that were invariant (Schmidt, 1985) across the members of the class. The idea was that this class of actions was governed by what was termed a generalized motor program (GMP), which had certain invariant features structured rigidly in it. The debate about what these invariant features are, and whether they are sufficiently invariant, has raged for several decades.

Chief among these invariants are sequencing and relative timing. Sequencing of the parts of an action simply refers to the order in which various events occur: For example, activity in Muscle 1 precedes Muscle 2, and both precede Muscle 3. It was assumed that all members of a class of actions have the same sequencing of elements. Relative timing refers to the temporal structure, or temporal pattern, of the action. If various muscle contractions were recorded, then we would see a temporal regularity in these elements. Specifically, for an action with several muscles participating, relative timing is invariant if the ratios among the contraction durations are constant across changes in, say, the overall duration of the action. Also, invariance requires that the duration of any contraction divided by the overall duration of the action must be constant across changes in overall duration. Another feature of the class of actions is relative force. Relative force refers to the patterning of forces among the various muscles: Muscle 1 always contracts with twice the force as Muscle 2, etc. This is another invariant feature structured in the GMP for the action.

According to the theory, when the performer attempts an action in the class, he or she retrieves the GMP for that class and then adds parameters to the program to suit the particular environmental demands. These parameters are proposed to be the overall duration of the action (throwing rapidly or slowly), the overall amplitude of the action (as in writing one's signature in different sizes), and the particular limbs to be used (writing with the fingers on a check, or with the arm/shoulder ten times larger on a blackboard). The selection of the parameters occurs prior to the action, and the GMP is initiated so that the invariant features of the movement emerge but with different surface features (such as amplitude or speed). Note that this kind of model helps to solve the problem of novelty, mentioned earlier, as a novel set of parameters will produce an action that the performer has never produced before. Also because there is only one program needed for the class, this eliminates the need to have a separate program for every different way that the action can be produced, and helps to solve the problem of storage. Note however, that the theory assumes the existence of the GMPs and is silent about how the programs are learned. This latter question remains one of the largest challenges facing schema theory (Shea and Wulf, 2005).

The theory does, however, provide a way for the parameter-selection process to be learned. When the performer produces an action such as throwing, he or she stores information from four sources: (1) the initial conditions (e.g., the required distance to throw, the weight of the object to be thrown, etc.), (2) the parameters that were used (absolute amounts of force, time, etc.), (3) the outcome in terms of the environmental (e.g., distance thrown) as provided by KR, and (4) the sensory consequences of the movement (how it looked, felt, sounded, etc.). This information is stored only long enough for the performer to update two schemas – rules or relationships among these stored values. The schemas are continuously updated with new information over many parameterizations for the class of actions.

The two schemas are the recall schema and the recognition schema. The recall schema is the relationship between the past parameters used and the past outcomes of the action, whereas the recognition schema is the relationship between the past sensory consequences of the action and the past outcomes. Given these schemas, when the person wants to produce a particular, perhaps novel, outcome with the action-class, he or she uses the recall schema to select the parameters based on the desired outcome. The recognition schema is used so that the performer can select the expected sensory consequences based on the desired outcome; this forms the basis for recognizing errors in performance after the action is produced. For example, knowing that on this occasion I want to throw 20 m, the recall schema estimates the parameter values needed (based on past experience with this class of tasks but with different outcomes), they are supplied to the GMP, and the action is triggered.

2.34.5.2.2 Fast versus slow actions

Finally, schema theory proposes that rapid actions (e.g., throwing, say 100 ms in duration) and slow

actions (e.g., linear positioning, say 3 s in duration) are produced systematically differently. Fast actions are produced by executing the GMP in an open-loop manner. After the action, the performer can compare the actual sensory consequences (response-produced feedback) with the expected sensory consequences from the recognition schema, providing a basis for the performer knowing about errors in movement production.

Slow actions are produced in a way analogous to Adams's (1971) proposal. When a slow action is required, the subject generates the expected sensory consequences from the recognition schema and then moves to that position such that the actual sensory consequences (movement-produced feedback) match the expected sensory consequences; the GMP is not involved. This leaves no capability for the performer to detect postperformance errors, as this error-detection process is used to produce the movement in the first place (see Schmidt and White, 1972). Thus, the overall generalization is that closed-loop processes produce slow actions and open-loop processes produce fast actions. There is considerable evidence supporting the distinction between motor control processes involved in fast and slow actions (see Schmidt and Lee, 2005: Chapter 13).

2.34.5.2.3 *Variability-in-practice effects*

One interesting implication of schema theory is that variability in practice among members of a class of actions, as compared to an equal amount of practice on any one of them, should be beneficial for learning (i.e., transfer performance on a novel variant). This is so because the schema for the class of actions – analogous to a regression line – is built up more reliably as the learner gains more variability in prior experiences. In this manner, the theory predicts that transfer performance to a novel variant is more accurate because the schema-rule is better defined. This somewhat counterintuitive prediction has been tested many times over the past 25 years, and the evidence generally supports it (McCracken and Stelmach, 1977; Shapiro and Schmidt, 1982; Lee et al., 1985; Van Rossum, 1990; see Schmidt and Lee, 2005: Chapters 11 and 13, for a summary and discussion). Another, even more counterintuitive prediction is that, for a task with, say, five variants (A, B, C, D, and E along some dimension such as speed), practice at A, B, D, and E is more beneficial for transfer to Variant C than is an equal amount of practice at Variant C itself. This prediction was supported in research by Shea and Kohl (1991), which is illustrated in **Figure 3**. In their research, Shea and Kohl asked participants to learn a force-production task, providing them with 100 trials to learn a criterion force of 150 N. The variable practice group received only 20 of these trials at the criterion force, and 80 trials were practiced at goals that were 50 or 100 N above or below the criterion force. Two control groups were included that received either 20 or 100 practice trials 'only' at the criterion force. A delayed retention test at the criterion force revealed that the variable practice group performed with less mean error than both the two criterion-only practice groups, supporting the schema-theory prediction. This variability-in-practice evidence is seen as contradictory to Adams's theory. The findings have strong implications for the structure of real-world practice.

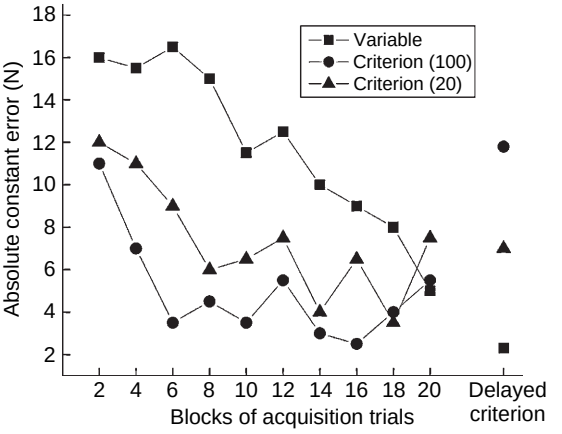

Figure 3 Variable practice (at goals surrounding and including a criterion target force) produces increased accuracy on a delayed criterion test than equal amounts of criterion-only task performance when controlling both for the number of criterion task trials (Criterion 20 group) and the total number of practice trials (Criterion 100 group). Data from Shea CH and Kohl RM (1991) Composition of practice: Influence on the retention of motor skills. *Res. Q. Exerc. Sport* 62: 187–195.

2.34.5.2.4 *Learning parameters versus learning programs*

In another line of research, investigators have found that movement errors arising from two sources can be separated. Errors stemming from the specification of parameters can be dissociated from errors resulting from the generation of the GMP itself (Wulf et al., 1993). Being able to separate these two kinds of errors makes it possible to study how various practice conditions affect these two theoretical processes. For

example, increased relative frequency of KR enhances parameter learning but degrades program learning (Wulf et al., 1993; Wulf and Schmidt, 1996). Several of the dissociations between factors that affect program versus parameter learning have been shown recently, and they tend to support the separation of these two constructs in memory (Wulf and Shea, 2002). Much more needs to be done in this area, in our view.

2.34.5.2.5 *Especial skills*

Recent findings by Keetch et al. (2005) seem to provide some difficulties for the schema view, however. One prediction arising from schema theory is that practice at any one variant of a class of actions exerts its effect by strengthening the schema for the whole class, not just for that particular variant. Thus, extensive practice at one of these members should not be evident on this particular member, but rather on the class as a whole. Keetch et al. studied set shots (where the feet do not leave the floor) made by skilled basketball players across various shot distances. They found that accuracy at the foul line (15 ft), which has had massive amounts of practice, was far better than predicted by performance at shot distances longer than or shorter than 15 ft (i.e., 9, 11, 13, 17, 19, and 21 ft). This effect can be seen in **Figure 4**. Keetch et al. interpreted these findings in terms of what they called an especial skill – an individual variant of a class of actions that, because it has received massive amounts of practice, stands out from other seemingly similar variants of the same class. These kinds of results suggest that learning is manifested not only in processes of generalization (as suggested by schema theory), but also by specificity in terms of these especial skills. Newer theories of motor learning will have to recognize both types of effects.

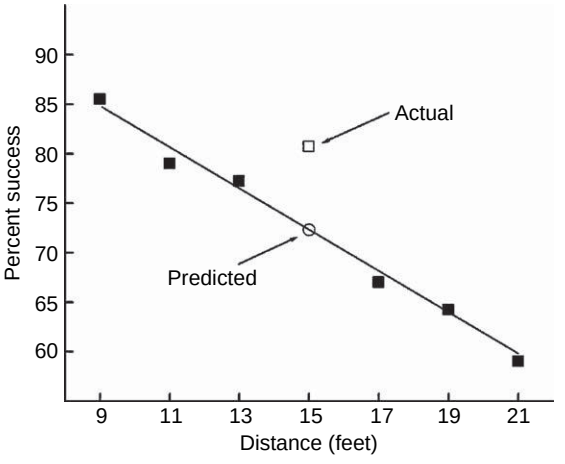

Figure 4 Shooting accuracy data at the free-throw line (open square symbol) exceeds predicted free-throw performance (unfilled circle symbol) based on set shot data from adjacent locations (filled square symbols). Data from Keetch KM, Schmidt RA, Lee TD, and Young DE (2005) Especial skills: Their emergence with massive amounts of practice. *J. Exp. Psychol. Hum. Percept. Perform.* 31: 970–978.

2.34.6 Cognitive Operations During Motor Learning

Motor control, the product in memory about which both Adams (1971) and Schmidt (1975) theorized, was critically dependent upon the interaction of movement and feedback (both sensory and augmented feedback). What was not considered important in these theories, however, was the role that cognitive operations served in the development of motor memories. As we mentioned earlier, it was the prominence of these theories, combined with their failure to explain the effects of cognitive operations during practice, that set the stage for the impact on learning research of the Shea and Morgan (1979) and Salmoni et al. (1984) papers.

2.34.6.1 Contextual Interference

The findings by Shea and Morgan (1979) on the effects of blocked and random practice were unexpected, given the views about memory that had been presented by Adams (1971) and Schmidt (1975). There was every reason to expect that blocked practice would be superior to random practice for learning, and this was confirmed by the acquisition data collected during practice. The reversal seen in retention and transfer might have been considered a set of data outliers, if it were not for the plausible theoretical rationale that was proposed to explain the results. Shea and Morgan found an ally in William Battig, and they relied heavily on some of Battig's ideas regarding elaborative and distinctive processing mechanisms to explain their results.

Battig had long been a proponent that learning comes at a cost. Earlier, he had suggested that "intertask facilitation is produced by intratask interference" (Battig, 1966: 227), referring to the finding that the difficulty in acquiring various items within the same category actually facilitated transfer when learning a different category of items. An expanded

view (Battig, 1979), which he termed contextual interference, further developed his stance regarding the relationship between interference and facilitation.

2.34.6.1.1 Elaborative and distinctive processing

An important part of Shea and Morgan's explanation for the differences in learning due to random and blocked practice was similar to Battig's (1979) views regarding elaborative and distinctive processing mechanisms. Consider first a typical trial in a blocked practice schedule. The learner prepares to perform one of the tasks to be learned, executes the action when cued to do so, and then evaluates the completed performance on the basis of movement-produced feedback and KR (if provided). By the very nature of blocked practice, the next trial requires essentially that same movement preparation and execution as the previous trial. In contrast, a typical random practice trial requires that the learner prepare, execute, and evaluate performance for a task that has a different goal on consecutive trials. Shea and Morgan (1979) proposed that the operations completed for one task, in the context of operations that may still reside in memory for a different task, provide more comparative and contrastive information about the tasks to be learned, compared to a blocked practice schedule.

In Shea and Morgan's view, the comparative and contrastive information during random practice would be explicit and verbalizable, and therefore should be available to report. This prediction was supported in a subsequent study by Shea and Zimny (1988), who found that subjects in a random-practice condition were more likely than those in blocked practice to provide information about the just-completed task in ways that set the task apart from the other tasks to be learned. Such information was argued to have made the explicit recall of the tasks more memorable, thus facilitating performance in retention tests.

The views of Shea and Morgan (1979) also suggest that any practice schedule (not necessarily a random schedule) that promotes comparative and contrastive processing should facilitate learning compared to a blocked schedule. A study by Wright (1991) provided strong support for this argument by combining different between-trial processing activities with a blocked practice schedule. One group of subjects was asked to describe the task that had just been performed, while another group was asked to describe a different task. A third group rested during the interval, and a fourth group was asked to explicitly describe the differences in task demands for the just-performed trials compared to one of the other tasks to-be-learned. The explicit processing of this latter group resulted in retention that was far superior to that of the other three groups, even though all groups had physically performed the tasks in a blocked schedule. The findings supported the proposition that random practice places more demands on the learner to prepare and evaluate performance in a manner that makes the information that has been learned about each of the tasks more memorable and distinctive.

2.34.6.1.2 Action reconstruction processing

An alternative view of the random/blocked effects emerged from research performed by Lee and Magill (1983). In their view, the differences between random and blocked practice schedules were similar to the effects seen in verbal memory when item repetitions are spaced during study, which has the effect of increasing performance in a retention test. According to Jacoby (1978; Cuddy and Jacoby, 1982), the spacing effect results from processing a repetition when the memory for the initial presentation has been forgotten (or at least degraded). Lee and Magill (1983, 1985) modified Jacoby's rationale to explain the contextual-interference effect. The processing requirements to learn a motor skill in a blocked-practice schedule are minimized because all repetitions of the task follow immediately after having just performed the same task. Processes associated with movement planning, execution, and evaluation all concern the very same goal-oriented action. Therefore, these processes need not be fully undertaken on each subsequent trial because the previous plan remains in memory. In contrast, a repetition of any single task in a random schedule may not occur until several trials later, during which the learner is required to abandon any previously constructed action plans in favor of an action plan that is appropriate for the current task demands. When a repetition of that earlier task is again required, the previously constructed action plan for that task is no longer in memory, thereby requiring that the learner reconstruct the action plans anew. It is practice at (re)constructing the action that gives random practice its benefits, according to this view.

Note that these processing requirements for random practice should be undermined if the action planning activities are obviated at the time that a

repetition is performed. Such a prediction was tested by Lee et al. (1997) in which modeled augmented information about the temporal components of a timing task was presented to the learner prior to each random practice trial. The powerful advantage of the modeled timing information was clearly evident in the acquisition trial blocks: This random group performed as well as the blocked group throughout acquisition, and both were better than a random group that did not have the modeled timing information. However, the fate of this random group that received modeled information during practice was revealed in learning tests. Despite having received random practice, the modeled information resulted in retention and transfer performance that was as poor as blocked practice, and was much worse than the random group that did not receive the modeled information. In terms of the reconstruction view, these data provided evidence that external reinstatement or augmentation of the action-plan information prior to a practice trial facilitates performance and is detrimental to learning, likely because it eliminates (or at least reduces) the need for the learner to practice the reconstruction of the action by him- or herself.

The findings of Lee et al. (1997) were replicated and extended in an experiment by Simon and Bjork (2002). In their study, groups of blocked and random subjects received modeled information that either matched the task to be performed or was appropriate for the timing requirements of one of the other tasks (mismatched). Their results appear in **Figure 5** and are straightforward. Matched timing models facilitated performance of both blocked and random practice groups (the filled symbols in **Figure 5**), relative to mismatched models (the unfilled symbols). Retention performance, however, benefited from the mismatched models, regardless of the physical order in which the practice trials were undertaken.

Note that the Simon and Bjork (2002) data do not distinguish between the elaboration and reconstruction views of contextual interference. Although the matching models may have provided important planning information that undermined learning, the information from the mismatching models could also have provided contrastive information that benefited both the random and blocked practice groups. Similarly, other studies reveal that processing activities are elevated in random practice relative to blocked, although these findings could be accounted for by either an elaboration or a reconstruction account. For instance, Immink and Wright (1998)

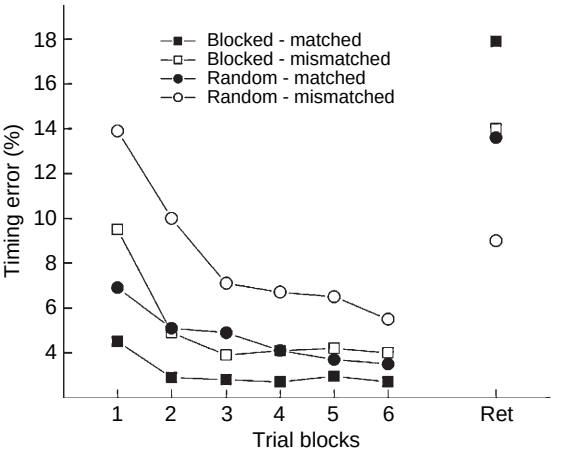

Figure 5 Matched timing models facilitate acquisition performance (relative timing error from target), but mismatched timing models facilitate retention performance, for both blocked and random groups. Data from Simon DA and Bjork RA (2002) Models of performance in learning multisegment movement tasks: Consequences for acquisition, retention, and judgments of learning. *J. Exp. Psychol. Appl.* 8: 222–232.

showed that preparation times taken prior to action are longer in random than in blocked practice schedules. As well, secondary tasks that are inserted into the interval during which a learner would be planning for an upcoming actions revealed longer probe reaction times for random than for blocked practice (Li and Wright, 2000). These data suggest that planning operations are likely to be elevated during random practice, although these could be result of enhanced elaboration or reconstruction processes.

More recent studies have identified more precisely what types of processing activities are likely to be affected by random and blocked practice schedules. Specifically, these studies have been designed to tease apart the nature of motor programming activities that have been undertaken in random and blocked schedules. In one experimental manipulation, Immink and Wright (2001) examined motor-programming operations using Morse code responses (dit and dah) within the experimental paradigm developed by Klapp (1996). According to Klapp, two programming operations are involved for this task: (a) organizing the internal components of motor program "chunks," and (b) sequencing the chunks. Further, it was argued that the internal process can be preprogrammed in advance, especially so if repeated trials on the same task are performed. In their experiments, Immink and Wright (2001) found that random practice facilitated the learning of the internal programming process

(relative to blocked practice), but not the sequencing process. They argued that, since the internal process can be preprogrammed, blocked practice required no practice at reconstructive processing, which degraded learning relative to random practice, as measured in retention tests.

An alternative view of the motor-programming process that is influenced by the effects of random and blocked practice was examined in an experiment by Lee et al. (1992). In this study, the nature of the reconstruction process was examined specifically using tasks that differed in terms of their relative timing structure. The rationale was that a set of tasks that shared the same underlying relative timing is based on a common GMP. Regardless of whether the practice sequence order was blocked or random, no reconstruction of the GMP would be required as long as the task variations that were practiced shared the common relative timing. In contrast, for a set of tasks that each had a distinct relative timing, random-practice schedules would require a new reconstruction of the programming process on each trial, but of course, not for blocked practice. Thus, based on reconstructive programming, the authors predicted, and found, large random-blocked differences for a set of different relative timing tasks, but no differences for tasks that shared a common relative timing (see also Magill and Hall, 1990). Although these findings supported the action-reconstruction view, other data suggest that task variations involving same GMPs can also result in a contextual interference effect (Sekiya et al., 1994; Hall and Magill, 1995; Sekiya et al., 1996). These latter findings suggest that simply reconstructing the parameterization of the GMP (same relative timing, but different overall duration) is sufficient to produce random versus blocked differences in learning.

2.34.6.1.3 Cognitive effort

The effect of task variations in blocked and random practice schedules have also been examined relative to the overall concept of cognitive effort – that random practice is overall, more effortful practice, which works optimally for tasks that are simple and which leads to boredom during practice (Guadagnoli and Lee, 2004). Tasks that are inherently more interesting, or perhaps more complex in nature, should be less amenable to contextual-interference effects because blocked practice engages the learner in more cognitive effort than would be the case for simple tasks. These predictions were supported in research by Albaret and Thon (1998), who found large contextual-interference effects for two simple versions of an arm-movement task, but no differences for the most complex task. Indeed, Wulf and Shea (2002) have suggested that contextual-interference effects are sometimes reversed for very complex tasks, and blocked practice can facilitate learning more than random practice. These suggestions are controversial, however, as large random-practice advantages have been found for complex tasks such as baseball batting (Hall et al., 1994) and handwriting skills in young children (Ste-Marie et al., 2004).

2.34.6.1.4 Meta-memory misattributions

We mentioned earlier that some of the attraction to this area of research might be attributed to the often-misunderstood distinction between performance and learning, and the counterintuitive conclusions about learning that resulted from these experiments. Two recent lines of research have continued to explore this issue directly. In one experiment, Simon and Bjork (2001) examined the effects of random and blocked practice on actual levels of performance and retention and contrasted these results with the participants' predicted levels of performance and retention. **Figure 6** illustrates their findings. The left panel reveals typical random/blocked effects in acquisition and retention. The right panel illustrates what the learners had predicted would be their level of performance. The findings are clear;

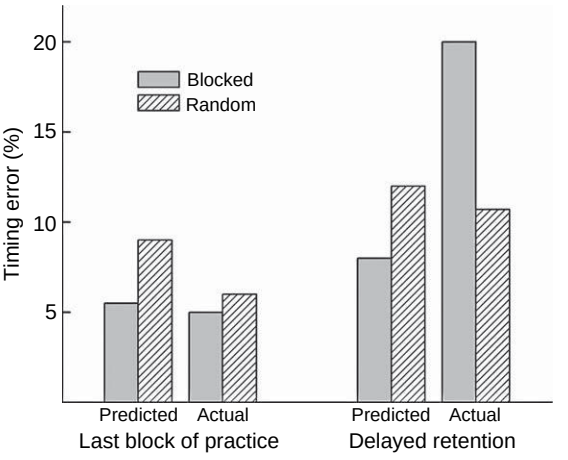

Figure 6 Blocked and random groups accurately predicted timing error performance at the end of acquisition. In retention, the blocked groups overestimated performance and the random group underestimated performance. Data from Simon DA and Bjork RA (2001) Metacognition in motor learning. *J. Exp. Psychol. Learn. Mem. Cogn.* 27: 907–912.

most importantly, the subjects in the blocked group overestimated their retention performance while the subjects in the random group underestimated retention.

These findings on self-estimations of learning are difficult to reconcile with recent research concerning the self-determination of practice schedules. Almost all studies of practice schedules in the modern era of motor learning research have investigated experimenter-determined schedules, in which the order of practice trials is beyond the learner's control. One would suspect, given the Simon and Bjork (2001) results, that learners would feel more confident with their progress in learning under a blocked schedule and would therefore self-select a schedule of practice that undermines their potential for learning. Surprisingly, such is not the case. New research is emerging (e.g., Keetch and Lee, in press), which suggests that self-determined schedules facilitate learning (relative to yoked controls), regardless of the degree to which the self-selected order is blocked or randomized. This finding might suggest that individual difference variables, in addition to the trial order, combine to influence the effectiveness of practice. We see this new paradigm on practice schedules as a having considerable potential in future motor-learning research.

2.34.6.2 Knowledge of Results

The role of knowledge results (KR) in the theories of Adams (1971) and Schmidt (1975) was rather mechanistic: KR served to guide the learner toward making correct movements, and these movements served to strengthen either the correct perceptual trace (in Adams's theory) or the schemas (in Schmidt's theory). This view was termed the guidance role by Salmoni et al. (1984) because KR served as a tool that guided the learner toward the target goal and therefore facilitated learning. However, in their reanalysis of the KR literature, Salmoni et al. discovered that the beneficial role of guidance could only be found in experiments when performance during acquisition trials was considered, i.e., during those trials in which the KR manipulation was ongoing. However, the effects on retention of these same variables were rather different. Salmoni et al. found a number of instances in which a KR variable that guided the learner to strong performance during acquisition was actually detrimental to learning as measured in retention. The conclusion that the guidance function of KR played a detrimental role in learning initiated a new round of research that continues today. Importantly, the guidance role of KR highlighted some cognitive factors that intervene during motor learning, which will be discussed in more detail later. First, we describe two KR variables that illustrate both the positive and negative roles for the guidance function of KR.

2.34.6.2.1 Timing of knowledge results

One of the most frequently studied variables in this area of motor learning research concerns the timing of the KR delivery following the performance of a task. Because augmented feedback refers to information about performance, it is delivered to the learner either concurrently with ongoing performance or following the completion of a performance. Typically, concurrent delivery of KR occurs when the performance of the task is of sufficiently long duration that perception and utilization of the provided information are possible (say, 0.5 s or longer). For tasks in which the movement duration is less than 0.5 s or so, KR is typically delivered after the completion of the movement. If the KR is not delivered instantaneously upon movement completion, then two variables may intercede; there is a time delay and the opportunity for other activities (e.g., motor, cognitive) to be conducted prior to the delivery of the KR. We highlight some research that documents the effects of the timing of KR.

A study by Schmidt and Wulf (1997) illustrates the guidance effects of concurrent feedback. The movement task was to displace a lever by making flexion and extension arm movements that matched precise spatial and temporal requirements. The overall movement time goal was almost 1 s, providing sufficient time for the learner to use concurrent augmented feedback to perform the task. A video monitor was used to provide one group with concurrent feedback by overlapping a trace of the learner's movement production with a template of the perfect (goal) movement. Discrepancies between the actual and goal movements were explicit from the images presented on the monitor. A second group received an image of the produced movement trace together with the goal trace 3 s after the movement had been completed; the monitor was blank during the movement. Schmidt and Wulf (1997) reported a number of spatial and temporal measures of average performance and performance variability. In general, these measures showed advantages during practice that favored the concurrent augmented feedback

group. This general trend was reversed in retention: the removal of KR resulted in drastic declines in performance for the concurrent feedback group, but not so for the delayed KR group.

A similar finding for acquisition and retention has been found when the augmented feedback is not presented concurrently, but rather is presented instantaneously on completion of the movement (Swinnen et al., 1990). These researchers found that a delay of 8 s prior to the delivery of KR improves retention, especially if learners are requested to estimate their feedback during this delay period prior to KR delivery. Thus, simply delaying the KR was sufficient to reduce the negative guidance effects of the instantaneous KR, and the estimation procedure provided an additional boost to the learning effect.

Another method by which the timing of augmented feedback can be manipulated has been termed the trials-delay method (Bilodeau, 1956). By this method, the presentation of KR for any specific trial may be delayed for a time period during which one or more intervening trials of the task are practiced. As illustrated in **Figure 7**, studies of this type (Lavery, 1962; Anderson et al., 2001, 2005) have typically found that the immediate delivery of KR (no trials-delay) resulted in superior acquisition performance compared to a trials-delay condition (e.g., Anderson et al., 2001, 2005, used a two-trials-delay condition). However, no-KR retention tests again support the efficacy for learning of the delayed KR practice conditions; in this case, the trials-delay condition typically produces better retention than the immediate KR conditions.

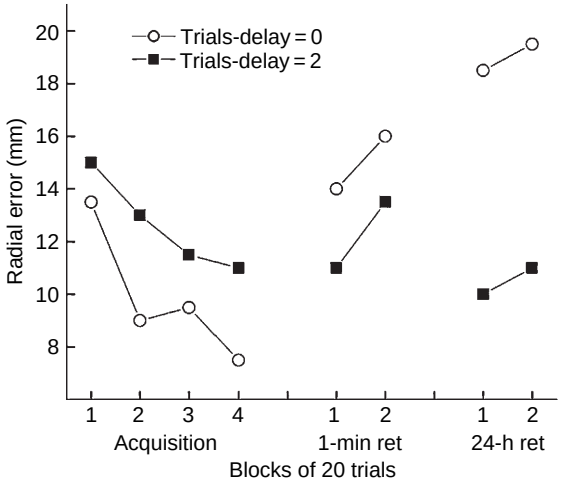

Figure 7 Immediate delivery of KR produces superior performance in acquisition (error in distance from target) but inferior retention (ret) compared to a KR trials-delay condition. Data from Anderson DI, Magill RA, and Sekiya H (2001) Motor learning as a function of KR schedule and characteristics of task-intrinsic feedback. *J. Mot. Behav.* 33: 59–66.

2.34.6.2.2 Frequency of knowledge results

Augmented feedback can also be manipulated by varying the relative frequency of its presentation in relation to the total number of trials practiced. One manipulation is to withhold KR after some trials (reduced relative frequency) and compare the effects in acquisition and retention to a condition that provides KR after every trial (100% relative frequency). The effects of a reduced relative frequency tend to be small, if any, in acquisition. However, retention performance is often enhanced by reduced relative frequency (compared to 100% frequency conditions; e.g., Winstein and Schmidt, 1990).

A variation of the feedback frequency manipulation provides information about every trial, but not directly after every trial. In this manner, the same amount of information is presented but it is done in such a manner that statistically summarizes KR over a series of previous trials. Studies have varied the manner in which summaries are presented: some have used graphs that plot performance for the previous series of trials; other studies have used statistical averages that summarize the average performance tendency for the series of trials. The effects are similar and produce contrasting effects in acquisition and retention, relative to a trial-by-trial KR delivery method. Relative to every-trial KR, summary KR degrades performance in practice but enhances retention (e.g., Schmidt et al., 1989). There appears to be a limit to the benefit of the size of summaries, however, with the optimal summary size dependent on the task demands (Schmidt et al., 1990; Guadagnoli et al., 1996). Yao et al. (1994) provided a convincing demonstration of the effect of both summary size and summary type; their results are illustrated in **Figure 8**. However, retention performance was facilitated by moderately sized (five-trial) summaries, compared to both the every-trial KR and larger (15-trial) summaries. This finding was present regardless of whether the summary information was presented as a graph of the individual trials or as a statistical average of the summarized trials.

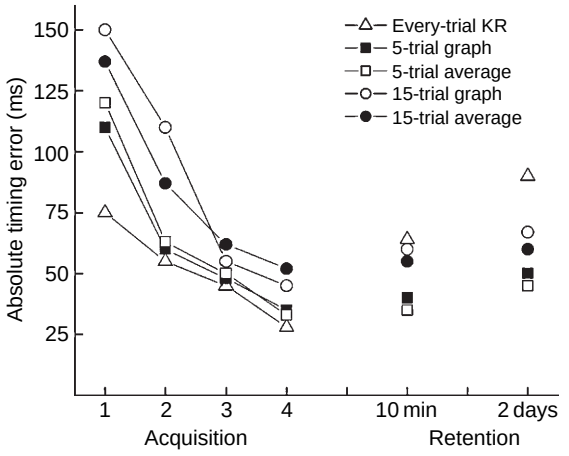

Figure 8 Moderately-sized (5-trial) summaries facilitated timing error in retention, relative to every-trial KR and larger (15-trial) summary sizes. Data from Yao W, Fischman MG, and Wang YT (1994) Motor skill acquisition and retention as a function of average feedback, summary feedback, and performance variability. *J. Mot. Behav.* 26: 273–282.

The product of learning under these conditions of the temporal delivery of KR suggests that memory is affected in different ways. Conditions of practice during which KR is delivered such that it guides the learner toward producing the correct, or optimal, movement solution, are suboptimal to performance in retention. Providing KR concurrently or instantaneously upon movement completion are both conditions that fulfill the guiding role of KR. In contrast, delaying the delivery of KR is likely to lessen its immediate guidance function and, possibly, allow time for other learning factors to intervene. Such factors include a better understanding of one's movement-produced feedback, which is enhanced under conditions where the learner is encouraged to produce magnitude estimates of feedback during the delay period prior to KR delivery.

2.34.6.2.3 The roles of knowledge results

Until the review of Salmoni et al. (1984), researchers had been content with the view that augmented information serves three roles in the acquisition of motor skills (Schmidt and Lee, 2005): (1) KR can be used as information by the learner to make permanent corrections in the actions being learned, (2) KR serves a motivating function that helps to maintain interest in the task and encourage continued practice, and (3) KR can provide a means to learn associations between motor commands and the sensory consequences of those commands. However, the finding that KR can serve a guidance role that has both positive effects (during practice) and negative learning consequences (i.e., in retention tests) suggested that the KR might be serving roles that had previously gone unnoticed.

Salmoni et al. (1984) and Schmidt (1991) speculated about two processes that might underlie these guidance effects. One idea is that successful motor learning involves the capability to understand and use the intrinsic feedback information that results from movement (such as proprioceptive and visual feedback). When KR is presented instantaneously and/or frequently, that information actively blocks or overshadows the processing of intrinsic feedback. Consequently, learning how to use intrinsic feedback when it is the only available source of information is degraded (e.g., when KR is removed in retention or otherwise is no longer available). An alternative view of these KR guidance effects suggests that the capability of a learner to correct errors is limited because of the natural, inherent variability of the motor system. However, often KR is provided without regard to what capabilities the learner possesses to use the information to make corrections to movement errors. In other words, the KR may encourage the learner to attempt to correct errors smaller than the learner's actual ability to control them; such attempts produce so-called maladaptive short-term corrections that may be beneficial for performance in practice, but are not beneficial for learning as measured on retention tests (Schmidt and Bjork, 1992).

In the previous section, we mentioned that one of the exciting, new directions in research concerns individual differences and the effects of self-determined schedules of practice. A similar line of investigation has also been undertaken with regard to the delivery of augmented feedback, and some intriguing findings are emerging. An early study (Janelle et al., 1997) revealed that retention was facilitated if learners were provided the control over the decision about whether or not to receive augmented feedback after a trial, relative to a yoked group (that controlled for the frequency, but not the decision to deliver feedback) and a group that received five-trial summaries. The benefit to learning for this self-determined group has been replicated in several experiments (e.g., Chiviacowsky and Wulf, 2002, 2005) and represents a curious effect. For example, in postexperiment interviews Chiviacowsky and Wulf (2002) found that individuals preferred to receive KR after trials on which they perceived that their performance had been relatively good. Additionally, Chiviacowsky and

Wulf (2005) found that the decision to receive feedback was more effective when made after the performance of a trial than when decided before a trial.

According to the guidance hypothesis, KR will have a detrimental effect on learning if it blocks or overshadows the learner's attempt to interpret his or her own movement-produced feedback relative to the information provided in the KR. The effects of self-determined KR are consistent with the guidance hypothesis to the extent that providing control of when KR is delivered gives the learner the opportunity to maximize the contrast between perceived KR and actual KR. However, these findings also contradict some basic theoretical arguments regarding how KR works. One consistent finding in the literature suggests that the usefulness of KR is optimized when it informs the learner about errors that had been made, not when it confirms to the learner that a trial had been performed well (Sherwood, 1988). Thus, similar to the research on practice schedules, individual differences in the learner's perception of ongoing performance, as well as their metacognitive strategies for how the delivery of KR is best suited to facilitate learning, are all likely to be important determiners of the effectiveness of augmented feedback.

2.34.7 Summary

Motor learning is the process by which the capability for skilled motor control becomes represented in memory. Motor memory is the product of learning. In this chapter, we have reviewed two theories regarding how memory for skill is developed. Both theories explain how the interaction of movement and feedback results in permanent representations that influence motor control. We suggest that future work needs to be done that further develops theories of motor learning that account for how and why cognitive factors influence the qualitative representations in memory.

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2.35 The Role of Sleep in Memory Consolidation

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2.35.1 The Role of Sleep in Memory Consolidation

We spend about a third of our lives sleeping, yet in spite of decades of scientific inquiry, the function of sleep remains an enigma. This is not to say that progress has not been made. From antiquity until the 1950s, sleep was generally believed to be a state of inactivity where the brain was turned off and the body rested, but we now know that the sleeping brain can be equally, and sometimes more, active than the brain in its awake state. Even during deep or slow wave sleep, when the brain is relatively quiescent compared to rapid eye movement sleep or wakefulness, it is still roughly 80% activated and thus capable of elaborate information processing (Steriade, 1999; Hobson, 2005).

Numerous hypotheses have been put forward to explain the functions of sleep, including energy conservation, brain detoxification, immune regulation, tissue restoration, and predator avoidance. More recently, the hypothesis that sleep plays a key role in the consolidation of memories has gained considerable attention (Smith, 1985; Maquet, 2001; Smith, 2001; Stickgold et al., 2001). Following two seminal papers in 1994 (Karni et al., 1994; Wilson and McNaughton, 1994), the publication rate on this topic increased fivefold over the next 10 years (Stickgold and Walker, 2005).

Despite this resurgence of attention, the question of how sleep contributes to memory consolidation is actually quite old. In the first century AD, the Roman rhetorician Quintilian, commenting on the benefits of sleep, noted that "what could not be repeated at first is readily put together on the following day; and the very time which is generally thought to cause forgetfulness is found to strengthen the memory."

In this chapter, we review the accumulating evidence supporting a sleep–memory connection, which

converges from studies at the molecular, cellular, physiological, and behavioral levels of analysis (Maquet, 2001; Smith, 2001; McNaughton et al., 2003; see also Gais and Born, 2004a; Stickgold, 2005; but see Vertes and Siegel, 2005). We begin with a précis of the field's history before turning to a review of its present status. We attempt to operationally define the terms sleep and memory, and offer our opinions on the field's strengths and shortcomings. In the first half of this chapter, we examine sleep's role in the strengthening of perceptual and procedural skills, and in the second half, we devote our attention to sleep's role in the consolidation of episodic memories. Our primary intention is to alert memory researchers to the growing field of sleep and memory, as well as to spark enthusiasm for a new way of researching memory systems that holds much promise for understanding both sleep and memory.

Although our review covers mainly behavioral and physiological evidence in humans, we point the interested reader to a growing animal literature on this topic. Numerous studies have examined the reactivation of neuronal patterns during post-training sleep, which show that neuronal activation sequences associated with various memory tasks are replayed during subsequent sleep. We touch briefly upon these studies toward the end of the chapter, but we refer the reader to the following articles for a deeper understanding of this fascinating topic (Wilson and McNaughton, 1994; Qin et al., 1997; McNaughton et al., 2003; Sirota et al., 2003; Ribeiro and Nicolelis, 2004). Other studies have begun to illuminate the molecular aspects of sleep-dependent memory consolidation, which we will not discuss here, but which certainly deserve attention as well (Smith et al., 1991; Nakanishi et al., 1997; Ribeiro et al., 1999; Graves et al., 2001; Benington and Frank, 2003).

2.35.2 Definitions of Sleep and Memory

Before turning to a discussion of the relationship between sleep and memory, we will first attempt to define both terms, as confusion has often arisen due to oversimplifications of one or both. We thus begin the chapter with a brief overview of the neurobiological characteristics associated with the various stages of sleep, and the different types of memory.

2.35.3 Stages of Sleep

Sleep progresses through a series of stages, which can be divided broadly into rapid eye movement or REM sleep (also called paradoxical sleep, due to the many wake-like features seen in this sleep stage), and non-rapid eye movement or NREM sleep. NREM sleep can be further subdivided into four NREM stages (1–4) corresponding, in that order, to increasing depth of sleep (Rechtschaffen and Kales, 1968). Slow wave sleep (stages 3 and 4) is the deepest of the NREM phases, and is the phase from which people have the most difficulty awakening.

In healthy adults, NREM and REM sleep alternate in approximately 90-min cycles throughout the night (so-called ultradian cycles). However, the relative contributions of NREM and REM sleep to these cycles varies across the night, with more of NREM stages 3 and 4 (slow wave sleep, SWS) early in the night, and more REM sleep late in the night. Thus, more than 80% of a night's SWS is concentrated in the first half of the night, while the second half of the night contains roughly twice as much REM sleep as the first half. This distribution of sleep stages has implications for some of the research paradigms described later in this chapter.

As NREM sleep progresses from stage 1 through stages 3 and 4 (SWS), electroencephalographic (EEG) activity steadily slows. In stage 1 sleep (drowsiness) there is an attenuation of the normally occurring alpha rhythm (8–13 Hz). In its place, a mixture of frequencies with a slower theta frequency (4–7 Hz) begin to emerge. Stage 2 NREM sleep is characterized by a continued reduction in EEG frequencies combined with two signature waveforms: large electrical sharp waves called K complexes and short synchronized bursts of 11- to 16-Hz oscillations called sleep spindles. Slow wave sleep is characterized by high-voltage, low-frequency (<4 Hz) EEG oscillations, which are an expression of underlying synchrony between the thalamus and cerebral cortex (Amzica and Steriade, 1995).

REM sleep, on the other hand, is characterized by low-amplitude, mixed-frequency EEG oscillations that are similar to the EEG patterns seen in wake. Periodic bursts of rapid eye movement also occur, along with a nearly complete loss of muscle tone.

As the brain passes through these sleep stages, it undergoes marked neurochemical alterations. In NREM sleep, acetylcholine neurons in the brainstem and forebrain become strikingly less active (Hobson

et al., 1975) and serotonergic and noradrenergic neurons also reduce their firing rates relative to waking levels. In REM sleep, both of these aminergic systems are strongly inhibited, while acetylcholine neurons become intensely active, in some cases more active than in wake (Marrosu et al., 1995). The brain in REM sleep is thus largely devoid of aminergic modulation and dominated by acetylcholine. Thus, sleep consists of myriad physiological states and many neurochemical and neurohormonal mechanisms. When considering the role of sleep in memory consolidation, one must take this dynamic model of sleep into account (Payne and Nadel, 2004; Walker and Stickgold, 2004).

2.35.4 Types of Memory

Like sleep, memory can be subdivided into different types. In contrast to earlier perspectives, in which memory was viewed as a single system subserved by a restricted part of the brain, most modern views posit several types of memory, each obeying different rules of operation, and each drawing on distinct neural systems that interact to produce the subjective sense of remembering. This insight is critical if we are to understand the role of sleep in human memory consolidation because it raises the possibility of many complex interactions among the dynamic processes of sleep and memory, where the neurochemistry associated with distinct sleep/brain states differentially influences the various types of memory.

Various taxonomies are used to classify the different memory systems (Schacter and Tulving, 1994), most of which agree on a distinction between two broad classes of memory. First, there are memories of the events in our lives and the knowledge of the world that we obtain from these events. Typically, this class of memories can be explicitly retrieved, and for this reason it is often referred to as explicit or declarative (i.e., that which can be declared). Second, there are memories for the various skills, procedures, and habits we acquire through experience – so-called 'how-to' memories. These memories are not so easily made explicit and are usually only evident through performance improvements in various behaviors. Thus, this class of memories is referred to as procedural or implicit. The neural mechanisms that support these memory systems appear to be partially dissociable; however, it is important to remember that they interact as well.

Explicit memory can be further subdivided into episodic and semantic memories (Tulving, 1972). Episodic memory concerns those aspects of explicit remembering that incorporate the specific context of an experienced event, including the time and place of its occurrence. Semantic memory, on the other hand, is concerned with the knowledge one acquires during events, but is itself separated from the specific event in question. Thus, our knowledge about the meaning of words and facts about the world, though acquired in the context of some specific experience, appears to be stored in a form that is context-independent (e.g., not bound to the originating context).

Beyond these, there is also evidence for an emotional memory system that mediates the encoding and consolidation of emotionally charged events (McGaugh et al., 1993; Cahill and McGaugh, 1996; McGaugh et al., 1996; Cahill, 2000; Packard and Cahill, 2001). This system is particularly concerned with learning about fearful and unpleasant stimuli, although growing evidence suggests it plays a role in memory for pleasant information as well (Hamann et al., 1999; Hamann et al., 2002; Hamann, 2003; Kensinger, 2004).

The explicit memory system is governed by the hippocampus and surrounding medial temporal areas, while procedural and implicit memory are thought to be independent of the hippocampal complex, relying instead on various subcortical and neocortical structures (Squire, 1992; Schacter and Tulving, 1994). The emotional memory system is critically modulated by the amygdala, a limbic structure located deep in the subcortical brain and richly connected to the hippocampus. It is important to note that because each of these memory systems is subserved by different brain areas, information dependent on each is open to differential processing during sleep. Thus, when attempting to answer the seemingly straightforward question – how does sleep influence memory? – we find that it quickly branches into numerous questions, depending on what kind of sleep and what kind of memory we are talking about.

Although memory consolidation is a complex, multistep process, we define it here as a slow process that converts a still-labile memory trace into a more stable or enhanced form (e.g., Dudai, 2004). As such, the benefits of sleep are sometimes seen as a reduction in the normal decay of a memory (assessed via performance on a memory task), while other times they are seen as actual enhancements in performance.

2.35.5 Procedural and Implicit Memory

Sleep appears to benefit both procedural/implicit and explicit memory. Since most of the recent work has focused on sleep and procedural memory, we begin our review here. A wide range of perceptual, motor, and cognitive abilities are gradually acquired through continuous interactions with the environment, and in many cases this occurs in the absence of conscious awareness. Converging data suggest that these abilities are acquired slowly and are not attained solely during the learning episode. While some learning certainly develops quickly, performance on various tasks improves further, and without additional practice, simply through the passage of time (so-called off-line improvement), suggesting that memory traces continue to be processed over long periods of time. Importantly for our purposes, these longer periods often contain sleep, and the consolidation occurring during them may be dependent on this sleep.

2.35.5.1 Visual Discrimination Learning

Early work investigating the effect of sleep on implicit learning used a visual texture discrimination task (VDT) that was originally developed by Karni and Sagi (1991). The task requires participants to determine the orientation (vertical or horizontal) of an array of diagonal bars that is embedded in one visual quadrant against a background of exclusively horizontal bars (**Figure 1**). At the center of the screen is the fixation target, which is either the letter T or L. This target screen is succeeded first by a blank screen for a variable interstimulus interval (ISI), and then by a mask (a screen covered with randomly oriented V letters, with a superimposed V and L in the center). Subjects must determine the orientation of the array, and the performance is estimated by the ISI corresponding to 80% correct responses (Karni and Sagi, 1993; Karni et al., 1994).

Amnesic patients with damage to the hippocampal complex, who cannot acquire knowledge explicitly, show normal performance improvements on the VDT. This was shown using a group of five densely amnesic patients. All five had extensive medial temporal lobe damage, including damage to the hippocampal formation. These patients were trained on the task on day 1 and retested on days 2 and 5. In spite of having no conscious recollection of having taken the test before, they showed substantially improved performance (Stickgold, 2003).

In neurologically normal subjects, improvement on the VDT develops slowly after training (Karni and Sagi, 1993; Stickgold et al., 2000a), with no improvement when retesting occurs on the same day as training (**Figure 2(a)**, open circles). Instead, improvement is only observed after a night of sleep (**Figure 2(a)**, filled circles).

This was true even for a group of subjects that were retested only 9 h after training. Importantly, there was not even a trend to greater improvement when the training–retest interval was increased from 9 to 22.5 h, suggesting that additional wake time after the night of sleep provided no additional benefit. While further wake time provided no benefit, additional nights of sleep did produce incremental improvement. When subjects were retested 2–7 days after training, 50% greater improvement was observed than after a single night of sleep (**Figure 2(b)**, green bars). Critically, another group of subjects was sleep-deprived on the first night after training. These subjects were allowed two full nights of recovery sleep before being retested 3 days later. They failed to show any residual learning, suggesting that performance enhancements are dependent on a normal first night of sleep (**Figure 2(b)**, red bar). Time alone is clearly not enough to produce

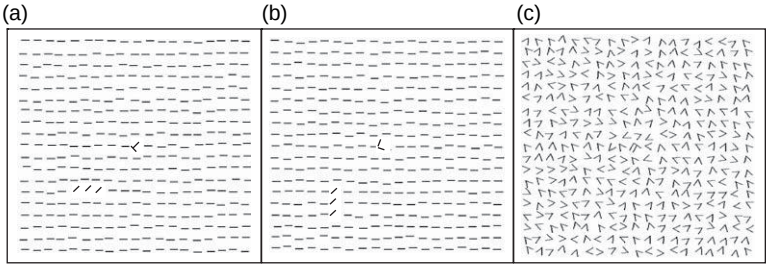

Figure 1 Sample screens from the visual texture discrimination task (VDT). See text for explanation.

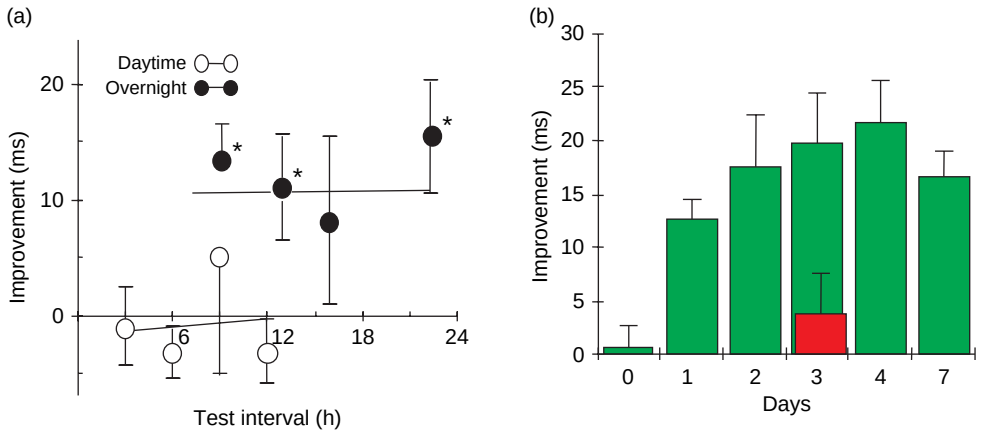

Figure 2 Sleep-dependent improvement on the VDT. All subjects were trained and then retested only a single time. Each point in (a) and each bar in (b) represents a separate group of subjects. Error bars in (a) and (b) are S.E.M.s. From Stickgold R, James L, and Hobson A (2000a) Visual discrimination learning requires post-training sleep. *Nat. Neurosci.* 2(12): 1237–1238; Stickgold R, Whidbee D, et al. (2000b) Visual discrimination task improvement: A multi-step process occurring during sleep. *J. Cogn. Neurosci.* 12: 246–254.

long-term benefits from VDT training. It appears that sleep is also required (Stickgold et al., 2000a).

Initially, improvement on this task appeared to depend solely on REM sleep, because subjects who underwent selective deprivation of REM sleep showed no improvement on the task (Karni et al., 1994). Later studies, however, showed that optimal performance on this task requires both SWS and REM sleep (Stickgold et al., 2000b).

When subjects were trained and their subsequent sleep monitored in the sleep laboratory, the amount of improvement was proportional to the amount of SWS during the first quarter of the night (**Figure 3(a)**), as well as to the amount of REM sleep in the last quarter (**Figure 3(b)**). Indeed, the product of these two sleep parameters explained more than 80% of the intersubject variance (**Figure 3(d)**). No significant correlations were found for sleep stages during other parts of the night (**Figure 3(c)**) or for the amount of Stage 2 sleep at any time during the night.

Gais et al. (2000) came to a similar conclusion by examining improvement after 3 h of sleep either early or late in the night. They found that 3 h of early night sleep, which was rich in SWS, produced an 8-ms improvement; but after a full night of sleep, which added REM-rich sleep late in the night, a 26-ms improvement was observed, three times that seen with early sleep alone. Interestingly, however, 3 h of REM-rich, late-night sleep actually produced deterioration in performance (Gais et al., 2000).

Daytime naps also lead to performance benefits on the VDT. To lay the groundwork for the nap studies, Mednick et al. (2002) showed that VDT performance suffers from repeated, same-day testing. When subjects were trained on the task and then tested at numerous time points throughout the day, their performance deteriorated (i.e., their ISI threshold was higher). **Figure 4** depicts tests given at 9.00 a.m., 12.00 p.m., 4.00 p.m., and 7.00 p.m., with performance worsening significantly on each successive test. However, if subjects are allowed to take an afternoon nap after the second test, their performance improves. Interestingly, 30-min naps prevented the normal deterioration seen during sessions 3 and 4 (Mednick et al., 2002), and longer naps ranging from 60 to 90 min, and containing both SWS and REM sleep, actually enhance performance (Mednick et al., 2003). Taken together, these studies suggest that both SWS and REM sleep play roles in the sleep-dependent memory consolidation of this task.

At this point, sleep's role in visual discrimination learning (as measured by VDT performance) is clear. But the VDT represents a very specific type of sensory memory that may or may not share its sleep dependency with other procedural tasks. This raises the question of whether the sleep effects observed with the VDT generalize to other forms of procedural memory. Studies of sleep-dependent auditory and motor skill learning strongly suggest that they do.

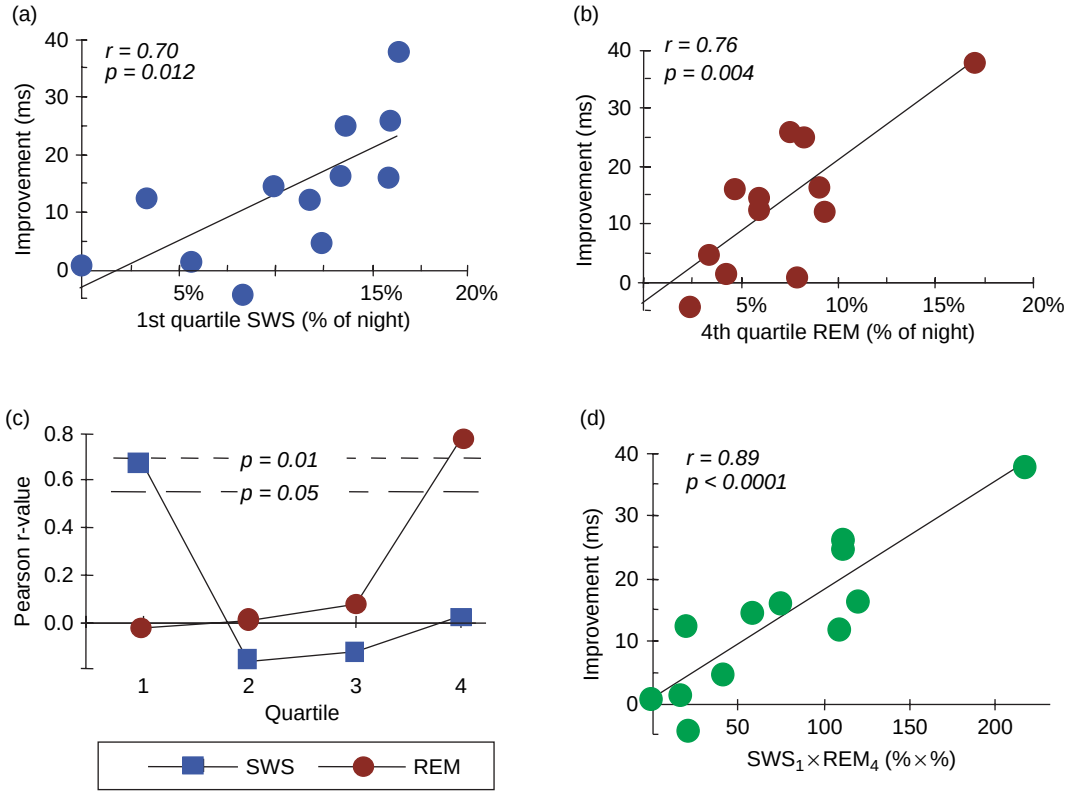

Figure 3 REM and SWS dependence of VDT learning. From Stickgold R, Whidbee D, Schirmer B, et al. (2000b) Visual discrimination task improvement: A multi-step process occurring during sleep. *J. Cogn. Neurosci.* 12: 246–254.

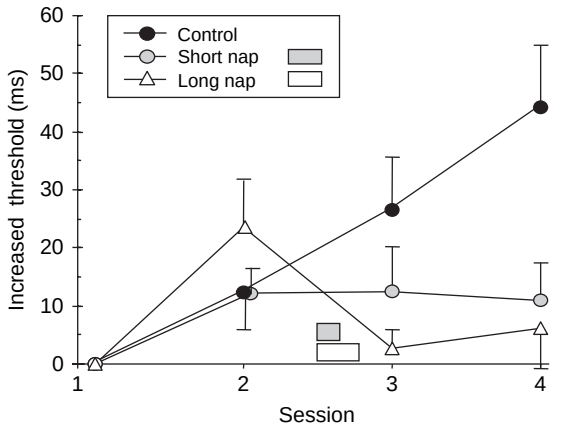

Figure 4 Deterioration in VDT performance with repeated same-day testing and recovery following napping. Note that the ordinate reflects changes in ISI threshold and, as such, higher values indicate worse performance. From Mednick SC, Nakayama K, Cantero JL, et al. (2002) The restorative effect of naps on perceptual deterioration. *Nat. Neurosci.* 5: 677–681.

2.35.5.2 Auditory Learning

Gaab et al. (2004) have shown that delayed performance improvements in memory for pitch develop only across periods of sleep and not across similar periods spent awake. Atienza and colleagues (Atienza et al., 2002, 2004) have also presented evidence of both time- and sleep-dependent auditory memory consolidation, including sleep-dependent changes in brain-evoked response potentials (ERPs). Although post-training sleep deprivation did not prevent continued behavioral improvements, ERP changes associated with the automatic shift of attention to relevant stimuli, which normally develop in the 24–72 h after training, failed to develop following a posttraining night of sleep deprivation. These findings highlight the danger of presuming that a lack of behavioral improvement is equivalent to an absence of beneficial plastic changes in the brain, and they demonstrate the importance of using combined behavioral and physiological analyses (Gaab et al., 2004; Walker and Stickgold, 2006).

Finally, Fenn et al. (2003) have demonstrated that sleep benefits learning on a synthetic speech-recognition task. Training on a small set of words improved performance on novel words that used the same phoneme but a different acoustic pattern.

Importantly, sleep benefited this ability to generalize phonological categories across different acoustic patterns. Time spent awake after initial training resulted in a degradation of performance on this task, but a subsequent night of sleep restored it. This suggests a process of sleep-dependent consolidation capable of reestablishing previously learned complex auditory skill memory, as well as a form of sleep-dependent generalization of learning, which is a hallmark of flexible learning in humans (Fenn et al., 2003). These studies suggest that, as with visual discrimination learning, sleep provides an important benefit to auditory skill learning. In the next section, we show that motor memory benefits from sleep as well.

2.35.5.3 Motor Memory

Numerous studies have demonstrated a relationship between sleep and various types of motor memory (Smith and MacNeill, 1994; Fischer et al., 2002; Walker et al., 2002; Maquet et al., 2003). As an example, Walker et al. (2002) have demonstrated sleep-dependent improvements on a finger-tapping task. The task requires subjects to type the numeric sequence 4-1-3-2-4 as quickly and accurately as possible. Training consisted of twelve 30-s trials, separated by 30-s rest periods. All subjects show considerable improvement during the 12 trials of the training session (a fast learning component), but 12 h later, subjects performed very differently depending on whether the 12-h interval was filled with time spent sleeping or time spent awake. When trained in the morning and retested 12 h later, only an additional nonsignificant 4% improvement was seen in performance, but when tested again the next morning, a large and robust (14%) improvement was seen (**Figure 5(a)**). The failure to improve during the daytime could not be due to interference from related motor activity because subjects who were required to wear mittens and refrain from fine motor activities during this time showed a similar pattern of wake/sleep improvement (**Figure 5(b)**).

In contrast, when subjects were trained in the evening, improvement was observed the following morning (after sleep), but not across an additional 12 h of wake (**Figure 5(c)**). Thus, improved performance resulted specifically from a night of sleep, as opposed to the simple passage of time. Curiously, unlike the findings for the VDT, overnight improvement on this task correlated with the amount of stage 2 NREM during the night, especially during the last quarter of the night. These findings are in agreement with those of Smith and colleagues (Smith and MacNeill, 1994; Tweed et al., 1999; Fogel et al., 2001), who have also shown that stage 2 sleep, and possibly the sleep spindles which reach peak density

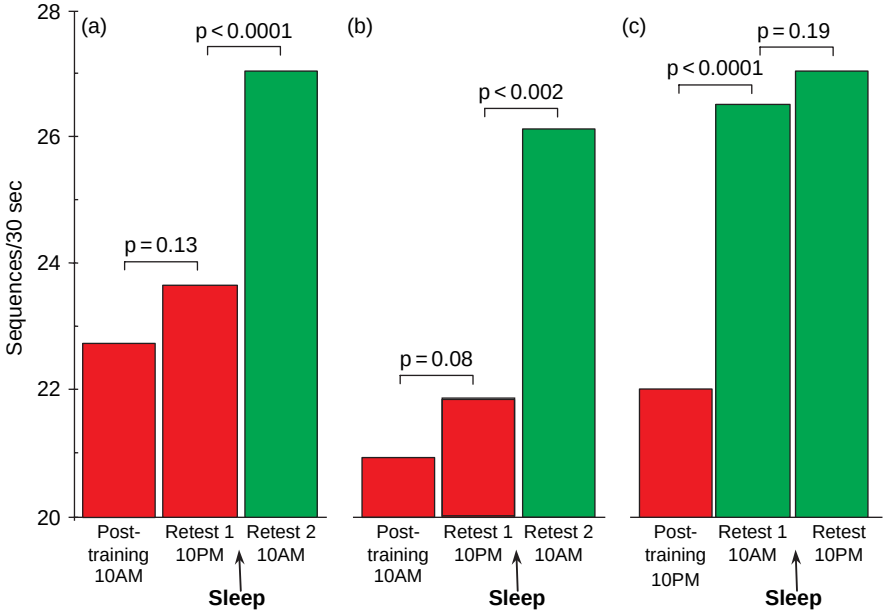

Figure 5 Sleep-dependent motor learning. Improvement in speed was seen in all three groups over a night of sleep, but not over 12 h of daytime wake. From Walker MP, Brakefield T, Hobson JA, and Stickgold R (2002) Practice with sleep makes perfect: Sleep dependent motor skill learning. *Neuron* 35(1): 205–211.

during late night stage 2 sleep, are critical for simple motor memory consolidation. This seems plausible, as sleep spindles have been proposed to trigger intracellular mechanisms that are required for synaptic plasticity (Sejnowski and Destexhe, 2000).

It is important to note that this sleep-based improvement was not due to a speed/accuracy trade-off. When the number of errors per 30-s trial was compared between evening and morning, the number of errors actually decreased, although not significantly (Walker et al., 2002). However, when error rates (i.e., errors per sequence) were calculated, a highly significant 43% decrease inthe error rate was seen overnight (**Figure 6**), while a 20% increase in the error rate was found across 12 h spent awake (Walker et al. 2003).

At least for a simple motor task then, sleep appears to benefit both speed and accuracy. More recent studies have shown that these sleep-dependent benefits appear to be specific to both the motor sequence learned and the hand used to perform the task (Fischer et al., 2002; Korman et al., 2003).

This motor sequence task has been examined to determine where precisely in the motor program the sleep-dependent improvement occurs (Kuriyama et al., 2004). In the sequence mentioned above (4-1-3-2-4), there are four unique key-press transitions; 4 to 1, 1 to 3, 3 to 2, and 2 to 4. When the speed between transitions was analyzed for individual subjects prior to sleep, sticking points emerged. While some transitions were easy (i.e., fast), others were problematic (i.e., slow), as if the sequence was being parsed or chunked into smaller bits during presleep learning (Walker and Stickgold, 2006). After a night of sleep, these problematic points were preferentially improved, whereas transitions that had already been mastered prior to sleep did not change. Subjects who were trained and retested after a daytime wake interval showed no such improvements.

These findings suggest that the sleep-dependent consolidation process involves the unification of smaller motor memory units into a single motor memory representation, thereby improving problem points in the sequence. This overnight process would therefore offer a greater degree of performance automation, effectively optimizing speed across the motor program, and would explain the sleep-dependent improvements in speed and accuracy previously reported (Walker and Stickgold, 2006). But importantly, it suggests that the role of sleep is subtle and complex and does more than simply strengthen memories; sleep may encourage the restructuring and reorganization of memories – an important and often overlooked aspect of memory consolidation. We will return to this idea later in the chapter.

Fisher et al. (2002), using a different sequential finger-tapping task, which involves finger-to-thumb movements instead of keyboard typing, have shown

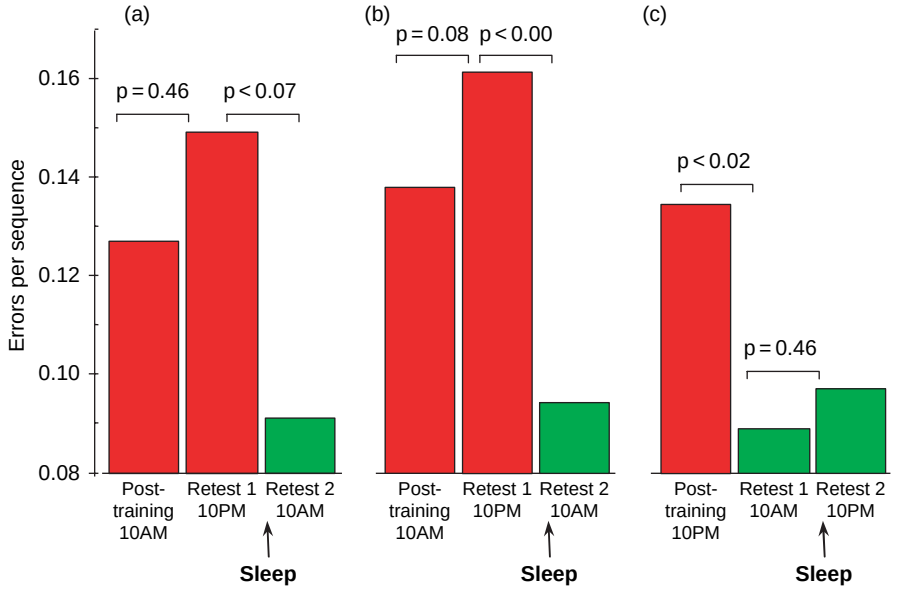

Figure 6 Sleep-dependent motor learning. Improvement in accuracy was seen in all three groups over a night of sleep, but not over 12 h of daytime wake. From Walker MP, Brakefield T, Hobson JA, and Stickgold R (2002) Practice with sleep makes perfect: Sleep dependent motor skill learning. *Neuron* 35(1): 205–211.

that sleep following training is critical for delayed performance improvements. However, they found this improvement to be most strongly correlated with REM sleep rather than stage 2 NREM sleep (see 'Stages of sleep' above).

This discrepancy in sleep stage correlations mirrors similar discrepancies in the declarative memory section below, and remains to be resolved. Nonetheless, it is possible that the more novel finger-to-thumb task requires REM sleep, whereas the keyboard typing task, so similar to the well-learned typing most of us do regularly, is consolidated during stage 2 NREM sleep. A similarly subtle distinction has been reported by Robertson et al. (2004), who found that sleep-dependent enhancement of performance on a perceptual-motor sequence task again correlated with NREM, but only when subjects were explicitly aware of the presence of a repeating sequence, and not when knowledge of the sequence was gained only implicitly (Robertson et al., 2004).

Moving to another type of motor memory – motor adaptation – Maquet et al. (2003) showed that sleep benefits performance on a pursuit task. Participants were trained on a task in which the target trajectory was only predictable on the horizontal axis. This meant that optimal performance could only be achieved by developing an implicit model of the motion characteristics of the learned trajectory. Half of the subjects were sleep deprived on the first post-training night, while the other half were allowed to sleep normally. Three days later, after 2 full days of recovery sleep, performance was superior in the sleep group compared to the sleep-deprived group, and fMRI revealed that the superior temporal sulcus (STS) was differentially more active for the learned trajectory in subjects who slept than in sleep-deprived subjects. Moreover, increased functional connectivity was observed between the STS and the cerebellum, and between the supplementary eye field and the frontal eye field, suggestive of sleep-related plastic changes during motor skill learning in areas involved in smooth pursuit and eye movements.

Similarly, Smith and MacNeill (1994) demonstrated that selective stage 2 NREM sleep deprivation impairs memory for a pursuit rotor task and Huber et al. (2004) demonstrated improved performance on a motor reaching-adaptation task across a night of sleep, but not across an equivalent period of time spent awake. Here, daytime motor skill practice was accompanied by a subsequent increase in NREM slow-wave EEG activity over parietal cortex. This increase was proportional to the amount of delayed learning that developed overnight. Subjects who showed the greatest increase in slow-wave activity in the parietal cortex during NREM sleep showed the largest benefit in motor skill performance the following day (Huber et al., 2004).

Taken together, these findings strongly suggest that sleep is fundamentally important for the development of motor skill memory. Initial daytime learning benefits are supplemented by a night of sleep, which triggers additional learning without the need for further training. Although the role of the various sleep stages in skill memory remains unclear, overnight memory improvements tend to exhibit a strong relationship to NREM sleep, and, in some cases, to specific NREM sleep stages at specific times of the night (Walker and Stickgold, 2006).

Admittedly, visual discrimination, finger tapping, and motor adaptation are all relatively basic, low-level procedural tasks that may become automated fairly quickly. What about more complex implicit and procedural tasks? Animal work clearly demonstrates that complex tasks (e.g., instrumental conditioning, avoidance or maze learning) benefit from sleep, with rats showing increases in REM sleep that continue until the tasks are mastered (Smith et al., 1980; Smith and Wong, 1991; Hennevin et al., 1995). For instance, Smith and Wong (1991) trained rats on a complex operant bar press task, on which only some rats demonstrated increases in REM sleep after training. This split successfully predicted which rats would improve on the task and which rats would fail. Furthermore, after training rats on an avoidance task, Datta (2000) observed an increase in PGO waves (waves of neural activity that are generated in the pons and activate the forebrain during REM sleep) during the first four post-training REM sleep episodes. Changes in REM density observed during the first three of these episodes were proportional to improvement in task performance. These data suggest that the activation of pontine cells that generate PGO waves during REM sleep lead in turn to the activation of forebrain and cortical structures involved in memory consolidation and perhaps to the initiation of these consolidation processes (Datta, 2000).

Recently, Datta and colleagues examined whether the activation of PGO waves could reverse the learning impairment seen after REM sleep deprivation. Rats were trained on a two-way avoidance-learning task and either slept normally or underwent REM sleep deprivation. In addition, they either received a saline injection (placebo) or a carbachol injection in

the P-wave generator. The rats that received both saline and REM sleep deprivation showed learning deficits when compared with the saline-injected rats that slept. But a carbachol-induced activation of PGO waves prevented this learning impairment in the sleep-deprived rats, suggesting that the PGO waves mediated the normal sleep-based memory consolidation (Datta et al., 2004).

Depriving rats of REM sleep after training also leads to performance deficits in complex skills, particularly if the deprivation occurs during so-called critical periods or paradoxical sleep windows (Smith et al., 1995; Smith and Rose, 1996). Two such critical periods emerged in rats attempting to learn a shuttle avoidance task when 20 trials per day were given over a 5-day period. The first occurred 9–12 h after training and the second occurred 17–20 h after training. If the rats were deprived of REM sleep during these windows, their memory for the task was significantly impaired. However, with more intensive training, the window appears earlier. When rats were given 100 training trials in a single session, the critical period appeared at 1–4 h after the end of the shuttle avoidance training (Smith and Butler, 1982).

Critical periods thus appear to vary depending on the task and the intensity of training, and hint at the complexity of sleep–memory relationships that we discuss later. Nonetheless, critical periods are thought to mirror the time after acquisition when REM sleep would typically increase over normal levels. These REM windows may not be prevalent in humans, however, who appear to be sensitive to deprivation during any REM period when trying to learn new complex skills (Smith, 1995).

REM sleep has been implicated in complex procedural learning in humans as well. In a PET (positron emission tomography) study of visuomotor skill memory using the serial reaction time task (SRTT) (Maquet et al., 2000), six spatially permanent position markers were shown on a computer screen and subjects watched for stimuli to appear below these markers. When a stimulus appeared in a particular position, subjects reacted as quickly as possible by pressing a corresponding key on the keyboard. Because the stimuli were generated in an order defined by a probabilistic finite-state grammar, improvement on the task (compared to randomly generated sequences) reflects implicitly acquired knowledge of this grammar (Maquet et al., 2000).

Neuroimaging was performed on three groups of subjects. One group was scanned while they were awake, both at rest and during performance of the task, providing information about which brain regions are typically activated by the task. A second group of subjects was trained on the task during the afternoon and then scanned the night after training, both while awake and during various sleep stages. Thus, group 2 was included to determine if similar brain regions were reactivated during sleep. A post-sleep session was also conducted to verify that learning had indeed occurred. Finally, a third group, never trained on the task, was scanned while sleeping to ensure that the pattern of activation present in natural sleep was different from the pattern of activation present after training.

Results showed that in REM sleep, as compared to resting wakefulness, several brain areas used during task performance were more active in trained than in nontrained subjects. These included occipital, parietal, anterior cingulate, motor and premotor cortices, and the cerebellum – all of which are consistent with the component processes involved in the visual and motor functioning involved in this task. Behavioral data confirmed that trained subjects improved significantly more across the night.

More recently, Peigneux et al. (2003), using the same task, showed that the level of acquisition of probabilistic rules attained prior to sleep was correlated with the increase in activation of task-related cortical areas during posttraining REM sleep. This suggests that cerebral reactivation is modulated by the strength of the memory traces developed during the learning episode, and as such, these data provide the first experimental evidence linking behavioral performance to reactivation during REM sleep (Peigneux et al., 2003). As with previously described animal studies (Datta, 2000), these findings suggest that it is not simply experiencing the task that modifies sleep physiology, but the process of memory consolidation associated with successful learning of the task.

These results support the hypothesis that implicit/procedural memory traces in humans can be reactivated during REM sleep, and that this reactivation is linked to improved consolidation. Indeed, looking a bit more closely at the literature, human REM sleep has also been linked to memory for complex logic games, foreign language acquisition, and to intensive studying (Smith, 2001). It is interesting that these more complex conceptual–procedural tasks often show REM sleep relationships, while more basic procedural tasks benefit mainly from NREM sleep. In order to understand these differences in sleep stage correlations, it is helpful to draw on a proposal by Greenberg and Pearlman (1974), who

suggested that habitual reactions may be REM sleep-independent, while activities involving the assimilation of unusual or unrelated information require REM sleep for optimal consolidation. Such a distinction would support Pearlman's suggestion that simpler tasks are learned without a REM sleep dependency, while the learning of more complex tasks is dependent on posttraining REM sleep (Pearlman, 1979; Greenberg and Pearlman, 1974).

The above findings provide encouraging evidence that sleep-based processes can aid in procedural memory consolidation, not only for basic forms of sensory and motor memory in humans, but for complex procedural and conceptual knowledge as well. Moreover, it argues that the consolidation of familiar skills, or those that are similar to other well-learned skills, may be reliant on NREM sleep stages (particularly stage 2 NREM sleep), whereas REM sleep may be required for the integration of new concepts or skills with pre-existing information that is already stored in memory. This is an important question that warrants future investigation.

Although much remains to be understood about the precise relationship of specific sleep stages to different procedural memory processes, we can say with confidence that sleep generally aids in the consolidation of implicit and procedural forms of memory. Evidence in support of this relationship is now so overwhelming that strong positions to the contrary will, at minimum, have to be revised (Vertes and Eastman, 2000; Siegel, 2001).

2.35.6 Episodic Memory

We turn next to the relationship between sleep and the consolidation of episodic memories. Interest in this relationship can be traced back to a landmark study by Jenkins and Dallenbach (1924), which showed that a period of sleep led to better retention of nonsense syllables than an equivalent period of wakefulness. They interpreted this work to mean that sleep, being an inactive state, transiently protected memory from interference, whereas reduction of recall during wakefulness was due to interference.

> "The results of our study as a whole indicate that forgetting is not so much a matter of the decay of old impressions and associations as it is a matter of interference, inhibition, or obliterations of the old by the new" (Jenkins and Dallenbach, 1924: p. 612).

This interpretation struck a serious blow to the then dominant trace decay theory of forgetting, which posited that the simple passage of time was responsible for forgetting.

Nonetheless, the fact that memories were protected during sleep led to increased interest in the topic (particularly among interference theorists), and Jenkins and Dallenbach's (1924) finding was quickly replicated in better-controlled studies (e.g. Lovatt and Warr, 1968; Benson and Feinberg, 1977). Researchers began to wonder if sleep was actively promoting memory formation, rather than simply reducing interference. Moreover, they began to hypothesize that some types of sleep played a bigger role in episodic memory consolidation than others (The relationship between sleep and the consolidation of semantic memory has received scant attention to date, although see Stickgold et al. (1999) and Brualla et al. (1998) for evidence of semantic memory processing during sleep.).

After the discovery of REM sleep by Aserinsky and Kleitman (1953), the prevailing hypothesis – inspired by psychoanalytic theory – was that memory content would show up in REM sleep, because this was the only stage of sleep in which dreams were thought to occur. (It is now clear that dreams and other types of mental content can occur in all sleep stages, including SWS (Foulkes, 1966; see also Payne and Nadel, 2004)). It made a great deal of intuitive sense that REM sleep should be the stage involved in the reprocessing and consolidation of episodic memories, because, as noted above, the brain during REM sleep is intensely active and looks like it is engaging in some sort of cognitive processing.

This hypothesis was initially supported by several REM sleep-deprivation studies, which showed that such deprivation interfered with memory for prose passages (Empson and Clarke, 1970) and increased the time interval over which memories remained fragile and susceptible to electroconvulsive shock (Fishbein et al., 1971). However, as summarized in Smith (2001), REM deprivation studies in humans provided mixed results on the whole (Chernik, 1972) (see Johnson et al., 1974; Lewin and Glaubman, 1975), which may not be surprising given that sleep deprivation suffers from many confounds, including disrupted natural sleep, decreased levels of arousal and motivation, and increased levels of stress (Maquet, 2001). The stress hormone cortisol, for example, often impairs memory at high levels but can facilitate some aspects of memory at low to

moderate levels (Payne and Nadel, 2004; Payne et al., 2004).

Seeking to avoid the confounds inherent in sleep deprivation studies, Ekstrand and colleagues developed a procedure that attempted to isolate SWS, which is prevalent early in the night, from REM sleep, which is maximal late in the night (see 'Stages of sleep' section above). These researchers were thus the first to systematically investigate the impact of different sleep stages on memory performance while controlling for the unspecific effects of REM sleep deprivation. Their findings implicated NREM, particularly stage 4 SWS, as the most beneficial sleep stage for episodic memory consolidation. Yaroush et al. (1971) required subjects to study a paired-associates list just before bedtime. Half of the subjects were awakened after 4 h of early sleep (dense in SWS) and tested for recall. The other half were allowed to sleep for 4 h prior to awakening; they then studied the list and returned to sleep for another 4 h late in the night (REM-rich sleep) before being awakened to recall the word pairs. A third group of subjects were trained during the day and returned 4 h later for the recall test. The early night group remembered more of the words than either the late night or wake groups in several tests of memory (paced and unpaced tests, matching, and relearning tests), suggesting that early sleep, rich in SWS, benefited episodic memory (Yaroush et al., 1971).

In a follow up-study, Barrett and Ekstrand (1972) replicated this effect while attempting to control for circadian differences. Here, all subjects were required to learn and recall at the same time of day; the retention interval was always between 3.00 a.m. to 7.00 a.m. One group remained awake until training at 3.00 a.m., slept for 4 h, and then were awakened at 7.00 a.m. for testing. Another group arrived in the lab at 10.00 p.m., slept for 4 h prior to training, awakened at 2.50 a.m. to train, returned to sleep for another 4 h and then awakened for testing at 6.50 a.m.. As in the Yaroush et al. (1971) study, recall of word pairs was better in the first-half sleep condition than in either the second-half sleep or wake conditions, thus replicating the early sleep effect while controlling for time of day (Barrett and Ekstrand, 1972).

Fowler et al. (1973) replicated the early sleep effect, this time in the sleep laboratory, where they showed that SWS was indeed most prevalent early in the night (first-half of sleep), while REM was maximal late in the night (second-half of sleep) in spite of the experimental awakenings. The authors pointed out that their findings were not easy to reconcile with an interference theory of forgetting. Subjects in the first and second half of night conditions slept for equivalent amounts of time during the retention interval, so simple protection against interference should have been equal in both groups. Unless one wanted to argue that dreaming is as much an interfering factor as a waking mental activity (and this remains to be determined), it seemed that early-night SWS, and perhaps particularly stage 4 sleep, was most important for episodic memory consolidation (Fowler et al., 1973).

More than 20 years later, Born and colleagues revived this procedure (Plihal and Born, 1997; Plihal and Born 1999a,b). In the first of their studies (Plihal and Born, 1997), both episodic (recall of semantically related paired associates) and procedural (performance on a mirror tracing task) memory were assessed within the same subjects. Participants were trained to criterion on both tasks and then retested after 3-h retention intervals, containing either early or late nocturnal sleep. The results showed that memory improvements were greater after sleep than after a corresponding period of wake, but more importantly, the different periods of sleep seemed to support consolidation of different types of memory. Recall of paired associates improved more after 3 h of early sleep rich in SWS than after 3 h of late sleep rich in REM, or after a 3-h period of wake. Conversely, mirror tracing improved more after 3 h of late, REM-rich sleep than after 3 h spent either in early sleep or awake.

In a related study, Plihal and Born (1999a) examined different measures of episodic and implicit memory in order to separate the effects of type of material (verbal vs. nonverbal) from type of memory (episodic vs. procedural). Thus, a nonverbal episodic memory task (spatial rotation) and a verbal implicit task (word-stem priming) were used in the same early versus late night sleep procedure, and the findings mirrored the previous results. Compared to wake, recall of spatial memory was enhanced after early retention sleep but not late retention sleep, while priming was enhanced more after late than early retention sleep.

It is important to note that this early-/late-night sleep procedure suffers not only from confounds associated with sleep deprivation, but also from an incomplete separation of REM and NREM sleep. Early sleep is an imperfect proxy for SWS, and similarly, late sleep is an imperfect proxy for REM sleep. SWS does appear in the second half of the night, and REM appears in the first half of the night, and thus one cannot exclude the possibility that REM and SWS during these periods contributed

to the noted consolidation effects. Moreover, the distribution of stage 2 NREM sleep is not entirely equal in both halves of the night. Thus, one cannot examine early versus late sleep and make definitive conclusions about SWS versus REM sleep.

In addition, SWS is tested by training subjects before they go to sleep (at around 10.00 or 11.00 p.m.) and then awakening them 3 h later for memory testing. REM sleep, on the other hand, is tested by awakening subjects to train in the middle of the night. These subjects then return to sleep before being awakened 3 h later for memory testing. Training in the middle of the night may well be less effective than training that occurs before subjects have slept at all, which means that the lack of improvement seen after REM awakenings in some experiments may be confounded; what looks like a failure to consolidate may simply reflect a difference in the quality of encoding and degree of attentional resources available for the task after being awakened in the middle of the night as opposed to in the evening prior to sleep. Finally, control groups that are awake for similar periods in the night are acutely sleep deprived, limiting the validity of the comparisons. Therefore, while the value of this creative procedure is that it manipulates sleep stages experimentally, a number of problems limits the clear interpretation of these findings.

In spite of these problems, two neuroimaging investigations of episodic memory consolidation have also suggested an important role for SWS. The first of these investigated performance on a hippocampally dependent virtual maze task (Peigneux et al., 2004). Daytime learning of the task was associated with hippocampal activity. Then, during posttraining sleep, there was a reemergence of hippocampal activation, and it occurred specifically during SWS. The most compelling finding, however, is that the increase in hippocampal activation seen during posttraining SWS was proportional to the amount of improvement seen the next day (see also Peigneux et al., 2003 described above). This suggests that the re-expression of hippocampal activation during SWS reflects the off-line reprocessing of spatial episodic memory traces, which in turn leads to the plastic changes underlying the improvement in memory performance seen the next day.

The second study (Takashima et al., 2006) investigated the time course of episodic memory consolidation across 90 days. Subjects studied 360 photographs of landscapes and were then tested on subsets of the photographs either after a nap the same day, or after 2, 30, or 90 days. Prior to each test, subjects studied 80 new pictures, and then were tested on 80 of the original pictures and the 80 new ones, as well as 80 pictures they had never seen before. All memory retrieval sessions occurred during fMRI scanning.

Following the initial 90-min nap, stage 2 sleep was positively correlated with successful recall of both remote and recent items, indicating a nonspecific benefit of stage 2 NREM sleep on episodic memory. This is an intriguing finding, given that stage 2 is where sleep spindles are most prominent (see the section titled 'Electrophysiological signatures'). Slow-wave sleep, on the other hand, was correlated only with memory for remote (but not recent) items. Because performance on remote items increased with longer SWS duration, but performance for recent items did not, the effect on memory performance for remote items cannot be explained by a general effect of SWS on memory retrieval processes. The authors also point out that this brief period of SWS may have had an even longer-lasting effect on memory, because there was a linear relationship between the amount of SWS during the nap and recognition memory performance after both 2 and 30 days, whereas there continued to be no such correlation for recent items. This finding is striking, given that this was a nap rather than a full night of sleep, and that only 15 of the 24 subjects reached SWS. Finally, longer SWS durations led to decreases in hippocampal activation when remote items were successfully retrieved (it should be noted that while this finding appears to support traditional consolidation theory (Squire and Cohen, 1984), the hippocampus remained active during successful retrieval throughout the study (up to the last test at 90 days), suggesting that episodic memories may never become completely independent of the hippocampus (Nadel and Moscovitch, 1997; Moscovitch et al., 2006). These findings strongly suggest that episodic memories can undergo initial consolidation within a rather short time frame and that this consolidation is promoted by SWS.

In another nap study, Tucker et al. (2006) found that naps containing only NREM sleep enhanced declarative memory for word pairs. Performance on episodic (paired-associates) and procedural (mirror tracing) memory tasks were tested 6 h after training, either with or without an intervening nap. While there was no difference between nap and wake subjects on the procedural memory task, the nap subjects performed significantly better on the paired associate task relative to the subjects who remained awake

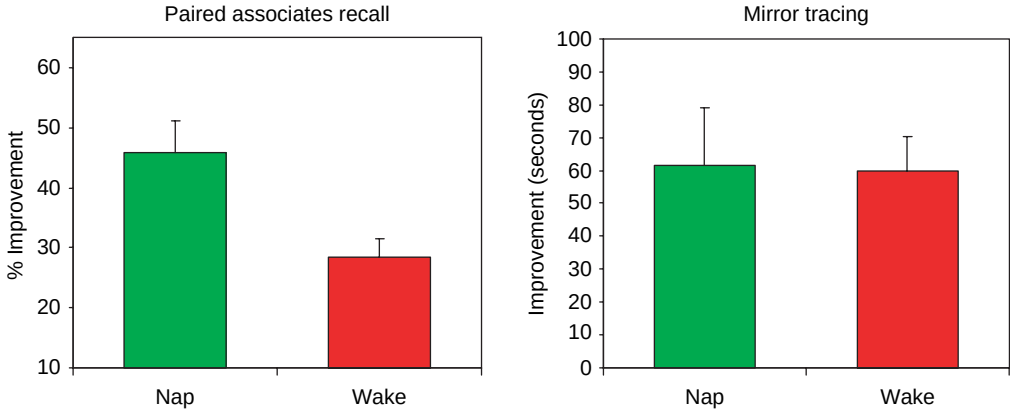

Figure 7 A brief daytime nap benefits episodic memory. Note that the nap (which did not contain REM sleep) benefited episodic, but not procedural, memory. From Tucker MA, Hirota Y, Wamsley EJ, et al. (2006) A daytime nap containing solely non-REM sleep enhances declarative but not procedural memory. *Neurobiol. Learn. Mem.* 86(2): 241–247.

(**Figure 7**). Subjects in the nap condition also showed a weak correlation between improved recall and the amount of SWS in the nap, further supporting the relationship between episodic memory and SWS (Tucker et al., 2006). It will be interesting to see a follow-up study in which the contribution of REM sleep physiology is assessed.

These results should not be taken to mean that REM sleep mediates the consolidation of procedural memories, whereas SWS mediates the consolidation of episodic memories. Matters are clearly not so simple. Recall that improvement on a visual discrimination task depended on SWS as well as REM (Gais et al., 2000), and improvement on a motor task correlated with stage 2 NREM sleep (Smith and MacNeill, 1994). Moreover, emotionally charged episodic memories may rely on REM sleep for their consolidation (see 'Emotional episodic memory' section below).

There are two possible interpretations of these apparent contradictions. First, the sleep stage dependency of these various memory tasks may depend on aspects of the task other than simply whether they are episodic or procedural, perhaps depending more on the intensity of training, the emotional salience of the task, or even the manner in which information is encoded (e.g., deep versus shallow encoding or implicit versus explicit). The second possibility involves an inherent oversimplification in correlating performance improvements with sleep stages as they are classically defined. Indeed, mounting evidence points to several electrophysiological, neurotransmitter, and neuroendocrine mechanisms that may underlie these effects and which do not necessarily correlate with any single sleep stage (see section below), and sleep staging, as it has been defined for 40 years, may not capture all of the key elements that lead to memory consolidation enhanced by sleep.

At an even more basic level, none of the verbal episodic memory studies described above demonstrated that a full night of sleep can produce the kinds of benefits seen following a half-night of sleep or an afternoon nap. The idea of early SWS-rich sleep, but not late REM-rich sleep, being linked to improvements in episodic memory performance, for example, loses much of its interest if these early-night benefits were lost across the second half of the night, such that they would not be available the following day.

Fortunately, several very recent reports dispel this concern (e.g., Ellenbogen et al., 2006; Gais et al., 2006b). These studies demonstrate lasting benefits of a full night of sleep on episodic memory. For example, Ellenbogen et al. (2006) showed that a full night of sleep not only strengthened memory for unrelated paired associates, but also made these memories more resistant to interference than an equivalent period of time spent awake.

Using a classic AB-AC interference paradigm (Barnes and Underwood, 1959), subjects first learned a list of paired associates, A_iB_i. After either a 12-h period including a night with 7–8 h of sleep, or an equivalent 12-h period of wakefulness during the day, half of the subjects in each group recalled the previously learned word pairs (cued recall). The other half learned a new list of paired associates, A_iC_i, before being tested for recall of the original list. To control for circadian effects and to demonstrate that the effects of sleep persist, an additional group of subjects was trained at the same circadian time as the sleep group (9.00 p.m.), and tested 24 rather than 12 h later.

While sleep provided modest protection against memory deterioration even in the absence of interference training, it provided a large and dramatic protection against post-sleep interference, and this benefit was sustained throughout the subsequent waking day (**Figure 8**). Thus, memories after sleep were highly resistant to interference and remained resistant across the subsequent day, demonstrating significantly better recall after 24 h than memories encoded in the morning and tested just 12 h later, without sleep. This study suggests that sleep does more than simply protect memories from interference while asleep: sleep stabilizes memories, making them resistant to future interference during the subsequent wake period.

A study by Gais et al. (2006b) provides additional evidence that sleep does more than protect memories against interference (see Wixted, 2004 for a full review of the interference argument). Subjects learned English–German vocabulary lists in the morning (8.00 a.m.) or in the evening (8.00 p.m.) and were tested immediately via cued recall to establish a baseline memory retention score. They were then retested after 24 or 36 h, either at the same circadian time or at a different circadian time (i.e., subjects trained at 8.00 a.m. and were retested at 8.00 p.m. or vice-versa). Subjects went to sleep either soon after training and initial testing (approximately 3 h in the 8.00 p.m. training condition) or after a significant delay (approximately 15 h in the 8.00 a.m. training condition).

Subjects who slept soon after training (and were retested either 24 or 36 h later) performed better on the retest session, suggesting that a night of sleep shortly after training benefited their performance on the task. In a second experiment, subjects were similarly trained in the evening either prior to sleep or to a night of sleep deprivation. Sleep-deprived subjects were allowed to sleep the following day, where they made up much of their lost sleep. However, the deprived subjects did not sleep until 10 h after training, whereas control subjects went to sleep a mere 3 h after training. In both conditions, recall testing took place 48 h after initial learning, again in the evening, to allow for recovery sleep in the deprivation condition. Although no differences emerged in the initial test on the first evening, there was a clear deficit after a night of sleep deprivation. Subjects remembered more words when they slept the night following training than when they remained awake, thus providing further evidence that sleep benefits memory consolidation. Importantly, both the 24 h groups (a.m. and p.m.

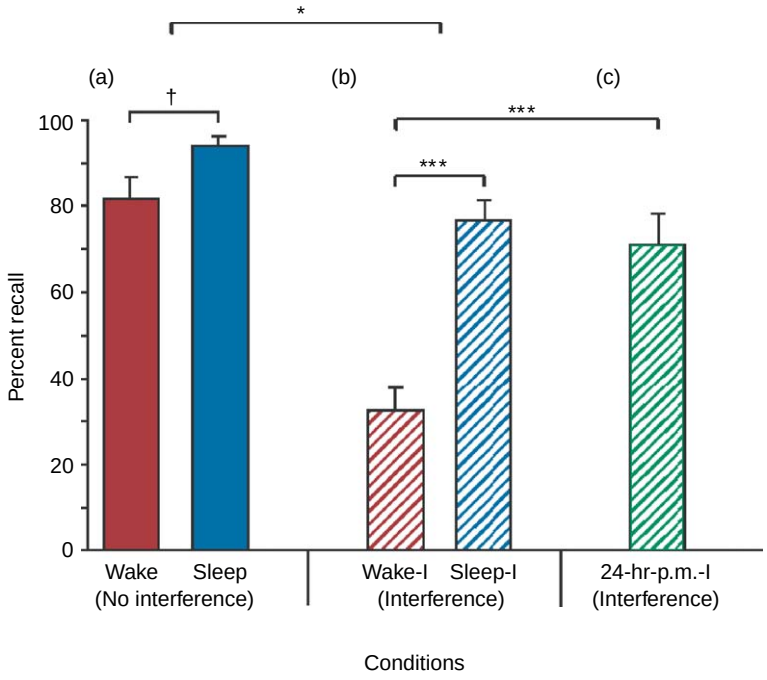

Figure 8 Sleep makes memory resistant to interference. Note that this beneficial effect of sleep was seen after 12 nighttime hours including sleep and remained 24 hours later. From Ellenbogen JM, Hulbert JC, Stickgold R, et al. (2006a) Interfering with theories of sleep and memory: Sleep, declarative memory, and associative interference. *Current Biology* 16(13): 1290–1294.

training) underwent identical amounts of waking interference, as did the two 48-h groups (controls and sleep-deprived), which strongly suggests that the sleep benefits cannot be explained by a decrease in waking interference.

Thus, it appears quite unlikely that sleep merely offers a permissive, interference-free environment for memory consolidation. It is plausible that sleep also activates unique neurobiological processes that play an active role in consolidation (for a recent review, see Ellenbogen et al., in press). This would suggest that there are sleep-specific neural processes that contribute to memory consolidation – an argument we review in the section titled 'Electrophysiological signatures'.

2.35.6.1 Emotional Episodic Memory

Sleep also appears to contribute to the consolidation of emotional episodic memories. This is interesting in light of the many early studies that demonstrated slow, time-dependent improvements in emotional memory, where memory for emotionally laden events, or emotional aspects of complex events, often continued to improve over days and even weeks (Kleinsmith and Kaplan, 1963; Kleinsmith et al., 1963; Kleinsmith and Kaplan, 1964). While it is well known that memories of emotional events are encoded and subsequently persist more strongly than memories for neutral events (McGaugh, 2000; Kensinger, 2004), only recently has sleep's contribution to this apparent consolidation effect been examined (Hu et al., 2006; Wagner et al., 2001).

Hu et al. (in press) examined the impact of a full night of sleep on both axes of emotional affect – valence (positive/negative) and arousal (high/low), across both remember and know measures of memory for pictures. Results showed that a night of sleep improved memory accuracy for emotionally arousing pictures relevant to an equivalent period of daytime wakefulness, but only for know judgments. No differences were observed for remember judgments. Moreover, memory bias changed across a night of sleep relative to wake, such that subjects became more conservative when making remember judgments, especially for emotionally arousing pictures. No bias differences were observed for know judgments between sleep and wake. These findings provide further evidence that the facilitation of memory for emotionally salient information may preferentially develop during sleep. Whether these effects emerge primarily after REM-rich, late night sleep, as in Wagner et al. (2001) (discussed in the section titled 'Neurohormones and neurotransmitters'), remains to be investigated. Nonetheless, the enhancing impact of sleep on the remembrance of emotional episodic information is becoming increasingly clear.

2.35.7 Electrophysiological Signatures

But what is it about sleep that leads to memory consolidation? Several electrophysiological signatures of sleep, recorded in animals with cortical electrodes as well as in the human EEG, reflect synchronized oscillatory patterns of neuronal activity in the cortex that may actively promote memory consolidation. There are still relatively few studies examining these proposed neurophysiological mechanisms. However, we expect the number to increase dramatically in the near future, and so we review what is currently known here.

2.35.7.1 Sleep Spindles

Sleep spindles are one example of such a mechanism. Sleep spindles are bursts of coherent brain activity visible on the EEG, which are most evident during stage 2 sleep. They consist of brief 11- to 16-Hz waves lasting 0.5–1.5 s. In animals, the initiation of cortical sleep spindles tends to occur with high-frequency (~200 Hz) ripples that ride on hippocampal sharp waves in NREM sleep (Siapas and Wilson, 1998). This co-occurrence of hippocampal sharp waves and cortical spindles may underlie the integration of information between the hippocampus and neocortex as memories are consolidated during sleep (Buzsáki, 1996).

In support of this hypothesis, several human studies have shown a correlation between hippocampally dependent episodic learning and cortical sleep spindles. In one such study (Gais et al., 2002), subjects studied a long list of unrelated word pairs 1 h prior to sleep, on two separate occasions, at least a week apart. In one case, they were instructed to imagine a relationship between the two nominally unrelated words, while in the other they were simply asked to count the number of letters containing curved lines in each word pair. Such instructions lead to deep, hippocampally mediated encoding and shallow, cortically mediated encoding, respectively. During the subsequent nights of sleep, subjects showed significantly higher spindle densities on the nights following deep encoding, averaging 34% more

spindles in the first 90 min of sleep. Moreover, sleep spindle density was positively correlated both with immediate recall tested in the final stage of training and with recall the next morning, after sleep. Thus, those who learned better had more spindles the following night, and those with more spindles showed better performance the next morning.

These findings mirror previous observations by Meier-Koll et al. (1999), who reported a similar increase in spindles following learning of a hippocampally dependent maze task, and by Clemens et al. (2005) who found a correlation between spindle density and overnight verbal memory retention (although not between spindle density and memory for faces). Interestingly, Smith and colleagues have reported increased spindle density after intensive training on a pursuit rotor task, and after combined training on several simple procedural motor tasks (Fogel and Smith, 2006; Fogel et al., 2007). Thus, spindles might contribute to the consolidation of both explicit and implicit memories (Meier-Koll et al., 1999; Clemens et al., 2005; Fogel and Smith, 2006).

But sleep spindles also appears to correlate strongly with IQ (Bodizs et al., 2005; Schabus et al., 2006), and it can thus be difficult to discern whether high spindle content correlates with overnight improvement in memory *per se*, or whether both reflect correlations with IQ. While this is not problematic when repeated measures or factorial designs compare nights with and without preceding memory encoding, the correlation with IQ confounds correlational studies that show more posttraining spindles in subjects who subsequently show better recall.

2.35.7.2 Slow Waves

Given the evidence for early night facilitation of episodic memory recall (e.g., Plihal and Born, 1997, 1999a,b), it is not surprising that neurophysiologic markers associated with slow-wave sleep (SWS) have also been implicated in memory consolidation. Slow-wave rhythms, including both classical delta activity (1–4 Hz), and the more recently characterized cortical slow oscillations (<1 Hz) increase as humans pass into SWS. Indeed, these cortical slow oscillations are now considered a hallmark of SWS (Steriade and Timofeev, 2003). Such slow oscillatory activity in neuronal networks allows distant ensembles to become synchronized in rats and has been hypothesized to facilitate the binding and consolidation of memories that are dispersed across distant brain regions (Buzsáki and Draguhn, 2004).

Cortical slow oscillations have recently been observed in humans in conjunction with increased EEG coherence. EEG coherence is a large-scale measure of the coactivation of distant brain regions. Molle et al. (2004) recently showed that increased EEG coherence, which was strong during the memorization of word pairs, reappeared with cortical slow oscillations during subsequent slow wave sleep. Interestingly, while there were only marginal increases in coherence when measured over all NREM sleep, this coherence was dramatically increased when the analysis was time-locked to the occurrence of cortical slow oscillations (Molle et al., 2004).

This finding suggests that slow oscillations are important for the reprocessing of memories during sleep, a conclusion that is based on two assumptions. First, it assumes that high coherence between EEG signals from different sites on the scalp reflect an increased interplay between the underlying neuronal networks, and second, it assumes that efficient encoding of associations in episodic memory is facilitated by the large-scale synchrony of cortical neuronal activity measured by EEG coherence. Given these assumptions, the finding suggests that cortical slow oscillations may be of particular functional significance for the reprocessing of newly acquired associative memories during human SWS.

Slow oscillations also appear to exert a grouping influence over spindle activity. Molle et al. (2002) examined the temporal dynamics between spindle activity and slow oscillations in the human EEG during NREM stage 2 and SWS. They found that during human SWS, rhythmic activity in the spindle frequency range correlated with periods of slow oscillations. They also showed that discrete spindles identified during NREM stage 2 sleep coincided with the depolarizing portion of the cortical slow oscillations and were preceded by pronounced hyperpolarizing half-waves (Molle et al., 2002). These results suggest that slow rhythmic depolarizations and hyperpolarizations in cortical neurons might alternately drive and inhibit thalamically generated spindle activity, thereby contributing indirectly to memory consolidation through their regulation of spindles.

There are two distinct mechanisms by which spindles might provide the conditions necessary to induce long-term synaptic changes. Relevant cortical neural networks may be selectively activated during

spindle activity as a result of previous learning, and, in turn, this activation may induce activity in, and thus modification of, related networks within the hippocampal complex. Alternatively, hippocampal activity driven by recent learning might selectively prime relevant cortical networks, which would then be activated and modified during subsequent sleep spindles (Siapas and Wilson, 1998). Either way, the spindles themselves may induce long-term synaptic changes in the neocortex.

Sleep spindles and slow oscillations represent promising candidate mechanisms for sleep-dependent memory consolidation, but it is important to note that a causal role for these electrophysiological signatures remains to be demonstrated. Nevertheless, they provide preliminary support, in humans, for the idea that the hippocampus and neocortex cooperate to integrate new information into long-term memory during sleep (Buzsáki, 1996).

2.35.7.3 Hippocampal and Cortical Replay

This hippocampal–neocortical communication paradigm is important, because it is intimately intertwined with theories of memory consolidation. New memories are at least initially dependent on connections between medial temporal and neocortical regions, and increased communication between these regions after training on a memory task may reflect consolidation of these recently acquired memories. A growing literature demonstrates precisely these effects in animals, where hippocampally dependent learning leads to post-training reactivations in brain areas involved in memory processing.

In the earliest studies, Pavlides and Winson (1989) demonstrated spontaneous neuronal replay of task-specific firing patterns during posttraining sleep, with individual hippocampal place cells that discharged during spatial exploration increasing their firing rates during subsequent sleep. Recording from large ensembles of place cells in the CA1 field of the hippocampus, Wilson and McNaughton (1994) showed that pairs of cells that fired together as rats passed through specific locations in an open field also fired together during subsequent SWS. This cellular activity during sleep mimicked the firing patterns seen when the task was performed, suggesting that information acquired during wake is re-expressed during sleep and that this reactivation forms a neurophysiological substrate of sleep-dependent memory consolidation (Pavlides and Winson, 1989; Wilson and McNaughton, 1994).

Since then, numerous studies have reported neuronal replay during both SWS (Skaggs and McNaughton, 1996; Kudrimoti et al., 1999; Lee and Wilson, 2002) and REM sleep (Poe et al., 2000; Louie and Wilson, 2001). Interestingly, the replay of temporal patterns of activity during SWS occurs on a time scale 20 times faster than the previous waking pattern (Lee and Wilson, 2002), while during REM it occurs in close to real time, averaging just 40% slower than in wake (Louie and Wilson, 2001).

The finding, discussed above (Siapas and Wilson, 1998), of temporal correlations during SWS between hippocampal sharp-wave/ripples and the initiation of individual prefrontal sleep spindles, along with similar correlations between the hippocampus and somatosensory cortex (Sirota et al., 2003) provides a mechanism by which such neuronal replay could lead to consolidation in both hippocampal and cortical networks.

2.35.7.4 Theta Rhythm

There is evidence in both humans and animals that theta frequency (4–7 Hz) oscillations are associated with enhanced learning and memory during the waking state (Bastiaansen and Hagoort, 2003), and it has been suggested that the integration of information within hippocampal and neocortical circuits may be mediated by theta activity. Although there is little theta activity during SWS, it is at waking levels during REM sleep, when hippocampal cell discharge is modulated at the theta frequency. This theta activity may aid memory reprocessing during REM sleep by enabling information to flow from the neocortex (through the superficial layers of the entorhinal cortex) into the hippocampus, where it can reverberate within hippocampal circuitry (i.e., replay). In contrast, during the sharp-wave and ripple activity of SWS, information may flow in the opposite direction, out of the hippocampus and back to the neocortex (through deep layers of the entorhinal cortex Buzsáki, 1996; Buzsáki, 1998), thus allowing information to flow throughout the complete neocortex–hippocampal circuit. Indeed, it has been proposed that high levels of the neurotransmitter acetylcholine and the neurohormone cortisol during REM sleep, and low levels during SWS, might modulate communication between hippocampus and neocortex as memories undergo consolidation (Payne and Nadel, 2004). In this view, as in others (Giuditta et al., 1995;Ficca et al., 2000), both SWS and REM sleep are thought to contribute to the

consolidation of episodic memories. In addition, for emotional memory processing, cooperative theta oscillations between hippocampal and amygdala regions during REM sleep may play an important role as well (Pare et al., 2002).

2.35.8 Neurohormones and Neurotransmitters

Many modulatory neurotransmitters contribute to memory formation. Acetylcholine, however, has received the most attention by the sleep community to date, most likely because it is critically involved in control of the NREM/REM cycle, and because it is present at particularly high levels during REM sleep and low levels during SWS (Hobson et al., 1998).

Acetylcholine, although mainly involved in memory encoding, appears to also play a role in the flow of information between memory systems during different stages of sleep. According to a model by Hasselmo (1999), acetylcholine inhibits feedback loops both within the hippocampus and between the hippocampus and neocortex. As a result, the high cholinergic activity seen during wakefulness minimizes consolidation and promotes encoding of new episodic memories, whereas the low cholinergic activity in SWS blocks new input and supports the replay of recently encoded information in the hippocampus. This replay may then lead to integration of this information within hippocampal and neocortical memory stores (Buzsáki, 1996; Hasselmo, 1999; Payne and Nadel, 2004).

To investigate the role of acetylcholine in the consolidation of episodic memory during sleep, Gais and Born (2004b) trained subjects on a list of paired associates, as well as a mirror tracing task, before 3 h of SWS-rich nocturnal sleep or wakefulness during which they received a placebo or an infusion of the cholinesterase inhibitor physostigmine (which increases cholinergic tone). When tested after 3 h of sleep, recall on the paired-associates task was impaired in the physostigmine group, while procedural memory performance was unaffected (Gais and Born, 2004b). This provides initial support for Hasselmo's (1999) model and suggests that the inhibition of cholinergic activity during SWS is critical for sleep-based episodic memory consolidation.

As with neurotransmitters, hormonal fluctuations across the sleep cycle may also help to explain why different sleep stages contribute differentially to the consolidation of episodic memories. Activation of the neuroendocrine hypothalamic–pituitary–adrenocortical (HPA) system, for instance, results in the release of the stress hormone cortisol from the adrenal glands. Cortisol then feeds back onto the brain, where the hippocampus and frontal cortex, arguably two of the most critical memory regions, contain the highest number of cortisol receptors in humans (Lupien and Lepage, 2001). Several studies have demonstrated cortisol-induced memory impairments with episodic memory tasks during wake (Kim and Diamond, 2002; Payne et al., 2002; Payne et al., 2006). Intriguingly, cortisol levels are at their lowest during early nocturnal sleep, while achieving a diurnal maximum during late night sleep (Plihal and Born 1999b; Born and Wagner, 2004).

Plihal and Born (1997, 1999a) have thus argued that the circadian suppression of cortisol release early in the night makes this SWS-rich sleep an ideal physiological environment for episodic memory consolidation. Naturally low cortisol levels during early sleep promote more efficient consolidation of episodic memories than is seen during late, REM-rich sleep, when cortisol levels are high. In support of this view, Plihal and Born (1999b) showed that artificially elevating cortisol levels during early sleep eradicated the normal episodic memory benefit seen during this period, suggesting that the salubrious environment provided by early sleep is a result, at least in part, of the naturally low levels of cortisol during this time.

Cortisol levels in the Plihal and Born (1999b) study were elevated to levels similar to those typically seen in response to mild to moderate stressors, that are sufficient to disrupt episodic memory function during wakefulness (Kirschbaum et al., 1996; de Quervain et al., 2003; de Quervain, 2006).

In another study suggestive of a cortisol-related influence on memory consolidation (Wagner et al., 2001), memory for emotionally laden narrative material was facilitated after late night, REM-rich sleep periods. At first blush, this result seems to contradict the evidence reviewed in the section titled 'Emotional episodic memory', which demonstrated that late night REM sleep does not support episodic memory consolidation, perhaps due to high cortisol levels. Yet studies have consistently shown that cortisol facilitates memory for emotional episodic materials, while impairing closely matched neutral materials during wakefulness (Buchanan and Lovallo, 2001; Payne et al., 2006). Given the role of cortisol in enhancing emotional episodic information, the late-night enhancement of emotional memory in this study is not surprising.

In addition to cortisol, other hormones (e.g., growth hormone) are known to impact memory function in the waking state, and also vary across sleep, suggesting that they might contribute to sleep-based memory consolidation. Although initial studies of growth hormone have failed to find such an effect (Gais et al., 2006), further investigation of the neurochemistry underlying the relationship between sleep and memory consolidation is a productive avenue for future research. Indeed, it seems especially important to forge ahead into precisely this neuromodulatory realm, where the chemical basis of the sleep/memory consolidation connection is examined.

2.35.9 Concluding Comments

Over the past 10 years, the field of sleep and memory has grown exponentially, with reports of sleep–memory interactions emerging from myriad disciplines, ranging form cellular and molecular studies in animals to behavioral and neuroimaging studies in humans. In our view, sleep undoubtedly mediates memory processes, but the way in which it does so remains largely unknown. This makes the future of the field truly exciting, but also challenging. Much remains to be done, from uncovering the mechanisms of brain plasticity that underlie sleep-based memory processing, to untangling the complex relationship between the various sleep stages and types of memory. In so doing, memory researchers may find a field in which some of the more recalcitrant problems of basic memory research can also be answered.

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2.36 Infant Memory

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2.36.1 Introduction

Infant memory is a burgeoning topic, difficult to comprehend and difficult to summarize, but one thing is clear: Infants from their inception have robust capacities to learn and remember. In the development of these capacities are hidden the secrets of adult learning and memory.

The infancy period extends from birth through 2 years of age. Over this period, the infant is dramatically transformed from a helpless, altricial organism who sleeps 99% of the time and depends on its parents for nourishment and protection into an active, highly social being who sleeps largely at night, locomotes independently, and exhibits unflagging curiosity (**Figure 1**). A central problem in cognition is understanding how the superior memory of adults evolves from the memory ability of infants. This is the question of how we came to be as we are. In this chapter, we review research that has shed some light on the solution to this problem. We conclude that memory processing in infants and adults is fundamentally the same.

2.36.1.1 Paradox of Infant Memory

Developmental scientists have long assumed that the effects of infants' early experiences gradually accrue and lay the foundation for their later cognitive development (Watson, 1930; Stevenson et al., 1967). Paradoxically, although this assumption requires

Figure 1 From left to right, infants are 2, 3, 6, 9, 12, 15, and 18 months of age. Note the dramatic physical and behavioral differences between the youngest and oldest infant.

that infants maintain a relatively enduring record of their early experiences, most also believe that infants lack the ability to do so. They think that the infant brain is too immature to encode or retrieve memories of specific events (Nelson, 1995; Liston and Kagan, 2002; Bauer et al., 2007), that infants younger than 18 months cannot form mental representations (Piaget, 1962; Anisfeld, 2005), or that the ability "to retain a memory over a long period of time [is] not possible" before the child can converse about it with others (Nelson, 1990: p. 307).

Researchers from different laboratories using different procedures, however, have amassed compelling evidence that preverbal infants can retain memories of their experiences for periods ranging from days and weeks to months and years (for reviews, see Rovee-Collier et al., 2001; Hayne, 2004, 2007).

2.36.1.2 Associative Memory and Retentive Memory

Associations play a central role in accounts of learning and memory phenomena. The formation of an association between two events is determined by their temporal contiguity. If an association is formed between two events that are separated by a delay, then the individual is said to have exhibited associative memory over the delay (Revusky, 1971). The upper limit of associative memory is the maximum interval between the conditional and unconditional stimuli that leads to classical conditioning and the maximum delay between a response and reinforcement that leads to operant conditioning.

Relative to associative memory, retentive memory is long lasting (Revusky, 1971). It is implicated when an individual exhibits the effect of prior training over an interval between training and testing. Watson (1984) referred to these forms of memory as memory in learning and memory of learning, respectively. This chapter focuses primarily on retentive (long-term) memory.

2.36.1.3 Historical Perspectives

The systematic study of infant long-term memory can be traced to Fagan's (1970) report of visual recognition memory with 5-month-olds and Rovee and Fagen's (1976) report of delayed recognition with operantly trained 3-month-olds. Earlier reports, often anecdotal, were of single subjects who were observed over long periods. In his autobiography, for example, John Stuart Mill said that his father, James Mill, taught him formal Greek at the age of 3, which he used throughout his life (Mill, 1909). In a single-subject study of the long-term maintenance of early memory, Burtt (1932, 1937, 1941) read the same Greek passages to his infant son once daily between 15 and 36 months of age, introducing a different set of passages every 3 months. When the child was 8.5, 14, and 18 years old, Burtt measured savings during relearning of the passages. Early exposure to the passages produced the largest savings at 8.5 years

(a 5-year retention interval), when the child required 30% fewer repetitions to relearn old passages than to learn new ones. Savings were smaller (8%) at 14 years and negligible at 18 years.

The classic study of Little Albert also provided evidence of long-term memory (Watson and Rayner, 1920). Here, the authors asked whether a conditioned emotional reaction (CER) could be established in an 11-month-old infant. Albert initially exhibited no aversive reactions to various novel stimuli, including a white rat. A loud noise was then sounded directly behind Albert's head on two occasions that he reached for the rat. The noise produced crying and hand withdrawal, which transferred to the rat. One week later, Albert still withdrew his hand when presented with the rat; in this session, he also received five more rat-noise pairings. Five days later, Albert generalized the CER to test objects that shared perceptually similar properties with the rat (a rabbit, dog, fur coat, Santa Claus mask, cotton) but did not generalize to perceptually different objects (wooden blocks). After an additional 10 days, Albert's CER to the rat had become muted and was freshened by another rat-noise presentation. The rabbit and dog were also explicitly paired with the noise during this session. One month later, Albert still exhibited strong CERs to the rat, dog, mask, and fur coat.

In another early study, Jones (1930) exposed a 7-month-old to repeated pairings of a tapping sound (the conditional stimulus, or CS) and an electrotactual stimulus (the unconditional stimulus, or US) for 5 days. The CR (conditional response), a galvanic skin reflex, was established in session 1. Despite receiving no additional conditioning trials in the interim, the infant still exhibited the CR 6 weeks later, and the CR had not completely disappeared after 7 weeks.

2.36.2 Methods of Study

The major impediment to research on the ontogeny of infant memory has always been methodological: The tasks commonly used with older infants are usually inappropriate for use with younger ones. This problem is hardly surprising when one considers the rapid physical and behavioral changes that infants undergo over the first 18 months of life (**Figure 1**). As a result, researchers used a hodgepodge of tasks with stimuli, task parameters, and task demands that varied nonsystematically with infants of different ages, making cross-age comparisons dubious at best. In addition, the use of verbal prompts biased results in favor of older, linguistically more competent infants. Within the last decade, new operant and deferred imitation tasks were developed to overcome this obstacle.

Most of what is known about infant memory has come from research with visual recognition memory, operant conditioning, and deferred imitation tasks. In visual recognition memory tasks, retention is measured indirectly, inferred from general reactions such as looking/orienting, electrophysiological and psychophysiological responses, facial expressions, and so forth. In these instances, the meaning of a response is a matter of interpretation (Lewis, 1967; Haith, 1972). In classical conditioning, operant conditioning, and deferred imitation tasks, retention is measured directly in the performance of a previously learned behavior.

2.36.2.1 Visual Recognition Memory

Visual recognition memory is studied using the visual paired-comparison (VPC) task (Fantz, 1956, 1964; Fagan, 1970) and the habituation task (Berlyne, 1958; Cohen and Gelber, 1975). Both exploit infants' propensity to look longer at novel than at familiar stimuli. The underlying assumption is that infants who exhibit a novelty preference must remember what they saw before. Recognition of a preexposed stimulus, therefore, is inferred from the relative extent to which infants look at a novel one.

In the VPC task, infants view two identical stimuli presented side by side (or occasionally only one) for a fixed duration. During the retention test, a novel stimulus replaces one of them (**Figure 2**).

Figure 2 The visual paired-comparison (VPC) test with a 6-month-old infant. Immediately before the test, the infant was shown two identical pictures of the woman's face. During the test, the infant is shown the previously exposed picture (the woman's face) and a novel one (the man's face). Proportionally longer looking at the novel picture is taken as evidence that the infant recognized the preexposed one.

Recognition of the original stimulus is inferred if the percent of total time spent looking at the novel stimulus significantly exceeds chance (50%). The upper limit of retention is the longest test delay at which infants fixate the novel stimulus more.

In the habituation task, infants are repeatedly exposed to one stimulus until looking time decreases by a specified amount, at which point they are successively tested with a novel stimulus and the original one. Increased looking at the novel stimulus indicates recognition of the original one. As the interval between the final habituation trial and testing increases, infants look increasingly longer at the original stimulus. The delay after which they fixate it for as long as they had on Trial 1 indicates forgetting.

Visual recognition memory tasks that are administered in one session provide a measure of short-term memory. In this situation, the upper limit of retention approximates 5–15 s at 3–4 months, 1 min at 6 months, and 10 min at 9–12 months (Diamond, 1990; Rose et al., 2004). Long-term memory is implicated when multiple sessions are administered. Using the VPC task over multiple daily sessions, Fagan (1973) found that 4–5-month-olds looked longer at a novel black-and-white pattern after 48 h and at a novel facial photograph after 2 weeks. Face recognition, however, may be special. Following habituation to faces, infants exhibit significant recognition after 2 min at 3 days of age (Pascalis and de Schonen, 1994) and after 24 h at 3 months of age (Pascalis et al., 1998).

2.36.2.2 Delayed Nonmatching-to-Sample

The delayed nonmatching-to-sample (DNMS) task is analogous to the VPC task but requires substantial motor coordination. Initially a reward (cereal, toy) is hidden in a well under a sample object, and infants retrieve the reward by displacing the sample. After a delay, the sample and a novel object are presented simultaneously, but the reward is under the novel one. To retrieve the reward, infants must recognize the sample object and displace the novel one. The upper limit of associative memory is the maximum delay after the sample trial when infants retrieve the reward. Infants younger than 15–21 months fail standard DNMS tasks after 5–10 s (Overman, 1990; Diamond et al., 1994). When the reward is the opportunity to play with the novel object, however, even 6-month-olds succeed after 10 min (Diamond, 1995).

2.36.2.3 Classical Conditioning

In classical conditioning, infants acquire an association between two stimuli, a CS and a US, that usually occur in succession. They must remember the CS until the US occurs (associative memory). The upper limit of associative memory is the maximum interval between the CS and US (ISI, interstimulus interval) that promotes learning.

In eyeblink conditioning, the optimal ISI is three to four times longer for infants than adults (Kimble, 1947). Using a delay conditioning procedure, Little (1970) presented 2-month-olds with a tone (CS) and airpuff (US) at ISIs of 500, 1000, 1500, or 2000 ms. Only the two longest ISIs promoted conditioning. Little et al. (1984) used 500-ms and 1500-ms ISIs with 10-, 20-, and 30-day-olds. At all ages, infants exhibited associative memory after the 1500-ms ISI only, and only 20- and 30-day-olds exhibited significant retention of the association (retentive memory) on a savings measure 10 days later (**Figure 3**). The optimal ISI decreases to 650 ms at 4–5 months of age (Ivkovich et al., 1999) and 500 ms in adults (Kimble, 1947). This decrease parallels an increase in synaptic efficacy (Kandel and Hawkins, 1992) and may reflect changes in infants' ability to perceive the CS and US as distinct events.

2.36.2.4 Operant Conditioning

In operant conditioning, infants acquire an association between a response and reinforcement. They must remember the response until reinforcement occurs (associative memory). The upper limit of associative memory is the maximum response-reinforcement interval that promotes learning. The upper limit is 0 s (immediate reinforcement) at 3 months (Ramey and Ourth, 1971), 1–2 s from 6 to 8 months (Millar, 1990), and 9 s from 9 to 16 months (Brody, 1981).

The most extensive analysis of infants' long-term memory (retentive memory) has come from operant research with the mobile conjugate reinforcement task (Rovee and Rovee, 1969) and its upward extension, the train task (Hartshorn and Rovee-Collier, 1997; for review, see Rovee-Collier et al., 2001). The logic behind using these tasks is straightforward: Because infants lack a verbal response to say what they recognize, they are taught a motoric one (a foot kick or lever press) that they can use instead. When tested with a display that is the same as or different from the training one, infants 'say' whether or not

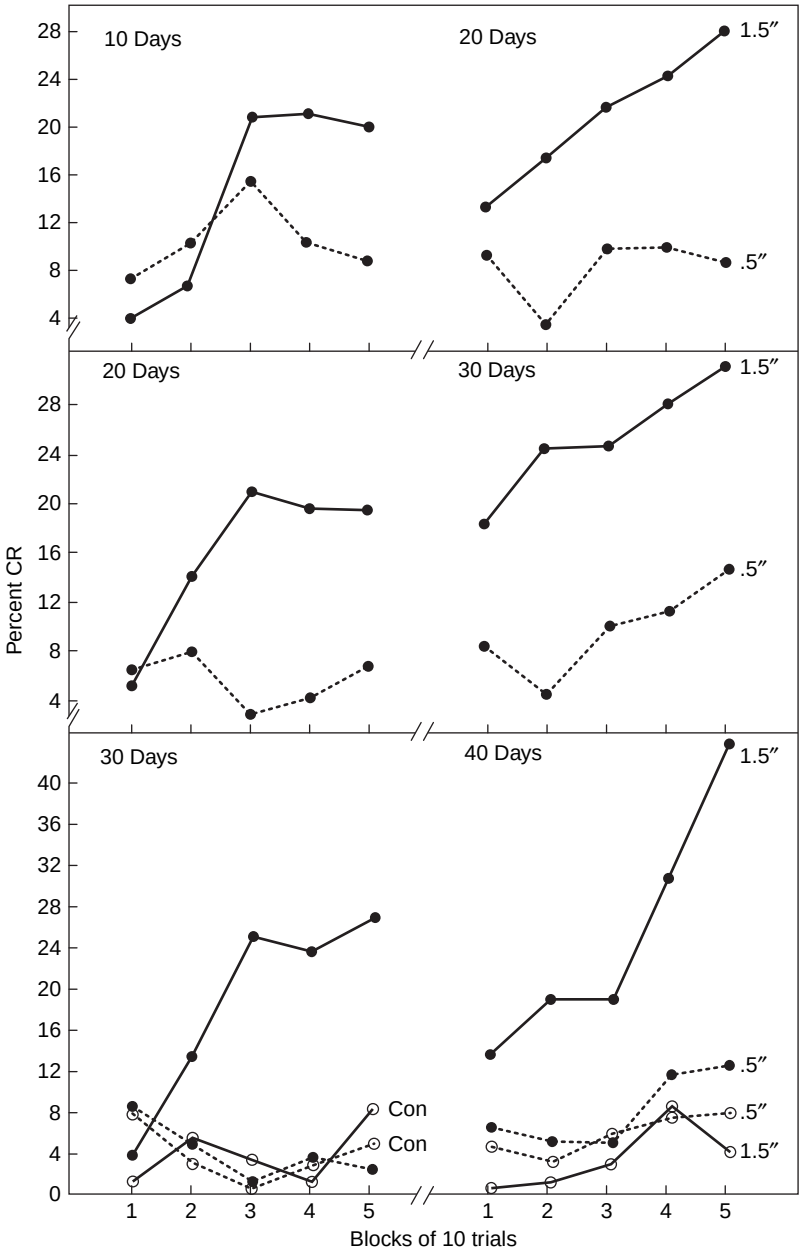


Figure 3 Percentage of responses on conditional stimulus–unconditional stimulus (CS–US) trials by infants who were classically conditioned at 10, 20, or 30 days of age (left panels) to blink their eyes to a tone (CS) that was paired with an air puff (US) and were retrained in a second session 10 days later (right panels). The CS and US were separated by either a 1500-ms or 500-ms interstimulus interval (ISI). Reprinted with permission from Little AH, Lipsitt LP, and Rovee-Collier C (1984) Classical conditioning and retention of the infant's eyelid response: Effects of age and interstimulus interval. *J. Exp. Child Psychol.* 37: 512–524. Copyright © Academic Press, Inc.

they recognize it by whether or not they produce the learned response.

In all studies, infants are trained in their homes for two sessions 24 h apart. At 2–6 months, the mobile task is used (**Figure (4a)–(4c)**). During the 9-min acquisition periods (6 min at 6 months) in each session, kicks are conjugately reinforced by mobile movement via a ribbon that is strung from the infant's ankle to the same suspension hook as an overhead crib mobile (**Figure 4(b)**). During 3-min nonreinforcement periods (2 min at 6 months) at the beginning and end of each session, the ankle ribbon is connected

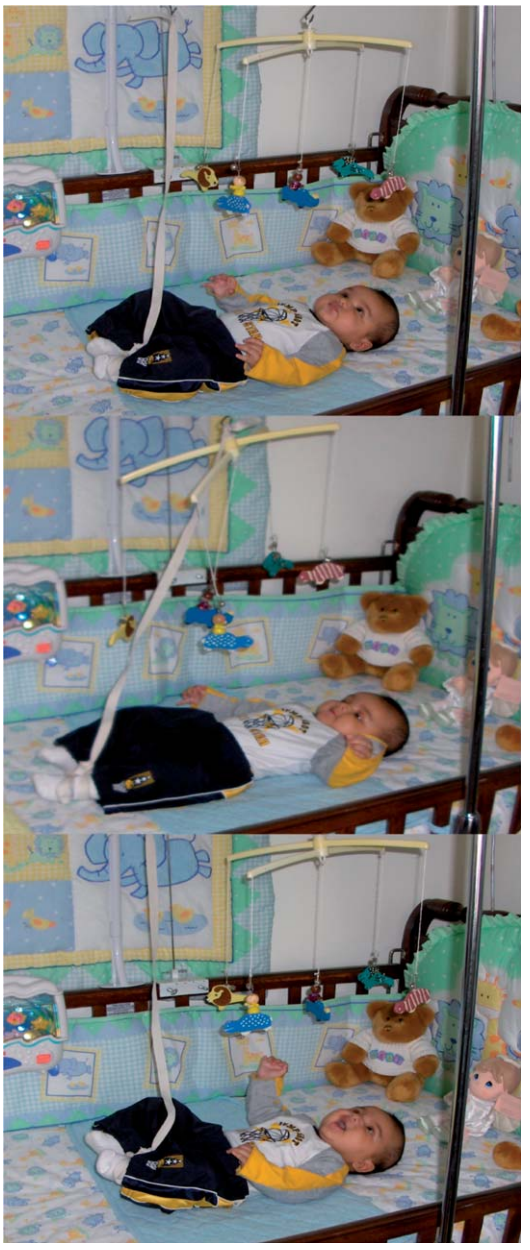


Figure 4 The experimental arrangement used with 2- to 6-month-olds in the operant mobile task, shown here with a 3-month-old. From top to bottom, (a) Baseline: The ankle ribbon and mobile are connected to different hooks, and kicks do not move the mobile; (b) Acquisition: Kicks conjugately move the mobile via an ankle ribbon connected to the mobile hook; (c) Immediate retention test, long-term retention test: The ankle ribbon and mobile are again connected to different hooks. Infants who recognize the mobile kick to move it during the test, even though they cannot.

to an empty suspension hook, and kicks cannot move the mobile (**Figures 4(a), 4(c)**). The baseline kick rate (operant level) is measured during the first nonreinforcement period in session 1; the response rate that indicates the final level of learning is measured during the last nonreinforcement period in session 2 (the immediate retention test). To proceed beyond the training phase, each infant must satisfy a learning criterion (responding 1.5 times above the baseline rate).

The long-term retention test occurs days to weeks later and is another 3-min (2 min at 6 months) nonreinforcement period when the response rate is measured again. Because individual operant levels vary widely, an infant's responding during the long-term retention test is expressed as a ratio of the same infant's responding during the baseline phase (the baseline ratio) and the immediate retention test (the retention ratio). A group baseline ratio that significantly exceeds 1.00 indicates retention; if the accompanying retention ratio is significantly below 1.00 (i.e., significant forgetting), however, then the group's retention is partial. Because long-term retention is assessed during a nonreinforcement period, savings (faster relearning) are not measured.

Because the mobile task cannot be used with infants older than 6–7 months, the train task was developed as an upward extension of the mobile task for infants between 6 and 24 months (**Figure 5**). Instead of moving a mobile by kicking, infants move a miniature train around a circular track by lever pressing. During reinforcement periods, each discrete lever press moves the train for 1 s (2 s at 6 months); during nonreinforcement periods, the lever is deactivated. At 6 months, all retention measures in the two tasks are identical, including the rate of forgetting before and after

Figure 5 The experimental arrangement used with 6- to 24-month-olds in the operant train task, shown here with a 6-month-old. Each lever press moves the toy train for 2 s (1 s at older ages) during acquisition; during baseline and all retention tests, the lever is deactivated, and presses do not move the train.

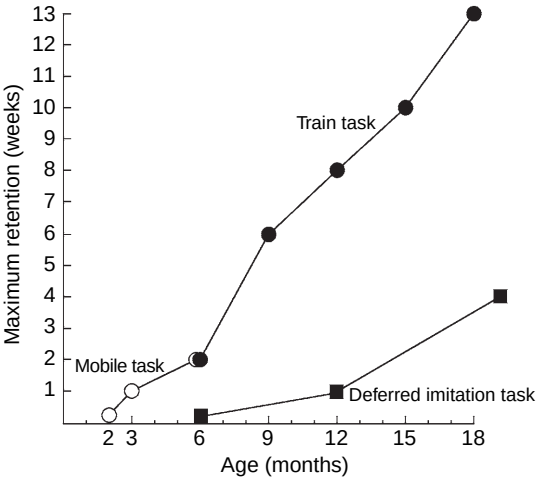


Figure 6 Standardized reference functions for the maximum duration of retention (in weeks) of infants who were trained and tested in standardized procedures with age-calibrated parameters. The experimental paradigms were the operant mobile and train tasks and the deferred imitation (puppet) task. Differences in the slopes of the two functions are solely the result of the use of different parameters. From Hartshorn K, Rovee-Collier C, Gerhardstein P, et al. (1998b) The ontogeny of long-term memory over the first year and a half of life. *Dev. Psychobiol.* 32: 69–89.

priming (Hildreth and Rovee-Collier, 2002), the latency of responding to a memory prime (Hildreth and Rovee-Collier, 1999), responding to cue or context changes before and after priming (DeFrancisco, in press; Hartshorn et al., 1998a), the minimum duration of an effective prime (Sweeney and Rovee-Collier, 2001), and the faster forgetting rate after a minimum-duration prime (Hsu et al., 2005). This equivalence permitted the mobile and train tasks to be used in combination to study memory development systematically over the entire infancy period.

Figure 6 shows that the maximum duration of retention increases linearly with age over the first year and a half of life. The overlapping points at 6 months show that infants' memory performance is not task specific. These data provide no hint that memory changes qualitatively at the end of the first year of life, when a late-maturing memory system is thought to emerge (Schacter and Moscovitch, 1984), or during the second year of life, when spoken language first appears (Best, 1984).

2.36.2.5 Deferred Imitation

In deferred imitation tasks, infants reproduce one or more target actions after a delay. Both Piaget (1962) and Meltzoff (1995) argued that true imitation prohibited performing the modeled behavior before the delayed test, so that imitation would reflect only the memory of what was seen and not what was done. Piaget claimed that infants younger than 18 months could not form mental representations and hence were incapable of true imitation. His claim was refuted by evidence that, with no opportunity to practice, 14-month-olds imitated a novel action (pulling apart a toy) 24 h later (Meltzoff, 1985), and 9-month-olds imitated three unique, single-step actions 24 h later (Meltzoff, 1988).

Barr et al. (1996) developed a task that permitted the systematic study of deferred imitation from 6 to 24 months of age. Here, infants watch an experimenter model three actions on an acrylic hand puppet wearing a same-color mitten (**Figure 7**). Modeling one sequence of actions (remove the mitten, shake it thereby ringing a jingle bell pinned inside during modeling, and replace the mitten) takes 10 s. Three repetitions (30 s) yield immediate imitation at 6 months and 24-h deferred imitation at 9–24 months; six repetitions (60 s) yield 24-h retention at 6 months. An infant's imitation score is the total number of actions copied within 90–120 s of touching the puppet. Between 6 and 24 months of age, the base rate of spontaneously producing the target actions is low (0.13–0.17). Older infants have higher imitation scores and remember progressively longer. The pattern of long-term retention in deferred imitation tasks is the same as in operant tasks except that the slope is flatter and the asymptote is lower (**Figure 6**).

Figure 7 The experimental arrangement used with 6- to 24-month-old infants in deferred imitation studies. Shown here is the experimenter modeling the three target actions to a 6-month-old.

These differences reflect task differences in training (30–60 s in one modeling session vs. 11–30 min over two conditioning sessions 24 h apart).

Development of the puppet imitation task spawned numerous studies with 6-month-olds (for review, see Hayne, 2004, 2007). Because infants younger than 6 months are motorically limited, whether they could exhibit deferred imitation was unknown until recently, when this problem was overcome by periodically reactivating the memory of the demonstration until they could finally perform the actions (Campanella and Rovee-Collier, 2005). Three-month-olds watched three target actions modeled six times (a total of 60 s) on a puppet. Over the next 3 months, infants received a total of six 30- to 60-s exposures to the stationary puppet. At 6 months of age, they successfully imitated the actions. These infants had not seen the target actions since they were modeled 3 months earlier and obviously had not imitated them immediately. A yoked reactivation control group received the same reminders but did not see the demonstration. It responded at the base rate during the long-term test. These results clearly indicate that the memory system of very young infants is sufficiently mature to support deferred imitation.

2.36.2.6 Elicited Imitation

Elicited imitation is a variant of deferred imitation in which infants imitate immediately (and sometimes during the retention interval) and experimenters provide verbal prompts. Both factors significantly affect infants' deferred imitation: Immediate practice facilitates generalization and priming (Hayne et al., 2003; Learmonth et al., 2004), and verbal cues facilitate imitation after long delays (Hayne, 2004; Hayne and Herbert, 2004).

Elicited imitation tasks have been used with infants between 9 and 32 months to assess their ability to imitate multistep actions (for review, see Bauer, 2004; Bauer et al., 2007). Researchers have consistently reported that the structure of an event influences imitation (Bauer et al., 1995). Imitation of actions that must be performed in a specific order (e.g., putting a toy bear in bed before covering it with a blanket) is superior to imitation of actions that can be performed in any order (e.g., removing the bear's coat and hat). The same result has been found with older children (Fivush et al., 1992) and adults (Ratner et al., 1986).

2.36.2.7 The Ruler Matters

Retention depends on how it is measured. Adults' retention of serial lists, for example, is better when measured by recognition than by recall or relearning (Postman and Rau, 1957, cited in Crowder, 1976). For infants, visual recognition measures of retention range from 5 s at 3 months to 10 min at 12 months (for review, see Rose et al., 2004), but operant measures fix retention from 5 days to 8 weeks over the same period (Hartshorn et al., 1998b). Even under the same conditions of testing, novelty preference and operant measures yield different estimates of retention. Wilk et al. (2001) trained 3- and 6-month-olds in the mobile task and gave them a paired-comparison test with the original mobile and a novel one 1–21 days later. In multiple experiments, infants consistently exhibited long-term retention on the operant measure but not on the VPC measure, looking longer at the training mobile. They concluded that infants look at what is predictive.

Using a split within-subjects design, Gross et al. (2002) exposed 6-month-olds to a picture in a visual recognition memory task and to either a hand puppet on which actions had been modeled or a mobile that they had moved by kicking. Immediately after modeling or operant training, the infants administered a VPC test with the original stimulus and a novel one, followed by a performance test with the original puppet or mobile. On VPC tests, infants looked longer at the novel picture but not at the novel puppet or mobile, indicating a failure to recognize them. On performance tests, however, the same infants imitated the modeled actions on the original puppet and kicked significantly above baseline to the mobile. Again, infants remembered at the predictive stimulus.

2.36.3 Reminders

Reinstatement and reactivation reminders have been used with infants of all ages to maintain, extend, or recover memories of earlier experiences. During reinstatement, infants are returned to the original training conditions during the retention interval and given a small amount of partial practice or repetition of the original event (Campbell and Jaynes, 1966). A reactivation procedure entails exposing infants to an isolated component or fragment of the original event at the end of the retention interval, after the memory was forgotten but before the long-term test

(Spear and Parsons, 1976). For both reminders, the essential control groups are the same: a forgetting control group that receives training but no reminder(s) and a reactivation or reinstatement control group that receives reminder(s) but no training.

Most scientists regard reinstatement and reactivation reminders as equivalent (e.g., Hudson and Sheffield, 1998; Mandler, 1998). Howe et al. (1993) wrote that the distinction between them "is artificial in that both . . . have similar (if not the same) memory-preserving effects" (Howe et al., 1993: p. 855), and Richardson et al. (1993) wrote, "there are some minor procedural differences between reactivation and reinstatement. . ., [but] the underlying process is the same in both cases" (Richardson et al., 1993: p. 2). Operant research with infants, however, has revealed that the two reminders differ functionally as well as procedurally. At 3 and 6 months of age, for example, retention is two times longer after reinstatement than reactivation when both are given midway through the forgetting function (Adler et al., 2000; Galluccio and Rovee-Collier, 2006) and ten times longer when both are given after the memory is forgotten (Hildreth et al., 2003). Also, three reinstatements protract retention longer than three reactivations (Hayne, 1990; Galluccio and Rovee-Collier, 1999).

What accounts for the superiority of reinstatement is unclear. Reinstatement is a partial learning trial, but it protracts retention longer than an equivalent amount of overtraining (Adler et al., 2000). Reactivation, on the other hand, is a retrieval trial that has been likened to rehearsal in nonverbal animals (Wagner, 1976). For now, this question remains unanswered.

2.36.3.1 Reactivation

Reactivation procedures used with infants and priming procedures used with amnesic adults are the same. Reactivation, like priming in amnesics, is an automatic, perceptual identification process that re-activates a preexisting memory representation and brings it to mind at a time when neither the prime nor the target item can be recognized (Schacter, 1990, 1992; Rovee-Collier, 1997). Because the time required for 3-month-olds to exhibit renewed retention after reactivation is so long (24 h), whereas amnesics respond to a prime (e.g., a word fragment) instantaneously, skeptics initially doubted that that reactivation and priming were the same. Hildreth and Rovee-Collier (1999), however, found that the latency of responding to a memory prime decreased linearly over the first year of life, from 24 h at 3 months to 1 h at 6 months and 1 min at 9 months. By 12 months, infants responded to the prime instantaneously, just like adults. These results confirmed that reactivation in infants and priming in adults are the same.

For both infants and adults, effective primes are hyperspecific to the original event, an extreme instance of encoding specificity (Tulving and Thomson, 1973). Effective primes for infants between 2 and 24 months include the reinforcer (Hsu and Rovee-Collier, 2006), the distinctive training context (Rovee-Collier et al., 1985; Hayne and Findlay, 1995), the demonstration hand puppet (Hayne et al., 2003; Campanella and Rovee-Collier, 2005), the occlusion event (Shuwairi and Johnson, 2006), a pre-exposed photograph (Cornell, 1979), the modeled actions (Barr et al., 2002), a subset of structured activities (Sheffield and Hudson, 1994), photographs of partially completed activities (Deocampo and Hudson, 2003), and a video of another child performing the activities (Sheffield and Hudson, 2006).

During reactivation in the mobile task, infants are in a sling-seat (to minimize activity) under the mobile (**Figure 8**). Instead of being connected to the ankle, the ribbon is used by the experimenter to move the mobile at the same rate that each infant had kicked to move it at the end of acquisition, thus ensuring that the prime is phenomenologically identical to what infants saw before. In the train task, the response lever is deactivated, and the computer is programmed to move the train accordingly.

Figure 8 The experimental arrangement during reactivation (priming), shown here with a 3-month-old. The ribbon was not connected to the infant's ankle but was held by the experimenter, who pulled it to move the mobile at the same rate that the infant had moved it by kicking during the last few training minutes. The infant seat minimized spontaneous kicking.

In the first reactivation study with infants, 3-month-olds were trained in the mobile task, allowed to forget it, and then were primed either 13 or 27 days later. Independent groups received a standard retention test 24 h later and after longer delays until they reforgot the task (Rovee-Collier et al., 1980). (Because kicking is also a general excitement behavior, priming occurred 24 h before the test so that any arousal it might induce would dissipate.) Priming restored responding to its original level, and infants forgot the reactivated memory at the same rate as the original one (**Figure 9**). Both results have since been obtained throughout the infancy period. The duration of retention increases linearly with age between 2 and 18 months (**Figure 10**, squares), and a reactivation reminder doubles it (**Figure 10**, diamonds; Hildreth and Rovee-Collier, 2002; Hsu et al., 2005). Thus, although reactivation does not protract retention as long as reinstatement, its consequences are nontrivial.

There is an upper limit to how long after training the memory can be primed successfully. Because retention is a monotonically increasing function of age, the absolute upper limit of reactivation increases linearly over the first year as well (**Figure 10**, triangles). The original memory can be reactivated after delays ranging from 1 month (3-month-olds) to 8 months (12-month-olds). When the upper limit of reactivation is expressed as a ratio of the maximum duration that infants typically remember at a given age, the relative upper limit is constant over ages, four times longer than the duration of original retention at a given age (Hildreth and Hill, 2003). The absolute upper limit of reactivation did not continue to increase beyond its peak at 12 months because infants trained at 15–18 months outgrew the train task by the time of testing. As a result, they could not be tested after relative delays longer than 1.5–2 times the duration of original retention (Hsu and Rovee-Collier, 2006). (The long-term retention test is an increasingly conservative test of retention as infants approach 2 years of age. Older infants often stop lever pressing when the train does not move and remark that it is broken or needs batteries.)

At 3 months of age, multiple reactivations flatten the forgetting function (Hayne, 1990), extend retention from 4 weeks with one reminder (Rovee-Collier et al., 1980) to at least 6 weeks with two reminders (Hayne, 1990), speed the memory recovery from 24 h with one reminder (Fagen and Rovee-Collier, 1983) to 1 h with two reminders (Hayne et al., 2000b), reduce the minimum duration exposure to a prime 1 week after forgetting from 2 min with one reminder (Joh et al., 2002) to 1 min with two reminders (Bearce and Rovee-Collier, 2006), and reduce the accessibility of

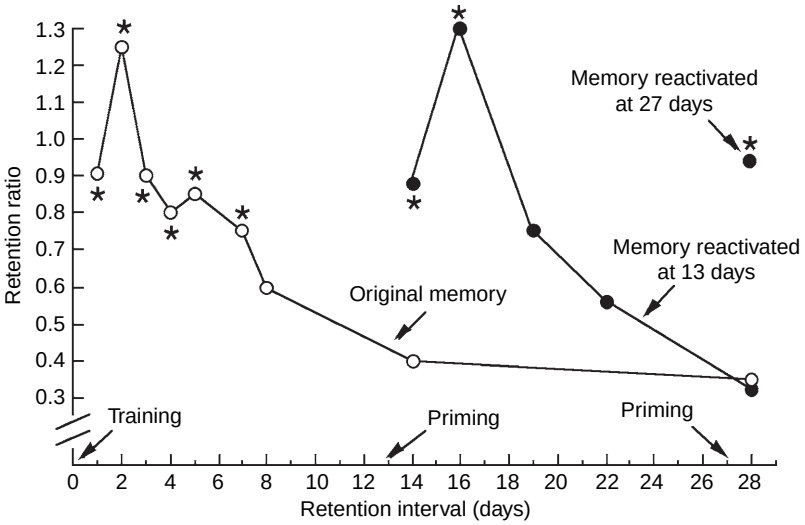


Figure 9 The forgetting and reforgetting functions of the original memory and the reactivated memory, respectively, as a function of the number of days after training or priming when retention was tested. Priming occurred either 13 or 27 days after training was over. Irrespective of priming delay, retention was renewed at the same level that it was 24 h after training. An asterisk indicates significant retention. Reprinted with permission from Rovee-Collier C, Sullivan MW, Enright MK, Lucas D, and Fagen JW (1980) Reactivation of infant memory. *Science* 208: 1159–1161. Copyright © 1980 by the American Association for the Advancement of Science.

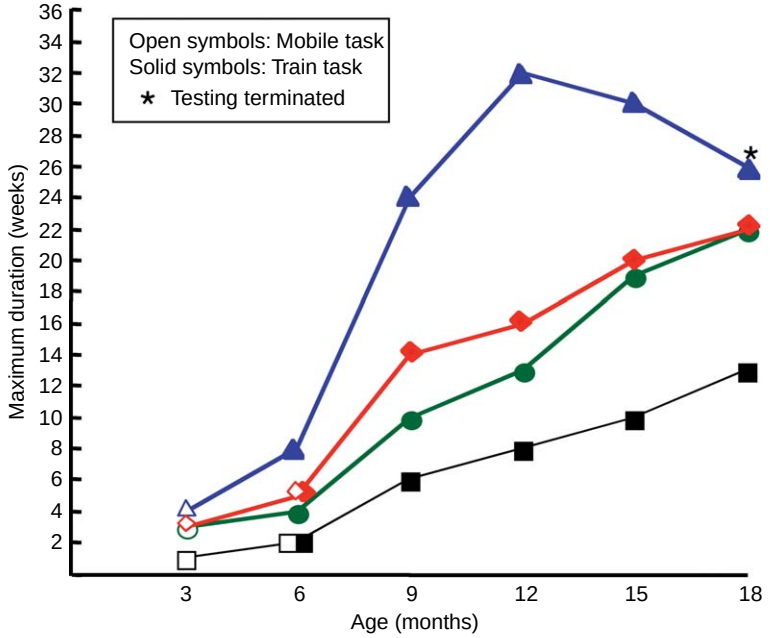


Figure 10 The absolute length of time that the memory (mobile task: open symbols; train task: solid symbols) was accessible between 3 and 18 months of age. The squares represent the duration of retention of the newly acquired memory of the operant task. Infants had a 1- or 2-week delay between forgetting of the task and reactivation with either the minimum-duration or full-length prime. In the functions, diamonds represent retention of the reactivated memory following a full-length prime, circles represent retention of the reactivated memory following a minimum-duration prime, and triangles represent retention of the memory that was reactivated near or at the upper limit of reactivation. An asterisk indicates that the experiment was terminated because older infants outgrew the task. Reprinted with permission from Hsu VC and Rovee-Collier C (2006) Memory reactivation in the second year of life. *Infant Behav. Dev.* 28: 1–17. Copyright © 2006 by Elsevier Inc.

memory attributes representing contextual details (Hitchcock and Rovee-Collier, 1996).

Finally, Shuwairi and Johnson (2006) used an innovative application of reactivation as a precue to probe whether explicit training with an occlusion event had an enduring effect on anticipatory eye movements. For either one or four training trials, 4-month-olds watched a ball move back and forth on a constant trajectory on a video monitor (**Figure 11(a)–(d)**). On test trials, its path was partially occluded. Immediately after training, the proportion of total eye movements that anticipated the ball's reemergence from behind the occluder significantly exceeded that of a baseline control group after four 30-s trials but not after one 30-s trial (**Figure 12**). Thirty minutes after training, when the memory was forgotten, infants received a single 30-s trial (a precue) immediately before the retention test. (Recall that one 30-s trial produced no learning.) The prime reactivated the training memory and restored anticipations to the previous level (**Figure 12**). This result indicates that early and relatively brief exposures to occlusion events produce stable and relatively enduring object representations that can be maintained and potentially strengthened by repeated reminders.

2.36.3.2 Reinstatement

During reinstatement in the mobile and train tasks, the ankle ribbon is connected to the mobile hook and the response lever is active, so that kicks and lever presses are reinforced. In both tasks, reinstatement is timed from the first response and lasts 3 min at 2–3 months and 2 min at 6–18 months. This regimen is insufficient to produce 24-h retention of new learning during reminding.

Hartshorn (2003) tested Campbell and Jaynes's (1966) original hypothesis that reinstatement is the mechanism by which early memories are maintained over significant periods of behavioral development. Recall that 6-month-olds typically remember the train task for 2 weeks. In three progressive replications, 6-month-olds learned the train task, received a 2-min reinstatement at 7, 8, 9, and 12 months of age, and were tested at 18 months of age. Prior to

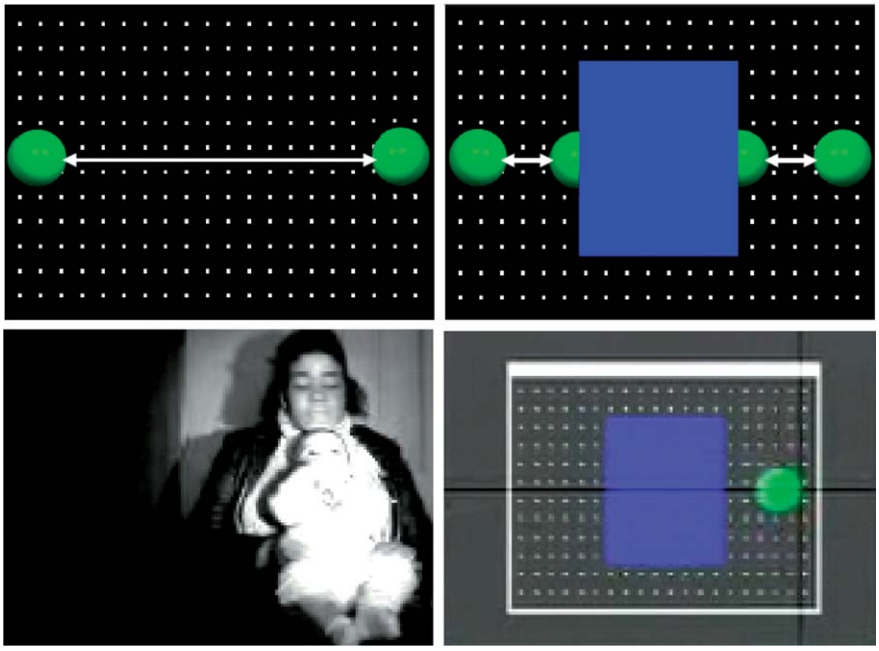


Figure 11 The experimental arrangement and stimuli used to study anticipatory eye movements in the eye-tracking paradigm. (a) Unoccluded (training) trial: The ball moved horizontally back and forth along an unoccluded trajectory. (b) Occluded (test) trial: The ball moved horizontally back and forth along an occluded trajectory. The ball moved behind a blue box and reemerged on the opposite side. (c) The experimental arrangement with a 4-month-old infant. (d) The corneal reflection eye tracker recorded the infant's visual fixation (the black cross to the right of the ball) as the ball emerged from behind the occluded object. Figure courtesy of Sarah M. Shuwairi and Scott P. Johnson.

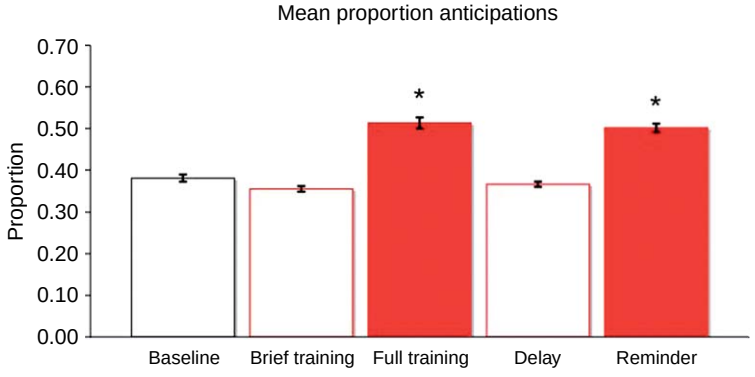


Figure 12 Mean proportion of anticipatory eye movements of independent groups of 4-month-old infants. The experimental groups received either a brief (Brief training) or full (Full training, Delay, Reminder) training regimen and were tested either immediately after training (Brief training, Full training) or after a 30-min delay (Delay, Reminder). The reminder condition received a brief training trial immediately prior to testing. An asterisk indicates significant anticipations relative to the baseline control group. Vertical bars indicate + 1 SE. Figures courtesy of Sarah M. Shuwairi and Scott P. Johnson.

reminding at 9 and 12 months, infants received a 2-min retention test as a memory probe. All infants not only remembered the task then but also remembered it during the 18-month test, 1 year after training. In each replication, yoked reinstatement control groups that received the same reinstatements but were not originally trained exhibited no retention after any delay. Immediately after the 18-month test, Hartshorn gave infants another reinstatement and retested them 6 months later, at 24 months of age. The infants also exhibited significant retention during the 24-month test, 1.5 years after they were

originally trained, despite receiving only one reinstatement in the preceding year.

In a concurrent study, Rovee-Collier et al. (1999) trained 2-month-olds in the mobile task and then administered either a reinstatement or a reactivation reminder every 3 weeks. Before each reminder, they presented a preliminary retention test as a memory probe to assess whether an infant remembered the task. Infants who did remember received a reinstatement, while those who did not received a reactivation reminder. Even though 2-month-olds typically remember the mobile task for only 1–2 days, they remembered the task for 21 weeks, through 7.25 months of age, after the periodic reminders. At that point, the experiment was discontinued because infants outgrew the task. Because periodic reminders maintained the memories of two equivalent tasks from 2 months to 2 years of age, the entire period presumably characterized by infantile amnesia, it seems likely that periodic reminders would also maintain a single memory over the same period, if not longer.

The preceding evidence casts serious doubt on popular accounts of infantile amnesia (see section 2.36.5). As long as infants periodically encounter an appropriate nonverbal reminder, their memory of an early experience will be maintained. Thus, whether or not an early experience will be remembered later is determined only by whether or not an appropriate reminder is periodically available in nature. In essence, the ultimate source of infant forgetting resides in the structure of the environment, not in the structure of the brain.

2.36.4 Ontogenetic Changes

2.36.4.1 Forgetting

Because adults of all species remember for so long, animal researchers have had to study forgetting with immature organisms. The first systematic study of the ontogeny of memory documented that forgetting by infant rats was inversely related to age (Campbell and Campbell, 1962). A parallel study with human infants, modeled after the Campbell and Campbell study, yielded the same result (Hartshorn et al., 1998b). Between 2 and 18 months, infants of all ages exhibited equivalent retention after the shortest test delay, but as the retention interval progressively increased, the younger infants forgot first (**Figure 13**).

The magnitude of the difference in retention between 2 months (1–2 days) and 18 months (13–14 weeks) has two major implications for interpretations of how different variables affect retention at different ages. First, effects on retention must be expressed in relative rather than absolute terms. Whereas 5 days is the longest interval that operantly trained 3-month-olds typically remember, for example, 5 days is a trivial retention interval for 18-month-olds. Second, age effects should not be expected after a short retention interval. Thus, claims that particular variables have no age effects must be treated skeptically unless the effects were also assessed at later points along the forgetting function.

Infants, like adults, forget and retrieve different memory attributes at different rates (Riccio et al., 1994). In operant and deferred imitation tasks, for example, infants forget object color faster than object form (Bhatt and Rovee-Collier, 1996; Hayne et al., 1997). They also forget the specific details of the training mobile before they forget its general features (Rovee-Collier and Sullivan, 1980), and after forgetting, a memory prime recovers its general features before its specific details (Rovee-Collier and Hayne, 1987). One day after priming, infants respond to both a novel test mobile and the original one; 3 days later, they discriminate the mobiles and respond only to the original one. This result is consistent with other evidence that more accessible memories are retrieved faster (see the section 2.36.4.2).

2.36.4.2 Accessibility

Memories that are inaccessible but available can still be retrieved; memories that are both inaccessible and unavailable cannot (Tulving, 1983). In both infants and adults, the rapidity with which a memory is retrieved is directly related to its accessibility and inversely related to both the strength or number of cues required to retrieve it and the length of time it has been forgotten. An important consequence of retrieving a memory is an increase in its accessibility (*See* Chapter 2.16). Paradoxically, after a less accessible memory is retrieved, both infants and adults subsequently remember it longer (Schmidt and Bjork, 1992).

Joh et al. (2002) found that the minimum duration of exposure to an effective prime is a linearly increasing function of how long the memory had been forgotten. At 3 months of age, when the memory had been forgotten for 1 day, the minimum duration of priming was 7.5 s; when it had been forgotten for 1

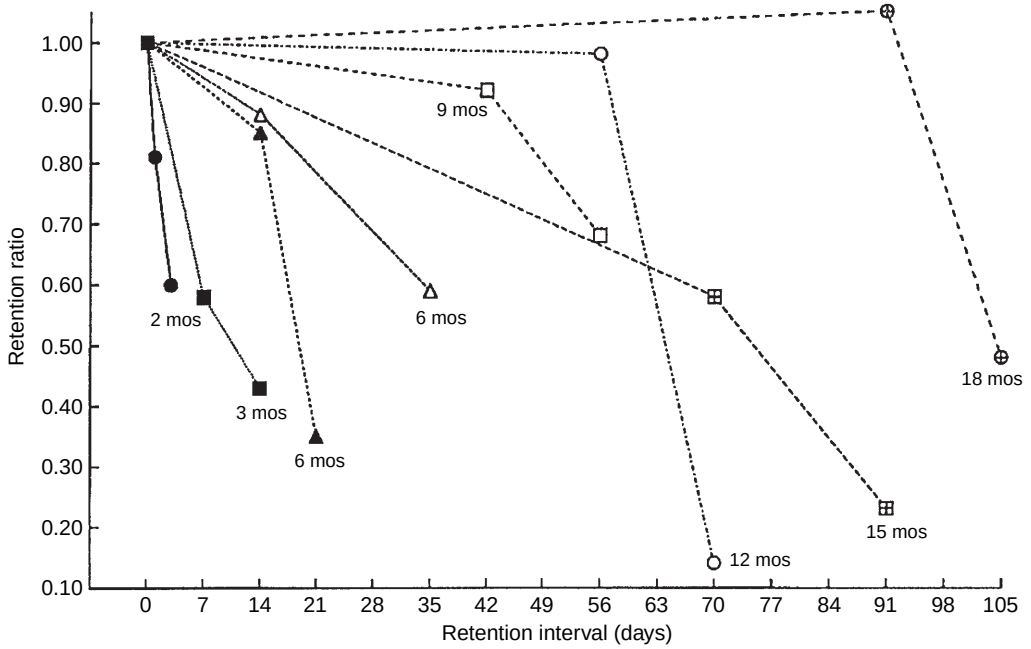


Figure 13 Forgetting as a function of infant age and test delay. Mean retention ratios of independent groups of infants between 2 and 18 months of age who were trained for 2 consecutive days in the mobile task (2–6 months) or the train task (6–18 months) and received an immediate retention test after the conclusion of acquisition on day 2. Six-month-olds were trained and tested in both tasks. Infants of each age received a delayed recognition test after different retention intervals until, as shown in the last point on each curve, they exhibited no retention. From Hartshorn K, Rovee-Collier C, Gerhardstein P, et al. (1998b) The ontogeny of long-term memory over the first year-and-a-half of life. *Dev. Psychobiol.* 32: 69–89.

week, the minimum duration was 120 s; and when it had been forgotten for 2 weeks, the minimum duration was 180 s. The finding that a longer prime is required to reactivate a memory that has been forgotten longer is theoretically important. In the past, there was no way to measure the accessibility of a memory that was not expressed. These data indicate that the minimum duration of exposure to a prime that can reactivate a latent memory is a direct measure of its accessibility.

A research problem with important applied and theoretical implications concerns manipulations that increase the accessibility of a forgotten memory. Because the latency of response to a memory prime (the priming latency) decreases logarithmically over the first year of life (Hildreth and Rovee-Collier, 1999), it is tempting to attribute the increased accessibility to maturational changes in the nervous system. Hayne et al. (2000b), however, reported that two reactivations decreased the priming latency of 3-month-olds from 24 h to 1 h, which is the typical priming latency of 6-month-olds given one reactivation. This result indicated that priming latency is experientially rather than maturationally based. They concluded that prior priming increased the accessibility of the forgotten memory.

Because prior priming increased the accessibility of a memory by decreasing priming latency (Hayne et al., 2000b), Bearce and Rovee-Collier (2006) hypothesized that prior priming would also increase its accessibility by reducing the minimum duration of exposure to the second prime. It did. The forgotten memory could be reactivated by a briefer prime after it had been primed before.

For infants initially trained between 3 and 18 months of age, the minimum duration of exposure to an effective prime presented 1 week after forgetting decreases logarithmically from 2 min at 3 months of age to 1.5 s at 18 months (**Figure 10**, circles; Hsu et al., 2005). In priming studies with adults, Schacter et al. (1991) reported that a 1-s exposure was not long enough to produce a priming effect, but a 5-s exposure was. Taken together, these findings indicate that successful priming of a preexisting memory representation requires attention that is longer than a mere glance, which takes 1 s. Even though exposure durations decreased by six log steps between 3 and 18 months, infants of all ages forgot twice as fast after

a minimum-duration prime as after a full-length one. Nonetheless, the total period over which infants given a minimum-duration prime could remember the training memory was still quite considerable, exceeding 5.5 months at the oldest age (**Figure 10**, circles).

These findings reveal that the minimum duration of exposure to an effective prime is the currency by which the accessibility of memories can be psychophysically scaled. That is, all latent memories that can be activated by primes of the same minimum duration are equally accessible. This principle applies to all memories irrespective of subject age, time since forgetting, memory content, speed of processing, number of prior retrievals, spacing of successive retrievals, and so forth.

2.36.4.3 Context

The incidental context refers to relatively invariant aspects of the setting in which an event occurs that do not affect the characteristics or demands of the task. Studies with animals and human adults have found that the context provides additional retrieval cues for the target memory (Riccio et al., 1984). Relatively little attention has been paid to the role of context in infant memory. This neglect has reflected the widespread assumption that infants' brains are too immature to store information about the place where learning occurs (e.g., Nelson, 1995). This assumption, however, is incorrect. Even 3-month-olds encode numerous aspects of the incidental training context, including location (room in home, laboratory), the immediate visual surroundings (a colored-and-patterned cloth covering the sides of the crib), the experimenter (social context), an ambient odor (Rubin et al., 1998), and background music (Fagen et al., 1997).

In order to assess contextual specificity at the same relative points along the forgetting functions of differently aged infants, Hartshorn et al. (1998a) compared retention at the first, middle, and last points on the forgetting functions of 3-, 6-, 9-, and 12-month-olds (see section 2.36.4.1). They found that a change in the testing context impaired retention only after delays near the end of the forgetting function at all ages except 6 months, when it impaired recognition only after relatively short delays. The latter result was attributed to infants' heightened sensitivity to context preceding independent locomotion (Borovsky and Rovee-Collier, 1990). Apparently, the context disambiguates infants' memory of the training cue when it becomes fuzzy and facilitates its recognition (Bouton and Bolles, 1985). The deleterious effect of a context change on recognition is overridden at 3 and 6 months of age by training infants in a different context each day (Amabile and Rovee-Collier, 1991; Rovee-Collier and DuFault, 1991).

After delays so long that the memory has been forgotten, if the memory has been reactivated in the original context, then infants can recognize the training cue in a different test context 24 h later by 9 months of age. By 12 months of age, the memory can also be reactivated in a different context (DeFrancisco, in press). Thereafter, reactivated memories become increasingly less context dependent with age. Thus, infants can transfer what they learn from one place (e.g., nursery school) to another (e.g., home) if asked to do so before too much time has elapsed.

Deferred imitation also increasingly generalizes across physically different contexts with age. Six-month-olds exhibit 24-h deferred imitation when either the test room or the mat they sit on during testing is different from the room or mat present during modeling but exhibit no deferred imitation when both differ (Learmonth et al., 2004). By 9 months, infants generalize when both the floor mat and the room differ. Also, a global context change (e.g., laboratory vs. home) impairs 24-h deferred imitation at 6 months but not at 12 and 18 months after test delays up to 28 days (Hanna and Meltzoff, 1993; Hayne et al., 2000a). Learmonth et al. (2005) found that a novel tester (the social context) disrupted 24-h retention of deferred imitation from 6 to 18 months of age, but preexposure to the novel tester in their home 2 days earlier allowed infants of all ages to generalize imitation. This finding parallels findings from operant studies with infants that novelty inhibits responding. These findings reveal that the similarity between the conditions of encoding and retrieval – not whether the task is deferred imitation or operant conditioning – determines whether young infants generalize.

Recent research on the renewal effect provides evidence that 3-month-olds can also associate the context with experimental contingencies (Cuevas et al., 2005). The renewal effect was originally described by Bouton and Bolles (1979) as the recovery (renewal) of acquisition performance when the contextual cues that were present during extinction are removed. Infants learned to kick to move the mobile in the presence of a distinctive context

(context A) and received an extinction manipulation (kicks did not move the mobile) with the original mobile in a different context (context B). Twenty-four hours later, infants tested with the original mobile in either the acquisition context (context A) or a neutral context (context C) exhibited retention, but infants tested in the extinction context (context B) exhibited none. Thus, the reduction of learned behavior via an extinction procedure in infants, as in adults, is context specific: In contexts other than the extinction context, infants will resume the behavior that was previously reinforced.

2.36.5 Latent Learning

Infants learn an enormous amount of information by merely observing their surroundings, but what they learn remains latent until they have a response and an opportunity to express it. Brogden (1939) introduced the sensory preconditioning (SPC) paradigm to study the latent learning of associations between neutral stimuli. The SPC paradigm has three phases. In phase 1, two stimuli (A, B) are repeatedly exposed in close temporal or spatial contiguity; in phase 2, a distinctive response is trained to one of the stimuli (A→R1); and in phase 3, the subject is tested with the other stimulus (B). The transfer of responding to the untrained stimulus (B→R1) but not to an equally familiar but unpaired stimulus is taken as evidence that an association was formed between A and B during the phase 1. Because the association that individuals form during phase 1 remains latent until it is expressed in phase 3, SPC is a form of behaviorally silent learning.

In the first infant study of SPC, Boller (1997) found that simultaneously preexposing 6-month-olds to two cloth panels (contexts) for 1 h daily for 7 days (phase 1) enabled them to associate the contexts. Twenty-four hours after phase 1, she operantly trained infants in one of the contexts (phase 2) and tested them in the other context 24 h later (phase 3). These infants transferred conditioned responding to the other context, but infants who were exposed to the contexts unpaired in phase 1 did not. Using a deferred imitation task, Barr et al. (2003) repeated Boller's procedure and preexposed 6-month-olds to puppets A and B either paired or unpaired for 1 h daily for 7 consecutive days (phase 1), modeled the target actions on puppet A 24 h later (phase 2), and tested infants with puppet B 24 h after that (phase 3). During the deferred imitation test, only the paired preexposure group imitated the target actions on puppet B. The same result was obtained when phase 1 lasted 2 days instead of 7.

Cuevas et al. (2006b) demonstrated that 6-month-olds could form an association between two objects that were neither physically present nor had ever occurred together. The association was formed when the memory representations of those objects were simultaneously activated by associated cues that the infants noticed. In phase 1, infants were exposed simultaneously to hand puppets A and B to establish an association between them. In phase 2, infants were trained to kick to move a crib mobile in a distinctive context to establish a mobile-context association. In phase 3, infants were exposed to puppet A in the distinctive context to establish a puppet B–mobile association. Presumably, puppet A would retrieve its associated memory of puppet B, and the distinctive context would retrieve its associated memory of the mobile. When the memory representations of puppet B and the mobile were simultaneously activated, then infants formed a new association, even though neither object was physically present.

Cuevas et al. subsequently demonstrated three target actions on puppet B to provide infants with an overt, measurable behavior that they could use to express the association. Typically, 6-month-olds remember the deferred imitation task for 1 day but not 3 days (Barr et al., 1996, 2001; **Figure 14**, solid circles), and they remember the mobile task for 14 days (Hill et al., 1988; **Figure 14**, triangles). If 6-month-olds had associated puppet B and the mobile *in absentia*, however, then they would be expected to imitate the actions on puppet B after the same test delays that they remember the mobile task. In fact, independent groups of infants successfully imitated the target actions on puppet B after delays up to 2 weeks, the same duration for which they remember the mobile (**Figure 14**, open circles). Two association control groups failed to imitate on puppet B even 1 week later (**Figure 14**, squares). These findings reveal that young infants form specific and enduring associations between the memory representations of stimuli that are simultaneously activated.

Townsend (2006) found that 6-month-olds are able to associate two puppets that they have never seen together. In phase 1, infants were preexposed to either two or three different pairs of puppets on 2 or 3 consecutive days (A–B, B–C or A–B, B–C, C–D; respectively). One day following their last exposure,

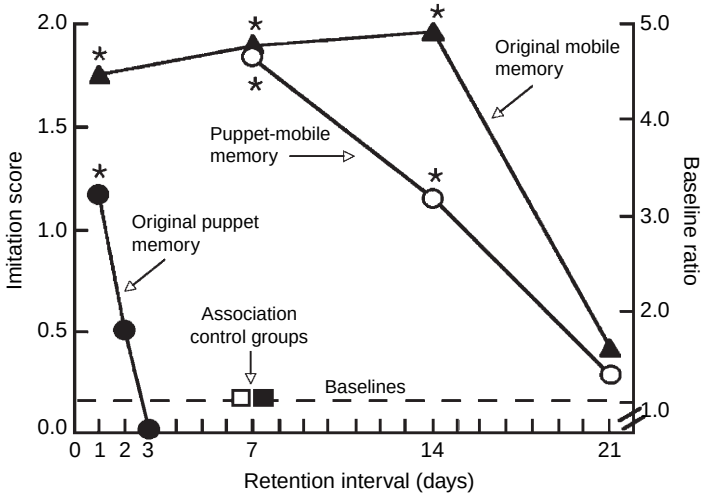


Figure 14 Mean imitation-test score (left *y*-axis) and mean baseline ratio, the index of operant retention (right *y*-axis), as a function of the retention interval. The deferred imitation function for the three experimental groups that associated the memory representations of puppet B and the mobile is presented along with the original deferred imitation function and the forgetting function for the operant mobile task. Also shown is the test performance of two association control groups. The dotted line indicates the mean base rate (baseline control group) for spontaneous production of the target actions in the deferred imitation task and the theoretical baseline for the mobile task. Asterisks indicate that test performance significantly exceeded the base rate or the theoretical baseline. Note that the form of the retention function of experimental groups in the deferred imitation task mirrors the form of the retention function of infants in the mobile task. Reprinted with permission from Cuevas K, Rovee-Collier C, and Learmonth AE (2006b) Infants form associations between memory representations of stimuli that are absent. *Psychol. Sci.* 17: 543–549. Copyright © 2006 by the Association for Psychological Science.

the target actions were modeled on the final puppet (C or D; phase 2). When tested with puppet A 24 h later (phase 3), infants in both groups imitated the modeled actions, whereas those tested with a novel puppet (puppet E) did not. Thus, 6-month-olds had apparently associated two puppets (A and C or D) that were never presented together. Infants who were preexposed to puppets A and B unpaired or to puppet B alone after the puppet A–B association was formed (an extinction procedure) did not imitate the modeled actions on puppet A, confirming that the A–B link was necessary for imitation.

Simultaneously preexposing two stimuli in phase 1 of SPC results in their association between 3 and 9 months of age (Barr et al., 2003; Campanella and Rovee-Collier, 2005; Bullman et al., 2006), but at 15 months, the same preexposure regimen is less successful (Bullman et al., 2006). Cuevas et al. (2006a) found that the extent of temporal contiguity required for two stimuli to be associated decreased with age from only simultaneous preexposure at 6 months to only sequential preexposure at 15 months. This result resembles age-related increases in associative memory in delay-of-reinforcement studies with infants.

Subsequent research has examined how long infants can remember an association before they finally express it. After one preexposure session at 6–9 months, infants remember the association between two puppets for only 2–3 days. After two preexposure sessions, infants remember the association for 1 week at 6 months and 2 weeks at 9 months (Bullman et al., 2006). The basis for the retention benefit afforded by the additional session results from the additional retrieval at the outset of session 2 and not from the longer exposure time afforded by two sessions. When the total duration of the two sessions was the same as that of one session, infants still remembered longer. These findings reveal that new learning acquired via mere observation can remain latent for a substantial period before it is finally used.

In the preceding studies, infants learned an association (correlation) between two different objects when they repeatedly saw those objects together, irrespective of the experimental paradigm within which they exhibited that knowledge. In all instances, however, the association that infants had picked up by merely looking remained latent until they were subsequently given an opportunity to demonstrate that knowledge through their direct actions.

2.36.6 Interference and Memory Updating

Retroactive interference is rare or nonexistent in studies of infant visual recognition memory (for review, see Rose et al., 2007) but common in conditioning studies of infant long-term memory (Rossi-George and Rovee-Collier, 1999; for review, see Rovee-Collier and Boller, 1995). Three-month-olds who were exposed to a novel mobile immediately after training, for example, recognized it but not the original one 24 h later. With the passage of time, the retroactive interference dissipated, and infants again recognized only the original mobile 48 h later (Gulya et al., 2002). In serial learning studies with adults, a recency effect after short test delays and a primacy effect after longer ones is also common.

Because we have never observed an instance of modification or updating that was not also accompanied by response suppression to the original cue, we conclude that retroactive interference is functionally adaptive, enabling memory updating. When responding to the original cue is suppressed and organisms respond to the more recently encountered one, if that recent cue is also predictive, then the original memory is modified or updated. If response suppression is necessary for subjects to respond to a recently encountered stimulus, then the opportunity for memory updating may be over when responding to the original stimulus resumes. Because retroactive interference dissipates if a new cue is not encountered, however, the time window within which updating can occur also decreases until the details of the original cue are forgotten (Rovee-Collier et al., 1994) or the memory is reactivated later (Galluccio and Rovee-Collier, 2005).

Suppressing response to the original stimulus is evidence of retroactive interference, which is temporary. Responding to a recently exposed stimulus instead of the original one, however, is evidence that the memory was permanently modified. Retroactive interference is common at the beginning of the retention interval, when the details of the original stimulus are highly memorable, whereas modification occurs readily at the end of the retention interval, when the details of the original stimulus have been forgotten. Reactivated memories are resistant to modification shortly after forgetting (Boller and Rovee-Collier, 1994; Galluccio, 2005) but are more readily modified when reactivation approaches its upper limit. At 3 months, forgetting is complete in 6 days. One week after training, exposure to a novel mobile immediately after reactivation did not affect infants' recognition of the original mobile; 2 weeks afterward, it interfered with recognition of the original one; and 4 weeks afterward (the upper limit of reactivation at 3 months), it both interfered with recognition of the original mobile and modified the reactivated memory, replacing the memory attributes of the original mobile with those of the novel one (Galluccio and Rovee-Collier, 2005). Findings that both original and reactivated memories are initially resistant to modification but become more malleable when they are older suggest that the same basic mechanism underlies the malleability of original and reactivated memories.

2.36.7 Spacing Effects

A general rule in the memory literature is that greater spacing between successive items during training (associative memory) produces memories that are more enduring (Cohen, 1985). The classic retention advantage of distributed over massed training (Crowder, 1976; Glenberg, 1979; Schmidt and Bjork, 1992) has also been obtained with infants. Using a visual recognition memory task. Cornell (1980) showed 5- to 6-month-olds a pair of identical photos of people of one sex for four trials and then tested them after delays of 5 s, 1 min, 5 min, or 1 h with a previously exposed photo paired with a photo of a person of the opposite sex. He found that greater spacing between successive items prolonged infants' retention. When intertrial intervals were 3 s (massed condition), infants recognized the familiar photo only after the 5-s delay; when intertrial intervals were 1 min (distributed condition), they recognized it after all test delays. Using the operant mobile task, Vander Linde et al. (1985) trained 2-month-olds for three 6-min sessions (distributed condition) or one 18-min session (massed condition). Infants given massed training remembered for 1 day, but infants given distributed training remembered for 2 weeks.

Recent research on spacing effects has focused on the interval between successive events (retentive memory) after the first event has been acquired. This research has been conducted within the conceptual framework of the time window construct, which specifies the conditions in which two successive events are integrated (Rovee-Collier, 1995). A time window is a limited period that begins with the onset of an event and ends when the event is forgotten. It specifies

when a second event can be integrated with the memory of the first one and when it cannot. New information encountered while the time window is open can be integrated with the initial event; information encountered after it has closed will be treated as unique. The integration is accomplished when the new event retrieves the representation of the initial event into primary or active memory. The time window construct specifies that each retrieval of a memory expands the time window (i.e., increases the period within which it can be retrieved again). Finally, the effects of retrieving the memory of the initial event at different points within the time window are nonuniform; retrieving it near the end of the time window expands the width of the time window more than retrieving it closer to when the time window opens.

In the first time-window study, Rovee-Collier et al. (1995) trained 3-month-olds in the mobile task for two sessions spaced by 1, 2, 3, or 4 days and tested them 8 days after session 1. The control group received session 1 only and the test. Groups exhibited significant retention when sessions were separated by 1–3 days. The group whose two sessions were separated by 4 days and the one-session control group exhibited no retention. Thus, the time window for integrating successive training sessions closed after 3 days. When the second session occurred after the time window closed, it was treated like a first-time event. In a follow-up study, groups received session 2 either inside the time window (2 days after session 1) or outside the time window (4 days after session 1) and a reactivation reminder 2 weeks later. Because memory reactivation requires two training sessions to be successful (Boller and Rovee-Collier, 1992; Richardson et al., 1993; Hayne et al., 2003), infants were expected to exhibit renewed retention after priming only if sessions 1 and 2 had been integrated. In fact, infants who received session 2 inside the time window exhibited retention, but infants who received session 2 outside the time window exhibited none. These results provide convergent evidence that successive events are integrated only if the second event occurs within the time window.

Hsu (2007) used the operant train task to study time window effects in infants between 6 and 18 months of age. The duration for which infants remembered a single training session defined the width of the time window at each age. Despite vast differences in the absolute durations of retention across ages, the pattern of results was remarkably uniform (**Figure 15**). Infants given session 2 just inside the time window remembered longer than infants given only one session, but infants given session 2 just outside the time window behaved as if they received only one session. Additionally, infants whose second session occurred at the end of the time window remembered longer than infants whose second session occurred at the beginning of the time window, 1 day after session 1.

The time window construct also predicts that retrieving the memory of the first event progressively later in its time window will produce an increasingly greater retention benefit. Using a reinstatement procedure, Galluccio and Rovee-Collier (2006) tested this prediction with 3-month-olds. Because 3-month-olds remember the mobile task for 5 days, they gave infants a single 3-min reinstatement 0, 3, or 5 days after mobile training. At the beginning of the time window (day 0), reinstatement afforded only a small retention benefit, 1 additional day. In the middle of the time window (day 3), reinstatement yielded a retention benefit of 5 additional days, or twice the duration of original retention. At the end of the time window (day 5), reinstatement yielded a retention benefit of 16 additional days, a duration of retention more than four times longer than 3-month-olds otherwise remember (**Figure 16**). The exponential increase in the retention benefit as a result of the timing of the reinstatement within the time window was particularly remarkable considering that the reinstatement lasted only 3 min, it was the same for all reinstatement groups, and the timing difference between the final reinstatement groups was only 2 days.

Because young infants' memories are so short-lived relative to the memories of older individuals, the consequences of the timing of a reinstatement within the time window are particularly dramatic. However, the differential retention benefit of presenting a reinstatement late in the time window is not unique to either the operant mobile task or 3-month-olds. A similar effect was obtained in a deferred imitation study with 6-month-olds. At 6 months, infants who imitated the actions immediately after the demonstration, when the time window opened, could defer imitation for 1 but not 2 days (Barr et al., 2001). In contrast, infants who first imitated the actions 1 day later, at the end of the time window, deferred imitation for 10 days after the demonstration (Barr et al., 2005).

Actively imitating the actions was not why infants' retention increased tenfold; infants who merely witnessed an adult model the actions again for 30 s 1 day later also deferred imitation after 10 days. Because

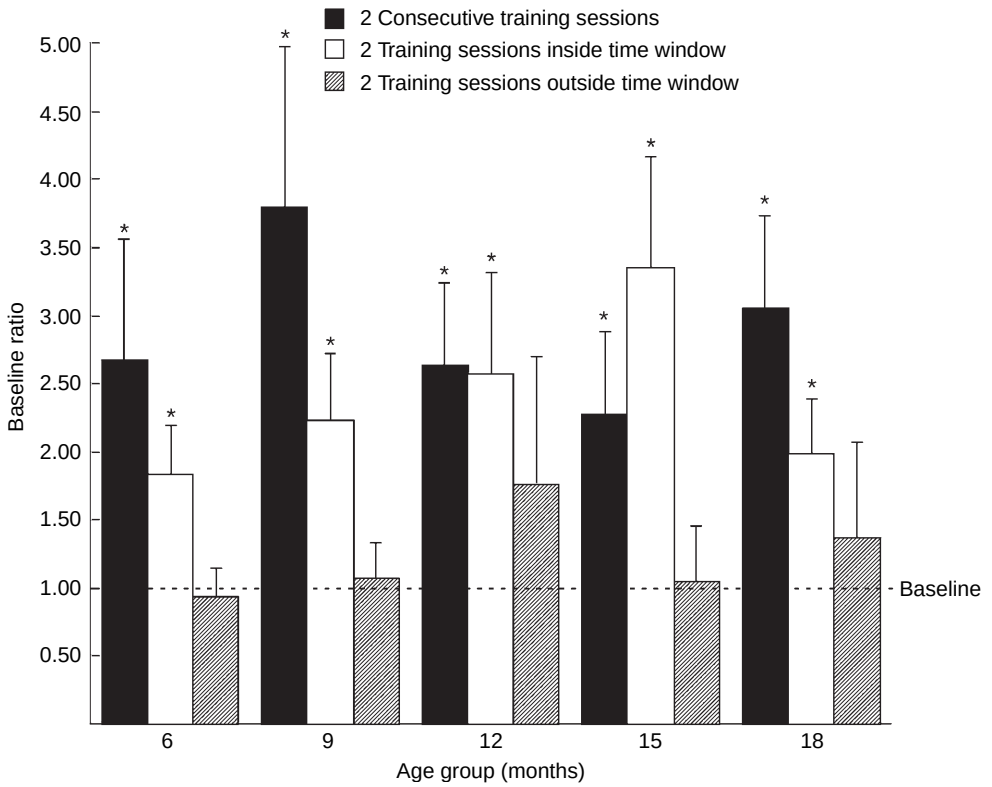
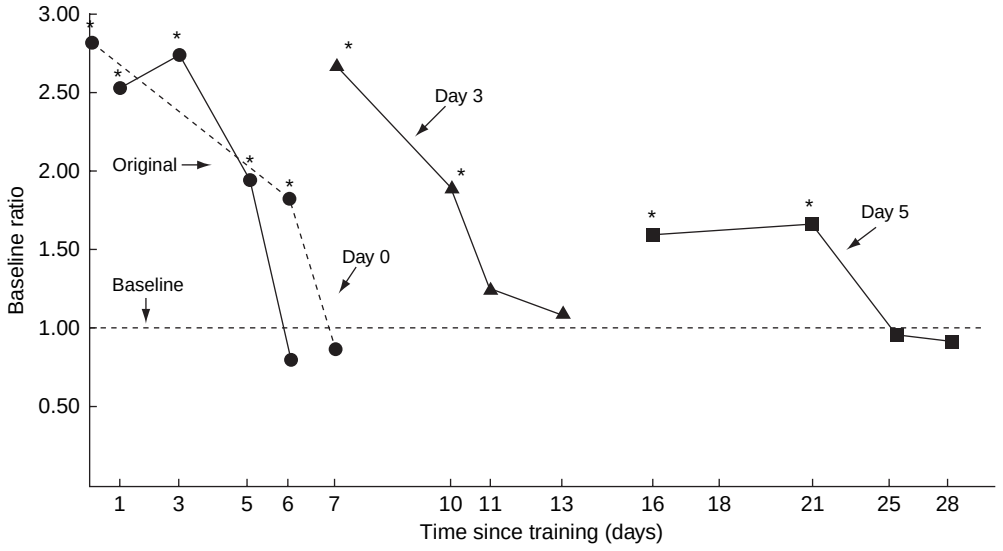


Figure 15 Mean baseline ratios of independent groups of 6-, 9-, 12-, 15-, and 18-month-olds who received a second training session either inside (black bars, white bars) or outside (gray bars) the time window (i.e., before or just after infants forgot the first training session). Infants given session 2 just inside the time window (white bars) remembered longer than infants who were given only one session, but infants given session 2 just outside the time window did not. The dotted line indicates the theoretical baseline ratio of 1.00 (i.e., no retention). An asterisk indicates significant retention. Vertical bars indicate + 1 SE. From Hsu VC (2007) *Time Window Effects on Retention over the First Year-and-a-Half of Life.* PhD Thesis, Rutgers University, New Brunswick, NJ.

6-month-olds who observe the original demonstration for 30 s cannot defer imitation 1 day later (Barr et al., 1996), merely retrieving the memory at the end of the time window prolonged its retention. In a follow-up experiment, 6-month-olds who repeatedly retrieved the memory at or near the end of the expanding time window on days 1, 10, 30, and 70 still exhibited significant deferred imitation after 10 weeks. Whether they might have done so after a longer delay was not determined. Given that infants otherwise exhibit deferred imitation of the same modeled actions for 1 day, this finding is compelling evidence of the effects of repeated retrievals near the end of the time window.

In a recent study, 6-month-olds' memory of the demonstration was associated with the 'retrieved' memory of the operant train task (Rovee-Collier and Barr, 2006). Six-month-olds first learned to move the train by lever pressing, and 7 days (the midpoint of the time window for the train task) or 14 days (the end of its time window) later, the target actions were modeled on a puppet in the presence of the stationary train. After both delays, the sight of the train cued retrieval of the memory of the train task, and the demonstration was then associated with the updated status of its memory representation. When the demonstration was associated with the retrieved memory 7 days after operant training, infants remembered the train task for 4 weeks instead of 2 weeks, and they also deferred imitation on the puppet for 4 weeks instead of 1 day. When the demonstration was associated with the retrieved memory 14 days after operant training, infants remembered the train task for 8 weeks instead of 2 weeks and deferred imitation on the puppet for 6 weeks instead of 1 day. A no-association control group that saw the demonstration and the stationary train unpaired 7 days after operant training failed to defer imitation 1 week later but continued to remember the train task for 14 days.



Note. *Indicates $p < .05$.

Figure 16 Mean baseline ratios of independent groups of 3-month-olds whose retention was tested after increasing delays since training (the simple forgetting function: circles, solid lines) and after receiving a reinstatement on day 0 (circles/dashed lines), day 3 (triangles/solid lines), and day 5 (squares/solid lines). The first point on the day-0 function is the retention of group 0/6 at the end of training, immediately before the reinstatement was administered. An asterisk indicates that a group exhibited significant retention. From Galluccio L and Rovee-Collier C (2006) Nonuniform effects of reinstatement within the time window. *Learn. Motiv.* 37: 1–17. Copyright © 2006 by Elsevier Inc.

What does all of this mean? Clearly, memory retrieval – particularly later in the time window – has a huge effect on the memory of what was retrieved as well as on the memory of what is associated with it, and the effects of multiple retrievals are even greater. Because most memory retrievals in real-world settings are probably latent, as are most of the associations into which retrieved (activated) memories enter, the extent of their contribution to the growth of the early knowledge base can never be known.

Time window effects have also been reported in infant studies of categorization, eyewitness testimony, postevent information, memory modification, and language acquisition (for review, see Rovee-Collier, 1995; Hsu, 2007). Although the applicability of the time window construct is not constrained by age or stage of development, its impact on the retention of very young infants, whose retention is initially so brief, may be most obvious.

2.36.8 Implicit and Explicit Memory

Many psychologists believe that adults possess two functionally independent and anatomically different memory systems that mature hierarchically (e.g., Schacter and Moscovitch, 1984; Bauer et al., 2007). By this account, infants possess only the primitive memory system (implicit memory) until late in their first year, when the higher-level system (explicit memory) matures. Proponents of dichotomous memory systems interpret functional dissociations as evidence for different memory systems (e.g., Tulving, 1983; Squire, 1987). A functional dissociation is seen when the same experimental manipulation that produces impaired performance by brain-damaged amnesic adults on recall or recognition tests does not affect their performance on priming tests. Recall and recognition tests are thought to tap the explicit memory system, which presumably processes information about a specific past experience; in contrast, priming tests are thought to tap the implicit memory system, which presumably processes information only about skills and procedures that can become habitual or automatized and general facts. A large literature has now amassed, however, documenting that even 3-month-olds exhibit all of the same functional dissociations on recognition and priming (reactivation) tests as adults (for review, see Rovee-Collier et al., 2001).

For proponents of dichotomous memory systems, however, the defining characteristic of explicit

memory is the conscious awareness of previously experiencing the event (Tulving, 1985), and they use conscious awareness to distinguish explicit from implicit memory. On a priming test, for example, amnesics (who presumably possess only an implicit memory system) respond with a word from a list they had just studied while being unaware that they had studied it. Amnesics also fail deferred imitation tests that healthy adults use conscious recollection to perform. As a result, proponents of dichotomous memory systems assume that infants who exhibit deferred imitation must also use conscious recollection to do so. Based on this assumption, they use deferred imitation as a benchmark that the explicit memory system is functionally mature (McDonough et al., 1995; Bauer, 1996; Bauer et al., 2007). If successful deferred imitation constitutes evidence that the explicit memory system has matured, however, then it must be mature by 3 months of age. Recall that infants who saw target actions modeled on a hand puppet at 3 months of age successfully imitated them once they were motorically capable of performing them (Campanella and Rovee-Collier, 2005).

Evidence that 3-month-olds exhibit both functional dissociations and deferred imitation disputes the notion that implicit and explicit memory develop hierarchically during the infancy period. If there are two memory systems, then they must develop in parallel from early in infancy. We note, however, that because scientists can neither define consciousness nor state what the function of consciousness in memory might be, it is not yet clear how memories that have it might be different from memories that do not (Willingham and Preuss, 1995).

2.36.9 Infantile Amnesia

Infantile amnesia refers to the fact that most people cannot remember events that occurred before the age of 3 or 4 (but see Fivush and Hamond, 1990; Usher and Neisser, 1993). There has been little agreement about the basis or even the ubiquity of this phenomenon (Mandler, 1990). Common explanations of infantile amnesia include the classical psychoanalytic account of repressed infantile memories, the immaturity of the infant's brain that prevents the encoding, storage, and retrieval of memories over the long term, young infants' exclusive reliance on a primitive memory system, and rapid forgetting within the infancy period. Most of these explanations were discounted by evidence reported earlier in this chapter.

Additionally, verbal cues are usually presented as retrieval cues in studies of infantile amnesia. The common conclusion is that "virtually no early memories slip through the barrier" (Nelson, 1990: p. 306). Simcock and Hayne (2002 see also Simcock and Hayne, 2003) questioned whether the development of language actually blocked early memories. To answer this, they developed a memory task using the Magic Shrinking Machine (**Figure 17**), in which 27-, 33-, and 39-month-olds participated in a highly unique, multistep event in their homes. Children learned a sequence of five target actions: Pull a lever to activate an array of lights and turn the machine on, pick a toy from the toy case (ball, teddy bear), drop the toy in a chute on top of the machine, turn the handle on the side of the machine (which produced noise and music from inside the box), and retrieve a smaller version of the toy from the front of the machine. Either 6 or 12 months later, infants' memory of the event was assessed with both verbal and nonverbal measures. Children of all ages exhibited retention on both measures after both test delays, but their memory performance on nonverbal measures was consistently superior. Importantly, children's verbal reports during the long-term test reflected their verbal skills at the time of encoding, even though the words that could be used to verbally recall the event were in their vocabularies at the time of testing. Thus, children with language could remember the prior event that had been encoded preverbally, but they could not translate what they had encoded into words.

Because the fundamental principles of memory processing in human infants and adults are the same, we conclude that the phenomenal experience of infantile amnesia can be understood within the existing framework of normal memory process. First, the encoding specificity principle (Tulving and Thomson, 1973) states that a match between the encoding and retrieval contexts is critical for retrieval. In infancy, this is especially true after long delays (Hartshorn et al., 1998a). As a result, the shift from nonverbal to verbal retrieval cues dramatically lessens the probability that a memory encoded in infancy would be retrieved later in life. From this perspective, words are retrieval cues whose status is no different than that of other potential retrieval cues.

Second, even if an appropriate retrieval cue were to recover an early memory later in life, a person

Figure 17 A child participating in the memory task with the Magic Shrinking Machine. The child places a large toy in a chute on top of the machine, turns the handle on the side of the box (presumably to shrink the toy), opens a side bin (where the shrunken toy has presumably dropped), and retrieves a miniature version of the toy. Photos courtesy of Harlene Hayne and Julien Gross.

would be unlikely to identify it as such. Because reactivated memories that are older are readily updated, for example, they may be impossible to identify as having originated early on (Galluccio and Rovee-Collier, 2005).

Third, as memories of healthy individuals become increasingly remote, they appear to become increasingly disconnected from their original temporal context and more semantic and fact-like (Bayley et al., 2003). Even in infants, specific contextual information is quite fragile and disappears from memories that are older or were previously reactivated (Hitchcock and Rovee-Collier, 1996; Galluccio and Rovee-Collier, 2005). As a result, people might actually remember many early-life events without knowing where or when they occurred. Alternatively, if an early memory was modified after a long delay, then it might differ substantially from the original one (Galluccio and Rovee-Collier, 2005). Even if an early memory were neither updated nor repeatedly retrieved, its recovery is ultimately constrained by the upper limit of reactivation (Hildreth and Hill, 2003; Hsu and Rovee-Collier, 2006).

2.36.10 Conclusions

Although the neurological mechanisms (the hardware) that underlie learning and memory change over development, the operating principles (the

software) that describe how individuals learn and remember do not. While the same variables and manipulations affect memory processing in the same ways in infants and adults, the temporal parameters of memory processing change dramatically but in an orderly fashion over the infancy period: (1) the duration of retention after original training increases, (2) the duration of retention after reminding increases, (3) the speed of responding to a memory prime increases, (4) the upper limit of reactivation increases, and (5) the minimum duration of exposure to a memory prime decreases. Most if not all of these changes can be produced at younger ages by retrieval experience. While later developments such as verbal and conversational skills, strategies for remembering, and the development of the self-concept may facilitate the efficiency of memory processing, they do not alter the fundamental mechanisms that underlie it.

Recent research with very young infants has expanded our knowledge of infant memory far beyond what was ever imagined possible, with equally dramatic implications for the infant's rapidly burgeoning knowledge base. The findings show that very young infants rapidly form new and relatively enduring associations between stimuli that are physically present in their visual surroundings and even between the activated memory representations of stimuli and events that are not. These new associations become linked with each other and with other members of a complex and rapidly growing associative network. When one member of an association is activated, the activation spreads to other members in the network and indirectly activates them as well. As a result, infants as young as 6 months of age exhibit bidirectional priming (Barr et al., 2002) and transitivity (Townsend, 2006) on deferred imitation tests and use correlated attributes to categorize novel stimuli on delayed recognition tests (Bhatt et al., 2004). The same processes may also be responsible for false memories and behavior that appears insightful in children and adults.

These findings necessitate a major revision in how we think about infant memory. Although some aspects of infant memory processing are age invariant, such as the effects of priming on various independent variables, other aspects of infant memory processing change with experience. Importantly, the fact that these changes are logarithmic and subject to Weber's Law indicates that memory processing is perceptually based. That said, the content of what is retrieved apparently results from activation that spreads nonlinearly through a growing web of associations, most of which will always remain latent. In short, infant memory is like other things in life: nothing is as simple as it once seemed.

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2.37 The Development of Skilled Remembering in Children

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2.37.1 The Development of Skilled Remembering in Children

A number of the intellectual giants of the nineteenth and early twentieth centuries thought seriously about the mnemonic abilities of young children. Initial considerations of children's remembering can be seen in Darwin's (1877) and Preyer's (1882/1889) diary case studies of their own children's memory skills, in Binet's (e.g., Binet and Henri, 1894a, Binet and Henri, 1894b) early experiments on children's memory for words and ideas, and in Freud's (1901) initial psychoanalytic writings about infantile amnesia. Sustained interest in children's memory was reflected in Hunter's (1913) basic studies of memory capacity and retention and in Stern and Stern's (1909) applied investigations of memory, suggestibility, and eyewitness testimony. Moreover, assessments of memory figured prominently in initial measures of intellectual capacity (e.g., Terman, 1916; Terman and

Merrill, 1937; Wechsler, 1939) – as they do today – but even this important early work did not prompt widespread interest in the development of memory. Indeed, reflecting to some extent the dominance of the behaviorist perspective in both experimental and developmental psychology, it would take more than 50 years for systematic research on children's memory to start to appear in the developmental literature. The first signs of renewed interest in the development of memory can be seen in Flavell's seminal explorations of children's use of strategies for remembering (e.g., Flavell, 1970; Flavell et al., 1996), and in a few years, there was sufficient critical mass in the area to justify a symposium on children's memory at the 1971 meeting of the Society for Research in Child Development. Flavell organized this now-celebrated symposium – "What is memory development the development of?" – to characterize what was then known about children's memory.

The last 35 years have witnessed a dramatic increase in research on children' abilities to remember (Kail and Hagen, 1977; Schneider and Pressley, 1997; Ornstein et al., 1998; Courage and Cowan, in press), but in many ways the research literature bears the imprint of the question posed at the 1971 symposium. Admittedly, the answers to this question have changed dramatically over the years as a function of changes in research paradigms, theoretical frameworks, and even the ages of the children being studied, but there has nonetheless been a consistent emphasis on characterizing that 'something' (e.g., strategies, underlying knowledge, basic capacity) thought to be changing with age (Ornstein and Haden, 2001; Ornstein et al., 2004). The net result is that a great deal is now known about the contrasting memory skills of children of different ages, as is documented in this chapter. However, in contrast to the progress that has been made in response to the 'what develops' question, relatively little is known about the process of development. For example, how do early manifestations of a skill (e.g., a naming strategy) give way to later and more sophisticated examples of that skill (e.g., a more complex rehearsal strategy), and what can be said about the rate of change that is observed? Most importantly, what factors are responsible for bringing about this developmental change? To a certain extent, researchers have focused remarkably well on issues of memory development but not very much on the development of memory (Ornstein and Haden, 2001; Ornstein et al., 2004).

In addition to this important distinction between memory development and the development of memory, the stage for this chapter is set by two pervasive themes in the now-voluminous research literature on children's remembering. First, a substantial corpus of work now documents the remarkable mnemonic competence of infants (e.g., Meltzoff, 1995; Rovee-Collier, 1995, Bauer et al., 2000; Bauer, 2006; *See* Chapter 2.36) and preschoolers (Baker-Ward et al., 1984; Goodman et al., 1990). Second, an equally impressive literature confirms the presence of substantial age differences in aspects of memory performance that include the degree of detail reflected in children's reports (e.g., Fivush and Hamond, 1990; Roebers and Schneider, 2001), the amount of forgetting observed (Brainerd et al., 1990; Howe and Courage, 1997), and the deployment and effectiveness of deliberate strategies for remembering (Ornstein et al., 1988; Bjorklund, 1990; Schlagmüller and Schneider, 2002). These two themes – the surprising competence of young children's memory on the one hand and clear age-related differences in performance on the other hand – represent a distillation of evidence stemming from research paradigms that range from elicited and deferred imitation (Meltzoff, 1995; Bauer et al., 2000, Bauer, 2006) and conditioning (Rovee-Collier and Shyi, 1992; Rovee-Collier, 1997) to those involving the production of narrative accounts of previous experiences (Fivush, 1991; McCabe and Peterson, 1991; Reese et al., 1993) and verbal measures of both strategy use and remembering (Baker-Ward et al., 1993; Folds et al., 1990; Schlagmüller and Schneider, 2002).

The aim of this chapter is to provide an overview of children's memory, focusing on age-related differences in the underlying processes of encoding, storage, retrieval, and reporting. With respect to the flow of information within the developing memory system, the emphasis is on early mnemonic competence and age-related changes in a range of memory skills, characterizing children's abilities at different points in time and exploring factors that serve to bring about change. However, reflecting the relative dearth of information in the literature on the development of memory, the bulk of the work reviewed here deals with memory development. To some extent, this state of affairs reflects the predominance of cross-sectional research designs, in which the performance of children of different ages is contrasted, and the infrequent use of longitudinal designs in which the same children are tracked over time. There are, of course, many reasons why cross-sectional designs have been favored, but

longitudinal research is certainly necessary for an account of developmental change within individuals, especially given the evidence to date that cross-sectional findings are not always replicated within a longitudinal framework. For example, although the cross-sectional literature would suggest a smooth age-related progression in the skill with which an organizational strategy is deployed, inspection of individual developmental trajectories available from the Munich Longitudinal Study reveals a markedly different pattern: Children's strategic deployment seems to be characterized by inconsistency and abrupt change across measurement points (Sodian and Schneider, 1999). As such, longitudinal designs are essential if the aim is to address issues concerning the development of memory, but these designs must be chosen so as to provide information concerning factors – within the child and within the environment – that may serve as mediators of the developmental change that is observed.

The bulk of the chapter is devoted to characterizing age differences in various aspects of children's memory performance, making extensive use of the cross-sectional literature. To the degree possible, longitudinal data are utilized to supplement this characterization of children's abilities in an attempt to move the discussion to (1) a description of the course of developmental change and (2) a treatment of potential mediators of this change. However, because longitudinal research designs are inherently correlational in nature, the treatment of longitudinal studies is combined, where possible, with parallel experimental investigations in which hypothesized mediators of change (e.g., mother–child communicative interactions) are brought under experimental control. These experimental interventions (e.g., Carr et al., 1989; Boland et al., 2003) are necessary if the aim is to make causal statements about factors that serve to bring about developmental change. In addition, the findings of these cross-sectional, longitudinal, and intervention studies are supplemented by a discussion of the few extant microgenetic studies of children's memory. In microgenetic studies (see Siegler and Crowley, 1991; Siegler, 2006), frequent observations are made of children's performance during periods in which their skills are thought to undergo rapid change and development (e.g., Schlagmüller and Schneider, 2002).

The sections that follow are devoted to a discussion of two basic literatures that are not often treated together: Children's memory for specific events that are typically experienced without intent to remember, as well as their deliberate memory for materials that are encoded with the expectation of a subsequent memory assessment. These different aspects of mnemonic competence are discussed together because the underlying processes of encoding, storage, retrieval, and reporting seem to operate in a similar manner in each of these domains (Baker-Ward et al., 1997; Ornstein et al., 2006b). Moreover, it seems likely that elemental skills in talking about past experiences set the stage for later accomplishments within the domain of deliberate remembering (Haden et al., 2001). After a consideration of research on the nonverbal memory skills of infants and young toddlers, the discussion turns to children's verbally based memory for events and autobiographical experiences, and then to a treatment of their strategic efforts in tasks that require deliberate remembering.

2.37.2 Nonverbal Memory

Given its developmental focus, the emphasis in this chapter is on the emergence and growth of children's verbal mnemonic skills. Nonetheless, it is important to appreciate the fact that the verbal skills that are described here are built upon a nonverbal foundation and that considerable attention has focused on characterizing this foundation (see, e.g., Barr and Hayne, 2000; Rovee-Collier et al., 2001; Bauer, 2006; Oakes and Bauer, 2007; Courage and Cowan, in press), with researchers using a wide variety of behavioral measures to piece together a picture of what infants can remember over varying delay intervals. Two caveats are in order, however, as we begin this brief treatment of early memory. First, the conclusions that one can reach about young children's memory seem to vary as a function of the measures used to assess remembering, and little is known about the extent to which the different measures converge to characterize children's skill at any one point in development. Second, little is also known about the ways in which children's nonverbal memory performance leads to (or predicts) subsequent performance on tasks that require verbal reports.

2.37.2.1 Estimates of Long-Term Retention

It is clear that infants evidence remarkable skills in being able to retain information over delays that increase dramatically over the first year and a half

of life. Early retention has been demonstrated in paradigms ranging from visual paired comparison and habituation to conditioning and imitation, with estimates of retention in neonates that range from a few minutes to weeks on visual habituation tasks (e.g., Slater et al., 1984; Pascalis et al., 1998), to months by the end of the first year on elicited imitation tasks (e.g., Carver and Bauer, 2001). But what can be said about the age-related changes in the nature and complexity of the information that is being retained? To illustrate current understanding related to this important question concerning early memory, we focus on studies of children's performance in the context of two tasks: operant conditioning (*See* Chapter 2.36) and elicited/deferred imitation procedures. (Meltzoff, 1985, 1995; Bauer, 2007). Systematic research with these behavioral tasks has enabled researchers to document infants' quite dramatic mnemonic skills and has also sparked a lively debate concerning the nature of early memory (e.g. Nelson, 1995; Bauer, 1996; Rovee-Collier, 1997).

2.37.2.1.1 Conjugate reinforcement paradigms

In the conjugate reinforcement paradigm, an infant – typically between 3 and 6 months of age – is placed on her back with a mobile overhead. After an operant period in which the infant's base level of kicking is measured, her leg is connected via a ribbon to the mobile. With this arrangement, each kick is followed by the reinforcement of observing the mobile move, and stable responding in its presence can easily be established. With the operant response acquired, remembering after varying intervals can readily be assessed under conditions of extinction in which the ribbon is disconnected from the mobile, so that no contingencies are in effect. Memory is then inferred if the rate of kicking observed in these test periods is greater than that seen in the baseline period, and under these conditions two fundamental patterns of age differences in performance in the first 6 months of life have been reported: Both speed of learning and length of retention increase with age. Thus, older infants acquire the kicking response more rapidly than younger children, and when trained to the same criterion of performance, they retain it longer than their younger peers (e.g., Hill et al., 1988).

Programmatic research with the mobile conjugate reinforcement task has also revealed two other important features of early memory. First, under some conditions, memories that would seem to be forgotten can be cued and recovered. Indeed, by using reinstatement, partial reminders of a previous experience (Campbell and Jaynes, 1966), and reactivation (Spear and Parsons, 1976) procedures in which a component of the original event is presented at the end of the delay interval, retention of the kicking response can be extended considerably (e.g., Sweeney and Rovee-Collier, 2001). Typically, exposure to the mobile or the context (e.g., the crib lining) can serve to maintain memory over an extended delay, but the timing of the reminder is of critical importance, with maximal facilitation occurring if it is administered shortly before the assessment of long-term memory, as long as the response has not yet been forgotten (Sullivan, 1982; Rovee-Collier et al., 1987; Rovee-Collier and Hayne, 1987). Second, Rovee-Collier and her colleagues have shown that the kicking response can be remarkably sensitive to changes in aspects of the mobile and/or the context, with maintenance of responding being dependent upon a complete overlap in the cues present during learning and subsequent testing. Even a change in a single element of the mobile or the decoration on the crib liner can lead to dramatic disruptions in performance (Hayne et al., 1986; Borovsky and Rovee-Collier, 1990; Rovee-Collier et al., 1992). These findings provide useful information about the precision of early memory and the specificity of the underlying representations in memory that have been established (*See* Chapter 2.36).

2.37.2.1.2 Imitation-based paradigms

In the elicited and deferred imitation paradigms, memory is demonstrated when an infant is able to use props to reproduce an action sequence that had previously been modeled by an examiner. Consider, for example, the acts involved in constructing a gong: putting a crossbar atop two posts, hanging a metal plate on the crossbar, and then hitting the plate with a plastic mallet. After a baseline period in which a young child interacts freely with these materials, an experimenter demonstrates the sequence that will lead to the construction of the gong one or two times while, under some conditions, providing simple labels for each of the actions. Typically, in the elicited but not the deferred imitation procedure, the modeling of these actions is accompanied by a verbal description of the target actions and the goal of the event sequence. Moreover, in the elicited imitation paradigm, an immediate assessment of memory is typically obtained, with the child being invited to

imitate the modeled sequence of actions: for example, "Now you show me how to make a gong." Memory is usually also assessed after a delay, with and without the verbal cue. In contrast, in the deferred paradigm, imitation is assessed, but without much verbal prompting, and only following a delay. As such, in the deferred imitation procedure, there is no immediate indication of remembering – and hence of initial encoding, or even of whether the child has the motor ability to reproduce the sequence – although control groups have been used to approximate children's ability to imitate the sequences following presentation (see Meltzoff, 1985; Barr et al., 1996). In tests of elicited imitation, children act as their own controls, such that memory is indexed by their better performance with previously modeled versus novel event sequences. It is worth noting that the procedural differences between the deferred imitation and elicited imitation tasks can make a difference in memory performance (e.g., Hayne et al., 2003), with exposure to language cues and the opportunity to imitate the action sequences immediately after modeling facilitating long-term retention.

As previously mentioned, converging evidence from the elicited and deferred imitation paradigms shows that the age-related changes that begin in infancy, to the extent to which information can be held in memory, continue during toddlerhood. For example, 6-month-olds are able to produce parts of a three-step sequence 1 day – but not 2 days – after it is modeled (Barr et al., 1996). Importantly, two features of this demonstration of early recall provide a foundation from which improvement in mnemonic skill can be observed across the first 2 years of life. First, recall at 24 h is dependent upon the amount of experience that the infants have with the modeled action sequence: Approximately two-thirds of the children who had seen the three-step sequence six times produced at least one of the actions 1 day later, whereas the children who had observed the sequence only three times did not differ from control participants who had not witnessed the modeling. Second, there is essentially no evidence that the children can produce the components of the sequence in order, either immediately or after the 24-h delay. In contrast, by 9 months of age, infants are able to recall individual components of novel two-step sequences after 5 weeks (Carver and Bauer, 2001). Approximately half of the 9-month-olds are able to produce the sequences in correct temporal order after a delay of 5 weeks (e.g., Bauer et al., 2003) but not after 3 months. To be sure, this is a period in which skills for remembering change in a dramatic fashion, as illustrated by the fact that by 10 months, children evidence ordered recall at delays of both 1 and 3 months (Carver and Bauer, 2001).

Although this improvement in performance is certainly impressive, it should nonetheless be emphasized that the temporally ordered recall of 9- and 10-month-olds is still rather limited. First, the children's recall is dependent upon multiple exposures to each modeled event sequence. Indeed, as Bauer (2006) indicates, ordered recall at these ages is observed if the infants observe the target sequence on two (and sometimes three) occasions before the onset of the delay. Under these conditions, approximately 45% of the infants evidence ordered recall after 1 month; however, if children view a to-be-remembered sequence at only one session, then these figures drop considerably, with only 7% providing ordered recall (Bauer et al., 2001; Bauer, 2006). Second, the size of the event sequences that are to be remembered is rather small, with 9- and 10-month-olds typically being able to remember two-step events, and third, the length of time over which information can be remembered is quite short.

Each of these limitations is overcome to a considerable extent over the course of the second year of life. For example, by 13 months of age, children no longer need multiple exposures to an event in order to remember it over a delay of several months (Bauer et al., 1995), and yet remembering is clearly enhanced by the opportunity to experience an event sequence several times. In addition, with increases in age, children are better able to remember longer sequences for greater periods of time. To illustrate, in contrast to the two-step events that are remembered by 9- and 10-month-olds, children at 24 months of age can produce sequences of five steps in length (Bauer and Travis, 1993). Finally, the length of time across which ordered recall can be observed increases dramatically during this time period; indeed, 100% of children at 20 months of age are able to recall in an ordered fashion after 1 month, with more than half evidencing memory for portions of the to-be-remembered sequences after delays as long as 1 year (Bauer et al., 2000). For additional information concerning imitation-based approaches to the exploration of young children's memory, see Bauer's recent reviews (2006, 2007).

2.37.2.2 Exploring the Underlying Representation

Research on young children's memory with the conjugate reinforcement and imitation-type paradigms provides information about age-related differences in the conditions under which representations in memory can be established and maintained over time. But what can children's behavior in these two types of situations tell us about the structure and contents of these representations? The conjugate reinforcement procedure is a recognition (as opposed to recall)-based assessment, in which the index of remembering is based on kicking in the presence of a previously experienced stimulus. The specificity of children's responding in these studies – with the response rate dropping off markedly as a function of changes in the mobile or crib context – would suggest that the representation is both detailed and specific. However, even though variation in kicking patterns provides a sensitive indicator of whether or not elements of the mobile or context have changed, the procedure is not informative about the ways in which component features may be organized sequentially in the underlying representation. Yet this type of information is available in the imitation paradigms because responding involves recall, albeit action-based – not verbally based – recall, as opposed to recognition. Admittedly, infants cannot generate long strings of actions, but those that they do produce include the elements of events that are being remembered. Moreover, with increases in age, children's productions become more and more sequentially organized, thus reflecting the structure of the events and the underlying organization of the representation (Bauer et al., 2000). Finally, 1- to 2-year-olds readily apply their prior knowledge to the task of remembering action sequences, as can be seen in their enhanced recall of enabling as opposed to arbitrary sequences (Bauer et al., 2000). With enabling sequences, each action must be performed in a temporally invariant pattern in order to reach the end state (e.g., making a rattle with a ball and a nesting cup by first placing the ball in one-half of the cup and then covering it with the other half before it is shaken); in contrast, in arbitrarily ordered sequences, there are no inherent constraints on the temporal position of the actions (e.g., in making a party hat, it does not matter if a pompom is put on top before a sticker is placed on the front).

2.37.2.3 Bridges to Verbally Based Remembering

Researchers using conjugate reinforcement and imitation-based tasks have provided alternative perspectives on the mnemonic skills of infants, but it is nonetheless clear that these views are complementary and indicate that an impressive memory system is in place before language is available for the encoding and reporting of information. Given these demonstrations of a mnemonic foundation, what can be said about linkages between early nonverbal memory and later verbally based skills for remembering information? At one level, statements about the extent to which young children's early (and rapidly changing) abilities are related to their later verbally based mnemonic skills are quite limited. These statements must be based on longitudinal studies in which children are assessed initially on nonverbal memory tasks and then later on verbally based procedures, and the necessary data have not yet been reported in the literature. At another level, however, questions about linkages between early nonverbal and later verbal memory can be addressed in terms of the types of memory systems that are in place at the two points in time, and from this systems perspective, there may indeed be evidence for developmental continuity. More specifically, a strong claim can be made that the imitation-based tasks tap explicit (as opposed to implicit) memory, and thus line up well with the explicit memory tasks that are employed in assessments of children's abilities to talk about past experiences and prepare for deliberate assessments of memory (Bauer, 1996, 2006).

In order to evaluate this claim, it is necessary to review the distinction between explicit and implicit memory. There certainly are many ways of characterizing memory, but a distinction between explicit (or declarative) and implicit (or nondeclarative) memory is widely accepted (Schacter, 1987; Squire, 1987; Moscovitch, 2000). These two types of memory are thought to differ on many dimensions. For example, in the type of information that is being remembered, in the speed with which it is acquired and lost over time, and in the degree to which remembering involves conscious recollection. To illustrate (and greatly simplify), consider the way in which an experience of visiting a friend may be processed by the explicit memory system. The features of this visit (e.g., names, facts, locations) are rapidly encoded, but specific information can also

be lost over time (and/or replaced in a constructive manner with related information). Yet, in any event, the telling of the tale certainly involves conscious recollection. Now, by way of contrast, consider the way in which a perceptual motor skill – such as driving a car or riding a bicycle – is acquired and represented in implicit memory. These skills require a great deal of practice and are literally honed over longer periods of time; but once mastered, there is little forgetting, and production does not entail conscious recollection. In addition, recent research suggests that in the latter half of the first year, it is possible to differentiate explicit and implicit memory systems structurally, with explicit memory relying on the hippocampus (in particular, the dentate gyrus and other supportive cortical structures), and implicit memory depending on the neostriatum and cerebellum (Eichenbaum, 2003).

From this vantage point, the types of memory that are the focus of this chapter – e.g., a child's report of a recently experienced event or recall of a list of words – would certainly be seen as involving the explicit memory system, but what can be said of the demonstrations of children's nonverbal memory prowess discussed above? To the extent to which any one of the nonverbal tasks used to assess memory in infancy can be viewed as tapping into the explicit memory system, there would be continuity across the nonverbal/verbal divide in terms of memory systems that are in place. In this regard, Bauer (2006, 2007) has argued convincingly that the imitation-based techniques capture the essence of explicit memory. She points out that the infants who are assessed with imitation-based procedures rapidly encode and learn the modeled event sequences, without extensive practice, but also that their memories are fallible, with considerable forgetting over time being observed. Moreover, the memory that is assessed with imitation procedures is clearly rather flexible in that it is preserved (or generalized) across variations in materials contexts. Admittedly, these tasks do not involve verbal reports, and it is impossible to know whether the infants whose performance is assessed experience a sense of conscious recollection, but one other source of evidence is relevant to the argument: Adult humans with amnesia that impairs their performance on explicit memory tasks have been shown to have deficits on the elicited imitation task (McDonough et al., 1995).

In contrast to these procedures, conjugate reinforcement has typically been viewed as reflecting implicit memory (Mandler, 1990, 1998; Schneider and Bjorklund, 1998; but see Rovee-Collier, 1997, and Chapter 2.36, for a contrasting perspective). As indicated in Section 2.37.2.1.1, these tasks are based on operant conditioning procedures, and both operant and classical conditioning have been taken – along with perceptual-motor skills and priming – to be indicators of implicit, as opposed to explicit, memory. Moreover, the contrast between conjugate mobile and imitation tasks can be seen in the basic features of performance: Learning in the conjugate reinforcement task takes a considerable amount of practice before stable levels of kicking are reached, and once the response is acquired, the memory seems to exhibit very high levels of specificity. Indeed, as suggested earlier, even minor changes in the mobile or the context are sufficient to disrupt performance considerably.

Given this view that deferred and elicited imitation tasks involve the same explicit memory system that is activated in verbally based tasks, we would expect that longitudinal analyses would reveal linkages between children's performance on the different procedures. Another reason for this expectation is that the imitation tasks seem to have greater face validity than does conjugate reinforcement, especially in terms of potential links both to language and to event memory as is reflected in assessments of older children's mnemonic skills. For example, although the evidence is admittedly mixed (Bauer et al., 2000; Bauer, 2006), under some conditions young children's elicited imitation is influenced positively by their language skills, and it is known that verbal ability plays a significant role in later events and autobiographical memory (Bauer and Wewerka, 1995; Welch-Ross, 1997; Boland et al., 2003). In addition, the task demands of the elicited imitation procedure seem similar in certain critical respects to those of tasks that are used to explore 2- and 3-year-olds' reports of their previous experiences. More specifically, the conversations between young children and their parents about recently experienced events that will be discussed below involve remembering and subsequently reporting the details of these experiences. As such, both elicited imitation and mother–child reminiscing procedures involve event recall, even though remembering is expressed motorically in one procedure and verbally in the other. Moreover, given that both procedures yield information about children's recollections about the component details of previously experienced events, they, in principle, provide insight into the underlying memory representations.

Given this discussion of conceptual linkages between the elicited imitation and verbally based assessments of children's memory, we turn now to a treatment of children's verbal reports of personally experienced events. In the section that follows, we provide an overview of children's memory for routine and unique experiences and discuss factors that impact developmental changes in remembering.

2.37.3 Learning to Remember

2.37.3.1 Remembering Previously Experienced Events

Starting with the work of Nelson and her colleagues (e.g., Nelson and Gruendel, 1979; see Nelson, 1986, for an overview) on children's memories for familiar and recurring events, the corpus of research on preschool-aged children's abilities to remember their personal experiences has expanded in an impressive fashion. Indeed, in addition to what we now know about children's abilities to produce scripts or generalized event representations for routine experiences such as going grocery shopping or dining at a restaurant, there is now a voluminous literature concerning their abilities to recall the details of specific, distinctive events that they have experienced. In providing a selective treatment of this work, we first discuss research on children's scripts, then review evidence concerning their memory for salient target events, and finally move to a description of studies that have emphasized event memories expressed in parent–child conversations about the past. We do so with a focus on the establishment, maintenance, and modification of event memories, emphasizing the role that knowledge plays in affecting the flow of information through the developing memory system.

2.37.3.1.1 Children's scripts

In their initial studies, Nelson and Gruendel (1981) conducted semistructured interviews with children as young as 3 years of age about what happens during familiar and routine events, such as eating at McDonald's, making cookies, and attending a birthday party. The results of these and later studies (Nelson, 1978; Nelson and Gruendel, 1979; Nelson et al., 1983; Fivush, 1984; Fivush and Slackman, 1986) demonstrate that preschoolers are able to give both veridical and consistent reports of what typically occurs during such events, although certainly older children's scripts are more detailed than those of younger children. Moreover, these script reports reflect the ways in which the events being described are structured in the world, just as the elicited imitation performance of infants studied by Bauer reflected the organization of the action sequences being remembered. To illustrate, in both settings some events are ordered in an enabling fashion, such that each component activity sets the stage for the next activity, whereas other components are arbitrary and variable in their temporal order. For example, in going to McDonald's, one must order food before one can eat it, whereas during a birthday celebration, one must open presents, but this does not have to happen at any particular time during the event. Children as young as 3 years of age are sensitive to these distinctions, recounting activities connected by enabling relations in their experienced order, whereas arbitrary activities are recalled in variable order (see, e.g., Fivush et al., 1992). Equally intriguing, children recount more information about events that are linked by enabling relations than those that are arbitrarily ordered, with some suggestion that this may be true even after the very first experience with the events (Slackman and Nelson, 1984; Ratner et al., 1990; Fivush et al., 1992; Murachver et al., 1996). The linkages between Bauer's elicited imitation studies and this research on verbal scripts lead to the basic conclusion that as early as 12 months of age, children are sensitive to the structure of events in the world, and that their memory reports of those events reflect this structure.

2.37.3.1.2 Memory for salient events

Supplementing research on children's generic representations of recurring events is a considerable body of work on young children's memory for unique personally experienced events. In some studies, children have been exposed to a range of specially crafted stimulus events, such as visiting a pirate (Murachver et al., 1996) or a pretend zoo (McGuigan and Salmon, 2004), whereas in others, the focus has been on naturally occurring routine visits to the doctor and other less familiar and more stressful medical experiences (Merritt et al., 1994; Peterson and Bell, 1996; Goodman et al., 1997; Ornstein et al., 1997a; Burgwyn-Bailes et al., 2001). This literature indicates the presence of substantial age differences in various aspects of memory performance. To illustrate, with increases in age, children demonstrate higher levels of overall recall of these experiences, recount more information in response to

open-ended questions, and thus show less dependence on yes/no questions to elicit memory (e.g., Fivush and Hammond, 1990; Baker-Ward et al., 1993; Ornstein et al., 1997). Moreover, older children evidence less forgetting over time (Brainerd et al., 1985, 1990; Ornstein, 1995) and are less susceptible to suggestive questions (Ceci and Bruck, 1995; Ornstein et al., 1997). Existing evidence also indicates that with age and increased experience in talking about the past, children's reports become more richly detailed and complex and less dependent on information being provided by adult conversational partners (e.g., Fivush et al., 1995; Haden et al., 1997).

In one illustration of this work, Baker-Ward et al. (1993) assessed 3-, 5-, and 7-year-olds' memory for details of a routine pediatric examination. Most children were interviewed two times, first immediately after the check-up and then after a delay of 1, 3, or 6 weeks. The interviews were structured in such a way that they began with open-ended questions (e.g., "Tell me about what happened during your check-up."), followed by more specific questions (e.g., "Did the doctor check any parts of your face?"), and, finally, yes/no probes (e.g., "Did she (he) check your eyes?"). The children were asked yes/no questions both about features that had not been volunteered in response to the open-ended probes as well as regarding activities that had not been included in the check-ups. As illustrated in the top panel (A) in **Figure 1**, even the 3-year-olds were able to report most (approximately 75%) of the features of the event. However, as illustrated in the lower panel (C) in **Figure 1**, there were clear age-related improvements in performance, such that the 7-year-olds reported the greatest number of features (approximately 90%). Moreover, even though the performance of the 3-year-olds was impressive, they nonetheless produced less information than the older children in response to open-ended probes – as shown in the black portion of the bars in the figure – and thus required more specific questions to provide information about the experience. A comparison of the bars across the three panels at each delay reveals that the younger children evidenced more forgetting than the older children over the 6 weeks of the study.

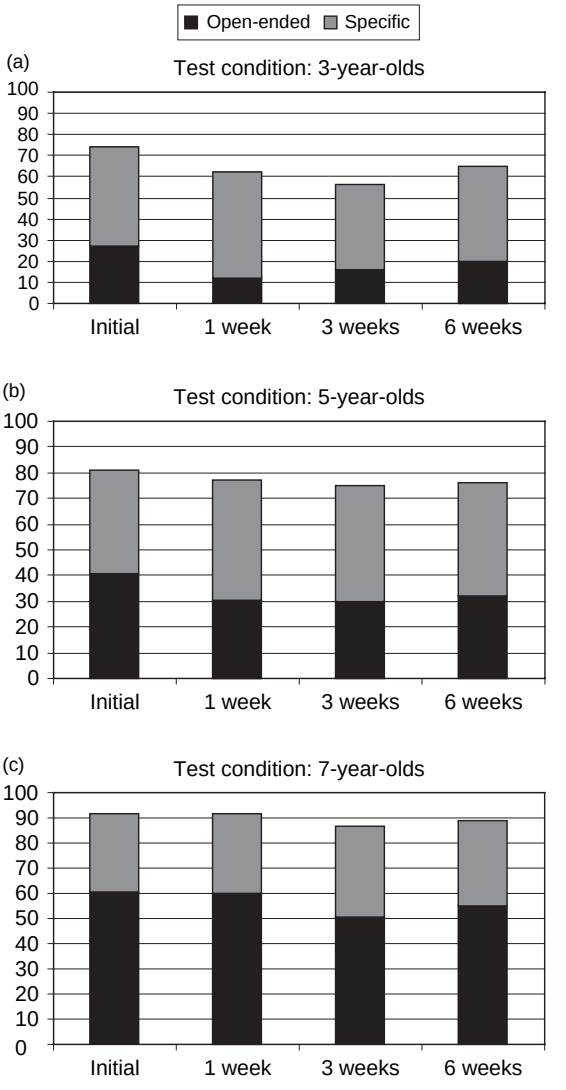


Figure 1 Percent of features correctly reported in response to open-ended and specific probes by test condition at ages 3 (panel A), 5 (panel B), and 7 (panel C) years. Note that the data presented for the initial test are averaged across the three delay groups. Adapted from Baker-Ward L, Gordon BN, Ornstein PA, Larus DM, and Clubb P (1993) Young children's long-term retention of a pediatric examination. *Child Dev.* 64: 1519–1533. Copyright © 1993, Blackwell.

2.37.3.2 The Role of Knowledge

The event memory literature has both challenged earlier views of young children's recall as being quite limited (e.g., Myers and Perlmutter, 1978) and raised important questions about how we are to understand the dramatic age-related changes in remembering in terms of the factors that contribute to the encoding, storage, retrieval, and reporting of information. Children's understanding of the events being experienced is one such factor, as age differences in knowledge can seriously affect the processing and retention of information in memory.

2.37.3.2.1 Prior knowledge

It is well known that prior knowledge enables people to create initial expectations that serve to focus their attention and make inferences that facilitate comprehension, so as to influence what gets into memory (Bjorklund, 1985; Chi and Ceci, 1987; Ornstein et al., 1997). In general, events about which children have significant prior knowledge are more readily encoded and subsequently retrieved than are those about which they have less knowledge. For example, studies that focus on the development of expertise in specific domains (e.g., chess, soccer) have demonstrated repeatedly that the highly organized and accessible knowledge of experts enables them to encode and remember domain-relevant information more effectively than novices (e.g., Chi, 1978; Schneider et al., 1989). In a similar manner, children's scripts (Nelson, 1986) that reflect their understanding of frequently occurring events can markedly affect their later memory of specific instances of these experiences (e.g., Farrar and Goodman, 1990).

An illustration of the impact of prior knowledge on memory can be seen in a reanalysis of the 5-year-olds' recall data from the Baker-Ward et al. (1993) study that was discussed in section 2.37.3.1.2. Clubb et al. (1993) rescored the protocols from the Baker-Ward et al. study to create memory scores representing the proportion of children who recalled each component of the check-up (e.g., blood pressure, eye check, urine specimen) in response to open-ended questions. These memory scores for each component of the office visit at each recall assessment were compared to knowledge scores that were constructed on the basis of interviews with a separate sample of 5-year-olds who responded to questions about what generally happens when they go to the doctor (e.g., "What does the doctor (nurse) do to check you?"). The knowledge scores were therefore based on the proportion of children in the Clubb et al. (1993) sample who nominated each component of the check-up in response to the interviewers' general knowledge probes. Given comparable memory and knowledge scores for individual features of the pediatric check-up, it was possible to determine the degree to which the recall of the 5-year-olds in the Baker-Ward et al. (1993) study could be predicted on the basis of Clubb et al.'s (1993) normative knowledge data.

Inspection of the data plotted in **Figure 2** indicates that there was considerable variability in the memorability of the components of the physical

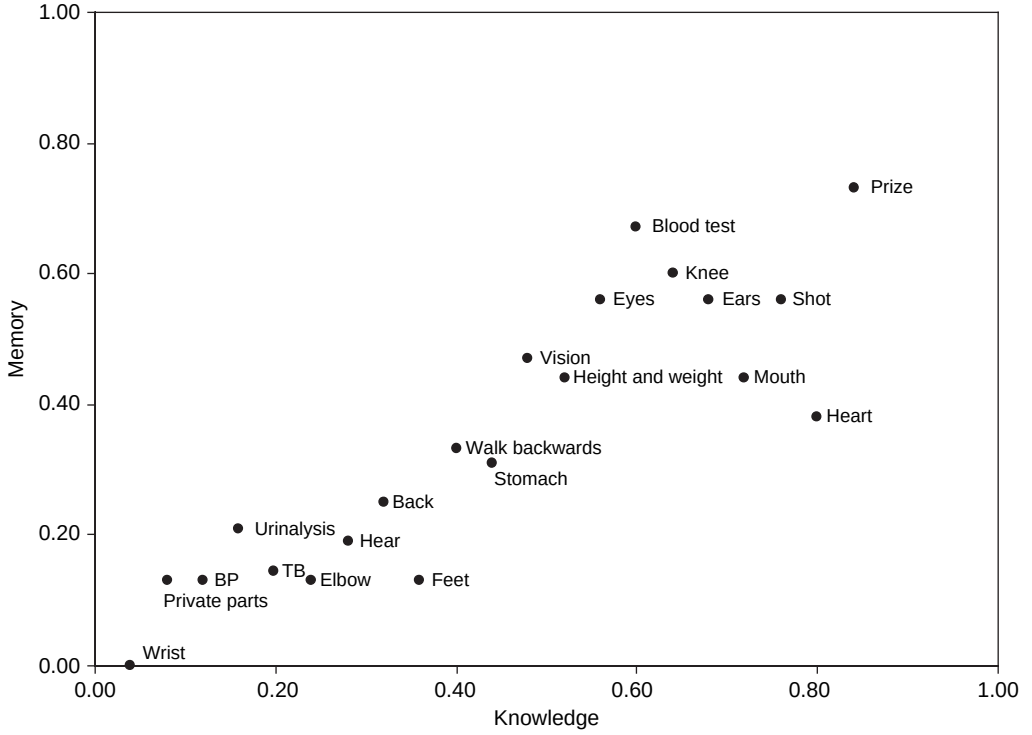


Figure 2 Scatterplot of knowledge and memory scores at 6 weeks for 5-year-olds. Reprinted with permission from Clubb PA, Nida RE, Merritt K, and Ornstein PA (1993) Visiting the doctor: Children's knowledge and memory. *Cogn. Dev.* 8: 361–372. Copyright © 1993, Elsevier.

examination. There was also variability in the children's knowledge of the individual features, but most interestingly, the knowledge and memory scores were highly correlated, indicating that increases in knowledge were associated with corresponding increases in remembering; indeed, the correlations were 0.68, 0.63, 0.64, and 0.74 for the initial, 1-, 3-, and 6-week (shown in **Figure 2**) interviews. These data (see also Ornstein et al., 1997b) and other findings with the subject as opposed to the feature as the unit of analysis (Ornstein et al., 2006a) strongly suggest that what a child knows about an event can seriously affect the extent to which information about the experience is coded and placed in memory.

Although prior knowledge certainly impacts children's memory performance positively, it is important to note that it also can have negative effects. To illustrate, in an effort to explore the extent to which an individual's general knowledge can, over time, lead to alterations in memory for a specific event, Ornstein et al. (1998) studied 4- and 6-year-olds' long-term memory for the details of a specifically constructed physical examination that was both consistent and inconsistent with knowledge-driven expectations. In this experiment, the stimulus event was a mock physical examination carried out by a licensed pediatrician that included some typical medical features (e.g., listening to the heart with a stethoscope) but omitted others that, on the basis of prior knowledge, would have been expected to occur (e.g., checking the mouth). These omitted features, moreover, were replaced by unexpected and quite atypical medical procedures (e.g., measuring head circumference). The findings indicated that prior knowledge had both positive and negative effects on performance. Expected features of the procedures were better remembered than atypical features at all assessments. Most interestingly, 12 weeks after their check-ups, the children made spontaneous errors of commission (i.e., they claimed that they experienced certain medical procedures that had not been administered) and evidenced high rates of false alarms to yes/no questions about medical features that had not been included in their check-ups. The spontaneous intrusions of omitted-but-expected features and the low rates of correct denials to explicit questions about these features that were observed by Ornstein and his colleagues are consistent with the view that the representations of the children's check-ups changed over the 12-week delay interval. More specifically, it would seem that as the children's memory for the check-ups faded over the course of the interval, aspects of their generic event representations for visits to the doctor were incorporated into their specific event representations.

2.37.3.2.2 Knowledge that is acquired during an event

When previously acquired knowledge is lacking, as in a situation in which a child experiences a novel event, facilitative effects can be observed when knowledge is gained while the experience is taking place. For example, Principe et al. (1996) used data from a study of 3- to 7-year-olds' memory for an invasive and novel radiological procedure (a voiding cystourethrogram, or VCUG; Merritt et al. 1994), to look at how the provision of information to children during the event about the stressful and unfamiliar medical procedure affected their subsequent remembering of the experience. Interestingly, the radiological technicians involved in the procedure naturally varied in the extent to which they provided medically relevant information to the children about the experience as it was ongoing. Therefore, whereas some children were provided with a verbal description of the catheter and its insertion, some mention of the contrast fluid going through the catheter, and information about the filling of the child's bladder with this fluid, other children did not receive this procedural narrative. Underscoring the dramatic impact of new knowledge on comprehension and memory, children in the procedural narrative group remembered more details immediately after the exam, as well as 6 weeks later, than children who were not given such a description. These differences could not be attributed to differences among the children in their age or their levels of stress during the procedure and suggest that information that is gained during an unfamiliar and stressful event enhances remembering.

2.37.3.2.3 Changes in knowledge

Once in the memory system, the status of information about an experience can be substantially altered during the period between the event and the later report of it. Indeed, a number of variables can contribute to changes in the representation, including the passage of time (Ornstein et al., 2006a) and intervening experiences (Principe et al., 2000), and these influences may vary as a function of age. Moreover, because knowledge does not remain constant over

time, it is important to ask what happens when knowledge itself changes. Ross (1989) has argued cogently that as memory for the details of an experience fades over time, one tends to 'fill in' on the basis of current understanding and knowledge. One demonstration of the ways in which changes in knowledge over time can lead to alterations in remembering was reported by Greenhoot (2000), who used a series of stories as stimulus material to lead children to develop certain assumptions about the protagonists. Over the course of several sessions, the 5- and 6-year-olds who participated in her study built up a knowledge base about the main story characters and their relationships, and hence the underlying motivation for certain acts that were depicted in the stories. Then, at later sessions, the children were given additional information that prompted some of them to reassess the relationships among the characters (and the motivation for various behaviors) that had been operative. Importantly, Greenhoot showed that the children's memory for prior episodes was distorted in the direction of the new information.

2.37.3.2.4 Recall in conversations about past events

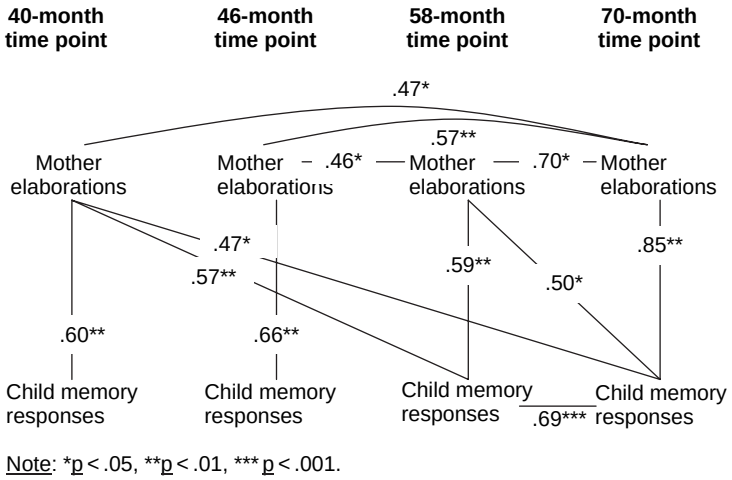
Although much has been learned about children's memory for salient events, a great deal needs to be done to understand how a variety of factors come to together to influence the establishment, maintenance, and modification of representations in memory. In this regard, it is clear that adults have a great role to play in facilitating children's understanding and remembering. Indeed, social–communicative interactions between parents and children provide opportunities for focusing children's attention on salient aspects of an event and thus increasing their understanding and memory, as well as facilitating the acquisition of generalized skills for remembering.

2.37.3.2.5 Parental reminiscing styles

Children begin to talk about past events almost as soon as they produce their first words, and the skills for recalling past experiences in parent–child conversations develop rapidly between 2 and 4 years of age. Nevertheless, as illustrated in this example of a mother and her 18-month-old, when children first begin to reminisce, it is the adult partner who provides most of the content and structure.

Mother: What else happened [at Taylor's house]?
Child: (no response)
Mother: We had dinner. What did you eat?
Child: (goes off task).
Mother: What did you do with Taylor?
Child: Barney.
Mother: Yeah, you watched a Barney video. What else did you do with Taylor? Did you guys fight about something?
Child: (shakes head no).
Mother: No? When you were watching Barney?
Child: (nods head yes).
Mother: Yeah. You guys got hungry and tired. Then what happened?
Child: Uh oh.
Mother: Yeah. What happened? Did you bite Taylor's finger?

A central focus in the literature on parent–child reminiscing has been on the marked individual differences in the reminiscing styles parents use to structure conversations about the past with their young children (see Fivush et al., 2006, for a review). In contrast to parents who use a low elaborative style, those who employ a high elaborative style – such as the mother in the example above – ask many questions, follow-in on their children's efforts to contribute to the conversation, and continue to add new information even when children do not. It is clear that these reminiscing styles generalize across different types of past event discussions (e.g., excursions and holidays, zoo or museum trips, entertainment outings) and tend to be consistent over several years with the same children (Reese et al., 1993) and across different-aged children in the same family (Haden, 1998). Most important, longitudinal data indicate that differences in maternal reminiscing styles are associated with later differences in children's abilities to recall personally experienced events. For example, as illustrated by the lagged correlations in **Figure 3**, Reese et al. (1993) demonstrated that mothers' elaborations during conversations with their 40-month-old children are associated positively with children's contributions of memory information in conversations with their mothers at 58 and 70 months of age. Moreover, the direction of the effect was more from mother to child over time than from child to mother. Although children did influence their mothers to a limited extent, as illustrated in the lower portion of the figure, the correlations for memory responses across age indicate that the children's own earlier skills for verbally recalling events were not directly related to their later abilities.



Note: *p < .05, **p < .01, *** p < .001.

Figure 3 Cross-lagged correlations between maternal elaborations and children's memory responses. Reprinted with permission from Reese E, Haden CA, and Fivush R (1993) Mother-child conversations about the past: Relationships of style and memory over time. *Cogn. Dev.* 8: 403–430. Copyright © 1993, Elsevier.

The finding that the more mothers engaged in highly elaborative talk about the past, the better their children's event memory skills even years later, has been widely replicated both in the United States and cross-nationally (e.g., Hudson, 1990; Flannagan et al., 1995; Welch-Ross, 1997; Haden, 1998; Harley and Reese, 1999; Peterson et al., 1999; Farrant and Reese, 2000; Leichtman et al., 2000; Low and Durkin, 2001; Welch-Ross, 2001; Fivush and Vasudeva, 2002; Bauer and Burch, 2004), such that it seems clear that mothers who are highly elaborative early in development facilitate their children's abilities to report on their past experiences in a detailed manner. Moreover, Peterson et al. (1999) were successful in manipulating mothers' conversational style when talking with their children about previously experienced events, finding that children of mothers who received their intervention produced longer memory reports that contained more details about past events than children of mothers who had not received reminiscing training.

Findings concerning the impact of maternal reminiscing styles on remembering have led to speculation about how early conversations about the past may change the way children organize and represent experiences (Fivush and Haden, 1997; Fivush et al., 2006). Interestingly, it has been suggested that children of mothers who use a highly elaborative reminiscing style may actually come to encode experiences in more richly detailed ways than children of less elaborative mothers, although presently no study has addressed this particular issue. Nevertheless, just as memories may be maintained, elaborated, or even modified through subsequent reminiscing, a growing body of evidence supports the idea that language-based interactions during events can be of critical importance in guiding initial encoding and the establishment of a representation in memory (Tessler and Nelson, 1994; Haden et al., 2001; Boland et al., 2003; McGuigan and Salmon, 2004; Ornstein et al., 2004; Hedrick, 2006). It is to this work that we now turn.

2.37.3.2.6 Conversation during events

The few studies to date that have examined mother–child talk during an event suggest that preschoolers produce longer and more detailed reports of these experiences if their mothers use elaborative Wh-questions and follow-in on and positively evaluate their children's verbal and nonverbal behaviors as events unfold (Haden et al., 2001; Boland et al., 2003; Ornstein et al., 2004). Moreover, joint linguistic interactions between parents and children during events are more strongly related to children's later memory than are interactions characterized as primarily involving mother-only talk, child-only talk, or no-talk (Tessler and Nelson, 1994; Haden et al., 2001). To illustrate, Haden et al. (2001) conducted a longitudinal investigation in which young children took part in three specially constructed activities with their mothers: At 30 months, a camping trip; at 36 months, a bird-watching adventure; at 42 months, the opening of an ice-cream shop. Within the confines of each family's living room, mother–child

interactions during the events were videotaped, providing a precise record of how each dyad interacted – both nonverbally and verbally – with each component feature of the event (e.g., in the camping event, hot dogs, marshmallows, backpack, sleeping bag) as it unfolded.

Given that the majority of features that were interacted with during the events were jointly handled (and thus jointly attended to), Haden et al. (2001) asked whether recall of these components varied as a function of the type of talk (e.g., joint verbal, mother-only verbal, child-only verbal, no verbal) that had been directed toward them during the activities. The children's recall of these experiences after delays of 1 day (upper panel) and 3 weeks (lower panel) is summarized in **Figure 4**. Inspection of the figure indicates the striking effect of joint talk as the events unfolded on the information the children provided in response to the open-ended questions of the interviewers. As can be seen, at both interviews and for each of the activities, the features that were handled and discussed by both the mother and the child jointly (solid bars) were better recalled than those that were jointly handled but talked about only by the mother (gray bars), which, in turn, were better recalled than those not discussed (white bars). Additional analyses indicated further that the features of the event (e.g., a spatula in the camping event) about which questions had been asked by the mothers during the activity that had been responded to by the children (e.g., the mother asks, "What is the spatula used for?" and the child responds "For flipping.") were better recalled than features about which mothers' questions did not result in the children's response (Ornstein et al., 2004). Thus, findings from this longitudinal study – as well as the work by Tessler and Nelson (1994) involving a sample of 4-year-olds – suggest that the nature of mother–child interaction as an event unfolds influences encoding and subsequent remembering.

Experimental work also supports this conclusion. For example, Boland et al. (2003) trained some mothers to use four specific conversational techniques to enhance their children's understanding of unfolding events: (1) Wh- questions to elicit their child's linguistic participation in the activity, (2) associations to relate that which was being experienced to what their child already knew, (3) follow-ins that

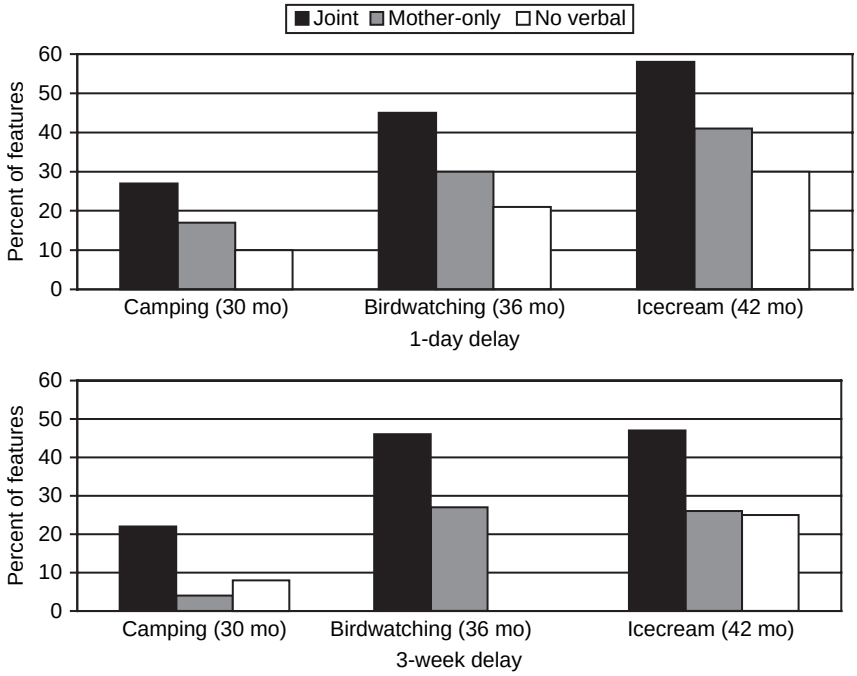


Figure 4 Proportion of features of the camping, bird-watching, and ice-cream-store events remembered in response to open-ended questions at the 1-day and 3-week interviews, as a function of the type of talk directed toward jointly handled features. Adapted from Haden CA, Ornstein PA, Eckerman CO, and Didow SM (2001) Mother-child conversational interactions as events unfold: Linkages to subsequent remembering. *Child Dev.* 72: 1016–1031. Copyright © 2001, Blackwell.

encouraged discussion of aspects of the event in which the child was showing interest, and (4) positive evaluations to praise their child's verbal and nonverbal contributions to the interaction. After this instruction, when observed engaging with their 4-year-old children in the context of the camping event, trained mothers produced significantly more of all four of the targeted conversational techniques than did untrained mothers. Moreover, the effects of the training did not vary as a function of the children's language skills and did not impact the mothers' use of untrained techniques (i.e., repetitions, yes-no questions, and statements). Of even greater interest, the children's recall of information about the camping event was affected by the training that their mothers received. For example, the children of trained mothers exceeded those whose mothers had not received training in the production of details of the event.

Given these demonstrations of the importance of adult–child talk for children's memory performance, interesting questions concerning the potentially additive effects of conversations about the present and the past on remembering are currently being explored (McGuigan and Salmon, 2004, 2005; Conroy, 2006; Hedrick, 2006). Moreover, relatively few studies have considered how talk prior to an event may set the stage for the development of children's representations (Hudson, 2002) and the linkages between children's performance in these event memory tasks and their subsequent use of deliberate techniques for remembering (Haden et al. 2001; Lange and Carroll, 2003). We turn now to a treatment of children's developing skill in the use of these mnemonic strategies.

2.37.4 Learning to Be Strategic

As young children develop expertise in talking about their past experiences, they also evidence increasing skill in the use of strategies for remembering information. To a considerable extent, their growing competency in discussing the past reflects age-related improvements in the incidental encoding of information – which in turn stem from children's greater understanding of the situations that they encounter – as well as improvements in retrieving and reporting information from memory. In contrast, however, the deployment of a specific strategy for remembering – such as naming or grouping – represents intentional preparation in the service of an expected assessment of memory (Ornstein et al., 1988; Wellman, 1988; Folds et al., 1990). Given this distinction between incidental and deliberate remembering, it is interesting that even young preschoolers can demonstrate "strategic" behavior under certain circumstances. For example, when asked to remember the location of a familiar stuffed animal that was hidden in a room, 18-month-olds utilized a number of rudimentary strategies (pointing, peeking, and naming) so that the toy could be retrieved after a delay (DeLoache et al., 1985). Although the deployment of these strategic behaviors was not unambiguously related to the memory performance, these responses to a memory request do indeed suggest that children enter the preschool years with a basic understanding that remembering requires action of some sort. Nonetheless, interpretation of this finding is complicated by the fact that similar behaviors are also exhibited – but to a lesser extent – in a variation of the hide-and-seek game in which remembering is not required (DeLoache et al., 1985, Experiment 3). Consistent with Wellman's (1988) treatment of intentionality, these early mnemonic skills can be viewed as protostrategies that emerge during enjoyable activities in highly salient and meaningful situations and may not necessarily be related to later strategy acquisition (see also Ornstein et al., 1988; Folds et al., 1990).

Older preschoolers may have a firmer understanding of the need to do something in order to prepare for an assessment of memory, but the effectiveness of their efforts is analogous to that of the 18-month-olds studied by DeLoache et al. (1985). Consider, for example, a study by Baker-Ward et al. (1984) in which 4-, 5-, and 6-year-olds made use of a set of similar techniques in a memorization task with common objects. These children were directed to interact with a set of objects and toys for a 2-min period and were placed in one of three conditions: Target Remember, Target Play, and Free Play. The children in the Target Remember condition were told that they could play with all of the objects but that they should try especially to remember a subset of the items (i.e., the target objects). In contrast, the participants in the Target Play group were given instructions that did not mention remembering but rather stressed playing with a subset of the target objects, whereas those in the Free Play condition were given general play instructions.

The use of an observational coding scheme during the activity period revealed that even at age 4, the children who were told to remember behaved

differently from those in the play conditions. For example, as can be seen in **Figure 5**, spontaneous labeling or naming occurred almost exclusively among the children in the target remember condition who were instructed to remember a subset of the objects, and it was found that these children also played less than the children in the free play and target play conditions. Moreover, as can be seen in **Figure 6**, the children who received instructions to remember also engaged in more visual inspection and evidenced more unfilled time than the children in the two play conditions. Unfilled time was coded when a child was not paying direct attention to the items but nonetheless did not seem to be off-task; informally, it seemed to involve reflection and self-testing. The memory instructions thus engendered a studious approach to the task among the 4-, 5-, and 6-year-olds alike, but it is important to note that only among the 6-year-olds were the strategic behaviors associated with higher levels of recall.

The literature now contains many demonstrations of what Miller (1990; see also Bjorklund and Coyle, 1995; Bjorklund et al., 1997) has termed utilization deficiencies in young children who are just beginning

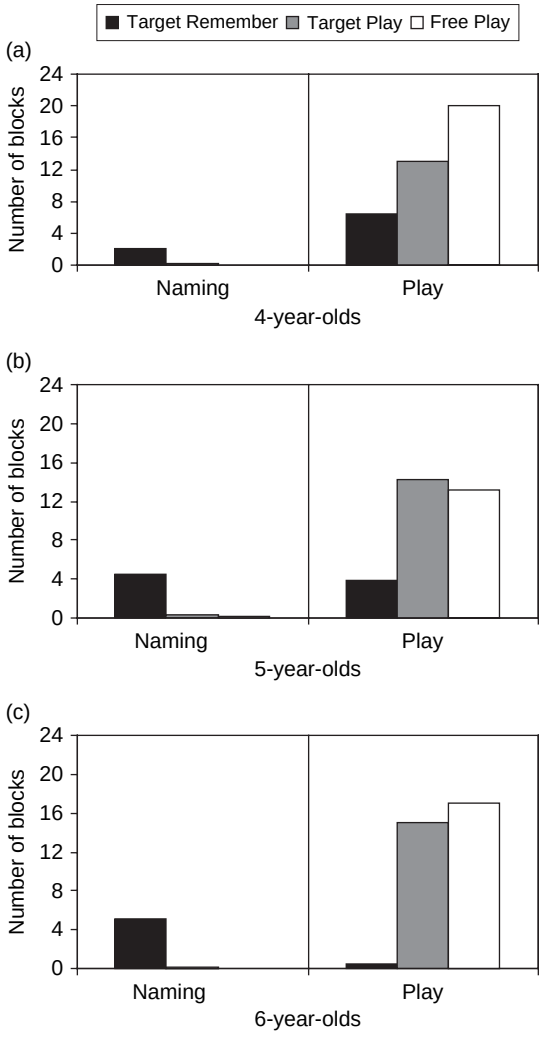


Figure 5 Mean number of 5-s blocks of the activity period in which each naming and play occurred for the 4-year-olds (panel A), 5-year-olds (panel B), and 6-year-olds (panel C) in each instructional condition. Adapted from Baker-Ward L, Ornstein PA, and Holden DJ (1984) The expression of memorization in early childhood. *J. Exp. Child Psychol.* 37: 555–575. Copyright © 1984, Elsevier.

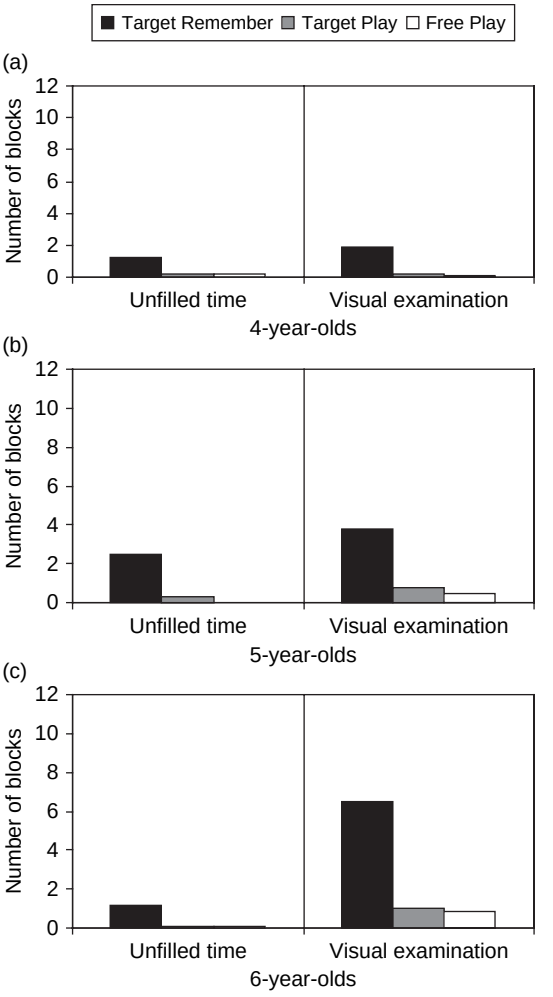


Figure 6 Mean number of 10-s blocks of the activity period characterized by unfilled time and visual examination for the 4-year-olds (panel A), 5-year-olds (panel B), and 6-year-olds (panel C) in each instructional condition. Adapted from Baker-Ward L, Ornstein PA, and Holden DJ (1984) The expression of memorization in early childhood. *J. Exp. Child Psychol.* 37: 555–575. Copyright © 1984, Elsevier.

to generate strategies in response to memory goals. As in the Baker-Ward et al. (1984) study, strategies are produced spontaneously, but they do not seem to initially correspond to improvements in the amount recalled. Why should this be the case? If Baker-Ward et al.'s 4-, 5-, and 6-year-olds were engaging in the same strategic behaviors, why did only the efforts of the 6-year-olds have a positive effect on their recall? Moreover, why should similar strategic activities differ in their mnemonic effectiveness? Of course, it is possible that even though the observable behaviors (e.g., naming, visual inspection) of the 4-, 5-, and 6-year-olds were similar, they may have been the external manifestation of quite different underlying strategies. As such, the similarity across age in strategic efforts may be illusory, with, for example, the children of different ages combining the observable behaviors into qualitatively different strategies. That this may have been the case is suggested by Baker-Ward et al.'s observation that the younger children seemed to combine verbal naming or labeling and manipulation, whereas the older children put naming together with visual examination. It thus seems worthwhile to develop higher-order coding schemes to capture these age-related changes adequately in the coordination of different mnemonic behaviors. Efforts of this kind may well result in more precise definitions of effective mnemonic techniques, but it is also possible that such fine-grained analyses will still leave open questions about the conditions under which the application of strategies may and may not impact remembering. As discussed here, there may be other factors – for example, age related changes in underlying knowledge (Bjorklund, 1985), speed of processing (Kail, 1991), and the effort requirements of strategy usage (Guttentag, 1984; Case, 1985) – that may influence whether or not a given strategy influences remembering.

At the very least, this brief treatment of early strategies that do not work serves to highlight the fact that intentionality is only one aspect of strategic behavior and that two others – consistency and effectiveness – must be considered in any account of the development of memory. This is especially the case when it is recognized that the developmental course of children's mastery of mnemonic skills extends through the end of the elementary school years. In terms of consistency, skilled strategy users have command over a broad repertoire of mnemonic techniques (e.g., rehearsal, organization, elaboration) and are able to apply them skillfully across a broad set of situations that call for remembering (Brown et al., 1983; Ornstein et al., 1988; Pressley et al., 1989). In contrast, novice strategy users not only have a limited set of techniques at their disposal, but the very application of any given procedure is often quite context-specific and not consistent across settings. Indeed, when young children are able to demonstrate sophisticated strategy use, it typically is only in highly supportive and salient settings (Ornstein et al., 1988; Ornstein and Myers, 1996). Moreover, in terms of effectiveness, work on utilization deficiencies, discussed above, indicates that the strategic efforts of young children often do not facilitate remembering. In addition, however, even when the application of strategies does influence recall, younger children may derive less benefit than do older children (Ornstein et al., 1988; Wellman, 1988; Folds et al., 1990).

Given these complexities, longitudinal data are necessary to track on a within-individual basis developmental progress in the acquisition and deployment of strategies for remembering. Ideally, young children's increasing sophistication in the use of these techniques would be charted over time with multiple indicators of strategic competence, under conditions that vary in terms of their effort and attentional demands. Data from microgenetic research designs (Siegler and Crowley, 1991; Schlagmüller and Schneider, 2002) in which children are followed intensively for limited periods of time are also very useful for developmental analyses of mnemonic skill. Unfortunately, longitudinal and microgenetic research designs are still quite rare in the area of memory development, and our understanding of age-related changes in strategy usage stems largely from the (admittedly rich) cross-sectional literature. To illustrate strategy development, we focus here on cross-sectional studies in which an age-related progression from passive to active memorization styles has been demonstrated (Ornstein and Naus, 1985; Ornstein et al., 1988).

2.37.4.1 Rehearsal and Organizational Strategies in the Elementary School Years

A comparison of children's performance on tasks that assess memory for personally experienced events and those that require deliberate remembering reveals substantial differences in the levels of demonstrated sophistication. Indeed, by 8 or 9 years of age, children are very adept at providing rich reports about their experiences, but at the same time their skills appear to be quite limited in situations that call for the deployment of complex deliberate mnemonic strategies.

To illustrate the relatively late emergence of these deliberate memory skills, consider the substantially different ways in which 9- and 14-year-olds behave when given a list of words to remember and are prompted to talk aloud as the items are presented. In this type of overt rehearsal task, 9-year-olds tend to rehearse each to-be-remembered item alone as it is displayed, whereas older children (and adults) rehearse each one with several previously presented stimuli (Ornstein et al., 1975; Ornstein and Naus, 1978). To illustrate, if the first three items on a to-be-remembered list are table, car, and flower, a typical third grader would rehearse table, table, table, when the first word is shown; car, car, car, when car is presented; and flower, flower, flower, when the third word is shown. In contrast, the average 14-year-old is likely to rehearse table, table, table, when table is presented; table, car, table, car, when car is presented; and table, car, flower, when flower is displayed. These children thus differ considerably in the extent to which rehearsal is limited (or passive) versus more cumulative (or active), and these differences in rehearsal style are related to substantial differences in recall. Indeed, with increases in age, not only does rehearsal become more active – with several different items being intermixed – but recall improves dramatically, especially that of the early list items. That is, children's increasingly active rehearsal styles are associated with improved recall of the primary section of the serial position curve (Ornstein et al., 1975).

These changes in the use of rehearsal are paralleled by comparable developments in the deployment of organizational strategies for remembering. Consider, for example, the performance of third and fourth graders on a sort-recall task in which they are given a set of low-associated words (or pictures) and asked to "form groups that will help you remember." Under these conditions, in which the items are sorted prior to each recall trial, children as old as 9 years of age tend not to form groups on the basis of semantic relations among the to-be-remembered materials but, rather, establish what seem to be randomly arranged groupings that vary considerably over trials (Liberty and Ornstein, 1973; Bjorklund et al., 1977). In dramatic contrast, children aged 12 and older routinely establish groups that are semantically constrained, even though the memory instructions do not prompt sorting on the basis of meaning. These older individuals seem to have the metacognitive understanding that sorting on the basis of meaning will facilitate recall, readily translating a remembering instruction into one that involves a search for a meaning-based organization (Ornstein et al., 1974). Moreover, consistent with the rehearsal literature, these age differences in the extent to which sorting is driven by the semantic organization of the materials are associated with corresponding differences in recall. However, it should be emphasized that younger children's failure to use a meaning-based grouping strategy does not imply that they lack understanding of the semantic linkages among the items, as they can readily sort even low-associated items on the basis of meaning when instructed to do so (Bjorklund et al., 1977; Corsale and Ornstein, 1980). As such, the age differences in performance would seem to reflect age differences in understanding how underlying knowledge can be applied strategically in the service of a memory goal.

2.37.4.2 Context Specificity in Strategy Development

These age-related differences in rehearsal and sorting represent a sampling from a now-extensive literature on children's developing skills in the use of a variety of strategies for remembering (Schneider and Pressley, 1997; Schneider and Bjorklund, 1998; Bjorklund et al., in press). Although the bulk of this literature is composed of cross-sectional studies, it is nonetheless clear that with increases in age there are corresponding increases in rehearsal, organization, elaboration, and other techniques that influence the encoding, storage, retrieval, and reporting of information. Further, demonstrations of the ways in which older and young children differ in the deployment of mnemonic strategies have been supplemented by training studies so as to document causal linkages between strategy use and remembering. To illustrate, the provision of minimal instructions to rehearse several items together is sufficient to increase the recall of younger children, and prompts to rehearse each item on a list alone or in relative isolation can reduce the recall of older children (Ornstein et al. 1977; Ornstein and Naus, 1978). Similarly, when young children are required by a yoking procedure to follow the more semantically constrained sorting pattern of older children or adults, their recall is facilitated, and when adults are yoked to these sorts of young children, their recall is reduced (Liberty and Ornstein, 1973; Bjorklund et al., 1977). Children's sorting of low-associated materials can also be manipulated – with corresponding effects on their remembering – by simply instructing them to sort on the basis of

meaning (Bjorklund et al., 1977; Corsale and Ornstein, 1980) or by exposing them to materials that are highly organized (Best and Ornstein, 1986).

It is thus clear that there are causal linkages between children's strategic efforts and their recall performance. However, it is also the case that there are limits to the success of these experimental interventions that shed light on other factors that contribute to effective strategy production. For example, although third graders can follow instructions to rehearse several items together, their use of such an active rehearsal strategy does not increase their recall to the level of sixth graders (Ornstein et al., 1977). This failure to eliminate age differences in remembering most likely stems from the fact that the use of an active rehearsal strategy requires that young children expend more of their attentional resources than is necessary for older children (Guttentag, 1984). Consistent with Guttentag's observation that the attentional demands of an active rehearsal strategy may vary at different points in development, it certainly is easier for young children to rehearse several items together when the effort demands of the task are reduced. Thus, for example, when instructions to rehearse actively are combined with a procedure in which children have continued visual access to each to-be-remembered item after its initial presentation, striking improvements in strategy use and subsequent recall are noted (Ornstein et al., 1985). Although effort demands are also important in the context of organizational strategies (see Bjorklund and Harnishfeger, 1987), when children of different ages have comparable understanding of the to-be-remembered items and are led by instructions to use this knowledge as a basis for their sorting, recall differences are generally eliminated (Corsale and Ornstein, 1980).

Although context can certainly affect the outcome of training manipulations, it can also influence the degree to which children will engage spontaneously in strategic activities, as well as the sophistication of their efforts. To illustrate, the manipulation mentioned above to reduce the effort demands of an instructed active rehearsal strategy – permitting children to view all previously presented items – has been shown to increase the likelihood of spontaneously making use of a cumulative rehearsal strategy. Indeed, Guttentag et al. (1987) observed that some third graders who typically rehearsed passively when the to-be-remembered items were presented in the typical fashion changed to a multi-item rehearsal strategy without prompting when given an opportunity to continue to see all items. In addition, consistent with the finding discussed above that prior knowledge can impact children's reports of previously experienced events, knowledge and understanding of the materials can dramatically influence children's use of mnemonic strategies when acting in the service of a memory goal (Bjorklund, 1985; Ornstein and Naus, 1985). Indeed, increases with age in the contents of the knowledge base and the ease with which stored information may be accessed have serious implications for the deployment of strategies. What a child knows about the items to be remembered can certainly determine just what can – and cannot – be done strategically with those materials. At one extreme, a child may not be able execute a grouping strategy at all if he or she does not know the category structure of the to-be-remembered materials, but even when children are familiar with the meaning of the materials, they may nonetheless appear to be strategic when given some items to remember and nonstrategic with others (Ornstein and Naus, 1985; Ornstein et al., 1988).

These demonstrations of the impact of the materials on remembering have led to the suggestion that the children's first expressions of deliberate memorization will be observed when they are presented with highly meaningful materials in very salient contexts (Ornstein et al., 1988). This was illustrated above in the discussion of Baker-Ward et al.'s (1984) study in which 4-, 5-, and 6-year-olds could interact with a set of toys and objects under remember- or play-based instructions. With these very salient materials, the 4-year-olds engaged in strategic behaviors when told to remember, even though their efforts did not facilitate remembering. In addition, although it is known that third graders do not sort low-associated items in terms of their underlying meaning when told to "form groups that will help you remember" (Bjorklund et al., 1977), as discussed earlier, when given more salient, categorically related items, they will readily group on the basis of meaning when given the typical memory-based instruction (Corsale, 1978). It seems likely that the saliency of the category structure is so powerful that it is difficult not to sort in an organized manner. Similar effects of the dependence of memory strategies on the stimulus properties of to-be-remembered materials can be seen in Tarkin's (1981; see also Ornstein et al., 1988) exploration of third graders' use of rehearsal strategies. Further, it is likely that the increasing articulation of the knowledge system with age and experience may facilitate information retrieval and thus reduce the amount of

attentional effort required to implement various subcomponents of memory strategies (Ornstein et al., 1988).

2.37.4.3 The Development of Effective Strategy Use

With increases in age, the context specificity that characterizes early strategy use is reduced, as children extend the range of settings in which they behave in a strategic fashion (Ornstein et al., 1988). In parallel with this generalization of strategy use, their strategic efforts become increasingly effective in facilitating remembering. Two features of this change in the efficacy of children's strategic efforts are discussed briefly, followed by a treatment of some of the factors that may underlie these age-related changes.

First, as already indicated, there are substantial age-related changes in what children actually do when confronted with a memory goal. Younger children, for example, are more likely than older children to select strategies that are inappropriate for a task, as, for example, when preschoolers implement deliberate strategies that do not facilitate performance in any way (see Wellman, 1988, for a treatment of faulty strategies). Moreover, when children of elementary school age are asked to remember verbal materials, there is a general progression from more passive, rote-type mnemonic techniques to more active strategies that seem to involve deliberate efforts at integrating the material being remembered with existing knowledge. Further, with increases in age, children have increased numbers of strategies at their disposal and are better able to make flexible use of this mnemonic repertoire (Folds et al., 1990; Schneider and Bjorklund, 1998). Second, even when the same strategy appears to be employed by children of different ages, the technique typically has a more facilitative effect on the memory of older as opposed to younger children. As indicated above, Baker-Ward et al. (1984) showed that 4-, 5-, and 6-year-olds all utilized strategies when trying to remember a set of objects, but these techniques only facilitated the memory performance of the oldest group of children. These data – and other demonstrations of utilization deficiencies (Miller 1990; Bjorklund and Coyle 1995) – again show that even very young children may be aware of the importance of implementing a strategy in the service of remembering, but that the strategies that they nominate may be largely ineffective.

2.37.4.4 Factors Underlying Developmental Changes in Strategic Memory

In an effort to understand changes in strategy deployment and effectiveness, we turn now to a brief treatment of several factors that may impact children's use of strategies and serve as mediators of the observed age-related progression: (1) Changes in the underlying knowledge base in memory, (2) reductions in the effort requirements of strategy implementation, (3) increases in metamnemonic understanding, and (4) experiences in formal schooling.

2.37.4.4.1 Prior knowledge

As discussed earlier in our treatment of knowledge and event memory, changes with age in both the content and structure of children's underlying knowledge in permanent memory can influence dramatically the flow of information within the memory system and thus affect overall performance (Chi, 1978; Bjorklund, 1985; Ornstein and Naus, 1985). Moreover, in recent years there has been a consensus among memory researchers that both knowledge and strategy use contribute in important ways to the development of children's deliberate memory skills (Ornstein et al., 1988; Muir-Broaddus and Bjorklund, 1990) and recognition that under some conditions, the impact of the knowledge base may be mediated by its effects on strategy implementation (Ornstein and Naus, 1985; Rabinowitz and McAuley, 1990). This emerging perspective emphasizes the impact that the current state of a child's knowledge system may have on both strategy selection and execution (Ornstein et al., 1988; Folds et al., 1990). Indeed, as illustrated previously in our treatment of context specificity, young children may appear to be quite strategic in supportive settings when presented with highly salient and meaningful sets of materials, but they may seem to be much more tentative, and even astrategic, when presented with less structured items.

How should these demonstrations of context specificity in strategy use be interpreted? As mentioned previously, one explanation for the sometimes dramatic differences in the performance of young children under contrasting task demands is that they may not have sufficient knowledge about the materials to carry out appropriate strategies. Indeed, knowing the meaning and categorical structure of a set of words is a necessary, albeit not sufficient, prerequisite for implementing a semantically based clustering strategy. A second explanation focuses on the beneficial effects of knowledge on the efficiency

of mnemonic processing. With increases in age and experience, the knowledge system becomes increasingly articulated, with rich interconnections among items, thereby contributing to the ease of access that is needed for the skillful execution of strategies such as active rehearsal and meaning-based sorting (Bjorklund, 1987; Ornstein et al., 1988; Bjorklund et al., 1990). Interestingly, these developments in the underlying knowledge base – with the increased likelihood of the automatic activation of strong associative links – may thus make young children's strategic efforts not entirely deliberate (Lange, 1978; Bjorklund, 1985). At the same time, however, these associative links may increase the efficiency and effectiveness of the strategy use of older children. Finally, these developments in the knowledge system may contribute to age-related increases in the likelihood that children will spontaneously use their underlying knowledge strategically when confronted with memory goals (Corsale and Ornstein, 1980).

2.37.4.4.2 Effort requirements of strategy use

Age-related changes in the effectiveness of children's strategies may also reflect corresponding differences in the attentional resources that are needed for the execution of mnemonic techniques. If one assumes a type of tradeoff between the processing and storage operations that are involved in carrying out any given cognitive task (Case, 1985), then in the early stages of acquiring a skill such as cumulative rehearsal, strategy execution may so tax the limited capacity system that little remains to be allocated to encoding and storage processes (Bjorklund and Harnishfeger, 1987). Consistent with this perspective, a child may be able to deploy a given memory strategy under some conditions, but the effort required to do so may be so great that the strategy does not actually facilitate performance. Indeed, as indicated earlier, Guttentag (1984) demonstrated that second graders may be capable of producing an active rehearsal strategy when so instructed, but that their deployment of this technique is more demanding of their limited capacity than is the case for older children or adults (see also Bjorklund and Harnishfeger, 1987; Kee and Davies, 1988). As suggested above, evidence consistent with this finding was reported by Guttentag et al. (1987), who found that some children who typically rehearsed passively were able to change to active rehearsal when the resource demands of this more complex strategy were reduced.

If we assume that young children may expend more of their cognitive resources on the processing component of strategy execution than older children, what factors underlie improvements with age in processing efficiency? Three possibilities can be mentioned. First, speed of information processing (e.g., Kail, 1991) increases markedly across the elementary school years, a change that is largely the result of maturation. Second, as indicated earlier, the development of the knowledge base – in terms of the greater coherence of the semantic network and increased ease of accessibility – may also contribute to increases in efficiency of strategy execution (Bjorklund, 1987). Third, the functional capacity of the system may increase with age because specific aspects of a task may come to require fewer resources, reflecting the increased automatization of skill that is associated with experience and practice (Case, 1985; Ornstein et al., 1988; Siegler, 1996).

2.37.4.4.3 Metamemory

It is well documented that with increases in age, there also are changes in children's metamemory, that is, in their understanding of the demands of various memory tasks and of the operation of the memory system (Flavell and Wellman, 1977; Cavanaugh and Perlmutter, 1982; Schneider, 1985). It must be indicated, however, that even though metamemory figures prominently in accounts of mnemonic growth (e.g., Cavanaugh and Borkowski, 1980; Schneider, 1985), the results of correlational studies in which both memory and metamemory have been assessed have been quite mixed. Examples of some of the difficulties encountered in providing evidence for the proposed linkage between metamnemonic understanding and strategic deployment and effectiveness include cases in which children can articulate awareness of a memory strategy but nonetheless fail to actually use it in practice (Sodian et al., 1986), and in contrast, situations in which children use what might be viewed as a deliberate strategy but are unable to demonstrate any corresponding metamnemonic knowledge (Bjorklund and Zeman, 1982). On the other hand, both early training studies in which strategy instruction was supplemented by the provision of metamnemonic information (e.g., Paris et al., 1982), and more recent studies involving improved methods of assessing young children's understanding (e.g., Schneider et al., 1998; Schlagmüller and Schneider, 2002), provide convincing empirical evidence for the linkage between metamemory and memory development. For example, in a short-term

longitudinal study, Schlagmüller and Schneider (2002) reported that children who acquired an organizational strategy over the course of the project actually showed increases in declarative metamemory well ahead of actually exhibiting the strategy.

2.37.4.4.4 Schooling

A number of lines of evidence lead to the inference that formal schooling may contribute to the development of children's increasing skill in the use of memory strategies. Consider, for example, comparative cultural investigations in which the performance of children who were matched in chronological age but who differed in terms of whether they had or had not participated in Western-style schooling have been contrasted. In studies carried out in Liberia (e.g., Scribner and Cole, 1978), Mexico (e.g., Rogoff, 1981), and Morocco (e.g., Wagner, 1978), children who attended school demonstrated superiority in the types of mnemonic skills that have typically been studied by Western psychologists and anthropologists. To illustrate, Rogoff (1981) reported that nonschooled children generally do not make use of organizational techniques for remembering unrelated items and that school seemed necessary for the acquisition of these skills. These findings, of course, do not in any way imply that schooled children outperform their unschooled peers on everyday memory tasks that are embedded in activities central to their culture. Nonetheless, they do suggest that something in the formal school context most likely is related to the emergence of skills that are important for success on tasks that involve deliberate memorization.

With comparative-cultural research indicating that something about formal schooling encourages the development of strategic behavior, the next question might be, When during a child's experience in school does this growth occur? First grade seems to be a strong possibility, as Morrison et al. (1995) showed that this grade is very important in terms of the development of memory skills. Morrison and his colleagues studied children who just made the mandated date for entry into first grade (a young first-grade group) and those who just missed the date (an old kindergarten group). As such, the children were basically matched in terms of age but nonetheless differed in their school experience, thus allowing for a comparison between a first-grade school experience and a kindergarten experience. To assess memory, Morrison et al. (1995) used a task (adapted from Baker-Ward, 1985) in which the children were asked to study a set of pictures of common objects. Taking performance at the start of the school year as a baseline, the young first graders evidenced substantial improvement in their memory skills. In contrast, the performance of the older kindergartners did not change over the year, although improvement was noted the next year, following their experience in the first grade. These findings imply that there is something in the first-grade context that is supportive of the development of children's memory skills. The potential importance of the first-grade experience is also suggested by Baker-Ward et al.'s (1984) finding, discussed above, that the strategic efforts of 4-, 5-, and 6-year-olds only facilitated the memory performance of the older children.

Given that the evidence points to formal schooling as a mediator of children's strategy development, Ornstein et al. (in press) have carried out a series of studies to characterize memory-relevant behaviors that teachers use that may support children's deliberate memory skills. Some of their findings are consistent with Moely et al.'s (1992) important report that it is quite rare to find explicit instruction in mnemonic techniques by teachers throughout the elementary school grades. However, even though mnemonic strategies are not generally taught by teachers in an explicit fashion, Ornstein and his colleagues find that first-grade teachers engage in a variety of memory-relevant behaviors in the course of whole-class instruction, including indirect requests for deliberate remembering, strategy suggestion, and metacognitive questioning. Moreover, children in first-grade classes taught by teachers who use more of this sort of memory-related language show a greater ability to take advantage of strategy training (meaning-based sorting and clustering in recall according to semantic categories) than those children with low-mnemonically oriented teachers (Coffman et al., 2003; see Moely et al., 1992, for similar results). In addition, teachers' mnemonic style in the first grade is linked to the organized sorting patterns on a sort-recall task with low-associated items that was administered to the children 3 years later, such that fourth graders who had been taught by high-mnemonic first-grade teachers sorted more semantically than did their peers who had been taught by low-mnemonic first-grade teachers. As such, this work suggests that just as 'parent talk' about events can impact preschoolers' developing abilities to remember (e.g., McCabe and Peterson, 1991; Reese et al., 1993; Haden et al., 2001; Boland et al., 2003), 'teacher talk' may also be relevant for the emergence and refinement of mnemonic skills.

2.37.4.5 Determinants of Performance and Development

The research on children's strategy development reviewed here suggests that knowledge, effort, metamemory, and schooling can be viewed as mediators of the performance of children at any given age. These determinants of memory performance may also underlie developmental changes in strategic deployment and effectiveness. Changes with age in children's knowledge of the materials being remembered, the cognitive effort they need to execute tasks that involve remembering, and their understanding of the operation of the memory system all can contribute to age-related increases in strategic effectiveness. However, we attach special status to schooling as a potential mediator of change because the available evidence suggests that school represents a critical context for the emergence and consolidation of children's mnemonic efforts. Further, as suggested in our discussion of schooling, it seems likely that teacher–child conversation in the classroom is of great relevance for the development of a repertoire of strategies.

2.37.5 Exploring the Development of Memory

The research literature reviewed here provides a picture of the quite remarkable mnemonic competence of young children, as well as clear age-related differences in many aspects of memory performance. Much is thus known about memory development, that is, the memory skills of children of different ages, but much less is known about the development of memory in the sense of understanding the ways in which early instantiations of skill give way to later competencies, and the factors that serve to explain these changes. It also must be admitted that even understanding of memory development, while substantial, is nonetheless limited and that much remains to be learned about children's skills at various ages.

Why are there substantial gaps in what we know about memory and its development? To a considerable extent, the problem stems from the methodological choices typically made by researchers. Consider first the paradigm-driven nature of work on children's memory. Most studies of memory deal with remembering in the context of one or another task (many of which have been discussed here), and as a result, relatively little is known about linkages across tasks that vary in terms of their processing demands and other important characteristics. Yet this is exactly the type of information that is necessary to characterize adequately children's skills at any given age and to identify just what is changing with age and experience. For example, just as Bauer (2006) compared infants' abilities to imitate enabling versus arbitrary action sequences, thus providing useful diagnostic information, within-subjects contrasts in strategy use under different conditions could yield valuable insights into the memory skills of elementary school children and their development. As an example, Guttentag et al.'s (1987) description of children's rehearsal under typical (i.e., each item removed after being presented) and scaffolded (i.e., each item remained visible after being presented) conditions provides important information about skills that are in transition.

A second methodological preference of researchers also seriously hinders our understanding of developmental change. As suggested earlier, because the bulk of the literature is based on cross-sectional experiments, little can be said about the course of developmental change within individual children. Cross-sectional studies present useful accounts of the average level of competence on specified tasks at particular age levels, and the impression one derives is that of a smooth developmental pattern. However, there is nothing in a cross-sectional study that enables inferences about the course of development of an individual child or contrasting patterns of change for different groups of children. Moreover, putting both methodological concerns together, the rich cross-sectional literature can say nothing about how early skill in, say, elicited imitation relates to later ability in talking about the past, and still later competence in settings in which deliberate memorization is required. For information of this sort, it is necessary to make use of longitudinal research designs in which the development of skill is traced over time, with children being assessed on a range of contrasting tasks. Microgenetic studies (Siegler, 2006) in which children are tested repeatedly over relatively restricted periods of time during which skills are undergoing change can also be invaluable in informing our understanding of development.

The challenge, then, is for a commitment to research designs that truly can facilitate our understanding of the development of memory. Such a commitment requires a willingness to move across the sometimes cherished conceptual boundaries of different subgroups of researchers, for example, those of the information

processing and the social constructivist traditions. It may be useful to think about the encoding, storage, and retrieval of information in information processing terms, and it may be equally productive to think about the forces that propel development from the perspective of social constructivism. This is especially the case when, as suggested above, children's developing memory skills may be fostered by social interaction with parents and teachers. By integrating these methods – and by including multiple assessments of children on tasks that are selected because of their contrasting information processing demands – it is possible to provide more precise cognitive diagnoses of children's changing skills as well as some insight into the social forces that drive development.

To illustrate the importance of longitudinal research for an understanding of development, consider first two longitudinal studies, one dealing with children's event recall in the context of the mother–child reminiscing work discussed above (Reese et al., 1993), and the other concerned with the development of active rehearsal strategies in deliberate memory tasks, also mentioned earlier (Guttentag et al., 1987). Admittedly, each of these studies is somewhat limited by a focus on only a single indicator of mnemonic competence and by the age range examined, but they nonetheless can serve to illustrate some of the benefits of this research strategy. For example, Reese et al. reported that the children of high-elaborative mothers showed higher levels of recall of the events under discussion (as assessed by their production of memory elaborations) than the children of low-elaborative mothers. However, what is unique about this study is the finding that, in the context of these mother–child interactions, the children acquired some generalized skills for remembering that had implications for their performance several years later. Thus, for example, levels of maternal elaboration early in development (at 40 months of age) are positively correlated with the children's skills in making independent contributions to these conversations at later points in time (e.g., at 58 and 70 months).

Guttentag et al.'s (1987) exploration of changes in verbal rehearsal from the third to the fourth grade complements Reese et al.'s (1993) event memory study. As suggested above, Guttentag and his colleagues were concerned with the effort requirements of active, cumulative rehearsal and reported that the rehearsal style of some third graders varied as a function of mode of presentation. In particular, some of the children who rehearsed in a passive fashion under the typical mode of presentation changed to a more active rehearsal pattern spontaneously when they were permitted visual access to the previously presented items. Turning this study into a short-term longitudinal investigation, Guttentag et al. assessed the children again after 1 year, when they were in the fourth grade. Interestingly, the researchers reported that the fourth graders' use of an active rehearsal technique under typical item-by-item presentation conditions was better predicted by what they could do as third graders in the scaffolded than the standard version of the task. They suggested that it was possible to view the children who evidenced active rehearsal as third graders when given visual access to the materials as being in a transitional stage of competence.

Other important insights into development are derived from two separate longitudinal studies of children's developing memory strategies that have been carried out by Schneider and his colleagues. In the first investigation (the Munich Longitudinal Study on the Genesis of Individual Competencies; Sodian and Schneider, 1999), children were tracked between 4 and 18 years of age, whereas in the second study (the Würzburg Longitudinal Memory Study; Schneider et al., 2004), a separate sample of children was observed multiple times between 6 and 9 years of age. Although the studies varied in a number of respects, a consistent pattern that emerges is that strategy development is not as gradual as the cross-sectional data discussed here would lead one to believe. In particular, in both investigations, the improvements that children showed in strategy use reflect a picture of dramatic leaps in performance and not gradual increases in sophistication over time.

Related to longitudinal investigations are microgenetic studies that feature frequent and intense observations of the same child across repeated sessions over relatively short intervals. Several unique insights have been gained from these types of investigation that provide new information concerning the emergence and consolidation of children's strategic efforts. First, Siegler's (2006) exploration of a variety of cognitive strategies suggests that children often use less effective techniques in tandem with more sophisticated and efficient strategies that have been recently acquired. In an important treatment of these patterns, Siegler (1996) describes strategy development in terms of an overlapping waves theory with elementary school children having mastery of a mix of strategies at any point in time, and development being viewed in terms of changes in the composition of this strategy mix. Consistent

with this position, in their longitudinal study of developmental changes in rehearsal, Lehman and Hasselhorn (2007) observed that more than half of the children utilized two or more strategies within each measurement point, suggesting that they are making use of multiple strategies (e.g. naming, cumulative rehearsal) for remembering. Second, consistent with the Munich and Würzburg investigations, the results of microgenetic analyses confirm the fact that children do not always transition from rudimentary to complex strategies in a gradual fashion over time (Kuhn, 1995). Consider, for example, Schlagmüller and Schneider's (2002) microgenetic study of the development of a categorization strategy in the context of a sort–recall task. Fourth- and fifth-grade children, who had been identified as nonstrategy users in the context of the Würzburg longitudinal study of memory development, were given nine sort–recall tasks over the course of an 11-week period. Importantly, those children who adopted the organizational strategy did so in an all-or-none fashion at different times, with some children never categorizing during the task. However, once children came to organize the materials, immediate improvements in recall were observed and were linked to metamnemonic insights immediately prior to strategy acquisition.

2.37.6 Closing Thoughts

It is clear that longitudinal and microgenetic analyses of children's memory can extend the cross-sectional database in critical ways by providing a truly developmental account of the acquisition of skill. Although cross-sectional studies can generate valuable information about the abilities of children of different ages, thus suggesting age-related trends, statements about development within individuals can only be made when researchers employ designs in which the changing abilities of the same children are tracked over time. However, to be truly informative, longitudinal studies must be designed so as to identify potential mediators – such as adult–child conversations – of developmental change. Nonetheless, these important features of longitudinal investigations notwithstanding, it must be emphasized, as well, that they are not without their limitations. Indeed, most explorations of cognitive development that incorporate repeated assessments of children are correlational in nature, and as such, it is difficult to make statements about causation. It is thus essential to supplement these within-subjects approaches with experimental interventions in which variables of theoretical importance – such as the nature of the conversation to which children are exposed – are brought under experimental control. In fact, in the ideal research world, we envision an integrated methodological approach in which longitudinal studies that enable us to track children's skills and identify potential determinants of development are paired with training studies in which these mediators are explored experimentally. In this way, it should be possible to study both memory development and the development of memory.

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2.38 Developmental Disorders of Learning*

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2.38.1 Developmental Disorders of Learning: What Do They Actually Mean?

Phenomenologically, the category of developmental disorders of learning refers to children who, for one reason or another, differ from their peers in acquisition of developmentally appropriate skills (e.g., speaking, counting, reading).

Conceptually, the category of developmental disorders of learning refers to deviations from typical development (1) that are substantial enough to qualify as disorders and (2) that affect learning. However, there is no single nosological category that brings these disorders together, and the two most established diagnostic manuals, the *Diagnostic and Statistical Manual of Mental Disorders* (DSM-IV, published by the American Psychiatric Association, 1994) and the *International Classification of Diseases and Related Health Problems* (IDC-10, published by the World Health Organization, 2005), present only a partial overlap in how these disorders are classified.

The diversity of the disorders commonly viewed as developmental disorders of learning is captured in the following paragraphs. This list is presented here not to overwhelm the reader (and the information is quite daunting!), but rather to demonstrate a lack of agreement of what disorders of learning actually are.

Specifically, DSM-IV distinguishes a large category of Disorders Usually First Diagnosed in Infancy, Childhood, or Adolescence. This category includes, among other subcategories, the disorders that directly involve and affect learning, specifically, Mental Retardation; Learning Disorders (Reading Disorder, Mathematics Disorder, Disorder of Written Expression, and Learning Disorder Not Otherwise Specified, NOS); Motor Skills Disorders; Communication Disorders (Expressive Language Disorder, Mixed Receptive-Expressive Language Disorder, Phonological Disorder, Stuttering, and Communication Disorder NOS); Pervasive Developmental Disorders (Autistic Disorder, Rett's Disorder, Childhood Disintegrative Disorder, Asperger's Disorder, and Pervasive Developmental Disorder NOS); and Attention-Deficit and Disruptive Behavior Disorders (Attention-Deficit/Hyperactivity Disorder (ADHD), Conduct Disorder, Oppositional Defiant Disorder, and Disorders in Both Categories NOS).

ICD-10's Chapter V presents Mental and Behavioural Disorders with subcategories referred to as (1) Disorders of Psychological Development and (2) Mental and Behavioural Disorders. The former category is subdivided into Specific Developmental

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Disorder of Speech and Language (Specific Speech Articulation Disorder, Expressive Language Disorder, Receptive Language Disorder, Acquired Aphasia with Epilepsy, Other Developmental Disorders of Speech and Language, and Developmental Disorder of Speech and Language, Unspecified); Specific Developmental Disorders of Scholastic Skills (Specific Reading Disorder, Specific Spelling Disorder, Specific Disorder of Arithmetic Skills, Mixed Disorder of Scholastic Skills, Other Developmental Disorder of Scholastic Skills, Developmental Disorder of Scholastic Skills, Unspecified); Specific Developmental Disorder of Motor Function; Mixed Specific Developmental Disorders; Pervasive Developmental Disorders (Pervasive Developmental Disorders, Childhood Autism, Atypical Autism, Rett's Syndrome, Other Childhood Disintegrative Disorder, Overactive Disorder Associated with Mental Retardation and Stereotyped Movements, Asperger's Syndrome, Other Pervasive Developmental Disorders, Pervasive Developmental Disorder, Unspecified), among other disorders. The latter category includes a cluster of disorders associated with hyperactivity and conduct problems (e.g., Hyperkinetic Disorder and Conduct Disorder), separating attention problems from problems of hyperactivity (with attention problems listed in the first category as a psychological problem) and including stuttering in this category, rather than as a disorder of speech and language.

To restate, there is no uniformly accepted approach in how developmental disorders of learning should be referred to or classified. Correspondingly, in staging the discussion that unfolds in this chapter, it is important to comment on the following three issues. First, it is clear that no single nosological category captures all developmental disorders of learning. There are many developmental disorders where learning is disrupted. Second, many of these developmental disorders are comorbid, that is, cooccur in the same individual. Thus, which disorder is diagnosed as primary and what other disorders are codiagnosed is variable. Third, although there are many disorders in which learning is disrupted, the 'label' that typically denotes challenged learning is Learning Disability (LD). As mentioned earlier, this category is not used as a diagnostic category. Yet, there is a mountain (or rather a mountain chain) of literature on this category. For the ensuing discussion, it is important to differentiate nonspecific (or general) and specific LDs. Conventionally, the term nonspecific LD is used to refer to generalized problems of learning, such as mental retardation, and the term specific LD (SLD) is used to refer to disorders in a particular domain of acquisition or learning, such as reading, writing, or mathematics.

In this chapter, I use the concept of LD even though, as mentioned earlier, it does not correspond directly to any particular nosological category in the two predominant diagnostic schemes of the developed world. Throughout the chapter, I argue that LD best captures the common thread of all developmental disorders of learning.

2.38.2 The Concept of Learning Disabilities

Fundamentally, the concept of LD encompasses society's capacity

> . . . to monitor (and recruit) children for unexplained school failure in a way that was not possible before the LD category was reified and passed into law in 1969. (Reid and Valle, 2004: 467)

The LD category replaced a variety of 'loose' definitional references to previously used qualifiers such as 'slow learner,' 'backward children' (Franklin, 1987), and 'minimal brain dysfunction' (Fletcher et al., 2002).

In terms of its 'realization' in the context of current practices, the LD label typically assumes the presence of the following process. Under normal circumstances, LDs are not diagnosable prior to a child's engagement with schooling and the opportunity to master key academic competencies. While in school, a child is assumed to be assigned grade-appropriate tasks. These tasks assume some degree of variability in children's performance; these theoretical ranges constrain the definitions of acceptable and worrisome variability in performance (*See* Chapter 2.40). It is when the child's performance consistently falls out of the acceptable range in one or more academic subjects that the child becomes the focus of intense observation and documentation and is referred for evaluation to appropriate professionals (e.g., educational psychologists, neuropsychologists, and clinicians such as pediatricians, clinical psychologists, or psychiatrists). An important qualifier here is that such observation, documentation, and evaluation are considered only for children whose performance is below that expected based on their general capacity to learn; thus, the concept of 'unexpected' school

failure is central to the definition of LD. When reports on the child's performance in the classroom, testing results, and clinical evaluations are compiled, the child and his or her family are referred to a special education committee, which determines eligibility for individualized special education services. If eligibility is established, an Individualized Education Program (IEP) is created. The IEP refers to a specific diagnostic label carried by the child and cites the proper category of public laws that guarantees services for an individual with such a diagnosis.

2.38.3 Definition

The definition that currently drives federal regulations was produced by the National Advisory Committee on Handicapped Children in 1968 and subsequently adopted by the U.S. Office of Education in 1977 (Mercer et al., 1996). According to this definition,

> Specific learning disability means a disorder in one or more of the basic psychological processes involved in understanding or in using language, spoken or written, which may manifest itself in an imperfect ability to listen, think, speak, read, write, spell, or to do mathematical calculations. The term includes such conditions as perceptual handicaps, brain injury, minimal brain dysfunction, dyslexia, and developmental aphasia. The term does not include children who have learning problems which are primarily the result of visual, hearing, or motor handicaps, of mental retardation, or emotional disturbance, or of environmental, cultural, or economic disadvantage. (U.S. Office of Education, 1977: 65083)

Again, neither DSM-IV nor ICD-10 uses the term learning disabilities. DSM-IV makes a reference to learning disorders (American Psychiatric Association, 1994), which, according to DSM-IV, can be diagnosed,

> . . .when the individual's achievement on individually administered, standardized tests in reading, mathematics, or written expression is substantially below that expected for age, schooling, and level of intelligence. (American Psychiatric Association, 1994: 46)

Of interest here is that this is one of the very few categories of DSM-IV where a reference is made explicitly to psychological tests, although DSM-IV does not provide specific guidelines as to what 'substantially below' means. Thus, DSM-IV implicitly refers to evidence-based practices (Fletcher et al., 2002) in the field. The problem, of course, is that there are multiple interpretations of these best practices (see discussion to follow). Yet, assuming there are consistent and coherent guidelines in place for establishing a diagnosis of LD, DSM-IV classifies types of LDs by referencing the primary academic areas of difficulty. The classification includes three specific categories and a residual diagnosis: Reading Disorder, Mathematics Disorder, Disorder of Written Expression, and Learning Disorder NOS. A common practice in the field is to view a diagnosis of a learning disorder as established by DSM-IV as an equivalent to 'specific learning disability,' which qualifies a child for special services under federal regulations (House, 2002).

2.38.4 History

The introduction of the concept of LD is typically credited to Samuel Kirk (then a professor of special education at the University of Illinois), who, while presenting at a parent meeting in Chicago on April 6, 1963, proposed the term learning disabled to refer to "children who have disorders in development of language, speech, reading, and associated communication skills" (Strydom and du Plessis, 2000). The category was well received by parents and promoted shortly thereafter by an established parent advocacy group known as the Association for Children with Learning Disabilities. Prior to the formal introduction of this concept, the literature had accumulated numerous descriptions of isolated cases and group analyses of children with specific deficits in isolated domains of academic performance (e.g., reading and math) whose profiles were later reinterpreted as those of individuals with specific LDs (e.g., specific reading and math disabilities). It is those examples in the literature and the experiences of many distressed parents who could not find adequate educational support for their struggling children that, in part, resulted in the creation of the field of LDs as a social reality and professional practice (Hallahan and Mercer, 2002). Subsequent accumulation of research evidence and experiential pressure led to the formulation of legislation protecting the rights of children with LDs.

Congress enacted the Education for All Handicapped Children Act (Public Law 94-142) in 1975 to support states and educational institutions in protecting the rights of, meeting the individual needs of, and improving the results of schooling for infants, toddlers, children, and youth with disabilities and their families. This landmark law is currently enacted as the Individuals with Disabilities Education Act (IDEA, Public Law 105-17; although the precise title of the law in its 2004 amendment is Individuals with Disabilities Education Improvement Act, it is still referred to as IDEA), as amended in 2004. The importance of this law is difficult to overstate: In 1970, U.S. schools provided education to only one in five children with disabilities (U.S. Office of Special Education Programs, 2000). By 2003–2004, the number of children aged 3–21 served under IDEA was more than 6.6 million (National Center for Education Statistics, 2005b).

SLDs make up 50% of all special education students served under IDEA. The term has proliferated very successfully and very quickly within the last two decades. There are multiple reasons why the concept of LD has enjoyed such success, among which are a lack of social stigma (i.e., parents are much more comfortable with the label of LD than with categories such as minimal brain dysfunction or brain injury), absence of implication of low intelligence or behavioral problems, and access to services (Zigmond, 1993).

In its 2004 amendment, IDEA recognized 13 categories under which a child can be identified as having a disability: autism; deaf–blindness; deafness; emotional disturbance; hearing impairment; mental retardation; multiple disabilities; orthopedic impairment; other health impairment; specific learning disability; speech or language impairment; traumatic brain injury; and visual impairment including blindness. It is notable that LDs as described in IDEA are referred to as 'specific learning disabilities' to emphasize the difference between children with SLDs and those with general learning difficulties characteristic of other IDEA categories (e.g., autism and mental retardation). The consensus in the field is that children with LDs possess average to above-average levels of intelligence across many domains of functioning but demonstrate specific deficits within a narrow range of academic skills. Finally, as stated earlier, exclusionary factors have been central to diagnoses of LDs since the authoritative definition of LD was introduced in 1977. As per these exclusionary criteria, a child cannot be diagnosed with an LD unless factors such as other disorders or lack of exposure to high-quality age-, language-, and culture-appropriate educational environments have been ruled out. It is the desire to rule out the exclusionary factor of lack of exposure to high-quality environments that prompted the introduction of the concept of Response to Treatment Intervention (RTI) (Deshler et al., 2005) in the 2004 amendment of IDEA. RTI signifies

> . . . individual, comprehensive student-centered assessment models that apply a problem-solving framework to identify and address a student's learning difficulties. (Deshler et al., 2005: 483)

It is important to note that RTI might appear counterintuitive at first: How can a disorder be defined through treatment if treatment is prescribed for a particular disorder? This 'circularity' of RTI, however, is only superficial. An implicit assumption behind RTI is that teaching is inadequate, and that is why schools 'produce' such a high level of LDs. A closer analogy would not be with treatment, but with prevention with vitamins; if vitamins are delivered properly, then many deficiencies can be avoided. Thus, if all children get extensive preventive instruction, the frequencies of LDs will diminish (see more detail on RTI in the section titled 'Presentation and diagnoses').

2.38.5 Epidemiology

Since the 1968 statutory introduction of LD as a legislated disability (i.e., within ~35 years of its existence as a category), approximately 50% of all students receiving special educational services across the nation have received them under the category of LD (Donovan and Cross, 2002). Among these students, the majority (80–90%) demonstrate substantial difficulties in reading (Kavale and Reese, 1992), and two of every five were identified because of persistent difficulties in reading acquisition (President's Commission on Excellence in Special Education, 2002).

There are two main sources for estimates of prevalence rates of LDs.

The first and most obvious one is linked to the number of children served under this category of IDEA. When this number is mapped on the total number of school-age children in the United States, although the number fluctuates from year to year,

the average estimates of prevalence rates for LDs are around 5–6% of the total school-age population. To illustrate, in 2003, 2.72 million children were identified as having LDs. This represents a 150–200% increase in the number of students aged 6–17 with LDs compared with that number in 1975.

Yet, it is important to note that prevalence rates vary substantially from district to district and from state to state. For example, in 2004, under the SLD category, in Kentucky, 1.8% of all students aged 6–21 received special education services, compared with 5.9% in Iowa. Thus, based on these numbers, the prevalence rates of LDs in Iowa are about 3.3 times as high as in Kentucky, two states in close geographic proximity! This observation stresses the mosaic-like situation of LD diagnosis – there is no unified approach to these diagnoses across different local education agencies in the United States.

When IDEA-related prevalence rates are considered, LDs are observed more frequently in boys than in girls (64.5% vs. 33.5% for boys and girls aged 6–17, respectively) and more frequently in underrepresented minority groups than in Asian Americans or Whites. Risk ratios (which compare the proportion of a particular racial/ethnic group served to the proportion of all other racial/ethnic groups combined) are 1.5, 0.4, 1.3, 1.1, and 0.9 for American Indian, Asian American, African American, Hispanic American, and White students, respectively. A risk ratio of 1.0 indicates no difference between the racial/ethnic groups.

The second source for these rates is research studies. Per results from these studies, it is assumed that, although 10–12% of school-age children show specific deficits in selected academic domains, high-quality classroom instruction and supplemental intensive small-group activities can reduce this number to ~6% of children. It is assumed that these 6% will meet strict criteria for LDs and require special education intervention.

It is important to note that most of the research in the field of LDs is currently conducted with reading and, correspondingly, Specific Reading Disability (SRD). There is little established evidence that reliably points to prevalence rates of disorders of math and writing.

To illustrate, according to the results of current research on early reading acquisition, 2–6% of children do not show expected progress even in the context of the highest quality evidence-based reading instructions. Based on U.S. national data, the risk for reading problems as defined through failure to reach age- and grade-adequate milestones ranges from 20–80%. Specifically, data from the 2005 National Assessment of Educational Progress show that 36% of fourth graders do not possess the adequate reading skills required for completion of grade-appropriate educational tasks (National Center for Education Statistics, 2005a). However, it is clear that far from all of these children have SRD. The majority of these children mostly likely underachieve because of inadequate educational experiences and causes other than SRD.

Some changes in the 2004 version of IDEA were invoked directly because of concerns regarding the overidentification of students as learning disabled. The category of LDs has often been the largest single category of children served under IDEA (for latest relevant statistics, see IDEA Data, 2006). The reality of everyday practices in school districts was such that most diagnoses prior to the 2004 reauthorization were based on so-called aptitude–achievement discrepancy criteria, which required a severe discrepancy between IQ and achievement scores (e.g., two standard deviations, 2 years of age equivalence), although IDEA had never specifically required a discrepancy formula (Mandlawitz, 2006). Correspondingly, it has been argued that these discrepancy-based approaches are flawed (Francis et al., 2005) and might have led to overidentification. In light of this hypothesis, IDEA 2004 emphasizes that there is no explicit IQ–achievement discrepancy requirement for diagnosis of LDs. As a possible alternative approach for identification and diagnosis, IDEA 2004 states that local educational agencies may use a child's RTI in lieu of classification processes (Council of Parent Attorneys and Advocates, 2004). A local educational agency (e.g., a school) may choose to administer to the child in question an evidence-based intervention program to determine his or her eligibility for special education services under IDEA based on the child's response to this program.

Specifically, the statutory language of IDEA 2004 (Public Law 108-446) states:

> (6) Specific Learning Disabilities.
> (A) In general.
> Notwithstanding section 607(b), when determining whether a child has a specific learning disability as defined in section 602, a local educational agency shall not be required to take into consideration whether a child has a severe discrepancy between achievement and intellectual ability in oral expression, listening comprehension, written expression,

> basic reading skill, reading comprehension, mathematical calculation, or mathematical reasoning.
> (B) Additional authority.
> In determining whether a child has a specific learning disability, a local educational agency may use a process that determines if the child responds to scientific, research-based intervention as a part of the evaluation procedures described in paragraphs (2) and (3). (§614(b) (6))

As a consequence of this language, although aptitude–achievement discrepancy has been and continues to remain the common, although not required, practice for local educational agencies, there is a new 'entry point' for RTI. Needless to say, these changes are of great theoretical and practical importance. The tradition and system of specific LD identification in the United States are now fluid, and rather few specific recommendations exist to help local educational agencies smoothly transition into the implementation of IDEA 2004.

2.38.6 Presentation and Diagnoses

As stated earlier, it is crucially important in a diagnosis of LD to establish a child's 'typical' intellectual performance and to document that the child's performance in the area of difficulty (e.g., reading or mathematics) does not correspond to what would be expected, given average intellectual functioning. Although this general principle is relatively easy to grasp, the field of LDs has struggled since its inception in the early 1960s to establish specific steps that should lead to the establishment of the diagnosis.

Prior to the 2004 reauthorization of IDEA, the most common way of establishing an LD diagnosis was the discrepancy criterion. The introduction of the discrepancy between ability and achievement criteria in the 1977 law was not based on empirical research, but rather driven by a need for a more objective approach to the diagnoses than those commonly used and largely discredited at the time (Gresham et al., 2004). Two decades of research and practical explorations of the discrepancy model resulted in its discreditation from points of view of theory (Sternberg and Grigorenko, 2002), reliability of diagnosis and classification (Francis et al., 2005), robustness of implementation (Haight et al., 2002), and treatment validity (Aaron, 1997). In response to the overwhelming amount of evidence for the inadequacy of the discrepancy model, however realized (through psychometric indices, age equivalences, regression approaches, or expert opinions), a number of alternative models have been proposed. The major dividing line between these new models and previous discrepancy-based models is in their theoretical orientation. Previous diagnostic models attempted to identify children diagnosable with LDs by looking for characteristic cognitive deficits, so that an intervention could be delivered to children with such deficits (Reschly, 1996), whereas the modern models argue for the need to deliver best pedagogical practices to all children and then best remediational-intervention approaches to those children who do not respond as well to good teaching (Reschly and Ysseldyke, 2002).

As per the 2004 reauthorization of IDEA, local educational agencies have some choice in selecting diagnostic models. At this point, the most widely discussed and evidence-supported model of LD identification is the Responsiveness/Response to Intervention (RTI) model (Vaughn and Fuchs, 2003). The RTI model has a number of features. First, the performance of the student in question is compared with the performance of his/her immediate peers on academic tasks. Specifically, RTI assumes tracking the academic performance and rate of its growth for all students within a given class, with a goal of identifying those students in a class whose performance differs from that of their peers both in absolute (i.e., level) and relative (i.e., rate of growth) terms. Second, the model is structured primarily by intervention, so students identified by these means are offered individualized accommodations and interventions with a goal of maximizing the effectiveness of the learning environment for a given student in need. Third, the model is multilayered, so that each layer offers an opportunity for further differentiation and individualization of education for students who need it. Typically, three layers are recommended: The first tier covers regular classroom environment; the second tier is characterized as 'supplemental' to tier 1; and the third tier is 'intensive,' 'individualized,' and 'strategic.' Fourth, only if these multilayered attempts to modify the regular classroom pedagogical environment are unsuccessful is the prospect of an LD diagnosis considered. In summary, a child could be identified as having an LD if he or she consistently failed to perform at a level and progress at a rate comparable with the child's peers in general education after having participated in an evidence-based intervention.

Although there is considerable agreement in the field on the promise of RTI as a diagnostic paradigm,

there are a variety of opinions regarding how, specifically, RTI should be quantified. Currently, the following paradigms are on trial: (1) Administer norm-referenced assessment batteries at the beginning and end of every school year to quantify the growth in response to intervention – students whose growth rate is below 'appropriate' should receive additional intervention, and (2) administer norm-referenced assessment batteries with a particular performance threshold (i.e., 25th percentile) – students whose performance is below this threshold should receive intensive interventions, and their performance should be monitored at least four times a year. There is also significant theoretical and experimental evidence suggesting the need for and importance of continuous progress monitoring with frequent (e.g., weekly) assessments of improvement. Currently, however, there are concerns about both approaches because of a lack of trained educational and practical professionals equipped to translate and implement research-based interventions into the everyday life of American schools. Since the 2004 reauthorization of IDEA, local education agencies have been in search of new robust solutions for identifying LDs that will meet the regulations of federal laws. RTI-based approaches to LD diagnosis present considerable challenges for all professionals involved in the realization of IDEA: general and special education teachers, diagnosticians (psychologists and psychiatrists), and school psychologists. The heart of this challenge is the lack of operationalization and practical guidelines that can be easily implemented at the 'frontiers' of diagnosing and treating children with LDs.

The majority of students with LDs are identified in middle and high school: Early years of schooling might simply be insufficient for exposing and making evident a deficit in a particular academic domain. As mentioned, the core conceptual piece of the LD definition is that the deficit could have not been predicted reliably prior to the child's school entry because a child with LDs demonstrates otherwise typical levels of cognitive functioning.

Previously when the discrepancy criteria were applied, the diagnosis of LD was different from other forms of learning difficulties because of its stress on the specificity of the deficit (i.e., a discrepancy was expected not in all academic domains, but in a specific academic domain). The introduction of RTI-based approaches to diagnosis makes the question of differential diagnosis somewhat difficult to address. In fact, students with mental retardation, emotional or behavior disorders, ADHD, and other childhood and adolescent disorders might also exhibit low responsiveness to intervention. Yet, their nonresponsiveness will occur for reasons very different from those of students with LDs. In other words, if RTI cannot differentiate LDs from other diagnoses where learning difficulties are present but nonspecific, can RTI even be considered as a classification/diagnostic instrument (Mastropieri and Scruggs, 2005)?

Although this question has been raised, it has not yet been answered. The pre-2004 conceptualization of LDs assumed that the texture of LDs was in deficient (or different, atypical) psychological processing of information. In other words, the field was driven by the assumption that LDs were likely to represent a dysfunction in one or more basic psychological processes (e.g., phonological processing, sustained attention, different types of memory, executive functioning). These deficient processes in turn can slow down or inhibit mastery of a particular academic domain (e.g., reading or mathematics). Under this assumption, intensive academic instruction could improve performance in specific academic domains but could not treat the disorder. Even if reading improves as a result of intervention, in this paradigm the disorder might remanifest as a deficiency in a bordering domain (e.g., writing). In other words, although reading skills might be enhanced, the deficient psychological skills might impede some other academic domain of functioning.

Throughout the existence of the category of specific LDs, there has been a consistent and strong drive from parents, researchers, and educators for differentiating these disorders from generic learning difficulties. In its current iteration, RTI does not differentiate nonspecific and specific learning difficulties, because nonresponsiveness to intervention can occur with a variety of developmental disorders. In sum, because IDEA preserved the category of SLDs, there is a new huge task to differentiate specific and nonspecific learning difficulties by means of RTI and possibly other methods in the field.

One of these 'other' methods has to do, of course, with psychological testing. Many researchers argue for the necessity of maintaining the role of psychoeducational and neuropsychological tests on a variety of indicators, including IQ, in establishing LD diagnosis (Mastropieri and Scruggs, 2005; Semrud-Clikeman, 2005).

2.38.7 Etiology

There is a consensus in the field that LDs arise from intrinsic factors and have neurobiological bases, specifically atypicalities of brain maturation and function. There is a substantial body of literature convincingly supporting this consensus and pointing to genetic factors as major etiological factors of LDs. The working assumption in the field is that these genetic factors affect the development, maturation, and functional structure of the brain and in turn influence cognitive processes associated with LDs. Yet the field is acutely aware that a number of external risk factors, such as poverty and lack of educational opportunities, affect patterns of brain development and function and, correspondingly, might worsen the prognosis for biological predisposition for LDs or act as a trigger in LD manifestation.

Although this model, in main strokes, appears to be relevant to all LDs, far more research on relevant genes and brain structure and function is available for children with SRD than for any other LD. Thus, illustrative findings are presented here from SRD (for a more comprehensive review, see Grigorenko, 2007).

Multiple methodological techniques, such as electroencephalograms, event-related potentials, functional resonance imaging, magnetoencephalography, positron emission tomography, and transcranial magnetic stimulation, have been used to elicit brain–reading relationships (for recent reviews, see Price and Mechelli, 2005; Shaywitz and Shaywitz, 2005; Simos et al., in press). When data from multiple sources are combined, it appears that a developed, automatized skill of reading engages a wide bilateral (but predominantly left-hemispheric) network of brain areas passing activation from occipitotemporal, through temporal (posterior), toward frontal (precentral and inferior frontal gyri) lobes. Clearly, the process of reading is multifaceted and involves evocation of orthographical, phonological, and semantic representations that in turn call for the activation of brain networks participating in visual, auditory, and conceptual processing. Correspondingly, it is expected that the areas of activation serve as anatomic substrates supporting all these types of representation and processing.

Somewhat surprisingly, per recent reviews, there appear to be only four areas of the brain of particular, specific interest with regard to reading. These areas are the fusiform gyrus (i.e., the occipitotemporal cortex in the ventral portion of Brodmann's area 37, BA 37), the posterior portion of the middle temporal gyrus (roughly BA 21, but possibly more specifically, the ventral border with BA 37 and the dorsal border of the superior temporal sulcus), the angular gyrus (BA 39), and the posterior portion of the superior temporal gyrus (BA 22).

It is also important to note the developmental changes in patterns of brain functioning that occur with increased mastery of reading skill: progressive, behaviorally modulated development of left-hemispheric 'versions' of these areas and progressive disengagement of right-hemispheric areas. In addition, there appears to be a shift of regional activation preferences. The frontal regions are used by fluent more than by beginning readers, and readers with difficulties activate the parietal and occipital regions more than the frontal regions.

In an attempt to understand the mechanism of the 'deficient' pattern of brain activation while engaged in reading, researchers are looking for genes that might be responsible, at least partially, for these observed differences in functional brain patterns. This search is supported by a set of convergent lines of evidence (for reviews, see Fisher and DeFries, 2002; Grigorenko, 2005; Barr and Couto, in press). First, SRD has been considered a familial disorder since the late nineteenth century. This consideration is grounded in years of research into the familiality of SRD (i.e., similarity on the skill of reading among relatives of different degree), characterized by studies that have engaged multiple genetic methodologies, specifically twin (Cardon et al., 1994, 1995; Byrne et al., 2005), family (Wolff and Melngailis, 1994; Grigorenko et al., 1997; Cope et al., 2005) and sib-pair designs (Francks et al., 2004; Ziegler et al., 2005). Although each of these methodologies has its own resolution power to explain similarities among relatives by referring to genes and environments as sources of these similarities and obtaining corresponding estimates of relative contributions of genes and environments, all methodologies have produced data that unanimously point to genetic similarities as the main source of familiality of SRD.

Today, it is assumed that multiple genes contribute to the biological risk factor that runs in families and forms the foundation for the development of SRD. Specifically, nine candidate regions of the human genome have been implicated (Grigorenko, 2005). These regions are recognized as SRD candidate regions; they are abbreviated as *DYX1–9*

(DYX for dyslexia, a term often used to refer to SRD) and refer to the regions on chromosomes 15q, 6p, 2p, 6q, 3cen, 18p, 11p, 1p, and Xq, respectively. Each of these regions harbors dozens of genes, so clearly, the field offers empirical validation that multiple genes contribute to the manifestation of SRD. A number of different research groups are actively at work on these genetic regions in an attempt to identify plausible candidate genes. Four successful attempts have been announced in the literature: one for the 15q region, the candidate gene known as *DYX1C1* (Taipale et al., 2003); two for the 6p region, the candidate gene known as *KIAA0319* (Francks et al., 2004; Cope et al., 2005) and the candidate gene knows as *DCDC2* (Meng et al., 2005; Schumacher et al., 2006); and one for the 3cen region, *ROBO1* (Hannula-Jouppi et al., 2005). Although the field has not yet converged on 'firm' candidates, it is remarkable and of great scientific interest that all four current candidate genes for SRD are involved with biological functions of neuronal migration and axonal crossing. Thus, all these genes are plausible candidates for understanding the pattern of brain functioning in SRD described earlier.

2.38.8 Relevant Theoretical Models and Considerations

As mentioned earlier, the literature on LDs is uneven, with the vast majority relating to SRD. Correspondingly, here I summarize the so-referred overarching model of LDs (Fletcher et al., 2007). Subsequently, I illustrate this model with detailed references to SRD. The overarching LD model delineates multiple levels of analyses and evidence.

According to this general model, LDs are anchored in a domain of particular academic difficulties (e.g., reading, spelling, computing, and writing). Correspondingly, the identification of an LD assumes that a diagnosis can be validly and reliably established on the basis of observed repeated patterns of weaknesses in a particular academic domain in the presence of strengths in all or some other academic domains. Thus, concerns, referrals, and diagnostic assessments are always centered on a particular academic domain that defines the content of LD. Correspondingly, the first step in LD identification is documenting the presence of a consistent failure or academic skill deficits, when compared with peer performance, on a set of specific tasks. Thus, behavioral presentation in a particular academic domain is the first level of analysis in the pyramid of LD diagnoses. However, the presence of an academic deficit is a necessary but insufficient condition for establishing an LD diagnosis.

The second level of analysis pertains to capturing individual characteristics of the child for whom an LD diagnosis is considered. Specifically, at this level, clusters of child characteristics are considered within the paradigm of inclusion and exclusion criteria of the LD category. Typically, at this level, the information is gathered in four directions: (1) pertaining to the academic domain of concern and cognitive processing known to be relevant to this particular domain, (2) pertaining to other academic domains in which the child demonstrates average or above-average levels of performance, (3) indicators of general cognitive functioning, and (4) other noncognitive and nonacademic domains of child's functioning (e.g., motivation, neurological and psychiatric indicators). Obviously, the information gathered at (1) is used within the context of inclusion and the information gathered in (2)–(4) within the context of exclusion criteria. It is critically important that there are well-developed psychological models available both for (1) and (2). For example, to identify LD in reading (SRD), it is important to know what cognitive processes constitute the texture of this academic skill. Similarly, since academic skills tend to correlate substantially in typically developing children, it is important to know what SRD and, for example, specific math disability (SMD) have in common and how they differ in terms of overlapping and specific psychological processes. To illustrate this level of analysis, I discuss modern psychological models of SRD below.

The third level of analysis involves both causal and associated etiological factors of LD. Specifically, a number of risk and protective factors rooted in the child's biology (e.g., gene and brain factors) and environment (e.g., school, neighborhood, and family environment factors) are considered at this level. The point here is to capitalize on the evidence in the field to differentiate LDs and underachievement, specific and nonspecific LDs, and specific LDs and comorbid conditions.

It is important to note that this model allows a diagnostician to move both up and down. The expectation is that the information converges across all three levels of analysis, and the diagnosis of LD is reliably established. However, it is possible, especially with young children, that the first 'level of entry' into the model is through cognitive processes

that constitute the texture of the skill and thus emerge prior to the acquisition of the skill; for example, a child having difficulty mastering rhymes and letters might be identified as at risk for reading failure prior to entering formal reading instruction (Lonigan, 2003). Similarly, it is possible to enter the model through the level of biological risk factors; for example, given that SRD appears to be genetic, a child whose parents both have difficulty reading is at higher risk for SRD than is a child from a risk-free family (Gallagher et al., 2000; Lyytinen and Lyytinen, 2004). But, again, no matter what level of analysis this overarching model is entered through, it is very important that there are evidence-based models of acquisition of a particular skill (e.g., reading or mathematics) that is challenged in an LD.

Although psychological models of other LDs have been developed, here only those for SRD are exemplified for illustration purposes.

So far, there have been only generic references to the disruption of both the acquisition and mastery of reading skills that constitute the texture of SRD. When this generic reference is closely considered, another massive body of literature materializes: (1) cognitive psychology literature on types of representation of information involved in reading (i.e., reading involves the translation of meaningful symbolic visual codes (orthographical representation) into pronounceable and distinguishable sounds of language (phonological representation) so a meaning (semantic representation) arises) (Harm and Seidenberg, 2004); (2) developmental psychology literature on when these representations develop and what might cause the development of a dysfunctional representational system (Karmiloff-Smith, 1998); and (3) educational psychology literature on how the formation of functional representations can be aided or corrected when at risk for malfunction (Blachman et al., 2004).

Here only brief commentaries relevant to these literatures are offered. Today, given the predominance of the phonology-based connectionist account of SRD, behavioral manifestation of SRD is captured through a collection of highly correlated psychological traits. Although different researchers use different terms for specific traits, these can be loosely structured into groups aimed at capturing different types of information representation, for example: (1) performance on orthographic choice or homonym choice judgment tasks for quantifying parameters of orthographical representation; (2) phonemic awareness, phonological decoding, and phonological memory for quantifying phonological representation; and (3) vocabulary and indices of comprehension at different levels of linguistic processing for quantifying semantic representation. Correspondingly, in studies of the etiology, development, and educational malleability of SRD, the quantification of the disorder is carried out through these various traits (or components of SRD). Thus, many studies attempt to subdivide SRD into its components and explore their etiological bases, developmental trajectories, and susceptibility to pedagogical interventions separately as well as jointly.

Of note is that similar developments with regard to dissection of an academic skill and differentiation of componential psychological processes contributing to this skill have been taking place in the studies of acquisition of other academic skills, for example, mathematics (Butterworth, 2005; Geary, 2005; Fuchs et al., 2006; Fletcher et al., 2007).

2.38.9 Manifestation and Life Course

There is an accepted understanding in the field that LDs are typically lifelong disorders, although their manifestations might and often do vary depending on developmental stage and demands of the environment (e.g., school, work, retirement) imposed on an individual at a particular time. This understanding assumes that LDs do not manifest themselves exclusively in academic settings. In fact, although it might be successfully remediated during schooling, a particular LD might need further assistance and remediation in later years (e.g., as a part of the workforce). Although the literature on adults with LDs is still somewhat limited, there is an accumulation of evidence that LDs constitute a serious public health problem even after schooling. Such evidence is particularly rich in the field of studies of SRD.

LDs are comorbid with a number of other disorders typically diagnosed in childhood or adolescence, especially attention deficit (Semrud-Clikeman et al., 1992) and disruptive behavior disorders (Grigorenko, 2006). LDs also often co-occur with anxiety and depression (Martinez and Semrud-Clikeman, 2004). Correspondingly, individuals with LDs are at higher risk for developing other mental health problems.

Yet, the main drawback for individuals with specific LDs has to do with their educational achievement. On average, only ~50% of students aged 14 and older diagnosed with LDs graduate with regular high school diplomas. The dropout rate among these students is very high (~45%), and

it is even higher for underrepresented minority students. The employment prospects of these students are also troublesome – only about 60% of student ages 14 and older diagnosed with LDs have paid jobs outside the home.

Thus, it is important to realize that the impact of LDs is not limited to any one academic domain (e.g., reading or mathematics); these are lifetime disorders with wide-ranging consequences.

2.38.10 Treatment, Remediation, Intervention, and Prevention

Currently, there are no approved medical treatments for children with LDs. There is a consensus in the field that children with LDs should be provided special education and related services upon establishment of their eligibility and determination of the necessity, content, duration, and desired outcomes of such education and services.

Yet, in much of the literature, many educators have expressed concern with the possible presence of faulty identification procedures in states and districts across the country, which has resulted in the possible abuse of the classification and service systems. The ever-growing number of children identified with LDs might indicate that this category has become a 'trap' for lower-performing students, irrespective of an LD diagnosis.

In response to this concern, the 2004 reauthorization of IDEA makes reference to a set of prevention mechanisms intended to establish a better classification strategy for identifying children with LDs. By law, schools need to implement systemic models of prevention that address (1) primary prevention: the provision of high-quality education for all children; (2) secondary prevention: targeted, scientifically based interventions for children who do not respond to primary prevention; and (3) tertiary prevention: the provision of intensive individualized services and interventions for those children who have not responded to high-quality instruction or subsequent intervention efforts. As per new regulations, it is assumed that this third group of children, namely those children who have failed to respond to age-, language-, and culture-appropriate, evidence-based, domain-specific instruction (e.g., in reading or mathematical cognition), can be identified as eligible for special education services. These prevention mechanisms are also assumed to be used as diagnostic mechanisms (see earlier discussion of RTI). This circular system of an outcome of intervention being also an entry point to diagnosis is currently creating significant turmoil in the literature and in practice.

In general, RTI approaches are conceived as a twofold simultaneous realization of high-quality, domain-specific instruction and continuous formative evaluation of students' performance and learning (Mellard et al., 2004a,b). In other words, RTI refers to ongoing assessment of students' response to evidence-based pedagogical interventions in particular academic domains. Thus, it is assumed that LDs can be identified only when underachievement related to poor instruction is ruled out. (It is also important to note that the primary diagnosis of LD is established only in the absence of other neuropsychaitric conditions.) Although it exists in a number of alternative forms, RTI includes eight central features and six common attributes. Among the central features linking all forms of RTI are: (1) high-quality classroom instruction, (2) research-based instruction, (3) classroom performance measures, (4) universal screening, (5) continuous progress monitoring, (6) research-based intervention, (7) progress monitoring during intervention, and (8) fidelity measures. Among common attributes of different RTI models, there are concepts of (1) multiple tiers; (2) transition from instruction for all to increasingly intense interventions; (3) implementation of differentiated curricula; (4) instruction delivered by staff other than the classroom teacher; (5) varied duration, time, and frequency of intervention; and (6) categorical or noncategorical placement decisions (Graner et al., 2005). Clearly, the concept of RTI is centered on the field's definition of high-quality research-validated instruction. It is important to note that, although there is growing consensus on the critical elements for effective reading instruction (e.g., Foorman et al., 2003), other domains of teaching for academic competencies are far from consensus-driven (*See* Chapters 2.37, 2.43).

There are numerous examples of RTI-based treatment of LDs; two often-cited ones are the Minneapolis Public School's Problem Solving Model, in action since 1994 (Marston et al., 2003), and the Heartland (Iowa) Area Education Association's Model, implemented in 1986 (Ikeda and Gustafson, 2002). The Minneapolis model is a three-tier intervention model where the referral to special education is made only after consecutive failures to benefit from instruction throughout all three tiers of pedagogical efforts. The Iowa model originally included four tiers, where the third tier was subdivided into two related steps, but it was then collapsed into one tier, similar to the

Minneapolis model. Unfortunately, neither model has published empirical data on its effectiveness. Yet, years of implementation have resulted in appreciation from the communities they serve and in a stable, relatively small special education population.

Currently, the concept of RTI is under careful examination by researchers supported by both the U.S. Department of Education and the National Institute of Child Health and Development. The future of RTI and its role in diagnosing and treating specific LDs is dependent on answers to critical questions: (1) whether an RTI model can be implemented on a large scale; (2) how an RTI model can be used for LD eligibility determination; (3) whether an RTI is an effective prevention system; and (4) whether RTI enhances LD determination and minimizes the number of false positives.

2.38.11 Conclusion

I began this chapter with a brief discussion of the concept of developmental disorders of learning and with the concern that there is no single definition of this concept. In fact, the discussion of the 'multirepresentativeness' of this concept in the two main diagnostic schemes (DSM-IV and ICD-10) led me to substitute it with the concept of LD. The discussion of the category of LD in this chapter hopefully stresses the importance of this concept and, indirectly, the concept of developmental disorders of learning. The LD concept is important because of its (1) prevalence, (2) implications for countless school-age students and adults, and (3) importance for the development of fundamental models of acquisition of cognitive skills and strategies of prevention and remediation of failure of acquisition.

Currently, students with specific LDs constitute about half of all students served under IDEA. Effective identification of such students and their efficacious and efficient remediation are crucial steps to address their individual educational needs and to provide them with adequate and equal life opportunities.

Given changes in IDEA 2004, it is no surprise that RTI is been central to current discourse on specific LDs. RTI is essential to the professional discussions of educators, diagnosticians, and policy makers because of its promise to alleviate many long-standing concerns with the IQ/aptitude–achievement discrepancy model predominant in the field of LDs for the last 30 years. At this point, however, RTI has yet to deliver on its promise. If RTI succeeds, numerous benefits to educational systems and individuals might be realized (Graner et al., 2005). Specifically, as for the system, many inappropriate referrals might be eliminated to increase the legitimacy and fair nature of 'true' referrals; the costs of special education services might be reduced; various gender and ethnicity biases might be minimized; and accountability for student learning might increase. As for individuals, because the 'labeling' criteria will change, there will be less time for a student to demonstrate a 'true' failure in achieving the stipulated discrepancy value – prevention and remediation efforts are expected to start as early as possible; instruction will be individualized; identification will be focused on achievement rather than on aptitude–achievement discrepancy; and minimized labeling should result in less social stigma.

Yet, these are only expectations for now, and the immediate future will show whether RTI is a viable replacement to the discrepancy criteria.

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2.39 Learning in Autism

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2.39.1 Introduction

Learning in autism is not a topic characterized by consensus. For example, the ability of autistics (see Sinclair, 1999, to appreciate our respectful use of the term 'autistic' rather than 'person with autism') to learn is considered nonexistent in the typical everyday environment (Lovaas and Smith, 2003) and fundamentally impaired (Klinger et al., 2006), but so astounding that the cognitive literature as a whole is insufficient to explain it (Atkin and Lorch, 2006). Autistic learning is recognized as distinctive (Volkmar et al., 2004) and singled out as subhuman (Tomasello et al., 2005), but is also considered unremarkable compared to nonautistic learning (Thioux et al., 2006). These apparently disparate accounts may be the result of autistic learning, in contrast to autistic perception, attention, and memory, being investigated in a piecemeal, *ad hoc* manner. This chapter will summarize a range of current and emerging proposals about autistic learning, examining each proposal's empirical basis and adding historical and thematic perspectives.

2.39.2 Autism: Classification and Description

Autism is a neurodevelopmental difference, classified as a pervasive developmental disorder in the DSM-IV (American Psychiatric Association, 1994) and diagnosed by atypical social interaction (e.g., "a lack of spontaneous seeking to share . . . achievements with other people," American Psychiatric Association, 1994: 70), atypical communication (e.g., difficulty "sustain[ing] a conversation," American Psychiatric Association, 1994: 70), focused interests (e.g., "persistent preoccupation with parts of objects", American Psychiatric Association, 1994: 70), and atypical body mannerisms (e.g., "hand or finger flapping", American Psychiatric Association, 1994: 70). While autism is innate, the overt behaviors used to diagnose autism may not appear until the second year of life, but always appear before age 3. Autism is polygenic (with as yet no agreed-upon loci) and highly heritable, with a male:female ratio of approximately 4:1 and a prevalence of approximately 20/10 000. Two less well-defined pervasive developmental disorders are considered, with autism, to form the autistic spectrum. The first is Asperger syndrome, which shares the behavioral characteristics of autism but presents with a different developmental trajectory, featuring no delay in the onset of speech and measured intelligence in the normal range (Szatmari et al., 2000). The second is Pervasive Developmental Disorder, Not Otherwise Specified (PDD-NOS), defined as a subthreshold presentation of the behaviors used to diagnose autism. Prevalence across the autistic spectrum is roughly 60/10 000, and has been shown to be stable over time, as has autism prevalence (Chakrabarti and Fombonne, 2001, 2005). This review will concentrate on autism itself, as the bulk of the relevant research concerns this specific diagnosis.

In research, autistics are often divided into high- and low-functioning subgroups, based on a snapshot

measurement of intelligence or developmental level. While this division is an efficient shorthand to denote whether participants fall into the range of diagnosable mental retardation, instruments normed for the nonautistic population are potentially misleading when applied to autistics (e.g., Mottron, 2004), and individuals' measured IQs may change dramatically over time, particularly before age 6 (e.g., Eaves and Ho, 2004; Gernsbacher, 2004). Autistics' average scores on intelligence test batteries (e.g., Wechsler scales) mask widely scattered subtest scores, raising the question of whether level of functioning can definitively be assigned even at any single point in time. The difficulty of assessing autistic intelligence is illustrated by recent epidemiology: The percentage of autistics who also meet current day criteria for mental retardation is reported as anywhere from 25–70% (Honda et al., 1996; Baird et al., 2000; Kielinen et al., 2000; Chakrabarti and Fombonne, 2001). The difficulty of assessing autistic intelligence is also illustrated via a speed-of-processing task known to be correlated with intelligence: Autistics assumed to be high- or low-functioning perform equally well, and as well as, nonautistics with Weschler IQs more than 2 or 3 standard deviations higher, respectively (Scheuffgen et al., 2000). Similarly, autistics' performance on Raven's Progressive Matrices, the preeminent measure of fluid intelligence, may significantly exceed their performance on Wechsler scales, suggesting that the high- versus low-functioning division is of questionable validity (Dawson et al., 2007).

Autism has no known etiology in the majority of cases, but in a minority of cases, an associated syndrome can be identified (e.g., tuberous sclerosis, West syndrome). In research, such syndromes are frequently cited as exclusion criteria or possible confounds, and the distinction between etiological autism (associated with such syndromes) and idiopathic autism (not associated with such syndromes) has been important in ascertaining whether epilepsy is associated with autism or with other conditions associated with autism. Indeed, evidence points to epilepsy not being associated with idiopathic autism (Pavone et al., 2004; Battaglia and Carey, 2006).

Another division is often drawn between savant autistics, whose uneven profile of abilities encompasses exceptional expertise in one or more characteristic areas (e.g., calendar calculation, drawing in perspective), and nonsavant autistics, whose uneven profile of abilities has not progressed to that level of atypical expertise. Savant abilities are far more prevalent in the autistic than in the nonautistic population (1 in 10 versus 1 in 2000; Hill, 1977; Rimland, 1978), and are consistently linked with autistic traits (Heaton and Wallace, 2004). Savant abilities and their significance for the study of autistic learning will be explored in later sections.

Few aspects of neurology have not been proposed as being atypical in autism. For example, regions of reported neurofunctional atypicalities range from the brainstem to the inferior frontal gyrus, while reported neuroanatomical atypicalities range from increased white and gray matter volume (e.g., Hazlett et al., 2005) to more densely packed cells and increased numbers of cortical minicolumns (Casanova et al., 2002). Neurofunctional connectivity has been suggested to be atypical (e.g., Just et al., 2004), and neural resources may be atypically allocated or rededicated (e.g., Turkeltaub et al., 2004; Koshino et al., 2005). Virtually every fundamental human cognitive and affective process, singly or as part of an overarching model, has been proposed to be dysfunctional or absent in autism, while persistent findings of superior performances by autistics are often interpreted as evidence of neurological and cognitive pathology (e.g., Langdell, 1978; Shah and Frith, 1983, 1993; Heaton et al., 1998; Beversdorf et al., 2000; Ropar and Mitchell, 2002; Toichi et al., 2002; Chawarska et al., 2003; Just et al., 2004; for analysis and perspective, see Baron-Cohen, 2005; Gernsbacher et al., 2006; Mottron et al., 2006). Thus, autism has been prolifically studied but remains poorly understood.

2.39.3 History and Background: Accounts of Autistic Learning

Accounts of recognizably autistic learning date back more than a century and precede the establishment of autism as a diagnosis. There are reports of individuals with an incongruous repertoire of abilities: Apparently general cognitive impairment coupled with outstanding performance in specific areas, such as music, drawing, calculation, and memory (see Treffert, 1988, for a review). The branding of these individuals as 'idiot savants,' a practice that endured until recently, is evidence of how autistic learning has been and may still be conceptualized.

Kanner (1943) first proposed autism as a distinct condition. His landmark description of 11 autistic children included observations about their unusual pattern of learning, evident from early development. The children precociously acquired quantities of

specific information, from the names of objects, people, and presidents; to numbers and the alphabet; to fine discriminations between musical compositions; to the texts of psalms, poems, and nursery rhymes (sometimes in several languages); to lists of plants and animals as well as "long and unusual words" (p. 243); to the contents of encyclopedias. Kanner characterized much of this learning, particularly in 2- and 3-year old children, as a "valueless" (p. 243) obstacle to genuine communication, but also reported excellent abilities in reading, spelling, and vocabulary. There were no difficulties with plurals, tenses, and grammar; an early reversal of pronouns (e.g., using 'you' for 'I') became less evident over time. The children were characterized as having strong and independent interests; one child "displayed astounding purposefulness in the pursuit of self-selected goals" (Kanner, 1943: 232).

Kanner observed that mute autistic children, a minority in his original sample, had "astounded their parents by uttering well-formed sentences in emergency situations"; he concluded that mute autistic children may demonstrate that they have, while apparently silent, accumulated a "considerable store" of information about language (Kanner, 1949: 417–418). In a later paper, Kanner observed that autistic children were extremely difficult to teach in conventional ways: They "learn while they resist being taught." For example, they remained unimpressed with persistent attempts to prompt them to walk, then spontaneously walked when this was "least expected." One autistic boy's parents undertook strenuous efforts, involving many hours per day, to teach and exhort him to speak. These efforts failed, but "at about $2\frac{1}{2}$ years of age, he spoke up and said 'Overalls,' a word which was decidedly not part of the teaching repertoire." (Kanner, 1951: 23–24).

Independently of Kanner, Asperger (1944/1991) also proposed autism as a distinct condition. In his seminal paper, Asperger recorded observations about autistic learning that were strikingly similar to Kanner's. Autistic children, some of whom were described as learning to read "particularly easily" (p. 75), were "almost impossible to teach" (p. 49) and could not "learn from adults in conventional ways" (p. 56) or "assimilate the ready-made knowledge and skill that others present" (p. 63). These children were poor in what Asperger called mechanical learning, or learning to do as others do automatically. However, they excelled in a kind of original thinking that Asperger called autistic intelligence. Asperger described an autistic child who spontaneously learned basic principles of geometry by age 3, and cubic roots shortly thereafter, but "learnt or did not learn as the whim took him" (p. 88), with unfortunate results in school.

Both Kanner's and Asperger's accounts resonate with earlier reports of 'idiot savants.' In 1945, Scheerer and colleagues discussed Kanner's observations (1943) within an extensive descriptive and empirical account of a child, L., who today would be considered an autistic savant. Alongside apparently comprehensive limitations in behavior and intelligence, L. had excellent abilities in calendar calculation and music, as well as in learning and recall of words, events, facts, and numbers. Interest in these areas first appeared when L. was 3. L. was reported to be incapable of learning by instruction; he had "an inherent difficulty in learning by following instructions and explanations in a systematic way" (p. 2) and "never absorbed or learned in a normal fashion" (p. 59). He had absolute pitch and enjoyed playing the piano "for hours without being taught" (p. 2). His unusual range of abilities was hypothesized to arise from impaired abstraction, which resulted in "abnormal concreteness" (p. 61) and a facility in acquiring and manipulating information that typical individuals would judge as "senseless or peripheral or irrelevant" (Scheerer et al., 1945: 61).

Kanner considered that the atypical strengths and not the obvious difficulties of autistic children reflected their true potential, but Kanner provided limited empirical evidence to support his position, which has accumulated opposition over the years. For example, Klin et al. (1997) contended that autistics' 'splinter skills' overestimated their true abilities, had little relevance to real life, and existed against a context of pervasive deficiency (see also DeMyer et al., 1974; Prior, 1979; Volkmar and Klin, 2005). Similarly, focused abilities and interests have been characterized primarily as interfering with learning in autism and Asperger syndrome, rather than representing it (Volkmar and Klin, 2000; Klin et al., 2005). Distinctly autistic learning and intelligence have thus been considered pathological, misleading, and uninformative, if not mythical (e.g., Epstein et al., 1985; Shah and Frith, 1993; Green, 1999). This judgment leaves no plausible explanation for the conspicuous success of some autistics (e.g., a child who "did phenomenally well in mathematics, was sent to an accelerated school, and is now finishing the eleventh grade with top marks"; Kanner and Eisenberg, 1956: 86).

Specific traits investigated in follow-up studies (e.g., speech fluency or measured intelligence) have not been consistently predictive of outcomes (Howlin, 2005) or explanatory of why some autistics have done notably well (Asperger, 1944; Kanner, 1973; Szatmari et al., 1989). Indeed, both Kanner (1973) and Szatmari et al. (1989) reported that fortunate outcomes were unexpected; they could not have been predicted from early presentation or development. The success in university, including one MBA, of half of Szatmari et al.'s sample (less than 70% of whom had useful speech before age 5) was achieved by individuals who, like the successful autistics reported by Kanner, grew up before the era of early intervention programs. Similarly, an individual who Asperger (1944) followed for 30 years was "grossly autistic" (p. 88) throughout his life, with "impossible behavior" (p. 89), failure, and ineptness in multiple areas (language, daily life, social behavior). This individual pursued his early interest in mathematics and rapidly became a successful, if unusual, academic. Like Asperger, Kanner (1973) underlined the importance of focused interests and abilities through development as the means by which autistics could participate in and contribute to society.

Less fortunate autistics were placed in institutions, denied education, subject to useless and harmful treatments (e.g., "tranquilizers ... pushed to the point of toxicity"; Kanner, 1971: 125), and were found to have poor outcomes (Rutter et al., 1967; Lockyer and Rutter, 1969; Kanner, 1971, 1973). In Rutter's (1966, 1970) sample, 56% of the 63 children had fewer than 2 years of school, and many had none at all, regardless of their measured abilities. More than half were institutionalized, and many endured deleterious or spurious treatments (e.g., electroconvulsive therapy, insulin coma, prefrontal lobotomy, prolonged psychoanalysis; from Rutter et al., 1967). Against this hazardous backdrop, many in Rutter's sample acquired reading abilities, several were employed, and some had academic achievements (e.g., in the areas of music and computer science). DeMyer et al. (1973) observed in their sample, 44% of whom were institutionalized, that a decrease over time in the performance IQs of poor-outcome autistics was related to an observed loss of their 'splinter skills.'

Descriptive and empirical accounts of autistics learning in unusual and successful ways have sporadically appeared and remained unexplained throughout the history of autism research. Autistics are no longer routinely institutionalized and are entitled to public education, but there continues to be a dearth of data linking early autism interventions to adult outcomes. Instead, there are data indicating that currently popular interventions may be unrelated to child outcomes (Gernsbacher, 2003; Eaves and Ho, 2004; Lord et al., 2006; Magiati et al., 2007). The educational and psychosocial intervention literature in autism, despite undeniable quantity and prominence, has failed to produce "a clear direct relationship between any particular intervention and children's progress" (National Research Council, 2001: 5).

2.39.4 Learning in the Autism Intervention Research

Comprehensive early intervention programs in autism have borrowed extensively from each other and have become progressively more similar (Dawson and Osterling, 1997; National Research Council, 2001; Kasari, 2006). A typical curriculum may, at the outset, involve series of trials for training eye contact ('look at me'), commands ('sit down,' 'stand up,' 'come here,' 'turn around'), motor imitation ('do this ...'), followed by commands to point ('point to the ...'), match, verbally imitate, and verbally label (see, e.g., Maurice et al., 1996). Comprehensive programs vary in their use of settings and structure (e.g., highly structured trials vs. more naturalistic approaches), in their use of procedures and techniques (e.g., prompting, reinforcement), in their incorporation of developmental and other theoretical considerations, etc. (Rogers and Ozonoff, 2006). Apart from their intensity (usually, more than 20 h per week) and their ideal of intervening as early as possible, they share the premise that autism represents a harmful deviation from (or multiple deviations from) typical development. They also share the goal of achieving, to the greatest extent possible, a typical developmental trajectory encompassing typical social, communicative, and adaptive behaviors. Failing to address presumed deviations or delays in early development is believed to result in autistics falling further and further behind, as autistic traits and abilities, which are seen as inadequate, inappropriate, or maladaptive, become entrenched obstacles to achieving the ideal typical trajectory. The promise that very early intervention will interrupt, reverse, prevent, and stop autism 'in its tracks' is avidly pursued (Cecil, 2004: 2).

The effectiveness of comprehensive early intervention programs is judged against autism's presumed

poor prognosis, and according to the extent to which typical skills have successfully been acquired and atypical autistic behaviors have successfully been extinguished (Smith, 1999; Handleman and Harris, 2001). The possibility that a typical developmental trajectory and repertoire of behaviors may not be adaptive for autistics or beneficial for autistic learning has not yet been considered. Researchers have "studied the effectiveness of programs, not the appropriateness of various goals" (National Research Council, 2001: 41), while as yet providing no empirical foundation for the popular contention that intensive early interventions result in successful, independent typical adults. The best adult outcomes in the peer-reviewed literature belong to autistics whose early development predates the availability of these interventions and was in no way typical (e.g., Kanner et al., 1972). Indeed, in Szatmari et al. (1989), all children retrospectively judged as only probable for a diagnosis of autism had poor outcomes as adults, while many children whose diagnosis – according to the strictest criteria for autism ever devised – was not in doubt went on to considerable achievement: "severity of early autistic behavior was a poor predictor of outcome" (p. 213).

Early interventions have been widely speculated both to prevent atypical brain activity in autism and to promote desirable typical activity (e.g., Lovaas and Smith, 1989; Perry et al., 1995; Mundy and Crowson, 1997; Smith and Lovaas, 1998; Howard et al., 2005). This speculation is as yet unsupported by studies involving measures of neural activity. The promotion of very early interventions to exploit neural plasticity in the developing brain (Dawson and Zanolli, 2003) appears to be supported solely by a report of a very early (starting at 14 months) applied behavior analysis-based intervention involving a child considered 'at risk' for autism (Green et al., 2002). However, such a young age (2 years) has been cited as an explanation for why other autistic participants failed, rather than succeeded, in another intervention study and why such young participants could not continue in an optimal applied behavior analysis-based intervention (Howard et al., 2005). Thus, promises that autistic brain activity and development can be altered by early interventions in controlled and predictable ways appear to be highly premature.

Training programs that involve older autistics (school-aged children, adolescents, and adults) and that target what are presumed to be core deficits in autism have also been speculated to correct faulty autistic neural mechanisms (Tanaka et al., 2005). However, the only empirical investigation to date found that autistics acquired the specific trained behaviors (labeling pictures expressing facial affect), but did so without producing the desired neurofunctional changes (increased task-related activity in the fusiform gyrus; Bölte et al., 2006). Demonstrations that untrained autistics display this desired brain activity when previous oversights in experimental design are addressed (e.g., Hadjikhani et al., 2004; Pierce et al., 2004) raise questions about the foundations of interventions that target core deficits and exploit task-related brain activity as outcomes.

A common finding arising from both targeted and comprehensive intervention studies is that autistics, when explicitly taught typical skills, fail to generalize those skills across contexts or to related typical skills (e.g., Lovaas et al., 1973; Lovaas and Smith, 1989; Ozonoff and Miller, 1995; Hwang and Hughes, 2000). This failure to generalize is widely regarded as an autistic learning deficit, but such a failure cannot always be attributed to specifically autistic limitations. Young so-called feminine boys who underwent early intensive behavioral interventions to impose stereotypically male behaviors also demonstrated a failure to generalize (Rekers and Lovaas, 1974; Rekers et al., 1974). Thus, the explicit teaching of typical behaviors may result in a failure to generalize in atypical individuals. Accordingly, autistics who fully understand typical, expected social behaviors (e.g., behaviors associated with pretend play or joint attention) may not spontaneously display these behaviors, which are adaptive for nonautistics but may not necessarily be adaptive for autistics (e.g., Boucher, 1989; Klin et al., 2002). Regulation of atypical autistic visual and auditory perception (Mottron et al., 2006; Samson et al., 2006) is currently the most plausible explanation for characteristic autistic behaviors (e.g., in the areas of eye contact, Gernsbacher and Frymiare, 2005; joint attention, Gernsbacher et al., in press; and orienting to stimuli, Mottron et al., 2007); therefore, attempts to train typical but less adaptive behaviors may not easily generalize. Further, Szatmari (2004) has argued that autistics' enhanced perception results in independent, spontaneous learning of which nonautistics are incapable.

2.39.5 Applied Behavior Analysis and Autistic Learning

The first reports of operant conditioning in autism in the early 1960s (e.g., Ferster and DeMyer, 1961) are considered by behavior analysts as the first

demonstrations that autistics could learn (Schreibman and Ingersoll, 2005). Behavior analysts henceforth characterized autistics as being governed by the same laws of learning as all other organisms, while being distinguished by failing to learn from the typical, everyday environment (e.g., Lovaas, 1987; Green, 1996; Smith and Lovaas, 1998; Koegel et al., 2001; Lovaas and Smith, 2003). Applied behavior analysis (ABA), summarized by Green (1996: 29) as employing procedures derived from the principles of behavior to "build socially useful repertoires" of observable behaviors and reduce or extinguish socially "problematic ones," has become the basis for an extensive autism intervention literature and service industry. The behavior analytic literature in autism presents autistics as having an extremely restricted behavioral repertoire that is not recognizably human, as lacking in human experience to the point of being *tabula rasa*, as requiring the explicit teaching of virtually every human behavior, and therefore as being an ideal proving ground for interventions based on learning theory (Lovaas et al., 1967; Lovaas and Newsom, 1976; Lovaas, 1977, 1993, 2003; Lovaas and Smith, 1989; Smith, 1999; Schreibman, 2005).

Stimulus overselectivity, wherein autistics "respond to only part of a relevant cue, or even to a minor, often irrelevant feature of the environment," has been identified by behavior analysts as underlying autistics' failure to learn and generalize (Lovaas et al., 1979: 1237; see also Schreibman, 1996). However, demonstrations of overselectivity in autistics (e.g., Lovaas et al., 1971b; Lovaas and Schreibman, 1971) exist alongside findings showing overselectivity in nonautistics, as well as the absence of overselectivity in autistics (e.g., Koegel and Wilhelm, 1973; Schover and Newsom, 1976; Litrownick et al., 1978; Gersten, 1983). An apparent failure of autistics to attend to and therefore learn from relevant social information using dolls as stimuli (Schreibman and Lovaas, 1973) contrasts with the empirical finding that autistic children (IQ ~60) perform better than age-matched typical controls in recognizing their classmates' faces (Langdell, 1978). Moreover, Lovaas and Schreibman's (1971) and Lovaas et al.'s (1979) overselectivity-based prediction that classical conditioning would be impaired in autism, with a consequent failure to acquire conditioned reinforcers, was found to be incorrect. In a classical eyeblink conditioning paradigm, autistics more rapidly learned an association between multimodal contiguous stimuli than did nonautistics (Sears et al., 1994). Regardless, overselectivity's enduring theoretical influence is demonstrated in the behavior analytic practice of breaking all skills down into small steps with each step being explicitly taught through repetition, and of minimizing and simplifying the information in an autistic's environment when teaching basic skills (Maurice et al., 1996; Leaf and McEachin, 1999; Lovaas, 2003).

The need to suppress the high prevalence of so-called self-stimulatory behaviors in autistics (e.g., rocking the torso, smelling objects) is a consistent theme across the behavior analytic literature. While it is believed that self-stimulatory behaviors interfere with learning explicitly taught behaviors (e.g., Lovaas et al., 1971a, 1987; Koegel and Covert, 1971), that is not always the case (e.g., Klier and Harris, 1977; Chock and Glahn, 1983; Dyer, 1987), and self-stimulatory interests (e.g., maps, calendars, movies) have also been used productively as reinforcement (e.g., Charlop et al., 1990). Self-stimulatory behaviors have not been consistently defined by behavior analysts; for example, immediate echolalia (repeating back what another person just said) was classified as self-stimulatory in one model (Epstein et al., 1985; Lovaas, 2003) but not in another (Gardenier et al., 2004; MacDonald et al., 2007).

Self-stimulatory behaviors are often defined as serving no obvious or apparent function (Gardenier et al., 2004; MacDonald et al., 2007), but in one extensive behavior analysis to understand the origin of self-stimulatory "ear covering that was reported by the [autistic] child's teachers to serve no identifiable function" the "[r]esults of a descriptive analysis revealed a correlation between ear covering and another child's screaming. An analog functional analysis showed that ear covering was emitted only when the screaming was present" (Tang et al., 2002: 95).

While self-stimulation has been defined as a subclass of stereotypy, characterized by its autonomy from social reinforcement (Lovaas et al., 1987), it has also been found to be socially mediated (Durand and Carr, 1987). Self-stimulation and stereotypy are sometimes regarded as interchangeable (e.g., Charlop-Christy and Haymes, 1996, in which 'stereotypy,' 'aberrant behaviors,' 'obsessions,' and 'self-stimulation' are equivalent terms), and self-stimulatory behaviors have been expanded to encompass all autistic focused interests and abilities. Absolute pitch, calendar calculation, hyperlexia, expertise in prime numbers, accurate drawing, and the like have been classified as self-stimulatory (Epstein et al.,

1985; Lovaas, 2003); autistics' spontaneous, untrained learning (in the absence of either teaching or reinforcement) has been classified as "generative self-stimulatory behavior" (Lovaas et al., 1987: 58). Epstein et al. (1985) described a 5-year-old autistic boy in an intensive ABA program who "suddenly emerged" (p. 292) with excellent calendar calculation skills; this and other spontaneous 'genius' behaviors were then discouraged and suppressed.

Indeed, exceptional and savant abilities are listed by behavior analysts as among autistics' abnormal behavioral deficits and excesses (e.g., Koegel and Koegel, 1995). Exceptional abilities in children who exhibit high levels of self-stimulatory behaviors, which are considered by behavior analysts to prevent autistics from learning, remain unexplained. For example, there is no explanation for how a 3-year-old autistic "engaged in lengthy periods of self-stimulatory behavior, such as lying down and sifting sand through his hands" learned to read at a grade 1 level (Koegel et al., 1997: 236), or how a 4-year-old autistic, with no basal score on standardized language measures and "high levels" of "stereotypic hand flapping, finger manipulation, body rocking and noise making" learned how to "decode written words" and "discriminate numerous varieties of automobiles" (Mason et al., 1989: 173). The behavior analytic observation that autistics have spontaneously learned various skills that they do not demonstrate on demand (e.g., Taylor and MacDonough, 1996) also remains unexplained, though the possibility that autistics' inconsistent responding in some situations results from 'boredom' has been raised (Dunlap and Koegel, 1980).

In attempting to address autistics' failure to learn, behavior analysts have created environments of extreme food deprivation (Lovaas et al., 1967); electric shock (Lichstein and Schreibman, 1976) or other contingent aversives (Lovaas, 1987; Lovaas et al., 1987); and extreme repetition (e.g., 90 000 discrete trials to teach an autistic boy one verbal discrimination; Lovaas, 1977). One autistic child underwent more than 24 000 discrete trials and failed to learn any receptive language (Eikeseth and Jahr, 2001). The same child acquired language skills in fewer than 100 trials when provided with text, rather than speech or signs, but environments created by behavior analysts to train some autistics (now deemed to be 'visual learners') with text have produced very limited results (Lovaas and Eikeseth, 2003). While physical punishment within behavior interventions became illegal in many jurisdictions and was replaced by other methods (but see Foxx, 2005), a nonrandomized controlled trial that depended on contingent aversives (Lovaas, 1987; McEachin et al., 1993) continues to be cited as the primary evidence that ABA-based interventions are effective. The only randomized controlled trial of an early comprehensive ABA program reported poor short-term results (Smith et al., 2000, 2001). When unmatched variables in a nonrandomized trial were accounted for, differences in outcome measures between the experimental and control groups (with the exception of classroom placement) were not significant (Cohen et al., 2006; see also Magiati et al., 2007). Further, none of the few existing small-sample controlled trials, in a vast literature dominated by single-subject designs, has reported a correlation between increased amount or intensity of treatment and better short-term outcome measures. Instead, data from an uncontrolled trial show that neither intensity nor quality of early ABA programs is related to short-term outcomes (Sallows and Graupner, 2005).

2.39.6 Autistic Learning in the Cognitive and Savant Literatures

The cognitive literature in autism provides few empirical findings directly related to learning, despite speculative claims about autistic learning impairments and 'learning style' (see Volkmar, et al., 2004, for a review). Among empirical findings, autistics have demonstrated enhanced discrimination of novel highly similar stimuli but an absence of a typical perceptual learning effect (Plaisted et al., 1998); and nonautistics, but not autistics, showed a training effect when copying drawings of objects and nonobjects, although overall performance of the two groups was equal (Mottron et al., 1999). In both cases (perception and procedural memory), procedures (e.g., repeated performance of tasks) that reliably resulted in learning in nonautistics appeared not to do so in autistics, while autistics appeared to learn in ways (e.g., apparently passive exposure to materials) that did not necessarily benefit nonautistics.

In the area of language, echolalia is common in typical development (e.g., a mother asks, 'Do you want a cookie?' and a child responds, 'a cookie?'), but echolalia occupies an atypical role in language acquisition in autism. Echolalia, which serves numerous functions (Prizant and Duchan, 1981; Prizant, 1983; Prizant and Rydell, 1984), is one example of how autistics atypically access the meaning of

language by first learning its complex structure, the reverse of the typical pattern (Dunn and Sebastian, 2000). For example, an autistic child, quite fond of the Teletubbies show on Public Broadcasting Service, initially repeated the scripted sentence, "One day in Teletubbie land, all of the Teletubbies were very busy when suddenly a big rain cloud appeared," and weeks later, using mitigated echolalia, stated, "One day in Bud's house, Mama and Bud were very busy when suddenly Daddy appeared" to express the construct of his father returning home. Initially, when this child wanted to play ball, he would approach his mother or father and say, "Quick, Dipsy. Help Laa Laa catch the ball." As his spoken language developed, the syntactic structure of echolalic sentences remained intact, but he replaced the nouns (e.g., "Quick, Daddy. Help Bud catch the ball"), and he eventually isolated single words and morphemes and began generating original phrases (e.g., "Daddy ball?" and "Dad, wanna play ball?"; Mom-NOS, 2006).

Hyperlexia (Silverberg and Silverberg, 1967), a spontaneous (uninstructed), precocious, interest-driven ability to decode written words, is also strongly associated with autism (Grigorenko et al., 2002). Atkin and Lorch (2006) extensively tested Paul, a 4-year-old autistic boy who intensively studied newspapers before age 2 and recited the alphabet and read printed words aloud by age 3. Paul's mental age was placed at 1 year and 5 months, and his comprehension of language was markedly delayed (though not absent), but he tested as having "extremely advanced decoding skills" (p. 266), including a reading vocabulary exceeding that of typical 9-year-olds. The authors concluded that these results "suggest the possibility of an atypical route to language acquisition" (p. 267) and that "existing cognitive accounts are inadequate to account for the development of literacy in this child" (p. 267).

With respect to the role of categories in learning, autistics may not necessarily use concepts to organize information (Hermelin and O'Connor, 1970; Bowler, 2007, for a review), but are able to do so, including the use of basic level and more abstract superordinate categories as well as prototypes (e.g., Tager-Flusberg, 1985a,b; Ungerer and Sigman, 1987). In a test of novel category learning, Klinger and Dawson (2001) found that autistics categorized using both explicit and implicit rules, but when answering an ambiguous question, failed to show the same response to prototypes as nonautistics. Molesworth et al. (2005), who instead used a false recognition procedure, found typical learning of novel categories in autistics, including typical prototype formation. At the level of perceptual categorization, autistics demonstrated typical category formation in a categorization task, but in contrast to typical controls, autistics showed no influence of categories in a discrimination task. The influence of categories may therefore be optional in autistics, while being mandatory in nonautistics (Soulières et al., 2007).

Klinger et al. (2006) have proposed a fundamental implicit learning (Reber, 1967, 1993; Frensch, 1998; Frensch and Rünger, 2003) impairment in autism based on the prototype paradigm in Klinger and Dawson (2001) and on preliminary data from two artificial grammar learning experiments. Their first study found equivalent autistic and nonautistic above-chance performance in the implicit learning of artificial grammars, while in their second study autistics with lower IQs than their nonautistic controls performed far above chance, but the nonautistic group performed significantly better. Reber (1967) reported a similar discrepancy between typical undergraduates and typical high-school students performing well above chance, without the latter being deemed impaired in implicit learning. Using a serial reaction time task (Nissen and Bullemer, 1987) involving a sequence of lighted circles, Mostofsky et al. (2000) found no evidence of implicit learning in autistics. However, using the same kind of task, Smith (2003) found robust implicit learning of a sequence of geometric figures in autistics, with response accuracy superior to that of typical controls. Results from Smith's (2003) second experiment using a sequence of emotional face images suggest that the presence of social information may demand more attentional resources from autistics than nonautistics, therefore disproportionately interfering with autistics' implicit learning of nonsocial material (in this case, a sequence).

Associative learning has been reported as intact in autism (e.g., Boucher and Warrington, 1976; Williams et al., 2006), but autistics were also found to associate paired stimuli more rapidly than nonautistics (Sears et al., 1994). Reviewing a wide range of evidence, Baron-Cohen (2003) posited systemizing, a form of intrinsically reinforced associative learning, as being a strength in autism, "a condition where unusual talents abound" (p. 138). Systemizing requires an exact mind and is motivated not by extrinsic reinforcement but by a drive to understand systems. Baron-Cohen (2003) describes an autistic 5-year-old boy whose mother accidentally discovered that, by walking down the same street every day, he had correctly associated hundreds of houses with the

hundreds of cars (parked on the street) of their occupants, along with the expiration dates and serial numbers of the cars' parking stickers.

In contrast, Tomasello et al. (1993, 2005; see also Tomasello, 2001) posited a form of social learning – cultural learning – as the defining achievement of uniquely human cognitive abilities, which autistics, along with apes, were deemed to lack. However, despite claims that the essential uniquely human ability is the learning of intentionality, which according to Tomasello autistics lack (Tomasello et al., 1993; Tomasello, 2001), empirical studies have demonstrated robust understanding of intentions in autistic children (Aldridge et al., 2000; Carpenter et al., 2001; Russell and Hill, 2001) and adults (Sebanz et al., 2005). The current model of cultural learning and cognition (Tomasello et al., 2005) is now founded not on the learning but the sharing of intentionality, which Tomasello has argued is absent in autistics and apes. The defining of humanity according to attributes that autistics are judged to lack is a hallmark of normocentrism (Mottron et al., in press).

After a long history of reductive explanations for savant abilities (e.g., photographic or phonographic memory), the savant literature largely recognizes that these abilities represent both spontaneous learning and creative manipulation of the structures and regularities underlying complex information (e.g., music, numbers, written language, visual proportions and perspective). Experimental studies of savants have concentrated on whether and how learned information and abilities are recalled, applied, modified, transformed, or transferred (Miller, 1999; Heaton and Wallace, 2004). Therefore, while savant abilities in autistics can be considered the equivalent of expertise in nonautistics (Mottron et al., 2006), there is only indirect evidence as to how this expertise is acquired. Overtraining with specific materials may (Howe et al., 1998) or may not (e.g., Selfe, 1977; Epstein et al., 1985) be observed prior to the full manifestation of exceptional abilities, which may also be discovered by accident (Sacks, 1985).

Thioux et al. (2006) proposed that savant abilities are driven by autistic focused interests, but depend on spared areas of typical learning abilities; in this model, as in Klinger et al. (2006), savant abilities are explicitly learned, with no role for implicit learning. However, implicit learning is widely considered to play an essential role in savant abilities (e.g., Hermelin and O'Connor, 1986; O'Connor, 1989; Miller, 1989, 1999; Spitz, 1995; Heaton and Wallace, 2004; Pring, 2005; Mottron et al., 2006). Miller (1999) has related the sophistication found in savant abilities to both enhanced processing at the perceptual level and the implicit learning of regularities, while suggesting that extensive exposure to materials may, for savants, be more effective than typical forms of teaching or rehearsal, which in turn may impede learning in savants. He concluded that "savants may provide a special perspective on the mixture of implicit and explicit learning that produces noteworthy performance" (p. 43).

Treffert (2000) has argued that savant abilities should be encouraged and nurtured; this results in a broadening of focused abilities and the flourishing of previously limited social abilities. For example, Miller (1989) denies that a young musical savant could be autistic, regardless of his fitting the relevant criteria, on the grounds that by age 5, he "showed obvious pleasure in social interaction" (p. 10). However, prior to the availability of a piano, the same boy was described as "not very responsive" (p. 9), "spending hour after hour gazing out the window" (p. 210), and "for a very long time, nonverbal and withdrawn" (Newman, 1989: 239). Further, autism does not preclude pleasure in social interaction, which for example is observed in autistics spontaneously sharing the same interest with each other (LeGoff, 2004).

Savant and nonsavant autistics are best considered as belonging to the same group, based on multiple behavioral and cognitive similarities. The performance of savants predicts the performance of nonsavant autistics in multiple areas. For example, savant musicians invariably have absolute pitch, while absolute pitch (Brown et al., 2003) and superior pitch labeling, pitch memory (Heaton et al., 1998; Heaton, 2003), and pitch discrimination and categorization (Bonnel et al., 2003) characterize nonsavant autistics. In a music imitation task, nonsavant autistic youths (mean IQ <70) with no musical experience performed as well as or better than age-matched controls who had considerable musical training (Applebaum et al., 1979), echoing the superior musical imitation found in savant autistics (e.g., Slodoba et al., 1985; Young and Nettlebeck, 1995). A savant draftsman (Mottron and Belleville, 1993) and nonsavant autistics (Mottron et al., 1999) shared a facility in copying impossible figures and a recognizable, locally oriented drawing strategy. Savant (Park, 1986; Steel et al., 1984; Hermelin and O'Connor, 1990; Young and Nettlebeck, 1995; Anderson et al., 1999) and nonsavant (Scheuffgen, 2000; Dawson

et al., 2007) autistics may present with exceptional performance in tests of processing speed and/or high-level abstract reasoning. Many other empirically documented similarities are available, but it is also true that regardless of being extensively studied, both autism and savant syndrome remain unexplained. So does the overlapping relationship between the two, along with the learning processes underlying both variations in neurological functioning and information analysis.

2.39.7 Summary: Characterizing Autistic Learning

Learning in autism is characterized both by spontaneous – sometimes exceptional – mastering of complex material and an apparent resistance to learning in conventional ways. Learning that appears to be implicit seems to be important in autism, but autistics' implicit learning may not map directly onto nonautistics' implicit learning or be governed by the same constraints. An understanding of autistic learning, of how and why autistics learn well and learn poorly, may therefore require a nonnormocentric approach and an investigation of the possibility that autistic and nonautistic cognition may be complementary in learning and advancing different aspects of knowledge.

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2.40 Individual Differences in Episodic Memory

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2.40.1 Introduction

> Wide individual differences in learning and memory abilities have long been noted, and have been addressed in psychometric research. Unfortunately, there has been little interplay between experimental and psychometric approaches to these areas, with the result that little can be said about the relations between processes studied in experimental investigations and the dimensions of individual differences isolated in psychometric research. (Carroll, 1993: 248)
>
> It is shortsighted to argue for one science to discover the general laws of mind or behavior and for a separate enterprise concerned with individual minds. (Cronbach, 1957: 673)

This chapter will selectively review the vast empirical terrain regarding normal individual differences in adult human memory for newly learned information. In so doing, we will consider research conducted within both the experimental and psychometric traditions (with the latter often referred to as 'correlational,' 'differential,' and 'individual'). These two research traditions have each yielded a wealth of data and theory about varieties of memory phenomena and their structural or taxonomic relations to one another (e.g., French, 1951; Atkinson and Shiffrin, 1968; Tulving, 1985; Sherry and Schacter, 1987; Carroll, 1993; Engle et al., 1999b; Baddeley, 2000), but the work in one domain has had regrettably little impact on the other. Indeed, one could argue the same for virtually all psychological phenomena that are studied by experiment on one hand and correlations among mental tests on the other (Cronbach, 1957).

Episodic memory, in particular, provides an interesting history regarding the hesitant dance between experimental and psychometric approaches to psychological science. For while the empirical study of human memory began by deriving nomothetic principles from the intense examination of one man (Ebbinghaus, 1885), the pursuit of individual differences in memory, and their relation to the broader intellect, was very close behind. Indeed, Jacobs's (1885) review of Ebbinghaus's book, and his subsequent (1886) call to create a Society for Experimental Psychology, predicted that the new science of memory would provide psychologists a window

into people's mental abilities and a means by which to rank them:

> May we hope to see the day when school registers will record that such and such a lad possesses 36 British Association units of memory-power. . .? If this be visionary, may we at least hope for much of interest and practical utility in the comparison of the varying powers of different minds which can now be laid down to scale. (Jacobs, 1885: 456)
>
> There is, I submit, a certain number of syllables up to which each person can repeat a nonsense word like *borg-nap-fil-trip* after only once hearing; and it is probable, though we cannot know for certain, that this number varies with different persons, giving a sort of test of their linguistic capacity. (Jacobs, 1886: 53)

Jacobs was right: People differ in their immediate memory capacity, and these differences are associated with other linguistic, and nonlinguistic, abilities. We know this, in part, because Jacobs (1887) invented the venerable memory span task for one of the first systematic individual-differences studies of memory. Schoolchildren heard and attempted to repeat lists of unrelated syllables, letters, or digits, and the largest set that each could perfectly reproduce was termed his or her span of prehension. Jacobs found this span to increase with chronological age and with higher school marks. The theoretical and practical links between memory span and intelligence, thus forged, persist to this day (albeit with controversy; Ackerman et al., 2005; Kane et al., 2005; Oberauer et al., 2005).

Ebbinghaus's and Jacobs's work represents the roots of parallel traditions of modern memory research. In caricature, the experimental approach discovers and explains lawful regularities of memory by analyzing the learning and remembering yielded by various laboratory conditions with univariate, ANOVA-based analyses; the differential approach discovers and explains lawful variation in memory by analyzing the learning and remembering yielded by a variety of valid and reliable tests with multivariate, regression-based analyses. The reality, of course, is more nuanced, with some cross-fertilization of theory, method, and analysis between experimental and psychometric traditions. We view such integration as critically important to the health of our discipline, and we argue that it is nowhere more prominent or promising today than in the study of working memory (WM). Thus, after discussing further the persisting tension between differential and experimental psychology in general, and then reviewing the largely atheoretical psychometric literature on individual differences in broad aspects of episodic memory, we will focus on theoretically motivated research that combines experimental and correlational methods to examine variation in WM (for a variety of theoretical views on WM variation, see Conway et al., 2007).

2.40.2 Psychology's 'Two Disciplines'

About once every generation, a prominent psychologist has called for a unification of experimental and psychometric psychology. As early as 1924, Terman argued against the looming notion that psychometric tests served mainly diagnostic and technological purposes, whereas experiments (and their 'tasks') sought discovery and theoretical advance. While acknowledging that mental tests often serve practical interests, Terman (1924) also highlighted the 'testing' research by theorists such as Spearman, Cattell, Hall, and Thorndike that firmly aimed to illuminate the basic nature of mental phenomena. He concluded that:

> One would probably be safe in predicting that the next decade will see a large interaction between the psychology of mental tests and the experimental psychology of thinking. On the one hand, the opening up of problems of individual differences by means of tests will inevitably lead to the more intensive study of such differences by the usual laboratory methods; and, on the other hand, the success attained by tests in the diagnosis of abilities for useful purposes is likely in turn to have a considerable effect upon the technique of experimental psychology. (Terman, 1924: 113)

2.40.2.1 Separate but Equal

If Terman (1924) had been right, of course, Cronbach's (1957) classic lament on the rift between "the two disciplines of scientific psychology," over a quarter century later, would have been unnecessary. Cronbach colorfully characterized the distinction between experimental and correlational psychology in their reactions to individual differences. For experimental psychology, they represent 'error

variance,' an intolerable nuisance to be minimized. For correlational psychology, in contrast, individual differences represent the results of important physiological, environmental, and developmental causes: "The correlational psychologist is in love with just those variables the experimenter left home to forget" (p. 674). Cronbach noted some examples of methodological integration with optimism, such as in experimental approaches to development, and, like Terman, he hoped that experimental and psychometric psychology would eventually capitalize on each other's strengths.

What can (and should) these disciplines offer one another? Cronbach (1957) suggested that, on one hand, experimental evidence can provide a source of construct validation for the psychometrician. If, for example, individual differences in some test scores are thought to reflect a particular construct, such as memory confidence, then scores should vary with experimental manipulations of confidence. On the other hand, the experimentalist would benefit from the psychometrician's multivariate methods, which yield optimally reliable and valid measures of hypothetical constructs. That is, experimental tests of theories about multifaceted phenomena need not focus on only a single, impure dependent variable, nor do ostensible classes of independent variables need to be categorized via intuition or tradition. Instead, correlational techniques such as factor analysis may provide simplification and organization of complex patterns of treatments and outcomes. Thus, for Cronbach, the experimentalists' study of variation among treatments and the psychometrician's study of variation among individuals should be combined in the pursuit of individual-by-treatment interactions. Such interactions are exactly what Melton later urged memory researchers to pursue:

> . . .the sooner our experiments on human memory and human learning consider the differences between individuals in our experimental analyses of component processes in memory and learning, the sooner we will have theories and experiments that have some substantial probability of reflecting the fundamental characteristics of those processes. (Melton, 1967: 250)

2.40.2.2 A Crucible for Theory Testing?

Unfortunately, no federation of psychological disciplines had yet been established by 1975 when Underwood found it necessary to convince experimental psychologists that an individual-differences approach could be useful to theory testing. A verbal-learning experimentalist, Underwood advocated that correlational studies could provide a useful 'go ahead' signal to new theories. His argument was that most nomothetic theories of mental process make idiographic predictions, and that these predictions should be tested early on to determine whether a theory warrants pursuit. That is, if individual-differences predictions fail to materialize, such falsification indicates that the theory requires revision. In Underwood's words, individual-differences research provides a 'crucible' in which to test general theory.

Cronbach's (1957) and Underwood's (1975) *American Psychologist* articles are both psychology classics, with over 1000 scientific citations combined. One might expect, then, that psychologists have finally gotten the message and are regularly testing their theories with individual-by-treatment interactions. On the contrary, this millennium saw yet another call for uniting experimental and psychometric disciplines, but here to bridge psychology and biology. Kosslyn et al. (2002) described studies in which experimental and correlational approaches to theoretical questions regarding mental imagery, avoidance, mood, stress, and immune function had yielded compelling theory confirmation, ruled out alternative explanations, clarified conflicting results, or illuminated biological mechanisms that had been obscured by averaging over subjects. They concluded that neither approach, by itself, would have accomplished these ends:

> Neither group nor individual differences research alone is sufficient; researchers need to combine the two. Indeed, by combining the two, one may discover that the group results reflect the combination of several strategies, each of which draws on a different (or partially different) system. Thus, the group and individual differences findings mutually inform each other, with the synergy between them illuminating the complex relations between psychology and biology. (Kosslyn et al., 2002: 348)

2.40.2.3 A Crucible for Theory Testing!

As an example of this combined experimental-psychometric approach to the study of memory, Underwood put his money where his mouth was in a large correlational study of episodic memory tasks. Underwood et al. (1978) tested whether the attributes of episodic memory that had been proposed to

account for experimental results (Underwood, 1969, 1982) also influenced individual differences in remembering. They administered, to 200 subjects, 24 different word-memory tests (e.g., free recall, paired-associate learning, serial recall, memory span, recognition), which measured six ostensible memory attributes: imagery, implicit associative, acoustic, temporal, affective, and frequency.

To test whether episodic memory could, in fact, be carved at its attributes, Underwood et al. (1978) factor analyzed the 24 memory tests. Factor analysis is a statistical procedure that reduces a large number of manifest test variables into a smaller number of unobserved, latent factors by examining the correlations between tests. Simply put, a factor represents the common variance among a group of measures. A test's factor loading indicates how much of its variance is captured by the factor, with higher loadings indicating a stronger association. Factors are easiest to interpret when tests are dominated by a single factor, that is, when they have high loadings on one factor and small loadings on others. Imagine, for example, that a collection of memory-span and immediate free-recall and recognition tests all load onto one factor, and a collection of span, free-recall, and recognition tasks requiring delayed memory all load onto another, with each task having near-zero loadings on the other factor. Such factor loadings would provide psychometric validation for the theoretical distinction between short- and long-term memory. In a sense, then, factors are theories about the tasks they represent (Carroll, 1993).

Five interpretable factors emerged from the Underwood et al. (1978) tasks, but they were disappointing for contemporary theory. First, the factors corresponded to the task categories rather than to attributes: factor 1 reflected primarily the paired-associate tasks, factor 2 the free-recall tasks, factor 3 the memory-span tasks, factor 4 the recognition and frequency-estimation tasks, and factor 5 the verbal discrimination tasks. None of the attributes of interest appreciably affected the factor loadings of tasks, despite some having significant and substantial experimental effects (e.g., concrete words were better remembered than abstract words). Second, the recognition and verbal discrimination tasks loaded onto separate factors. This was surprising because verbal discrimination and recognition memory were both explained by frequency theory, according to which the cue used to discriminate between two words is the perceived difference in linguistic frequency. Verbal discrimination and recognition tests, therefore, should have loaded on the same factor, but they did not. As Underwood et al. put it,

> Perhaps never before has a theory that evolved from experimental work been so savagely attacked by a correlational approach. (Underwood et al., 1978: 416)

Of course, this is just as Underwood (1975) would have had it.

(For Underwood et al. (1978), these findings did not falsify the claim that memory attributes are distinguishable and important to memory theory. Subtleties in their data suggested that subjects could strategically attend to particular attributes depending on their prior experiences and task demands, and so memory attributes were important phenomena to continue considering.)

Before considering more recent research that has attempted to meet the appeals of Terman, Cronbach, Underwood, and Kosslyn, by uniting the correlational and experimental approaches to memory research, we first highlight the key findings and conclusions drawn from the psychometric study of healthy young adults. The rest of this volume will amply review the fruits of experimental approaches, and individual chapters by Naveh-Benjamin (*See* Chapter 2.41), Ornstein (*See* Chapter 2.37), and Rovee-Collier (*See* Chapter 2.36) will consider memory's lifespan development.

2.40.3 The Psychometric Approach to Memory

Memory tasks have appeared within standardized intelligence test batteries since their inception (e.g., Cattell and Galton, 1890; Binet and Simon, 1905; Wechsler, 1997; Roid, 2003). Until recently, however, particular memory processes have only sporadically been linked theoretically to aspects of 'intelligent' behavior, such as complex learning, comprehension, or reasoning (e.g., Blankenship, 1938; Bachelder and Denny, 1977a,b; Dempster, 1981, 1991). In fact, most of the twentieth century saw research on memory variation take a distinctly bottom-up, atheoretical approach, by 'throwing into the hopper' a number of different mental tests, including some involving memory, and examining the resulting factor structure. We discuss these studies before reviewing more theoretical, top-down approaches to individual differences in memory.

2.40.3.1 The Structure of Memory, from the Bottom Up

Prototypical studies tested between 30 and 200 subjects on a battery of popular, standardized tests assessing a range of cognitive abilities, from pitch perception, to motor speed, to mental rotation, to mechanical knowledge, to reading comprehension, to abstract reasoning. Fewer than five memory tests usually appeared in these batteries, selected more for their availability than for their utility in evaluating theoretical questions. Indeed, most of these studies assessed the structure of intellect, broadly defined, rather than examining the structure or processes of memory, proper. The memory tests that were used most often were memory span, paired-associates, recognition, and free recall. We described memory span earlier, and subsequent studies did not veer far from Jacobs's (1887) original methods. Paired-associate tests usually tested subjects on novel stimulus pairings, such as between unrelated words, words and digits, or first and last names, with the test presented almost immediately after learning. Recognition and free-recall tasks varied widely in their methodological details, but most presented verbal material and imposed only brief study–test delays.

2.40.3.1.1 Factor-analytic findings

French (1951) and Carroll (1993) have reported comprehensive reviews of factor-analytic studies of intelligence (the former qualitative, the latter quantitative). Because Carroll's analyses subsumed most of French's, our discussion relies heavily on Carroll (1993).

2.40.3.1.1(i) First-stratum memory factors

'First-stratum' memory factors refer to those that represent associations among individual psychometric tests assessing relatively narrow cognitive abilities. Carroll's (1993) review, which included reanalyses (via exploratory factor analysis) of 117 datasets on intelligence, found strong evidence for four distinct memory factors: memory span, associative memory, free-recall memory, and meaningful memory (other potential factors, such as visual memory, were identified more provisionally).

Seventy datasets provided strong evidence that memory-span tests comprised a separate factor from other memory tests, usually as a single factor regardless of stimulus type or modality. At the same time, some studies indicated modest separation between verbal and nonverbal tests, and most did not include enough tests to draw strong conclusions about the unity of memory span. Limited evidence also suggested that the use of supraspan lists and the induction of interference by interpolation of lists yielded a factor separate from the standard span test (e.g., Hunt et al., 1973, 1975). We will discuss related findings in our subsequent treatment of theoretically motivated psychometric research.

Although their respective datasets were fewer (51 and 12, respectively), Carroll also provided evidence that associative-memory and free-recall factors were separate from memory span. As in French's (1951) earlier review, paired-associate tests were the best indicators of the associative-memory factor, but it also loaded (more weakly) on recognition tests and serial-recall tests. Associative processes may thus be involved in learning for, and cuing in, both recognition and serial recall. Stimulus type did not appear to affect the correlations among paired-associate tests, as was true for memory span. Free-recall tests were also frequently discriminable from both memory span and associative memory, thus forming a separate factor of their own, and this was especially so when the tests presented supraspan lists. For example, in Games (1962), letter-span tests with list lengths of ten loaded with other free-recall tests rather than with traditional span tests.

2.40.3.1.1(ii) Higher-stratum memory factors

Individual differences in memory span, paired-associate recall, and free recall are psychometrically separable. Should we therefore propose that they measure discrete mental abilities and cognitive processes? The answer is yes and no, depending on the stratum we consider within memory's hierarchical structure. Carroll (1993) showed that these factors emerge reliably at the first stratum, reflecting the correlations among individual memory tests. But their factorial separation does not imply stochastic independence. This is because memory tests of all types tend to correlate positively, and studies that include enough tests of each first-stratum memory factor find that their factors correlate, too. Indeed, Carroll's analyses yielded a higher-stratum 'general memory' factor, that is, a single memory factor at a higher level of the hierarchy that subsumed all the memory factors at the lower level. So, while it is true that people's performance on a memory-span test correlates more strongly with other span tests than it does with paired-associate tests or free-recall tests, these different memory tests all correlate more

strongly with one another than they do with other kinds of mental tests. Individual differences thus suggest a general ability to remember recently experienced events or newly learned information.

Of importance, the finding of a general memory factor comprising memory span, associative/recognition memory, and free recall replicates across multiple investigators, using a variety of statistical techniques, working under different hierarchical theories of intelligence. For example, the Horn-Cattell theory of general fluid and crystallized intelligence (Gf-Gc theory; Horn and Cattell, 1966), which is based on a wealth of lifespan development data, proposes a 'short-term acquisition and retrieval' (SAR) factor at a second stratum that bears striking resemblance to Carroll's (1993) general memory factor. As described by Horn (1988), SAR factors typically comprise memory-span, associative-memory, and free-recall memory tests that impose brief delays between study and test. Horn thus describes SAR as reflecting attention to, and maintenance of, information for use in other cognitive processes (by analogy to Baddeley and Hitch's (1974) notion of WM).

The reliable presence of a higher-order memory factor that is general to many varieties of episodic memory tests indicates that, despite differences that are indicated by separate first-order factors, some abilities or processes are common to episodic memory tasks, and they vary reliably among adults. As such, any compelling theory of performance in an episodic-memory task must explain both the processes that are unique to it and the processes that are shared with other episodic memory tasks (Carroll, 1993).

2.40.3.1.2 Summary

Early factor-analytic studies of intelligence tested no particular theory of memory and selected tasks more for their convenience than for representativeness or utility in testing claims about memory process or function. Despite this important limitation, we can draw a few broad conclusions about individual differences in memory. First, paired-associate and recognition-memory tests measure some common processes or abilities that cause them to correlate strongly with one another, and more strongly with one another than with memory-span and free-recall tests, which are also discriminable from each another. Second, despite their differences, these memory tests all correlate more strongly with one another than they do with other, nonmemory tests, indicating some common processes or abilities across tests that assess memory for information learned some seconds or minutes ago.

2.40.3.2 The Structure of Memory, from the Top Down

The last decade has witnessed a surge in the application of individual-differences analyses to theoretical questions about memory, and we are especially interested in research on the ostensible distinction between short-term memory (STM) and WM, in part because a growing and controversial literature suggests that WM, more than STM, provides clues about the nature of general intelligence (e.g., Ackerman et al., 2005; Kane et al., 2005; Oberauer, et al., 2005). Before we review this research, it is worth noting that the recent WM literature is not unique in taking a top-down psychometric approach to theory testing. Recall the Underwood et al. (1978) use of correlational data in testing frequency theory as well as some broader claims regarding memory attributes. Moreover, factor analyses of putative STM versus long-term memory (LTM) measures have provided evidence for their conceptual, if not structural, distinction (e.g., Robertson-Tchabo and Arenberg, 1976; Geiselman et al., 1982), as have analyses of tests reflecting episodic versus semantic memory (e.g., Carroll, 1993; Nyberg, 1994).

2.40.3.2.1 A distinction between STM and WM

The terms 'STM' and 'WM' are sometimes used interchangeably as generic labels for phenomena or tasks where little time intervenes between the study and test of a limited amount of information. Other times they are used to represent competing theoretical conceptions of immediate memory, with STM commonly designating a monolithic limited-capacity memory structure involved in active rehearsal of information and its transfer into LTM (e.g., Atkinson and Shiffrin, 1968), and WM referring to a joint memory and attention system that keeps information accessible in the service of ongoing and complex cognitive activities, such as comprehension or problem solving, with separate representational and rehearsal systems for different kinds of information (e.g., Baddeley, 2000). Recent psychometric research has asked the simple question: Do individual differences distinguish these hypothetical constructs?

Cantor et al. (1991) seem to have first addressed this question rigorously. They tested 49 undergraduates in

three kinds of immediate serial-recall tasks with verbal or numerical stimuli. Two probe-recall tasks presented lists of nine items, with each list followed by a cue to recall either the first, second, or third three items from the list (with the latter two assessing immediate memory). Two traditional memory-span tests (or 'simple-span,' or 'STM-span' tests) presented sequences of three to nine items for immediate recall. Two complex memory-span tests (or 'WM span') presented to-be-recalled sequences of two to seven stimuli interpolated with a secondary processing task; both presented a sentence to be read aloud between memory items. The complex span tasks were thought to reflect WM, as conceived by Baddeley and Hitch (1974), because they demanded subjects to do more than simply retain information within a storage buffer via overlearned rehearsal strategies. Their requirement that subjects maintain access to stimuli in the face of a simultaneous processing demand sought to bring attentional, executive processes to bear on maintenance (see Daneman and Carpenter, 1980). Factor analyses yielded two factors, one representing the variance common to simple span and probe-recall tasks ('STM,' with factor loadings from .54 to .95), and one representing variance common to complex span ('WM,' with factor loadings of .74 and .82). It thus appeared that tasks requiring the immediate serial recall of stimuli without the imposition of secondary tasks (i.e., STM tasks) measured at least some different cognitive processes than did those with the imposition of secondary tasks (i.e., WM tasks).

Engle et al. (1999b) took a more sophisticated analytic approach to this issue. They tested 133 subjects on three simple and three complex span tasks (all with verbal or numerical stimuli), and used confirmatory factor analysis (CFA) to test a one- versus two-factor solution to the data. CFA allows researchers to impose a theoretically informed factor model on the data and statistically test whether it fits; it also allows for statistical comparison of competing models. Here, a single-factor model did not fit the data, but a two-factor model distinguishing simple (STM) from complex (WM) span did (and the two-factor model fit significantly better than the unitary model). STM and WM span factors were correlated, but not strongly enough to yield a single memory factor. Thus, with verbal materials, simple and complex span tasks measure some independent mental processes and support the view that WM is, in part, a separate cognitive system from STM. Although we cannot review the relevant findings in detail, the WM-STM distinction is reinforced by numerous reports of WM span factors correlating more strongly and broadly with factors representing complex cognitive abilities (e.g., comprehension and reasoning) than do STM span factors (e.g., Engle et al., 1999a; Conway et al., 2002; Bayliss et al., 2003).

Engle and his colleagues have argued that, even though both WM and STM tasks involve some shared processes such as storage, rehearsal, and executive-attention control, WM span's imposition of secondary tasks increases the executive-attention contribution relative to that in STM span (e.g., Engle et al., 1999a; Engle and Kane, 2004; Kane et al., 2005). These executive-attention processes help maintain access to memoranda in the face of attention shifts away from their representations and toward the execution of the secondary task; that is, WM span tasks bring executive control to bear on keeping representations accessible when they are outside conscious focus. STM span tasks, because they provide no secondary task to interfere with stimulus maintenance and rehearsal, require less executive involvement.

2.40.3.2.2 A distinction between STM and WM?

But wait – subsequent research using visuospatial materials suggests a different conclusion, namely that STM and WM may be inseparable (or, at least, less separable than verbal STM and WM). Miyake et al. (2001) tested 167 undergraduates in two STM span tasks presenting sequences of visuospatial stimuli (e.g., dots within different locations of a grid), and two WM tasks interpolating a spatial processing task (e.g., mental rotation of letters) between the spatial memoranda. CFAs indicated that a model separating STM from WM fit the data well, but the correlation between these factors was very high (.86) with a 95% confidence interval including 1.0. Fixing the correlation between these factors to 1.0, thus deriving a unitary memory factor, did not significantly hurt model fit, indicating that, in the spatial domain, STM and WM were equivalent as measured by span tasks.

Although subsequent studies have not found spatial STM and WM to be indistinguishable, they have found STM and WM to be somewhat more strongly associated in the spatial than in the verbal domain (Park et al., 2002; Kane et al., 2004). One interpretation of these findings is that, because people have fewer and less-practiced rehearsal strategies for visuospatial than for verbal sequences, even 'simple' STM tasks with spatial materials draw

heavily on executive-attention processes for effective maintenance. Given our emphasis in this chapter on convergence between psychometric and experimental findings, we note that the 'executive' interpretation of spatial STM-WM correlations dovetails nicely with conclusions drawn from dual-task experimental studies, which have suggested domain-general executive and attention processes are more important to short-term retention of spatial than verbal stimuli (e.g., Klauer and Stegmaier, 1997; Awh et al., 1998).

In any case, the tidy distinction between STM and WM is rapidly becoming more complicated. First, as mentioned, spatial STM and WM tasks are highly correlated and may sometimes be indistinguishable. Second, Oberauer and colleagues have demonstrated that immediate-memory tasks need not involve secondary-processing tasks to correlate strongly with WM span tasks; for example, tasks that require the updating of mental representations of several stimuli, without the imposition of irrelevant information, are just about indistinguishable psychometrically from WM span (e.g., Süß et al., 2002; Oberauer, 2005). At the same time, Unsworth and Engle (2007) have shown that non-recency portions of immediate-free-recall lists correlate so strongly with WM span that they are also psychometrically indistinguishable from each another. If WM can be measured without dual tasks, then what really distinguishes it from STM? Third, Colom and colleagues have recently argued, based on reanalyses of old datasets and analyses of new ones, that WM and STM span, even in the verbal domain, are much more highly correlated than has been recognized, and that the shared variance between WM and STM is more important to their broad power to predict cognitive individual differences than is the variance that is unique to WM (e.g., Colom et al., 2006a,b).

It now appears that the key to understanding the WM-STM relation, as assessed by span and other tasks, is to resist the reification of tasks onto hypothetical constructs. Instead, we must consider more carefully the shared and unique mental processes that determine performance. Although Engle and colleagues have emphasized the multiply determined nature of both STM and WM span (e.g., Engle et al., 1999a; Engle and Kane, 2004; Kane et al., 2005), noting that storage, rehearsal, and executive processes contribute to both tasks, many researchers assume their view to be that 'STM' tasks simply measure storage and rehearsal and 'WM' tasks measure exclusively executive control (e.g., Ackerman et al., 2005; Colom et al., 2006a).

Fortunately, Unsworth and Engle (2006, 2007) have clarified the mappings of these tasks to their underlying constructs. In short, WM span tasks, STM span tasks with long (more than four item) lists, and nonrecency portions of free-recall lists all measure just about the same thing ('WM'), and all seem to account for similar variance in higher-order cognitive abilities. Why should this be? Unsworth and Engle argue that, in all of these cases, subjects must recover some or all of the target information from 'secondary,' inactive memory in the face of competition from other memory representations. To do so, subjects use cues to guide memory search and delimit a search set that discriminates target from competing information. Higher WM subjects appear to generate and use better cues, or to use the same cues more effectively, than do lower WM subjects, and this accounts for their better performance on these varied memory tests.

2.40.3.2.3 Summary

The individual-differences literature on STM and WM yields an irony. Tasks such as STM and WM span are assumed to measure immediate-memory and attention-related processes that are quite different from those involved in LTM encoding and retrieval. However, current evidence suggests that search and retrieval from inactive secondary memory (or LTM), rather than maintenance within active primary memory, drives individual differences in memory-span and span–ability correlations. These conclusions seem quite consistent with those from experimental studies suggesting that, aside from a very limited number of highly active and accessible representations we might call primary memory, retention and recall over both the short and long term is driven primarily from the cue-driven search and retrieval of inactive memory (e.g., Wickelgren et al., 1980; Wixted and Rohrer, 1994; McElree, 1998; Cowan, 2001; Nairne, 2002; Davelaar et al., 2005). The clear lesson is that we should not assume that the processes engaged by tasks follow from the labels, such as 'STM span,' that we have traditionally affixed to them.

2.40.4 Individual-by-Treatment Interactions in Memory

> In both applied and general scientific work, psychology requires combined, not parallel, labors from our two historic disciplines. . . . In the search for

> interactions we will invent new treatment dimensions and discover new dimensions of the organism. We will come to realize that organism and treatment are an inseparable pair and that no psychologist can dismiss one or the other as error variance. (Cronbach, 1957: 683)

Cronbach (1957) called for a united psychology to bring the strengths of experimental and psychometric methods to bear on theoretical and practical problems of human behavior, in particular by seeking interactions among individuals and experimental treatments. We believe that the literature on individual differences in WM is rich with examples of this approach, and so we review briefly two categories of such studies. In the first, experimental manipulations of the WM span task, itself, are tested for their effect on correlations between WM span and some criterion variables, such as language comprehension or reasoning. In this way, researchers isolate some of the cognitive processes that are most important and least important to the predictive power of WM span (e.g., retrieval of information in the face of proactive interference, and engaging in particular mnemonic strategies, respectively). In the second category, WM-related individual differences are assessed within other, nonspan tasks that feature various experimental manipulations designed to be more or less vulnerable to WM variation. This correlational technique may often yield significant advances in general theory about cognitive tasks and task domains.

2.40.4.1 Individual-by-Treatment Interactions within WM Span Tasks

WM span tasks are complex. They require timesharing between two unrelated tasks, such as memorizing letters and solving equations, they afford numerous strategies for managing the dual-task requirement and for encoding memoranda, and they present processing material that may be more or less demanding for subjects with different skills. So which of these variables, if any, are important to the correlations between WM span and various complex cognitive abilities?

Individual differences in skill on the processing task (Conway and Engle, 1996), or in strategy use (Dunlosky and Kane, in press), are not. Let us consider strategy use in more detail. If differential strategy use (or efficiency) were largely responsible for WM span correlating with, say, reading comprehension scores, then experimentally manipulating strategy use should alter the span–comprehension association. What actually happens? When subjects are allowed to pace themselves through WM span tasks, thus allowing more time to study the items and employ complex mnemonics, mean scores go up. This experimental effect is not surprising. What is more important is the individual-by-treatment interaction: the span–comprehension correlations either remain unchanged, or get weaker, as a result of this experimental manipulation (Engle et al., 1992; Friedman and Miyake, 2004).

The fact that, if anything, span correlations get weaker when we allow subjects more freedom in their approaches to the task suggests that normal individual differences in these approaches or strategies are not responsible for the strong correlations normally observed. In fact, strategic variation appears to contribute noise to span correlations rather than causing them. If strategic variation were actually causal, then allowing strategies to vary more freely should increase the correlations, not decrease them. Indeed, in studies that teach subjects to use one particular memory strategy within WM span, the correlations get stronger compared to those from uninstructed subjects (Turley-Ames and Whitfield, 2003). By forcing all subjects to use the same strategy, nuisance variation in strategy selection is reduced, and the true (stronger) span–ability association is revealed.

What do individual-by-treatment interactions tell us about what is important to WM span variation? They tell us that the buildup of proactive interference (PI) is important. Like most immediate-memory tasks, WM span tasks present many different lists of similar items within a single test. As experimental studies of memory have long indicated, this is a recipe for PI and rapid forgetting (e.g., Underwood, 1957; Keppel and Underwood, 1962). We might expect, then, that experimental manipulations of PI should affect mean span scores, driving them up or down. Like the effect of strategy use on mean span scores discussed earlier, this would not be surprising. The really interesting question, again, is about the individual-by-treatment interaction: Do experimental manipulations of PI affect individual differences in WM span and its correlation with other tasks?

Indeed they do. May et al. (1999) presented WM span trials in one of two orders to younger and older adults. In ascending orders, smaller memory sets were presented before larger sets. Thus, larger sets, which by convention contribute more to span scores than do smaller sets, were encountered only after PI

had built up from prior trials. In descending orders, the high-impact larger sets were presented first and, therefore, before PI had much effect. May et al. found not only that descending administrations yielded higher mean scores than did ascending, but also that age differences in WM span, which are usually robust, arose only in the ascending administration. By minimizing the effect of PI on subjects' scores via descending administration, the ubiquitous age-by-span correlation was eliminated. Lustig et al. (2001) and Bunting (2006) have further demonstrated that experimentally reducing PI during span tasks dramatically reduces their correlations with comprehension and reasoning tests. Likewise, Gray et al. (2003) used neuroimaging techniques to show not only that increasing the contribution of interference to WM tasks increases their correlation to reasoning tests, but also that this correlation is almost completely accounted for by individual differences in the recruitment of brain areas that are important to executive control. The fact that reducing interference reduces WM span correlations, and increasing interference increases span correlations, suggests that PI and individual differences in the ability to resist it are important causal variables in the relation between WM span and higher-order cognition. Only investigations of individual-by-treatment interactions could have led to such conclusions.

2.40.4.2 Individual-by-Treatment Interactions in the Effects of WM on Other Tasks

As we have seen, one effective method to investigate the strong empirical associations between WM span and various cognitive abilities is to manipulate variables within span tasks and then measure their effects on span–ability correlations. Another common strategy in this literature is to test for WM-related individual differences in the performance of other tasks under varying experimental conditions. In the domain of complex cognitive abilities, for example, WM span and language comprehension correlate significantly under some experimental conditions, but not others, suggesting theoretical distinctions between more resource-demanding and more automatic referential and parsing processes (e.g., Just and Carpenter, 1992; Caplan et al., 2007). Regarding somewhat more simple cognitive processes, such as selective attention and visual search, WM span also correlates strongly and selectively with performance in only some task contexts, and these differential correlations have fueled novel theoretical proposals for how such attention tasks are performed (e.g., Kane and Engle, 2003; Kane et al., 2006; Heitz and Engle, 2007).

As we emphasized the importance of PI to span–ability correlations earlier, let us consider here what WM-by-treatment interactions tell us about PI and the executive control of memory processes, quite generally. Nomothetic theory derived from experiments has suggested that executive selection and inhibitory processes play significant roles in producing and combating PI (e.g., Postman et al., 1968; Anderson, 2003). Because WM variation has been thought to reflect, in part, variation in such executive-control processes, Kane and Engle (2000) investigated the relation between WM span and PI buildup in a variant of the Brown-Peterson task. Subjects who had been previously identified as having high WM span scores (i.e., from the top quartile of a large university distribution) or low WM scores (i.e., from the bottom quartile) studied and recalled three consecutive lists of ten words drawn from one semantic category (animals, occupations, or world nations). Immediately following each list, subjects engaged in a rehearsal-prevention task for 16 s before recalling the list. High- and low-WM subjects did not differ in recall of the first list but, as expected, WM span predicted PI susceptibility, with low-WM subjects showing greater PI on lists 2 and 3 than did high-WM subjects. Although this finding represents an individual-by-treatment interaction, with WM span predicting recall across late but not early lists, more generally important findings came from a higher-order interaction.

While Kane and Engle (2000) tested for WM-related individual differences in PI susceptibility, they also manipulated subjects' capacity for executive control by dividing their attention. During either the encoding or retrieval of each list, subjects continuously tapped a pseudorandom finger sequence under time pressure. The logic here was similar to that in neuropsychological studies of brain injury: task components that normally elicited executive control would suffer under dual-task conditions, as those executive processes were thwarted by the tapping task. Two relevant results warrant mention. First, divided attention increased the PI effects for high-WM subjects and had no effect on their list 1 recall, suggesting that high spans engaged attention-control processes only to meet the increased encoding and retrieval demands of PI-vulnerable lists. In fact, high WM performance in the dual-task

conditions matched low WM performance under single-task conditions, and so dividing attention turned high-WM subjects into functional low-WM subjects. Second, and in contrast, divided attention hurt low-WM subjects' list 1 recall while having no subsequent effect on their PI susceptibility. This suggests that low-WM subjects exhausted their attention-control processes to meet the basic encoding and retrieval requirements of single lists with little PI potential, leaving nothing additional to exert against the interference on subsequent lists. These findings suggest some interesting hypotheses about the nature of WM-related individual differences, but they also paint a much more complicated picture regarding the attentional demands of encoding and retrieval than is typically considered (e.g., Craik et al., 1996). We suggest that further investigation of span-by-treatment interactions would be informative to nomothetic theoretical pursuits regarding the nature of episodic memory and retrieval and their interaction with attentional processes.

2.40.5 Summary and Conclusions

The psychological investigation of memory, like psychological investigations of other behavioral and cognitive phenomena, has been slow to integrate the experimental and correlational approaches to theory testing. We hope to have convinced experimentally inclined cognitive researchers that they should consider the psychometric literature on individual differences in memory in forming hypotheses about memory processes and tasks. Moreover, they should consider incorporating individual differences into their own experimental tests of such hypotheses. Our plea, like Terman's, Cronbach's, Melton's, Underwood's, and Kosslyn's before us, is that students of memory whose interests span traditional areas of memory research (so well represented in this volume) will recognize the potential benefits of a united experimental–psychometric approach to important theoretical questions about memory structure and process.

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2.41 Aging and Memory

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This chapter is a review of studies assessing age-related changes in memory – that is, the changes that occur in different memory systems and processes from young adulthood to old age – with the intention of establishing the major empirical findings reported in the literature, the theoretical interpretations of these findings, and directions for future research. Most of the chapter is based on the comparison of performance of young people in their twenties to older adults between 65 and 85 years old. We limit the discussion to normal age-related changes, focusing on behavioral studies in which the older participants have no apparent pathological changes related to dementia or other similar conditions (*See* Chapter 3.28 for a discussion of some of the mediating brain structures of these changes). As this research field has gone through a vast expansion in the last 25 years, the review is necessarily selective, covering several representative topics studied in the literature that use different types of conceptualizations in terms of memory stores, memory systems, and memory processes.

2.41.1 Introduction

One of the most intriguing aspects regarding age-related changes in memory is the variability in the changes; whereas some memory abilities decline significantly in old age, others are held fairly constant throughout older adults' years. Explaining this variability in performance is a major purpose of research in the area, and although we still lack comprehensive explanations, several promising approaches have been suggested to explain components of this variability.

Many studies have addressed questions regarding changes in different types of memory established in general memory research. For example, one distinction is between declarative (explicit) and nondeclarative (implicit) memory systems (e.g., Squire, 1986). The former involves conscious intentional retrieval (e.g., trying to remember the name of the person you met last night), whereas the latter involves memories that can be inferred by subsequent behavior without any intention to be retrieved (e.g., riding a bike, or responding faster to a stimulus because you have seen it several times before). Within the domain of declarative memory, a distinction has been made between episodic memory – that related to a particular time and place in an individual's personal history (e.g., remembering whom you met last Thursday for lunch) – and semantic memory, which involves knowledge of general facts not related to a particular time and place (e.g., knowing the capital of France). Another distinction is based on memory stores; short-term memory, for example, involves the ability to retain a recently experienced event for a brief period (such as listening over the phone for a meeting time and then writing it down), whereas long-term memory involves the retention of information for an extended period of time (e.g., remembering the names of the people you met last night for dinner). A further distinction is between retrospective memory – memory for a past event – and prospective memory – memory to perform a future action (such as remembering to show up for a dental appointment). Finally, viewing memory in terms of processing phases allows for the assessment of age-related differences in learning (encoding) the information, in maintaining it during the retention interval, and in accessing (retrieving) it when necessary.

As mentioned above, research shows differential patterns of age-related changes in tasks involving these different memory types. For example, whereas semantic memory seems to be maintained relatively well into old age, episodic memory shows an appreciable decline (**Figure 1(f)**). In addition, in contrast to explicit (declarative) episodic memory, implicit memory, as one type of nondeclarative memory, shows very few changes in old age.

We begin by describing age-related patterns in different types of memory based on empirical findings and then discuss theoretical frameworks that have been suggested to explain these patterns. We end with a discussion of some further issues, including the uniqueness of memory changes within the larger realm of general cognitive changes, tasks, and circumstances in which older adults seem to show adequate memory performance, and limitations on conclusions based mostly on laboratory research.

2.41.2 Empirical Findings

In order to establish reliable conclusions based on numerous studies, we have resorted, wherever possible, to meta-analytical studies of age-related differences in various memory domains. Such studies involve quantitative summaries of a large number of studies investigating specific questions and, hence, help us to draw conclusions based on replicable results. After establishing the patterns in a given domain based on each meta-analysis, we describe a few illustrative studies to provide the reader with characteristic methods and representative results for each domain. Among other issues, we discuss age-related changes in short and long-term memory, explicit (semantic and episodic) and implicit memory, and prospective memory. We also assess age-related changes in autobiographical and false memory and in encoding and retrieval processes. Most of the research is based on experimental studies, although some studies use multivariate correlational approaches.

It should be noted that most of the meta-analytical studies representing the type of studies conducted on memory and aging are based on cross-sectional designs, in which different age groups are tested at a given point in time. This method usually shows larger age-related differences than are obtained in the rather less frequently used longitudinal studies, in which groups of people are followed over time (e.g., Rönnlund et al., 2005). This point is further discussed at the end of this chapter.

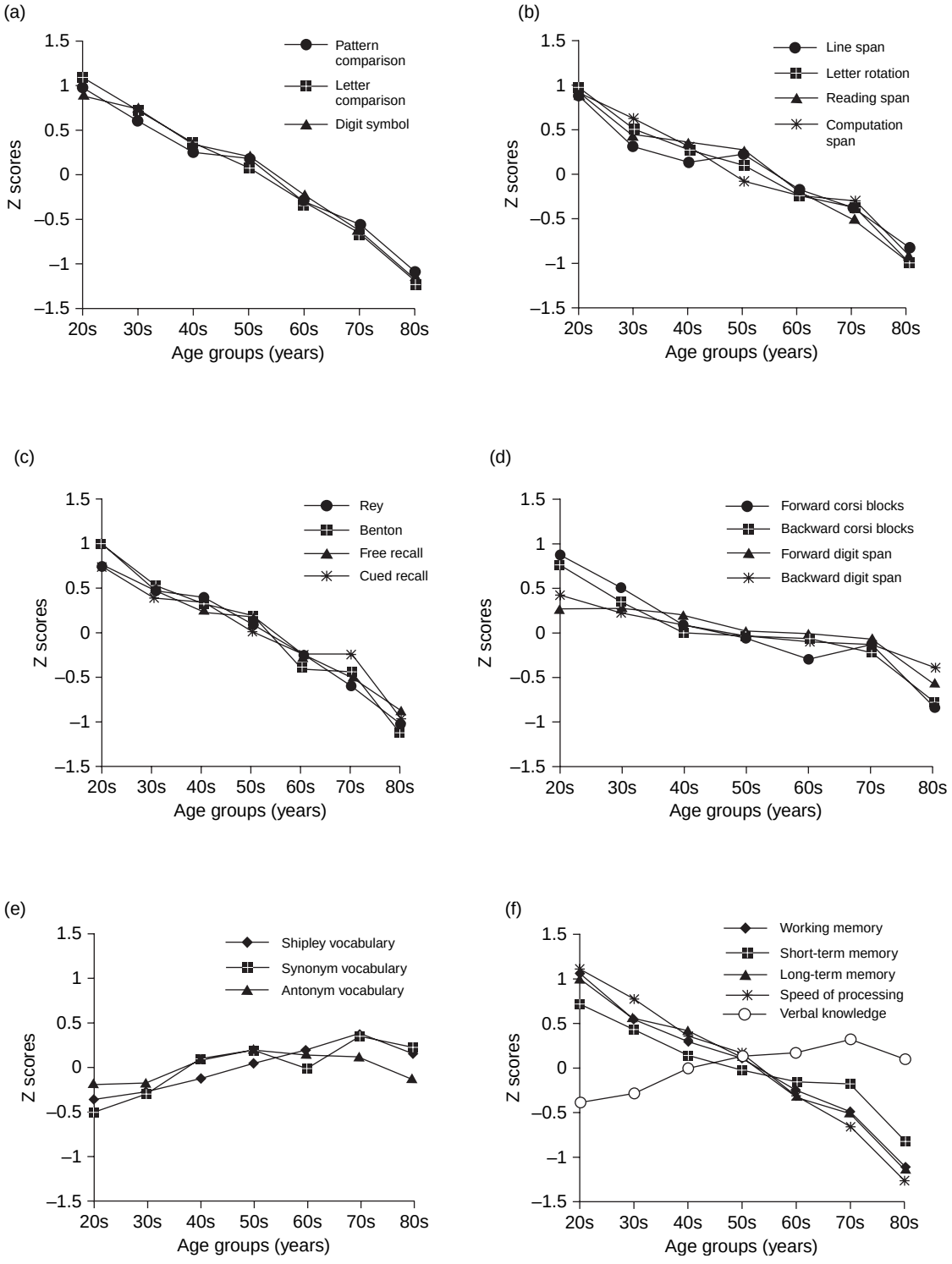

Figure 1 Life span performance measures. (a) Speed of processing. (b) Working memory. (c) Long-term memory. (d) Short-term memory. (e) Knowledge-based verbal ability. (f) A composite view. From Park DC, Lautenschlager G, Hedden T, Davidson NS, Smith AD, and Smith PK (2002) Models of visuospatial and verbal memory across the adult life span. *Psychol. Aging* 17: 299–320. Copyright 2002 by the American Psychological Association.

2.41.2.1 Implicit/Indirect Memory Versus Explicit/Direct Memory

One area of memory research that received substantial attention in the 1980s and 1990s is related to the distinction between tasks in which participants are aware of the fact that their memory is being probed (explicit/direct) and those in which they are unaware of performing a memory task (implicit/indirect/procedural). Interestingly, most of the research in this area indicates different patterns of age-related changes in these two classes of memory tasks. A meta-analysis carried out by Light and La Voie (1993), based on 33 experiments, concluded that there are small age-related declines on implicit memory measures ($d = -0.18$, where d is the mean effect size in terms of unit standard deviations over all the studies included). However, these are much smaller than the decline shown for explicit measures such as recognition or recall, in which d ranges from -0.5 to -1.5, as found in other meta-analytical studies reviewed here (see **Figure 1(c)**).

One example of such a pattern was reported by Light and Singh (1987). In their study, older and younger adults viewed lists of words and rated each word by its pleasantness. Later, subjects were provided with the first three letters of various words, some of which had been presented earlier. Half of the participants (the implicit group) were told simply to complete each stem with the first word that came to mind, whereas the others (the explicit group) were asked to try to fill in each stem with a word they had seen earlier. Finally, all subjects completed a recognition test, in which they chose studied words from a list of both targets and distractors. The results indicated that, whereas cued recall scores and recognition scores were impaired in old age, the priming effect (i.e., providing a previously rated word following implicit instructions) did not differ significantly between age groups. Similar results were obtained by Light and Albertson (1989), who used lists of semantically categorized words with explicit cued recall and implicit exemplar generation tasks (see **Figure 2**).

Differential age-related decline in implicit versus explicit memory measures has been further supported by a longitudinal design employed by Fleischman et al. (2004). Results of several implicit and explicit tests administered once a year for 4 consecutive years showed that explicit memory declined significantly over the four assessment periods, and this decline was largest in participants who were the oldest. Implicit memory, however, was unrelated to age at baseline and did not decline over the 4-year period.

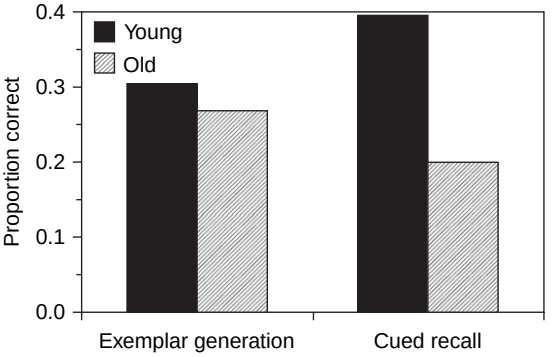

Figure 2 Performance of older and younger adults on an implicit exemplar generation task and an explicit cued recall task. From Light LL and Albertson SA (1989) Direct and indirect tests of memory for category exemplars in young and older adults. *Psychol. Aging* 4: 487–492. Copyright 1989 by the American Psychological Association.

Altogether, older adults are impaired more on explicit than on implicit memory measures. Thus, it is important to consider whether a task requires deliberate or nondeliberate memory when evaluating and predicting age-related declines.

2.41.2.2 Short-Term and Working Memory

Short-term memory (STM) was originally suggested as the mechanism that allows temporary storage of information (Atkinson and Shiffrin, 1968). Later, Baddeley and Hitch (1974) proposed the concept of working memory (WM), which involves both the temporary storage and simultaneous manipulation of information. For example, when listening to a conversation, we must maintain individual words while concurrently relating them together into a coherent message. Aging seems to differentially affect these two functions of passive maintenance and active on-line processing. A meta-analysis by Bopp and Verhaeghen (2005) examined age differences in several verbal STM and WM tasks. Relatively small age differences were found in tasks that require simple temporary maintenance of materials. For example, forward digit span tasks, which involve storage without processing, showed a relatively modest age-related decline ($d = -0.53$). In contrast, tasks requiring both storage and processing showed robust age differences, and the impairment of older adults was largest when the processing component of the task became more dominant relative to the maintenance one (e.g., $d = -1.01$, -1.27, and -1.54, for sentence span, listening span, and computation span, respectively).

An example of a relevant study was conducted by Li (1999), who assessed age-related differences in storage and processing of verbal materials. In the STM condition of this experiment, older and younger adults were simply required to recall lists of digits. In the WM condition, subjects recalled similar lists of digits, but each digit was presented following a math problem in which subjects indicated whether the provided answer was correct or incorrect. Thus, the STM and WM tasks were similar, except that the former required only storage, whereas the latter required both storage and processing. Results showed that older adults were impaired more on the WM span than on the STM span measure. The authors conclude that older adults were more impaired, relative to younger adults, on the task that required both processing and storage than on the task that required only storage. This pattern of results seems to characterize memory not only for verbal materials but also for visuospatial materials, as shown by Vecchi and Cornoldi (1999).

2.41.2.3 Long-Term Memory: Semantic Versus Episodic Memory

One interesting question addressed in previous research is the degree to which there are age-related changes in memory for general knowledge (semantic memory) and whether such changes are different than those involving memory for specific events (episodes). Are older adults worse than younger adults in their ability to name the capital of Italy or to indicate whether a whale is a mammal or TOTBASHI is a word in English? Moreover, are age-related differences in the ability to respond to such questions unlike age-related differences in the ability to answer questions about specific events that tap episodic memory?

A relevant indicator for the differential patterns of performance in younger and older adults in episodic and semantic memory tasks comes from a recent large-scale study by Park et al. (2002). The study used tests of both episodic and semantic memory given to 345 people from 20 up to 92 years of age. Measures of episodic memory included visuospatial memory tests, such as the Rey visual design learning test and the Benton visual retention test, both of which required the viewing and reproduction of a specific drawing. Verbal tests involving free and cued recall of words were also included. The semantic memory tasks concentrated on verbal abilities and included the vocabulary section of the Shipley Institute of Living Scale, a synonym vocabulary test, and an antonym vocabulary test. The results, which can be seen in **Figure 1**, indicated that the age-related pattern observed was very different for episodic versus semantic memory. There was a substantial age-related decline in all measures of episodic LTM (see **Figure 1(c)**). In contrast, all three semantic memory tests showed a significant increase with age (see **Figure 1(e)**). The conclusion reached by the authors was that semantic memory does not decline – and may in fact increase – with age, whereas episodic memory exhibits a substantial age-related decline. Similar results were reported in a longitudinal study by Lövdén et al. (2004).

Several other studies also indicate that semantic memory is relatively stable over the adult lifespan. Semantic priming effects measure the amount of activation that occurs in the organized system of concepts that are connected together by associative networks (Collins and Loftus, 1975; *See* Chapter 2.28) and, as such, can provide information about age-related changes in people's structure of knowledge. Studies comparing age-related differences in semantic priming have shown either no differences or a larger semantic priming effect in the old. For example, a meta-analysis by Laver and Burke (1993) concluded that semantic priming effects showed a pattern of an increase in old age ($d = +0.10$).

One relevant study was conducted by Madden (1988), who asked older and younger adults to read sentences with one word presented at a time. The last item of each sentence, presented in all capital letters, was a target item for a lexical decision task; subjects indicated whether it was a word or a non-word as quickly as possible. Two factors were varied: one was the clarity of the target word, with some target items presented in a normal fashion and others degraded by the separation of letters by asterisks (e.g., B*O*O*K*S). The other factor was congruency, with some of the target words being congruent with the sentence context (e.g., "The accountant balanced the BOOKS"), others being incongruent (e.g., "The train went over the CLOUDS"), and still others being neutral in terms of congruence. The results showed that for nondegraded target words, neither age group responded more quickly to target words when they were congruent versus neutral in relationship with the sentences. For degraded target words, however, both age groups benefited from sentence context. Interestingly, this benefit was larger for older (117 ms) than for younger (52 ms) adults. There was

also an overall context cost (i.e., the slowing of RTs for incongruous compared with neutral-context trials), which was larger for older (336 ms) than for young (167 ms) subjects. The authors conclude that these results indicate that older adults show an "increase in the influence from the semantic information that is activated automatically by a sentence context" (Madden, 1988: 171).

Another study, by Balota and Duchek (1988), manipulated the strength of the association between the prime and the target. For trials with a short delay between the prime and the target (200 ms), younger and older adults showed the same advantage in responding faster to highly associated pairs. However, when the delay between the prime and the target was longer (800 ms), older adults showed smaller benefits from the relatedness of the pairs in comparison to younger adults. Balota and Ducheck interpreted this finding to reflect intact automatic spread of activation within the semantic memory network in older adults (in the 200-ms delay condition), coupled with some decline under conditions that demand more attention (when the activation has to be maintained over a longer period of time).

There are, however, some indications that access to certain forms of semantic memory may suffer in old age. For example, it has been shown that, while they are able to name uncommon objects relatively well, older adults exhibit an impairment in naming famous people shown in pictures (e.g., Rendell et al., 2005). Overall, however, it seems that semantic memory is mostly spared of age-related changes. Episodic memory, in contrast, seems to be highly affected by advancing age; we discuss this in more detail in the next section.

2.41.2.4 Episodic Memory

In this section we survey research on different aspects of episodic memory changes, including memory for context versus content, the effects of intentional and incidental learning, the effects of reliance on semantic memory, performance on different episodic memory tasks, and encoding and retrieval factors.

2.41.2.4.1 Memory for context versus content

One hallmark of episodic memory is its relation to time and place. That is, major characteristics of an episode involve when and where it occurred. If I am asked about what I had last night for dinner, unless I eat the same dish every night, I have to go back in my memory and use different contextual aspects of the episode, including the time (last night) and the place (a specific restaurant) where I had dinner, in order to retrieve the relevant information about the meal. Research indicates that aging impairs memory for such contextual elements as time and place to a greater degree than memory for the content of an event. For example, Spencer and Raz (1995) reviewed evidence from 46 studies. They found that the magnitude of age-related changes in context ($d = -0.90$) was significantly larger than for content ($d = -0.72$).

One representative empirical study was conducted by Puglisi et al. (1985), who compared age-related differences in memory for individual items (content) to that for occupied spatial locations (which served as context). Older and younger adults viewed target objects placed within a grid. During test, subjects were given a recall and then a recognition test over the objects and were then placed given objects in their studied location within the grid. The results showed an age-related impairment in memory for item location but not in item recognition. The authors concluded that older adults are able to recognize objects as well as younger adults but are less able to remember the spatial location of those objects (i.e., the context). Similar results were obtained by Kausler and Puckett (1980), who looked at age-related differences in memory for another contextual element – the case (upper or lower) in which a given word appeared. Their results provide support for the notion that aging has a greater effect on memory for contextual information (case of a given word) than on memory for content information (a word itself).

2.41.2.4.2 Intentional versus incidental learning

A question relevant to both theory development and everyday life performance is whether age-related differences in memory are mediated by the type of learning used. Specifically, are there differences between younger and older adults in memory if, during encoding (learning), they do not expect any memory tests (as happens, e.g., when one is introduced to new people on a social occasion)? Furthermore, are these age-related differences larger or smaller than when learning occurs intentionally (such as when one learns a chapter in a textbook over which a later test is expected)?

The meta-analysis by Spencer and Raz (1995), mentioned earlier, looked at this issue and found larger age effects in studies involving memory for

content and context that used intentional learning (d = −0.62) than those using incidental learning (d = −0.41). Similar results were reported in a meta-analysis by Verhaeghen et al. (1993), based on 120 studies, which found a trend toward larger age differences in list recall under intentional learning (d = −1.00) than incidental learning (d = −0.87). Finally, a meta-analysis by Johnson (2003) showed that when subjects were provided during study with advance knowledge about the upcoming test over textual information, age differences were larger (d = −0.85) than when this information was not provided (d = −0.55).

One representative study was conducted by Hogan et al. (2006). In this study, older and younger adults viewed several series of nouns and made simple judgments about each one. The word 'learn' appeared above some of the presented words, which made participants aware that memory for those specified words would be tested. Participants completed a recognition test, in which they indicated whether or not given words had been presented previously. This test included words from both the 'learn' (i.e., intentional learning) condition and words presented without 'learn' instructions (i.e., incidental learning). Results showed an interaction between age and instructions, with older adults showing greater impairment in the intentional than in the incidental learning condition. Similar results were reported by Troyer et al. (2006), who found age-related impairments in memory for people's names when encoding was intentional (the 'learn' condition), but not when encoding occurred incidentally (through physical, phonemic, or semantic processing; see **Figure 3**).

The results of the aforementioned studies indicate that older adults can encode information incidentally quite well relative to younger adults. However, when they have to encode information intentionally, older adults show a disadvantage relative to younger adults. This differential effect could stem from an age-related impairment in the spontaneous use of effective strategies at encoding and retrieval (e.g., Dunlosky and Hertzog, 2001). This issue is further discussed later in the chapter.

2.41.2.4.3 Episodic memory support by semantic memory

As previously mentioned, older adults seem to retain their semantic memory quite well. An important issue involves the degree to which this intact semantic knowledge can be used to support episodic memories. A meta-analysis by Verhaeghen et al. (1993) provides an indication that increasing categorizability (the ability to categorize new information into previously learned semantic categories) of to-be-remembered information leads to a decrease in age differences in memory (d = −0.78 and −1.07 for lists high and low in categorizability, respectively). Similarly, a more recent meta-analysis by Johnson (2003) showed larger age differences in memory for unconnected sentences (d = −0.89) than for textual passages, which are easier to connect to previously learned semantic knowledge (d = −0.62).

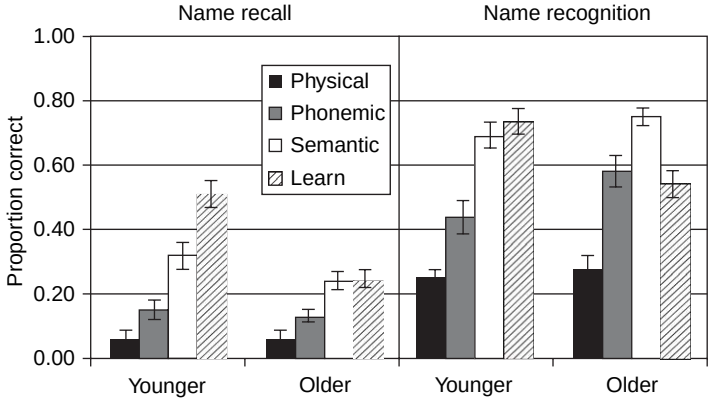

Figure 3 Memory for names following three types of incidental learning instructions (physical, phonemic, or semantic processing) and intentional learning instructions. From Troyer AK, Häfliger A, Cadieux MJ, and Craik FIM (2006) Name and face learning in older adults: Effects of level of processing, self-generation, and intention to learn. *J. Gerontol. Psychol. Sci.* 61B: P67–P74. Copyright 2006 by The Gerontological Society of America.

One relevant study was reported by Wingfield et al. (1998), who examined age-related differences by looking at temporal patterns of free recall from categories. Older and younger adults studied lists containing words from each of several semantic categories (e.g., the animal category included bear, cat, cow, dog, and horse), until they were able to freely recall each list perfectly. At that point, participants' recall times were recorded. Results showed that nearly all responses by both younger and older adults were clustered, that is, once one word from a category was recalled, all words from that category were recalled before the participant moved to the next category. Furthermore, older adults' within-category inter-response times were similar to those of younger adults. Thus, once they were able to retrieve category names, older adults were just as fast as younger adults. However, between-category inter-response intervals were generally longer for older than for younger adults (see **Figure 4**). The authors conclude that when words are from a single semantic category, older adults' memories are just as effective as those of younger adults, indicating that older adults can in fact make use of semantic knowledge to support episodic memory performance.

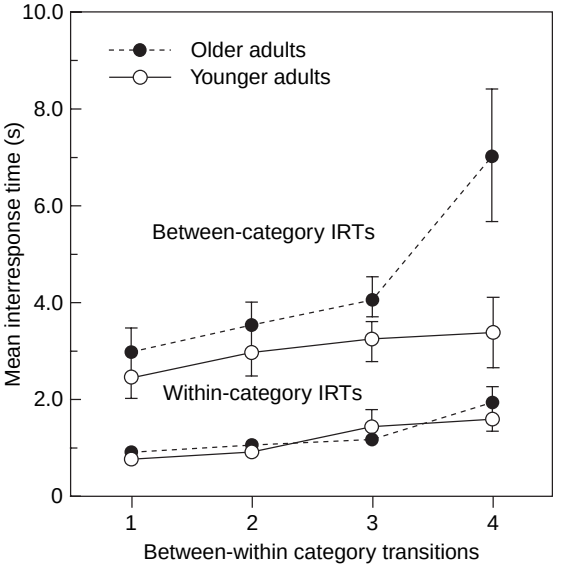

Figure 4 Mean between-category interresponse times (IRTs) for younger and older adults over four transitions between five recalled categories, and mean within-category IRTs averaged across the five categories recalled. From Wingfield A, Lindfield KC, and Kahana MJ (1998) Adult age differences in the temporal characteristics of category free recall. *Psychol. Aging* 13: 256–266. Copyright 1998 by the American Psychological Association.

Another study, by Naveh-Benjamin (2000), assessed this issue by testing memory for related and unrelated pairs of words. After studying these pairs, participants were given a recognition, cued recall, or free recall test. Results showed that, overall, age differences were much larger in memory for unrelated word pairs than for related word pairs, and this was the case for each of the memory tasks (see **Figure 5**). The author concluded that older adults are more disadvantaged in memory for unrelated pairs but can benefit from previously learned information (schematic support, Craik, 1986; see section titled 'Attentional resource limitations') that can be utilized in related pairs to bring their episodic memory performance close to that of the younger adults.

2.41.2.4.4 Retrieval from memory

As discussed, processes involved in the encoding of information are important; however, processes used to access the information (i.e., retrieval processes) are also crucial to explicit memory tasks. One factor shown to be an important facilitator at retrieval is the number and quality of the cues available. The issue of whether there are age-related differences in the ability to use those cues can be studied by employing different types of memory tasks. For example, by comparing age-related differences in a free recall task (in which no cues are provided), those in a cued-recall task (in which some cuing is provided), and those in a recognition test (in which copies of the original events serve as cues), we can assess the degree of cue utilization by younger and older adults.

2.41.2.4.4.1 Test type In a meta-analysis of studies on memory using different types of tests, Johnson (2003) showed (with the analysis including one mean effect size per study) that age effects were smaller in recognition tests ($d = -0.67$) than in free recall ($d = -0.82$) or cued recall ($d = -0.88$) tests. Similarly, Spencer and Raz (1995), in their meta-analysis, found larger age differences in tests of recall ($d = -1.01$; including both free and cued recall) than in recognition tests ($d = -0.57$).

An example of a study that directly compared age-related differences in recall and recognition is one by Craik and McDowd (1987). Old and young adults studied lists of phrases with target words (e.g., A body of water – pond), then completed two auditory tests, one using cued recall and the other testing recognition. In addition, during retrieval, all subjects carried out an additional reaction time (RT)

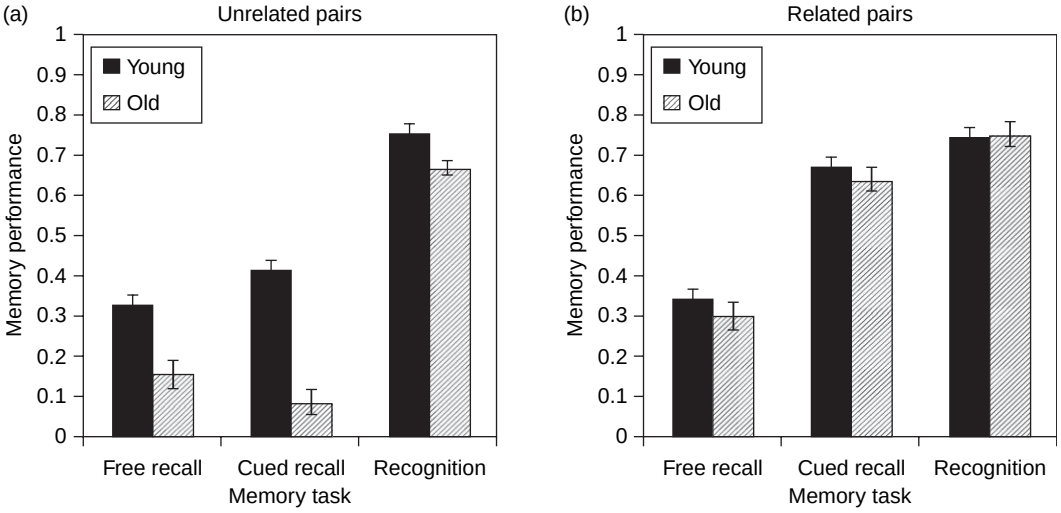

Figure 5 Memory performance of older and younger adults for unrelated (a) and related (b) word pairs. From Naveh-Benjamin M (2000) Adult age differences in memory performance: Tests of an associative deficit hypothesis. *J. Exp. Psychol. Learn. Mem. Cogn.* 26: 1170–1187. Copyright 2000 by the American Psychological Association.

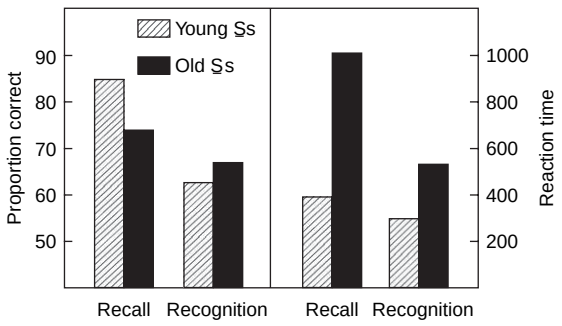

Figure 6 Left panel: Cued recall scores (proportion correct) and recognition scores (hits minus false alarms) as a function of age; right panel: Reaction time (RT) costs (in milliseconds; mean dual-task RT minus mean baseline RT) as a function of age and retrieval task. From Craik FIM and McDowd JM (1987) Age differences in recall and recognition. *J. Exp. Psychol. Learn. Mem. Cogn.* 13: 474–479. Copyright 1987 by the American Psychological Association.

task in which they pressed one of four keys according to whether a visually presented stimulus was a vowel, consonant, odd digit, or even digit. Results showed that older adults did significantly worse than young in recall test performance but performed similarly to young on the recognition test (**Figure 6**, left). In terms of RTs on the concurrent task, younger adults performed similarly during the recall and recognition tests, whereas older adults were slower in responding to the concurrent task while they were performing recall than recognition (**Figure 6**, right). Thus, recall may be especially taxing for older adults. Similar results were obtained using recall and recognition of names (Troyer et al., 2006), showing a significant age-related decline in recall performance but no differences in recognition.

Overall, older adults seem to be impaired more on tasks requiring recall than on those using recognition. Craik and McDowd (1987) use these patterns of results to support the notion that recall requires more processing resources than does recognition, and that older adults exhibit a decline in these resources. These resources are less necessary when environmental support is provided in the form of retrieval cues (see further discussion of this issue in the section titled 'Attentional resource limitations').

2.41.2.4.4.2 Recollection and familiarity Recent discussions of memory retrieval distinguish between two types of retrievals, one based on the process of recollection and the other on processes driven by familiarity (e.g., Yonelinas, 2002). Recollection is required in order to retrieve contextual and other details of an episode (e.g., when, where), whereas familiarity is based on the feeling of having previously experienced the information without remembering any specific contextual details about the event.

One major paradigm used to assess recollection and familiarity is the process dissociation procedure

(PDP; Jacoby, 1991), which generally indicates an age-related decline in retrievals based on recollection but not on familiarity (e.g., Jacoby et al., 1996; Jacoby and Hay, 1998); this conclusion was supported by Prull et al.'s summary of 13 studies (as cited by Hoyer and Verhaeghen, 2006) using this procedure. An example of such a study was conducted by Jennings and Jacoby (1993). Older and younger adults, unaware of an upcoming memory test, read aloud a list containing names of fictitious, nonfamous people. Participants then took two recognition tests, each including old and new nonfamous names, as well as real famous names. In the inclusion test, subjects were to indicate whether or not a presented name was that of a famous person. They were told, incorrectly, that any name they recognized from the earlier phase was famous, and that they should respond 'yes' to such names. In the exclusion test, they were again asked to identify famous names but were told that names they recognized from the first phase were nonfamous, so they should respond 'no' to such names. The PDP was used to calculate estimates of familiarity and of recollection for each group of subjects, based upon the idea that yes responses to studied nonfamous names could indicate either recollection or familiarity on the inclusion test but could indicate only familiarity in the absence of recollection on the exclusion test. Results showed that aging produced large declines in recollection, but familiarity estimates did not differ significantly between the groups.

Other methods used to measure recollection and familiarity include assessment of receiver operating characteristics (ROC) curves and remember/know (R/K) judgments. The former procedure involves plotting hit rates against false alarm rates at various levels of confidence, and the latter provides estimates of recollection from items judged as 'remembered' and familiarity from items judged as 'known.' Both methods, like the PDP, show a definite age-related decline in recollection, but the picture regarding familiarity is less clear. For example, Prull et al. (2006) used all three procedures and showed clear age-related differences in recollection. The different methods, however, yielded different results in regard to familiarity estimates; the R/K and ROC methods showed an age-related deficit in familiarity, whereas the PDP method did not. Light et al. (2000) reported similar patterns in a summary of relevant literature.

To summarize, there is a great deal of evidence for an age-related decline in recollection. However, such a clear conclusion cannot be drawn about familiarity, as aging effects seem to depend on the method used to measure them.

2.41.2.4.5 False memory

Memory research has been slowly moving from an interest in variables that affect accurate performance to assessment of the errors that people make in their memory reports and the sources of these errors. One area of such research involves eyewitness testimony, with studies repeatedly showing that postevent information can be mistakenly thought to have happened in the original event (e.g., Loftus and Palmer, 1974). A review article by Jacoby and Rhodes (2006) concludes that older adults are more prone than younger adults to reporting inaccurate memories. Aging seems to lead to high susceptibility to misinformation, accompanied by unawareness of this susceptibility, as older adults are relatively confident in the accuracy of their false memories (Dodson et al., 2007).

An example of a study that assessed age-related differences in susceptibility to misinformation is one by Karpel et al. (2001). In this study, older and younger adults viewed slides showing a theft and were told to try to remember the objects and events shown in the slides. Fifteen minutes after study, subjects were questioned over the slides; two of the included questions contained misleading information, mentioning objects not actually seen in the slides (called critical objects; e.g., "Did the thief pick up the bottle of Elmer's glue that was on the second desk in front of the can of Coke?" when a can of Coke was not actually shown). Five minutes later, subjects took a final test, in which they indicated whether they had seen various objects in the slides; the two critical objects were included in this test. Subjects also provided a confidence rating for each response. Results showed that older adults were less able to reject critical objects but more confident in their incorrect endorsement of those items compared with younger adults. The authors concluded that older adults are more susceptible to misleading information than are younger adults. A recent study by Roediger and Geraci (2007) showed similar susceptibility of older adults to the misinformation effect. The age-related deficit, however, was smaller for those participating in a source-monitoring test condition who needed to indicate whether the tested information was derived from the original slides, the following misleading text, both, or neither, suggesting that older adults' vulnerability to misleading information may be reduced when source information is made especially salient.

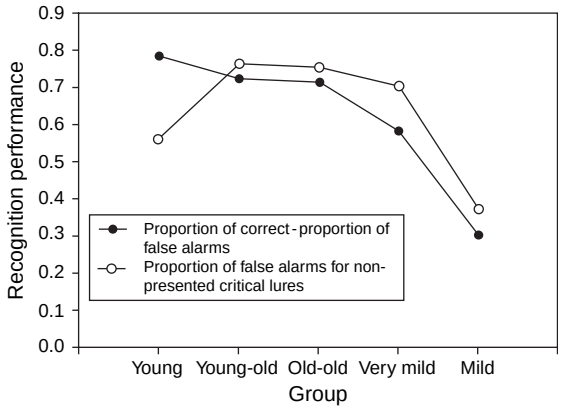

Figure 7 Mean recognition performance as a function of group. From Balota DA, Cortese MJ, Duchek JM, et al (1999) Veridical and false memories in healthy older adults and in dementia of the Alzheimer's type. *Cogn. Neuropsychol.* 16: 361–384. Copyright 1999 by Psychology Press.

Another paradigm used in the study of false memories is the DRM (named after its creators: Deese, 1959; Roediger and McDermott, 1995). Balota et al. (1999) used this paradigm, presenting lists of items related to a critical, unpresented word. For example, 'desk,' 'computer,' 'phone,' 'books,' and so on might be presented, all being associated with the unpresented critical word 'office.' Five groups of people were tested in this study, including young adults, healthy young-old (aged 60–79 years), healthy old-old (aged 80–96 years), and adults with mild or very mild dementia of the Alzheimer's type (DAT; aged 56–91 years). At test, both groups of healthy older adults tended to falsely recall or recognize more critical words than younger adults, as indicated in **Figure 7**.

These results and others indicate that in addition to having poorer memory for the presented information, under certain conditions older adults also show an increase in intrusions and false memory more than younger adults. Some explanations for these patterns – for example, intact activation in semantic memory coupled with poor source memory – are discussed later in this chapter.

2.41.2.4.6 Autobiographical memory

A question that has received more attention recently involves the degree to which people at different ages can remember events in their personal past. These autobiographical memories provide information about what people remember from their own past with respect to the frequency of these memories, their type and nature, and the ease with which they are retrieved. The procedures used in these studies are different from most of the studies mentioned so far, in that researchers assess memories that have already been established but cannot be experimentally controlled. As a result, we cannot be as confident of the authenticity of these memories. Nevertheless, these are important memories, as they provide people with a sense of continuity over their lifespan.

Research on autobiographical memories shows that an important factor, in addition to the age of the participants during retrieval, is the age at which the memories were established. Not surprisingly, several studies have shown that people tend to remember recent events. Events that occurred between the ages of 10 and 30, however, are also especially well remembered. This effect, known as the reminiscence bump, has been attributed to "privileged encoding of experiences highly relevant to an individual during a critical phase of development and consolidation of the self" (Holmes and Conway, 1999: 30).

Fromholt et al. (2003) assessed age and cohort effects on autobiographical memory by comparing life narratives produced by centenarians with those of younger seniors. Fifteen centenarians, 30 healthy younger seniors (mean age, 78 years), depressed seniors (mean age, 80 years), and demented seniors (mean age, 81 years) were interviewed, being given 15 minutes to freely tell about important events from their lives. Although the healthy younger senior group reported more memories than the other groups, all groups exhibited a reminiscence bump, with a relatively high percentage of their memories being from their teens and twenties, as can be seen in **Figure 8**. These researchers also found a reminiscence bump in a sample of 22 centenarians using the cued recall method. Interestingly, Fromholt et al. further found that historical events (such as World War II) were more often reported if they had occurred during one's bump period than if they had taken place shortly after that period. This study, and others, highlight the importance of the age at which experiences are learned, in addition to their age at retrieval.

2.41.2.4.7 Prospective memory

One type of memory important in everyday life involves remembering to perform future actions. This ability, termed prospective memory (PM), is required when we must remember to show up for an appointment, to meet a friend for lunch, or to take certain medications at specific times. Results of several studies conducted on age-related differences in

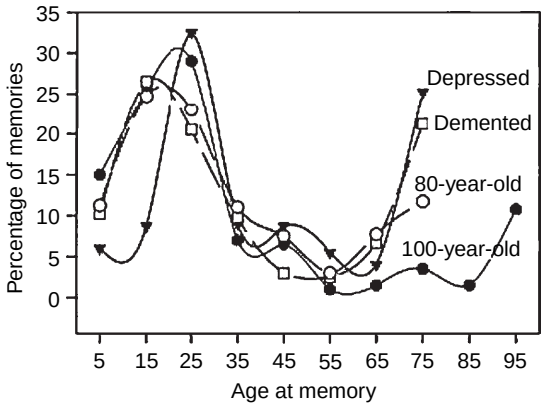

Figure 8 The percentage of life-narrative autobiographical memories reported from each decade of life as a function of group. From Fromholt P, Mortensen DB, Torpdahl P, Bender L, Larsen P, and Rubin DC (2003) Life-narrative and word-cued autobiographical memories in centenarians: Comparisons with 80-year-old control, depressed, and dementia groups. *Memory* 11: 81–88. Copyright 2003 by Psychology Press.

PM are summarized in a meta-analysis reported by Henry et al. (2004), which indicates several patterns.

First, there seems to be a pattern indicating somewhat larger age-related declines in time-based (having to perform a specific activity at a certain hour) than in event-based (those with retrieval cues, such as having to give someone a message upon meeting him/her) PM tasks. This may be related to the former type of task relying more heavily on self-initiated processing (Craik, 1986). However, the specific results in a given task also depend on several other variables, including subject and task characteristics. For example, within event-based PM tasks, those tasks that create high strategic demands for controlled processing related to cue type, monitoring of the ongoing task, and retrieval demands, are more age sensitive than those tasks based on more automatic processing (e.g., Einstein et al., 1997; Park et al., 1997; Einstein and McDaniel, 2005). A second pattern of results within PM tasks is that older adults seem to show a larger impairment in remembering 'to' perform an action than in remembering 'which' action to perform (e.g., Cohen et al., 2001). Finally, in naturalistic studies (e.g., when people report about their performance in real-life situations, or when they have to respond to a meaningful prospective event in a laboratory situation, e.g., Rendell and Craik, 2000), rather than in typical laboratory studies, older adults do not show poorer PM than younger adults, and in some cases they even outperform younger adults. Possible reasons for this finding involve the higher relevance of ecologically valid tasks for older adults (to be discussed in the final section of the chapter) or their common use of external memory aids (e.g., writing appointments in a calendar and relying on it, as opposed to internal aids, which younger adults may use well in laboratory situations).

2.41.3 Theoretical Views

Considering the wealth of empirical evidence related to age-related changes in memory, some of which are reviewed above, it is not surprising that various theoretical accounts have been offered to explain the underlying mechanisms and processes that give rise to the different phenomena. Some of these mechanisms are more distal and relate to phenomena in cognitive aging in general, whereas others are more specific to age-related changes in memory. Following, we outline some of the major approaches and the way they explain the phenomena at hand. We then suggest an integrated approach.

2.41.3.1 General Mechanisms: Processing Resources Limitations

Several suggested frameworks claim that age-related changes in memory in particular, and in cognition in general, are the result of a decline in mental processing resources associated with age. These resources may be related to attention, speed, or capacity.

2.41.3.1.1 Attentional resource limitations

Several researchers (e.g., Hasher and Zacks, 1979; Craik and Byrd, 1982; Craik, 1983, 1986) have suggested that the pool of attentional resources needed to perform a given task is reduced in old age, and that this reduction is the cause of many age-related changes in cognitive tasks. This view predicts that older adults will be especially impaired on difficult tasks that require a significant amount of attentional resources. Evidence from Craik and Byrd (1982), Craik (1983, 1986), Rabinowitz et al. (1982), and Craik and McDowd (1987) is consistent with this notion, suggesting that a shortage of available attentional resources in older adults results in poorer memory because effortful cognitive operations, such

as elaboration and effortful retrieval, require substantial attentional resources.

Furthermore, whereas recurring aspects of items can be encoded in a relatively automatic fashion, changing contextual elements of a situation demand a large amount of attentional resources. Thus, when these attentional resources are low – as in old age – there will be a tendency to encode items in terms of their general, stereotyped features, resulting in poor memory for the event. Results from several studies are consistent with this notion (e.g., Rabinowitz et al., 1982; Hess et al., 1989; Hashtroudi et al., 1990). Moreover, Craik and Byrd (1982) and Craik and Simon (1980) have shown that general retrieval cues (which require fewer attentional resources, as measured by secondary task performance) are relatively more effective for older subjects, whereas more specific contextual cues are relatively more effective for young adults.

Similar patterns have been reported for retrieval, as well. For example, as mentioned earlier, Craik and McDowd (1987) showed that older adults' performance was poorer on cued recall than on recognition tasks compared with younger adults. Such results can be interpreted in terms of the amount of attentional resources required by each type of task (as indicated by secondary task performance); cued recall requires substantial resources to search for the target word, whereas recognition, in which subjects are provided with a copy of the original target, requires fewer attentional resources.

Another line of research supporting the reduced attentional resources notion involves the effects of divided attention (DA) on memory. Several studies indicate that under DA conditions involving memory, older adults demonstrate a larger reduction in the combined performance on both the primary and the secondary tasks, relative to younger adults (e.g., Anderson et al., 1998; Li et al., 2001). Furthermore, several studies have found that younger adults whose available attentional resources are reduced by performing a secondary task exhibit a pattern of memory performance decline similar to that of older adults (e.g., Craik and Byrd, 1982; Rabinowitz et al., 1982; Jennings and Jacoby, 1993; Chen and Blanchard-Fields, 2000).

The notion of an age-related decline in attentional resources is in line with other memory impairments described in the previous sections. For example, the larger age-related decline in working memory than in short-term memory (e.g., Bopp and Verhaeghen, 2005) could be caused by a greater demand on attentional resources by the former. Likewise, explicit memory tasks require more attentional resources at retrieval than do implicit memory tasks, which usually do not require deliberate memory search. Furthermore, the encoding and explicit retrieval of contextual details and processes involved in recollection require more attentional resources than do familiarity responses (e.g., Troyer et al., 1999; Gardiner et al., 2006).

Craik (1986) elaborated on the notion of attentional resources to make the distinction between tasks that rely on environmental support and those that require self-initiation. Tasks that rely more on environmental support – for example, recognition memory, in which a copy of the presented stimulus is provided as a cue – require less self-initiation/attentional resources and hence should not show large age-related declines. In contrast, tasks that require more self-initiation, such as free recall, should show larger age decrements. Both notions are in line with empirical evidence (e.g., Craik and McDowd, 1987).

A related view of the age-related decline in memory addresses the distinction between controlled and automatic processes. According to this view, older adults will show a decline in memory tasks that require controlled, effortful processes but will exhibit no or only a small decline in tasks mediated by more automatic processes (Hasher and Zacks, 1979). This suggestion relates to the depleted attentional resources view, as tasks based on automatic processes should not require substantial attentional resources, whereas tasks that require controlled effortful processes should draw heavily on attentional resources; thus, age-related impairments should be largest on tasks that demand controlled processing. Such a position can explain why implicit memory, for example, seems to be intact in older adults, as it is driven largely by automatic processes. Likewise, recall tasks, which require relatively controlled processes, should be more affected by aging than recognition tasks. The controlled-automatic distinction is also consistent with results of studies suggesting that older adults' episodic memory impairments may be partially the result of the failure to spontaneously use effective strategies at encoding and at retrieval. Interestingly, these studies suggest that older adults can improve their performance by receiving appropriate instructions to use strategy, although this generally does not eliminate age differences altogether (e.g., Verhaeghen et al., 1992; Dunlosky and Hertzog, 2001; Naveh-Benjamin et al., 2005).

While the distinction between tasks based on automatic and controlled processes seems to have heuristic value, there are problems regarding the definition of each type of process and in task analyses to determine which processes are involved in a given task. Similarly, some researchers (e.g., Salthouse, 1988; 1991b) claim that the notion of attentional resources is too vague, at a framework level only, without enough details to be considered a model or a theory and is not well operationally defined.

To summarize, although there have been criticisms of the reduced attentional capacity framework on the grounds of vagueness, it seems to provide a heuristic functional explanation to age-related decline in memory performance. Further studies should employ different tasks to assess the construct validity of attentional resources, as well as to specify the mechanisms underlying depleted resources, potentially in terms of specific brain-related changes (see Carpenter et al., 1999).

2.41.3.1.2 Speed of processing limitations

Another idea involving limited resources of older adults is in terms of the speed with which mental processes can be carried out. Several researchers have suggested that the execution of mental processes slows down in old age (e.g., Birren, 1965; Birren et al., 1979). Additionally, Salthouse (1996) suggested that slowing of basic information processing at the microlevel may result in poorer performance at a more macrolevel. For example, if a task involves several processing components, and the first few are slowed down with age, the input of these components will not arrive in time to feed later processing units, resulting in failure to complete the task. Using tools of multivariate statistics, such as path analysis and large-scale psychometric studies, Salthouse (1996) has shown that measures of basic speed mediate a significant age-related variance in several memory tasks. Verhaeghen and Salthouse (1997) conducted a meta-analysis of cross-sectional studies and found, for example, that speed can account for over 70% of age-related variance in episodic memory measures.

Although the slowing down notion seems reasonable, future studies using the speed approach should provide for a better understanding of the relations of speed to the specific differential decline patterns characterizing memory and aging. For example, the approach must be able to explain why intentional learning is more harmed by age than is incidental learning, why semantically related information is better remembered by older adults than is unrelated information, why context memory is especially poor in older age, and why recollection is more affected by age than is familiarity.

2.41.3.1.3 Capacity (working memory) limitations

Some researchers (e.g., Welford, 1980; Parkinson, 1982; Hasher and Zacks, 1988; Puckett and Lawson, 1989) claim that reduced WM capacity is a major factor in age-related declines in many memory and other cognitive tasks. The notion is that to engage in any online processing required for different memory tasks – for example, encoding of spoken sentences – people must use processes to coordinate the interplay between temporary storage of the information and its integration into a cohesive message (Baddeley, 1986). According to this idea, older adults possess less efficient control processes, making, for example, the encoding of text more demanding and difficult (e.g., Light and Albertson, 1988). In support of this notion, Salthouse (1991a) has shown that when performance on measures of WM is statistically controlled, age-related declines in memory tasks are reduced by a substantial amount.

Whereas the original notion of WM capacity was somewhat similar to that of depleted attentional resources, mentioned above, more recently, more specific executive processes involved in WM have been suggested as the loci of memory decline in older adults. In particular, Hasher and Zacks (1988) and Zacks and Hasher (1997) claimed that older adults have a special problem in the recruitment of efficient inhibitory processes, making it difficult to block irrelevant information from entering WM. In this sense, it is not necessarily that WM capacity is smaller in old age but that it is cluttered with irrelevant information that was not appropriately filtered. This, in turn, hinders efficient processing – such as that involved in encoding and retrieval operations – of ongoing information. Support for the inhibition notion comes from studies showing, for example, that in contrast to young adults, irrelevant information is still held in older adults' WM despite being disconfirmed (e.g., Hartman and Hasher, 1991). Chapter 3.28 in this volume discusses such failures in terms of an age-related decrease in frontal lobe efficiency. Other relevant executive processes are those involved in task switching. Recent results indicate that older adults have difficulty rapidly switching between different aspects of a given task (e.g., Mayr et al., 2001).

The recent trend toward assessing the specific WM processes that decline as people age seems to be a fruitful direction that should be followed up in future research. This avenue of study could potentially explain one underlying root of the age-related decline in general cognition and in memory processes in particular.

Overall, although the above suggestions for the mediating role of age-related decline in processing resources in cognition are reasonable, for some their direct effects on memory performance have not yet been demonstrated. Further studies are needed to demonstrate empirically the precise way in which these different suggestions of decline in processing resources are responsible for the complex pattern of both decline and stability in memory in old age.

2.41.3.2 Memory-Specific Mechanisms

Whereas the above views are more general and relate not only to performance on memory tasks but also to cognitive processes in general, there have been some attempts to specify the particular mechanisms involved in age-related patterns in memory tasks.

2.41.3.2.1 The source-contextual deficit approach

According to this approach, older adults have problems at retrieval in distinguishing between different sources of original events (e.g., Johnson et al., 1993). In the eyewitness memory paradigm, for example, the claim made is that older adults, relative to young ones, cannot distinguish between the original information and related information that is subsequently presented. This idea can be extended to context in general, with the claim that one reason for older adults' memory decline is their inability to remember different aspects of the encoding context, including the time when an event took place, where it happened, the associated internal psychological context, and the social circumstances involved. The notion is that young people can better encode these contextual details, which can later serve as retrieval cues when a particular episode, or its components, must be remembered.

The source-contextual deficit suggestion is in line with studies reviewed earlier in this chapter that indicate a differential age-related decline in episodic versus semantic memory, as only the former relies on specific contextual details. This may help to explain the findings on eyewitness testimony, discussed above in the false memory section, in that older adults may fail to remember the source of specific information, thus leaving them susceptible to misinformation. Furthermore, as reviewed earlier, older adults show a particularly large deficit in memory for contextual/source details relative to their memory for the content/focal events. Finally, free and cued recall are more affected by contextual details than is recognition memory (e.g., Godden and Baddeley, 1975, 1980), and as mentioned earlier, older adults show more of a decline on recall tests.

2.41.3.2.2 Associative-binding deficit approach

Another suggestion in the literature is that older adults have a special problem in associating/binding different components of an episode into a cohesive unit (Chalfonte and Johnson, 1996; Mitchell et al., 2000; Naveh-Benjamin, 2000; Naveh-Benjamin et al., 2003). Naveh-Benjamin termed this the associative deficit hypothesis (ADH), which suggests that older adults have a relatively intact mechanism to encode and retrieve components of an episode but have a special problem in binding together the different components into a cohesive episode and in explicitly retrieving these associations. The ADH is more general than the source/context deficit notion, suggesting that the older adults have problems whenever explicit memory requiring the binding together of different components is involved. These bindings could be between an item and its context or between two items or objects.

The ADH is supported by evidence showing that older adults perform significantly more poorly than young adults on tasks requiring memory for associations but that age differences are relatively small when memory for individual components is tested. For example, Naveh-Benjamin et al. (2004) presented young and old participants with faces paired with names, with the task of trying to learn the names, the faces, and the associations between the name and the face in each pair. Three recognition tests were given. On the name test and the face test, participants were presented with either two names or two faces, respectively, and were asked to choose the stimulus that they had seen at study. The third test was an associative recognition test, in which subjects chose between two previously presented names that belonged with a given face or between two previously presented faces that belonged with a given name. Results, which can be seen in **Figure 9**, showed no age-related difference in name recognition and only a small age-related difference in face

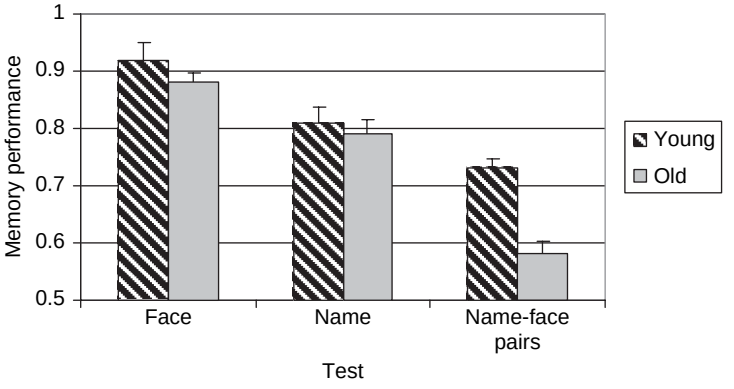

Figure 9 Memory performance in recognition of faces, names, and name–face pairs, as a function of age group. From Naveh-Benjamin M, Guez J, Kilb A, and Reedy S (2004) The associative memory deficit of older adults: Further support using face-name associations. *Psychol. Aging* 19: 541–546. Copyright 2004 by the American Psychological Association.

recognition but a large age-related decline in the associative tests of names and faces. This associative deficit has been observed using various types of stimuli, including associations between Snodgrass drawings and their arbitrary colors (Chalfonte and Johnson, 1996; Mitchell, et al., 2000), pairings of words (Naveh-Benjamin, 2000, Experiment 2; Castel and Craik, 2003; Light et al., 2004; Healy et al., 2005), pairings between words and their fonts (Naveh-Benjamin, 2000, Experiment 3), and pairings of pictures (Naveh-Benjamin et al., 2003).

This ADH can accommodate several of the results reviewed earlier. For example, implicit memory should not be affected as much by aging as explicit memory, as it requires to a much lesser degree explicit retrieval processes involved in recovering information about binding of elements in an episode. In addition, semantic memory should be affected to a lesser degree than episodic memory because it does not require the specific binding of information to a place and time. There are also some indications that part of the deficit is due to the inability of older adults to spontaneously produce and efficiently use adequate associative strategies to bind together information. Studies inducing the use of a connective strategy (a sentence or an interactive mental image) during encoding and during retrieval have shown an increase in older adults' performance on memory tasks requiring associative information, in some cases more so than younger adults (Naveh-Benjamin et al., 2005; Naveh-Benjamin et al., 2007).

2.41.3.2.3 Recollection deficit approach

This hypothesis, for which empirical results were cited earlier in this chapter (e.g., Jacoby et al., 1996; Jacoby and Hay, 1998), claims that older adults' memory deficit is largely a result of their inability during retrieval to access the details of an episode, resulting in recollection failure. According to this suggestion, the ability to remember via familiarity – for example, to notice that something was already presented before, without remembering detailed information about the event – is left mostly intact in old age. This hypothesis (e.g., Jacoby, 1999) is supported by many studies that used the process dissociation paradigm or the R/K procedure, which were reviewed earlier in the chapter. The hypothesis can explain the relatively adequate performance of older adults in implicit memory tasks and in those requiring semantic memory. In both cases, no detailed conscious recollection of the original event is required. Likewise, source memory, which requires recollection of detailed information, is especially prone to the effects of age.

2.41.3.3 An Integrated View

The above-suggested source-contextual, associative-binding, and recollection deficit hypotheses seem to have different emphases. For example, the source-contextual and the associative-binding approaches emphasize failure more at the encoding phase, whereas the recollection perspective deals more with processes that happen at retrieval. In addition, the paradigms used by each of the approaches are quite different. Nevertheless, there are some fundamental similarities between the three approaches. The common assumption held by all three is that age-related memory declines are caused by a lack of efficient episodic encoding and retrieval of detailed

bound information. Difficulty in encoding specific features of an episode and in establishing associations among those features may result in, for example, the inability of an older adult to recall the name of a new acquaintance upon encountering that person, despite the fact that he or she may look familiar. This difficulty may be caused by a failure of conscious recollection of the contextual details or circumstances (e.g., time and place) regarding the original encounter with this person and/or to the fact that this person and not someone else they met on the same occasion is named, for example, George. The poor encoding of source-contextual features, as well as a reduced ability to bind together the different features of the event and its contextual elements, will increase the chances of a conscious recollection failure of the detailed information at the retrieval phase. In this sense, the three approaches are somewhat similar and complement each other.

Such an integrated approach can explain several of the empirical lines of evidence for age-related memory changes reviewed earlier in this chapter. For example, the fact that semantic memory is held relatively intact during adulthood and old age could be a result of the encoding of contextual details and their recollection being crucial in episodic – but not in semantic – memory. This is because the multiple repetitions of facts in semantic memory increase their familiarity, which is relatively intact with age. Such an integrated view can also explain the relatively minor age-related decrements in implicit memory, since this system is based more on familiarity and fluency and does not require detailed encoding and binding of contextual details or the recollection of specific encoded features (e.g., Roediger, 1990; Schacter et al., 1993).

This integrated approach can also explain age-related changes in false memory. As we reviewed earlier in the chapter, older adults tend to show an increase in the retrieval of incorrect memories. This finding could be caused by an age-related reduction in the ability to bind together bits of information from the original event and to keep these bindings separate from information included in the postevent episode. If during retrieval, older adults cannot recollect the contextual details of the original event, they may integrate bits of later-learned information into their memory of the original event, mistaking, for example, a person viewed in a mugshot at a police station for the true culprit. Similarly, in the DRM paradigm of false memory, older adults tend to recall or recognize, more so than young, the critical lure – the related event that did not happen. According to the suggested view here, this may be a consequence of their inability to bind together the content words and the context – either external (presented) or internal (generated). When the critical word is later presented in a recognition test, the reduced ability of older adults to recall contextual features related to the critical and target words, coupled with feelings of familiarity, could lead to false recognition.

One potential task of future research on memory and aging is to test specific predictions based on this integrated approach. For example, it seems reasonable that the more features and their bindings involved in an event, the more difficult it would be for older adults to encode and bind together the features and to recollect them during retrieval. Furthermore, if some of the features, for example, the contextual elements, are common to the different episodes, this might result in a nondistinct contextual encoding that will make recollection by older adults more difficult later on (see Hunt and McDaniel, 1993, for a discussion of distinctiveness). On the other hand, if the features of each event are unique and distinctive from each other, older adults might be better able to bind together and to recall those features (e.g., Johnson et al., 1995; Degl'Innocenti and Backman, 1996).

Such a unified view can be incorporated with the depleted resources framework reviewed earlier to provide the following hypothesis: It may be that the lack of resources in older adults – for example, reduced attentional resources both at encoding and at retrieval (e.g., Anderson et al., 1998; Naveh-Benjamin et al., 2005) – could mediate the source/context/binding failure at encoding, as well as recollection failure at retrieval. This suggestion makes several assumptions. First, the encoding of contextual elements and the binding together of features is more effortful and requires more resources than the encoding of the focal elements and each feature separately (Troyer, et al., 1999; Castel and Craik, 2003; but see different results by Naveh-Benjamin, et al., 2003). Second, the retrieval and recollection of bound and contextually detailed information requires more attentional resources than that of contextually impoverished information (e.g., Troyer, et al., 1999). Future studies could assess this suggestion, for example, by measuring the attentional resources associated with different types of feature, context, and associative (feature-binding) encoding and retrieval.

2.41.4 Further Issues

2.41.4.1 Uniqueness of Age-Related Memory Changes in General Cognitive Decline

One question that has consequences for the interpretation of age-related differences in memory is whether these differences constitute unique declines in the memory system or whether they are just a manifestation of more general changes in cognition. One statistical control approach to studying this question was employed by Salthouse et al. (2002), who assessed performance of different age groups on several variables, some related to memory and others to other cognitive tasks. Salthouse et al. then looked at the variance accounted for by age in a given memory task while statistically controlling the variance in some of the other cognitive tasks. The results showed that the proportion of age-related variance in a free recall memory task declined from 30% to less than 20% after statistically controlling the variance in a block design task (taken from the WAIS III, Wechsler, 1997) and to less than 5% after statistically controlling the variance in performance on the digit symbol task (also taken from the WAIS III). Similar findings were shown by Siedlecki et al. (2005), who administered a battery of different cognitive tests to 330 adults between the ages of 18 and 89, along with several tests of source memory. Using structural equation modeling, the researchers found no indication of a unique age-related variance on source memory only. Similar results have been obtained for other episodic memory measures (e.g., Salthouse et al., 2006). Such findings imply that age-related effects on memory measures and on other cognitive variables are not independent of each other.

These results raise interesting questions regarding the different approaches to studying the effects of age on memory and their respective outcomes and contributions. One approach, discussed throughout most of this chapter, is experimental, assessing the effects of specific manipulations on the memory performance of young and older adults. This approach reveals a variety of differential effects of aging on memory. The approach discussed in the previous paragraph, based on psychometric studies of individual differences, looks simultaneously at relationships between age and several memory and cognitive indices and often shows that the effects of age on memory are not unique but are shared with other cognitive variables. It seems that the research on age-related differences in memory would benefit from the integration of both approaches in order to provide a better understanding of the absolute changes in different tasks on one hand and the degree to which differences in one condition are statistically independent from differences in another condition.

2.41.4.2 Positive Modulators of Older Adults' Episodic Memory Performance

Even though older adults seem to show poorer episodic memory than younger adults, there are nevertheless variables that seem to positively modulate older adults' episodic memory. For example, studies indicate that older adults tend to remember emotional material relatively well, often performing just as well as younger adults on positively valenced material. This has been found both in working memory (e.g., Mikels et al., 2005) and in long-term episodic memory (see, e.g., Carstensen et al., 2006, for a review). The suggestion raised (e.g., Carstensen et al., 1999) is that socioemotional regulation becomes more important as people age, changing their motivation, which then leads to successful encoding and retrieval of emotional information, especially when it is positive. Such changes in priorities also result in the tendency to remember autobiographical events more positively as people age (see, e.g., Mather and Carstensen, 2005 for a review).

In addition, although older adults are relatively impaired in memory for verbatim information, they remember well the gist of information and add their interpretation to it, creating a richer narrative. For example, studies regarding memory of information presented in stories indicate that older adults do well on remembering the underlying messages and gist of the stories (e.g., Adams et al., 1997; Stine-Morrow et al., 2004).

Finally, time of day seems to make a difference in memory performance, with older adults doing relatively well when they are tested in their 'prime' time – in the morning. Yoon et al. (1999) administered the Morningness-Eveningness Questionnaire (MEQ) to over 2000 older and younger adults and report that approximately 75% of the older sample, but less than 10% of the young sample, could be classified as morning types; conversely, very few older adults and nearly 40% of younger adults were evening types. Interestingly, May et al. (2005) found that evening-type younger adults and morning-type older adults performed better on an explicit memory

task – but worse on an implicit task – during their peak hours. Furthermore, it has been suggested that different circadian patterns in young and old can artificially enhance age-related declines in memory (May et al., 1993). This occurs because participants are often tested in the afternoon (the convenient time for the young research assistants), when younger adults, by their arousal patterns, are performing at their highest levels. When testing is done in the morning, older adults often perform quite well, for example, on working memory tasks (e.g., West et al., 2002).

2.41.4.3 Negative Modulators of Older Adults' Episodic Memory Performance

There are several other factors that may have inflated some of the reviewed age-related declines in episodic memory. For example, older adults might not be as motivated as younger students to perform at their best in laboratory situations. The materials used in many episodic memory tasks (such as lists of words) may seem contrived and of little relevance to older adults, therefore decreasing their motivation to do well (e.g., Henry et al., 2004). This may be one reason why they perform relatively well on semantic memory tasks, which by their nature employ more ecologically valid, relevant, and interesting materials.

Another mediating factor might be a lack of recent practice in testing situations and the anxiety produced by such testing in the laboratory. Older adults usually do not frequently encounter explicit testing situations in their lives, as do younger adults, who are often university students and who take tests very frequently. This lack of recent testing practice may put older adults at a disadvantage. In addition, it may create higher anxiety in older adults (but see Birren, 1964), which in turn is known to reduce cognitive performance (Wetherell et al., 2002). Although this factor may not straightforwardly explain interaction effects – cases where older adults are doing worse on one episodic task than on another – it can increase the overall differences observed between young and old. An intriguing demonstration of the potentially mediating effect of anxiety on age-related differences in memory is a study by Rahhal et al. (2001), who found that age differences in recognition memory were eliminated when the terms 'memory' and 'testing' were completely omitted from task instructions. One interpretation of these results is that the latter condition did not induce the usual level of anxiety in older adults, which, in turn, helped them to perform well. Alternatively, knowing that they are in a memory experiment might evoke older adults' negative stereotypes about memory and aging, which might negatively affect their performance. These stereotypes are presumably not induced when the task does not seem related to memory.

Finally, as we mentioned at the outset, most of the studies reviewed in this chapter used a cross-sectional design and, as such, might have been affected by various factors, most notably, cohort effects (e.g., Schaie, 2000). It is important to note that, when data on the same memory phenomenon are collected using both cross-sectional and longitudinal designs, age-related differences tend to be smaller in longitudinal studies, with peak performance later in life (e.g., Rönnlund et al., 2005). Thus, the patterns of decline reported in this chapter, which are mostly based on cross-sectional designs, may be an overestimation of actual age-related declines in memory.

2.41.5 Summary

The evidence reviewed in this chapter indicates interesting differential patterns of development in adulthood and old age that depend on different factors, including the task and the memory type involved. Whereas implicit and semantic memory show little decline with age, tasks based on episodic memory, especially those that require the encoding and the explicit retrieval of detailed bound information about a given event, show appreciable decline. It seems that as long as the task does not require remembering specific details, and as long as performance can be supported by previous knowledge or environmental cues, older adults do quite well.

Several theoretical accounts at different levels of generality have been suggested to explain the age-related patterns of memory development, and especially of episodic memory deficits. A unified approach characterizes old age as being associated with a lack of detailed encoding and binding together of both contextual and focal components of an episode, coupled with difficulty in accessing and recollecting these details during retrieval, possibly because of the high demand of these processes for attentional resources. Such an approach may serve as a departure point to further investigate these important issues using both the manipulative and the psychometric approaches to advance our knowledge of age-related changes in memory.

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2.42 Superior Memory of Mnemonists and Experts in Various Domains

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2.42.1 Superior Memory of Mnemonists and Experts in Various Domains

One of the most striking individual differences between people concerns their ability to recall information and everyday events. Some people seem to remember an exceptional amount of details of past events – much more than others who also were present and thus presumably had a similar experience. It is difficult to validate any superior memory accounts because there are rarely objective records, such as video recordings. As a consequence, the recall of people with reputation for having a good memory is often simply accepted, even when other people cannot recall the same information. There are, however, bodies of cultural and professional knowledge, which are publicly accessible in the form of periodicals, books, and encyclopedias. Memory for this type of knowledge is measured regularly by schools, universities, and professional licensing boards. Based on such tests, we know that experts have a greater body of knowledge about their domain of expertise than other individuals. These tests also show substantial individual differences between experts who have the same educational background and thus similar opportunities to acquire the same knowledge. How is it possible to explain these large differences in memory and accumulated knowledge?

Ideas of individual differences in memory depend on one's general conception of the process of memory formation. Plato viewed the formation of memories as akin to the impressions made by a signet ring on a wax tablet (Yates, 1966). Due to differences in the hardness and quality of the wax, some people would be able to make sharp impressions (accurate memory), whereas others were diffuse and rough (poor memory). This account matches many people's introspective impressions that their memories are clear and vivid and sometimes even approach the vividness of the original experiences. Some people are even believed to possess the ability to store completely accurate visual images (photographic memory) (La Brecque, 1972). From this conception of individual differences in memory we would expect a superiority of memory that would generalize across all sorts of domains.

There are two types of evidence on superior memory. One type concerns the domain-specific superiority of experts to remember new information and experiences in their domain of expertise. Some elite athletes can, after a sports event, discuss the play-by-play action. Expert chess players can readily recall details of chess positions from their matches in recent tournaments. There are even numerous anecdotes that were collected as evidence of an unusual ability to store presented information rapidly in their domain of specialization. For example, Mozart was supposed to be able to reproduce a presented piece of music after hearing it a single time. Although these feats could be explained by a general superiority of memory, it is rarely supported

by reliable evidence. Most experts' strikingly superior memory appears to be limited to information in their domain of expertise, with, if anything, poorer memory for other information and particularly more mundane material, such as information about people, appointments, and other everyday issues. This preoccupation with some domain does not preclude their having a generally superior memory when they attend to information with the intent to commit it to memory. Scientists realized that it is necessary to find experts who are willing to be tested to assess whether they have fundamentally different and generally superior memories. Their controlled memory studies have found that the memory advantage of experts was surprisingly specific to the domain of expertise, as will be described in more detail later in this entry.

The other type of evidence for superior memory is found when some individuals are able to memorize seemingly meaningless information, such as names of people, lists of digits and unrelated words, and verbatim text in an unfamiliar foreign language. Superior memory for this type of seemingly meaningless information was initially assumed to be mediated by some superior general capacity to store information in memory that would generalize to all types of materials. Laboratory studies testing the memory of people with this type of superior memory found that any time the performance was exceptional – outside the range of random sample of adults, then the performance was limited to a small number of types of material, such as digits, letters, and playing cards. Furthermore, these individuals were found to have engaged in extended memory training using various mnemonic memory strategies that allow seemingly meaningless information to be encoded with meaningful associates already in memory. For example, 9631492177 could be encoded as [1]963 (death of Kennedy) 1492 (Columbus) and 177[6] (American Revolution). In sum, recent research (Ericsson, 2003; Wilding and Valentine, 2006) has rejected the hypothesis of an exceptional innate memory capacity that generalizes across materials and has demonstrated that exceptionally superior memory is limited to some particular types of materials and domains of expertise and reflects the result of acquired skills and knowledge relevant to each specific domain.

2.42.2 Brief Historical Background

Sir Francis Galton (1883) innovated the method of interviewing many people by sending out a list of questions about mental imagery—said to be the first questionnaire. He was interested in how the memory and imagery of exceptional people, such as scientists, judges, and other famous people of his time, differed from regular people. He had been intrigued by reports of photographic memory and asked questions of the acuity of specific memories, such as the clarity and brightness of their memory for specific things such as their breakfast table. He found striking individual differences in the clarity or vividness, but no clear superiority of the eminent scientists: for example, Darwin reported having weak visual images. Now over a hundred years later, it is still unclear what these large individual differences in reported vividness of memory images reflect in objective performance, as will be discussed later in this section.

A few years later Ebbinghaus (1885/1964) published his seminal work on laboratory studies of memory, where he argued that individual differences in memory between adults in everyday life were primarily caused by differences between individuals' knowledge and prior experience. He consequently suggested that psychologists should present unfamiliar materials, such as nonsense syllables, under controlled conditions in the laboratory and then assess the number of repetitions necessary for successful reproduction. Ebbinghaus designed his studies so he could be a participant and based on the data from a single participant (himself) to uncover the general laws of human associative memory. All of his findings have been replicated thousands of times with large groups of naive participants (Slamecka, 1985a,b). One of the findings relevant to superior memory was that Ebbinghaus found that memory for nonsense syllables is much worse than memory for typical information encountered in everyday life – in fact, he needed ten times more time to memorize lists of nonsense syllables compared to the poems with the same number of syllables. Other contemporary psychologists, most notably Alfred Binet (1894), raised doubts about whether memory for nonsense syllables was mediated by the same types of processes as memory in everyday and exceptional memory by mental calculators and chess players.

Binet's (1893/1966) report on chess players' 'mnemonic virtuosity' was arguably the first published study on memory and expertise. Binet interviewed chess players about their ability to play chess blindfolded without seeing a chessboard. The ability required to maintain chess position in memory during blindfolded play did not appear to reflect a basic memory capacity

to store complex visual images, but a deeper understanding of the structure of chess. Hence, to play chess blindfolded requires knowledge and skill to understand the reasoning that led to each chess move. It is the ability to discover the meaningful connections between these ideas that provides the basis for the superior memory and the ability to maintain a chess position in memory. However, Binet found that the verbal descriptions of the visual images of the mental chess positions differed enormously between blindfolded chess players. Some claimed to see the board as clearly as if it was shown perceptually with all the details and even shadows. Other chess players reported seeing no visual images during blindfolded play and claimed to rely on abstract characteristics of the chess position. Unfortunately, there was no independent evidence to support or question the validity of these diverse introspective reports. Binet (1893/1966) also studied mental calculators, who could multiply large numbers mentally, and other people with exceptional memory. He was able to show that memorization of a matrix of digits did not involve forming a visual image because subsequent retrieval was only rapid for retrieval of digits presented in the same row of the matrix (Ericsson and Chase, 1982).

Subsequent research was guided by the hypothesis that superior memory was mediated by innate talent and general basic capacities. In the early part of the twentieth century Djakow et al. (1927) measured the basic abilities of world-class chess players and compared their test performance to the average of a large sample of nonchess players. Contrary to the assumed importance of natural gifts, the international players were superior only on a single test – a test involving memory for chess positions. A few decades later de Groot (1946/1978) found that when chess players were instructed to select the best move for a briefly presented chess position, their ability to recall the positions of the chess pieces was closely related to chess skill. International chess masters were able to recall virtually all the pieces on the chessboard, whereas beginners could recall only a few pieces. Taken together, these two findings suggest that exceptional memory of chess masters is constrained to superior memory of meaningful chess positions – a claim that has been validated by subsequent research in the laboratory.

In parallel with the studies of chess experts' exceptional memory, several researchers conducted case studies of individuals with exceptional memory (for reviews, see Wilding and Valentine, 1997, 2006). The most influential series of studies was conducted by Luria (1968) on a newspaper reporter referred to as Subject S – an abbreviation for the last name of the participant, namely, Shereshevski. Luria (1968) found that S could memorize a wide range of materials, such as list of nonsense syllables and poetry in a foreign language, by forming eidetic images of meaningful associations. For example, to memorize the first few words in the first line of Dante's *The Divine Comedy*, namely, "Nel mezzo del cammin di nostre vita," S reported that he associated **Nel** by thinking, "I was paying my membership dues when there in the corridor, I caught sight of the ballerina **Nel**'skaya" (Luria, 1968: 47), and then associating mezzo with an image in the same context and then associating **del** by thinking, "There is a pack of **Del**i cigarettes near them' and so on" (Luria, 1968: 47). Although Luria attributed S's exceptional memory to the vividness of his eidetic memory, subsequent researchers argue that S was using a variant of the ancient method of loci (Yates, 1966), where presented information, such as a text in a foreign language, is recoded into meaningful images (see preceding example), which in turn are associated to familiar locations, such as the concert hall and adjacent streets and buildings. Skilled memory experts (mnemonists) who use the method of loci have acquired long lists of journeys of locations, such as locations encountered with visiting one's own house – the mailbox, driveway, garage door, garage, door at main entrance, and so on. Most importantly, Luria (1968) found that S was able to recall matrices of digits without the need to retrieve meaningful associations and was able to commit a matrix of 50 digits to memory in only 3 min. Later, S was able to recall the digits in an arbitrary manner, in columns and zigzag patterns, which led Luria (1968) to infer that digits were directly available as a visual image. Other studies of exceptional individuals, such as the mathematics professor Rückle (Müller, 1911) and the Japanese mnemonist Isihara (Susukita, 1933), showed similar findings and found that mnemonic encoding methods played a central role in their superior performance. Scientists also examined the memories of individuals who were able to make mathematical calculations mentally (mental calculators), such as the Polish mental calculator Dr. Finkelstein (Bousfield and Barry, 1933) and the mathematics professor Aiken (Hunter, 1962, 1977), and were able to establish both calculation methods and exceptional memory performances for numbers.

These case studies of exceptional memory relied extensively on the individuals' introspective descriptions of their experiences during interviews and the characteristics of their images and, in particular, their

vividness. Other contemporary researchers studied children with eidetic imagery. These children reported having detailed visual images of presented complex stimuli even after they had been removed from view. Many researchers, including Luria (1968), argued that individuals with exceptional memory must be relying on a similar basic capacity to form and maintain visual images. After decades of research on describing the reports of eidetic imagery, researchers started measuring the memory performance of eidetic and normal children. To everyone's surprise there was no difference in memory performance between children who reported seeing an image of the presented stimuli and other children (Haber, 1979). More recently, researchers have studied ratings of the reported vividness of memory for an experience and the level of accurate recall of presented pictures (Richardson, 1988; McKelvie, 1995). Most surprisingly, the amount of recall was not found to differ between people who rated their memory image as very vivid (almost as clear as still seeing the picture) and people who reported having little visual image of the stimulus at all. These findings supported the opinions of many experimental psychologists, who held that introspective judgments about experience were frequently misleading and sometimes even inconsistent with measures of performance.

A scientific analysis of exceptional memory would need to be based on reliably superior performance that could be repeatedly reproduced in the laboratory. Similarly, introspective descriptions by individuals are not acceptable as valid data and should be discarded in favor of concurrent verbal reports of participants' thinking (Ericsson and Simon, 1993). These reports can be analyzed and explained as are other types of data on cognitive processes (e.g., eye fixations, latencies, and recordings of brain activity).

The following sections of this chapter will discuss experimental studies of exceptional memory performance that assess its scope and structure, the development of exceptional memory ability, the structure of superior memory of domain experts, and some recent developments in study of the pattern of brain activation during exceptional memory performance.

2.42.3 Experimental Studies of Exceptional Memory: Generalizability and Mediating Mechanisms

When somebody demonstrates exceptional memory for a list of digits or a chess position, it is only natural that one would expect that superior memory would generalize to any aspect of memory. It is not possible to assess the generalizability of the exceptional memory simply by observing the individuals' behavior involved in committing particular types of information of the person's own choosing under everyday life conditions. It is necessary to get their consent to participate in experiments where possible to vary the presented types of materials and conditions of memory. Some of the early investigations of exceptional memory showed that the formation of memory was not instantaneous, and although much faster than required by regular adults, it took considerable time. Even more important, exceptional memory is primarily demonstrated for seemingly meaningless or arbitrary types of materials, such as list of digits, nonsense syllables, and texts in a foreign language. In fact, some of the early laboratory studies showed that the dramatically superior memory performance was often limited to lists of a certain types of materials, such as lists of digits and words. For example, the Japanese memory expert Isahara did not show exceptional memory for presented color patches (Susukita and Heindl, 1935). In his review of mental calculators, Smith (1983) found that their superior memory was invariably limited to numbers and digits. The most influential study demonstrating the domain-specific nature of mechanisms involved in exceptional memory was conducted by Bill Chase and Herb Simon (Chase and Simon, 1973) on chess players' memory for chess configurations in the 1970s.

2.42.4 The Role of Meaningful Associations in Superior Memory Performance

In their pioneering studies Chase and Simon (1973) replicated the superior memory for chess positions by chess experts found previously by Djakow et al. (1927) and de Groot (1946/1978). Chess players ranging from a beginner to an international master were shown a position from an actual chess game (such as the one illustrated in **Figure 1(a)**) for a brief time (normally 5 s) and then asked to recall the locations of all the chess pieces. The ability to recall increased as a function of chess skill. Beginners at chess were able to recall the correct location of about four pieces, whereas international-level players recalled virtually all of the more than 20 pieces.

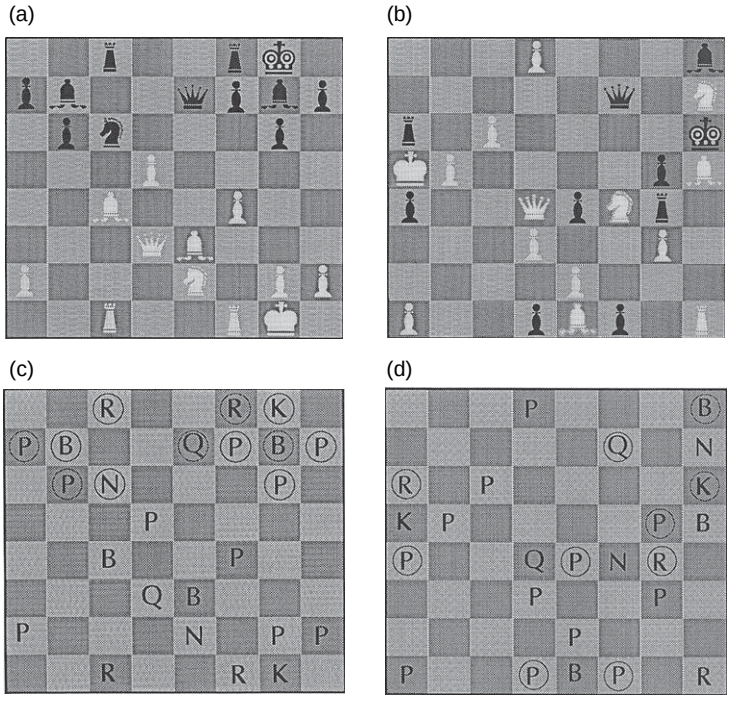

Figure 1 Standard diagrams of an actual chess position (a) and a chessboard with randomly arranged pieces (b). Nonstandard representations of the same information using the first letter of the names of pieces are shown in (c) and (d).

To rule out that the superior memory of chess experts reflects a general superior ability to store any kind of visual information, Chase and Simon (1973) had chess players recall chessboards with randomly placed pieces (as illustrated in **Figure 1(b)**). With briefly presented random chessboards, players at all levels of skill had a similar poor recall performance and were able to recall the correct location of only about four pieces – a performance comparable with that of chess beginners for actual positions from chess games. Further, Chase and Simon (1973) showed that when an actual chess position was shown using an unfamiliar notation (see **Figure 1(c)** for an encoding of the meaningful positions and **Figure 1(d)** for the random positions), the chess expert was able to display a similar level of superior memory performance after a brief period of adjustment. These results imply that the superior memory of chess experts is not photographic and depends on seeing arrangements of chess pieces that can be encoded using associations to the experts' extensive knowledge of chess. Since Chase and Simon's classic study, investigators have replicated these findings for chess and found a slight yet reliable advantage of chess experts to recall more chess pieces even from random chess configurations (Gobet and Charness, 2006). Numerous other studies have also shown that level of expertise is related to superior memory performance for representative stimuli in the associated domain, such as computer programming, basketball, and dance, and that this superiority is mediated by increased knowledge and domain-specific skills (for a review, see Ericsson et al., 2000).

Unless one has the knowledge of the expert, it is difficult – indeed, impossible – to grasp the meaningful relations between chess pieces perceived by the expert in **Figure 1**(a) and **1(c)**. If, on the other hand, the availability of knowledge providing meaning to a stimulus is critical to superior memory, it should be possible to demonstrate the same effect in a domain where all adults are proficient, such as language. Human adults are able to recall verbatim meaningful sentences of 20 or more words after a brief presentation (Chase and Ericsson, 1982). An example of such a sentence would be: The woman in front of him was eating peanuts that smelled so good that he could barely contain his hunger. If the words of the sentence are randomly rearranged (analogous to Chase and Simon's procedure for generating random chessboards), accurate verbatim recall drops to around six words. An example of a random rearrangement of the preceding sentence would be: Was

smelled front that his the peanuts he good hunger eating barely woman of so in could that him contain. For random lists of words, the recall of subjects is limited by the small number of words they can keep rehearsing, and once they stop rehearsal, the words are quickly forgotten. In contrast, once meaningful sentences are understood, their meaning is well retained in long-term memory. For example, during normal comprehension of a text the essential information in each sentence is efficiently stored in memory so it can be integrated with related information presented later in the text (Ericsson and Kintsch, 1995).

Stimuli from an unfamiliar domain of expertise, such as diagrams of chess positions and medical diagnoses, are often about as meaningless to most adults as random lists of words and digits. Studies described how people with exceptional memory for meaningless information actively sought out meaningful associations for the meaningless material, such as Subject S's encoding of the words of the Italian poem and remembering digits by noticing familiar dates, like 1945 – the year of the end of World War II. Individuals exhibiting truly exceptional memory performance for numbers, names, and pictures have been found to rely on some kind of mnemonics, relying on associations with previous knowledge, patterns, and acquired cognitive structures (Wilding and Valentine, 1997, 2006). There was always a question of whether individuals with exceptional memory were innately different and special or whether ordinary adults would be able to acquire exceptional memory through training.

2.42.5 Acquisition of Exceptional Memory through Practice and Training

The first study to trace the development of exceptional memory from average performance to the best memory performance in the world (in some memory tasks) was conducted in a training study by Chase and Ericsson (1981, 1982; Ericsson et al., 1980). We studied a college student (SF), whose initial immediate memory for rapidly presented digits was around 7, in correspondence with the typical average (Miller, 1956), but he eventually acquired exceptional performance for immediate memory and after 200 hours of practice was able to recall over 80 digits in the digit-span task. During this extended training period, we monitored any changes in SF's cognitive processes by having him give retrospective reports on his thought processes after most memory trials (this methodology is qualitatively different from the accounts given in interviews reported in the introduction of this article (cf. Ericsson and Simon, 1993). As his memory performance started to increase, he reported segmenting the presented lists into three-digit groups and whenever possible encoded them as running times for various races, because SF was an avid cross-country runner. For example, SF would encode 358 as a very fast mile time, 3 min and 58 s, just below the 4-min mile. The central question concerning any type of verbal reports is whether we can trust the validity of these reports and whether the ability to generate mnemonic running-time encodings influences memory.

To address that issue, we designed an experiment to test the effects of mnemonic encodings and presented SF with special types of lists of constrained digits. In addition to the list of random digits, we presented other lists that were constructed to contain only three-digit groups that could not be encoded as running times, such as 364 as 3 min and 64 s, in a list (364 895 481…). As predicted, his performance decreased reliably. In another experiment, we designed digit sequences where all three-digit groups could be encoded as running times (412 637 524 …) with a reliable increase in his associated performance. In over a dozen specially designed experiments, it was possible to validate several aspects of SF's acquired memory skill (Chase and Ericsson, 1981, 1982; Ericsson, 1985). A similar methodology was applied to two other college students, who both attained exceptional memory after 50–200 h of training. In fact, one of the participants reached a digit span of over 100 digits (Richman et al., 1995). Other investigators, such as Wenger and Payne (1995), have also relied on protocol analysis and other process tracing data to assess the mechanisms of individuals who increased their memory performance dramatically with practice on a list learning task.

More generally, this method has been extended to any individual with exceptional memory performance. During the first step, the exceptional individuals are given memory tasks where they could exhibit their exceptional performance while giving concurrent and/or retrospective verbal reports. These reports are then analyzed to identify the mediating encoding and retrieval mechanisms of each exceptional individual. The validity of these accounts is then evaluated experimentally by presenting each individual with specially designed memory tasks that would predictably reduce that individuals' memory performance in a decisive

manner (Ericsson, 1985; Wilding and Valentine, 1997). With this methodology, verbal reported mechanisms of superior performance have been validated with designed experiments in a wide range of domains, such as a waiter's superior memory for dinner orders (Ericsson and Polson, 1988), mental calculators (Chase and Ericsson, 1982), and other individuals with exceptional memory performance (Ericsson et al., 2004; Thompson et al., 1993). Several studies even demonstrated impressive memory improvements in large samples of participants after extended practice with instructions to use mnemonic encodings (Kliegl et al., 1987; Kliegl et al., 1989; Higbee, 1997).

2.42.6 Superior Memory of Experts and Their Superior Performance on Representative Tasks

Memory experts have been found to improve their memory performance by acquiring mnemonic techniques through extended practice. In contrast, chess experts and medical doctors attain superior memory for representative stimuli from the domain without training their memory deliberately. The primary goal for all experts is to excel at the representative tasks in their particular domain of expertise. For example, chess experts need to find the best moves to win chess matches, and medical experts have to diagnose sick patients to give them the best treatment. Their superior memory ability must thus be an indirect consequence of their improved performance on representative tasks (Vicente and Wang, 1998). Furthermore, experts appear to store task-relevant information in memory when they normally perform representative tasks in their domain, because if they are unexpectedly asked to recall information about a performed task, their memory is typically much superior to that of less-skilled individuals. In fact, experts' incidental memory of the relevant information is frequently so good that instructing them to intentionally memorize the information does not reliably improve their memory. For example, when chess experts analyze a position to find the best move, their memory of the position is just as good whether they were informed about an upcoming memory test or not. As part of performing the representative task of selecting the best move, the experts encode the important features of the presented information and store them in accessible form in memory. In contrast, when subjects, after training based on mnemonics and knowledge unrelated to chess, attain a recall performance comparable with that of the chess experts, they still lack the ability to extract the information important for selecting the best move. Hence, the remarkable characteristic of expert memory is not just the amount recalled, which can often be matched by training, but the rapid extraction and storage of important patterns and relevant information that allows the experts to perform better the representative task, such as selecting moves in chess (Ericsson, 2006a,b).

An analysis of expert performance shows that it is not sufficient to have merely stored the knowledge in memory; it is also critical that relevant knowledge is well organized and can be efficiently retrieved when it is relevant to the ongoing processes. In fact, the principal challenge of expertise is to acquire and organize the vast body of domain knowledge (Chi et al., 1981; Chi, 2006) such that all relevant prior knowledge can be immediately accessed to guide action in encountered situations. For example, with the superior organization of knowledge, a chess expert can rapidly perceive a promising move, or a medical expert can rapidly notice an inconsistency in a suggested diagnosis.

Efficient and reliable storage of relevant information in memory is especially important to experts when they engage in planning and complex reasoning that mediate their superior performance. During planning, experts have to mentally compare many alternative sequences of actions, which produces a great deal of information in working memory. Consequently, beginning chess players do not generate long plans, and it takes years of chess study before chess experts are able to plan long sequences of future moves reliably (Charness, 1989; Gobet and Charness, 2006). Chess masters eventually improve their memory skills for planning so much that they are even able to play chess without seeing the chessboard (blindfold chess), thus having to represent the locations of all the pieces on the board during the entire game in their mind. Analyses of the superior ability to plan suggest that experts acquire memory skills, which allow them to rely on long-term memory for storage of generated information (Ericsson and Kintsch, 1995). Recent research on expertise is making it increasingly clear that the vast knowledge of experts has to be well organized and supplemented with special memory skills so as to support memory-demanding planning, design, and reasoning.

Recent research has revealed the complex and intricate structure of expert performance and its associated memory skills. These skills are not attained

automatically with experience but require the engagement in deliberate practice, typically designed by teachers. Even the most talented individuals have spent around 10 years of intense preparation before attaining an international level of performance in many domains, such as sports, chess, and arts (Ericsson, 2006b).

2.42.7 Research on Brain Structure and Activation Associated with Superior Memory Performance

Do the brains of people with exceptional memory differ in their anatomical features or their pattern of activation while exhibiting their superior memory performance? It is difficult to determine whether the brain of a single individual with exceptional memory differs from those of other people with similar gender, age, education, and ethnic background. Differences in the anatomical structure of brains exhibiting exceptional memory compared to a control sample have been found for taxi drivers in London, who have spent several years before they have successfully memorized the map of London with its massive number of streets, hotels, and significant landmarks (Maguire et al., 2003a). Most interestingly, the differences in the taxi drivers' hippocampus appear to be a consequence of the extended initial memorization of the London map as well as the daily work as a cab driver, rather than any innate differences.

More recently, Maguire et al. (2003b) examined the brains of ten of the world's foremost memory performers and compared them to control subjects with matched spatial ability and intelligence. They found no systematic anatomical differences between the two groups. This study also recorded the brain activity (functional magnetic resonance imaging) of both groups of participants while they were engaged in memorizing different types of stimuli for which the memory expert either exhibited clearly superior memory, namely three-digit numbers; intermediate superiority, namely faces; or no superior memory, namely snow crystals. After completing the memory tests, the participants gave detailed descriptions of their encoding strategies during the memorization. All of the memory performers reported using previously acquired techniques for generating associations, such as mnemonics, to make the presented information more memorable. All but one of the memory performers reported using the method of loci. In sharp contrast, none of the control group reported using any of the standard mnemonic techniques. These reported differences in strategies were sufficient to explain the regional differences in brain activation observed during memorization.

Recent brain-imaging studies of exceptional performers show that they activate brain regions that are different from those activated by control subjects. Consistent with accounts of memory experts, their differential brain activation is consistent with cognitive processes reflecting acquired memory skill. For example, exceptional mental calculators rely on storage in long-term memory (Presenti et al., 2001), and expert mental abacus calculators encode numbers in a manner qualitatively different from that of controls (Tanaka et al., 2002).

2.42.8 Conclusion and Future Directions

The emerging research on exceptional memory does not support the traditional views that only some uniquely gifted individuals endowed with an innately different memory system can attain exceptionally superior memory performance for particular types of information. Instead, the accumulated evidence supports the plasticity of the memory system in response to practice. The evidence supports the potential for ordinary healthy individuals to improve their memory performance with appropriate strategies and practice. However, future research is required to understand the specific processes of physiological adaptation of the brain and detailed modification of skills that occurs during extended skill acquisition. This research will need to combine cognitive and brain-imaging methods to study the process of skill acquisition and the changes required to reach exceptional levels of memory performance.

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2.43 Cognition, Memory, and Education

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Our focus in this chapter rests on the observation that much learning in the classroom consists of the acquisition of factual information. Consider college level biology. Students have to know the names and functions of typical animal and plant cell organelles, the mechanism by which plants convert light energy to chemical energy, and the way genetic information flows from DNA to RNA to protein. As well, in introductory geology, students must master knowledge of multiple characteristics of types of rocks; in political science, students need to thoroughly learn a number of characteristics associated with each of a few types of political systems; and in developmental psychology, students must learn the attributes of a variety of theories of development (Pressley et al., 1988).

To enhance educational effectiveness, current trends in educational psychology are focusing on classroom methods that stimulate active engagement for students (e.g., clicker systems), engender collaborative problem solving (see Wieman, 2004), and create inquiry-based environments. These are exciting and worthwhile advances. However, it remains that students are required to commit to memory tremendous amounts of information, and many struggle with this challenge. For the most part, after engaging, inquiry-based classroom activities, educators leave the student to figure out how to remember the material for the ubiquitous examination. Students can flounder at this point. Indeed, some instructors in large college introductory biology classes find that students feel that biology is a difficult and complicated subject precisely because it requires so much memorization (discussed at the Biology Leadership Conference, March, 2006).

The premise of this chapter is that the science of learning and memory offers rich and extensive literatures that provide a foundation to assist students in the challenge of remembering the vast amounts of information they are required to learn. We highlight principles from basic memory and text comprehension research that potentially carry significant implications for improving student learning. From each principle, we identify techniques and approaches that could be implemented to assist students' acquisition of material in fact-laden courses. We first examine rereading, which is a common study method used by students. The chapter then discusses various techniques to engage the learner and improve retention of the material. With basic

research on elaborative processing as a springboard, we describe and evaluate educationally realistic approaches to stimulate effective elaboration, including elaborative interrogation, advanced organizers, and imagery mnemonics.

We next discuss the concept of desirable difficulty, which involves injecting difficulty into the learning environment to stimulate effective processing on the part of the learner. We review evidence that supports the desirability of difficulty but also cautions that all difficulty is not desirable. From this work we develop theoretical guidelines for determining what kinds of difficulty are indeed desirable. Finally, we address the issues of comprehension ability and individual differences (such as the amount of prior knowledge a reader possesses) and how these factors interact with the basic learning techniques.

Throughout the chapter we highlight both the principles that potentially carry significant implications for improving student learning and the techniques that could be implemented to assist students. This cannot be an exhaustive review. Accordingly, we focus on principles and techniques for which there is translational research using educational materials so that we can evaluate the effectiveness of those principles and techniques for educational practice (cf. Naveh-Benjamin, 1990). First, however, based on theoretical and empirical work we examine the soundness of a study activity that appears to be predominant among students.

2.43.1 Is Rereading an Effective Way to Learn?

Many students report that their typical study activities involve rereading text (Goetz and Palmer, 1991; Carrier, 2003) and lecture notes (see Sikorski et al., 2002; Karpicke et al., unpublished data), and rereading is advocated as a study method by researchers (Howe and Singer, 1975; Mayer, 1983; Amlund et al., 1986; Barnett and Seefeldt, 1989; Krug et al., 1990;). Based on some theoretical approaches to text comprehension, rereading would be expected to increase learning and memory for the read material. The basic premise is that different processing occurs during a second reading than the first (Millis et al., 1998; Stine-Morrow et al., 2004).

More specifically, the initial reading is presumably used to establish the surface (lexical and syntactic features) and textbase (network of propositions) levels of representation, and the second reading is used to construct a situation model (a representation integrating text content, relevant knowledge, inferences, summary statements, casual relations) of what the text is about (Millis et al., 1998). The general idea is that a limited pool of resources is available while reading, and during the initial reading, those resources are allocated to lower level processes such as word recognition, extraction of propositions, and establishing coherence among propositions. The second time the text is read resources are freed and able to be allocated to higher level (situation model) processing (see Millis et al., 1998; Stine-Morrow et al., 2004 for findings supporting this theory).

This theory is not without dispute, however. Dunlosky and Rawson (2005) measured resource allocation to particular levels of representation, using word frequency (corresponding to the surface level), the number of propositions and new arguments nouns (corresponding to the textbase) and sentence importance (corresponding to the situation model) to predict reading times. Based on Millis et al. (1998), reading times should increase for the situation model measures but not for the surface level measures on a second reading. Dunlosky and Rawson, though, found that the regression coefficients for the sentence importance (situation model) predictor actually decreased for the second reading.

Additionally, researchers have found that a situation model changes very minimally with additional processing time (labeled a rigid situation model). Along these lines, Mannes (1994) found that when the same text is read multiple times, the same inferences are made, and the same connections between topics are formed. In a similar vein, some theorists believe that most readers adopt a lazy approach to constructing a representation from read material, avoiding processing that is not straightforwardly afforded by the text (e.g., see Fletcher and Bloom, 1988). Consequently, improvements in learning from texts may require more than rereading: additional presentations of the material may need to force the student to generate information not ordinarily extracted from reading alone. We examine this possibility in detail in a subsequent section (titled 'Generation') of the chapter.

The educational psychology literature reflects the cloudiness of the theoretical positions on this issue. Generally, the conclusion is that while it is better to use methods that engage the reader more than simply a second reading of the text, rereading can improve performance on tests of comprehension and memory

for the text. This conclusion, however, should be accepted cautiously, as not all of the evidence is supportive of this conclusion.

Some research has found that rereading improves performance on summative assessments. For instance, Amlund et al. (1985) found a benefit of rereading a text on cued and free recall tests over main ideas and details. Participants read a text either one, two, or three times (a between-subject factor) and took an immediate test over the text, as detailed above. The immediate test revealed that reading twice improved performance as compared to reading once for both main ideas and details (as evidenced by both criterial tests). Likewise, Durgonoglu et al. (1993) investigated rereading of fables and found that when answering text-based comprehension questions, reading the text twice in a spaced manner produced better performance than reading once or reading the text twice in succession. However, not all the results support the idea that rereading improves performance on comprehension tests, as there was no difference between the read-twice groups and the read-once group on questions requiring inferencing and integrating background knowledge with the text to answer the question. Further, Rawson and Kintsch (2005) showed in two experiments that massed rereading improves performance on an immediate test and spaced rereading improves performance on a delayed test. Interestingly, there was no difference between spaced and once-read on the immediate test, and no difference between massed and once-read on the delayed test.

More surprising results were reported by Callender and McDaniel (2007a). In a series of four experiments, five different texts were read and reread by participants and multiple-choice and short-answer questions were answered (or in one experiment, a summary was generated). Only one text showed a small benefit of rereading on multiple-choice questions, a benefit that was not replicated in other experiments of the study.

One difference between Callender and McDaniel (2007a) and the studies reviewed above is the texts used in the studies. Callender and McDaniel attempted to increase the educational relevance of the study by using textbook chapters and assessments like those used by educators. Previous studies have been more limited in their applicability to educational settings, particularly with respect to three aspects of the research: the assessment/criterial test used, text characteristics, and learning instructions.

With respect to the assessment or criterial test used, educational assessments use multiple-choice or short-answer assessments (Bol and Strage, 1996; Jackson, 2005) rather than free recall (Mayer, 1983; Amlund et al., 1986; Krug et al., 1990). Additionally, educational assessments focus on assessing gist-based representations rather than verbatim recall of text (Bol and Strage, 1996) as tested in the laboratory using a cloze test (filling in missing words from a text; Rothkopf, 1968) or verifying if a particular sentence was in the original text (Howe and Singer, 1975; Mayer, 1983; Haenggi and Perfetti, 1992). Educational texts tend to be longer than texts used in the laboratory (99 words [Glover and Corkill, 1987] to 1770 words [Rawson and Kintsch, 2005], with most texts much shorter than 1770 words [see Howe and Singer, 1975; Mayer, 1983; Amlund et al., 1986]). Finally, learning in educational settings is usually intentional rather than incidental (Mayer, 1983; Amlund et al., 1986; Durgunoglu et al., 1993). Comprehension processes could differ when the reader has knowledge of test and study method as opposed to when the reader is uninformed.

A simple conclusion from this research is that rereading has not been established as a beneficial study method in educational settings. Considering that rereading is time intensive and could provide little benefit (Callender and McDaniel, 2007a), there may be superior methods for students to use in educational settings.

2.43.2 Improving Learning and Retention in Education: Elaborative Processing

Memory research has established that elaborative rehearsal produces learning and retention. Elaborative rehearsal is an umbrella term that encompasses a variety of processes including verbal elaboration (Craik and Tulving, 1975), organization (e.g., Bower, 1972; Bellezza et al., 1977), and visual imagery (McDaniel and Pressley, 1987). The following sections will focus on each of these processes in terms of their potential educational applications. Again, by necessity this is a selective presentation that emphasizes reasonably direct translation of basic memory findings to learning with educationally relevant materials and content.

2.43.2.1 Verbal Elaboration

A long-standing issue in the literature on verbal elaboration concerns the characteristics of elaboration that facilitate learning. In this regard, one line of work that seems especially pertinent for education stems from the observation that many facts seem arbitrary to students, because students do not have the expert knowledge base to understand the significance of the facts (Bransford et al., 1982). To mimic this situation in the laboratory, Stein et al. (1978; Stein and Bransford, 1979) required subjects to study a set of sentences that paired certain identifying attributes to specific actions:

1. The diamond was too expensive for the slow man.
2. The child was comforted by the short man.

To increase memory for the association between a particular man and a particular action, one general approach could be to stimulate elaboration by expanding the target information in a semantically congruous fashion (cf. Craik and Tulving, 1975) as follows:

1. The diamond was too expensive for the slow man to hand down to his son.
2. The child was comforted by the short man who sat around a lot.

However, these semantically congruous elaborations did not improve memory for the associations presented in the base sentences (33% recall with semantic elaborations versus 49% recall with base sentences). Instead, Stein et al. (1978) demonstrated that elaboration of these arbitrary associations was effective when it reduced the arbitrary nature of target information – when it precisely specified the nature of the target relations. Specifically, the elaborations below, which specified the significance of the relations in the sentences, significantly improved memory for the 'man' sentences to 69% correct (see Bradshaw and Anderson, 1982, for similar findings with real-world target facts).

1. The diamond was too expensive for the slow man who was fired from his job.
2. The child was comforted by the short man who looked the child in the eye.

In relation to education, consider that many of the facts presented to students may appear as arbitrary as the base 'man' sentences were to subjects in the laboratory experiments. For example, in a biology class in primary school, students may be required to learn that veins have thin walls and arteries have thick, elastic walls, or that plant cells have mitochondria. At a conference on improving biology education, one of us recently overheard a biology instructor lament, "We beat it over students' heads, 'plant cells have mitochondria.' Why can't they learn?". By the present analysis, it is because these relations are essentially arbitrary to the student. Accordingly, a key implication from the basic memory literature is that to enhance learning in educational settings, students should be encouraged to engage in elaborative processing that reduces the arbitrary nature of the target material. One avenue would be for instructional material or the teacher to provide elaborations for the student. For instance a teacher may elaborate that arteries are elastic to withstand the spurts of blood pumped by the heart and to act as a kind of valve to keep blood from flowing backwards. In many settings, however, from grade school social studies to a college-level course, students may have to generate their own elaborations for much of the content because the books and teachers do not do so. Basic memory work suggests at least one technique for prompting students to generate elaborations that reduce the arbitrary nature of target content, a technique termed elaborative interrogation (Pressley et al., 1988).

Using laboratory materials, Pressley et al. (1987b) prompted learners to generate elaborations that would reduce the arbitrary nature of the association by asking learners to explain why the association might be exist. In one experiment, subjects were presented with the 'man' sentences, and for each sentence subjects were required to provide an elaboration that would answer the question 'Why did that particular man do that?'. The group that generated answers to the why questions remembered significantly more of the man sentences than did a group receiving the base sentences and even a group that was provided with precise elaborations. In a subsequent experiment, Pressley et al. (1988) applied the elaborative interrogation technique to a set of Canadian civics facts that students would be expected to learn. Students who attempted to answer why a particular fact might be true (an example target fact is 'Apples were first cultivated in Nova Scotia') showed over a 50% increase in retention of those facts relative to students who read the facts (with study time kept constant across the conditions).

The elaborative interrogation technique has been extended to more complex materials and contrasted against more traditional study techniques. McDaniel

and Donnelly (1996, Experiment 2) presented students with short didactic passages culled from high school and college textbooks that presented astrophysics concepts such as the conservation of angular momentum. In one experimental condition, each short text was followed by a why question about the concept (Why does an object speed up as its radius gets smaller (as in conservation of angular momentum)?). This condition produced significant improvements on multiple-choice assessments focused on factual information presented in the text and on the ability to draw inferences from the presented information. By contrast, conditions in which (a) the texts highlighted key words, (b) a labeled schematic was provided to display how the key concepts in each text were related, and (c) subjects were required to generate their own schematic did not improve performance on the multiple-choice assessments relative to the basic text condition (in all cases, performance was nominally lower relative to the basic text condition).

Elaborative interrogation has also been applied to connected prose. Seifert (1993) hypothesized that elaborative interrogation may not be as effective with prose as with factual statements because once the facts are embedded in paragraphs, the context provides meaning. The arbitrariness of the facts is removed by the surrounding information. Elaborative interrogation, however, did improve memory for facts in the text, although the effect size was smaller than when elaborative interrogation was used with lists of facts. Similarly, Ozgungor and Guthrie (2004) found a benefit of elaborative interrogation on recall and the ability to generate inferences about the text. This finding is not universal, as Boudreau et al. (1999) found no benefit of elaborative interrogation over self-selected strategies or underlining on memory for prose.

It is possible that the benefits of elaborative interrogation (and other elaboration techniques) are most prominent for individuals with lower learning and reading abilities. Along these lines, Callender and McDaniel (2007b) found that elaborative interrogation provided no benefit for high ability comprehenders (as measured with the Multimedia Comprehension Battery; Gernsbacher and Varner, 1988) when answering questions about a text they had studied, but it did prove helpful for low ability comprehenders when answering test questions that targeted information specific to the 'why' questions. Successful learners may spontaneously produce effective elaborations when studying and therefore need less guidance to promote elaboration (i.e., generating why) than less successful learners.

2.43.2.2 Organization

An understanding of the organization of a text is also important in both comprehension processes and later retention of the text (Caverly et al., 2000). When a reader constructs a good mental representation of the organizational structure of the text, the reader is able to determine what information is essential to understanding the text, and what information is irrelevant (see Gernsbacher, 1990). This is particularly true when the text does not encourage the reader to process the organization of the individual propositions within the text (Einstein et al., 1990).

Two methods have been used to aid readers in organizing and identifying the macrostructure of the text: outlining and advance organizers. Outlining is the traditional task of creating a structure (usually hierarchical) out of the text, and advance organizers are generally short paragraphs or diagrams that introduce the concepts in the text to be read. Both of these methods provide a schema into which the text can be integrated.

The rationale for using outlining (and organization in general) stems from basic work on word list recall. When seemingly unrelated words were presented and participants were forced to organize the words into categories (e.g., things that are green, liquids, and things made of wood; Einstein and Hunt, 1980), recall of the list was higher than when no organizational strategy was used (see Mandler, 1967, for similar findings). Thus, it is apparent that forcing students to organize information may lead to better recall. While categorizing words may not be used in educational settings, outlining text is a commonly used method.

In a series of experiments, Einstein et al. (1990) showed that outlining improved readers' recall of expository texts. Further, similar tasks that encouraged relational processing of the text significantly improved recall a week later over a read-only control. Outlining, however, may not always result in improved performance. Some assert that outlining can improve performance on criterial tests only if the reader is taught how to outline appropriately (Caverly et al., 2000). Additionally, outlining does not automatically prompt a reader to integrate the text with prior knowledge, which is often necessary for improved performance.

One way to encourage connecting the text with prior knowledge, and possibly to provide knowledge when none is possessed, is using an advance organizer (Mayer, 1987). Ausubel (1963) first developed the advance organizer as a way to provide information and orient the reader to the contents of the text (Kirby and Cantwell, 1985). The organizer serves to encourage integration of new information in the text with the reader's existing schema. For those who have no schema in place, the advance organizer provides a schema (Mayer, 1978; Tyler et al., 1983).

One view of the advance organizer is that while it provides the reader with the important information in the text, it presents it in a more abstract and general way than, for example, a summary would (Tyler et al., 1983). Others suggest that advance organizers are more effective when they provide a concrete model of the information in the text. This type of organizer does not present additional information or add anything substantive to the text, but does provide a way for the reader to organize the text. In this way, it is thought that advance organizers work as though they are just an additional presentation of the text (Mayer, 1987).

In two experiments, Mayer (1983) showed the benefits of using advance organizers with unfamiliar text. Mayer provided participants with a diagram that explained the key principles in the text, as well as a way to organize them, and allowed the students to study the diagram for 60 s before listening to the passage. The advance organizer increased overall recall of the text and improved performance on tasks that required application of the concepts (but not for tasks that required verbatim memory of the text; see **Figure 1**).

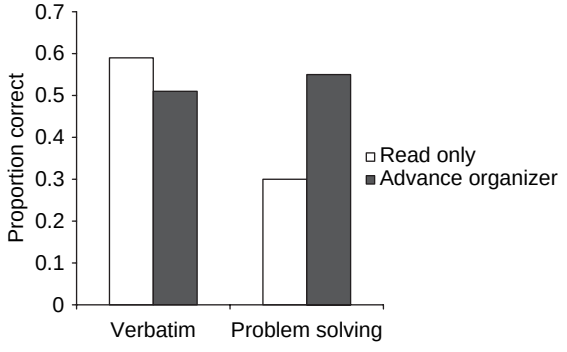

Figure 1 Effects of advanced organizer's verbatim memory and problem solving in Experiment 1 from Mayer RE (1983) Can you repeat that? Qualitative effects of repetition and advance organizers on learning from science prose. *J. Educat. Psychol.* 75: 40–49. Reprinted with permission.

One important aspect of organizational methods is that individual differences greatly affect performance. The Structure Building Framework (Gernsbacher, 1990) is based on the differing abilities of readers to form a hierarchical structure out of the text and to construct substructures of information related to the central information in the text. Those who have difficulty determining the organizational structure of the text when reading develop a poor mental representation of the text, which adversely affects both immediate comprehension of the text and later recall of it.

Outlining is particularly sensitive to individual differences. When outlining is taught appropriately, it improves performance of low- and average-ability readers, but adversely affects the spontaneous processing of high ability readers. When not taught, outlining only benefits high-ability readers and does not affect performance of low- or average-ability readers (Caverly et al., 2000).

Effectiveness of advance organizers is also moderated by individual differences, as they are most effective when the reader lacks prior knowledge for the content of the text (Mayer, 2003). Other organizational methods may also be sensitive to individual differences. Concept maps, or graphical representations of the text that represent both content and structure of the text, are generally more effective with low-ability readers than high-ability readers (Nesbit and Adesope, 2006). However, a recent experiment conducted by the authors (in collaboration with Kathy Wildman) showed that when low- and high-ability readers were asked to complete partially constructed concept maps after every two pages of a lengthy text, completing the concept maps improved recall for low- and high-ability readers. A good understanding of the organizational structure of the text and ways to integrate this structure with existing knowledge are both key components to understanding and remembering text. Outlining and advance organizers are two simple and effective ways of accomplishing this task.

2.43.2.3 Imagery

A plethora of basic memory work has demonstrated that visual elaboration (visual imagery; pictorial presentations) of the referents of target material improves memory performance (see McDaniel and Pressley, 1987; Paivio, 1986, for books on the topic). For educational content, if the referent is concrete enough to generate visual imagery, then instructing

learners to form visual images can produce significant gains in retention relative to a condition in which learners are simply presented the facts to learn. For instance, for learning Canadian civics facts, subjects instructed to use visual imagery recalled as many facts as those in an elaborative interrogation elaboration condition and more facts than those in the control condition (Pressley et al., 1988). Educational material with an associative component can also be depicted pictorially with potent benefits for learning (see Levin, 1985, for a review).

In the basic literature, imagery has been integrated as a key component into successful mnemonic devices, with one of these devices in particular – the keyword method – having direct application to education. The keyword method has been most touted as a method to assist in learning the meanings of vocabulary items, either in one's primary language or in second language learning. In the keyword method, a familiar word embedded in, or sounding like, the vocabulary item is identified as the keyword, and then a visual image is constructed of the referents of the keyword and the vocabulary item interacting. For example, to learn that the Spanish word *carta* means *letter*, a familiar sound alike English word is identified such as cart (one could also use art or car) and an interactive image of a cart and a letter is constructed (such as a cart carrying a giant letter). On a subsequent test for the meaning of *carta*, the learner identifies the keyword and reconstructs the image, from which the meaning letter can be extracted. A wealth of evidence confirms that the keyword method produces better retention of new vocabulary meaning than does a control in which learners are given equivalent amounts of study time to learn the vocabulary items (but without instruction in any particular study method) or in conditions in which the students are given semantic-based (enriched context) strategies (McDaniel and Pressley, 1984; Pressley et al., 1987a). This pattern holds for college students learning foreign vocabulary meaning (Raugh and Atkinson, 1975), unfamiliar English vocabulary items (McDaniel and Pressley 1984, 1989), and technical terms in a college psychology course (Balch, 2005). The pattern is also found with children learning new vocabulary (Levin et al., 1982) and when the keyword strategy is implemented with mature learners in in-class settings (Pressley et al., 1982).

The keyword method is also effective for acquisition of other associative factual content that students must learn. For instance, some elementary school social studies curricula require children to learn the states and their capitals. Levin et al. (1980) examined learning this material using a keyword method in which pictures were presented to link the keywords, instead of mental imagery. For example, to learn that Annapolis is the capital of Maryland, elementary school children were presented with a picture of two apples (keyword for Annapolis) getting married (keyword for Maryland) (see **Figure 2**). This method was compared against a control in which children studied the state–capital pairings on their own. On an initial test learning was better with keyword instruction (78%) than self-study (66%), and impressively 3 days later a retest showed 96% retention of the items learned with the keyword method and only 51% retention of the items (of which there were fewer) learned through self-study. Moreover, the students indicated the keyword materials made the work more fun and they actively requested that the experimenter leave the materials in the classroom once the study was concluded. There are excellent reviews of the experimental work that detail the range and robustness of the benefits of the keyword learning method (e.g., Pressley and Woloshyn, 1995, see also Pressley et al., 1987). These uniformly positive results raise the issue of why the keyword technique has seemingly enjoyed limited penetration into educational contexts. We will conclude the present section by addressing this issue.

The criticism raised against the keyword method by some educators is that it is an artificial way to learn vocabulary that produces at least two possible

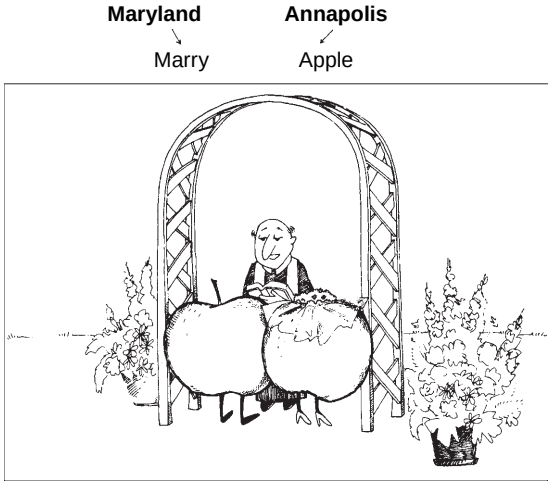

Figure 2 Sample illustration to support keyword method of learning states and their capitals (from Levin JR, Shriberg LK, Miller GE, McCormick CB, and Levin BB (1980) The keyword method in the classroom: How to remember the states and their capitals. *The Elementary School Journal* 80: 185–191.

negative consequences (a) The keyword method creates an artificial link that disrupts speed of translation and therefore slows comprehension. A particularly strong objection is that such interference in fluency perhaps could persist throughout the learner's usage of the target vocabulary. (b) It does not support acquisition of the nuances in which a vocabulary item can be used in a language, especially relative to alternative techniques of learning definitions from embedding target vocabulary in semantic contexts. However, evidence relevant to these concerns, though currently limited, does not support the above claims.

To provide initial data regarding the first concern, which is an interesting basic issue in its own right, Crutcher and Ericsson (2000) required subjects to learn 45 Spanish–English translations to 100% accuracy using the keyword method. Subjects were then timed on the speed with which they could produce the English translation given the Spanish word, and English translation given the keyword. The time to generate the English translation was significantly longer when given the Spanish word than when given the keyword, implying that when given the Spanish word subjects were retrieving two consecutive items: the keyword followed by the English translation. That is, the keyword method appeared to produce an intermediate (artificial) retrieval step. However, after extended practice at directly retrieving the English meaning, the extra retrieval step was eliminated as evidenced by (a) faster times to produce the English word when given the Spanish word than when given the keyword and (b) self-reports that before practice 80% of the learners used the keyword to mediate retrieval but after the practice period less than 15% mentioned the use of any mediator. The implication here is that if the learner continues to use the vocabulary word, the keyword mediator will be eventually eliminated from the retrieval link.

To more directly contrast the consequences of vocabulary usage following keyword versus semantic-context methods of vocabulary acquisition, McDaniel and Pressley (1989) compared the keyword condition with a semantic context-learning condition in which subjects first read the usage of the vocabulary item (an unfamiliar English word) in a three-sentence passage, then tried to infer the meaning of the item, and afterwards were given the definition. Note that this semantic context condition had a number of theoretically positive features: learners could get a sense of how the word is used in language, they processed the semantic context to attempt to infer (generate) the word's meaning, and they received direct feedback on the accuracy of their inference (e.g., see Metcalfe and Kornell, 2007). Nevertheless, the mnemonic keyword method increased recall of the definition (when prompted with the word) by over 50% relative to levels achieved in the semantic context condition.

More importantly for the present purposes, after the vocabulary acquisition session, subjects were given a text to read in which 15 of the new target vocabulary items were used (none of these items had been tested in the definition recall task). Disfavoring the concern that keyword learning would slow comprehension, reading time for phrases containing the new vocabulary was equivalent for keyword and semantic context conditions. Also, the keyword method did not penalize the accuracy of comprehension: learners in the keyword condition scored slightly better on a true/false comprehension test for the material involving the new vocabulary than did learners in the semantic context condition. In another experiment with similar learning conditions, subjects produced sentences using the new vocabulary items with as much facility after keyword learning as after semantic context learning; indeed, more sentences were correctly produced after keyword learning because more meanings were retained (McDaniel and Pressley, 1984, Experiment 2).

To take stock, the experimental work shows that (a) the keyword method significantly reduces the time to reach a high degree of accuracy for meaning recall relative to self-study strategies (McDaniel et al., 1987); (b) the method does not penalize comprehension and production with the new vocabulary relative to typical semantic-context learning conditions (and may enhance comprehension because more meanings are remembered after keyword learning); and (c) learning via the keyword method appears not to foster permanent reliance on the keyword link to retrieve meaning. Further, the evidence suggests that children (students) like the method. Given these positive outcomes, it is noteworthy that vocabulary learning curricula in at least some schools would be quite compatible with a keyword approach. For instance, in a public school near the authors' university, the students are given a list of new vocabulary words weekly to study for definition learning, and after several weeks the students are tested on the meanings of the target vocabulary. Here the keyword method could be straightforwardly implemented to assist the students' learning.

Accordingly, educators' reservations concerning the keyword method may be misplaced. We suggest that this basic mnemonic technique could be profitably integrated into educational practice to assist learners with the burden of learning associative factual material required in many curricula.

2.43.3 The Paradox of Difficulty: Its Desirability for Learning and Retention

Students likely prefer instruction in which acquisition seems rapid and learning is easy. Similarly, instructors may evaluate their effectiveness based on the extent to which students are able to quickly demonstrate understanding of the material (and students may evaluate such instruction very positively). For instance, students and instructors may favor massed presentation of content over distributed presentation because massed presentation typically yields clear and immediate gains in performance relative to distributed practice (see Bjork, 1994). This bias in massing practice, again perhaps because it seems to foster easy, rapid learning, can be seen in standard instructional materials in elementary schools. As an example, some workbooks for handwriting repeatedly present the identical letter (see **Figure 3**), resulting in blocked practice (Ste-Marie et al., 2004).

In contrast to these practices, the experimental memory literature has established that distributed presentation is superior to massed presentation for long-term performance or recall. Indeed, a range of basic memory findings suggests that introducing difficulty or challenge into the learning environment can promote retention. Basic work reflecting this pattern includes mnemonic benefits of creating interference in the learning environment with concurrent presentation of distracting information (Battig, 1972; Einstein, 1976; Shea and Morgan, 1979), spacing of content (rather than massed presentations; see Cepada et al., 2006, for a review), and generation of material (relative to reading material; Slamecka and Graf, 1978; McDaniel et al., 1988; McNamara and Healy, 1995).

With regard to educational implications, Bjork (1994) has synthesized these findings into the general notion that instructional effectiveness is significantly improved through desirable difficulties – arrangement of instruction and study activities that create difficulty for the learner. These difficulties, though

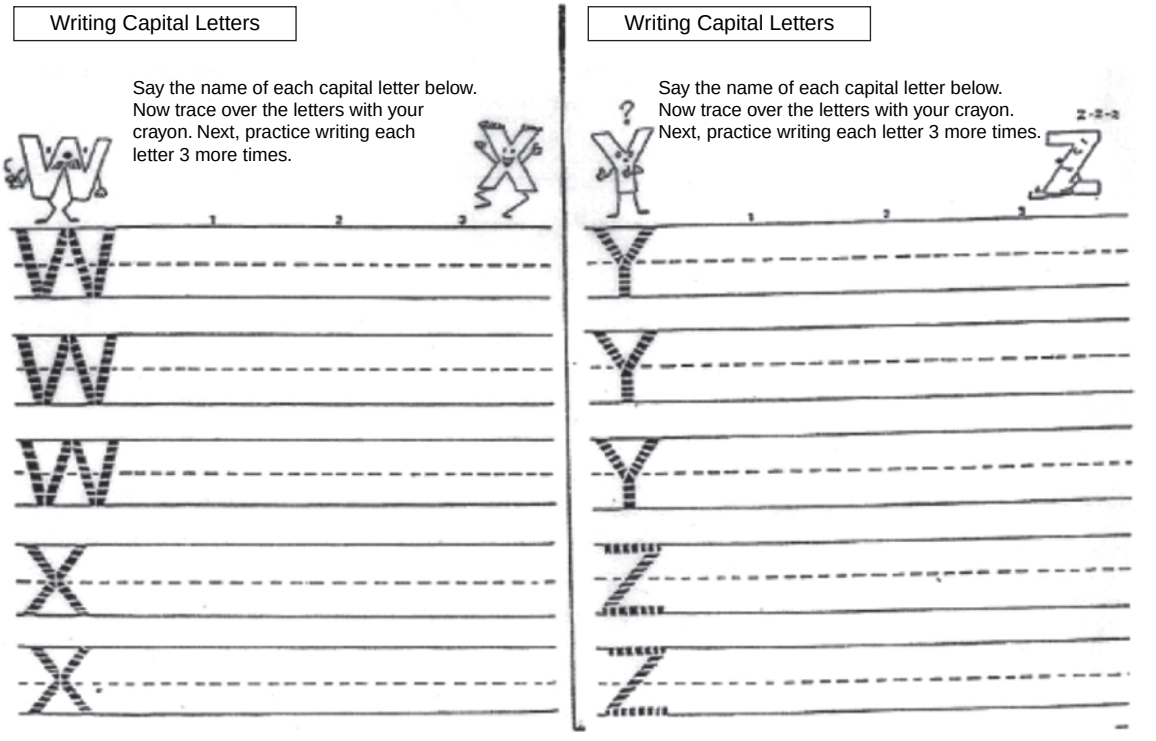

Figure 3 Example of handwriting workbook with massed practice. From *My Creative Preschool Workbook* by Preschool Press, New York, New York: Playmore Inc., Publishers and Waldman Publishing Corp.

slowing initial acquisition (but not preventing acquisition), are desirable in that they promote retention and transfer. The claim here is standard goals of education, such as fostering long-term retention of content, accessibility of material as contexts change, and transfer of knowledge to new situations, may be better achieved through creating difficulty for the learner – not by arranging instruction to make learning easy.

However, encouraging desirable difficulty in educational practice is a particularly provocative idea that runs counter to many instructors' intuitions and students' expectations. Accordingly, marshaling evidence for the benefits of desirable difficultly with educationally relevant content and material and developing a theoretical understanding of the kinds of difficulty that are desirable are critical steps for compelling its acceptance in educational application. To this end, we first present research examining manipulations of difficulty with educationally relevant material (see also Richland et al., 2007), and then we review theoretical work that provides a framework for specifying difficulty that will and will not be desirable.

2.43.3.1 Create Interference

Basic research with paired-associate lists (e.g., lists of consonant-vowel-consonant pairs such as *SIF-TOG* and *RAB-SOC*) has shown that arrangements of materials that produce interference, thereby slowing learning, can increase retention of the studied material (see Battig, 1972). Schneider et al. (2002) examined the general implication of this basic finding for learning English–French vocabulary. They varied the difficulty during training in two ways. First, the vocabulary items (and translations) were either blocked by category (five vehicles, five school items, five body parts, and so on) or mixed (one vehicle, one school item, one body part, etc.). The idea was that mixed presentation should create interference relative to blocked presentation. Second, translation direction was varied, with some learning conditions requiring that the French term be translated into English (French–English), and other conditions requiring the reverse (English–French). The latter condition is more difficult as the learner has to learn the response terms (the foreign vocabulary items) and the associative relation between the original and new language terms.

During the learning, subjects studied the vocabulary items several times and then took an immediate test to assess learning. Then one week later, subjects were given a retention test. The format of the retention test was varied such that some conditions were the same as during learning (intermixing/blocking of items and direction of translation) and some were reversed. The results were complex, but the general pattern was that difficulty (e.g., translation direction) impeded initial levels of learning but benefited retention and transfer.

This pattern was most clearly evident for the translation direction manipulation, with the results displayed in **Figure 4**. On the immediate retention test, the more difficult English–French learning condition showed substantially reduced performance (about 50% correct) relative to the French–English condition (just under 80% correct). However, after a 1-week delay, the pattern reversed: students in the difficult English–French condition remembered more than those in the French–English condition. When considered in terms of forgetting, there was little forgetting in the English–French condition (approximately 10% was forgotten) and dramatic forgetting in the French-English learning condition (approximately 60% was forgotten).

Moreover, as shown in **Figure 5**, when the English–French condition was transferred on the delayed test to translate French items into English (the unfilled bar on the left side of the figure), performance was actually slightly better than for conditions that were trained to translate French items into English (the filled bar on the left side).

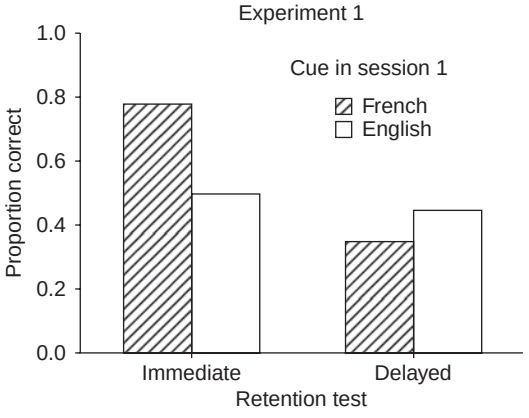

Figure 4 Immediate and delayed translation of foreign vocabulary as a function of trained translation direction in Experiment 1 of Schneider VI, Healy AF, and Bourne LE (2002) What is learned under difficult conditions is hard to forget: Contextual interference effects in foreign vocabulary acquisition, retention, and transfer. *J. Mem. Lang.* 46: 419–440.

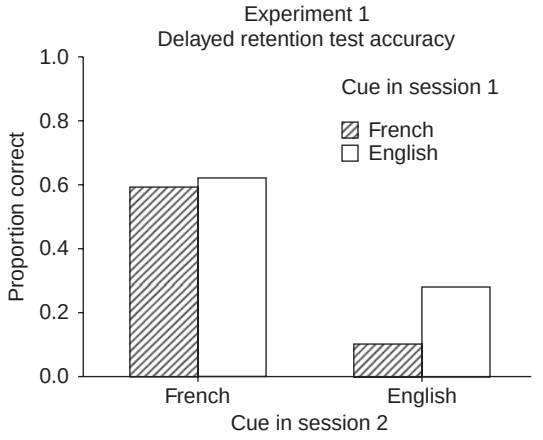

Figure 5 Delayed translation as a function of both trained and tested translation direction in Experiment 1 of Schneider VI, Healy AF, and Bourne LE (2002) What is learned under difficult conditions is hard to forget: Contextual interference effects in foreign vocabulary acquisition, retention, and transfer. *J. Mem. Lang.* 46: 419–440.

By contrast, there was exceptionally poor transfer to the reverse translation direction for French–English training (performance was under 10% correct; see the filled bar on the right side). Thus, these findings with educationally relevant associative learning materials converge with the basic memory research showing that difficult learning conditions can produce desirable educational outcomes of better retention and transfer.

Creating interference during learning with more complex materials has produced similar results. Prior to reading an article on the industrial uses of microbes, learners were provided with an outline that was consistent with the structure of the text or with an outline that was inconsistent with the sequence and organization in which the content was presented in the text (Mannes and Kintsch, 1987). Subsequent to reading the text, learning was assessed with true/false, recall, and problem-solving tests, which together yielded measures of verbatim learning and inference. Learners given the consistent outline showed more verbatim learning than those given the inconsistent outline. By contrast, learners given the inconsistent outline were better able to draw correct inferences from the information than those given the consistent outline. In this case then, interference was not effective for increasing verbatim learning but was effective for enhancing inference and application of the content, arguably a key objective for education.

2.43.3.2 Massed Versus Spaced Practice

A standard educational practice to improve learning is to repeat target material, both with content presented in lectures as well as content presented in student workbooks and homework assignments (e.g., see **Figure 3**; Ste-Marie et al., 2004). An extensive basic memory literature suggests that spacing repetitions of content is preferred to massing repetitions (see Cepeda et al., 2006, for a review, though massed repetitions may confer the impression that the material is being well understood and learned; Bjork, 1994). Experimental evaluations of the value of spacing in educational contexts have been sparser, but the results are encouraging.

In a heroic effort, Reynolds and Glaser (1964) manipulated spacing for content in a junior high general science class for which a sequenced instructional program administered by teaching machines was being used. Students completed the programmed instruction lessons (covering 10 topics in biology) during 40-min science periods, and the experiment was conducted over the course of four weeks of classes. The lessons covered topics on cells, organs and systems, green plants, and so on. The experimental manipulation focused on the lesson on mitosis. This lesson was the sixth topic in a span of 10 topics covered during the four-week period. The lesson described 11 new technical terms and required that students learn the meanings of the terms and how the terms were used to describe and understand the process of mitosis. Throughout the lesson, there were repetitions of these technical terms. In the spaced condition, this base lesson was reduced such that there were fewer repetitions of the terms, and the remaining repetitions were spaced in small review units interspersed after topic 7 (plant reproduction) and topic 8 (animal reproduction). Tests on the mitosis content were administered two days after (fill in the missing terms in 15 sentences) and three weeks after (fill in the blank, essay, and multiple-choice tests) the completion of the four-week program. It is worth mentioning that in one experiment, to ensure an equivalent delay between the most recent coverage of mitosis in both conditions (i.e., there was a review presented after topic 8 in the spaced condition), at the end of four weeks extra content was added to the spaced-condition program.

On the 2-day delayed test, the spaced presentation condition produced over a 50% gain in correct responding relative to the standard lesson. For testing

after a 3-week delay, once again spaced presentation resulted in a 50% increase in performance for both the fill-in-the blank and the essay ('describe and illustrate the mitosis process') tests. On the multiple-choice test, for which more retrieval cues are provided, spaced presentation still produced a 33% gain in performance relative to the standard lesson. Overall then, spacing the presentation of the technical terms throughout other content produced striking benefits in learning and retention. Unfortunately, such spaced presentation may seem antithetical to the demands that most instructors face to cover increasing amounts of material in a course. Note, however, that the above study suggests that repetition within a lesson could be reduced, thereby saving time with which to space presentation of the content across other lessons.

For basic content areas (such as arithmetic, geography facts, and reading skills taught in lower grade levels) massed repeated presentations may be rarer, with spacing being the typical practice. For example, in some first-grade curricula, phonics instruction is spaced across short daily instructional periods (Seabrook et al., 2005). Even so, spacing could be more extended such that the single daily lesson is divided into three short sessions throughout the day. A small experiment involving 34 children (in two classrooms) tested this possibility. For two weeks, one group of first-grade children was given daily 6-min phonics lessons, and another group was given three 2-min phonics lessons distributed throughout each class day. Improvement from pre- to posttest on phonics skills was substantially better for the instruction spaced both within and across days (three 2-min sessions per day) relative to instruction spaced only across days (Seabrook et al., 2005). Accordingly, even curricula that already implement spacing might be improved with even finer grained spacing of the material. Much remains to be learned about the variety of educational content that would benefit from spaced presentations and about the time grain over which spacing is most effective, but clearly spacing is a technique that warrants serious consideration in educational practice (see also Smith and Rothkopf, 1984, for additional evidence).

2.43.3.3 Generation

Another technique to increase the difficulty of encoding target material, and thereby to presumably increase elaboration, is to require the learner to generate the material rather than read the material. An abundance of experiments in the basic memory literature have reported that generation of target items produces better memory for those items than does reading. For instance, subjects remember words that they generated from word fragments better than words that they read (e.g., Jacoby, 1978; McDaniel et al., 1988). Similarly, solutions to multiplication problems are remembered better when they are generated than read (McNamara and Healy, 1995). These basic results have a straightforward implication for education: engineer instructional materials that require the student to generate the content rather than just read it.

Some laboratory-based research has investigated the effects of generation with educationally relevant materials. In one experiment, some key terms in several paragraphs from an introductory psychology text were presented as word fragments (and learners generated the term) and other key terms were presented as intact words (deWinstanley and Bjork, 2004). The phrases in which all key terms were embedded were presented in red type. An example of a phrase with a fragmented key term is: 'the emotional or aff_ct_v_ part'. Learners studied one paragraph followed by a fill in the blank test for the key term, and then studied a second paragraph followed by a fill in the blank test. For the first paragraph, generating produced better memory for the target terms than did reading. Unexpectedly, for the second paragraph no generation effect was obtained because memory for the read terms increased to levels produced by generation. This provocative result suggests that if learners observe the mnemonic benefits of generating relative to reading, then learners will spontaneously develop more effective strategies for learning the read target terms. However, the experiment was conducted at a highly selective liberal arts college, possibly limiting this positive metamemory effect of generation to high-ability learners.

In another line of work, the generation task required learners to reorder a set of randomly presented sentences into a coherent text (McDaniel et al., 1986; Einstein et al., 1990; Thomas and McDaniel, 2007a). In the read condition, the text was presented in normal fashion. The texts were didactic passages (e.g., describing avalanches in the Kanjenchunga Mountains) adopted from reading-training programs used in classrooms. Learners who were required to generate the texts recalled significantly more of the passage than learners who read the texts (as measured by both free recall, McDaniel

et al., and cued recall for conceptual information in the text, Thomas and McDaniel). Of course, those in the generation condition also needed significantly more time to process the texts than those in the read condition. However, even when differences in processing time were statistically partialled out, the generation effect remained (in free recall; McDaniel et al.).

To successfully integrate generation into educational environments, we believe that several challenges must be surmounted. One straightforward challenge is to design generation tasks that are acceptable for the classroom. That is, requiring students to generate a coherent didactic text from randomly ordered sentences will not likely be embraced by teachers. However, there may be some limited use for this kind of generative task. In a sixth-grade class that MAM's child attended, as a learning exercise the students were given a history chronology that was scrambled, and they had to unscramble it and put it in chronological order. As well, textbooks replete with fragmented words are unlikely to become commonplace.

Some intriguing possibilities for implementing generation techniques in classroom settings are starting to appear, and at least one publisher is attempting to develop a prototype interactive textbook, in which interactive visuals interleaved with text require students to generate material. An even greater challenge is to gain a more complete theoretical understanding of when generation is desirable and when it is not in order to allow for effective prescription of desirable difficulty. That basic progress on this front is underscored by the disappointing effects of some classroom generation tasks (Metcalfe and Kornell, 2007) and by recent findings in our laboratory (conducted with Keith Lyle, Andrea Young, and Robin Heyden) that learners given an interactive text (requiring generation of information through click and drag procedures to complete visual/spatial presentation of material) showed significantly diminished learning of definitions relative to those learners reading a comparable text (once or twice) with no visuals (see **Figure 6** for results of once-read text, twice-read text, and interactive text conditions). For multiple-choice questions on conceptual content, the interactive text produced slightly better performance than the standard text read once and worse performance than the standard text read twice (even though reading twice took slightly less time than processing the interactive version of the text). In this study, it appears that the interactive component of the text was distracting rather than facilitating for learners. Accordingly, in the next section we by presenting a framework that illuminates key factors in determining the desirability of difficulty and in particular of generation.

2.43.4 A Contextualistic Framework of Desirable Difficulty

Contextualistic accounts of memory (e.g., Jenkins, 1979) assume that memory performance will be determined not only by the kind of processing the learner directs at the target material but also by the type of test task, the type of materials, and characteristics of the learner. These factors can be critical in determining whether introducing particular difficulties into the learning environment are desirable. We briefly present a framework that specifies in a principled way how parameters of the test task, materials, and learner characteristics will affect the desirability of difficulty (see McDaniel and Einstein, 1989, 2005). In concert, we will provide illustrative support for each component of the framework by presenting laboratory research using educationally relevant materials. We emphasize that this framework assumes that a necessary feature of any desirable difficulty is that the learner must be able to overcome the particular difficulty (see Bjork, 1994).

2.43.4.1 Test tasks and transfer-appropriate processing

Basic memory work has firmly established the transfer-appropriate processing (TAP) principle, which asserts that memory performance is determined by the degree to which the learning activity stimulates processing that is appropriate for the memory test (e.g., Morris et al., 1977; McDaniel et al., 1978; Roediger et al., 1989). Applied to the desirability of difficulty, the implication is that one must consider the type of processing stimulated by the particular difficulty that is embedded into the learning task in relation to the type of test administered. Specifically, we expect that types of difficult processing that simulate the sort of processing required by the criterial test will enhance learning (desirable difficulty), whereas difficulty that stimulates processing not appropriate for the test will have little or no positive impact (an undesirable difficulty). Note that by our framework a particular type of difficulty cannot be identified as desirable in an absolute sense. Rather, a particular difficulty can be desirable when followed

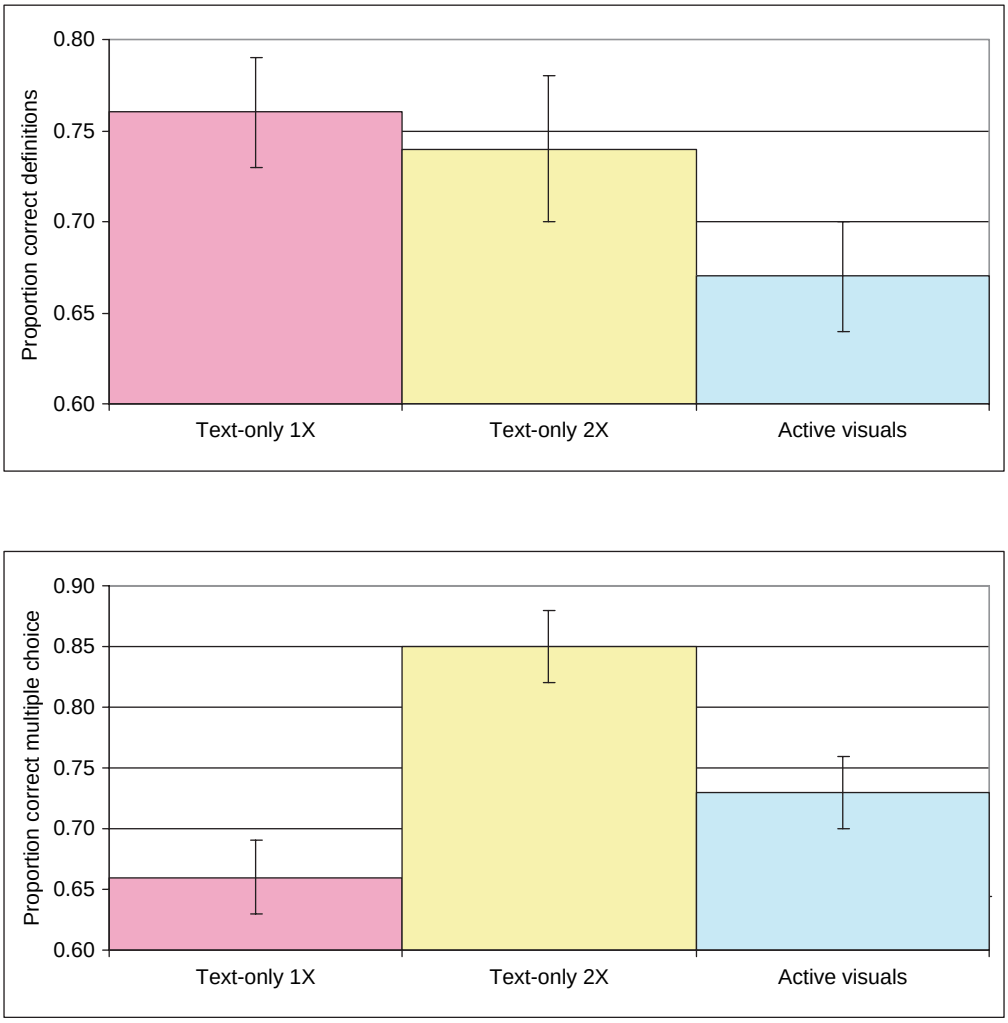

Figure 6 Definition recall (top panel) and multiple-choice test performances (bottom panel) after standard textbook presentations and interactive visually enhanced textbook presentations of biology content (from an unpublished experiment by McDaniel, Lyle, Young, and Heyden).

by one type of test and undesirable when followed by a different type of test (as we will see, the same holds for materials and individual learner characteristics).

Using generation tasks and didactic texts (e.g., discussing why leaves turn color in autumn, avalanches in the Himalayas, or spiders), Thomas and McDaniel (2007a) provided strong support for the above expectations. For each passage, two kinds of tests were prepared. One test was composed of questions focusing on details in the passage ("The walls of ice in Kanchenjunga range from _____ feet high"), and the other test was composed of questions focusing on global thematic information (information that was presented across multiple sentences: "The downward speed of snow is much faster in Kanchenjunga than the Alps because _____"). Two generation (desirable difficulty) tasks were also implemented. One generation task was the sentence-reordering task described in the previous section. For the other generation task, participants had to fill in missing letters from fragmented words (letter reinsertion task). Sentence reordering has been shown to encourage relational processing of the material, and the letter reinsertion task has been shown to promote processing of details (McDaniel et al., 1994, Experiment 1).

Thomas and McDaniel (2007a) reasoned that sentence reordering would be a desirable difficulty when testing focused on thematic information but not specific details. On the other hand, letter reinsertion

would be a desirable difficulty when testing focused on specific details but not thematic information. The results were even more striking than anticipated. Sentence reordering improved performance relative to a control that studied the passage without a generative task but only for the thematic test (0.63 vs. 0.49, respectively; detailed test performance was identical). In contrast, letter reinsertion improved performance on the detailed test relative to the study control (0.60 vs. 0.43, respectively) and significantly disrupted performance on the thematic test (0.30 vs. 0.49)!

Moreover, TAP effects for desirable difficulties extended to learners' metamemory accuracy (see Thomas and McDaniel, 2007b, for more extensive discussion). Participants in the Thomas and McDaniel (2007a) study were asked to provide a judgment of how well they would remember information presented in each paragraph of the passage (termed a judgment of learning). Participants were informed about the type of test that would be administered and prior to the experimental passages, participants were given examples of each type of test. **Figure 7** displays the metamemory resolution (correlation of judgments of learning with actual memory performance) associated with each of the conditions. As is typically reported, metamemory resolution was modest in the study-control (real) condition. Metamemory resolution substantially improved when the generative activity was appropriate for the test (e.g., letter reinsertion and a detailed test). But when the generative activity was inappropriate for the test, metamemory was rendered inaccurate. The correlations between predicted performance and actual performance were zero or even negative. These values indicate that in these conditions participants judged they could remember content that they did not and judged they would not remember information that they were able to remember.

These results have important implications in educational settings because metamemory is presumed to guide the control of learners' study activities (Son and Metcalfe, 2000; Dunlosky et al., 2005). Thus, when a difficulty is desirable it will not only improve memory performance but also may support more effective control of subsequent study activity for the target material. However, when the difficulty is inappropriate for the test task, metamemory is abysmal and thus becomes essentially useless for effectively guiding subsequent study. Thomas and McDaniel (2007a, Experiment 2) confirmed these implications by allowing learners to restudy the passages (presented in normal format) after completing the generation task (either letter reinsertion or sentence reordering). All learners tended to spend more time studying the content that they judged they would not remember well (in line with Thiede and Dunlosky, 1999). But because learners' impressions of what needed to be studied and what did not need to be studied were inaccurate when the generation task was inappropriate for the test task, the additional studying failed to improve performance (relative to when no additional study was allowed; see **Table 1**). By contrast, the additional study boosted performance (relative to no additional study) when the initial generation task was appropriate for the test task.

Taken together, the above theoretical insights and associated empirical results have profound implications for educational practice, both in terms of

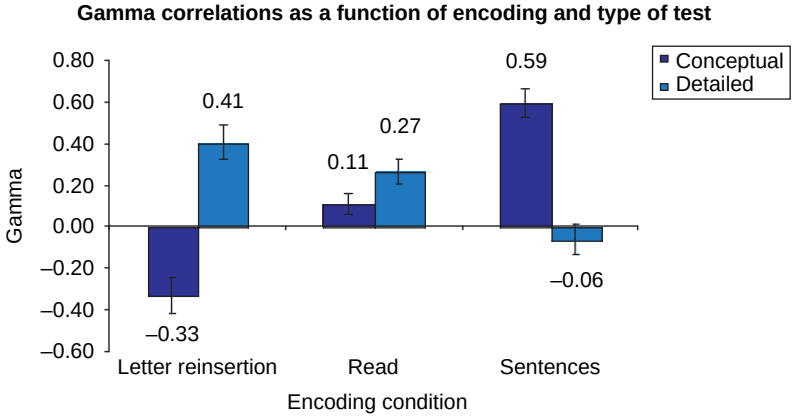

Figure 7 Correlations between judgments of learning and memory performances as a function of study and testing conditions in Experiment 1 of Thomas and McDaniel (2007a) The negative cascade of incongruent generative study-test processing in memory and metacomprehension. *Mem. Cogn.* 35: 668–678.

Table 1 Cued recall performance with and without restudy as a function of generation and test task

		Generation task	
	Type of test	*Letter reinsertion*	*Sentence sorting*
Without restudy	Conceptual	**0.30**	0.63
	Detailed	0.60	**0.43**
With restudy	Conceptual	**0.25**	0.84
	Detailed	0.81	**0.40**

The means for the inappropriate generation—test task conditions are in bold font.
Reproduced from Thomas and McDaniel (2007a) The negative cascade of incongruent generative study-test processing in memory and metacomprehension. *Mem. Cogn.* 35: 668–678.

specifying the desirability of difficulty and more generally in terms of appreciating the importance of transfer-appropriate processing for students' study activities. Our initial work suggests that transfer-inappropriate study activities produce a negative cascade of effects that can penalize the learner in terms of test performance, metamemory accuracy, and control of study activities. Unfortunately educational practice does not always reflect an appreciation of the consequences of transfer-appropriate and inappropriate processing. In one situation related by a colleague, the study activity advocated in his son's ancient history class was to compare and contrast cultures in terms of political, economic, religious, scientific, and other dimensions (see McDaniel, 2007). One would expect this study activity to produce integrative, thematic processing, and thus many educators might embrace its use. Yet the instructor gave multiple-choice tests to the class, which focused on specific facts. To the extent that this supposedly good study scheme produced processing inappropriate for the test, it likely harmed students' memory and metacomprehension for the tested material rather than enhancing it.

2.43.4.2 Material-appropriate processing

A second contextualistic factor that influences the desirability of difficulty is the to-be-learned material. With regard to texts, it is clear that there is variation across texts in terms of the kinds of processing afforded by the text. For instance, texts for which readers' prior knowledge about conventional frames and scripts (knowledge about structures of episodes and causal structures that relate events, as well as knowledge of the canonical structures of particular text forms) can be activated in the service of comprehension to allow readers to readily organize the content (van Dijk and Kintsch, 1983; Trabasso and van den Broek, 1985; Fletcher and Bloom, 1988). In contrast, texts that do not have such organizational properties appear to force readers to focus on individual propositions to try to gain some understanding of the content (see McDaniel and Einstein, 1989, for details). The desirability of a particular difficulty will be a function of the overlap between the type of processing encouraged by the material (e.g., the text) and the type of processing stimulated by the difficulty task (e.g., generation).

Evidence for the above principle has been gleaned using the generation tasks discussed in the previous section. Because the sentence-reordering task stimulates organization of the ideas within the text, this generation task is desirable for a text that encourages focus on individual propositions (a didactic text for which the learner has little prior knowledge); however, this processing is redundant for a text that already encourages organization (e.g., a fairy tale) and thus is not desirable for this kind of material. Conversely, because the letter insertion generation task presumably stimulates focus on individual concepts and propositions, this task is desirable for a text that fosters organization, but it is not desirable for a text that encourages focus on individual propositions. These predicted patterns have been reported using free recall as the criterial test (a test for which both elaboration of individual elements and organization of the elements produces optimal performance) (McDaniel et al., 1986; Einstein et al., 1990; see McDaniel and Einstein, 2005, for a summary). The striking feature of these results is that there is no significant benefit to recall (and sometimes not even any nominal benefit; McDaniel et al., Experiment 1) when the particular generation task is redundant with the processing encouraged by the text itself, despite five- to eightfold increases in the time needed to comprehend the text.

The above results are partially limited in their direct generalization to education because fairy tales were used for some of the materials. At a general level, however, the essential lesson here is that any particular generation (or difficulty) task cannot be identified as desirable *per se*; depending on the context, incorporating a difficulty could be useless and inefficient. As applied to education, this point cautions against sweeping recommendations concerning introducing particular sources of difficulty into instruction. Instead, determination of desirable difficulty will necessitate a careful analysis of the processing stimulated by the type of difficulty and its appropriateness for the criterial test and the materials being learned. For instance, even within a text genre, the rated interest of the text seems to modify the processing invited by the text and thus the desirability of a particular difficulty (generation) task (McDaniel et al., 2000).

2.43.4.3 Differences in learners

Even the foregoing analysis is incomplete because it does not acknowledge a third important contextual factor, individual differences in learners. Learner characteristics (comprehension ability) have also been shown to modulate the desirability of a particular difficulty manipulation. For example, learners who are high in structure-building ability (those who readily form coherent, organized representations of the content of text, Gernsbacher, 1990) may not benefit from a difficult task that stimulates organization (sentence reordering), whereas low-ability structure builders (who do not ordinarily form well-organized representations) do show improved recall when such difficulty (sentence reordering) is introduced (McDaniel et al., 2002).

In another line of work, McNamara and Kinstch (1996) found that increasing the difficulty of text by reducing its coherence improved criterial performance (relative to the more coherent version) for learners high in background knowledge but not for learners with little prior knowledge of the content. These initial studies suggest that further investigation of how individual learner characteristics influence the desirability of difficulty could translate into fruitful application to education. In this vein, we turn now to a consideration of individual differences and their interaction with study adjuncts that have received more attention in the educational literature.

2.43.5 Comprehension

2.43.5.1 Individual Differences

As mentioned in previous sections, individual differences in reading comprehension moderate the benefits of various study methods (though individual differences have largely been ignored in basic laboratory work; Naveh-Benjamin, 1990). One conceptualization of comprehension based on laboratory materials (narratives) that we have found fruitful is Gernsbacher's (1990) Structure Building Framework. In this framework, it is suggested that readers build a mental representation of the text. The representation is built on a foundation of the initial, important information encountered in the text. As new information is encountered, it is either incorporated into the existing framework, or if it is not conceptually related to the existing framework, a new substructure is built out of the new information. It is theorized that poor comprehenders are not able to inhibit irrelevant information. Further, they have difficulty incorporating information into the existing structure. This results in the poor reader shifting multiple times and constructing many more substructures than a good comprehender. A good comprehender, however, is able to inhibit the irrelevant information and build a coherent, well-organized mental representation of the text.

We have examined several different adjuncts (study methods) to try to help poor comprehenders build better representations of the text. One long-standing method used both by researchers and educators is embedded questions, questions placed throughout the text targeting specific content, which have been shown to be generally beneficial (Hamilton, 1985). We showed that answering embedded questions improved performance on both multiple-choice and short-answer questions requiring application of target material for low-ability but not for high-ability comprehenders (Callender and McDaniel, 2007b). Further, this benefit of embedded questions was significant over a read-twice control (which equated the amount of time spent with the text for those who only read and those who answered the questions). By targeting specific points in the text, it appears that embedded questions provide low comprehenders with an anchor around which students can build their representation (cf. Mayer, 2003). This adjunct helps to improve the mental representation of poor comprehenders more than simply rereading a text. Also, as mentioned earlier, low comprehenders (but not high comprehenders) showed learning benefits (relative to the read-twice

control) when 'why' questions to prompt explanatory elaboration were inserted into the text.

Other research investigating study adjuncts has also revealed the importance of attending to individual differences in comprehension (Mayer and Gallini, 1990; McNamara, 2004). One type of individual difference that affects comprehension processes is the amount of prior knowledge one has for the text content. Mayer and Gallini (1990) showed that some adjuncts, like illustrations, improve performance of low-knowledge readers but not high-knowledge readers. Using three different texts to establish generality of the benefits of illustrations, the authors showed that in specific instances, illustrations can improve test performance. When an explanative text is used (that is, a text that contains cause and effect) with illustrations that convey both parts of the system as well as the function of the parts, the illustrations can improve performance on questions requiring application of knowledge. Illustrations do not improve performance for fact-based questions or questions that require verbatim memory for the text. However, these findings only hold for low-knowledge readers. The illustrations bring the low-knowledge readers close to the level of high-knowledge readers on conceptually based questions, but high-knowledge readers do not benefit from the illustrations (although their performance is not harmed by the illustrations, either; see **Figure 8**).

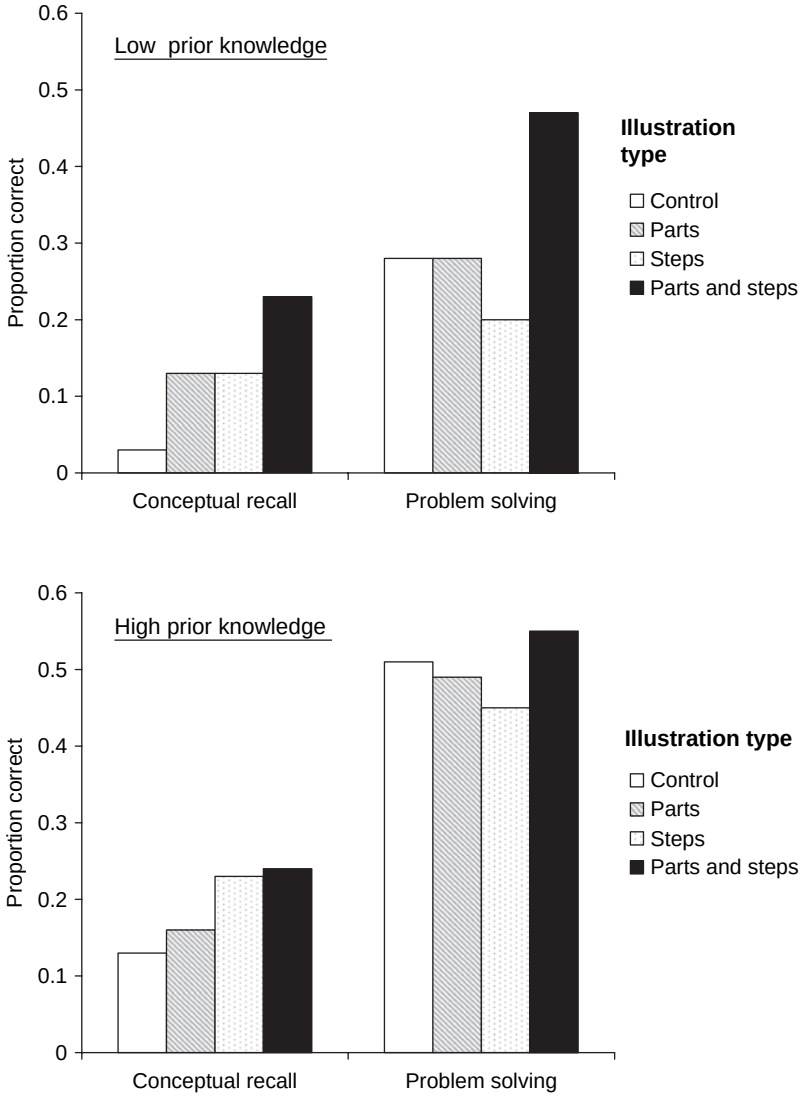

Figure 8 Recall and problem solving as a function of illustration type and prior knowledge in Experiment 1 of Mayer RE and Gallini JK (1990) When is an illustration worth ten thousand words? *J. Educat. Psychol.* 82: 715–726. Reprinted with permission.

Individual differences in knowledge level have also been connected with moderating benefits of comprehension training (McNamara, 2004). McNamara developed a comprehension training system called Self-Explanation Reading Training (SERT). The basis of SERT is that those who explain the text are able to build better mental representations of the text. The goal of SERT is to improve the self-explanations of readers to further enhance the benefits of self-explanation. Specific strategies used by SERT include comprehension monitoring, paraphrasing, making bridging inferences, elaborating, using logic, and predicting (see **Table 2** for examples). These strategies enabled low-knowledge readers to use general world knowledge or logic to compensate for their lack of knowledge specific to the domain being studied. When answering text-based questions, training significantly improved performance of low-knowledge readers, but not of high-knowledge readers. Questions requiring inferencing were not influenced by training; rather, only prior knowledge affected performance. Thus, again we see that question type and prior knowledge influence performance on final tests, although, in this case, individual differences in prior knowledge interacted with training on text-base questions (rather than conceptually based questions as in Mayer and Gallini, 1990). Most striking is that the low-knowledge readers' performance doubled with the training. Indeed all three adjuncts addressed in this discussion of individual differences of the low-comprehension or low-knowledge readers greatly improved performance: Performance increased either to the same level as the high-ability/-knowledge readers or doubled with the use of the method.

In sum, comprehension (and memory) of educational materials can be improved by study adjuncts. Importantly, though, both educators and researchers should be cognizant that individual differences may interact with various study adjunct and text characteristics (such as coherence or genre) such that benefits will not extend to all students. In this regard, knowledge and reading ability have so far been identified as important individual differences.

2.43.5.2 The Importance of Text Coherence

One central process in text comprehension is the construction of a structured representation of the text. A cognitive theory of this process has important implications for improving comprehension in educational situations. We first sketch this theory and then examine research investigating the educational application of the theory. In Kintsch and van Dijk's (1978) theory, the reader builds a structured representation

Table 2 Examples of students' self explanation strategies

Strategy	*Self-explanation*
Comprehension monitoring	Example 1: ''I don't remember what DNA stands for.'' ''So I guess daughter cells are part of a larger cell or came from a larger cell – I don't know.''
Paraphrase	Example 1: ''So each daughter cell will receive a duplicate copy of the same strand of DNA from the parent cell.'' Example 2: ''OK through this process of mitosis all the genetic information belongs in the DNA of the parent cell and that is transferred over to the daughter cell.''
Bridging inference	Example 1: ''So mitosis – the first stage of cell division where each set of chromosomes goes to each daughter cell will contain DNA.'' Example 2: ''So, yeah, so all the genetic information is in the chromosomes and each cell gets a complete set, so that's mitosis – when each cell has just as much DNA as the first mother cell – main cell – parent cell.''
Elaboration	Example 1: ''OK so there's the daughter cell and then there's a parent cell – mitosis it has to do with genetic information so when I'm thinking of cell division I'm thinking of maybe how a baby is made and how it's developing.'' Example 2: ''So by mitosis it guarantees that the chromosomes will get passed on so that the traits or whatever will be able to live on or whatever.''
Using logic	Example 1: ''OK what they're saying is that mitosis will make sure that an equal amounts of genetic information will go to each of the cells – equal amount will go to each daughter cell that way. They will develop basically the same – multiply the same.'' Example 2: ''Ok, so the genetic information that must be the chromosomes because the chromosomes are going into each of the cells. And that is made up of the DNA. So a part of . . . a part of each of the . . . a part of genetic information which is the DNA goes into each of the two cells that come out of this.''
Prediction	Example 1: ''OK this is the separation of the cell – the DNA – the next one should be the RNA.'' Example 2: ''So that's the first stage, now they'll give the second one.''

Note. The above responses were generated in response to this sentence: ''Mitosis guarantees that all the genetic information in the nuclear DNA of the parent cell will go to each daughter cell.''
Reproduced from McNamara DS (2004) SERT: Self-explanation reading training. *Discourse Process.* 38: 1–30.

of the text by attempting to establish connections among the propositions (ideas) expressed in the text. These connections are based on referential overlap: formally, referential overlap is present when propositions share at least one identical concept. Working memory plays a fundamental role here, because during reading several propositions from previously read portions of the passage are held in mind while reading the next chunk of text (e.g., a sentence). The next chunk of text is integrated into the developing text representation if any one of the propositions in working memory has referential overlap with the new propositions (from the incoming chunk of text). If referential overlap cannot be established between the propositions in working memory and the incoming propositions, then the reader must search the text representation in long-term memory to find a proposition that does overlap with incoming content. This search of long-term memory is termed a reinstatement search, and such searches significantly slow comprehension (Kintsch and van Dijk, 1978).

An even more serious challenge to comprehension is when the reinstatement search fails (these are termed text breaks). At this point, standard coherence processes cannot continue, and an inference must be generated to connect the incoming propositions (new sentence) with propositions in working memory. The problem is that readers may not try to generate a linking inference (see for example, Noordman et al., 1992) or readers may attempt to generate a linking inference but fail. In either case, the text representation becomes less coherent and comprehension (and memory) accordingly suffer (see Britton et al., 1990). Critically with regard to education, a survey of social science texts showed that typical textbooks tended to have numerous text breaks (Beck et al., 1989), and perhaps as a consequence, students' ability to comprehend textbooks is hampered (Snow, 2002). The intriguing implication of the theory, however, is that students' comprehension could be improved if the text breaks in the textbooks were eliminated.

Britton and Gulgoz (1991) conducted a study to test this implication. Based on the theory just described, they revised a section of a chapter from a college history text. For every text break in the passage, they inserted material to ensure referential overlap and to make explicit important inferences so that text coherence could be established (without inference generation or costly reinstatement searches). This theory-based revision was contrasted against a revision prepared by a writing expert, who attempted to produce the best possible revision that he could. In addition, a readability revision was prepared that lowered the difficulty of the original by two grade levels. In a first experiment, college students read one of these three revisions or the original passage at their own pace, and then were given free recall, a fact test, and an inference test. All students were forewarned about these tests. Students given the theory-based and the expert-based revisions performed comparably on the comprehension and memory tests and significantly better than did the students given the readability revision or the original passage.

In a second experiment, modeling analyses were used to assess the network structure of the mental representations formed by students after reading the theory-based revision, expert-based revision, or the original passage. Students' mental networks after reading either of these two revisions (but not after reading the original) were correlated with the textbook author's knowledge network of that content. Thus, the revisions enabled the students to better extract a more expert-like representation of the content than the original version.

Two important implications follow from these results. First, principled revisions can substantially improve the didactic value of at least some textbooks. Second, and most pertinent for this chapter's theme, a cognitive model of text comprehension can be successfully applied to diagnose problems in educational texts and prescribe repairs to the text that substantially improve students' representation and memory of the text. Further, as Britton and Gulgoz (1991) note, this theory-guided revision is preferred over expert-guided revision because it provides concrete, well-operationalized guidelines to repair educational text and it is easy to implement. With approximately 2 h of training on the Kintsch and van Dijk (1978) model, a technician can begin to effectively revise textbook material.

2.43.6 Using Testing to Enhance Learning

Generally, educators use testing to assess student learning. However, basic memory research has shown that testing itself promotes learning and retention, and that often the mnemonic benefits of testing are superior to additional study of the target material (e.g., Hogan and Kintsch, 1971; see Roediger and Karpicke, 2006b, for an extensive review). These

findings are theoretically important because they imply that processes involved in retrieval are potent modifiers of memory, that is, that the testing effect is not merely a re-exposure effect (Bjork, 1975; McDaniel and Masson, 1985; Carpenter and DeLosh, 2006). The implication for education is striking: Instead of using testing solely as an evaluation procedure, testing (i.e., requiring retrieval of to-be-learned material) could be exploited to enhance learning and retention. Educational psychologists have sporadically noted this possibility and provided empirical support for the testing effect (Spitzer, 1939; Glover, 1989), but it has largely been ignored as evidenced by the title of Glover's (1989) paper 'The 'testing' phenomenon: Not gone but nearly forgotten.'

Recent research in the laboratory that has attempted to simulate classroom contexts and research conducted in an actual course has reinforced the value of using tests (quizzes) to enhance learning and retention. We briefly describe two examples here, but note these are not exhaustive (e.g., see Roediger and Karpicke, 2006a; Karpicke, 2007; Kang et al., 2007). In the laboratory, Butler and Roediger (2007; see also McDaniel et al., 2007b) presented students art history lectures that were commercially available as continuing education DVD courses. After students viewed a lecture, they were either given a short-answer test on some of the target facts, a multiple-choice test on the facts, or focused restudy of the facts. Thirty days after the lecture, students were given a final criterial test of short-answer questions (e.g., 'What aspect of Morisot's art could be used to date her paintings?'). Performance on the final test was better for target facts that had been presented for restudy or presented on the multiple-choice quiz relative to control facts (those not restudied or quizzed). Further (and significant) gains in final performance were evident for facts that had appeared on the initial short-answer quiz. Assuming that the short-answer quiz required more retrieval processing than the multiple-choice quiz (i.e., more reliance on recall than recognition; cf. McDaniel and Masson, 1985), these results support the idea that requiring learners to retrieve target information enhances learning even more than additional study.

Parallel findings have been reported in an experiment conducted in a college biopsychology course (McDaniel et al., 2007a). For each assigned textbook chapter in the course, some target facts were presented for restudy, some were quizzed with a multiple-choice test, some were quizzed with a short-answer test, and some facts were neither presented for restudy nor quizzed. The quizzes (and restudy presentation) were presented on the course web site and corrective feedback was given for the quizzes. Students were able to take the quizzes anytime they wanted prior to unit exams, which were composed of multiple-choice questions (administered every three weeks). On the unit test, unlike previous work (e.g., Butler and Roediger, 2007; Kang et al., 2007), the stems for the unit questions were changed from those appearing on the quizzes. For example, if the quiz used the stem, 'All preganglionic axons, whether sympathetic or parasympathetic, release _____ as a transmitter,' then the item would appear on the unit test as, 'All _____ axons, whether sympathetic or parasympathetic, release acetylcholine as a neurotransmitter.' Accordingly, this experiment was sensitive to effects of testing on learning of integrated facts or concepts, rather than learning of a particular answer.

Performance on the unit tests showed that items that were not previously exposed (i.e., not seen in restudy, multiple-choice, or short-answer quizzing) fared as well as items that were exposed for restudy. Multiple-choice quizzing produced a slight but significant improvement on the unit test relative to performance on the not previously exposed items. Short-answer quizzes (with feedback) produced the largest increase in performance on the unit exam, relative to the non-preexposed items and better performance than for items quizzed with multiple choice. On a cumulative final multiple-choice test administered approximately five weeks after the last unit exam (for which the experimental conditions were in effect), the significant benefit of initial short-answer quizzes persisted, while the multiple-choice format benefit did not remain significant. However, it may be that with repeated quizzing that even multiple-choice quizzing would be more mnemonically potent (see Roediger et al., 2007).

Thus, under the variable conditions found in a course setting, such as student differences in completing assignments, amount of study, motivation, and various delays between taking quizzes and exams (when students self-schedule the quizzes), testing (especially testing that challenges retrieval, such as recall tests) enhances learning and retention. These findings are even more impressive in that transfer was evident across different question frames from the quizzes to the examinations. The strong implication is that, at least in courses that

are heavily fact-based, using testing to enhance learning should be seriously considered.

2.43.7 Summary

Over the years, a number of researchers have advocated application of cognitive research to improve educational practice (e.g., Rothkopf and Bisbicos, 1967; Hudgins, 1975; Mayer, 1987; Naveh-Benjamin, 1990; Bjork, 1994; Mayer, 2003, to name a few). In this chapter, we have attempted to translate established principles from the memory and comprehension literature into fairly specific implications and techniques that could be implemented in educational settings. Based on current basic research, we were able to identify an array of techniques, including stimulating explanative elaborations, mnemonic use of imagery, organizational devices, desirable difficulties, and repairs to text (for example, increasing the coherence of the text) to improve learning and retention (and certainly, this is not an exhaustive list). A prominent objective here was to evaluate the potency of these candidate techniques from experimental investigations that approximated the materials, test tasks, and other contextual variables present in educational settings. The evidence was quite encouraging in this regard, and we believe it provides the foundation for effective implementation into educational practice.

In support of this claim, we close with a brief description of recently reported work that has combined several techniques discussed herein into a computer-based learning program implemented in a game format to assist students' vocabulary learning in a challenging inner city school setting (Metcalfe and Kornell, 2007; Metcalfe et al., 2007). The computer-based program implemented some of the principles reviewed in this chapter such as spaced practice, generation, and testing to enhance learning. Grade 6 children in an inner-city public middle school in New York City's South Bronx either were assisted with learning necessary vocabulary by the computer-based program, or they attempted to learn the vocabulary in a self-study condition. After seven weeks, test performance on the vocabulary learned in the computer condition was over 400% of that observed for vocabulary learned by self-study. We think that these impressive results strongly compel continued efforts to translate basic work in memory and cognition into effective educational applications.

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2.44 Eyewitness Identification

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In the course of a criminal trial, eyewitness testimony can be very persuasive evidence (e.g., Wells et al., 1981; Fox and Walters, 1986; Cutler et al., 1990). Although the law makes no distinction between the relative weights juries should give to various types of evidence (United States v. Ramirez-Rodriguez, 1977), eyewitness evidence is most influential and salient to jurors even when paired with direct evidence that is inconsistent with the eyewitness identification (Fox and Walters, 1986; Cutler et al., 1990). Eyewitness identification of crime perpetrators is a common form of eyewitness testimony, forming the primary and sometimes the sole evidence in criminal cases. Eyewitness identification, however, is frequently inaccurate (Rattner, 1988; Wells, 1993; Wells and Bradfield, 1999; Wells et al., 1998).

Two independent phenomena combined in the late 1990s to create a perfect storm which resulted in a movement to reform the procedures by which eyewitness identifications are typically obtained, the lineup. First, research on eyewitness identification grew and matured throughout the 1980s and 1990s. By the late 1990s, effects of lineup instructions, filler selection, lineup presentation, and confidence assessment were fairly well understood based on a substantial body of research. Second, technical advances in DNA testing of identity and solid investigative work by the Innocence Project led to the growing realization that many innocent people had been convicted of serious felonies and had spent years in prison (Sheck et al., 2000). For example, the Innocence Project (groups of attorneys and law students operating out of law schools and legal clinics) has to date identified 200 cases of erroneous conviction. Mistaken identification is cited as the single leading cause of these erroneous convictions (Cutler and Penrod, 1995). Many of these erroneous conviction cases involve vivid, dramatic accounts of convicted felons who steadfastly maintained their innocence and traumatized and sincere eyewitnesses who with equal vehemence stood by their identifications (e.g., Junkin and Bloodsworth, 2004; Transcript of Penny Beernsten's speech, 2006; Transcript of Jennifer

Thompson Cannino's speech, 2006). Because eyewitness testimony is so prevalent and because such testimony can have dire consequences for the defendant, it has inspired volumes of research.

Given the voluminous research on eyewitness identification, an exhaustive review is untenable, even as a handbook chapter. Instead, we have adopted the following, more achievable objectives for this chapter. First, we provide an overview of the breadth of research on eyewitness memory. Second, we select two subsets of this research for more in-depth review: Estimator and system variables affecting eyewitness identification. Within each subset we review a sample of specific factors that have well-established effects on identification accuracy. For each of these variables, we provide a description of the effect on eyewitness identification accuracy and review a sample experiment to illustrate the science behind the research conclusions. We then summarize the state of knowledge regarding the factor, usually based on a published meta-analysis. We describe existing theory or at least speculation concerning the causes of the underlying effects of the factor. Following each subset of variables we discuss the practical implications of the knowledge gleaned from the research and how this knowledge influences actual policy and practices within the criminal justice system.

As mentioned, we divide this chapter into two subsets, estimator and system variables, a dichotomy that was developed nearly 30 years ago by Gary Wells (1978) and that continues to serve as a useful and oft-cited guiding principle today. Estimator variables are variables over which the police and criminal justice system exert no control. Many of these variables are those associated with the encoding stage of memory, such as the length of time the culprit is in view, whether the culprit wears a disguise or covers his face, whether the culprit has a gun, and the amount of stress experienced by the witness. They are called estimator variables because their main utility is to be used to estimate the reliability of an eyewitness's identification. System variables, by contrast, are under the control of the judicial system. These variables can therefore be used to enhance the likelihood of correct identification or minimize the likelihood of false identification. Examples of system variables include the instructions given to the eyewitness prior to a lineup, the selection of fillers for the lineup, and the manner in which the lineup is presented to the eyewitness. Research on estimator and system variables contributes to our understanding of the myriad of factors influencing identification accuracy, and both sets of variables have led to practical applications.

2.44.1 Breadth of Research on Eyewitness Memory

In addition to the distinction between estimator and system (independent) variables, the research on eyewitness identification can also be divided into two general categories, identifiable by the primary dependent variables: eyewitness recall and eyewitness identification. The estimator-system variable distinction applies to both categories of dependent variables. With respect to eyewitness recall, examples of estimator variable research programs include the impact of misleading postevent information on eyewitness memory, the suggestibility of child witnesses, the conditions under which people form false memories, and factors affecting peoples' abilities to describe perpetrators and crimes. System variable research on eyewitness recall has focused on such issues as the development of interview practices that increase the amount of accurate information recalled by eyewitnesses (e.g., the cognitive interview), and interviewing techniques for minimizing errors in children's recall.

A list of estimator and system variables examined in eyewitness recall and identification is presented in **Table 1** (column 1), followed by the percentage of experts in the field that agree that phenomenon is reliable (column 2), descriptions of their general effects (column 3), citations to sample studies (column 4), and citations to review papers – meta-analyses if available (column 5). As one can see, the number and range of variables examined are substantial. Column 2 of **Table 1** is an interesting index and requires some explanation. Kassin and colleagues (2001) authored an article describing a study of the general acceptance of factors affecting eyewitness identification. Kassin et al. surveyed 64 eyewitness researchers (mainly cognitive and social psychologists who had authored published research on eyewitness memory) for their opinions about the extent to which the factors listed (and some not listed) are reliable enough to testify about in court. The primary purpose of this study was to empirically address a concern that frequently arises in courts when expert witnesses are proffered. Our purpose in including this information is to provide another

Table 1 List of system and estimator variables with representative and review studies

Variables	*Expert agreement*[a]	*Major results*	*Representative study*	*Review study*
Weapon focus	87%	Reduced ID and description accuracy when weapon is present	Loftus et al., 1987	Steblay, 1992
Disguise		Reduced ID accuracy with disguises and physical transformations	Cutler et al., 1987a, b	Cutler, 2006
Stress	60%	Extreme stress impairs ID accuracy	Morgan et al., 2004	Deffenbacher et al., 2004
Own-race bias		Reduced ID with other-race than same-race identifications	Platz and Hosch, 1988	Meissner and Brigham, 2001a
Exposure time	81%	Longer viewing times increase identification accuracy	Valentine et al., 2003	Shapiro and Penrod, 1986
Speed of identification	40%	Faster identifications lead to more accurate identifications	Sporer et al., 1993	Weber et al., 2004
Unconscious transference	81%	False identification of person who seems familiar because he/she was encountered near scene of crime	Read et al., 1990a	Ross et al., 1990
Misleading postevent information	94%	Reduces witness accuracy	Loftus et al., 1978	Loftus, 1996
Retention interval	83%	Longer retention intervals (>1 week) lead to less accurate identifications	Shepherd, 1983	Shapiro and Penrod, 1986
Confidence malleability	95%	Witness confidence is affected by social factors unrelated to accuracy	Bradfield and Wells, 2000	Leippe, 2006
Confidence accuracy	87%	Relationship strong only when choosers are considered	Cutler et al., 1987a	Sporer et al., 1995
Alcohol intoxication	90%	Alcohol impairs witness identification	Morgan et al., 2004	Birnbaum and Parker, 1977
Hypnotic suggestibility	91%	Increases false reports	Karlin and Orne, 1996	Lynn et al., 1997
Hypnotic accuracy	45%	Small increases in witness accuracy	Geiselman and Machlovitz, 1987	Scheflin et al., 1999
Witness age: children	94%	Increases likelihood of false IDs in young witnesses	Pozullo and Lindsay, 1998	Dickenson et al., 2005
Witness age: adults	50%	Increases likelihood of false IDs in old witnesses	Memon and Gabbert, 2003	Mueller-Johnson and Ceci, 2004
Verbal overshadowing		Reduces identification accuracy	Schooler and Engslter-Schooler, 1990	Meissner and Brigham, 2001b
Distinctiveness of culprit	32%	Increases accuracy with distinctive faces	Vokey and Read, 1992	Shapiro and Penrod, 1986
Lineup instructions	98%	Unbiased instructions lower false identifications from TA lineup with lowering accuracy from TP lineups	Malpass and Devine, 1981a	Steblay, 1997
Double-blind lineups		Increase lineup identification accuracy	Haw and Fisher, 2004	Russano et al., 2006
Foil selection	71%	Match-to-description strategies increase ID accuracy	Luus and Wells, 1991	Wells and Olson, 2003
Lineup presentations	81%	Sequential lineups reduce false IDs	Malpass and Devine, 1981a	Steblay et al., 2001
Showups	81%	Increased risk of misidentification	Yarmey et al., 1996	Steblay et al., 2003

(*Continued*)

Table 1 (Continued)

Variables	*Expert agreement*[a]	*Major results*	*Representative study*	*Review study*
Postidentification feedback		Increases witness confidence without increasing accuracy	Wells and Bradfield, 1998	Douglass and Steblay, 2006
Suspicion inducement		Reduces the inflation effect of postidentification feedback	Neuschatz et al., 2007	Fein et al., 1990
Mug shot exposure	95%	Exposure to mug shots reduces identification accuracy	Gorenstein and Ellsworth, 1980	Deffenbacher et al., 2006
Cognitive interview		Increases in witness accuracy without increases in inaccurate information	Fisher et al., 1989	Powell et al., 2005

[a]This column represents the percentage of experts that agree that research on the variable is reliable. Those variables that do not have percentages in the testify and agreement columns were not included in the Kassin et al. (2001) survey.

index of the extent to which the findings are generally accepted in the scientific community.

Although this chapter focuses on estimator and system variables in eyewitness identification, note that these subsets of variables do not fully capture the breadth of research on eyewitness identification. Considerable research has also explored the effectiveness of safeguards designed to protect defendants from erroneous conviction resulting from mistaken eyewitness identification. This research has examined, for instance, the effectiveness of the presence of counsel at lineups, motions to suppress identification testimony, cross-examination, expert psychological testimony, and judges' instructions (see Van Wallendael et al., 2007, for a review). Underlying much of this research are assumptions concerning the extent to which laypeople (jurors) and legal practitioners (attorneys and judges) are sensitive to the factors that are known from the research to influence the accuracy of eyewitness identification, and the studies test these assumptions.

2.44.2 Estimator Variables Affecting Eyewitness Identification

2.44.2.1 Exposure Time

Witnesses typically identify suspects based on appearance, so it is reasonable to expect that longer and clearer viewing times lead to better memory and, therefore, enhanced identification accuracy. The literature on facial recognition certainly supports this assertion, as it has been consistently found that longer exposure leads to enhanced facial recognition (Ellis et al., 1975; Shapiro and Penrod, 1986; MacLin et al., 2001). To illustrate, Memon et al. (2003) exposed 64 young adults (ages 17–25) and older adults (ages 59–81) to a videotaped reconstruction of a robbery in which the perpetrator's face could be seen for either 12 s or 45 s. Each witness then attempted to identify the robber from a robber-present or robber-absent photoarray. Exposure duration had a significant impact on identification accuracy. Ninety-five percent of the young adults and 85% of the older adults made correct identifications from the robber-present photoarrays when the robber was exposed for 45 s, but only 29% of the young adults and 35% of the older adults made correct identifications when the robber was exposed for 12 s. Similarly, 41% of the younger adults and 50% of the older adults made false identifications from the robber-absent photoarrays when the target was exposed for 45 s, but 90% of the younger adults and 80% of the older adults made false identifications when the robber was exposed for only 12 s.

Shapiro and Penrod (1986) meta-analyzed 128 studies of face recognition and eyewitness identification involving over 17,000 participants. They examined the effects of exposure time in two different ways: They coded each study for exposure time and examined the impact of exposure time across studies (while controlling for other study characteristics), and they examined the average effects of exposure time within the subset of studies within which exposure time was directly manipulated. Both approaches revealed the expected relations between exposure time and identification accuracy. The respective hit rates for the long and short exposure time conditions were 69% and 57%. The respective false alarm rates were 34% and 38%. As exposure time increased there was a concomitant increase in the hit rate but no increase in false alarms.

Nevertheless, there are some qualifications to the effect of exposure time. Read et al. (1990b), for example, found that increased exposure time enhanced facial recognition when the face did not change from study to test. When slight variations in facial features occurred from study to testing, increased exposure did not improve recognition accuracy. They explained these findings by suggesting that increased exposure allows for encoding of more specific facial features, and the reliance on this information is only helpful at test if those features are still present in the target face at the time of retrieval. To the extent that features cue changes, more exposure time was found by Read et al. to be deleterious of facial recognition accuracy.

2.44.2.2 Changes of Appearance and Disguises

To what extent does a perpetrator's change in appearance from the time of the crime to the identification influence a witness's ability to identify him? The influence of changes in appearance on identification accuracy has been examined in several ways. The second author and his colleagues (Cutler et al., 1986, 1987a,b; Cutler and Penrod, 1988; O'Rourke et al., 1989) examined the effect of wearing a hood or baseball cap to cover the hairline of the culprit. In these studies, witnesses watched videotaped crimes and later attempted lineup identifications. In half of the videotaped crimes, the perpetrator wore a cap, while in the other half, his head was uncovered. In each of six studies the percent of correct identification decisions (including both target-present and target-absent lineups) was higher when the perpetrator's head was uncovered. The average performance levels across the six studies, which involved over 1300 eyewitness identifications, was 57% correct when uncovered versus 44% when a hat was worn (Cutler, 2006).

Whereas Cutler and colleagues examined deliberate attempts to disguise one's physical characteristics, Read (1995) examined the impact of more moderate and less invasive changes in appearance in a field study. In this study, two women, at separate times, approached a sales clerk. The first woman asked if her daughter had been in the store because they had arranged to meet at a specified time. The daughter entered the store 15 minutes later and asked the same store clerk if her mother had been in the store and asked for her. The daughter wore her glasses and had her hair pulled back from her face when she interacted with the clerk. Two days later the clerks were asked to identify the daughter from one of two lineups. In one lineup, the daughter's appearance was not changed, while in the other, her appearance was altered (no glasses, hair loose). It is important to note that even though the daughter's appearance was changed she still matched the general description given by the clerks. Consistent with the results reported by Cutler and colleagues, the changed appearance impaired subsequent lineup identification. Read found similar results in a subsequent study (Read et al., 1989)with a male confederate who changed his appearance by removing his facial hair between the crime and the lineup. In further support of these results, Hockley et al. (1999) found that presence or absence of glasses impaired facial recognition to the extent that there was a mismatch at study and test.

Appearance changes can occur naturally as a result of aging, for example, when a suspect is apprehended several months or years after a crime. Read et al. (1990b) examined the effect of such age changes on identification accuracy. Participants viewed black-and-white high school yearbook photos of students taken 2 years apart, in grades 10 and 12. Matched pictures were rated for appearance change by independent raters, and appearance change ratings were inversely associated with recognition accuracy. In Experiment 1, recognition accuracy was highest in the pairs of high similarity (54.5% correct) and lowest in the low-similarity pairs (32.6% correct). Furthermore, when two photos were highly similar, performance increased with exposure time.

Together, these results demonstrate that appearance changes resulting from deliberate attempts to mask features, modest changes in hairstyles and facial hair, and the natural processes of aging tend to impair identification accuracy. Conclusions from the studies of appearance change conform to the encoding specificity principle (Hunt and Ellis, 1974; Tulving, 1983). The encoding specificity principle states that the best memory performance occurs when the stimulus material at encoding matches the items at retrieval. When applied to appearance changes, we would predict, in accordance with this principle, that changes in appearance from the event (encoding) to the identification (retrieval) negatively impact identification accuracy.

2.44.2.3 Own-Race Bias

The own-race bias (ORB), also known as cross-race effect or other-race effect, refers to the conclusion

that members of one's own race are more accurately recognized when compared to members of other races (Kassin et al., 1989). Though the majority of studies of the ORB have utilized White and Black participants, the consistency of this effect has been demonstrated across various racial and ethnic groups (Luce, 1974; Carroo, 1986). To illustrate, Platz and Hosch (1988) conducted a field study in which White, Black, and Hispanic customers visited convenience clerks and interacted with 90 White, Black, or Hispanic clerks. Within 2–3 h following each visit, an experimenter asked each clerk to identify the customers from photoarrays. Witnesses of each racial group demonstrated the ORB. White clerks were more likely to correctly recognize White customers (53.2%) than Black (40.4%) or Mexican (34%) customers. Black clerks were more likely to correctly recognize Black (63.6%) than White (54.6) or Hispanic (45.4%) customers. Hispanic clerks were more likely to correctly recognize Hispanic (53.6%) than White (35.7%) or Black (25%) customers.

The reliability of the ORB was examined by Meissner and Brigham (2001a), who meta-analyzed the results of 31 separate studies involving 91 separate experimental tests of own-race versus same-race identifications. The studies included over 5000 participants. Across all studies, eyewitnesses were 1.4 times more likely to correctly identify members of their own race than members of other races, and they were 1.56 times more likely to falsely identify members of other races than members of their own race. White participants demonstrated a significantly larger ORB when compared with Black participants but only with respect to false identifications. With respect to correct identifications, Whites and Blacks showed the same ORB. Exposure time moderated the ORB such that the magnitude of the ORB was inversely associated with exposure time.

Scholars with interests in the ORB have drawn upon various social-cognitive approaches (including racial attitudes, physiognomic homogeneity, interracial contact, schema theory, and perceptual learning theory) in their attempts to better understand its causal mechanisms. This research casts doubt on the underlying roles of racial prejudice and physiognomic homogeneity as explanations. Although racial attitudes may not have a direct influence on the ORB, several studies have found a correlation between racial attitudes and amount of interracial contact, a factor that, as noted later, does seem to influence the ORB effect (Brigham, 1993; Slone et al., 2000).

Considerable ORB research has examined the role interracial contact plays in reducing the ORB effect. Contact has been hypothesized to lessen the need to rely on stereotypical responses and to motivate people to accurately recognize members of other racial groups (Malpass, 1981b, 1990). The majority of the studies examining the contact hypothesis give some evidence in support of these predictions. For example, Cross et al. (1971) found that segregated neighborhood residents displayed a significantly greater degree of ORB than residents from desegregated neighborhoods. Further support for the contact hypothesis was obtained in the aforementioned meta-analysis (Meissner and Brigham, 2001a).

The contact hypothesis enlightens our understanding of certain social aspects that influence the ORB effect. Nevertheless, the contact hypothesis fails to account for the cognitive mechanisms by which it operates. To understand the cognitive mechanisms behind the ORB effect, researchers (e.g., Malpass, 1981a) have drawn upon Gibson's (1969) perceptual learning hypothesis. Perceptual learning and differentiation are acquired skills that enable an individual to efficiently extract pertinent information from the environment by experience and practice through focused attention toward invariant cues within certain stimulus sets (Gibson, 1969). Applied to the ORB, people are able to discriminate own-race faces more accurately because they can more efficiently extract invariant cues from own-race faces than other-race faces. Furthermore, these invariant cues are not necessarily predominant in other-race faces, resulting in decreased performance in recognition of other-race faces (Meissner and Brigham, 2001a).

2.44.2.4 Stress Experienced by the Eyewitness

Witnessing a crime can be a highly stressful event, particularly if the witness is a victim or is in serious danger. Stress is commonly identified as a potentially influential factor affecting identification accuracy. Interestingly, lay opinion about the effect of stress is mixed. Many people think extreme duress improves identification accuracy, while others believe it impairs memory (Schmechel et al., 2006). This prevalence of conflicting lay opinions underscores the need for empirical research examining the impact of stress.

Stress is defined as the perception of the potential threat of injury or death to oneself or to another person (Thompson et al., 1998). Empirical

examinations of the impact of extreme stress are made difficult for obvious reasons: A proposal to simulate extreme stress in the eyewitness laboratory would be risky business and would understandably meet with considerable resistance by university ethics review committees. Researchers have therefore had to rely on less evasive – and less ecologically valid – manipulations of stress. Some researchers have manipulated stress by exposing witnesses to violent versus nonviolent videotaped crimes (e.g., Clifford and Hollin, 1981). Others have conducted field research and examined actual witnesses to crime. Yuille and Cutshall (1986) interviewed witnesses to a murder and found that those who reported being under the greatest amount of stress were more accurate than those who reported experiencing less stress. Complementing this finding, Reisburg et al. (1988) and Wagenaar and Groeneweg (1990) interviewed participants about recently experienced ordinary events and traumatic events. When describing traumatic events, participants expressed more vivid memories, including the specifics of the event, than when describing ordinary events. Unfortunately, because the experimenter was not privy to the actual events described, the accuracy of these flashbulb memories could not be verified.

Morgan et al. (2004) investigated the impact of extreme versus mild stress on identification accuracy in a sample of 530 active-duty military personnel who participated in military survival school training. The training protocol required some participants to undergo a high-stress interrogation with real physical confrontation and a low-stress interrogation without real physical confrontation. Others experienced either high- or low-stress interrogations. At the end of training, all participants attempted to identify their interrogators from lineups which were either live (simultaneous presentation) or photographic (simultaneous or sequential presentation). Among eyewitnesses shown live, interrogator-present lineups, correct identification rates were much higher following low-stress interrogation (62%) than following the high-stress condition (27%). The same pattern held for simultaneously presented perpetrator-present photographic lineups (76% vs. 36%) and sequentially presented perpetrator-present photographic lineups (75% vs. 49%, respectively).

The effects of stress were further confirmed in a meta-analysis (Deffenbacher et al., 2004) that examined 27 separate tests of the impact of stress on identification accuracy and 36 tests on the impact of description accuracy. Across all studies of identification accuracy, stress inversely and significantly affected the likelihood of correct identification ($h = -0.52$, corresponding to correct identification rates of 0.19 in the high-stress conditions and 0.34 in the low-stress conditions). Stress had a negligible impact on performance in target-absent lineups ($h = 0.01$, corresponding to a 1% difference in false identification rates). Stress also impaired description accuracy across studies ($d = -0.31$).

Although the meta-analysis shows that extreme stress impairs eyewitness memory, the relation between stress and eyewitness memory is not thought to be linear. In one of the earliest reviews of the research on stress and eyewitness memory, Deffenbacher (1980) concluded that the research across 21 studies conformed to the Yerkes-Dodson law (Yerkes and Dodson, 1908). This law states that the effects of stress can be graphed as an inverted U so that with slightly increased levels of stress, there is improvement with memory. However, as stress increases, it reaches an apex and then begins to have a negative impact, resulting in poor encoding and recollection.

Easterbrook's cue-utilization theory has been posited as an explanation for the impact of stress on memory. According to this theory, as stress surpasses moderate levels, attention is diverted from the details of the event to the anxiety (Christianson, 1992; Easterbrook, 1959). It is further hypothesized that because less attention is available for a stressful event, there is a narrowing of focus so that the person allocates more attention to the most informative data (e.g., a weapon in a crime). This leads to the centralization of focus that occurs during highly arousing events. While there has been some debate as to the effects that stress has on the memory of an event, it is clear that information is encoded differently during a stressful event than during a nonstressful event, so that stressful events improve memory for certain aspects of the event while impairing memory for other aspects of the event.

2.44.2.5 Weapon Focus

The presence of a weapon is thought to lead an eyewitness to attend to the weapon, leaving less attention to deploy toward other information (Loftus et al., 1987; Mitchell et al., 1998; Pickel, 1998). Weapon focus, therefore, should impair the eyewitness's ability to identify the perpetrator. Put another way, the presence of a weapon is thought to create competition between the weapon and the assailant's face and

physical characteristics. Does the presence of a weapon actually impair identification accuracy? In a study by O'Rourke et al. (1989), 120 community members (members of a church group, parents of a local Boy Scouts troop, and undergraduate summer school students) viewed a videotaped enactment of a crime. In half of the crimes, a handgun was visually present, and in the other half, the gun was hidden from view in the perpetrator's coat. One week later, each eyewitness attempted to identify the perpetrator from a lineup. Percent of correct decisions on the lineup test was 55% among witnesses who did not see the weapon but 37% for those who did see the weapon – a statistically significant difference. The weapon focus effect increased the likelihood of false identification and decreased the probability of correct identification.

Steblay (1992) meta-analyzed 19 empirical studies of the weapon focus effect. Of the 19 studies she examined, six demonstrated a significant weapon focus effect while 13 found null results. No study revealed enhanced memory for the presence of a weapon. When the results of these studies were combined, the weapon focus effect for identification accuracy was significant but relatively small in magnitude (corresponding to a difference of about 10% in identification accuracy rates). The effect was larger on description accuracy, however. The weapon focus effect was larger among studies that used more ecologically valid research designs.

The most direct support for the weapon focus effect comes from a study by Loftus et al. (1987). They employed a corneal reflection device which tracks both the direction and duration of eye movements. Participants watched slides that included a person approaching a restaurant counter with either a check or a weapon. They found that people looked at the weapon longer and more often than they looked at the check. In addition, participants' memories were significantly worse in the weapon condition compared to the check condition.

One of the more popular explanations for the weapon focus effect is that people focus their attention on information that is meaningful and not on the weapon *per se*. When a weapon is involved, it becomes the meaningful information (Loftus et al., 1987; Brown, 2003). Thus, the eyewitness focuses on the gun or the central details of the crime, while largely ignoring the peripheral information such as clothing or facial features of the perpetrator. Other researchers, in contrast, have theorized that the weapon focus effect occurs not because the weapon is stressful or highly arousing but rather because the weapon is unusual. To examine this hypothesis, Pickel (1998) had participants view a videotape in which a target was empty-handed or carrying something threatening or unusual. Her results indicated that it was the unusual object – not the dangerous object – that drew witnesses' attention. In other words, the appearance of a gun in a situation in which one would expect its presence, such as a hunting trip, would not lead to the traditional weapon focus effect. Pickel's explanation received further support in a study (Pickel, 1999) showing that the same weapon elicited a larger weapon focus effect when carried by a preacher (an unusual event) than when carried by a police officer (a typical event). Thus, Pickel's research supports the idea that unusualness of the object and situation, and not the mere presence of a weapon, are critical for obtaining the weapon focus effect.

2.44.2.6 Eyewitness Confidence

The confidence accuracy relationship (CA) is one of the most studied variables in eyewitness research. Common sense tells us that highly confident witnesses are more likely to be accurate than less confident witnesses (Schmechel et al., 2006). The courts have gone so far as to establish witness confidence as one of the indicators jurors should use to evaluate the accuracy of eyewitness identifications (Neil v. Biggers, 1972).

Does the relation between confidence and accuracy conform to common sense? Many studies of eyewitness identification have assessed witness confidence as a primary or secondary variable. Correlations between confidence and accuracy (or comparable indices of association) are reported in many studies. For example, in a study conducted by Cutler et al. (1987a), 165 students watched a videotaped enactment of a robbery and later attempted to identify the thief from videotaped lineups. Various aspects of the viewing conditions and lineup were manipulated. In all conditions, participants rated their confidence in the accuracy of their identifications immediately following their identification decisions. Across all conditions, the correlation between confidence and accuracy was significant but relatively weak ($r = 0.20$; $p < 0.05$) in magnitude.

The correlations between confidence and identification accuracy have been subjected to several meta-analyses over time (Deffenbacher, 1980; Wells and Murray, 1984; Shapiro and Penrod, 1986; Bothwell et al., 1987; Cutler and Penrod, 1989; Sporer et al.,

1995). Sporer et al.'s (1995) was the most recent and comprehensive meta-analysis. Their analysis included 30 studies and over 4000 witnesses. The average correlation across studies was 0.29. The correlation was significantly higher among witnesses who made positive identifications than among witnesses who rejected their lineups ($r = 0.41$ vs. 0.12).

The oft-found modest correlation between confidence and accuracy begs explanation, as it is inconsistent with theoretical decision-making models (e.g., signal detection theory) that would predict a strong relation between confidence and accuracy (Brewer et al., 2005; Libuser and Ebbesen, unpublished data; Ebbesen and Wixted, unpublished data). The explanation, we believe, is that although eyewitnesses are somewhat sensitive to the accuracy of their identifications, their expressions of confidence are influenced by cognitive, personality, and social factors that are independent of identification accuracy. Any factor that influences confidence independent of accuracy should attenuate the relation between confidence and accuracy.

This very general explanation has received support in the eyewitness literature. For example, with respect to cognitive factors, Deffenbacher (1980) offered the optimality hypothesis as an explanation for the weak relation between confidence and accuracy he observed in his review. Optimal viewing conditions, according to this hypothesis, improve both accuracy and the relation between confidence and accuracy, for under optimal conditions, witnesses should give more accurate meta-cognitive judgments. The optimality hypothesis has received support in meta-analytic reviews (Deffenbacher, 1980; Shapiro and Penrod, 1986; Sporer et al., 1995), but support is not universal (Penrod and Cutler, 1995).

Kassin (1985) reasoned that the failure to find a relation between confidence and accuracy might reflect an inability to successfully employ the intrinsic and extrinsic cues that exist with memory traces. More specifically, witnesses may not be aware of the diagnosticity of the cues that exist in remembering and therefore cannot successfully apply those cues to gauge confidence estimates. For example, in their seminal article, Nisbett and Wilson (1977) have shown that people are poor at both identifying reasons for their behaviors and expressing their thought processes. Kassin (1985) believed that making participants aware of their meta-cognitive cues by showing them videos of their own identifications would in turn increase the confidence-accuracy relation. To this end, Kassin (1985) recorded participants during a lineup procedure and subsequently had participants report their confidence. Some of the participants watched a video of their identification before they made their confidence estimates, and others did not. The results indicated that those who watched the identification videos of themselves demonstrated a higher confidence-accuracy correlation.

2.44.2.7 The Application of Estimator Variables: Expert Testimony

The primary application of estimator variable research is expert testimony about the psychology of eyewitness memory. With increasing frequency, psychologists are called upon to testify in criminal cases about the reliability of eyewitness identification. In the aforementioned survey of eyewitness experts (Kassin et al., 2001), the 64 experts surveyed reported being invited to testify on 3370 occasions. They agreed to testify in 1373 trials and actually testified in 960 trials. This activity represents a substantial increase over the results obtained in a previously published survey (Kassin et al., 1989). In this previous survey, experts reported being invited to testify in 1268 trials.

The typical content of expert testimony varies from jurisdiction to jurisdiction, and even from courtroom to courtroom within a jurisdiction, for judges have considerable discretion in determining what testimony will be allowed in a given trial. Generally speaking, experts discuss how memory works (e.g., the stages of memory, reconstructive processes), dispel myths about memory (e.g., memory does not work like a video recorder), and describe relevant estimator and system variables in the case that could influence memory. For example, an expert in a given case might discuss the influence of high stress, weapon focus, the ORB, and suggestive lineup instructions. Experts are not permitted to comment on the accuracy of the eyewitness.

Expert testimony about the psychology of eyewitness memory is in some respects controversial. Admissibility of the expert testimony varies considerably from state to state and within the federal court system. When expert testimony is not admitted, the single most common reason given is that the content of the testimony is merely a matter of common sense – a conclusion that is seriously challenged by empirical research (Schmechel et al., 2006). Some scholars (e.g., Konecni and Ebbesen, 1986; Elliott, 1993) have questioned the extent to which eyewitness studies, which are mainly conducted in the laboratory, generalize to actual crimes and therefore challenge the

appropriateness of expert testimony. Such critics have found themselves in the role of opposing experts on occasion. Although the vast majority of cases in which experts testify are criminal cases, and the expert is almost always proffered by the defense (Kassin et al., 2001), occasionally the prosecution will offer an opposing eyewitness expert. The experts surveyed in Kassin et al.'s study reported that in the 960 trials in which they testified, an opposing expert testified in 76 cases (8%). In such cases, the opposing expert might challenge the generalizability of the research, question the extent of expert agreement about certain factors, or challenge the defense expert's conclusions based on the literature. Another concern about expert testimony is its actual effect on the jury. Empirical investigations of the impact of expert testimony on juror decisions show a range of effects, including making jurors more skeptical about eyewitness identification (Leippe, 1995), enhancing juror sensitivity to some of the factors that influence identification accuracy (Cutler et al., 1990), and no effect at all (Devenport and Cutler, 2004). In all probability, the effects of expert testimony are complex and qualified by other factors (e.g., Leippe et al., 2004).

Controversial issues notwithstanding, expert testimony is becoming an increasingly popular safeguard against erroneous conviction in cases in which eyewitness testimony figures prominently. The quality of testimony rests on the foundation of eyewitness memory research on estimator and system variables.

2.44.3 System Variables Affecting Eyewitness Identification

2.44.3.1 Lineup Instructions

Considerable attention has been devoted to understanding the impact of lineup instructions on identification accuracy. In their seminal article, Malpass and Devine (1981a) examined the effect of biased and unbiased lineup instructions on identifications from target-present and target-absent lineups. By not containing an explicit option to reject the lineup, biased instructions implied that participants were to choose someone from the lineup, whereas unbiased instructions provided a no-choice option. Malpass and Devine found that accurate identifications were not affected by instruction type when the target was in the lineup (75% vs. 83% accurate for biased and unbiased instructions, respectively). In contrast, false identifications from target-absent lineups were significantly higher in the biased-instructions condition (78%) than in the unbiased condition (33%). Thus, biased instructions increased the likelihood of false identification without influencing correct identification rates.

Steblay (1997) meta-analyzed the studies examining the effects of lineup instructions on identification accuracy. Employing 18 different studies in her analysis, she found a clear, consistent pattern that replicated the results of Malpass and Devine (1981a). That is, with target-absent lineups, unbiased instructions led to fewer false identifications (35%) than did biased lineup instructions (60%). The rates of correct identification from target-present lineups were virtually identical for unbiased (54%) and biased (53%) instruction conditions. While the impact of biased lineup instructions on false identifications is generally accepted (Kassin et al., 2001), the effect of biased lineup instructions on correct identifications is less clear, as noted by Clark (2005), who re-analyzed the studies examined by Steblay (1997) and reached a different conclusion.

2.44.3.2 Blind Administration of Lineups

According to Wells and Olson (2003), the person who typically conducts the lineup is the police officer assigned to the case. This officer usually constructs the lineup as well (i.e., chooses the fillers and the position of the suspect). Although having the investigator assigned to the case conduct the lineup test may seem perfectly reasonable from the perspective of efficiency and police administration, many years of psychological research on experimenter bias and expectancy effects call into question the value of this practice. Put succinctly, the investigator, who knows which lineup member is the suspect, can advertently or inadvertently convey the correct answer to the eyewitness and therefore influence her identification decision. When evaluating a positive identification under these circumstances, it is impossible to know whether the identification is the product of the witness's memory for the perpetrator, influence by the investigator, or some combination of the two. Given that the purpose of the lineup is to test the hypothesis that the suspect is the perpetrator, ruling out alternative explanations for positive identifications, such as influence by the investigator, is highly desirable. Thus, for the same reasons that we ensure that our experimenters are blind to the participant's experimental condition – or our physicians are blind to the assignment of patients to treatment

versus placebo conditions in clinical trials – investigators would be well advised to ensure that lineups are conducted by investigators who do not know which lineup member is the suspect. This blind administration procedure allows the authorities to rule out investigator influence as an explanation for a witness's identification.

Although the need for blind lineups can be sufficiently established based on the vast literature on expectancy effects (Haw and Fisher, 2004), some eyewitness researchers have nevertheless empirically compared eyewitness identifications from blind versus nonblind procedures. In a study conducted by Garrioch and Brimacombe (2001), participants arrived in pairs and were assigned the roles of lineup administrator and witness to a crime. The lineup administrator was instructed on how to conduct the lineup, was told the position of the suspect, and was then instructed not to communicate this position to the witness. Unbeknownst to the lineup administrator, the lineups did not contain the perpetrator. Witnesses watched a videotaped crime and were reunited with their respective lineup administrators for the identification task. The results indicated that participants were more confident in their selection when they chose the target consistent with the administrators' expectations as compared to participants who chose an alternative lineup member. When asked if there was any outside influence that affected their decision, witnesses responded that there was none. Thus, even when lineup administrators were explicitly asked not to influence the witness, they were still able in some way to cue and influence the witness's identification decisions.

Phillips et al. (1999) compared blind versus nonblind administration in simultaneous and sequential lineups. Their study demonstrated the biasing influence of nonblind administration, but only for sequentially presented lineups. Haw and Fisher (2004) varied the amount of contact between the lineup administrator and the witness during the lineup test. They found that the witness was less likely to make a decision that was consistent with the lineup administrator's expectations if the amount of contact between the two parties was limited (the administrator was present but did not speak). This was true regardless of whether the lineup was simultaneous or sequential or whether the target was present or absent.

The use of blind administration raises some additional questions. For example, if a blind administrator shows a lineup to a witness and the witness makes a positive identification, is that investigator still blind? Should that investigator be allowed to show the lineup to another witness? There are often multiple eyewitnesses, so the impact of a positive identification on subsequent lineups is worthy of examination. Douglass et al. (2005) empirically examined this issue. They had participants, in the roles of lineup administrators, conduct the same target-absent lineup twice, once to a confederate witness and once to a participant witness. The authors were interested in determining if the lineup administrator's knowledge of the confederate selection would influence the selection of the second witness. Their results revealed that if the confederate witness selected a suspect with low confidence, then the lineup administrator influenced the participant witness to select the same person. Furthermore, even though the participant witness's selection was influenced, the influence was so subtle that independent observers could not detect it when watching the tainted identification procedure. Thus, it is clear that not only should lineup administrators be blind to the identity of the suspect, but they should also be replaced with another blind administrator after an identification is made, for then the administrator of the first lineup is no longer blind to the identity of the suspect.

In their recent review of both published and unpublished research on blind administration, Russano et al. (2006) concluded that the results are mixed, with some studies demonstrating the biasing effects of nonblind procedures and others failing to do so. Given the general acceptance of expectancy research, these mixed results are somewhat surprising. Russano et al. posit that the mixed results are in part due to the difficulty of effectively simulating investigations in the laboratory, the subtlety of the influence, the magnitude of the effect (which might be small), the use of students posing as lineup administrators (as opposed to more experienced police investigators), and, more generally, the dearth of research on the phenomenon.

2.44.3.3 Filler Selection

Fillers, which are sometimes referred to as foils or distractors, are innocent people included in the lineup with the suspect. Luus and Wells (1991) outlined some of the major functions of fillers in a well-constructed, fair lineup. Given that fillers, by definition, are known to be innocent, any selection of a filler is a known error, thereby giving the lineup administrator information regarding the accuracy of the eyewitness. The filler also serves as a control for

guessing: the suspect should not be chosen more often than each of the fillers if the witness has no memory of the culprit. A witness with no memory of the culprit should choose the suspect at a rate of 1/N (where N is the total number of lineup members) if the lineup is not biased. Another function of the fillers is to ensure that the identification is made based on memory rather than on logical deduction (e.g., the culprit had a beard and only one person in the lineup has a beard so that must be him).

There are at least two strategies utilized in selecting fillers: match-to-similarity-of-suspect (MS) and match-to-description-of-culprit (MD) (Luus and Wells, 1991). The former involves selecting fillers who physically resemble the suspect. The latter involves selecting fillers based on their match to the witness's description of the perpetrator. How do the different strategies for choosing fillers achieve these aforementioned purposes of having fillers in the lineup? Wells (Luus and Wells, 1991) explains the benefits of the MD approach over the MS approach. The MD approach protects against the witness identifying the suspect based merely on her memory for her description of the perpetrator. For example, if the witness remembers some unique features of the perpetrator and the suspect is the only one in the lineup that possesses those features, the witness can deduce which one is the suspect. Deduction, like guessing, is not the preferred cause of a positive identification. By contrast, using the MD strategy, fillers are chosen because they possess those unique features, and the witness cannot identify the perpetrator merely on the basis of those features, and the witness is therefore required to rely on memory when making an identification (the preferred cause of a positive identification).

Using the MS strategy does not provide this same protection against mistaken identification. In theory, the MS strategy poses great risk to the innocent suspect who was arrested because he looks like the perpetrator. Consider a situation in which an innocent suspect becomes a suspect because he matched the description of the perpetrator. Of course, the suspect will not perfectly resemble the perpetrator. There will be some natural variation in their physical characteristics. Now suppose that fillers are selected because they match the suspect (i.e., the MS strategy). Because the suspect did not perfectly resemble the perpetrator and the fillers did not perfectly match the suspect, it is reasonable to infer that the fillers will on average look less like the perpetrator than does the suspect. If the witness seeks to identify the suspect who looks most like the perpetrator, it will usually be the innocent suspect. Clark and Tunnicliff (2001) refer to this ironic consequence of the MS strategy as the backfire effect.

According to Luus and Wells (1991), the MD strategy does not suffer from the backfire effect because fillers are matched on the relevant descriptors provided by the witness. In the target-absent lineup, all the relevant features (i.e., those mentioned in the description of the perpetrator) should appear in all members. Thus, the MD strategy leads to similar amounts of correct identifications when the target is present and fewer false identifications when the target is not present, as opposed to the MS strategy.

Beneficial effects of the MD strategy, however, are not universally obtained. Lindsay et al. (1994) noted that the MD strategy is less effective when the description of the culprit is incomplete. This study tested three types of lineups: MS, MD, and biased MD. In the biased condition, the fillers were chosen so that they matched the features mentioned in the witness description but were maximally different in appearance to the culprit. For example, if hair color was not mentioned as a feature by the witness, then the distracters could have a hair color that differed from that of the suspect. This biased condition produced more false identifications of the suspect than the other two conditions. Thus, it appears that the MD is the best strategy as long as the fillers selected match the description and also match the appearance of the culprit on some general overall characteristics such as race, hair color, or presence or absence of facial hair.

2.44.3.4 Lineup Presentation

Considerable research has been devoted to the examination of various methods for presenting lineups. The most commonly examined presentation methods are simultaneous and sequential. In simultaneous lineups the witness is shown all of the lineup members at the same time and is asked which one, if any, is the perpetrator. In the sequential lineup the witness views lineup members one at a time and makes an identification decision (yes/no) for each lineup member as he is presented. The witness is not told in advance how many lineup members are in the lineup.

Why should performance differ as a function of lineup presentation method? Wells (1984) hypothesized

that the simultaneous method induces a cognitive process, known as relative judgment, in which the witness compares each of the lineup members in order to determine which one 'most' resembles his memory of the perpetrator. This process would lead a witness to compare each lineup member to the next using a process of elimination (e.g., "Number 2 looks more like the perpetrator than Number 1 does"). Given that one lineup member will inevitably better resemble the perpetrator than the other lineup members, the relative judgment strategy tends to produce positive identifications, whether the actual perpetrator is present or absent, thus increasing the likelihood of false identifications from target-absent lineups.

Recognizing the limitations of the relative judgment strategy, Lindsay and Wells (1985) developed the sequential method as a means of discouraging relative judgment processing and encouraging absolute judgment processing. In absolute judgment processing, the witness compared each lineup member in the sequence to his memory for the perpetrator and makes an identification decision on the basis of the memory-lineup member match. To test their hypotheses concerning simultaneous and sequential lineups, Lindsay and Wells (1985) staged thefts before 243 undergraduates and in the same sessions had eyewitnesses attempt identifications from thief-present or -absent photoarrays. Half were shown simultaneous and half were shown sequential photoarrays. When the thief was present, the percentage of correct identifications was comparable for simultaneous and sequential lineups (58% vs. 50%, respectively). When the thief was absent, simultaneous lineups produced more false identifications than did sequential lineups (43% vs. 17%).

To further test this theory, Lindsay (1991) compared each of the lineups and then asked people to report if they used a process of elimination (relative) or if the perpetrator popped out (absolute). He found that the accurate participants were more likely to indicate that they used absolute judgment in their decision process. In addition, some research has suggested that self-reported use of an absolute judgment process as opposed to a relative judgment process postdicts identification accuracy (Dunning and Stern, 1994).

How reliable is the effect of presentation method on identification accuracy? Steblay et al. (2001) addressed this question by conducting a meta-analysis of 23 studies comparing simultaneous and sequential presentation, nine of which were published and 14 of which were unpublished. In data from target-present lineups, participants were more likely to correctly choose the target (50% vs. 35%) and less likely to make a false rejection (26% vs. 46%) from simultaneous lineups. There were no differences between the two lineup presentation methods for filler choices. In target-absent lineups, sequential lineups garnered more correct rejections (72% vs. 49%) and fewer false identifications (28% vs. 51%). Overall, participants were more likely to positively identify a suspect from a simultaneously presented lineup than from a sequentially presented lineup (74% vs. 54%). Note that the effect of presentation was considerably larger on false identifications than on correct identifications. This means that, overall, sequential presentation produced identifications that were more diagnostic than did simultaneous presentation.

Although Steblay et al.'s (2001) meta-analysis reflects the state of the science concerning the effects of simultaneous and sequential presentation, the conclusion is not universally accepted. A critique of the meta-analysis by McQuiston-Surrett et al. (2006) identified some potentially important methodological issues that deserve consideration. Specifically, these authors observed that most of the significant results between the two procedures were produced in the same psychological laboratory. The inclusion of unpublished studies in the meta-analysis was also a concern for these authors. They call for greater attention to factors that may qualify the impact of sequential and simultaneous presentation.

The benefits of sequential lineups notwithstanding, not all researchers agree that the gains in lineup accuracy are due to relative versus absolute judgment processing, as originally proposed by Lindsay and Wells (1985). Clark and Davey's (2005) research found that identification decisions did not conform to predictions derived from relative- and absolute-judgment strategies. They included in the comparisons of simultaneous and sequential presentation conditions in which the targets were removed from the lineups without replacement. Clark and Davey reasoned that witnesses who adopt relative-judgment strategies would shift their identifications from the target to other fillers, whereas witnesses who adopt absolute-judgment strategies would shift their identifications from the target to lineup rejections. They found comparable degree of shifts from target identifications to filler identifications from simultaneously and sequentially presented lineups, thus casting doubt on the absolute-relative judgment explanations for the differences observed

due to simultaneous and sequential lineups. By contrast, Meissner et al. (2005) demonstrated that the benefits of sequential lineup may be explained by a criterion shift. More specifically, sequential lineups induce the use of stricter criteria, which results in fewer false identifications without lowering correct identifications.

2.44.3.5 Showups

A showup is a one-to-one confrontation between the witness and the suspect in which the witness is asked if the suspect was the person who committed the crime. Psychologists have typically suggested that showups are suggestive and unreliable (Yarmey et al., 1994, 1996; Lindsay et al., 1997). In the aforementioned survey by Kassin et al. (2001) 74% of the eyewitness experts agreed with the statement "The use of a one-person showup instead of a full lineup increased the risk of misidentification" (see **Table 1**).

There are two theoretical arguments against using showup. First, a showup, by its very nature, provides only one option to the witness, making it difficult to distinguish identifications made from memory versus guessing, deduction, or social influence. A properly conducted photoarray provides better safeguards against these alternative explanations for positive identifications.

The second argument is that innocent fillers in the lineup that closely match the witnesses' description of the culprit improve lineup performance (Luus and Wells, 1991; Wells et al., 1994), as described earlier. It is thought that having fillers in the lineup that are reasonable alternatives forces the witness to closely scrutinize the lineup members and make more accurate decisions.

Do showups produce more false identifications than lineups? Gonzales et al. (1993) had a perpetrator enter the classroom and sit in the back row and steal the purse off of the instructor's desk. Participants were later shown either a live lineup or a live showup. In the showup condition, 30% correctly identified the perpetrator, where 67% correctly chose the perpetrator in the lineup condition. Furthermore, in the target-absent condition 92% in the showup condition correctly stated the perpetrator was not there, as compared to 38% in the lineup condition. Thus, the showups did not result in more mistaken identifications than lineups; however, Yarmey et al. (1994) have argued that when you take into account the guessing rate (15% vs. 16% showups and lineups, respectively), lineups produce more accurate lineup identifications with no differences in false identifications.

To further examine the difference in lineup versus showup performance, Steblay et al. (2003) meta-analyzed the existing 12 studies, which included 3013 participants. Overall, they found nonsignificant differences in identification performance between showups and photoarrays. Indeed, the rates of correct identifications from target-present lineups were nearly identical (47% and 45%, respectively). Contrary to expectation, there were significantly more correct rejections from showups than from lineups (85% and 57%, respectively). At least based on these data, therefore, the conclusion that showups produce more false identifications than lineups is not supported. Nevertheless, the first argument raised still holds: Showups provide poor safeguards against guessing, deduction, and social influence, all of which can explain positive identifications.

2.44.3.6 Postidentification Feedback

Postidentification feedback is any statement made to an eyewitness after he or she has selected a suspect from a lineup (Wells and Bradfield, 1998). For example, if Eileen Eyewitness picks out Scottie Suspect from a lineup, the administrator may say something like "Great, you got 'em." This comment may seem innocuous, but is it? Luus and Wells (1994) conducted one of the first studies that systematically investigated the effect of postevent feedback on retrospective certainty. Pairs of subjects watched a staged theft, made individual lineup identifications from a photo spread, and were subsequently given bogus postidentification feedback regarding their co-witnesses' alleged identification decisions. It is important to note that all of the witnesses who made positive identifications were incorrect, because they were all exposed to target-absent lineups. Although Luus and Wells gave nine different types of feedback, for the sake of brevity we will only address the confirmatory feedback (i.e., when the witness was told that they had selected the same person as the co-witness). Participants who were given confirmatory feedback expressed more confidence in their identification than witnesses given no feedback (8.77 vs. 6.90 on a nine-point scale) when later interviewed by confederates posing as police officers. It is important to note that this confidence inflation occurred even though participants were given no indication that the identification

was correct. In the second experiment, participants role-playing jurors rated the (inaccurate) witnesses who received the confirmatory feedback as the most credible. Thus, confirmatory feedback increased witness confidence in their identifications and made their testimony seem more believable to jurors, and this occurred independently of the accuracy of the eyewitnesses.

In a related study, Wells and Bradfield (1998) examined the effects of postidentification feedback from a lineup administrator on retrospective eyewitness certainty. Their participants watched a clip from a security camera video and attempted to identify the target from a target-absent lineup. Following their identifications, some participants were told that they had selected the right person, while others were given no such feedback concerning their identifications. Participants who were given feedback reported that they paid more attention to the perpetrator, were more certain in their identification, and had a clearer view when compared to the subjects who were not given feedback.

The effects of postidentification feedback are robust and reliable. They have been demonstrated using a wide variety of dependent measures, as noted. The effects have been found to persist using a 1-week retention interval (Neuschatz et al., 2005) and have been observed in witnesses of varying ages (Hafstad et al., 2004).

Can the detrimental impact of postidentification feedback be mitigated? Wells and Bradfield (1998) suggested that eyewitnesses, at the time they make their identifications, do not make online judgments about how good of a view they had, how much attention they paid, or how certain they are in their identifications. When they are later asked about these issues, they base their responses on what is accessible in memory. Because participants do not make online judgments, they are forced to infer their confidence from the feedback that was given to them. To illustrate, an eyewitness might infer that if she was told that she was correct, she must have had a good view, paid attention, and been confident in her decision. Wells and Bradfield proposed that the postidentification feedback effect could be eliminated by forcing participants to think about their confidence, attention, and view at the time of the identification, thus giving them memory traces for these internal cues without having to rely on feedback from an external source.

To test this notion, Wells and Bradfield had participants give confidence ratings before and after the feedback manipulation. They argued that the confidence question would force participants to think about how certain they were and how good a view they had before they received the postidentification feedback. The results indicated that the feedback effect was mitigated in those participants who received the confidence question prior to the feedback. Wells and Bradfield referred to this as the confidence prophylactic effect. One problem with this effect is that, although it works if eyewitnesses are questioned immediately, its preventative effects seem to be short-lived. Neuschatz et al. (2007) found that the confidence prophylactic effect worked immediately but not after a 1-week retention interval. Given that the length of time between identification and trial is normally much longer than 1 week, the confidence prophylactic effect might not be an adequate safeguard against confidence inflation due to postidentification feedback.

Neuschatz et al. (2007) examined whether inducing suspicion about the postidentification feedback weakened its effect. Suspicion is the orientation in which the perceiver maintains the possibility that multiple causes may be influencing the actor's behavior and that the actor may be hiding something that might discredit the meaning of that action (Fein et al., 1990). Suspicion inducement has been found to reduce biases in studies examining the impact of prejudicial pretrial publicity and inadmissible evidence on jury decision making (Fein et al., 1997). In the experiment conducted by Neuschatz et al. (2007), participants viewed a video and were asked to identify a suspect from a target-absent photo lineup. Afterwards, some participants were informed that they selected the actual suspect, while others were given no information. Either immediately or after a 1-week retention interval, participants were led to another room by a different experimenter. Some participants who received feedback were given reasons to entertain suspicion regarding the motives of the lineup administrator. Subsequently, participants answered a questionnaire regarding their identification. The authors hypothesized that making the participant suspicious would lead the participant to question the validity of the feedback, thus adjusting their confidence in their witnessing experience. Neuschatz et al. found that suspicious perceivers did not demonstrate the confidence inflation effects typically associated with confirming feedback.

In summary, research converges on the conclusion that postidentification feedback influences

eyewitness confidence and retrospective reports of the conditions under which they witnessed the event. Postidentification feedback, therefore, should serve to further attenuate the relation between eyewitness confidence and identification accuracy. Some research suggests that the postidentification feedback effect can be mitigated by assessments of confidence prior to postidentification feedback (at least when retention intervals are brief) and the inducement of suspicion concerning the source of the postidentification feedback.

2.44.3.7 The Application of System Variables: Lineup Reform

The aforementioned perfect storm resulted from, on the one hand, growing documentation of miscarriages of justice resulting from mistaken eyewitness identification and, on the other hand, a readiness on the part of eyewitness researchers to offer practical advice based on a large body of research on lineup techniques. What followed was a series of published recommendations, including the first white paper endorsed by the American Psychology-Law Society (Division 41, American Psychological Association) (Wells et al., 1998) and an influential United States Department of Justice report commissioned by Janet Reno, Attorney General at the time (Technical Working Group on Eyewitness Evidence, 1999). Following these published recommendations, the State of New Jersey was the first in the nation to adopt new guidelines for lineups. The guidelines adopted by the New Jersey Attorney General included such recommendations as (1) instructions that warn jurors that the perpetrator might not be in the lineup; (2) use of the match-to-description technique for selecting fillers; (3) sequential presentation of photoarrays; (4) the use of blind lineup administration; and (5) the assessment of eyewitness confidence immediately after the lineup and before witnesses are given confirming or disconfirming evidence about their identifications. These recommendations follow directly from the research literature. The State of North Carolina was the second state to develop and adopt new recommendations, and theirs were very similar to those adopted by New Jersey. Other states (Illinois, Minnesota) have experimented with these new techniques. Other states and cities (e.g., Virginia, Florida, Wisconsin, Boston, Seattle) are in the process of studying or implementing lineup reform.

Just as continuing research on estimator variables continues to inform expert witnesses and therefore lawyers, judges, and juries, research on eyewitness identification continues to inform psychologists who work with police and prosecutors to reform their identification procedures. Consider, for example, the influence of two recently published findings and the implications of these findings for practice. First, based on the research of Haw and Fisher (2004) described earlier, the amount of interaction between the lineup administrator and the witness should be held to a minimum. Second, the lineup administrator should not conduct the same lineup for witnesses tested in sequence. Being privy to the selection of one eyewitness can compromise the protection afforded by having administrators blind to the identity of the suspect, as the first identification may allow them to develop their own hypothesis as to the suspect's identity (Douglass et al., 2005).

2.44.4 General Conclusions

The foregoing review serves to illustrate how basic research on human memory can be applied to real-world phenomena, eyewitness memory in this case. Many years of research on eyewitness memory have positioned eyewitness researchers to offer practical advice in courtrooms and to police investigators charged with the responsibility of creating and administering lineups. The eyewitness research draws from traditional theories and methods of cognitive and social psychology and ultimately informs those disciplines in return.

Ideally, the next generation of eyewitness research will draw upon lessons learned in the field. The adoption of techniques developed and tested in the laboratory for use in the field should reveal new problems and questions that could not have been anticipated by laboratory researchers. For example, both authors of this chapter have served as expert witnesses in cases involving eyewitness identification. When providing these services, both authors have encountered new questions – potential variables – that can be brought back to the laboratory for further investigation. To illustrate, the first author recently worked a case in which the witness was given six simultaneous lineups, each with a different suspect, in order to identify two culprits. The witness chose four suspects out of the six lineups with varying degrees of confidence, even though there were only

two culprits. This case raises many interesting questions. What effects do multiple lineups with multiple suspects have on the accuracy of the witness? How does the presence of two false identifications affect investigators', attorneys', and jurors' evaluations of the eyewitness? How 'should' these known false identifications affect evaluations of the eyewitness? Similarly, field studies and actual implementation of new lineup techniques can reveal questions and issues that have not been examined in the laboratory, thus suggesting new directions for laboratory research. With respect to the implications for justice, the application of eyewitness research to police practices and trials should serve to reduce identification errors and ultimately reduce the likelihood of erroneous conviction. Though much of the identification research seems to focus on one type of error (false identification), the research also has implications for improving the likelihood of correct identification and improving the extent to which positive identifications are diagnostic of guilt as well.

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2.45 Prospective Memory: Processes, Lifespan Changes, and Neuroscience

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Prospective memory involves remembering to perform actions in the future. Thus, remembering to buy a loaf of bread on the way home, remembering to go to the dentist for an appointment, and remembering to actually attach an attachment to an email all are examples of prospective memory. Prospective memory has often been contrasted with retrospective memory (we explore this distinction in more detail in a subsequent section), which is what is typically studied in the laboratory. Remembering the plot of a movie that you saw 2 weeks ago and remembering a list of words presented in an experiment are examples of retrospective memory.

2.45.1 The Importance of Prospective Memory in Everyday Life

An interesting feature of prospective memory is that it is prevalent in everyday life and central to normal functioning, and yet it is an area that until recently has been neglected by memory researchers. In

reflecting on the activities in our typical day, it is easy to realize the enormous number of prospective memory demands that permeate our lives. From remembering to take vitamins and medication in the morning to remembering meetings, appointments, and errands throughout the day, our lives are full of prospective memory demands. Consistent with this impression that prospective memory demands are ubiquitous, Crovitz and Daniel (1984), in a study in which they asked students to record in a diary all instances of forgetting over a 1-week period, found that about half of the reported instances of forgetting were prospective in nature.

Not only do prospective memory demands permeate our lives, but successful remembering is also critical to normal and efficient functioning. Consider that one-third of older adults take three or more medications on a regular basis (Morell et al., 1997). Problems in remembering to take these medications could have serious health consequences and could threaten independent living. Consider also that prospective memory demands are often the cause of mistakes and accidents at work (Reason, 1990). Indeed, Nowinski et al. (2003), in examining voluntary reports of cockpit incidents from pilots to the Aviation Safety Reporting System, found that 74 of the 75 memory failures in their sample were prospective in nature. From the other side of airline safety, imagine the consequences of prospective memory failure for a busy air traffic controller, who gets the thought to reroute an airplane but cannot do so immediately because she is engaged in another activity and therefore must hold on to the intention until she is free. As another example, despite the best intentions of conscientious surgical teams, roughly once a year in a large hospital, they accidentally leave foreign instruments such as sponges and clamps in a patient. The patient shown in **Figure 1** complained of abdominal pain and nausea 8 months after a hernia surgery. As you can see, a scan revealed that the surgical team had forgotten to remove a 16-cm clamp from his abdominal area.

More generally, Tulving (2004) theorizes that a forward-looking mind that is capable of imagining and anticipating the future is critical to human survival. He assumes that this subjective and conscious apprehension of the future is mediated by the episodic memory system, and he labels this ability proscopic chronesthesia. Moreover, he believes that this ability is unique to humans and that the evolution of this ability was necessary for the creation of human culture. Prospective memory is among the important functions of chronesthetic consciousness. The idea here is that basic survival as well as rich human-like social relationships benefit from those who are capable of appreciating the future, planning for it, and later remembering to perform planned actions.

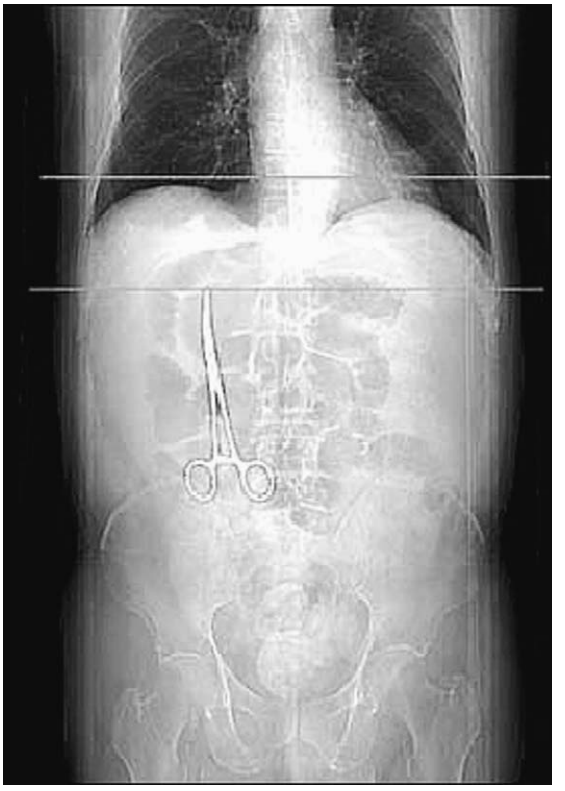

Figure 1 Scan showing a 16-cm clamp left in the abdominal area of a patient. From Dembitzer A and Lai EJ (2003) Retained surgical instrument. *N. Engl. J. Med.* 348–228.

In the 1980s, a few researchers (e.g., Harris, 1984; Craik, 1986) started proposing that the retrospective memory literature had not addressed fundamental issues in prospective memory and, as such, alerted researchers to the gap in our understanding of prospective memory. As can be seen in **Figure 2**, the number of articles and chapters on prospective memory (collapsed over 2-year intervals) has risen dramatically since that time. The increased interest has been driven by a number of factors, including the realization that prospective remembering is critical to our everyday leisure and work lives, the growing awareness that important components of prospective memory tend not to be studied in the typical retrospective memory experiment or captured in conventional neuropsychological assessments of memory, the development of laboratory paradigms for studying prospective

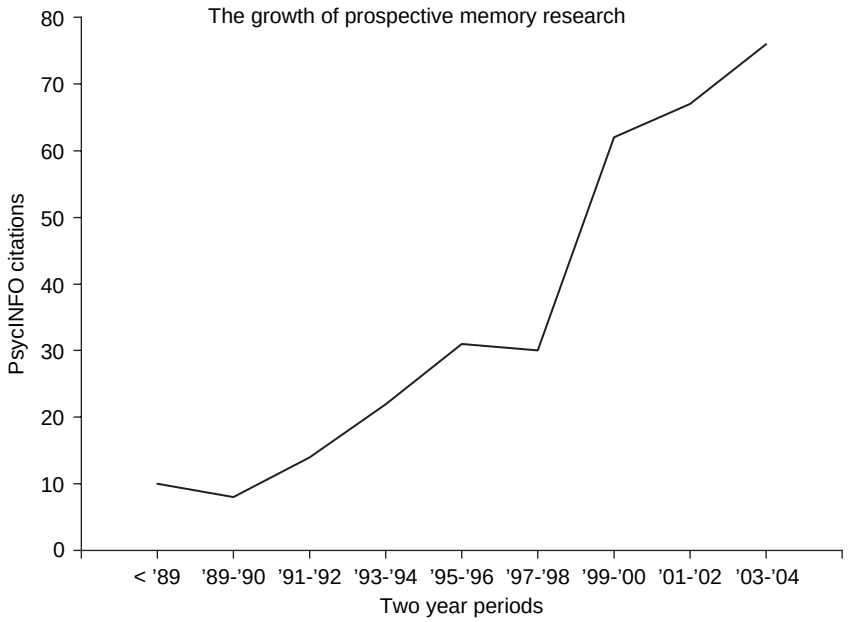

Figure 2 References to prospective memory over recent years in the PsychINFO database. From Marsh RL, Cook GI, and Hicks JL (2006a) An analysis of prospective memory. In: Ross BH (ed.) *The Psychology of Learning and Motivation*, Vol. 46, pp. 115–153. San Diego: Academic Press.

memory, theoretical progress on the cognitive processes that support prospective memory as well as interest in how these processes change across the lifespan, and the development of imaging techniques for understanding the neural basis of prospective memory. We focus on these factors in this chapter.

2.45.2 Paradigms for Studying Prospective Memory

By and large, explicit retrospective memory tasks involve presenting people with materials to learn and then, at some later point, putting the participants in what Tulving (1983) calls a retrieval mode and directing them to search memory for the previously learned information. For example, in the standard cued recall task, participants might be asked to learn pairs of items (e.g., dog/grass, table/binder, etc.). After a delay, the experimenter presents the first member of the pair and explicitly asks the participants to search memory for the associated second member of each pair. On the surface, many prospective memory tasks resemble this cued-recall scenario. Consider, for example, remembering to give your friend Patty a message. It is likely that you form an association between Patty and the message (i.e., Patty/message), and then after a delay, when Patty occurs, you need to retrieve the message. A major difference between this and the retrospective cued recall task, however, is that in the prospective memory task no one puts the participant in a retrieval mode and asks her or him to search memory when the target cue occurs (i.e., Patty). Instead, upon seeing Patty, successful remembering requires that the participant remember to retrieve the intention on her or his own. It is this feature of prospective memory that led Craik (1986) to characterize prospective memory as being especially high in self-initiated retrieval. Thus, in designing a research paradigm for studying prospective memory, it is critical to include this self-initiated component of requiring subjects to remember on their own (see McDaniel and Einstein, 2007, Chapter 1, for additional defining features of prospective memory tasks).

2.45.2.1 Nonlaboratory Paradigms

The earliest methods for investigating prospective memory were conducted outside of the laboratory. For example, Meacham and Singer (1977) asked college students to return postcards on specified days and found, among other results, that stronger incentives led to better prospective memory. Other studies (West, 1988; Maylor, 1990) asked subjects to telephone the experimenter at particular times. Another

approach is to examine the success with which people remember to carry out their own intentions. For example, Marsh et al. (1998b) asked subjects to list their planned activities for the next week (along with the importance of each). One week later, they asked them to indicate which intentions had been carried out and which had not (and to try to explain why these were not performed). In the medication adherence literature, researchers have asked people to adhere to their medication regimen and then put their pills in electronic medication bottles that record the date and time (over a 6-month period) every time the bottle cap is removed (see Park and Kidder, 1996, for a description). As reflected in these studies, nonlaboratory paradigms have the potential to examine prospective memory under highly naturalistic conditions.

One limitation of this approach, however, is that it is difficult to assess and/or control the strategies that subjects use in particular situations. For example, in the Meacham and Singer (1977) postcard study, some of the student subjects may have remembered using purely cognitive strategies, others may have used calendars, and still others may have given the post-cards to their parents to return for them. Thus, it is difficult to hone in on the mechanism by which incentives improve prospective remembering. Imagine also comparing older and younger adults and finding that the older adults remember more often than the younger adults (a typical finding in naturalistic studies; see Henry et al., 2004). This type of paradigm does not allow you to determine whether the better prospective memory for older adults was a result of more effective cognitive processes related to prospective memory, greater use of external aids, or both.

In recent years, researchers have been creative in elaborating nonlaboratory paradigms, and this has enabled them to begin exploring these processes. Kvavilashvili and Fisher (2007), for example, asked subjects to remember to call the experimenter 1 week later. They additionally asked them to record in a diary all thoughts related to the prospective memory intention over the 1-week period. Among other findings, Kvavilashvili and Fisher found that related cues (such as walking past a telephone pole) tended to spontaneously trigger thoughts of the intention. Sellen et al. (1997) gave their participants, all of whom were employees working in a single building, a prospective memory intention to perform for several days (e.g., to perform an action whenever they were in a particular room in the building). Moreover, the participants wore badges and were instructed to click their badge whenever they thought of the intention. There were sensors in the building that enabled the researchers to determine the location of the badge when it was clicked. Interestingly, participants were more likely to think of their intention when they were in transition (e.g., walking from one room to another) than when they were settled in a particular room (i.e., engaged by a task). Although these nonlaboratory paradigms have advantages over laboratory techniques in the sense that they tend to more closely approximate real-world prospective memory demands, ultimately they do not allow the precise control over independent and extraneous variables that is afforded by laboratory techniques. We now outline the basic laboratory paradigm that has been used.

2.45.2.2 Laboratory Paradigms

The essence of laboratory tests of prospective memory has been to busily engage participants in an ongoing task and to give them an intention to perform at some later time (see **Table 1** for the major

Table 1 A typical laboratory paradigm for studying prospective memory

1	Present participants with instructions and practice trials for an ongoing task (e.g., pleasantness rating).
2	Present participants with the prospective memory (PM) instructions (e.g., press a designated key whenever you see the word 'rake' in the context of the ongoing task).
3	A delay is introduced during which participants perform other activities (e.g., do other memory tasks and/or fill out demographics forms).
4	Reintroduce the ongoing task (pleasantness rating) without reminding participants of the PM task.
5	The PM target ('rake') occurs several times in the ongoing task, and PM performance is measured by the proportion of times participants remember to press the designated key when the target occurs.
6	To verify that forgetting was a result of PM failure rather than retrospective memory failure, participants are queried at the end of the experiment for their memory of the task demands.

From Einstein GO and McDaniel MA (2005) Prospective memory: Multiple retrieval processes. *Curr. Dir. Psychol. Sci.* 14: 286–290.

phases of a typical experiment, and see Einstein and McDaniel, 1990, for a specific example). Successful prospective memory requires that one remembers to perform an intended action (the prospective component) and also the contents of the intention (i.e., that the target item is 'rake' and the particular response key to press; the retrospective component). In explicit retrospective memory tasks, experimenters challenge the retrospective component. That is, they present participants with a body of material and then test how much is retained. In prospective memory tasks, the retrospective content is usually kept simple, and the question is whether participants will remember to perform the action at the appropriate moment or time period. This is done so that one can be fairly certain that omissions are the result of prospective memory failures as opposed to forgetting the contents of the intention. Indeed, it is important to verify this by testing participants at the end of the experiment for their memory of the prospective memory task demands. Prospective memory failures occur when participants fail to perform the intended action and yet later show complete memory for the task demands (i.e., the retrospective component).

This basic paradigm seems to capture the processes that are involved in many everyday prospective memory demands. For example, consider the prospective memory task described earlier: the task of remembering to give your friend Patty a message. After forming the intention, there is a delay during which we become engaged by the demands of life (i.e., the ongoing task), and the interest is in whether we will remember to give the message when we later see Patty (i.e., the prospective memory target). Within this general paradigm, researchers have manipulated a number of variables, including the emphasis on the ongoing and prospective memory task (Marsh et al., 2005), whether the cue for initiating the action is an event, a time, or an activity (e.g., Einstein et al., 1995), the nature of the cue (e.g., whether the cue is distinctive; McDaniel and Einstein, 1993), the length of the delay (e.g., Hicks et al., 2000), and the demands on the participant while encoding the intention and at the point of retrieval (e.g., Einstein et al., 1997; Marsh and Hicks, 1998).

Despite the widespread use of some variation of this basic laboratory paradigm, it is important to realize that it does not capture all real-world prospective memory processes. For example, planning is minimized as the experimenter tells the subject when to perform the action. Also, Dismukes (2007) points out that many everyday prospective memory demands, unlike those in laboratory tasks, are embedded in well-learned and highly sequenced routines. For instance, a pilot's typical sequence of actions prior to take off may be to perform a checklist of actions, then set the flaps to take-off position, and then taxi to the runway. For an experienced pilot, this sequence has been performed thousands of times in just this order, and the completion of the checklist and the perceptual environment prior to taxiing are strong cues for setting the flaps to the take-off position. There has been little research examining this kind of heavily cued habitual prospective memory task or what happens on those rare occasions when the action must be performed out of sequence (e.g., when weather conditions require that the pilot delay the setting of the flaps until after taxiing, when the normal kinesthetic and perceptual cues are no longer present). In theory, however, these kinds of conditions can be created in the laboratory either through training or by taking advantage of long-standing habits. Thus, while it is clear that existing tasks have been and continue to be useful for understanding basic processes involved in the encoding, storage, and retrieval of prospective memories, we look forward to the development of other laboratory paradigms for examining prospective memory under a broader set of conditions.

2.45.3 Varieties of Prospective Memory Tasks and How They Are Measured

2.45.3.1 Event-Based Prospective Memory

Although there are some grey areas when defining prospective memory tasks (see McDaniel and Einstein, 2007, Chapter 1), the field seems to have focused the research on three main types of tasks. The lion's share of the research has examined event-based prospective memory in which the rememberer offloads the intention onto some external environmental cue or cues. An example of an event-based task is the one described in the previous section of pressing a key when the target word 'rake' is encountered while performing an ongoing task. A real-world example would be the task of stopping to buy stamps when driving by the post office. Usually performance on an event-based task is measured as the proportion of cues detected and responded to in the manner requested when the intention was formed. Cues can either be specific or general, such as responding to particular words and constructs or to more general categories of items such as fruits or U.S. presidents. The responses could be as simple as marking a response sheet in a particular way, pressing a special key on a keyboard, or rapping on the table when an item is detected.

2.45.3.2 Time-Based Prospective Memory

Another form of prospective memory has been labeled time-based intentions because the intended actions relate to time in some way. The intention could be a relative measure, such as returning a phone call in 30 min, or it could be related to clock time, as in attending a meeting at 1.30 p.m. (note that if the target time were associated with an event or activity and one of these features triggered the intention rather than the monitoring of time, it would not be classified as a time-based task). Not much is known about the mechanisms underlying time-based prospective memory. Most of the work that has been done appeals to a test-wait-test-exit model (see Kvavilashvili and Fisher, 2007). Here the rememberer executes a time check, which is presumably on the first several occasions too early and thus necessitates a cyclical waiting period before another time check is made. As Kvavilashvili and Fisher have so eloquently stated, the problem with this model is that it does not specify what causes a person to engage in a time check in the first place. They conducted a diary study with a long-term time-based intention and found that many remindings were related to chance encounters with objects and language that were direct reminders of the time-based intention. They also found that many such retrievals of the time-based intention came to mind unbidden. We do know that the most successful individuals at time-based prospective memory tasks check the clock frequently in the period just prior to a required response (Einstein et al., 1995). However, that still does not specify what psychological process is responsible for the clock check in the first place, and this is especially true when a participant records in a diary that there was not an external or internal trigger of the time-based intention. Like event-based prospective memory, how many responses are successful is the usual dependent variable, although some metric of being early versus late is also a common variation. Of course, when measured, the distribution of clock checking can also be very informative as well.

2.45.3.3 Activity-Based Prospective Memory

Finally, the third common form of prospective memory measures what is called activity-based prospective memory (e.g., Schaeffer et al., 1998). With this type of intention, people intend on doing one activity after finishing another one. For example, intending on walking the dog after the evening news would represent an activity-based intention. Although this might seem to be a habitual intention, whether something is novel or habitual depends on the frequency with which it is carried out, and this applies equally well to event-based and time-based tasks. There have not been many experimentally based studies on activity-based prospective memory, probably because there is some theoretical ambiguity about whether this is just a special form of an event-based task, with the conclusion of one task serving as the event that signals responding. However, this ambiguity highlights a very important point concerning prospective memory, namely, the rememberer can form an intention in any of these three different ways, and each will have varying success depending on tasks and conditions that prevail on that occasion. Take the simple intention of purchasing a birthday card. One could write oneself a note and hope that seeing the note was sufficient to accomplish the task (an event-based task). One could formulate the intention to run to the store right after lunch in order to carry out the purchase (an activity-based intention). Or, one could plan a specific deviation of one's day and commit to leaving the office at 5.00 p.m. to carry out the task (a time-based task). All three formulations are prospective memory tasks, but they will vary in the success rate depending on the individual and the conditions surrounding the performance interval (e.g., seeing the note but being late for work or for a class). The important point is that a desire to accomplish some goal can be linked to various future contexts in a variety of ways depending on whether the rememberer gives some serious consideration to what formulation will be best (for a more detailed treatment of prospective memory and contextual associations see Marsh et al., in press).

2.45.4 Retrieval of Prospective Memories: Retrieval Without an Explicit Request to Remember

A central problem in prospective memory is in understanding how we initiate an intended action at the appropriate moment. This is an interesting question because models of retrospective memory retrieval (e.g., recall and recognition) start with the assumption that people have been put in a retrieval mode and have been explicitly directed to search their memory for previously encoded information. As discussed earlier, prospective memory is different

in the sense that we form an intention to perform an action at some later point and then get busily involved in other activities. How, then, do we remember to perform the action in response to that event? Because the majority of the research has investigated retrieval on event-based tasks, this is our focus in this section. Following, we consider two opposing theories that address this question and then present a compromise view. (For those interested in research and theorizing on retrieval of time-based prospective memories, we recommend the following sources: Harris and Wilkins, 1982; Harris, 1984; Ceci and Bronfenbrenner, 1985; Block and Zakay, 2006; Kvavilashvili and Fisher, 2007.).

2.45.4.1 Attentional Monitoring Theory

The attentional monitoring view assumes that some of our attentional and/or working memory resources need to be devoted to monitoring the environment for the target event in order for retrieval to occur. According to this view, successful prospective memory requires that an attentional system like Shallice and Burgess's (1991) supervisory attentional system monitors the environment in light of our prospective memory demands. When a target event is detected, this system interrupts the ongoing activity, evaluates whether the conditions for performing the action are appropriate, and if so initiates the appropriate actions.

The most thoroughly developed statement of this view is Smith and Bayen's (Smith, 2003; Smith and Bayen, 2004, 2006) preparatory attentional and memory (PAM) theory. According to this theory, when we form an intention, we initiate a capacity-consuming preparatory attentional process that monitors environmental events by initiating recognition checks to determine whether the events are instances of the prospective memory target. For example, consider the task presented in **Table 1**, in which the prospective memory task is to press the slash key when the target word 'rake' occurs while performing the ongoing pleasantness rating task. According to the PAM theory, preparatory processes involve initiating a recognition check for each item to determine whether it is an instance of the target event and could also include rehearsing the target event. According to this theory, forgetting occurs when people fail to maintain their attention on the intention and therefore fail to initiate recognition checks, or when there is a recognition failure (see Smith and Bayan 2004, 2006, for a multinomial model that measures these two parameters). Smith takes a strong position on the necessity of monitoring for successful prospective memory, arguing that "capacity must be devoted to the prospective memory task in the form of monitoring before a target event occurs if the target is to be recognized as a signal or an opportunity to perform the prospective memory action" (Smith, 2003, p. 359).

Because this view assumes that people are using attentional resources to monitor the environment for target events when they have a prospective memory intention, this view clearly predicts that adding a prospective memory task to an ongoing task should produce task interference (i.e., slowing on the ongoing task). Continuing with the example, the idea is that the pleasantness ratings for nontarget items will be slower because subjects are additionally monitoring these items for the prospective memory target event while they are performing the pleasantness ratings. Smith (2003, Experiment 1) provided strong support for this view when she found that participants were approximately 300 ms slower in performing a lexical decision task when they were also performing a prospective memory task compared with when they were performing the lexical decision task alone. Task interference to the ongoing task has been found repeatedly and with other types of ongoing tasks (Smith and Bayen, 2004) and in other labs (Marsh et al., 2003; Einstein et al., 2005). Moreover, Smith (2003) found that individuals who showed more task interference (i.e., more slowing on the ongoing task as a result of performing the prospective memory task) had higher prospective memory, thereby indicating that monitoring is important for prospective memory retrieval.

The monitoring view is also supported by research showing that dividing attention during retrieval lowers prospective memory (Einstein et al., 1997; Park et al., 1997; Marsh and Hicks, 1998). Marsh and Hicks have shown that divided attention tasks that required central executive resources, but not ones that increased the demands of articulatory suppression or visuospatial involvement, reduced prospective memory performance. A straightforward interpretation of these results is that dividing attention compromises monitoring processes that are needed to identify prospective memory targets.

2.45.4.2 Spontaneous Retrieval Theory

A different way to think about prospective memory retrieval is to assume that the occurrence of the target event can trigger remembering even when no

resources are devoted to the intention at the time that the target event occurs (Einstein and McDaniel, 1996; McDaniel and Einstein, 2000; McDaniel et al., 2004; Einstein et al., 2005). Much like walking by a friend can reflexively trigger the recollection of an amusing past episode with the friend (when there was no prior intention to remember that episode prior to encountering the friend), according to the spontaneous view, it is the occurrence of the cue that triggers processes that lead to retrieval of the intended action. Within this view, then, monitoring or preparatory attentional processes are not necessary for successful retrieval.

In this section, we briefly review two theoretical mechanisms by which spontaneous retrieval can be accomplished. One theory, called the reflexive-associative theory (Einstein and McDaniel, 1996; McDaniel and Einstein, 2000; McDaniel et al., 2004), assumes that relatively automatic processes can underlie prospective memory retrieval. The idea is that during planning, people form an association between the target cue and the intended action (e.g., an association between the target word 'rake' and the action to press the slash key). Later, when the target event is processed in the context of the ongoing task, an automatic associative system (like the one proposed by Moscovitch [1994] and presumed to be mediated by the hippocampal system) retrieves the intended action and delivers it to consciousness. According to Moscovitch, the hippocampal system is an associative module that mediates associative encoding and associative retrieval. If we have formed a good association between the target cue and the intended action, and if the cue is fully processed at retrieval, then this associative module should rapidly, obligatorily, and with few cognitive resources deliver the intended action (press the slash key) to consciousness.

There are several results that are consistent with this theory. One comes from introspective reports of participants who often remark that the thought to perform the intended action appeared to pop into their mind while performing the ongoing task (Einstein and McDaniel, 1990). Also, Reese and Cherry (2002) found very little evidence that participants were monitoring while performing an ongoing task. They probed participants at various points during the ongoing task and asked them to indicate what they were thinking about. Both younger and older adults rarely indicated thinking about the prospective memory task (less than 5% of the time, compared with reporting thoughts of the ongoing task about 69% of the time). Even so, prospective memory performance was at a reasonable level (about 60%). Also consistent with the spontaneous retrieval theory is the finding that subjects who demonstrate no costs or task interference when performing an ongoing task (and are therefore unlikely to be monitoring) can still exhibit very high levels (93%) of prospective memory (Einstein et al., 2005, Experiment 4). We should also note that the previously described findings of negative effects of dividing attention on prospective memory do not unambiguously argue against spontaneous retrieval. At this point in our research, it is not clear exactly how dividing attention affects performance. For example, dividing attention may interfere with full processing of the target event, which may be essential for good spontaneous or associative retrieval (Moscovitch, 1994). Or, it may not interfere with retrieval of the intention but, instead, may increase working memory demands to such a degree that participants have difficulty selecting the retrieved intention and scheduling the intended action while it is still activated in working memory (Einstein et al., 1997).

The discrepancy plus attribution theory also explains how retrieval can occur spontaneously and in the absence of monitoring. It assumes that the processing of the target event leads to a feeling that there is something significant about the event, and this in turn leads to a search of memory for an explanation of its significance. Depending on how well the intention was encoded, this search at retrieval can lead to the realization that the event is a cue for an intended action. According to Whittlesea and Williams (2001a,b), people chronically evaluate the quality and coherence of their processing, and they are sometimes sensitive to the discrepancy between the actual quality of processing and the expected quality of processing in that context. This sense of discrepancy is alerting and begs for an explanation, which in the context of a recognition task could lead to the interpretation that the item has been seen before. McDaniel et al. (2004) proposed that these processes can also explain prospective memory retrieval. Specifically, the idea is that the target event, on the basis of its initial processing during the encoding of the intention, will, when it appears again in the context of the ongoing task, be processed more fluently (than other items in the ongoing task), and this discrepancy is likely to elicit a sense of significance. In turn, this noticing can lead to a search of memory for the source of the significance, and this can lead to

the realization that the event is a cue for an intended action.

There has been recent support for this theory from research manipulating the prior exposure of the target item relative to the ongoing task items. McDaniel et al (2004) created a high-discrepancy condition by presenting the target words during the initial instruction and not preexposing the nontarget items. That is, none of the ongoing task items appeared in a preceding list-learning task, but the prospective memory target item appeared during the instructions for the prospective memory task items. In this condition, the target item should have been processed more fluently relative to the ongoing task items and thus should have produced discrepancy and a sense of significance. In the low-discrepancy condition, the ongoing task items were preexposed in a preceding list learning task. Thus, there should have been less discrepancy in the fluency of processing the target items relative to ongoing-task items. Consistent with the discrepancy attribution theory, prospective memory performance was better in the high-discrepancy condition than in the low-discrepancy condition.

Breneiser and McDaniel (2006) have recently provided additional support for this theory by showing that it is not simply greater familiarity for the target item but, rather, the discrepancy between the actual quality of processing and the expected quality of processing that is critical for determining a sense of significance. They found that preexposing ongoing task items four times each in a preceding list-learning task relative to one preexposure for the target items (high-discrepancy condition) led to better prospective memory performance than one preexposure of both the target and ongoing task items (low-discrepancy condition). Other evidence consistent with the general idea that discrepancy can stimulate a search for significance comes from research showing that manipulations that increase the noticing of the target event, such as making the target event distinctive (e.g., a target word presented in upper case letters with the ongoing task items in lower case letters), produced very high prospective memory performance (McDaniel and Einstein, 1993; Brandemonte and Passolunghi, 1994).

2.45.4.3 Multiprocess Theory

So, which processes do we rely on for prospective remembering? According to the multiprocess view (McDaniel and Einstein, 2000; see also Einstein and McDaniel, 2005; Einstein et al., 2005; McDaniel and Einstein, 2007: Chapter 4), there are many reasons to believe that the human cognitive system uses both monitoring and spontaneous retrieval processes for prospective remembering. First, given the prevalence and importance of prospective memory demands in the real world, it would be adaptive to have a system that relies on multiple processes for prospective memory retrieval and thus increases the chances that we will remember under a variety of conditions. Second, given that the delays between forming an intention and the opportunity to execute the intention are often substantial (on the order of several hours or more), it would seem maladaptive to have a system that relied exclusively on capacity consuming monitoring processes for successful retrieval. In Smith's (2003) research, for example, monitoring for target events slowed down lexical decision times by about 45% (Experiment 1). If we relied entirely on monitoring processes for successful prospective remembering, then the efficiency with which we performed the intervening ongoing activities in our lives would be severely compromised. Third, the view that participants sometimes (perhaps most often) rely on spontaneous retrieval processes fits with Bargh and Chartrand's (1999) theory that we have a limited capacity for conscious control over behavior and therefore much prefer to rely on automatic or unconscious processes. Consistent with this idea, several studies have shown that exerting conscious control over behavior in one phase of an experiment leads subjects to expend less conscious effort in a later phase (e.g., Baumeister et al., 1998). From this perspective, the cognitive system is limited in the extent to which it is able to maintain controlled monitoring of the environment for target events.

Fourth, the idea that we sometimes rely on spontaneous retrieval processes and sometimes augment these processes with capacity-consuming monitoring processes has the potential to explain some apparently inconsistent results. For example, although dividing attention often interferes with prospective memory, there are conditions under which dividing attention has no effect on performance (McDaniel et al., 2004). An explanation of this pattern of results is that dividing attention will interfere with prospective memory primarily in those conditions in which monitoring is useful for prospective memory retrieval but will have minimal effects under those conditions in which spontaneous retrieval processes are effective in producing retrieval.

There are three assumptions to the multiprocess view. One is that prospective remembering can be supported by several different kinds of processes ranging from strategic monitoring of the environment to spontaneous retrieval processes. A second assumption is that the process that people rely on in a particular situation and the effectiveness of that process for producing retrieval depend on a host of factors, including the nature of the prospective memory demand, the demands and characteristics of the ongoing task, and the characteristics of the individual. For example, if people can anticipate that they will later encounter a salient retrieval cue for prospective memory, they are more likely to rely on spontaneous retrieval processes. If, on the other hand, it would be catastrophic to forget the intended action and the delays are fairly brief, people may initiate and maintain an active monitoring strategy over the delay interval. A third assumption, and in line with the theory of Bargh and Chartrand (1999) noted above, the multiprocess theory assumes that people have a bias to rely on spontaneous retrieval processes.

As just noted, according to the multiprocess theory, certain conditions make it more likely that the presence of the target event spontaneously triggers retrieval of the intention (McDaniel and Einstein, 2000; see McDaniel and Einstein, 2007: Chapter 4, for further discussion of relevant variables). Assuming good encoding of the intention, an important variable, for example, is the extent to which the ongoing task encourages focal processing of the target event. This idea, which is an extension of the retrospective memory theory of transfer-appropriate processing (see more about this later, and see Morris et al., 1977), is that spontaneous retrieval is likely to occur to the extent that the ongoing task directs attention to the target event and encourages processing of those features that were processed at encoding or planning (see **Table 2** for examples of conditions McDaniel and Einstein believe do and do not encourage focal processing). On the other hand, with nonfocal cues, spontaneous retrieval is less likely, and successful retrieval is more dependent on monitoring for the target event.

The multiprocess theory assumes that subjects are generally sensitive to the conditions that lead to spontaneous retrieval (e.g., focal cues) and those that demand active monitoring for the target event (e.g., nonfocal cues). This idea is similar to that of Marsh et al. (2003; see also Marsh et al., 2006), who believe that subjects develop an allocation policy that is based on their perceived difficulty of performing the ongoing task and the prospective memory activities. For example, if subjects believe that the prospective memory task will be difficult, they will allocate attentional resources to monitoring for the

Table 2 Representative examples of task conditions, some of which have been used in published research, that we assume are high and low in focal processing

Processing	*Ongoing task*	*Prospective memory task*
Nonfocal	Words were presented in the center of a computer monitor and participants had to learn them for recall tests that occurred at unpredictable times.	Respond when you see a particular background pattern (background pattern changes every 3 s).
Focal	Participants had to keep track of the number of occurrences of each background screen pattern.	Respond when you see a particular background pattern (background pattern is changed every 3 s).
Nonfocal	Lexical decision task	Respond to items from the animal category.
Focal	Lexical decision task	Respond to the word 'cat.'
Nonfocal	Pairs of words were presented, and participants decided whether the word on the left was a member of the category on the right.	Respond to the syllable 'tor.'
Focal	Pairs of words were presented, and participants decided whether the word on the left was a member of the category on the right.	Respond to the word 'tortoise.'
Nonfocal	Pictures of famous faces were presented, and the task was to name the face.	Respond when you see a face with eyeglasses.
Focal	Pictures of famous faces were presented, and the task was to name the face.	Respond when you see a face with the first name of John.

From Einstein GO and McDaniel MA (2005) Prospective memory: Multiple retrieval processes. *Curr. Dir. Psychol. Sci.* 14: 286–290.

target event. Marsh et al. believe that this allocation policy is dynamic and varies in response to subjects' changing perceptions of the difficulty of prospective memory and ongoing tasks change.

2.45.5 Storage of Prospective Memories: Do They Enjoy a Privileged Status in Memory?

Because prospective memories are formed with the goal of later retrieving them in the appropriate context, some researchers have suggested that they may have special storage properties. Indeed, Goschke and Kuhl's (1993) seminal work suggests that intentions may hold privileged status in memory. In their work, participants were asked to learn pairs of scripted actions for, say, clearing a messy desk or setting a table (e.g., actions might be: distribute the cutlery, polish the glass, light the candle, etc.). After learning, participants were told that one script in the pair would have to be performed later. In an immediate recognition test, latencies were faster to words coming from the to-be-performed script as opposed to the neutral script about which there was no prospective intention. Marsh et al. (1998a) replicated this decreased latency effect using a lexical decision task but also discovered that if the assessment of activation came after performing the script, then latencies were slower to the already-performed script as compared with the neutral script. Applying the standard interpretation that faster latencies are associated with information being more accessible in memory, Goschke and Kuhl concluded that prospective memories enjoy a privileged status in memory, whereas Marsh et al. concluded additionally that a prospective memory, once completed, goes into a state of being temporarily inhibited, which could be ecologically adaptive in planning what activities one has to do next.

There are converging reports to suggest that prospective memories reside in a privileged state. For example, Maylor et al. (2000) asked younger and older adults to list their plans for the coming week and also to list what they completed in the previous week. The conditions under which they did so were speeded, and participants were asked to write two or three words to describe each future intention or each completed intention. Consistent with the intention superiority effect (ISE), younger adults listed more future plans than they did completed activities, ostensibly because the future plans were more available in memory. In contrast, the older adults did not. Lebiere and Lee (2002) modeled Marsh et al.'s (1998) data using the ACT-R assumptions (Anderson and Lebiere, 1998) that prospective memories represent a goal node in that model. According to ACT-R, goal nodes receive constant sources of activation, and this facet of the model would account for their higher accessibility in memory. In measuring cue interference, Marsh et al. (2002b) found that prospective memory cues that were missed (i.e., that were not detected and that had received no prospective memory response) in a lexical decision task were responded to more quickly than control-matched items. Because they used a categorical intention to respond to animals, Marsh et al. assumed that they had found a more specific version of the ISE. That is, because intention-related material has privileged accessibility, it is processed more quickly even when the cue does not elicit a prospective memory response (but see West et al., 2005).

Marsh et al. (2008) have recently reported a related, very provocative finding. In this study, participants were asked to pay attention to a visual stream of words and actively ignore the information presented in an auditory channel. The participants were also given the prospective memory task of responding to a categorical intention (e.g., vegetables) when an exemplar appeared in the visual channel of information. Participants were then tested on their memory for only information presented in the to-be-ignored auditory channel. Recognition of intention-related material in the to-be-ignored channel was significantly better than control-matched material in the same channel. Marsh et al. interpreted these results as consistent with the ISE, in which intention-related material gains more obligatory access to consciousness than comparable material about which there is no intentionality.

As an alternative to the ACT-R account, if one assumes a network model of memory, then whenever plans and intentions are considered or otherwise brought into consciousness, some small amount of activation might accrue to other plans and intentions, and this process could keep them in a higher baseline resting state. Combined with a view that prospective memories are revisited from time to time, whereas retrospective memories probably receive fewer such rehearsals, then perhaps some confluence of these different explanations is what actually confers a special status on prospective memories. Of course, this general perspective is not without its opponents. In their original report, Marsh et al. (1998; see also

Marsh et al., 1999) were somewhat skeptical that a critic would necessarily adopt the notion that prospective memories were stored in a more accessible fashion. They argued that an alternative conception of the ISE is that prospective memories reside as declarative representations with the same level of baseline activation in their resting state as do retrospective memories. As such, prospective memories may be able to be revived faster because they are more elaborately encoded or because they are related to one's self schema. Also, Freeman and Ellis (2003) report boundary conditions on the ISE. In their report, they used subject-performed tasks (e.g., clap your hands), and they found an ISE only with verbal encoding and not if people learned the tasks motorically. Consequently, the ISE may be a verbal learning phenomenon. If so, this finding may not really constrain the generality of the effect because most of our everyday intentions are self-generated from thoughts.

There are no published reports contravening the ISE other than Maylor et al.'s (2000) failure to find the effect in older adults and Freeman and Ellis's (2003) failure to find it with motoric encoding. Unfortunately, this does not mean that the ISE is not a major contributor to the file drawer problem. After the Marsh et al. (1998) article appeared, Richard Marsh was contacted by many people for stimulus materials expressing an interest in testing older adult populations in order to assess whether older adults fail to inhibit after completing a prospective memory task. Because none of these reports have appeared in the last decade, one cannot help but wonder just how robust the ISE truly is. Of the many effects found in prospective memory, the ISE stands alone because it is a tantalizing proposition that the human memory system would have evolved to single out our ancestors' intentions as privileged material. Of course, from an evolutionary perspective, it would be advantageous if our ancestors who were proactive about finding food, water, shelter, and a mate survived and thrived more readily as opposed to being reactive toward these basic needs. The ISE is one of those phenomena in the realm of prospective memory that needs to be scrutinized more carefully than it has been in the scientific record to date. Only a handful of reports have been published on the effect, but any theoretical influence the ISE has on clock checking or the probability of an event-based cue being recognized needs to be based on a deeper understanding of the basic phenomenon and why it occurs.

2.45.6 Encoding of Prospective Memories

The work on encoding has reflected two general orientations: (1) the influence on prospective memory performance of instructions or experimental conditions that guide encoding of the intention to perform an action in the future (including encoding of the target event that signals the appropriateness of performing the intention) and (2) the nature of planning processes that people display in the absence of instructions directing specific encodings. Most of the research has centered on the first topic, and accordingly our review concentrates on that research.

2.45.6.1 Associative Encoding

A primary finding is that instructions or conditions that foster associative encoding of the target cue and the intended action tend to improve prospective memory performance. This finding resonates well with the reflexive associative theory presented earlier. Several aspects of this finding merit amplification. First, as is elaborated later, associative-encoding manipulations do not always produce improvements in prospective memory, and this may be because of the nature of laboratory tasks. In laboratory prospective memory tasks, where the participants are instructed to perform an intended action in the presence of a particular cue event, it is especially likely that people are spontaneously encoding a target cue–intended action association. Consequently, instructions specifically designed to augment such associative encoding could be redundant with the encoding already engaged by participants.

A second key pattern is that prospective memory effects of at least one type of associative-encoding manipulation may be accompanied by signatures of spontaneous retrieval. This pattern is consistent with the reflexive-associative theory described earlier that assumes that retrieval of an encoded target cue–intention association can be mediated by an automatic associative memory system.

One general technique to stimulate associative encoding that people can be instructed to use is an implementation intention (Gollwitzer, 1999). An implementation intention specifies situational cues for initiating an intended action and a technique to link these specific cues to the intention by using a condition-action statement such as: If situation *x* arises, I will perform *y*. However, in the experimental

work on prospective memory, laboratory instantiations of an implementation intention have varied. Cohen and Gollwitzer's (2007) implementation intention required subjects to write down three times the implementation intention (e.g., "If I see the word *window* at any point in the task [lexical decision], I will say *wrapper* as fast as possible!"). The implementation intention produced a significant advantage in prospective memory over a standard prospective memory instruction that simply told participants to say a response word upon seeing the cue word (but without repetitive writing of the instruction), even though the standard instruction group displayed relatively high prospective memory performance.

In other experiments, the implementation intention involved both saying aloud the condition–action statement and a period of encoding (typically 30 s) during which subjects imagined themselves performing the intended action upon seeing the target cue (Chasteen et al., 2001). Prospective memory performance improved under these conditions relative to a control not given implementation intention instructions for both younger adults (Howard et al., 2006) and older adults (Chasteen et al., 2001). Furthermore, it appears that the imagery encoding alone is not sufficient to produce the benefits (Einstein et al., 2003, Experiment 3; Howard et al., 2006, Experiment 2), even though imagery encoding would presumably be fostering associative linkages between the target event and the behavior (McDaniel and Pressley, 1987). Thus, based on current evidence, it seems that the full implementation intention procedure (imagery plus the if ... then statement) is most likely to create positive effects of this kind of associative-encoding instruction.

It is important to note, however, that the full implementation intention procedure does not always yield improvements in prospective memory (Kardiasmenos et al., 2004; Bennett et al., 2005; see also Chasteen et al., 2001; Howard et al., 2006, for other instances of null effects with implementation intentions). These findings dovetail with the first point made above. Participants under standard prospective memory instructions may at least sometimes spontaneously form good associative encodings of the target cue–intention action, thereby rendering experimenter-instructed associative encoding procedures unnecessary.

Another possibility is that even when implementation intentions do not affect levels of prospective memory, the processes underlying prospective memory retrieval may be altered. Under standard prospective memory instructions, attention-demanding retrieval processes (e.g., monitoring) might be recruited (processes that in some cases support relatively high levels of prospective memory; McDaniel et al., 2006; Cohen and Gollwitzer, 2007), whereas with an implementation intention, encoding relatively automatic retrieval processes may prevail (see section 2.45.4 for details of these processes). The limited evidence is consistent with this possibility. For instance, in Cohen and Gollwitzer (2007), response times to the ongoing activity (lexical decision) did not differ between the implementation intention condition and a control for which there was no prospective memory task (implicating relatively spontaneous retrieval processes), yet in the standard prospective memory condition, the response times were significantly longer relative to the no-prospective memory control (this cost implicating a demanding process for prospective memory). Further, Howard et al. (2006, Experiment 2) substantially increased the demands of the ongoing activity (by requiring random number generation as a secondary task). Prospective memory performance significantly declined relative to a condition without the demanding ongoing activity (random number generation was not required) with standard prospective memory instructions but not with implementation intention instructions.

The benefits of focusing encoding on the association between the target cue and the intended action are underscored by another type of finding. In one paradigm, after encoding the prospective memory intention, participants were interrupted several times during the ongoing task and re-presented with aspects of the prospective memory instructions (Guynn et al., 1998, Experiment 3). Some participants were presented with only the target cues, others were presented with the intention, and still others were presented with the target cues and the associated intended action. In all cases, participants were instructed to think only about the information presented. Thus, these conditions reflect additional encoding of target cues, the intention, or both. The differences in prospective memory performance as a function of the type of additional encoding were pronounced. Additional encoding of target cues alone and intention alone produced relatively low performance (36% and 56% prospective memory responding, respectively), whereas additional encoding of the target cue–intention pairs promoted high prospective remembering (82%).

2.45.6.2 Target Cue Encoding

An interesting aspect of the above results is that repeated encoding of target cues produced no increases in prospective remembering relative to a single-encoding control condition. Ample evidence indicates, however, that the quality of the target-cue encoding plays a role in successful prospective remembering. Paralleling the retrospective memory literature, semantic encoding of the target cue tends to improve prospective memory performance relative to nonsemantic encoding (McDaniel et al., 1998, Experiment 3), generating the target cue at encoding improves prospective remembering relative to reading the target cue (Matthews, 1992; Robinson-Riegler, 1994, Experiment 1), and presenting the referent of the target cue as a picture at encoding produces better prospective memory performance than presenting the cue as a word (even when the presentation of the cue during retrieval is in a different modality than at encoding; McDaniel et al., 1998, Experiment 2). Similarly, dividing attention during encoding of the target-cue significantly attenuates prospective remembering (Einstein et al., 1997).

Further, elaboration of the target-cue prior to its specification as a prospective memory target event appears to enhance prospective remembering. In one study, prior to the prospective memory instructions, some participants repeatedly generated the target cue (from word fragments or anagrams). These participants evidenced high levels of prospective memory under both standard and demanding ongoing task demands. In contrast, participants who generated words that were not subsequently used as targets displayed a significant reduction in prospective memory when ongoing task demands became more challenging (Guynn and McDaniel, in press; see Mantyla, 1994, for a similar finding).

It is worth noting that the positive effects of elaborative encoding of the target cue are entirely compatible with the theories of prospective memory retrieval reviewed in the preceding section. Such encoding would be expected to lead to better recognition of the cue during the retrieval period (assuming the PAM theory) or to create more discrepancy between subsequent processing of the target cue and nontarget events (assuming the discrepancy-attribution processes), thereby facilitating noticing of the target cue in the retrieval context. Even the reflexive-associative approach assumes that interaction of the cue with a memory trace (e.g., the intended action) is facilitated by initial encodings that are more semantic or distinctive (Moscovitch, 1994).

2.45.7 Similarities and Differences Between Prospective and Retrospective Memory

Given the formal distinction between prospective and retrospective memory, it may be tempting to focus on their differences and perhaps even to appeal to different memory systems; however, this approach would overlook many similarities as well as undercut our exploration of how our rich conceptualization of retrospective memory can help us understand prospective memory (see Marsh et al., 2006, for a more detailed treatment of the similarities and differences between retrospective and prospective memory). As a fundamental starting point, consider that prospective memories share three basic stage-like histories with retrospective memories, namely, encoding, retrieval, and any changes that occur over a retention interval (as reflected in the content of the previous sections; cf. Ellis, 1996). Intentions occur as a function of direct requests from others, or they are self-initiated. No work to date has experimentally examined the fate of these two basic types of intentions. However, even a cursory analysis or Gedanken experiment would suggest that the former type should go unfulfilled more frequently than the latter (with notable exceptions arising such as not breaking social contracts). The reason for this is twofold. First, self-generated information may undergo more rehearsals because it is self-referential in nature. Second, prospective memories that are self-referential may be more elaborately encoded and better linked to present and future contexts. More generally, for both of the same reasons that self-defined intentions may be completed more often than requests from others, prospective memories may be more durable than retrospective memories as a consequence of the manner in which they are encoded and/or rehearsed (see West and Krompinger, 2005, for an empirical approach designed to maximize similarities in order to identify fundamental differences).

To elaborate, when an intention is formed, a host of self-referential information is stored, such as why we want to complete the task, the costs and benefits of doing (or not doing) so, the current context, and the future context we might be in at the time of completion. Because material that is related to one's self is better remembered (e.g., Klein and Kihlstrom,

1986), prospective memories may be more durable than otherwise equivalent retrospective memories. More elaborated intentions that are stored more durably in memory also should have a higher probability of coming to mind during the retention interval. Just like a retrospective memory, the more frequently a memory is rehearsed, the better it will be recalled on a subsequent occasion (called retrieval sensitivity by Mäntylä, 1994). So, based on the properties of encoding, one cannot make a blanket statement that all prospective memories will be remembered more faithfully than retrospective memories, only that on average, the amount of effort expended in creating a prospective memory could be greater than in creating a simple, everyday retrospective memory. In addition, the contextual details surrounding retrospective memories are usually lost quite quickly (e.g., Bornstein and LeCompte, 1995), whereas they often form the core of a prospective memory. For example, we often plan to fulfill a prospective memory in a particular context, and therefore, a prospective memory will have linked with it at least two contexts (the environment during formation and the one in which we expect to do it). These can serve as important retrieval cues to fulfilling intentions, and when contexts mismatch our expectations, then the consequences can be very grave indeed for intention completion (Cook et al., 2005).

As we said earlier, retrieving intentions is usually a self-initiated act, whereas many times retrieving retrospective memories is not. Of course, exceptions to this rule exist, such as when a third party queries you about your intentions (e.g., "Got plans for this weekend?"). Nevertheless, when we rely on retrieval cues, many of the principles of prospective memory appear to mimic what has been found with retrospective memory. For example, if one has the intention to respond to a word such as 'bat' (as in baseball), then receiving the cue as bat (as in mammal) leads to much worse prospective memory (McDaniel et al., 1998). A form of transfer-appropriate processing is also found in what is known as task-appropriate processing (Marsh et al., 2000; West and Craik, 2001; Maylor et al., 2002). If the features of the ongoing task focus one on the correct aspects of the prospective memory cue, intention retrieval is more successful. As such, a semantic intention to respond to words denoting animals is more successful if the ongoing task encourages semantic, as opposed to orthographic, processing of the items. Also, resource sharing during retrieval appears to have similar effects on prospective and retrospective memories. Dividing attention during either encoding or retrieval generally reduces retrospective memory (Baddeley et al., 1984; Craik et al., 1996) and prospective memory (e.g., Einstein et al., 1997, 1998; McDaniel et al., 2004). One possible difference is that some forms of event-based prospective memory require difficult, centrally mediated divided attention tasks to observe lower rates of responding to prospective memory tasks (Marsh and Hicks, 1998).

Finally, prospective and retrospective memories both share the property that they will change over the course of a retention interval. Obviously, an unrehearsed memory will grow weaker over time and eventually be forgotten. However, most people review their intentions periodically as a part of their daily mental life. Alternatively, cues in the environment can remind us of intentions, such as the sight of one's vehicle serving as a reminder to have the oil changed. These periodic reminders of intentions only serve to strengthen their representation, as we argued earlier. Most retrospective memories do not enjoy such periodic revisitation and more likely fall into desuetude, thereby requiring increasingly stronger retrieval cues over time to recover that information.

2.45.8 Development and Prospective Memory

Although there has been much interest in examining prospective memory from developmental perspectives, most of it has been conducted with older adults. This focus on older adults is probably a result of the obvious practical importance of understanding how aging affects prospective memory (e.g., to help inform health care issues related to prospective memory such as medication adherence), but also in response to compelling theoretical issues. We briefly review first the literature with children and then the research with older adults. As might be expected, the research generally shows that older children outperform younger children and younger adults outperform older adults on prospective memory tasks. However, it is also clear that the age differences vary greatly across prospective memory tasks and that there are some tasks on which no age differences are found. Thus, an interesting theoretical and applied challenge for prospective memory researchers is to understand those conditions that are and are not especially difficult for younger children and older adults.

Before reviewing this literature, we briefly raise a methodological issue that is important to consider when examining developmental trends in prospective memory. Given that prospective memory tasks are embedded in ongoing tasks and that demanding ongoing tasks have been shown to interfere with prospective memory (Marsh and Hicks, 1998), it is important to control the demands of the ongoing task across age groups. Otherwise, differences in prospective memory could stem from the ongoing task being functionally more demanding for younger children or for older adults (see Einstein et al., 1997; Kvavilashvili and Fisher, 2007, for discussion of this issue).

2.45.8.1 Prospective Memory in Children

Even though there is not a plethora of existing research on prospective memory in children, several interesting results have emerged, and these seem to be stimulating increasing interest (e.g., see Kliegel et al., in press; Kvavilashvili et al., in press). Recent studies examining event-based prospective memory in 5- and 7-year old children generally suggest that age differences are larger on tasks that require more controlled or strategic processes. All of these studies used a variation of Kvavilashvili et al.'s (2001) prospective memory task of asking children to name pictures from stacks of pictures for Morris the Mole because he does not see very well (the ongoing task). The prospective memory task was to hide any picture of animals from Morris because he was scared of them. Kvavilashvili et al. varied whether the animal pictures appeared in the middle or the end of the stack. Five- and 7-year old children both remembered about 75% of the time when the target was at the end of the stack, but the older children did much better than the younger children when the target was in the middle. Thus, the older children were better able to inhibit the ongoing activity in order to perform the intended action. When inhibition was not needed, however, the younger children were as capable of remembering as the older children.

To directly study strategic processes, Stokes et al. (2007) manipulated whether the target event was focal or nonfocal. Children in their study were presented with cards (with four pictures on each card) and asked to name the circled picture on each card (the ongoing task). The prospective memory task was to hide the card if there was an animal on it. In the focal condition, animals always appeared as the circled picture, and thus the ongoing task requirement to name the picture forced processing of the target pictures, which in turn could trigger spontaneous retrieval. In the nonfocal condition, the target picture always occurred in a noncircled location, and thus subjects had to remember to monitor the other locations for the target picture. Whereas the prospective memory performance of the older children was nearly perfect regardless of the cuing condition (around 95%), prospective memory was much higher for the younger children in the focal condition (68%) than in the nonfocal condition (20%). Consistent with this pattern, McGann et al. (2005) found high performance and no differences between 5- and 7-year old children with salient target pictures (when the pictures were larger than others) but higher performance for the older children (relative to the younger children) with nonsalient target pictures. All of these studies suggest that some prospective memory conditions are more difficult for younger children than others. Consistent with the general developmental trend showing that younger children have more limited attentional and working memory resources (e.g., Guttentag, 1984) and with the multiprocess theory (McDaniel and Einstein, 2000), younger children seem to have greater difficulty with prospective memory tasks that require active monitoring of the environment and inhibiting the demands of the ongoing task.

Very little research has examined time-based prospective memory in children. An interesting question with this kind of task is whether children can develop and maintain a clock checking strategy in the absence of a cue to trigger remembering. Ceci and Bronfenbrenner (1985) asked 10- and 14-year-old children either to remember to remove cupcakes from the oven or to remove cables from a battery charger exactly 30 min later. The children performed these tasks either at home or in the laboratory. During the 30-min interval, they were engaged in an entertaining video game, and there was a wall clock at their back. This arrangement allowed the researchers to record monitoring of the clock. Interestingly, most children developed a monitoring strategy, but the strategy varied across the laboratory and home contexts. In the lab, the children monitored the time increasingly more often as the target time approached. In the home setting, children tended to adopt what Ceci and Bronfenbrenner described as a more adaptive U-shaped monitoring pattern. That is, they monitored frequently initially (presumably to calibrate the passage of time) and then very little after that except for the last 5 min before the target

time (at which point they monitored frequently). This strategy is adaptive in the sense that it frees up resources for the ongoing video task. Although the large majority of both 10- and 14-year-olds remembered on time, late responding was associated with less strategic monitoring. In light of the surprising finding that very young children (2 years old) can show very good prospective memory for tasks that they consider important (e.g., buying candy at the store; Sommerville et al., 1983), it would be interesting to explore the conditions and age at which strategic monitoring develops.

2.45.8.2 Prospective Memory in Older Adults

As noted earlier, the majority of the developmental research has focused on aging issues (for recent papers, see Henry et al., 2004; McDaniel and Einstein, 2007: Chapter 7; McDaniel et al., in press; Phillips et al., 2007; Wilson and Park, 2007). This interest was motivated by both practical and theoretical considerations. The applied concerns included that good prospective memory may be especially important for older adults who often have health-related prospective memory needs like remembering to take medication. Craik's (1986) theory, suggesting that prospective memory should be very difficult for older adults, provided the theoretical thrust. Noting that aging affects some retrospective memory tasks more than others, Craik proposed that aging disrupts self-initiated retrieval processes, and therefore that older adults need greater environmental support or external cuing for accomplishing retrieval. This theory helps explain why age differences are often larger with free recall than recognition tasks. Because prospective memory is not accompanied by an external request to remember (i.e., subjects are not put in a retrieval mode), Craik theorized that prospective memory should be especially demanding in terms of self-initiated retrieval and thus particularly difficult for older adults.

The findings remind us that prospective memory is not a unitary concept and that age differences vary as a function of the nature of the task demands and the contexts in which they are performed. One pattern is what Phillips et al. (2007) describe as the age prospective memory paradox, which is the finding that older adults generally perform more poorly on prospective memory tasks in the lab but perform as well as or better than younger adults in naturalistic settings (e.g., remembering to mail postcards or to call the experimenter on designated days). Indeed, Wilson and Park (2007) discuss the high medication adherence of older adults in the face of declining cognitive functioning as another paradox (but see Insel et al., 2006, for evidence of modest levels of medication adherence by older adults). It is not currently clear what produces this reversal of performance across naturalistic and laboratory settings, but Phillips et al. and others (e.g., Kvavilashvili and Fisher, 2007) have suggested several possible explanations including age differences in conscientiousness, views regarding the importance of punctuality, busyness and structure of lifestyle, perceptions of task importance, and use of reminders (see also Wilson and Park, 2007). Another possible explanation is that older adults have greater control over the pacing of their ongoing activities in natural settings (McDaniel et al., in press).

Even in laboratory settings, however, there is a large range of age effects. Many studies show large age-related deficits in prospective memory (e.g., Maylor et al., 1999), whereas some show modest or no age-related declines in prospective memory (e.g., Einstein and McDaniel, 1990; Cherry and LeCompte, 1999). Henry et al.'s (2004) meta-analysis revealed an interesting pattern that prospective memory tasks that required greater degrees of controlled or strategic processing (i.e., ones with less external support, and thus ones that required greater monitoring) were associated with larger age effects than those that could be accomplished by relatively automatic retrieval processes (i.e., those with good external cues that could support spontaneous retrieval processes). From a cursory interpretation of Craik's (1986) theory, this should not happen; all prospective memory tasks should be difficult for older adults. From a deeper analysis, however, if one considers prospective memory to be a general label for a variety of specific tasks that differ in the extent to which they are cued by environmental events, the data may be consistent with the theory. The data also appear consistent with the multiprocess theory, which assumes that, depending on the conditions, people rely on monitoring versus spontaneous retrieval processes to different degrees in different kinds of prospective memory tasks. This is important as it relates to aging because working memory and attentional resources that are assumed to be needed for monitoring are thought to decline with age (Craik, 1986), whereas relatively automatic retrieval processes may remain relatively intact with age (McDaniel et al., in press).

To evaluate this interpretation, Reese (2004) tested younger and older adults and varied whether the prospective memory cues were focal or nonfocal (see **Table 2**; recall that focal cues are thought to stimulate spontaneous retrieval processes, whereas nonfocal cues are thought to require monitoring for successful retrieval). The ongoing task involved remembering short lists of words, and as every new word appeared, the background pattern of the screen changed. In the focal condition, subjects were asked to press a designated key whenever they saw a particular word, whereas in the nonfocal condition subjects were asked to press a designated key whenever a particular background pattern occurred. Consistent with the multiprocess theory prediction, Reese found that the size of the age difference depended on the type of prospective memory cue such that the age difference was smaller with the focal cue (80% for younger vs. 49% for older) than for the nonfocal cue (80% younger vs. 17% older). Sometimes there is no age difference with focal cues (e.g., Einstein and McDaniel, 1990; Cherry and LeCompte, 1999; McDaniel et al., in press) and sometimes, as in this particular experiment, the age difference is reduced but not eliminated (see also Rendell et al., 2007, Experiment 1). Possible explanations for the existence of age differences in some experiments even with a focal cue conclude that younger adults may be more likely to engage in monitoring and thereby increase the chances of retrieval, and that spontaneous retrieval processes may not be entirely spared with age.

In addition to tasks with nonfocal cues, prospective memory tasks that seem to pose special problems for older adults seem to be time-based tasks (Henry et al., 2004), habitual prospective memory tasks (ones in which the intended action is performed repeatedly; Einstein et al., 1998), and those in which the retrieved intention cannot be performed immediately and must be delayed (as when a person remembers to take her/his medication in the bathroom but then needs to maintain the intention until she/he gets to the kitchen; see McDaniel et al., 2003). In closing this section, we note again the striking finding that the magnitude of the age differences varies greatly across studies. We suspect that we will better understand this pattern as we examine the processes that are recruited for different prospective memory tasks and how aging affects these processes.

2.45.9 Cognitive Neuroscience of Prospective Memory

Building upon the considerable advances that have been made in our understanding of the cognitive processes underlying the realization of delayed intentions, significant progress has been made in identifying the functional neuroanatomy of prospective memory. The neural basis of prospective memory has been investigated using complimentary methodologies within the neuropsychological, functional neuroimaging, and electrophysiological traditions. Study in these domains has revealed a number of neurological and psychiatric conditions that are associated with impaired prospective memory as well as illuminating the temporal dynamics of the functional neuroanatomy of prospective memory.

2.45.9.1 Neuropsychology

Studies using the neuropsychological approach reveal that impairments of prospective memory are observed in a variety of neurological and psychiatric disorders including traumatic brain injury (TBI; Shum et al., 1999), stroke (Cockburn, 1995), epilepsy (Palmer and McDonald, 2000), multiple sclerosis (Bravin et al., 2000), Parkinson's disease (Kliegel et al., 2005), schizophrenia (Shum et al., 2004), and substance abuse (Hefferman et al., 2001). Additionally, other evidence has revealed individual differences in prospective memory associated with genetic expression (Driscoll et al., 2005; Singer et al., 2006). Together, work in the area of neuropsychology converges with several themes that arise from the cognitive psychological literature. A number of studies reveal that damage to or disruption of neural networks involving the prefrontal cortex results in impaired prospective memory (Cockburn, 1995; Burgess et al., 2000). This finding is consistent with evidence revealing that prospective memory covaries with the efficiency of executive functions (typically thought to be dependent on the functional integrity of the prefrontal cortex) and the availability of working memory capacity (Marsh and Hicks, 1998). Also, disruption of the medial temporal lobe memory network results in impaired prospective memory (Palmer and McDonald, 2000). This finding is consistent with theoretical models of prospective memory wherein similar processes are thought to support prospective memory and explicit episodic

memory (Einstein and McDaniel, 1996; Guynn et al., 2001).

The effects of mild to severe TBI on prospective memory have been considered in a number of studies. This research reveals negative effects of TBI on measures of time-, event-, and activity-based prospective memory (Shum et al., 1999) that increase with the severity of the injury (McCauley and Levin, 2004). TBI has an adverse effect on multiple phases of prospective memory including intention formation, re-instantiation, and execution, and it may have a lesser effect on intention retention (Kliegel et al., 2004). Consistent with this finding, individuals with TBI can benefit from reminders that are inserted in the middle of task performance (McCauley and Levin, 2004). The magnitude of the effect of TBI on prospective memory is equivalent when focal and nonfocal prospective cues are used (Schmitter-Edgecombe and Wright, 2004), possibly indicating that patients do not benefit from spontaneous processes underlying the recognition of prospective cues (Einstein et al., 2005). There is also some evidence that indices of monitoring for prospective cues may be relatively intact in patients with TBI (Shum et al., 1999; McCauley and Levin, 2004). Studies examining the effects of TBI on prospective memory have revealed a mixed neuropsychological profile, with some, but not other, groups of patients demonstrating impairments of episodic or declarative memory, processing speed, and executive functions (Kliegel et al., 2004; Schmitter-Edgecombe and Wright, 2004; Mathias and Mansfield, 2005), making it difficult to ascertain whether there is a core deficit underlying the effects of TBI on prospective memory.

There is growing evidence that disruption of the frontostriatal dopamine system leads to impaired prospective memory. At least two studies reveal that schizophrenia can produce deficits of time-, event-, and activity-based prospective memory (Shum et al., 2004; Kumar et al., 2005). The effect of schizophrenia may result from a disruption of the representation of intentions as the intention superiority effect is reduced or absent in patients with this disorder (Kondel, 2002), or from a reduction in the efficiency of strategic monitoring processes (Elvevag et al., 2003; Shum et al., 2004). There is also some evidence that prospective memory is disrupted in Parkinson's disease (PD; Katai et al., 2003). Furthermore, the effect of PD on prospective memory may result from a reduction in the efficiency of processes supporting the formation and realization of intentions rather than processes supporting the representation of an intention in memory (Katai et al., 2003; Kliegel et al., 2005). Finally, data from two studies indicate that the recreational abuse of MDMA, or Ecstasy – which is known to be toxic to dopaminergic and serotonergic neurons (Ricaurte et al., 2002) – results in both self-reported (Heffernan et al., 2001) and laboratory-based (Zakzanis et al., 2003) prospective memory deficits.

2.45.9.2 Functional Neuroimaging

Data from studies using functional neuroimaging methods generally converge with those from the neuropsychological literature. Specifically, PET and fMRI studies reveal activation of a broadly distributed neural network during the performance of prospective memory tasks that includes the rostral and lateral frontal cortex, structures within the medial temporal lobe, parietal cortex, and the thalamus (Okuda et al., 1998; Burgess et al., 2001; Simons et al., 2006). Functional neuroimaging techniques also reveal neural correlates of processes that may distinguish prospective memory from working memory, vigilance, and divided attention (Reynolds et al., 2003; De Bruycker et al., 2005).

Evidence from one line of research reveals that the recruitment of rostral frontal cortex is important for the realization of delayed intentions (**Figure 3**;

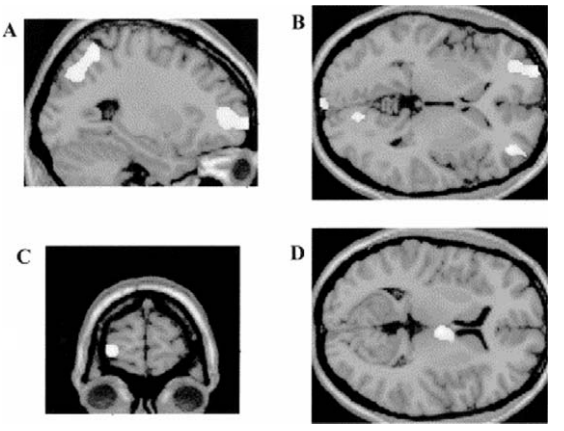

Figure 3 Functional activation differentiating (execution + expectation) – ongoing alone (a–c) and execution – expectation (d) conditions. Parts (a) and (b) portray activation within lateral rostral PFC, (c) portrays activation in right lateral prefrontal cortex, and (d) portrays thalamic activation. Adapted from Burgess PW, Quayle A, and Frith CD (2001) Brain regions involved in prospective memory as determined by positron emission tomography. *Neuropsychologia* 39: 545–555.

Burgess et al., 2001, 2003). In tasks requiring event-based prospective memory, the lateral rostral frontal cortex is consistently activated, while the medial rostral frontal cortex is often deactivated (Burgess et al., 2003). In contrast, in tasks requiring time-based prospective memory, this pattern may be reversed, revealing activation of medial rostral frontal cortex and deactivation of lateral rostral frontal cortex (Okuda et al., 2001). Variation in the respective roles of the lateral and medial rostral frontal cortex across a variety of tasks has served as the impetus for the development of the Gateway hypothesis, wherein rostral frontal cortex is believed to play a role in switching the focus of one's attention between stimulus-dependent and stimulus-independent aspects of information processing that may be critical for the realization of delayed intentions (Burgess et al., 2005). For instance, as applied to the typical prospective memory paradigm, the rostral frontal cortex may support the ability to switch from a focus on attributes of a stimulus that are relevant to performance of the ongoing activity to a focus on attributes of a stimulus that are internally represented, such as the cue-intention association.

Using PET, Burgess et al. (2001, 2003) sought to determine whether the rostral frontal cortex was involved in the maintenance or realization of delayed intentions. In the baseline condition of these studies, individuals simply performed one of three ongoing activities; in the expectation condition, individuals anticipated the presentation of prospective cues, but cues were never presented; in the execution condition, individuals anticipated prospective cues – and cues were in fact presented. A comparison of neural recruitment in the expectation + execution conditions versus the baseline condition revealed bilateral recruitment in lateral rostral frontal cortex, right parietal cortex, and the precuneus region, while a comparison of neural recruitment in the expectation and execution conditions did not reveal activation in these regions. This finding led to the suggestion that rostral frontal cortex was associated with cognitive processes that support the maintenance of an intention during the delay period (e.g., preparatory processing, Smith, 2003), rather than processes related to the realization of an intention once the prospective cue was detected (Burgess et al., 2001).

fMRI has also been used to examine item-level or event-related neural recruitment associated with processes underlying prospective memory. Evidence from one study reveals what may reflect a neural correlate of item checking described in the strategic monitoring account of prospective memory (Guynn, 2003; Smith, 2003). De Bruycker et al. (2005) compared neural activity for ongoing activity stimuli when the ongoing activity was performed in isolation or when it was performed in the context of a prospective memory task. This comparison revealed increased activation in the medial and lateral extrastriate cortex for ongoing activity stimuli presented during the prospective memory condition relative to the ongoing activity condition. This basic finding was replicated by Reynolds et al. (2003), who observed decreased activation for prospective cues that were presented in a prospective memory condition relative to a simple vigilance condition. The decrease in activation for prospective memory cues from the prospective memory condition to the vigilance condition is consistent with the idea that the addition of a prospective memory component to a task may require the reallocation of processing resources between the prospective and ongoing components of the task (Smith, 2003; Marsh et al., 2006b).

2.45.9.3 Electrophysiology

Studies incorporating the event-related potential (ERP) methodology have sought to address three fundamental issues related to the neural basis of event-based prospective memory. First, work in this area has sought to identify the temporal dynamics of the neural correlates of prospective memory. Second, investigations in this area have sought to determine whether the neural correlates of prospective memory can be distinguished from other modulations of the ERPs related to target processing. Third, other investigations have sought to link the neural correlates of prospective memory to cognitive processes described in theories of prospective memory.

Work examining the temporal dynamics of the neural correlates of prospective memory has consistently revealed three modulations of the ERPs that are associated with the realization of delayed intentions (**Figure 4**; N300, parietal old–new effect, and prospective positivity; West et al., 2001; West and Krompinger, 2005). The N300 reflects a phasic negativity over the occipital-parietal region of the scalp that typically emerges between 300 and 400 ms after onset of the prospective cue and is often accompanied by a positivity over the midline frontal region of the scalp (West et al., 2001; West and Ross-Munroe, 2002). The amplitude of the N300 is greater for prospective hits than for prospective misses, leading to the suggestion that it is associated with processes

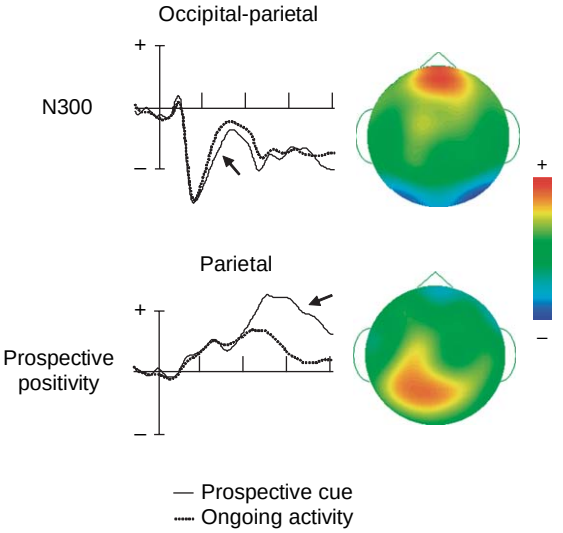

Figure 4 Grand average ERPs and scalp topography maps as viewed from above, demonstrating the time course and topography of the N300 and prospective positivity. Adapted from West and Wymbs (2004) and West and Covell (2001).

supporting the detection of prospective cues (West and Ross-Monroe, 2002). The N300 is elicited by prospective cues that are defined by letter case, color, and word identity, indicating that it reflects a relatively generic process that is associated with prospective memory (West et al., 2001; West et al., 2003; West and Krompinger, 2005). The parietal old–new effect and prospective positivity reflect enhanced positivity over the parietal region of the scalp between 400 and 1000 ms after stimulus onset (West et al., 2001; West and Krompinger, 2005). The parietal old–new effect reflects a relatively general process that is associated with item recognition in recognition memory (Rugg, 2004) and prospective memory paradigms; the prospective positivity is more specific to prospective memory and may reflect processes that serve to coordinate the prospective and ongoing components of the task once the prospective cue is detected and the intention is retrieved from memory (West and Krompinger, 2005).

An example of research addressing the second issue is portrayed in a study comparing the prospective positivity and the P3 component. Given similarities between the time course and topography of the prospective positivity and P3 component, one might wonder whether the prospective positivity reflects a general index of target categorization in prospective memory paradigms (West et al., 2003). To examine this question, West et al. (2006) examined the effects of working memory load on the amplitude of the prospective positivity and the P3 component. The logic of the study was this: If the prospective positivity and P3 arise from the activity of similar processes, then both should be sensitive to working memory load (Gevins et al., 1996); in contrast, if the prospective positivity and P3 reflect distinct processes, then there may be differential effects of working memory load on these two modulations of the ERPs. The data from this study support the latter hypothesis, as the amplitude of the P3 for target stimuli decreased with increasing working memory load (**Figure 5**), while the amplitude of the prospective positivity was unaffected by increasing working memory load (West and Bowry, 2005; West et al., 2006). These data demonstrate that that the neural correlates of prospective memory, in this case the prospective positivity, can be dissociated from processes that are more generally related to target categorization or selection.

Following from work examining the temporal dynamics of processes underlying prospective

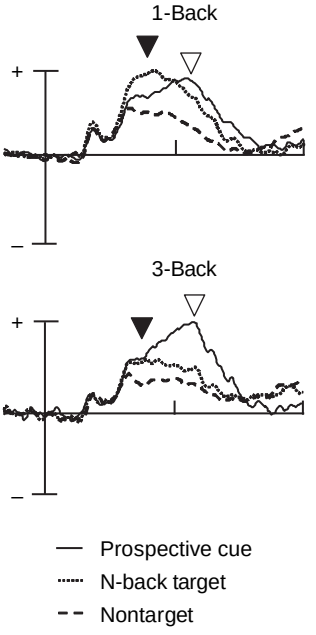

Figure 5 Grand average ERPs for prospective cue, N-back target, and nontarget trials at electrode, Pz demonstrating the effect of N-back load on the P3, but not the prospective positivity. The filled arrow marks the P3, and the unfilled arrow marks the prospective positivity. Adapted from West R, Bowry R, and Krompinger J (2006) The effects of working memory demands on the neural correlates of prospective memory. *Neuropsychologia* 44: 197–207.

memory, other investigations have sought to determine whether modulations of the ERPs associated with the realization of delayed intentions possess the characteristics of cognitive processes described in theories of prospective memory. Two such studies have examined the influence of the working memory demands of the ongoing activity and strategic monitoring on the N300 (West et al., 2006; West, in press a). Based on strategic monitoring accounts of prospective memory, the amplitude of the N300 was expected to decrease as the working memory demands of the ongoing activity increased; in contrast, based on the discrepancy plus search account, the N300 was not expected to be sensitive to working memory load. The application of partial least squares analysis (McIntosh et al., 1996) – which allows one to decompose the effects of different experimental manipulations on the ERPs into a set of orthogonal latent variables – revealed that the N300 was expressed by two latent variables (West et al., 2006): one that was sensitive to N-back load and expressed the N300, but not the prospective positivity, and one that was insensitive to N-back load and expressed the N300 and prospective positivity. The results of this study reveal two important findings. First, consistent with the multiprocess view of prospective memory, these data reveal that both relatively automatic and more resource demands processes contribute to the detection of prospective cues. Second, these data reveal that the N300 and prospective positivity may be coupled to one another, a finding that is consistent with the general architecture of the discrepancy plus search theory (West, in press b).

2.45.10 Summary

Although ignored for many years, and indeed characterized as a forgotten topic 25 years ago (Harris, 1984, p. 71), research since that time has proven prospective memory to be an experimentally tractable and theoretically exciting area. Laboratory and nonlaboratory paradigms have been developed to examine prospective remembering under a variety of situations, and theoretical issues are stimulating rich understanding of the cognitive processes and neural mechanisms underlying prospective memory. Because the memory literature has focused on memory tasks in which experimenters initiate retrieval by putting subjects in a retrieval mode, it has ignored the important capability of humans to plan for future events and then later perform them in the appropriate circumstance. It appears that this self-initiated characteristic of prospective memory has important implications for considering optimal encoding, storage, and retrieval processes (see Ellis, 1996; Dobbs and Reeves, 1996). As our understanding of prospective memory has developed, and consistent with contextualistic views of memory (Jenkins, 1979), it also appears that prospective memory is not a unitary concept and, instead, that different processes are involved in different prospective memory tasks. We believe that it will be important, for both theoretical and applied concerns, to carefully examine these processes and the extent to which they are prominent in different prospective memory tasks.

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2.46 Autobiographical Memory

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2.46.1 Introduction

The term autobiographical memory refers to our memory for specific episodes, episodic memory, and to our conceptual, generic, and schematic knowledge of our lives, autobiographical knowledge. Typically these two types of long-term memory representation are brought together in an act of remembering where they form a specific memory. Consider the following example:

> My earliest memories relate to a time in my childhood when we were living in Malta. I was about four years old. We lived in the most glorious Italian house on the sea which had a great big flagstone hall and shutters looking out to the sea and a sweeping staircase that led up to the first floor and, I think this is true, but it seems wrong somehow because my parents were very kind to me. I remember having to stand looking at the wall halfway up the stairs because I couldn't remember the days of the week and I was taught them with reference to the gods, you know, Thor's day, Woden's day and so forth, and that I remember very vividly. One lunchtime I was asked to repeat them and I couldn't remember them and my father told me to go and stand halfway up this great big sweeping staircase and just look at the wall. (Taken from the BBC Radio 4 Memory Survey, July 2006, which collected 11,000 memories from the general public.)

There are various segments of autobiographical knowledge in this memory, e.g., when we lived in Malta, my parents were kind to me, some generic visual imagery, e.g., how various features of the house looked, and some highly specific knowledge of time, locations, and actions. Autobiographical memories very frequently come to mind as these compilations of different types of knowledge are configured into a memory in a specific act of remembering. As such they clearly illustrate the highly constructive nature of autobiographical remembering. We will return to memory construction in a later section, but now that we have some idea of what is meant by the term autobiographical memory, we might ask about how it has been studied. After all, autobiographical memories are personally important memory representations. They are the content of the self and define who we are, who we have been, and, importantly, who we can yet become. They enable us to have a past, present, and future in which we exist as individuals. They are, therefore, one of our most important bodies of knowledge and because of that would have been, it might be thought, the focus of memory research for many decades.

2.46.2 A Brief Biography of Autobiographical Memory Research

Remarkably, however, the study of autobiographical memory has mainly taken place over the last 2 decades, whereas as the formal scientific study of memory itself is at least over a century old, dating, arguably, to the seminal work of Herman Ebbinghaus (1885). Ebbinghaus famously studied memory for relatively meaningless items, such as short lists of constant-vowel-constant (CVC) letter strings. Less well known is that he also studied memory for meaningful materials such as passages of prose, poetry, etc. Ebbinghaus concluded that memory for these latter

materials was influenced by too many factors beyond the experimenter's control and because of this the scientific or experimental study of memory would be more surely advanced using materials that the experimenter had powerful control over, such as CVC strings. Ebbinghaus's view held sway and the experimental study of memory in the laboratory has generally used to-be-remembered materials generated and controlled by the experimenter. Almost by definition this excludes autobiographical memories, as these are formed outside the laboratory in our everyday lives in response to complicated meaningful experiences – experiences over which the experimenter has no control.

Given the dominance of experimental studies of memory, it is perhaps not so surprising that it is only in relatively recent times that autobiographical memory has received any attention at all. According to one view, science moves from the simple to the complex and perhaps it was the case that some understanding of memory, deriving from experimental studies, had to be attained before the field could grapple with the complexities of autobiographical memory and the inevitable role in memory of mysterious entities such as the self, goals, and emotion. There is no doubt some truth in this but, as with all history including personal history, the story is more complicated. So, for instance, at the time Ebbinghaus was writing his field-defining book, another great nineteenth-century scientist, Sir Francis Galton (1883), was reporting his seminal work into memory. One aspect of this research focused on the recall of autobiographical memories. Galton was interested in how many memories we have and developed a technique that 100 years later became known as the cue word technique. In this procedure, Galton revealed to himself, one at a time, words he had previously arranged into an alphabetical list. In response to each word, he noted what thoughts passed through his mind. So when reading abasement, abhorrence, etc. (remember this was Victorian England), he would write out his thoughts. He carried out this procedure for the fairly long list of words on several separate occasions. There were a wide range of findings but one striking outcome was that many of his thoughts were (autobiographical) memories and they often came to mind in the form of visual mental images. Galton was rather disappointed to discover that there was not an endless variety in his thoughts or memories and that he often recalled the same thoughts/memories on subsequent occasions of testing. He concluded that we probably have far fewer memories than we imagine we have – about 6500 according to one researcher who tried to recall all her memories (Smith, 1952).

An obvious problem with Galton's method is that once a subject has recalled a memory, then that memory became associated with the cue word and as such was much more likely to be recalled on subsequent occasions. If so, then Galton may well have underestimated the extent of his autobiographical memories. Nonetheless, the cue word method has proved especially useful in more contemporary studies of autobiographical memory and Galton's original work remains a rewarding read for memory researchers, as does Ebbinghaus's important book.

Another book from this period that remains significant is Theodore Ribot's (1882) classic case studies of memory distortions and malfunction following brain injury. This work also contains one of the first theories of autobiographical memory and is worth consulting for that alone. Other memory researchers from the late nineteenth century also studied autobiographical memory (see Conway, 1990, 2004, for reviews), and among them Henri and Henri (1896, 1898) conducted the first autobiographical memory survey. However, psychology came to be dominated by behaviorism, at the heart of which was the belief that all psychological theory should be built upon that which was observable. As memories are internal mental states, they cannot be studied by direct observation but can only be inferred by their effects upon behavior, i.e., upon what can be recalled in an experiment where the conditions of learning, retention, and remembering are highly controlled. This approach became known as verbal learning. Indeed, the dominant journal in the area was called the *Journal of Verbal Learning and Verbal Behavior* (renamed in the 1980s the *Journal of Memory and Language*). For many decades, verbal learning dominated memory research and in many respects still does. A lone voice during this period was the British researcher Sir Fredrick Bartlett, whose famous book *Remembering: A Study in Experimental and Social Psychology* (1932) is generally credited with having created and maintained a different tradition in memory research. In this tradition, the concept of a schema (some sort of general representation of similar experiences, narrative, and cultural conventions) was central and social interactions and culture played important roles in remembering. Bartlett was, however, largely uninterested in detailed memories of specific experiences – what we now call episodic

memories. Because of this, his work did not reinvigorate the study of autobiographical memory.

Instead the reemergence of the study of autobiographical memory after 100 years of silence (Cohen, 1989) started to take place in the 1970s and gathered pace in the 1980s. **Figure 1** shows the cumulative frequency of papers, by year since 1970, that have used the phrase autobiographical memory. This admittedly is a crude index of research activity into the topic, but as crude as it is, it nonetheless depicts very strikingly how autobiographical memory research has rapidly increased and developed in the last 35 years. So what happened to end the century of silence? There were, arguably, two main forces that led to renewed interest in this important aspect of memory. The first was the gradual emergence of neuropsychology as a distinct research area and within it the study of malfunctions of human memory following brain damage. One of the striking symptoms of patients with memory impairments caused by brain damage is that they virtually always have disrupted autobiographical memory. In a particularly important paper Crovitz and Schiffman (1974) reintroduced the Galton cue word method as a way of eliciting autobiographical memories in normal populations and later in patients with closed head injuries suffering from various degrees of amnesia, thus simultaneously rediscovering both Galton and Ribot. The second force was the developing interest within cognitive science in how to model and represent stories and memories. An important paper here that demonstrated how autobiographical memory might be studied under laboratory conditions was that of Robinson (1976), who also used the cue word method to investigate differences between memories with different types of affect. Add to this Brown and Kulik's (1977) original survey of flashbulb memories, a rather timely reminder from Neisser (1978) about the narrowness of memory research in the 1970s and preceding decades, and the highly significant volume edited by Neisser (1982), *Memory Observed*, which reprinted many of the papers of earlier researchers on autobiographical memory and other then-neglected areas of memory, and a strong impetus was in place to rejuvenate research into autobiographical memory. It is, perhaps, important to note that the renewed interest, reflected in **Figure 1**, had its roots in a rediscovery of the original work of Galton, Ribot, and others (see too Rapaport, 1950, for an especially interesting review of emotion and memory). It might be noted that the methods used by these early researchers – studying one's own memory, investigating malfunctions and distortions of memories, and surveying memories – also re-emerged in the contemporary study of autobiographical memory, and it is to the findings of these more recent studies we now turn.

2.46.3 The Representation of Autobiographical Knowledge in Long-Term Memory

This section reviews current thinking about the nature of autobiographical knowledge. It is important

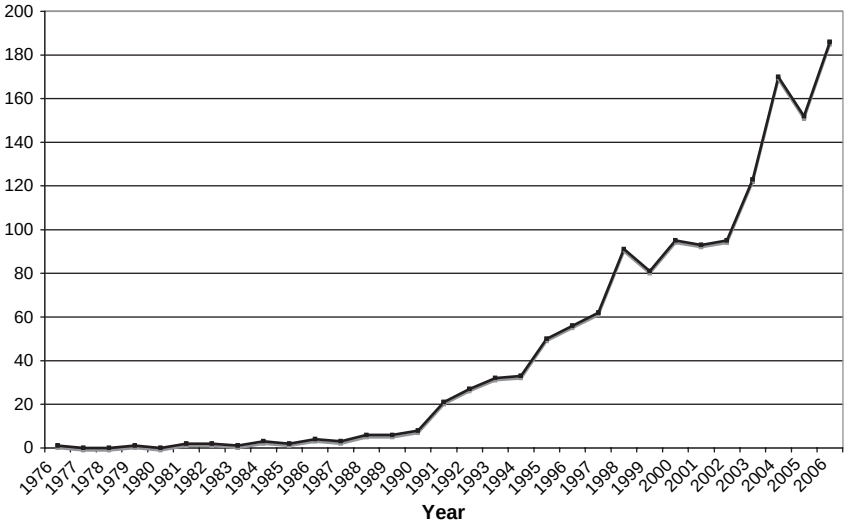

Figure 1 Frequency of articles or research reviews published using the term autobiographical memory in the article title, abstract, or key words from 1970 to 2006. Data obtained from ISI Web of Knowledge, January 2007.

to note that a full review of findings is not undertaken here and instead only main findings and their implications are considered. One current model proposes that autobiographical memories are generated in the self-memory system or SMS (Conway and Pleydell-Pearce, 2000). Very briefly, the SMS is considered to be a virtual memory system consisting of a temporary interaction between control or executive processing systems with a complex multilayered long-term memory knowledge base. Another way to conceive of this is as an interaction between currently active, dynamic, or fluid aspects of the self with more permanent, long-term, or crystallized representations of the self and attributes of the self. The dynamic or executive aspect of the self is termed the working self. The working self consists of a complex hierarchy of currently active goals (Conway and Pleydell-Pearce, 2000) through which memories are encoded and retrieved. The working self also contains what Conway et al. (2004) termed the conceptual self, which in turn consists of beliefs, evaluations, and currently active self-images of what the self has been in the past, currently is considered to be, and what it may become in the future.

The working self regulates the construction of new memories in the SMS, at both encoding and during retrieval, by controlling access to the autobiographical memory knowledge base. **Figure 2** illustrates this relation between the working self and the knowledge base. The working self modulates memory by controlling the cues that are used to activate knowledge in the knowledge base. This is achieved by shaping cues so that particular types of information are activated. For example, a person asked to recall a memory of childhood might recall their earliest memory. Thus, elaborating the cue from 'recall a memory from childhood' into the cue 'recall my earliest memory.' This elaboration may take place several times as a cue is fine-tuned to access the information sought. An idea central to the SMS model is that specific autobiographical memories are formed when stable patterns of activation exist over interconnected representations of autobiographical knowledge and associated episodic memories. Thus, when conceptual and generic knowledge of the attributes of a house one lived in as a child, the relationship one had with one's parents, and a specific (episodic) memory of a moment in time are all activated together and interlinked, then the rememberer has the experience of remembering and their consciousness is dominated by a specific memory – as in the example we started with. It is these different types of autobiographical knowledge and their organization in long-term memory that we are concerned with next and we return to considering the process of constructing memories in a subsequent section.

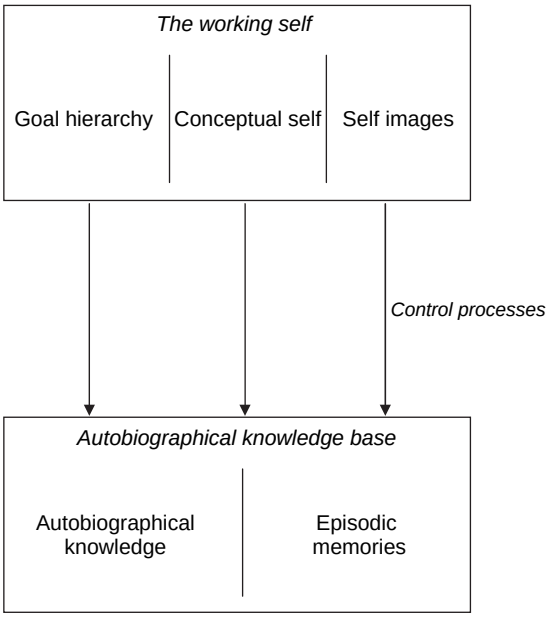

Figure 2 The relationship between the working self and the autobiographical knowledge base.

According to the SMS model, long-term memory contains two distinct types of autobiographical representation: autobiographical knowledge and episodic memories. Autobiographical knowledge is organized in partonomic hierarchical knowledge structures (Conway and Bekerian, 1987; Barsalou, 1988; Conway, 1993, 1996; Lancaster and Barsalou, 1997; Burt et al. 2003) that range from highly abstract and conceptual knowledge (such as that contained in the conceptual self) to conceptual knowledge that is event-specific and experience-near. Autobiographical memory knowledge structures terminate in episodic memories, the second type of autobiographical representation contained in the autobiographical knowledge base. **Figure 3** illustrates how these complex autobiographical memory knowledge structures might be represented in long-term memory.

The upper part of **Figure 3** focuses on autobiographical knowledge and specifically on the life story, lifetime periods, and general events (Conway, 2005). These divisions of autobiographical knowledge are on a dimension of specificity, and at the most abstract level is a structure termed the life story

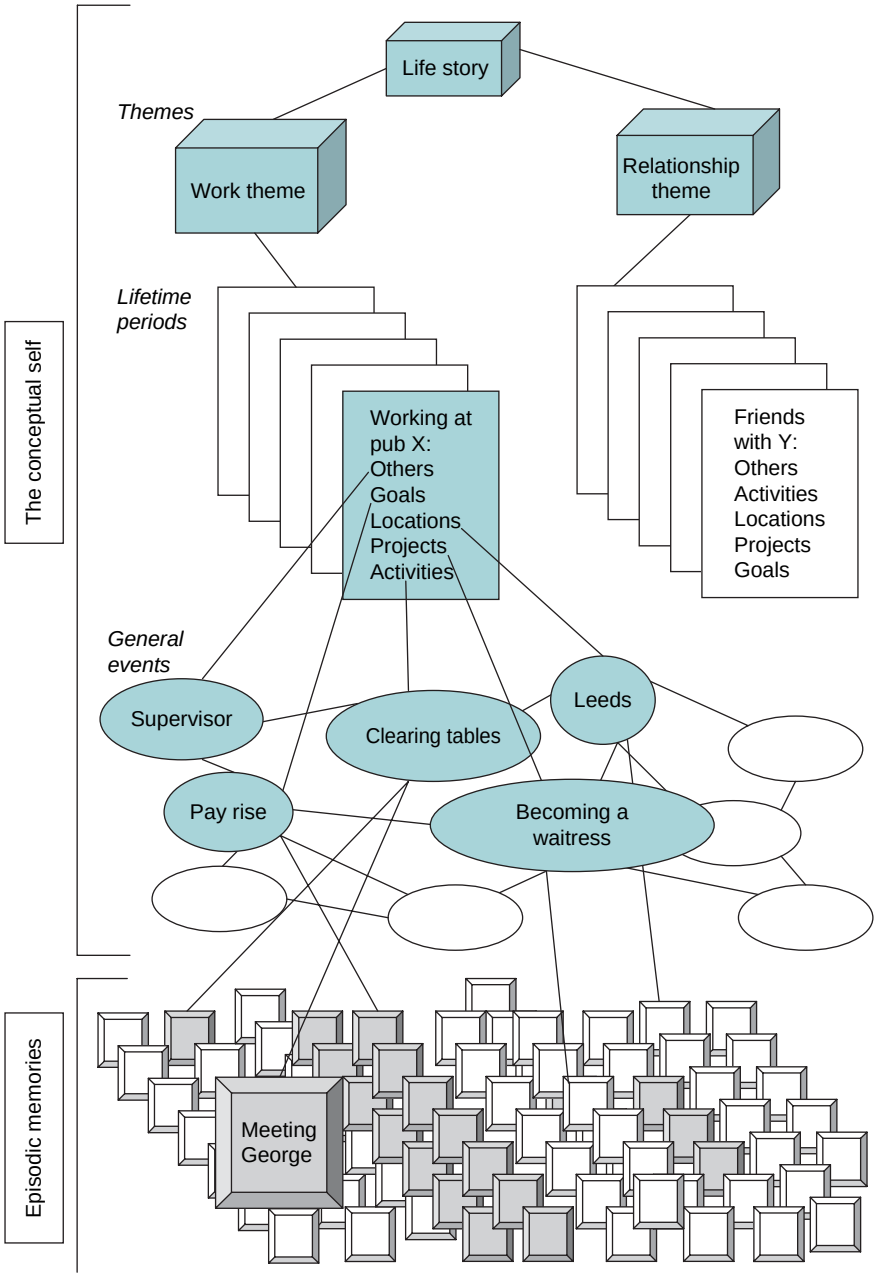

Figure 3 Knowledge structures in autobiographical memory. Adapted from Conway MA (2005) Memory and the self. *J. Mem. Lang.* 53(4): 594–628.

(Pillemer, 1998; Bluck and Habermas, 2001; Bluck, 2003). The life story contains general factual and evaluative knowledge about the individual. It may also contain self-images that divide and separate the self into several different selves. It is represented in more or less coherent sets of themes that characterize, identify, and give meaning to a whole life (Bluck and Habermas, 2000, 2001). Divisions in the life story may be supported by the way in which different self-images contain cues that differentially access other knowledge in the autobiographical knowledge base. For example, a self that accesses a particular lifetime period (see **Figure 3**) will have cues that are channeled by knowledge represented as part of the lifetime period, which in turn can be used to access particular sets of general events that contain cues to

specific episodic memories. It in this way that a memory can be gradually formed or constructed.

Lifetime periods contain representations of locations, people, activities, feelings, and goals common to the period they represent. They effectively encapsulate a period in memory and in so doing provide further ways in which access to autobiographical knowledge is channeled, or directed. Lifetime periods have been found to contain evaluative knowledge, negative and positive, of progress in goal attainment (Beike and Landoll, 2000), and lifetime periods may play an important role in the life story. For instance, lifetime periods may provide autobiographical knowledge that can be used to form life story schema and thus support the generation of themes. Lifetime periods may be particularly appropriate for this because of the goal-evaluative information they contain. For example, a lifetime period such as 'when I was at university,' will consist of representations of people, locations, activities, feelings, and goals common to the period but will also contain some general evaluation of the period, i.e., this was an anxious time for me, living away from home was difficult, I was lonely, I found the work too difficult, etc. (see Cantor and Kihlstrom, 1985).

The life story and lifetime periods are part of the conceptual self where they represent a summary account of the self and its history, and where they can be used to initiate and focus searches of the autobiographical knowledge base. General events, on the other hand, are more clearly part of the knowledge base itself and have been found to play important roles in organizing personal knowledge. General events are more strongly event-specific than lifetime periods but not as event-specific as sensory-perceptual episodic memories, which are directly derived from actual experience (Conway, 2001, 2005). General events refer to a variety of autobiographical knowledge structures such as single events, e.g., the day we went to London; repeated events, e.g., work meetings; and extended events, e.g., our holiday in Spain (Barsalou, 1988). General events are organized in several different ways. For example, they can take the form of mini-histories structured around detailed and sometimes vivid episodic memories of goal attainment in developing skills, knowledge, and personal relationships (Robinson, 1992). Some general events may be of experiences of particular significance for the self and act as reference points for other associated general events (Singer and Salovey, 1993; Pillemer, 1998). Yet other general events may be grouped together because of their emotional similarity (McAdams et al., 2001), and it is likely that there are yet other forms of organization at this level which await investigation(see for example, Brown and Schopflocher, 1998). However, the research currently available indicates that organization of autobiographical knowledge at the level of general events is extensive and it appears to virtually always refer to progress in the attainment of highly self-relevant goals. General event knowledge then represents information highly relevant to the goal hierarchy of the working self.

In one study of this type of knowledge, Robinson (1992) examined people's memories for the acquisition of skills, e.g., riding a bicycle, driving a car, and for aspects of personal relationships. These general events were found to be organized around sets of vivid memories relating to goal attainment. Consider two examples from Robinson's study:

> Ever agreeable, and eager to do anything that would get me out of the doldrums of inferiority, my father rented a bike and undertook to help me to learn to ride it. I shall always remember those first few glorious seconds when I realized I was riding on my own... (Quinn, 1990, cited in Robinson, 1992: 224.)

> The first time I flew an airplane was one of the best firsts. It marked a sense of accomplishment for myself, and it also started me on the career path I have always wanted to follow. The day was warm and hazy, much as summer days in Louisville are. My nervousness didn't help the situation, as I perspired profusely. But as we took off from runway 6 the feeling of total euphoria took over, and I was no longer nervous or afraid. We cruised at 2500 feet and I worked on some basic manoeuvres for approximately 45 minutes. We then returned to the airport, where I realized that this will soon be a career. (Robinson, 1992: 226.)

These first-time memories cue other related memories and the whole general event carries powerful self-defining evaluations that persist over long periods of time.

Relatively recent experiences, particularly those occurring during the current lifetime period, that give rise to sets of multiply related general events and associated episodic memories must be represented in terms of the currently active goals of the working self that dominate at the time. Burt et al. (2003) investigated this for several extended events, e.g., Christmas shopping. In these studies, events were sorted into groups by participants, and from these groupings currently active themes were identified. **Figure 4** shows the organization of a series of episodic memories associated

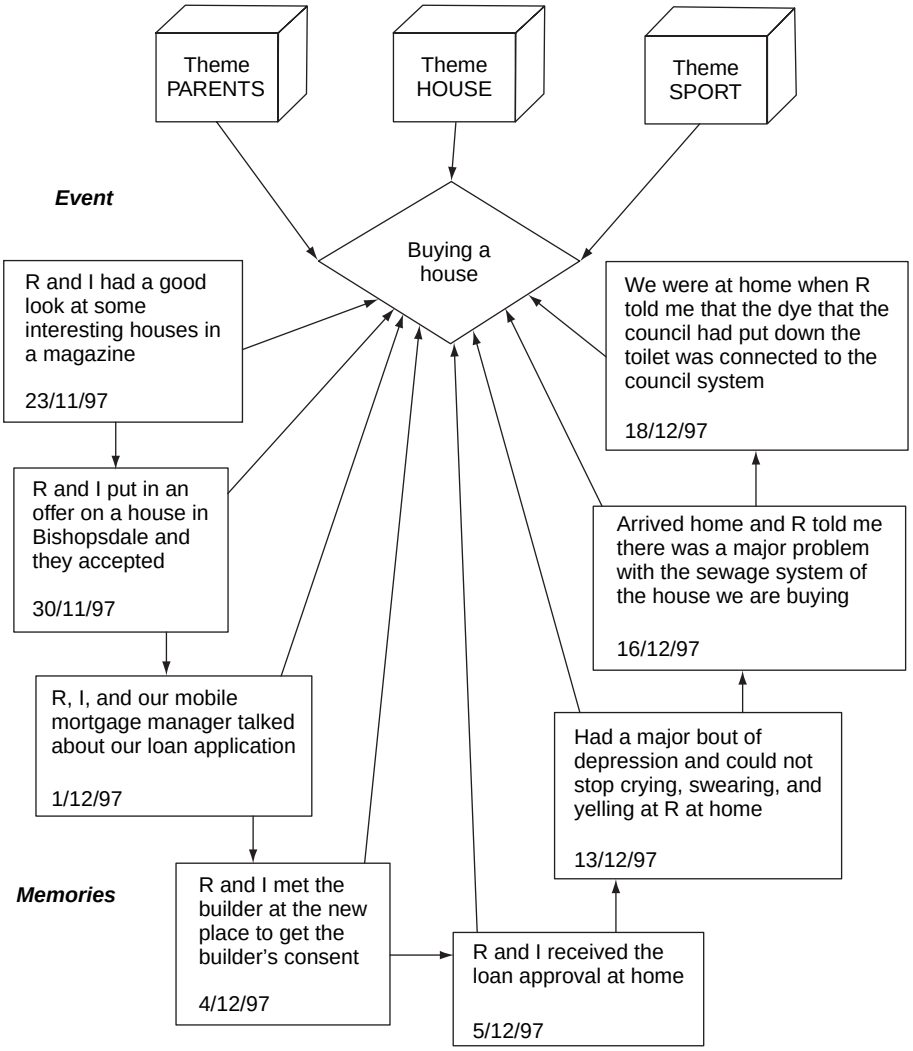

Figure 4 Episodic memories associated with the general event of buying a house. From Burt CDB, Kemp S, and Conway MA (2003) Themes, events, and episodes in autobiographical memory. *Mem. Cogn.* 31: 317–325.

with the general event of buying a house (Burt et al., 2003). The themes shown in **Figure 4** are all associated with other memories as well and with lifetime periods in which the themes were present. The findings of Burt et al. (2003) demonstrate that general events typically access groups of episodic memories that connect the general event to unique and specific moments in time. One important property of this organization is that when goals change and new themes and lifetime periods become central to the working self, a record of the past concerns of an older version of the working self exists in the form of general events and the colonies of episodic memories they access. Thus, even if no goal information is explicitly encoded, it can, to at least some extent, be inferred from the groupings of general events and the associated episodic memories. Indeed, Robinson found that many memories featured goal-related evaluative knowledge or self-defining memories (Singer and Salovey, 1993) along with more general knowledge and specific episodic memories. General events provide, then, records of complicated and extended goal-related activities. These have powerful implications for the self, especially the conceptual self, and how a person evaluates their self.

2.46.4 Episodic Memory

So far we have been concerned with autobiographical knowledge, but specific autobiographical memories

Table 1 Eight characteristics of episodic memory

I	They retain summary records of sensory-perceptual-conceptual-affective processing derived from working memory.
II	They are predominantly represented in the form of (visual) images.
III	They represent short time slices, determined by changes in goal processing.
IV	They are only retained in a durable form if they become linked to conceptual autobiographical knowledge. Otherwise they are rapidly forgotten.
V	Their main function is to provide a short-term record of progress in current goal processing.
VI	They are recollectively experienced when accessed.
VII	When included as part of an autobiographical memory construction, they provide specificity.
VIII	Neuroanatomically they may be represented in brain regions separate from other (conceptual) autobiographical knowledge networks.

consist of autobiographical knowledge and episodic memories. Episodic memories are, however, rather different types of representations. **Table 1** lists eight characteristics of episodic memories (from Conway, 2005, Table 4), and each of these is now considered in turn. The first three characteristics of episodic memories (numbered I, II, and III) in **Table 1** concern properties of episodic memories. First, the content of episodic memories is highly event-related and consists of detailed records of sensory-perceptual and conceptual-affective processing that was prominent during the original experience. Note that these are summary rather than literal representations, although they may occasionally contain some exact representations of processing that occurred during an experience (see the last paragraph of this section). Second, although they can, and indeed do, contain information from all the sensory modalities, they have been found to be predominantly visual in nature (see Brewer, 1988, for an interesting early study of the content of episodic memories). Finally they represent short time-slices of experience highly related to the moment-by-moment segmentation of experience into events (Williams et al., 2007b; Zacks et al., 2007).

Clearly, many episodic memories will be formed every day and simply casting one's mind back over the events of the day will bring to mind many highly detailed and specific episodic memories of events which occurred earlier in the day (see Williams et al., 2007b). In subsequent days, however, as the retention interval lengthens, many of these episodic memories, which are often of rather low self-relevance, routine events, become inaccessible. Even those that are retained over longer retention intervals are often not as detailed as they were close to the point of their formation. It has been suggested that only those episodic memories that are linked in some way to currently active goals become integrated with autobiographical knowledge in long-term memory. Episodic memories that become integrated in this way are retained over long retention intervals measured in months, years, decades, and even a lifetime (point IV in **Table 1**). Relatedly, the SMS model posits that one of the main functions of episodic memories is to provide a record of recent goal-processing episodes. Episodic memories provide a way in which to rapidly and effectively check that goal-related actions have been executed. They let the rememberer know that they did, for instance, lock the door, post the letter, have a coffee, and so on. If one of these routine events mapped onto an important goal or set of goals, then the episodic memory might become integrated with other knowledge in the autobiographical knowledge base and so become an enduring episodic memory. The study of self-defining experiences, the experience of trauma, and vivid memories generally provide many examples of how episodic memories become important parts of the autobiographical knowledge base, where they endure for many years (see Pillemer, 1998; Ehlers and Clark, 2000; Singer, 2005).

Points VI and VII in **Table 1** focus on another important aspect of episodic memories – that they are very highly associated with the experience of remembering. This is often referred to as recollective experience, and this and other forms of memory awareness have been the focus of many contemporary memory studies (see Tulving, 1985; Gardiner and Richardson-Klavehn, 2000, for reviews). Memory awareness in autobiographical remembering appears to be triggered or activated when an episodic memory enters conscious awareness (Conway, 2001, 2005), although it can also occur in other ways (cf. Moulin et al., 2005). Episodic memories, when they enter the construction of an autobiographical memory, cause the experience of remembering and also provided the constructed memory with specificity. As we will

see, the specificity of the memory is important and is a quality that can be lost when memory malfunctions in, for example, psychological illness. Specificity provides a link to the experience of the world, and episodic memories are experience-near representations and stand in contrast to autobiographical and other conceptual knowledge which is experience-distant. Thus, the experience of remembering and memory specificity are important qualities of episodic memories. Finally, in **Table 1** (see VII), it is suggested that episodic memories might be represented in a separate brain region from more autobiographical conceptual knowledge (this is elaborated in Conway, 2005). We will return to this issue in the closing section of this chapter, but we might note here one general and intriguing finding that seems to support it: patients who suffer brain damage which has led to amnesia for much of their preinjury life, and especially amnesia for preinjury episodic memories, have nonetheless been found to retain often extensive autobiographical knowledge (Conway and Fthenaki, 2000).

2.46.5 Self-Defining Memories

The autobiographical knowledge base is complex and represents the personal history of an individual in different ways, i.e., as knowledge and as specific memories. Because of this complexity, the knowledge base is highly organized and some parts are more accessible than other parts. Generally, those autobiographical knowledge structures that are strongly associated with current goals and current images of the self are in a more accessible state than knowledge structures that are currently less self-relevant. In this section, we consider how the relation to the self can shape and organize autobiographical memory.

One important type of personal knowledge that appears to be highly accessible to the self is that of self-defining memories (SDMs). An SDM is a specific type of autobiographical memory that has the following attributes: affective intensity, vividness, high levels of rehearsal, linkage to similar memories, and connection to an enduring concern or unresolved conflict (Singer and Moffitt, 1991/1992; Singer and Salovey, 1993; Singer, 2005). Self-defining memories can be distinguished from other types of vivid memories. For example, flashbulb memories, as originally defined by Brown and Kulik (1977), are a particularly vivid and affective form of personal event memory (Pillemer, 1998), often about important public events. They have been found to be associated with four interrelated variables: surprise, consequentiality, importance, and emotion (Conway, 1995). Having these qualities does not necessarily indicate, however, that the memory is central to enduring goals of the self, and it is certainly possible to have highly vivid memories of events that are low in self-relevance (Conway et al., 2004). Importantly then, the two distinguishing criteria for self-defining memories that differentiate them from other vivid memories are, first, their linkage to other memories within the individual that share similar personal themes and, second, their relevance to the individual's enduring concerns or unresolved conflicts.

Both of these features – linkage of similar memories and relevance to concerns and conflicts – have been investigated in research into individuals' motivations and goals. For example, Thorne et al. (1998) looked at young adults' important relationship memories generated in two interviews over a 6-month period of time. Participants had freedom to describe similar or different relationship episodes in the second interview. Thorne et al. scored the memories for social motives for the memories that varied from time 1 to time 2, as well as the points of emphasis in the twice-told memories. For both unique memories and repeated memories, the authors found "moderate thematic consistency" (Thorne et al., 1998: 258), indicating that these memories, even when varying in content, reflected similar motivational themes and narrative structures. In a related study, Demorest and Alexander (1992) had raters code individuals' significant personal memories for overarching interpersonal scripts. A month later, these same individuals generated a set of fictional scenarios. Raters coded the themes of these scenarios and found striking overlap in terms of thematic continuity between the original memories and the imaginary stories. These results, along with those of Thorne et al. (1998), suggest that individuals link remembered and imagined experiences through personally significant themes. These themes originate, according to the SMS model, from the goals of the working self, but later can also serve to influence its ongoing goal processing.

Further evidence of the relationship of self-defining memories to individuals' enduring conflicts and concerns comes from the work of Singer and colleagues (Singer, 1990; Moffitt and Singer, 1994; Singer, 2005). These researchers found the affective quality of self-defining memories to be a function of the relevance of the memories to the attainment of a person's most desired goals. Moreover, this was found

to be the case not only for memories relevant to the attainment of approach goals (desired goals), but also for memories about active efforts to avoid the consequences of undesired outcomes (Moffitt and Singer, 1994). Singer et al. (2002) additionally reported that the more personal growth students attributed to memories that grew out of community service experiences, the more likely these students were to place an overall emphasis on generative goal pursuits in their lives (see also de St. Aubin and McAdams, 1995). Similarly, in examining the relationship of turning-point and other significant personal memories to overall themes of the personality, McAdams (McAdams, 1982; McAdams et al., 1996) has consistently found power-oriented memories to be linked to agentic or individualistic motives, while intimacy-oriented memories reflected communal, social, and relationship motives. Jardine (1999) found that women counselors who experienced life transitions during their clinical training associated themes from their self-defining memories with their set of possible selves (Markus and Nurius, 1986). In a series of clinical case studies involving both individual and couples in psychotherapy, Singer found self-defining memories to be linked to critical relationship themes which were expressed in both clients' intimate relationships and in the transference dynamics of the therapy (Singer and Singer, 1992, 1994; Singer and Salovey, 1996; Singer, 2001; Singer and Blagov, 2004).

In addition to their linkage to goals, SDMs also can play directive and mood regulatory functions for the self (Pillemer, 1998, 2003; Bluck, 2003). For example, SDMs have been found to play a role in providing life lessons or integrative meanings that help individuals in optimal adjustment and personal growth. This is what Bluck (2003) termed the directive function of autobiographical memories. Blagov and Singer (2004) demonstrated that individuals with larger numbers of SDMs that contained reflective themes or messages, as reliably coded by three raters (see Singer and Blagov (2000) for an SDM coding manual), displayed optimal levels of self-restraint and emotional expression, as measured by the Weinberger Adjustment Inventory Short Form (Weinberger, 1997, 1998). Thorne et al. (2004) found that, compared to other types of personal memories, individuals were more likely to rely on SDMs involving tension or goal conflict to provide insights and life lessons.

SDMs provide information that can guide and direct the individual in everyday life. One specific form of directive function is the regulation of mood. Josephson et al. (1996) found that nondepressed individuals enlisted positive memories to repair negative moods, while mildly depressed individuals were less likely to recruit positive memories after a negative mood had been induced. Similarly, Moffitt et al. (1994) found that depressed individuals were less likely to recall SDMs when asked to retrieve a positive memory, while they did not differ in memory specificity for negative memories. Williams (1996), though not specifically addressing SDMs, has argued that a lack of memory specificity in depressed and suicidal individuals reflects a cognitive deficit generalized from a learned defense against encoding and retrieving affectively threatening self-relevant experiences. In summary, the findings from a broad range of studies converge on the view that SDMs are central to goals and conflicts within the individual (see Singer, 2005); they provide important integrative lessons, insights, or directives for the working self (see especially Pillemer, 1998); and they may regulate mood in important ways.

2.46.6 Self-Images

Conway et al. (2004) describe what they termed the conceptual self. One important knowledge structure in the conceptual self are self-images. It is proposed that self-images are knowledge structures that summarize complex sets of interlinked autobiographical knowledge and episodic memories that cumulatively support a particular view or version of the self. (Note that self-images can be permanent stable representations or more transitory, fleeting mental representations.) Conway (2005) proposes that these summary representations may often be experienced as images and hence the term self-images. A question of some interest here is how self-images are related to selective sets of memories. Rathbone et al. (2006; described in Conway, 2005) studied this by having a group of middle-aged participants complete a short questionnaire in which they completed six 'I am…' statements (Kuhn and McPartland, 1954). An 'I am…' could be anything, for example, I am bad, I am sociable, I am a banker, I am a mother, etc. Later each person recalled specific autobiographical memories to each of their 'I am…' statements. The dates of the memories, expressed in age at encoding, and the dates of the emergence of the 'I am…' statement were then compared; **Figure 5** shows the distribution of age at encoding of the memories relative to age of emergence of the 'I ams…'. **Figure 5** strikingly shows that age at encoding clusters around the date of emergence of the 'I ams…,' strongly

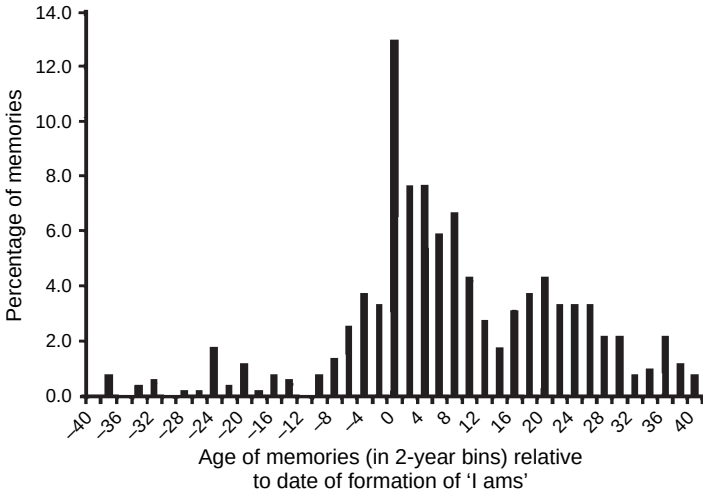

Figure 5 Distribution of memories recalled to "I ams. . ." (Rathbone et al., 2006).

suggesting that 'I ams. . .' or self-images are grounded in sets of memories of formative experiences.

Further work found that the 'I ams. . .' could be categorized into two broad classes: roles and traits, e.g., I am a student versus I am charming. However, both types of 'I ams. . .,' role and trait, gave rise to the same distribution as that shown for 'I ams. . .' overall in **Figure 5**. Both role and trait 'I ams. . .' seem then to be marked in memory by highly accessible specific memories that come first to mind when the 'I am. . .' is processed. This may reflect the grounding of these aspects of the conceptual self, self-images, in subsets of memories and knowledge that define and provide the content for that self-image. This differentiation of the self, supported by the organization of autobiographical memory into self-images, might be particularly important in the development of the self – a point we return to after considering the distribution of memories over the life span and the significance of this for the self.

2.46.7 The Life Span Distribution of Autobiographical Memories

Important periods of development of the self are reflected in the life span retrieval curve which is observed when older adults (about 35 years and older) recall autobiographical memories in free recall or in a variety of cued recall conditions (Franklin and Holding, 1977; Fitzgerald and Lawrence, 1984; Rubin et al., 1986, 1998). Memories are plotted in terms of age at encoding of the remembered experiences, and the resulting life span retrieval curve typically takes a form similar to that shown in **Figure 6** (this is an

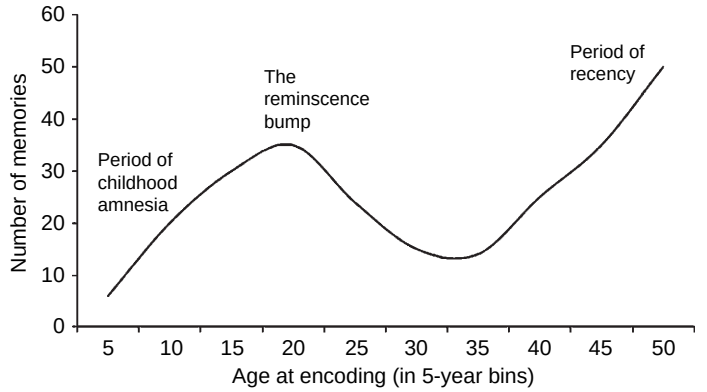

Figure 6 Idealized representation of the life span retrieval curve. From Conway MA (2005) Memory and the self. *J. Mem. Lang.* 53(4): 594–628.

idealized representation derived from many studies and not based on specific data).

As **Figure 6** shows, the life span retrieval curve consists of three components: the period of childhood amnesia (from birth to approximately 5 years of age), the period of the reminiscence bump (from 10 to 30 years), and the period of recency (from the present declining back to the period of the reminiscence bump). The pattern of the life span retrieval curve is extremely robust and has been observed in many studies – to such an extent that it led Rubin to conclude that it was one of the most reliable phenomena of contemporary memory research (Conway and Rubin, 1993). This reliability is remarkably striking. In a recent study, Conway et al. (2005) sampled groups from five different countries: the United States, the United Kingdom, Bangladesh, Japan, and China. **Figure 7** shows the life span retrieval curves for each of these countries. (Note that participants were instructed not to recall events from the previous year to eliminate the recency portion of the curve.)

It can be seen from **Figure 7** that there were highly similar periods of childhood amnesia and reminiscence bump across countries. This further demonstrates the robustness of the life span retrieval curve and perhaps its universality. If the data for the five countries are collapsed together and an overall life span retrieval curve plotted, then the remarkably consistent distribution shown in the idealized curve of **Figure 6** is observed.

There are many theoretical explanations of the period of childhood amnesia (see Pillemer and White 1989; Wang, 2003, for reviews), but most flounder on the fact that children below the age of 5 years have a wide range of specific and detailed autosbiographical memories (Fivush et al., 1996; Bauer, 1997). Explanations that postulate childhood amnesia to be related to general developmental changes in intellect, language, emotion, etc., fail simply because apparently normal autobiographical memories were in fact accessible when the individual was in the period of childhood amnesia. It seems unlikely that an increase in general functioning would make unavailable previously accessible memories. From the SMS perspective, this period is seen as reflecting changes in the working self goal hierarchy, the idea being that the goals of the infant and young child, through which experience is encoded into memory, are so different, so disjunct, from those of the adult that the adult working self is unable to access those memories (see also Howe and Courage, 1997, for a particularly interesting account of childhood amnesia in terms of development of the self). Other accounts emphasize mother/child interactions, the role of language development, and emergence of narrative abilities (Fivush and Nelson, 2004).

Socialization and culture must play some role in the development of memory, although it seems that the infant/child capacity to actually have episodic memories may predate these developments (Rovee-Collier, 1997). If this is the case, then presumably the effects of socialization, culture, and language are largely on the organization of memory and perhaps on memory content as well, rather than on the processes that mediate the actual formation of episodic memories. For instance, the finding of Conway et al. (2005) that U.S. participants retrieved earlier earliest memories than all other groups might relate to the

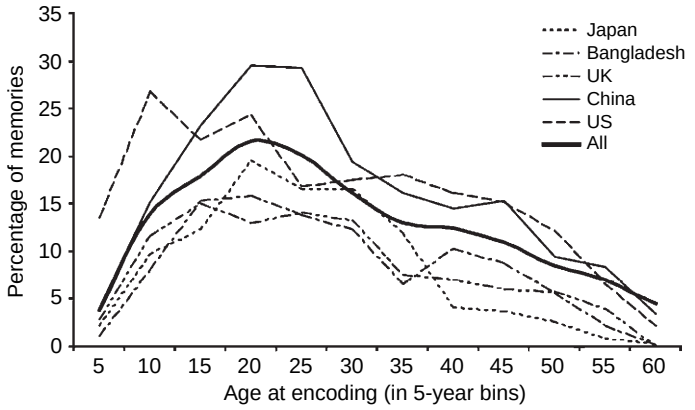

Figure 7 Life span retrieval curves from five countries. From Conway MA (2005) Memory and the self. *J. Mem. Lang.* 53(4): 594–628.

observation that U.S. mothers undertake more memory talk with their children than mothers from other countries. Moreover, Wang and her colleagues (e.g. Wang, 2001) have found powerful cross-cultural differences in the focus and content of memories. Childhood memories from people in cultures with interdependent self-focus (Markus and Kitayama, 1991) such as China tend to be less oriented to the individual, less emotional, and more socially oriented than the childhood memories of people from cultures with independent self-focus, for example, Northern European or North American cultures (see Wang, 2001). Thus, socialization experiences and the self-focus that predominates in a culture may influence the accessibility of earliest memories and their content.

The second component of the life span retrieval curve is the period when rememberers were aged 10 to 30 years, known as the reminiscence bump (Rubin et al., 1986). The reminiscence bump is distinguished by an increase in recall of memories relative to the periods that precede and follow it. The reminiscence bump is present not just in the recall of specific autobiographical memories but also emerges in a range of different types of autobiographical knowledge. For example, the reminiscence bump has been observed in the recall of films (Sehulster, 1996), music (cf. Rubin et al., 1998), books (Larsen, 1998), and public events (Schuman et al., 1997; Holmes and Conway, 1999). Memories recalled from the period of the reminiscence bump are more accurate (Rubin et al., 1998), are judged more important than memories from other time periods, and are rated as highly likely to be included in one's autobiography (Fitzgerald, 1988; Fromholt and Larsen, 1991, 1992; Fitzgerald, 1996; Rubin and Schulkind, 1997). The reminiscence bump is only observed in people over the age of about 35 years and some recent findings suggest that it might only be present, or is much more prominent, in memories of positive experiences (Rubin and Bernsten, 2003).

Many of the more obvious explanations of the reminiscence bump have been rejected, e.g., that the memories are of first-time experiences and that is why they are memorable, as in fact it has been found that less than 20% are typically of first-time experiences (Fitzgerald, 1988). Rubin et al. (1998) reviewed a series of potential explanations and argued in favor of an explanation in terms of novelty. According to this view, the period when people are aged 10–30 years, and especially 15–25 years, is distinguished by novel experiences, occurring during a period of rapid change that gives way to a period of stability. It is assumed that memories from the period of rapid change are more distinct than those from the period of stability and this is why they are comparatively more frequently accessed. By this account, a period of rapid change taking place at some other point in the life cycle should also lead to raised accessibility of memories from that period relative to more stable periods, and there is some evidence that this is the case (Conway and Haque, 1999). However, periods of (goal) change and experiences of novelty always involve the self and a related but alternative explanation is that the high accessibility of memories from this period (and other periods as well) may be related to their enduring relation to the self (Conway and Pleydell-Pearce, 2000). Possibly, many memories from the period of the reminiscence bump are memories of self-defining experiences (see Fitzgerald, 1988) and have a powerful effect in cohering the working self into a particular form. The novelty of reminiscence bump experiences lies in their newness and uniqueness for the self and they may play a crucial role in the final formation of a stable self system and identity formation during late adolescence and early adulthood. The raised accessibility of these memories might then serve processes relating to the coherence of self through time.

Thus, the period of the reminiscence bump might be a period in which a sole 'I am. . .,' or self-image, develops into multiple 'I ams. . .,' e.g., I am a son, I am a student, I am a boyfriend, etc. Also, at this point multiple 'I will becomes. . .' may be formed, supported by the differentiation of 'I ams. . .' and the final emergence of a complete working self goal hierarchy and conceptual self grounded in autobiographical knowledge and memories (the SMS). Finally it might be noted that older patients with schizophrenia have been found to show an early and disorganized reminiscence bump, with an impairment of conscious recollection associated with memories highly relevant to personal identity (Cuevo-Lombard et al., 2007). This suggests that a developmental failure present in schizophrenia is the consolidation of personal identity in late adolescence/early adulthood. Possibly, one of the features of the abnormal SMS associated with this is a failure or weakening of the grounding of conceptual autobiographical knowledge in episodic memories of formative experiences, further demonstrating the importance of an integrated self with self-images strongly embedded in sets of defining episodic memories.

2.46.8 Closing Section: Why Do We Have Autobiographical Memory?

In many respects this may seem a pointless or rhetorical question; after all, if we did not have autobiographical memory there would be little in the way of individuality, personality, culture, society, literature, etc. Much that differentiates humanity from other species would be absent (see Tulving, 1983). At the level of the individual, disruption to or loss of autobiographical memory leads to people who typically cannot function in society. For example, clinically depressed patients often have severely impaired autobiographical memories in which they can no longer generate specific memories, their memories lack detail, they are overly general (Williams, 1996). Such patients cannot operate in the social world and, moreover, have unspecific futures in which they cannot visualize specific plans and goals (Williams et al., 2007a). Similarly, with amnesic patients whose memory disorders arise from organic brain damage, having multiple self-images in a specific future in which goals and plans originating from memories of the past are realized is no longer possible. Thus, one good reason to have an intact and functioning autobiographical memory is that it allows the individual to have a future in which a continuous self operates.

But what does this mean? The future is, of course, a time where new experiences, some anticipated, will take place. But we cannot know we have arrived at the future without a memory – that is, without knowledge of a past. The concept of future makes no sense, conceptually or psychologically, without a past. One way to think about this is to conceive of the future as a place where new goal processing will take place and the past as some sort of record of previous episodes of goal processing. To achieve future goals it is essential to have a record of how one has progressed with the same or related goals in the past. Consider very recent goals. In order to know that one locked the car after parking it this morning, we simply remember that episode. The events of the current day can typically be recalled (on that day) at length and in highly specific detail. Thus, checking on progress with goals, locking the car, making a call, mailing a paper, etc., can be verified. However, within a few days, access to these sorts of detailed memories is lost. No doubt this is useful as retaining a highly detailed record of every action would lead to an overloaded and unworkable memory. Nonetheless, keeping a detailed record in the short term is highly adaptive and prevents the repetition of actions and the adoption of courses of actions that have a high probability of failing.

Conway (2005) argues that episodic memory is the memory system that keeps a record of very recent goal-related activities. It is a system that has evolved highly specific memory representations that facilitate the type of short-term goal processing that can keep goals focused and environmentally relevant. It is suggested that this is a species-wide adaptation and, consequently, episodic memory is common to many species. As such it is probably a phylogenetically older memory system and may be represented in neural networks located toward the middle and posterior of the brain (a temporal-occipital network; see Conway, 2005). In contrast, humans have developed conceptual knowledge that forms complex knowledge structures that endure over long periods of time, even over a lifetime. This, it is suggested, is a more recent evolutionary development and is mediated by neural networks toward the front of the brain: fronto-temporal regions. The conceptual memory system supports long-term goal processing, for example, relationships, work projects, etc. Episodic memories that are retained become attached to conceptual knowledge and provide highly specific instances of goal processing related to the more general or generic goals of the conceptual self and self-images. Autobiographical memory then allows us to have both short- and long-term goals and to integrate these in coherent ways that facilitate goal processing in the future.

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2.47 Social Memory Processes

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In 1932, Bartlett published a classic book on memory in which he rebelled against the prevailing Ebbinghaus tradition that focused on people's ability to reproduce lists of words or nonsense syllables. Bartlett argued for the importance of studying memory of more complex and meaningful material. He also opposed the idea, still common today (e.g., Kandel, 2006), that remembering is analogous to mental time travel. This analogy implies that people can go back in time and recapture their original experiences. The invalidity of this assumption is perhaps evident to most people with memory for emotionally tinged events. The negative emotions associated with adverse events fade markedly with time. Similar fading of emotional intensity occurs for positive events, but at a much slower rate (Walker et al., 2003).

Bartlett emphasized that memory of more complex information often involves active reconstruction, in addition to the reactivation of lasting traces in nerve cells in the brain. He suggested that remembering is guided by present knowledge and goals, with the result being that a reconstruction can differ from the original experience. Bartlett also noted that remembering can be a collaborative as well as an individual activity, and that people develop particular memory skills in response to the demands of their social, cultural, and physical environments. Bartlett highlighted the importance of social factors by entitling his book *Remembering: A Study in Experimental and Social Psychology*.

Today, most psychologists studying memory focus on the cognitive and neurological factors underlying individual recall. It is social psychologists who have picked up Bartlett's themes regarding the social aspects of remembering. Social psychologists study topics such as self-conceptions, attitude formation, relationships, conformity, and conflict. They do not typically study memory *per se*. Nevertheless, memory, and especially autobiographical memory, plays an active role in many phenomena of interest to social psychologists. Autobiographical memories and self-conceptions are closely related (James, 1950; Singer and Salovey, 1993; Pillemer, 1998; Conway and Pleydell-Pearce, 2000; McAdams, 2001; Bluck, 2003; Ross and Wilson, 2003). People can use their memories to assess their beliefs, traits, self-worth, and social acceptance, as well as to guide their actions and decisions.

According to Bartlett (1932) and Neisser (1967), people often remember only a few elements of an episode; they then use their current knowledge and beliefs to fill in gaps and resolve ambiguities in their memories. Rememberers reconstruct what should (or must) have happened and confuse their reconstruction with recollection: They suppose that what

should have happened did happen (Bartlett, 1932; Mead, 1964; Neisser, 1967; Ross, 1989). Although people are better than Bartlett proposed at distinguishing their suppositions from their recall (Roediger et al., 2001), his analysis provides a useful conceptual tool for examining when and how the present affects recollections.

The reconstructive aspect of memory has perhaps been overemphasized relative to another important characteristic of memory: its selectivity. In remembering and reconstructing the past, people focus on some episodes and not others (e.g., memory of a particular wedding), and on some aspects of episodes rather than others (Rogers et al., 1977; Bellezza, 1984; Symons and Johnson, 1997). People's current goals and motives can influence both the selection of past episodes and their reconstruction. Past experiences are often sufficiently numerous, contradictory, and ambiguous that people can use selection, reconstruction, or both to remember what they need or prefer to recall. A man motivated to recall happy times in his marriage will typically remember at least some pleasant memories involving his partner. When motivated to recall unhappy times, the same man would probably remember some unpleasant memories. As we shall see, however, there are constraints on memory: Remembering is not just wish fulfillment.

In the present chapter, we discuss research on the effects of present knowledge, goals, and motivation on individual recall. Because people often remember as pairs or groups in real life, we also review research on two important forms of joint remembering. People collaborate to recall dates, names, and past events. We examine research on the effects of collaboration on accuracy of recall. Also, people often exchange memories with other individuals. Indeed, much of people's knowledge of events comes from others, including friends, family, teachers, and the media. We examine how memories change as they are transmitted from one person to another, as well as how these changes affect people's beliefs and attitudes.

2.47.1 The Effects of the Present on Recall

In everyday life, the hindsight bias, or the I-knew-it-all-along effect, is perhaps the most widely recognized example of the influence of the present on recall. According to a popular cliché, hindsight is 20-20. In research on the hindsight bias, psychologists compare hindsight judgments made with knowledge of an outcome (e.g., the winner of an obscure military battle) to foresight judgments made without such knowledge. Participants in the hindsight condition typically regard the actual outcome as more likely than do those in the foresight condition (Fischhoff and Beyth, 1975; Slovic and Fischhoff, 1977; Hoffrage et al., 2000). For example, Fischhoff and Beyth (1975) asked university students to predict the likelihood of various events before President Nixon's visits to Beijing and Moscow. After Nixon's trips, these students remembered assigning higher probabilities than they originally did to events that actually occurred. Presumably participants had a difficult time recalling the exact probabilities that they had assigned. In reconstructing their predictions, they used their present knowledge to estimate their prior probabilities (Hoffrage et al., 2000).

The hindsight bias has potentially important implications for people's assessments of behavior and individuals. When people evaluate past performances, they often know the outcomes (e.g., whether a medical diagnosis was valid or a military tactic was successful). The hindsight bias can inappropriately lead people to criticize individuals who fail and admire those who succeed. For example, physicians informed of both a patient's symptoms and autopsy results indicating the cause of death are surprised that other physicians could have made an incorrect diagnosis prior to the autopsy. Physicians told of the symptoms but not of the autopsy results are less certain of the diagnosis (Dawson et al., 1988). Alternatively, when events turn out well, successful people are sometimes credited with too much foresight. In *War and Peace*, Tolstoy accuses Russian historians of making this error in judgment (cited in Hoffrage et al., 2000). Historians wrote that the Russian army defeated Napoleon by tricking him into marching toward Moscow. However, the Russian victory was probably more attributable to luck than to foresight.

Researchers have extended investigations of hindsight by examining how people's current knowledge and beliefs influence their recall of their earlier attitudes, feelings, and behaviors. While recalling the past, people are often very aware of the present (Ross, 1989). For example, people know how they currently feel about a politician but may be less certain how they felt years earlier. Unless they have a compelling reason to think that they have changed, they often presume personal consistency, supposing that their earlier opinions resemble their current beliefs (Ross, 1989). The perception of consistency

helps people to sustain a sense of personal identity: They are the same individuals that they were yesterday or last year (Erikson, 1968; Epstein, 1973; James, 1950). When people assume stability in the face of actual change, they exaggerate the similarity of the past to the present and evidence a consistency bias in recall.

2.47.2 A Consistency Bias in Recall

Much of the research demonstrating a consistency bias in recall examines people's memories of their earlier attitudes and feelings. Goethals and Reckman (1973) studied the effect of an experimentally induced change in attitudes on recall of earlier opinions. At an initial session, high school students completed a questionnaire indicating their attitudes on a variety of issues, including bussing students to achieve racial integration in the schools. At a second session 4–14 days later, the students received information that led them to change their views on bussing. They were then asked to recall their attitude responses on the initial questionnaire. They remembered responding more in harmony with their new position on bussing than they actually had. Following a change in attitudes, individuals tend to underestimate the degree to which they have altered their opinions.

Several researchers have examined this same phenomenon in the context of naturally occurring changes in evaluations. McFarland and Ross (1987) asked undergraduates to evaluate their dating partners at an initial session on a series of rating scales and then again months later. At the second session, participants also attempted to recall their earlier ratings. Presumably they could not easily remember precisely where they placed their x on the rating scales. As a result, they had to reconstruct their earlier evaluations. Apparently, participants based their reconstructions, in part, on their current impressions of their partners. Participants who became more favorable over time recalled more positive evaluations than they had provided originally; those who became less favorable recalled more negative evaluations.

Levine (1997) studied changes in feelings reported by supporters of Ross Perot, an independent candidate for the US presidency. Perot withdrew from the presidential race in July 1992 and then re-entered in October 1992. Levine polled Perot supporters immediately following his withdrawal and again following the election in November of the same year. After the election, supporters recalled the emotional reactions they had reported immediately following Perot's withdrawal 5 months earlier. Respondents' recall of their emotions following the withdrawal was consistent with their current political ideology. Those who continued to support Perot recalled feeling less anger and more hope than they had reported earlier.

Taking the consistency bias in recall one step further, Ross et al. (1981) examined how people's current attitudes affect their recall of their own past actions. To show a cause and effect relation between attitudes and behavioral recall, Ross et al. (1981) provided participants with communications that challenged their beliefs concerning certain health issues. For example, some participants learned of scientific evidence that frequent tooth brushing is potentially harmful to gums and tooth enamel. Shortly after reading the communication designed to change their attitudes toward tooth brushing, participants completed a questionnaire that assessed their frequency of engaging in various health-related behaviors in the previous 2 weeks. As anticipated, those who now believed that frequent tooth brushing was harmful recalled brushing less often than did participants not exposed to the anti–tooth brushing message.

If people recall behavioral histories that are consistent with their new beliefs, then the act of remembering past behavior could increase people's commitment to their new beliefs. On looking back, people 'discover' that they have behaved consistently with their new beliefs; this memory should support the validity of these beliefs. To test whether behavioral recall increases support for new attitudes, Ross et al. (1983) induced participants to change their attitudes. Some participants were then asked to recall past behavior relevant to the attitude domain in question. Their recall was not constrained. They were free to recall behaviors congruent or incongruent with their new opinions. Other participants did not engage in behavioral recall. Participants in the behavioral recall condition seemed to be more committed to their new attitudes. They were more likely to report intentions to act consistently with their new attitudes and more resistant to a message attacking their new attitudes than were participants who were not prompted to recall past behaviors. These findings suggest a reverberating circuit in which new attitudes affect behavioral recall, which in turn affects commitment to the new attitudes.

Studies of memory reconstruction point to the dangers of assuming the validity of people's recall. Recent research in cross-cultural psychology supports this concern. There is an extensive literature on cultural differences in the experience and determinants of emotion (e.g., Kitayama and Markus, 1994; Oishi, 2002). Much of this research on emotion is retrospective. For example, people are asked how frequently they felt various emotions in the last month (e.g., Eid and Diener, 2001). Researchers typically find that American and Canadians of European heritage recall experiencing many more positive (e.g., happy) than negative (e.g., sad) emotions in their everyday lives. In contrast, Americans and Canadians of East Asian heritage as well as respondents living in Japan report experiencing about the same number of positive and negative emotions (Markus and Kitayama, 1994; Oishi, 2002; Ross et al., 2002), and fewer positive emotions than European Americans and Canadians.

These research findings can be interpreted as indicating that Westerners are happier on a daily basis than are their East Asian counterparts. Alternatively, perhaps Western and East Asian individuals experience about the same number of pleasant and unpleasant episodes, but Westerners recall a greater number of their pleasant experiences. Such a recall bias in Western cultures could reflect a Western cultural schema that happiness is important and common, a cultural schema that is less evident among Eastern cultures (Oishi, 2002). Conceivably, Westerners and East Asians recruit memories that support their differing beliefs, with the result that Westerners recall more happy experiences.

Oishi (2002) compared retrospective accounts to daily diary and online reports of emotional experiences. European and Asian Americans did not differ in daily diary reports of the quality of their day, or in online reports of current positive and negative moods. Interestingly, cultural differences clearly emerged at the end of the week when participants were asked how good or bad the week was, or how often they had experienced positive and negative moods. European Americans retrospectively reported greater satisfaction with the week and a higher frequency of positive moods compared to Asian Americans.

Are East Asians less happy than their Western counterparts? The answer depends on whether the focus is on current or retrospective reports. As Oishi observed, "retrospective judgments seem to be as important as actual experiences in understanding subjective experiences of well-being . . . online and global reports capture different but equally important aspects of well-being" (Oishi, 2002: p. 1405).

Oishi's study is unlikely to be the final word on cultural differences in emotional experience. Researchers using other samples and procedures might find evidence of online differences. From the present perspective, the Oishi study is important for two reasons. First, it reminds us that recall should never be assumed to be an exact replica of earlier experience. Second, as Oishi notes, retrospective reports can be psychologically significant, even when they do not mirror online reports.

2.47.3 Motivated Recall

In presenting research on the effects of current beliefs on recall, we depicted the recall process as a rather dispassionate cognitive exercise. A new belief can be more than a recall cue, however; it can also motivate biased recall (Greenwald, 1980; Kunda, 1990). For example, in the tooth brushing study reported by Ross et al. (1981), people presumably preferred to believe that they had been behaving in a way that would not cause their teeth to rot or their gums to fall apart. Researchers examining the relation between motives and recall have studied both chronic motives and experimentally induced motives. The research evidence indicates that the content of autobiographical recall reflects people's persistent motives (e.g., McAdams, 1982; McAdams et al., 1996; Woike et al., 2003; Gramzow and Willard, 2006; Sahdra and Ross, 2007). For example, Sahdra and Ross (2007) examined how degree of identification with a religious group influenced people's recall of harms committed by members of that group. High identifiers (who are motivated to view their group favorably) were less likely than low identifiers to recall episodes in which members of their group acted violently toward members of a different religious group.

Other researchers have altered people's beliefs about the desirability of specific traits or behaviors and then assessed people's memories of their past actions. In studies conducted by Sanitioso and his associates, individuals were led to believe that a particular trait such as extraversion was or was not related to success in life. Participants who believed that extraversion is desirable were able to recall their own extraverted behaviors more quickly and easily than were participants who supposed that

introversion is preferable (Sanitioso et al., 1990; Sanitioso and Niedenthal, 2006).

Murray and Holmes (1993) asked undergraduates in dating relationships to report the amount of conflict they had with their partner while deciding on joint activities. Participants in the experimental condition then read a bogus psychological article that argued that the development of intimacy in a relationship depended on people's willingness to express disagreement. Thus, experimental participants who had reported that they and their partner experienced little conflict now learned, much to their surprise, that low conflict was actually bad for a relationship. A control condition contained participants who had also reported low conflict with their partners, but who did not read the bogus article.

How did experimental participants deal with their new understanding that conflict was desirable? Many altered their views of their partner's past behaviors. When asked to assess their relationships on a number of dimensions, experimental participants were more likely than controls to endorse items such as "My partner clearly expresses his/her needs even when he/she knows that these needs conflict with my needs." In short, they discovered evidence that their relationship was appropriately conflict-ridden.

Baumeister and his colleagues (Baumeister et al., 1990, 1993; Stillwell and Baumeister, 1997) have studied how people who anger someone else (perpetrators) remember a dispute compared to individuals who are provoked (victims). Generally, perpetrators regard their behavior as less harmful and more justifiable than victims do. Along the same lines, young children recall disputes with their siblings in a manner that tends to absolve them of blame. They remember more harmful actions by their siblings than by themselves and portray their own actions as justifiable and their siblings' behavior as arbitrary and incomprehensible (Ross et al., 1999).

Studies of mood regulation provide further evidence of motivated remembering. Several theorists have suggested that individuals who are feeling dejected may attempt to improve their moods by selectively retrieving pleasant memories (Clark and Isen, 1982; Isen, 1987; Singer and Salovey, 1988). For example, Parrott and Sabini (1990) found that participants experiencing negative moods were more likely to recall pleasant events from their lives than were participants experiencing positive moods. Subsequent researchers suggested that certain personality traits may predispose individuals to alleviate negative affect by engaging in mood-incongruent recall (Smith and Petty, 1995; Boden and Baumeister, 1997; McFarland and Buehler, 1997). McFarland and Buehler found that only individuals who are especially inclined to focus on their feelings recruited more pleasant memories after a negative mood induction than after a neutral mood induction.

The research on motivation and recall might seem to imply that people can readily create a preferred past. If a woman wants to believe that she is shy, she can recall introverted behaviors. If the same person prefers to believe that she is outgoing, then she can readily recall extraverted behaviors. George Herbert Mead (1964) argued that memory is indeed this malleable and compared people's recollections to "escape fancies ... in which we rebuild the world according to our hearts' desires" (pp. 348–349).

More recent research suggests that there are limits to people's ability to recall pasts consistent with their heart's desires. People's autobiographical memory includes more general memories as well as specific episodic memories (Conway and Playdell-Pearce, 2000; Klein et al., 2001, 2002). These general memories are summaries of repeated behaviors and events (e.g., going to nightclubs) and include personality traits (e.g., 'I am an introvert'). A person who arrives at an experiment with the generalized memory that she is an introvert is unlikely to discover suddenly that she is outgoing, even if she learns that extraversion is highly desirable (Sanitioso et al., 1990). Her remembering is constrained in two interrelated ways. She possesses a generalized belief that she is an introvert, and her stock of accessible episodic memories likely reflects her generalized belief. A woman who believes strongly that she is shy should be more able to access introverted behaviors, regardless of the experimenter's claims regarding the desirability of the trait.

Constraints on the effect of preferences on memory are clearly evident in research that assesses recall accuracy. Consider, for example, a study of university freshmen and sophomores' recollections of their grades over all 4 years of high school (Bahrick, 1998; Bahrick et al., 1996). Overall, their recall was quite good: Participants recalled 71% of their grades accurately. In short, students did not rewrite history to create a past in which they received straight As. Nonetheless, the errors they did make were systematic. Of the errors in recall, 81% were inflations of the actual grades (Bahrick, 1998). Also, participants' errors reflected their general academic ability. Students with high grade point averages recalled more of their Bs as As than did students in the lowest grade point average quartile. Thus outstanding

students were more likely than mediocre students to infer a grade of A on those relatively few occasions that they misremembered their performance.

Studies of mood recall also provide evidence of both accuracy and bias. Retrospective reports of mood are accurate, in that recollections are correlated with earlier online reports of mood (e.g., Feldman Barrett, 1997) but biased by the rememberers' personality and beliefs about emotional experiences (Feldman Barrett, 1997; Robinson and Clore, 2002; Christensen et al., 2003).

Some theorists (e.g., Bahrick, 1998) associate schema-consistent errors with memory reconstruction and accurate recall with reproductive memory. Neither of these claims is necessarily true. Memory can be schema consistent because it is selective rather than reconstructive. For example, a person who is motivated to believe that she is outgoing may accurately retrieve episodes from her memory store (reproductive memory) but selectively retrieve memories that imply extroversion rather than introversion. As well, accurate recall can reflect memory reconstruction rather than the direct retrieval of information from memory. Suppose, for example, that some participants in the Bahrick et al. (1996) study believe that they are outstanding students. Also suppose that they cannot readily recall the grades that they received in several courses. These students might infer that they received high grades in these courses, because they view themselves as good students. If their academic self-assessment is reasonably valid, then they will be accurate in inferring high grades.

As Bartlett and others (e.g., Neisser, 1967) have argued, most autobiographical recall is probably a combination of reconstruction and reproductive remembering. The degree to which reconstruction or reproduction dominate will depend on a variety of factors, including the strength of encoding of the original events, the length of the time period between the event and the recall, the motivation to remember accurately, and the accessibility of relevant cognitive schemata (e.g., beliefs about of one's academic ability, personality, etc.) to guide recall.

2.47.4 Perceiving Change

We have argued that people are often cognitive conservatives who underestimate the degree to which their past feelings and beliefs differ from their present judgments. But people commonly perceive change in themselves on other dimensions, especially ability- and personality-related attributes on which improvement is possible. They also see changes in the world around them. Next, we review research showing that people are particularly inclined to see themselves as improving and the world as getting worse.

2.47.4.1 The Perception of Self-Improvement

In his autobiography, Arthur Koestler (1961) remarked that people are critical of their adolescent past self: "The gauche adolescent, the foolish young man that one has been, appears so grotesque in retrospect and so detached from one's own identity that one automatically treats him with amused derision. It is a callous betrayal, yet one cannot help being a traitor to one's past" (Koestler, 1961: p. 96). Wilson and Ross (2000, 2001) obtained research support for Koestler's observation but found that retrospective criticism of past selves extends well beyond adolescent selves. Across various samples (e.g., university students, middle-aged individuals, celebrity interviews) and in a variety of dimensions, people viewed themselves as steadily improving (Wilson and Ross, 2000, 2001). Moreover, people rated themselves as having improved more since a particular time in the past when that point was manipulated to seem long ago rather than recent (Wilson and Ross, 2001).

This perception of improvement is due, in part, to a retrospective tendency to find fault with earlier selves. The self that seems impressive today appears less remarkable in retrospect. Ironically, the tendency to disparage earlier selves seems to reflect concerns for self-enhancement (Ross and Wilson, 2000, 2001). People who are motivated to evaluate themselves favorably (e.g., those with high self-esteem) are particularly inclined to recall an inferior past self. Also, people do not see the same steady improvement in their peers that they see in themselves. By criticizing their own earlier selves, people can view their current self favorably by contrast.

Most of the studies of perceived self-improvement involve younger people. Would people in their 60s and 70s be more likely to see themselves as declining physically and cognitively? The answer is a qualified yes (McFarland et al., 1992; Ross and Wilson, 2001). Older people do view themselves as declining, but at a much slower rate than their peers (Ross and Wilson, 2002). When people cannot use retrospective comparisons to feel good about their current selves,

they apparently engage in downward social comparisons to accomplish the same end.

If people are concerned with self-enhancement, why do they not simply praise their present selves rather than derogating past selves? There are advantages to deflating the past rather than inflating the present. If people continually boosted their current selves, their present self-regard might become so overstated as to be inconsistent with objective indicators (Baumeister, 1989). Appropriate judgments and choices depend on an accurate view of one's strengths and weaknesses. By derogating the past, individuals create an impression of improvement without greatly misrepresenting their present strengths and weaknesses.

Researchers have examined perceived improvement in a number of different contexts. Karney and his associates examined people's retrospective evaluations of satisfaction with their marriage (Karney and Coombs, 2000; Karney and Frye, 2002). Spouses underestimated their past contentment and often recalled it as lower than their present satisfaction. Although marital satisfaction decreased over the early years of marriage, individuals created the illusion of improvement by underestimating their former satisfaction levels. Studying prospective and retrospective trajectories of newlyweds' relationship satisfaction, Karney and Frye (2002) showed that this perception of improvement is linked to other indicants of relationship success. Spouses' retrospective reports of increases in relationship satisfaction predicted optimism about their relationship's future, independent of any actual change in satisfaction. In contrast, absolute levels of relationship satisfaction were unrelated to expectations. By derogating earlier aspects of themselves and their relationships, people can make their current state seem superior by comparison and foster optimism about the future.

People can also use retrospective reevaluation to detect a silver lining in their personal tragedies. McFarland and Alvaro (2000) asked individuals who had experienced a tragedy to evaluate what they were like prior to the episode. Some participants were reminded of the disturbing episode before completing the evaluation, and others were not reminded. Participants who were reminded provided lower evaluations of their earlier, pretrauma selves. In addition, people were more critical of former selves after being reminded of severely rather than mildly disturbing experiences. Individuals may reduce the negative impact of a trauma by focusing on how it led to growth or positive outcomes for the self.

Retrospective overestimation of change is especially likely when people experience a circumstance that they expect to produce change on certain dimensions, but that in reality has minimal impact on those qualities. Self-help programs are a context in which people's hopes of change are likely to be disappointed. Participants tend to suppose that self-help programs are beneficial, but formal evaluations that include placebo control conditions typically show the programs to be of little value (Polivy and Herman, 1983; Ross and Conway, 1986). Conway and Ross (1984) studied the relation between memory and expectations for change in the context of a study-skills program. They asked university students to evaluate their study skills and then randomly assigned half of them to a study-skills program that lasted several weeks and the remaining half to a control condition. Although participants in the treatment program expected to improve their grades, their program, like most other study-skills courses, was ineffective. At the conclusion of the course, participants in the treatment and control conditions were asked to recall their original ratings of their study skills. They were reminded that the researcher had their initial ratings and would assess the accuracy of their recall. Participants who took the course remembered their preprogram ratings as being worse than they had reported initially. In contrast, control participants, who had not received the program, exhibited no systematic bias in recall. The biased recollections of participants in the study skills course would support their belief that the program had improved their skills. More generally, a tendency to revise the past in order to claim personal improvement may explain why many individuals report that they benefit from ineffective therapies and self-improvement programs (Conway and Ross, 1984).

2.47.5 Perceiving Change in Society

2.47.5.1 Mistaking Change in Self for Change in the World

Although people tend to see themselves as improving, they regard their society as deteriorating. Moral standards are weakening, crime rates are rocketing, and the quality of popular music and films is declining (Eibach et al., 2003). This view of societal deterioration can be found in most cultures and eras: "Virtually every culture past or present has believed that men and women are not up to the standards of their parents and forbears" (Herman,

1997: p. 13). There are a host of plausible explanations for the perceived decline (Eibach et al., 2003; Ross, 1989), but Eibach and his colleagues tested a particularly intriguing one: Change in the self is mistaken for change in the world. According Eibach et al. (2003), when people experience life changes their concerns and interests shift. For example, new parents are more sensitive to possible dangers to children, including crimes. In the face of an actual decline in crime rates, new parents are more likely to perceive crime rates as increasing than are respondents who did not become parents during that interval (Eibach et al., 2003). Similarly, dieters perceive an increase in advertising for unhealthy foods in the previous decade, relative to nondieters (Eibach et al., 2003).

Why are the perceived social changes so often negative? Eibach et al. (2003) provide a number of possible answers to this question. For example, they quote Herman (1997), who notes that as older people's cognitive and physical abilities decline with age, they might confuse their own diminishing powers with decline in the world. Also, older people's greater familiarity with the social mores, films, and music of their youth might enhance their appreciation of the good old days.

2.47.5.2 Group Status and the Perception of Social Change

Eibach and his associates have conducted other research on how different groups in society, especially the haves and have-nots, evaluate progress toward social equality. Generally, privileged groups see more progress than disadvantaged groups do. Men report that the income gap between men and women has declined more in the previous decade than women do; White Americans view the conditions for Blacks as improving more over the previous few years than Blacks do (Eibach and Keegan, 2006). Eibach and Ehrlinger (2006) suggest that advantaged and disadvantaged groups use different reference points to evaluate change. White respondents compare the treatment of Blacks in the past to the treatment of Blacks in the present and report progress. Black respondents compare their current outcomes to the outcomes they should receive (equality with Whites) and report that they still have a long way to go. Both groups are right: a half full glass is also half empty.

Why do more privileged and less privileged groups select different reference points? One answer is that members of the more privileged group are concerned about losing their advantages, and so are especially sensitive to how each group's status has changed over time. In contrast, members of less privileged groups are primarily interested in achieving equality; therefore they focus on the gap between where they are and where they need to be to attain equality (Eibach and Keegan, 2006).

2.47.6 Subjective Time and Point of View

Although most studies have concerned the content of autobiographical memory, researchers have examined two additional properties of memory with social psychological implications: People's feelings of temporal proximity to past events and their visual perspective on the remembered events.

2.47.6.1 Subjective Time

The subjective experience of time is related to clock and calendar time – last week feels farther away than yesterday – but it is not the same thing (James, 1950; Brown et al., 1985; Block, 1989; Wilson and Ross, 2003). Of particular interest here is that differences in the evaluative implications of past episodes influence people's feelings of proximity to those events (Ross and Wilson, 2000, 2002). To protect their current self-regard, people are motivated to feel farther from past failings than from achievements. In one study (Ross and Wilson, 2002), university students were asked to remember the course in the previous semester in which they received either their best or worst grade. After reporting their grade, participants indicated whether the course 'felt' recent or far away. Participants felt farther away from a course in which they obtained a relatively low grade, even though the actual passage of time did not differ in the two conditions. Subsequent research indicated that this asymmetry reveals both a tendency to pull favorable outcomes forward in subjective time and push inauspicious outcomes backward (Ross and Wilson, 2002), though the latter effect may be somewhat stronger.

Meichenbaum (2006a,b) examined the relation of the subjective experience of time to psychological disorders such as posttraumatic stress disorder (PTSD). According to Meichenbaum, these disorders reflect a flawed self-narrative. A self-narrative is the internal autobiography people construct to make sense of the life they have lived so far.

Meichenbaum suggests that the trauma memories of individuals with PTSD are stuck in the present. Individuals with persistent PTSD engage in internal conversations about ongoing threats, ruminate on the negative impact of the trauma, and actively try to suppress thoughts and emotions related to the trauma. The trauma takes center stage and is characterized as the most important theme in the life story.

To address the effects of PTSD, Meichenbaum advises therapists to help patients reframe their traumatic memories into historical narratives that have a distinct beginning, middle, and end. Patients with PTSD should incorporate traumatic memories into the self-narrative in such a way that the events are seen as a small part of the life story, rather than an ongoing theme. Meichenbaum also suggests that therapists emphasize the distinction between present and past and position the traumatic memory firmly in the past.

2.47.6.2 Point of View

Nigro and Neisser (1983) reported that individuals recall events from either a first-person or a third-person visual perspective. When people adopt a first person perspective, they view the event through their own eyes. When people assume a third-person point of view, they view the event as an outside observer who is watching the actions of the past self. The fundamental attribute of a third-person memory is that individuals can see themselves in the recollection.

Like subjective time, point of view is a variable that relates to 'how' people remember, rather than 'what' they remember. Moreover, like subjective time, point of view is associated with actual temporal distance, memory content, and self-concept. Older memories are more likely to be viewed from a third-person point of view (Nigro and Neisser, 1983). Memory perspective is flexible, however, and the content of the memory also affects the perspective adopted (Nigro and Neisser, 1983). For example, participants were more likely to use a first-person perspective when they focused on the emotional content of a memory, rather than its objective circumstances (Nigro and Neisser, 1983).

Libby and Eibach (2002; Libby et al., 2005) related the visual perspective of autobiographical memories to the self-concept. Individuals were more likely to invoke a first-person perspective when recalling actions consistent with their current self-concept. For example, participants who were induced to feel religious (by means of a biased questionnaire) were highly likely to recall a religious memory from a first-person perspective. Participants who were encouraged to feel irreligious were significantly more likely to report that they viewed a religious memory from a third-person perspective (Libby and Eibach, 2002). In another study (Libby et al., 2005), participants were randomly assigned to recall the same episode from either a first-person or third-person perspective. Participants who invoked a third-person perspective reported that they had changed more since the time of the episode. A third-person perspective seems to operate as a distancing mechanism, leading individuals to perceive that a past self is a different person than the current self.

Some clinicians have advocated use of the third-person perspective in therapy. Lawrence (1990) suggested that a patient speaking in the third person is able to adopt a more detached perspective on memories. Lawrence claimed that, as a result, third-person analysis yields less guilt and fewer defensive justifications. Similar to pushing events back in subjective time, the use of a third-person perspective involves reducing the psychological threat of negative experiences through distancing, rather than through forgetting or denial.

2.47.7 Memory in a Social Context

2.47.7.1 Collaborative Memory

Although psychologists generally study remembering as a solitary cognitive activity, everyday remembering is frequently collaborative. For example, spouses depend on each other's memories as they try to recall phone numbers or names (Wegner et al., 1991). Intuitively, it seems likely that collaboration would improve recall, and research confirms this belief: Two people, remembering together, recall more than either individual would recall alone (e.g., Vollrath et al., 1989; Weldon, 2001).

There are two obvious reasons why collaboration might improve memory. First, group memory might be better simply because two individuals are remembering rather than one. Alternatively, collaboration might bring forth memories that would not arise during solitary remembering. If such synergy occurs, two individuals would remember more together than they would if they pooled their individual recollections. To clarify this distinction, consider two spouses independently remembering a shopping list. On his

own, the man remembers items a, b, c, and d; meanwhile, his wife remembers a, b, e, and f. Alone, they each remember four items. If they pooled their individual recollections, they would together recall six nonredundant items (a, b, c, d, e, f). What if, instead of recalling the shopping list separately, they had recalled it together? Collaboration provides the opportunity for cross-cuing: The recollections of one person can offer cues that help another person remember information (Meudell et al., 1995). It seems plausible, then, that collaborative recall would generally exceed the sum of individual recollections provided by group members.

To examine whether collaboration produces better memory than pooled individual recollections, researchers include three conditions: individuals remembering alone, individuals remembering together, and nominal group recall. Nominal groups are groups in name only. Participants remembering alone are coupled, often randomly, and their recall is pooled. The recall score of a nominal group is the total amount of nonredundant information in the pooled recall. When these procedures are followed, the findings are consistent: Nominal group recall exceeds collaborative recall, which in turn surpasses individual memory (e.g., Basden et al., 1997; Weldon and Bellinger, 1997; Finlay et al., 2000; Weldon, 2001; Ross et al., 2004).

Labeled collaborative inhibition (Weldon and Bellinger, 1997), the finding that nominal recall outstrips collaborative recall has been obtained in dyads of strangers, friends, and married couples, and in elderly couples as well as college students. Although collaborative inhibition sometimes declines in well-acquainted groups, it is not reversed. Collaborating friends or spouses recall no more information than nominal groups, and they usually recall less (Andersson and Ronnberg, 1995; Johansson et al., 2000; Gould et al., 2002; Ross et al., 2004). Evidently, the cross-cuing that occurs during collaboration produces inadequate retrieval cues and interference rather than emergent memories (Meudell et al., 1995; Finlay et al., 2000). While listening to someone else's recollections, group members might forget their own memories or be prevented from trying to remember (Diehl and Stroebe, 1987). Also, idiosyncratic, self-generated retrieval cues are often better triggers for one's own memories than cues provided by another person (Basden et al., 1977; Meudell et al., 1995; Andersson et al., 2006). To the extent that collaboration inhibits self-generated retrieval cues, recall is likely to suffer.

So should spouses or work colleagues collaborate when trying to remember information in everyday settings, for example, the items in a list? The answer is yes for three reasons. First, collaborative recall is better than individual recall, even if it is not superior to pooled nominal group recall. Second, over time well-acquainted groups develop integrated systems of memory storage and retrieval for some everyday memory tasks (Wegner, 1986). They learn to divide the labor on memory tasks based on personal expertise (e.g., the travel agent spouse remembers to book summer vacations) and gender-role stereotypes (e.g., the man remembers when the car needs an oil change). Because of this division, there is no reason for spouses to try to remember everything. As long as they know what type of information their partners know, they can call on them when needed.

Finally, collaboration is useful because it can help reduce mistakes in memory even when it does not increase the amount of true memory recalled. A measure of mistakes, or false positives, is omitted in many studies of collaborative recall because the frequency of errors is low in the types of memory tasks typically used in this research (Ross et al., 2004). Using everyday memory tasks in which false memories were quite common, Ross et al. (2004) found that collaborative groups of older adults reported fewer errors in free recall than did nominal groups or individuals recalling alone. A recent study shows that collaboration produces a similar reduction in errors of younger (under age 40) participants (Ross et al., unpublished data).

Why might collaboration reduce memory errors? Any particular error is often unique to an individual, reflecting his or her knowledge, beliefs, and associative linkages between items in long-term memory (Ross et al., 2004). When errors are idiosyncratic, a rememberer's partner can exercise a kind of quality control by inspecting the memory, assessing its accuracy, and expunging false recall.

A reduction in false recall could be especially important for older people, who tend to recall more false memories than their younger counterparts (Jacoby and Rhodes, 2006). Relative to younger adults, older individuals are more likely to be misled by false information, more prone to source memory errors, and more confident of the accuracy of their false memories (Hashtroudi et al., 1989; Jacoby, 1999; Karpel et al., 2001; McCabe and Smith, 2002; Kelley and Sahakyan, 2003; Jacoby et al., 2005). There is not much research on techniques that might help older people reduce such errors. Collaboration has the advantage of being a readily available strategy in everyday life.

2.47.7.2 Controlling and Transmitting Memories

The research discussed in the preceding section on collaborative memory features two individuals remembering together at the same time. But memory is often collaborative in another sense. Memories are transmitted from person to person and from generation to generation. Much of what we remember we have learned from others rather than experienced directly. This type of remembering is evident in people's knowledge of the history and origins of their countries. As with individual memory, prior events or beliefs that contradict current ideas and values are sometimes erased from the history or altered so as to be consistent with present understandings (Goody and Watt, 1968; Ong, 1982). For example, when the British arrived in Ghana in the early part of the twentieth century, they found that the state of Gonja was divided into seven territories, each ruled by its own chief (Goody and Watt, 1968). When British authorities asked them to explain their system, the Gonja revealed that the founder of their state, Ndewura Jakpa, had fathered seven sons. Jakpa divided the land so that each son ruled one territory. Shortly after the British arrived, two of the seven states in Gonja disappeared as a result of changes in boundaries. Sixty years later, oral historians again recorded the myths of state. In the updated version, Ndewura Jakpa had only five sons; the Gonja made no mention of the founders of the two territories that had vanished from the scene.

In literate societies, individuals also revise history, especially in response to changing knowledge, goals, and political regimes (Greenwald, 1980). People interpret the past in terms of the present, and therefore "every generation rewrites its history" (Mead, 1964: p. 351). Some of the revisions involve efforts to improve the past. Stories of atrocities and wrongs committed by compatriots are often excluded from a nation's history textbooks and from popular culture (Hein and Selden, 2000). As Blight (2001) remarked with respect to memory of the American Civil War, "deflections and evasions, careful remembering and necessary forgetting, and embittered and irreconcilable versions of experience are all the stuff of historical memory" (Blight, 2001: p. 5).

We do not have to look to history to find archival evidence of people's efforts to improve the past. We can look at our own discipline, psychology. Research findings constitute the core of scientific psychology. Are past results described accurately in secondary sources, such as review articles and book chapters of the sort you are reading? Not always. When writing about past research, psychologists sometimes describe the results or procedures in ways that magnify the strength of the findings (Berkowitz, 1971; Loftus, 1974; Harris, 1979; Vicente and Brewer, 1993), as well as allow their theoretical preferences to guide their summaries and interpretations of past research (Berkowitz, 1971; Harris, 1979; Vicente and Brewer, 1993). Comparable distortions occur in the literature of other sciences (Vicente and Brewer, 1993).

It is also not difficult to find evidence of distortions in the media that could contribute to distortions in memory. The media play a role in communicating the words of famous individuals to society at large and therefore in producing memories of their statements. Misquotations in newspapers and other print media provide intriguing examples of historical revision (Keyes, 1992). For example, baseball manager Leo Durocher is credited with saying "Nice guys finish last." He really said, "The nice guys are all over there. In seventh place." The quote became punchier and pithier with repeated retelling. A second notable misquotation is associated with the comedian W. C. Fields. Fields is renowned for saying, "Any man who hates dogs and children can't be all bad." Fields did not say it. Leo Rosten said it about Fields when he introduced the comedian at a banquet. Keyes (1992) provided many other examples of quotations that change or are ascribed to the wrong person over time. Keyes was able to trace the source of the various quotations because he had access to documentary records.

Ordinary people do not write textbooks or journal articles or get quoted in the media all that often. But they do transmit information and memories across generations through socialization, stories, and teaching. Sometimes this information is transmitted precisely and remembered verbatim. For example, children memorize the times tables and the spelling of words, while anatomy students memorize the names and locations of brain structures. In these cases, approximations are not good enough. Even though the words are very similar, you cannot write golf if you mean gulf. In most contexts, however, children and adults are not required to recall or transmit a verbatim memory. Daily remembering typically involves recalling the meaningful gist of episodes rather than exquisite detail. Moreover, as information is transmitted from individual to individual, it changes. People adjust their reports of their memories in response to their listeners' status, age, interests, knowledge, and attitudes (Brown and

Levinson, 1978; Higgins and Rholes, 1978; Cansler and Stiles, 1981; DePaulo and Coleman, 1986; Schlenker and Weigold, 1992; Kashima, 2000; Thompson et al., 2000; Lyons and Kashima, 2003). The recipients of the information then take ownership of it by making it more consistent with their current beliefs and knowledge (Bartlett, 1932).

The study of rumors provides an intriguing example of how information is constructed and transmitted. Rumors often emerge when information is scarce but people feel a need to know (Allport and Postman, 1947). Consider, for example, rumors that occurred after Hurricane Katrina struck the Gulf Coast region of the United States (Rosenblatt and Rainey, 2005). The hurricane destroyed lines of communication and the associated ability to obtain direct news reports from the Gulf Coast. Most news reports were based, at best, on secondhand accounts. News agencies reported grossly exaggerated stories. In the first few days after the hurricane struck, news reports implied that the city had descended into chaos: Senseless looting was prevalent, snipers were randomly shooting people from rooftops, and gangs were roving the streets in murderous rampages. Most of these rumors were either exaggerations or completely false (Rosenblatt and Rainey, 2005).

Bartlett (1932) attempted to capture this process of rumor transmission with his method of serial reproduction. In Bartlett's research, the first participant in a chain read a fairly obscure and confusing passage (e.g., 'The War of the Ghosts') and recalled the passage after a 15-min delay. A second person read the first person's account and recalled it; a third person read the second person's account, and so on. Bartlett found that verbatim recall of the passage was rare. Participants commonly omitted unusual elements and added connections to make sense of the material. Bartlett took these findings as evidence for a reconstructive view of memory. It is also likely that the process of communication influenced recall of the story. To be clear and comprehensible, participants might alter the information that they transmit.

Along the same lines, Allport and Postman (1947) had the first participant in a six- or seven-person chain view a picture. The first person served as an "eyewitness" and was the only person in the chain to see the picture. The initial person described the picture (while viewing it) to a second person. This second person then transmitted what he or she remembered to a third person and so forth. Participants were told to listen carefully and transmit what they heard as "exactly as possible" (Allport and Postman, 1947: p. 66).

In contrast to Bartlett, who used obscure stories as stimuli in his studies, Allport and Postman used pictures that were not difficult to understand. The pictures contained everyday scenes such as people seated on a subway. Nonetheless, Allport and Postman obtained results similar to Bartlett's. They found that descriptions of the pictures were leveled and sharpened as recall moved along the chain. Leveling involves reducing the amount of detail, and sharpening refers to emphasizing just a few core elements. As rumors are transmitted, they change from more elaborate stories (e.g., desperate residents of New Orleans breaking into stores seeking supplies) to a core theme (e.g., senseless looting).

Allport and Postman (1947) used several pictures, but their most famous scene portrayed a Black and White man having a confrontation on a subway train. In the original scene, the White man held a knife. Early in the chains, participants accurately recalled that the White man possessed the knife. As the description of the picture was passed along the chains, however, the knife sometimes ended up in the hands of the Black man. The stereotype of aggressive Black men apparently influenced memory further down the chain. Similar to Barlett, Allport and Postman did not provide detailed descriptions of their findings. It is unclear in how many chains the knife changed hands.

Recent media reporting of world events may have replicated this finding. Many have argued that the news reports of senseless looting in New Orleans reflect similar racial stereotyping (Rosenblatt and Rainey, 2005). News reports portrayed Black Americans breaking into stores looking for necessary supplies and, occasionally, expensive consumer items (Rosenblatt and Rainey, 2005). Often incidents of people stealing expensive products such as plasma televisions were emphasized more than incidents of people appropriating diapers, toothbrushes, and canned goods (Rosenblatt and Rainey, 2005). Negative aspects of the Black stereotype likely contributed to how events were reported in the media and remembered by a non-Black audience.

Gilovich (1987; Study 1) applied the method of serial reproduction to person perception. The first person in the chain watched a videotape of a male or female student describing transgressions they had committed, along with mitigating circumstances that helped explain their actions. Participants then evaluated the target on several dimensions (e.g., generous/selfish) and described what they had seen on the videotape to another person. They were told to provide a

description that would permit a listener "to determine what this person did and what this person was like" (Gilovich, 1987: p. 63). The second-generation person evaluated the target after hearing the description. Interestingly, second-generation participants evaluated the transgressor more negatively than did the first people in the chain. This difference arose because first-generation participants were more likely to take mitigating circumstances into account. In leveling and sharpening their report, however, first-generation participants underemphasized mitigating circumstances; as a result, such conditions had less of an impact on the judgments of second-generation participants.

The Gilovich study has intriguing practical implications. Because the public rarely has direct exposure to world events, public opinion is influenced by how events are depicted in the media. And the leveling and sharpening that occurs in the process of reporting is likely to affect public opinion. There is evidence, for example, that media reports affect judgments of the criminal justice system. Public opinion in Canada and elsewhere often evaluates criminal sentences as too light (Roberts and Doob, 1990). In examining this phenomenon, Roberts and Doob found that media reports of criminal trials are leveled and sharpened. Journalists often omit mitigating circumstances that judges take into account during sentencing. In one study conducted by Roberts and Doob (1990), individuals who read actual court documents supported a lighter sentencing decision than did those who read a newspaper description of the sentencing decision.

2.47.8 Creating Memories

The issues that we have discussed concerning the transmission of memories come into focus in media portrayals of historical figures. Novelists, dramatists, and screenwriters sometimes deliberately blur the line between historical truth and fiction. They may sometimes do this for political reasons; for example, Shakespeare tailored the facts in his histories to suit the preferences of the Tudor monarchs of his day. Authors may also rewrite history to increase dramatic tension and maintain an audience's interest. In his play *Amadeus*, Peter Shaffer's depiction of the characters of Mozart and Salieri, and of Salieri's possible complicity in Mozart's death, is effective drama but questionable history. For many members of the audience, Shaffer's account may provide the primary source of information on Mozart's life story.

Presumably most people attend films or live theater to be entertained rather than to obtain a history lesson. When the tale is set in an historical context, however, they may believe that they have received both. Audiences who are unaware of alternative accounts may accept such stories as authentic. In this manner, the media help shape people's collective memories.

The revision of history to accomplish particular objectives is not limited to professional writers, the media, or scientists. As we have emphasized in this chapter, it is also a hallmark of everybody's efforts to recall and communicate their pasts. In comparison to professional writers who derive their historical stories from written records, individuals may be less aware of their alterations as they use their present knowledge, beliefs, and goals to construct their own histories. In this sense, people's personal recollections are comparable to the oral traditions of nonliterate societies.

It is useful and normal for people to create pasts that satisfy their current needs. As Bartlett (1932) and Mead (1964) stressed, the past is a resource that people can use and adapt for current purposes. People can get into trouble, however, if they underestimate the fallibility of their own memories. Perhaps the lesson of social psychological research on memory is not that people should be less creative but that individuals should be aware of the degree to which they rewrite their own histories.

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2.48 Collective Memory

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Collective memory is a representation of the past that is shared by members of a group such as a generation or nation-state. Instead of focusing on individual experience and memory, the study of collective memory examines social phenomena such as commemoration, history education, and mass media to understand how they give rise to shared accounts of the past. In some cases, the events in collective memory have occurred during the lifetime of members of the group, and in others they are from decades or centuries earlier, but in all instances the emphasis is on the social, cultural, and psychological processes that give rise to shared representations.

Instead of neutral knowledge, collective remembering typically involves beliefs – often strongly held – that are tied to identity, and hence they may evoke strong emotions when challenged. The fact that different groups can have quite different accounts of the past means that social identity and the politics of identity typically must be taken into account.

The concept of collective memory is often traced to writings of the French sociologist Halbwachs (1887–1945), who argued that remembering is shaped by participation in collective life and that there are as many accounts of the past as there are collectives (Halbwachs, 1992) (The two major works by Halbwachs in English – *On Collective Memory* (1992) and *The Collective Memory* (1980) – are compilations of French publications from the 1920s, 1930s, and early 1940s. He died in Buchenwald concentration camp shortly before the end of World War II.). In recent decades, related terms such as public memory (Bodnar, 1992) and cultural memory (Assmann, 2006; Lotman, 1990) have emerged alongside of collective memory and are now part of the memory industry (Klein, 2000) in the humanities and social sciences. A concern with these topics can be found in academic disciplines such as psychology (Pennebaker et al., 1997), anthropology (Cole, 2001), history (e.g., Novick, 1999), and sociology (e.g., Schuman and Rodgers, 2004), and it is also widely encountered in public discussions of issues such as the Holocaust and the Vietnam War.

Despite – or perhaps because of – the fact that collective memory is so widely discussed in the public sphere and academic disciplines, there is little agreement on its definition. In contrast to the study of individual memory, where some concurrence exists on basic constructs and methods, definitions of collective memory, let alone the methods for studying it, vary widely. Indeed investigators usually seem to be quite unaware of the work of others, even when they employ the same terms.

In an attempt to lay out a conceptual map for this field of inquiry, I shall use collective memory as a general term to discuss findings from a range of disciplines and theoretical traditions. Scholars such as Gedi and Elam (1996) object to this, viewing collective memory as a poor substitute for older terms such as political tradition and myth. For them, using the term is "an act of intrusion . . . forcing itself like a molten rock into an earlier formation . . . unavoidably obliterating fine distinctions" (Gedi and Elam, 1996: 30). The problem with such critiques is that they do not deal with the issue of how various strands of inquiry can be related to one another. Instead of assuming that these strands will remain isolated, the emphasis in what follows is on how they can be connected into a larger whole.

This review will be organized around sections concerned with: (1) collective memory as social framing, (2) collective memory in the social construction of groups, and (3) collective memory as semiotic distribution. These three topics are not so much competing perspectives on the same set of issues as different research traditions that have had little contact, each with its own theoretical and methodological starting point. The tradition concerned with memory as social framing focuses on how individuals'

memories are shaped by social forces. Its main emphasis is on the influence of these social forces on individuals in a group, rather than the origin and nature of the groups themselves. The tradition concerned with the social construction of groups focuses on processes that create and maintain groups, and it tends to treat memory in an instrumental role in these processes. And the tradition concerned with collective memory as semiotic mediation draws on yet other disciplines to examine how language and other cultural tools mediate social and individual processes.

These three traditions do not line up neatly along disciplinary lines, nor do they constitute anything as coherent as schools of thought. They have, however, remained relatively self-contained and isolated lines of inquiry. This is due not so much to contradictions among them as to the fact that different communities of scholars, using different concepts and methods, are often unaware of the others' existence. However, the terms memory and collective memory are widely used across approaches, and for this reason these traditions of inquiry can be viewed as providing different answers to a basic question that guides this chapter: What makes collective memory collective?

All three traditions I shall outline are united in their acceptance of a psychological dimension of the larger picture, a point worth noting since this means they avoid the pitfalls of a strong version (Wertsch, 2002) that sometimes emerges in discussions of collective memory. Strong versions in one way or another assume the existence of memory in some sort of group mind, an assumption that is usually grounded in parallels between individual and collective processes. Such ideas – often in the form of unexamined assumption – have been criticized at least since Bartlett objected to positing a "more or less absolute likeness . . . between social groups and the human individual" thereby assuming that "whatever is attributed to the latter has been ascribed to the former" (Bartlett, 1932: 293).

Bartlett, who used the term social psychology quite strategically in the subtitle of his landmark monograph *Remembering*, rejected a strong version of collective memory (social memory in his terminology). He did so by stressing the difference between "memory *in* the group," which he embraced as the focus of his research, and "memory *of* the group" (Bartlett, 1932: 296, italics in the original), which he rejected as being a fundamentally misguided notion. In his view "Social direction and control of recall – memory within the group – are obvious; but a literal memory *of* the group cannot, at present at least, be demonstrated" (Bartlett, 1932: 298). As will be argued, the tradition of inquiry concerned with collective memory as semiotic distribution provides the clearest means for avoiding a strong version of collective memory, but all three traditions avoid the pitfalls of a strong version of collective memory in one way or another.

These traditions also share a preference for focusing on remembering as a process rather than memory as a static object or body of information. For reasons similar to those that motivated Bartlett to title his 1932 text *Remembering*, it is would be preferable to speak of collective remembering rather than collective memory. Using the former emphasizes the centrality of what Bartlett called the "effort after meaning." In the case of collective remembering, using the term furthermore highlights the active social and political processes involved, processes of contestation and conflict between visions of the past. But collective memory is a term that is so widely used in academic and popular discourse that any attempt to ban it would be futile. Hence, I shall use collective memory and collective remembering interchangeably in what follows.

2.48.1 Collective Memory as Social Framing

At least since the 1920s scholars have argued that remembering must be viewed as a socially framed or situated activity. When Halbwachs said that there are as many memories as groups, he often had such ideas in mind, a point that is reflected in his assertion that "it is individuals as group members who remember" (Halbwach, 1980: 48), and as already noted, Bartlett made similar claims. Both of these founders of memory studies took it as a given that individuals – but socially situated individuals – remember. As Olick (1999) has noted, Halbwachs sometimes appeared to be ambivalent on this issue, and at some points he even seemed to accept a strong version of collective memory (Wertsch, 2002). However, many passages in his writings are quite similar to what can be found in Bartlett.

The social framing of memory has continued to be an important thread of research in psychology and sociology, even shaping the study of forms of remembering that appear to be prototypically individual. This can be seen in the research on flashbulb memory. Since being introduced by Brown and Kulik

(1977), this phenomenon has been the object of widespread interest both in psychology and the broader public. Neisser (1982) defines it as "a subjectively compelling recollection of an occasion when we heard an important piece of news" (Neisser, 1982: 43). It appears to be intensely personal since the memory involved focuses on the individual's own experience of the event rather than the event itself.

Some flashbulb memories are about specific events experienced by individuals and derive from "the sundry private shocks in each person's life" (Brown and Kulik, 1977: 75) such as hearing about the death of a family member. However, most cases examined in the literature concern events that are jointly experienced, and it is in this connection that they have a collective dimension. The assassination of President John F. Kennedy in 1963 provides the classic example of this. Virtually everyone in the United States above early childhood at the time has a vivid and subjectively compelling recollection of where she was and how she heard about it. Along with other events such as the assassination of Martin Luther King, Jr., the 1986 Challenger shuttle explosion, and the terrorist attacks of September 11, 2001, such instances of flashbulb memory are widely shared in the United States.

From the very outset of flashbulb memory studies there have been attempts to address its collective dimensions. Brown and Kulik, for example, reported group differences between African Americans and White Americans in flashbulb memories for the assassinations of civil rights leaders. A tendency to view flashbulb memories as individual phenomena runs throughout the literature, however, largely because the research has focused on individuals' personal impressions and reactions rather than attention to the shared historical event. As Brown and Kulik noted, flashbulb memory is above all "memory for the *circumstances* in which one first heard the news" (Brown and Kulik, 1977: 95), vivid images of precisely where we were, what we were doing, who told us the news, and so forth, instead of memory for the historical event itself.

In more recent research, additional claims about the collective framing of flashbulb memories have been pursued. For example, in two studies of flashbulb memories for earthquakes (Er, 2003; Neisser et al., 1996), investigators found differences between groups of subjects who experienced the event directly and those who found out about it indirectly through media reports. A more elaborated account of group differences having to do with social identity can be found in a study by Berntsen and Thomsen (2005). Concerned with "the accuracy and clarity of [flashbulb memories] as a function of emotional and social factors" (Berntsen and Thomsen, 2005: 242), they examined Danes' memories for the German invasion of their country in 1940 and its liberation in 1945. Specifically, they examined differences between the memory of Danes who had ties to the resistance movement in World War II and those who did not and found that those in the first group had more vivid and accurate memories than subjects who did not.

In another study, Bohn and Berntsen (2007) examined additional aspects of the impact of group membership on flashbulb memory. In this case, the object of memory was the fall of the Berlin Wall in 1989. Germans who viewed the event either negatively or positively participated in the study. Those in the positive group rated their memories higher in terms of reliving and sensory imagery, but their memories were actually less accurate than participants in the negative group, something that Bohn and Berntsen attribute to a more "detail-oriented, bottom-up processing strategy related to negative affect" (Bohn and Bernsten, 2007: 571) for the negative group. They also note that differences in accuracy may be due to increased levels of discussing and rehearsing the event by the positive group, practices typically grounded in schema-based organization that derives from narrative retelling.

In sum, although flashbulb memories need not be about events experienced by a group, most of the cases studied to date are in fact of this sort. This has led some investigators to be concerned with how memory processes in one group may differ from those in another. Findings indicate that such differences can be complex, involving more than the amount of information recalled. This is part of the larger set of issues originally raised by Bartlett about the social framing of memory, issues that occasionally make it into psychological studies. While interesting, these issues "have not been systematically pursued" (Roediger, 2000: 155) to date.

2.48.2 Collective Memory in the Social Construction of Groups

In the view of Olick (1999), the sort of social framing discussed in the previous section is concerned with collect*ed*, as opposed to collect*ive*, memory since it amounts to "the aggregation of socially framed individual memories" (Olick, 1999: 333). In such cases, the social dimension enters the picture as a kind of

independent variable having to do with objective facts about group membership. In some instances, this group membership is a matter of culturally salient categories such as race or political affiliation, and in others it amounts to little more than happening to be part of a group that experienced a major event together. From this perspective, group membership is viewed primarily as a predictor of individual memory performance.

In contrast, several investigators in sociology and psychology have outlined alternative proposals concerned with what Olick considers to be genuinely collective memory. A hallmark of this approach is that collective remembering involves social facts (Durkheim, 1982) and processes that cannot be reduced to individual psychological phenomena. As a student of Durkheim, whose ideas have done much to shape contemporary discussions in sociology, much of what Halbwachs (1980, 1992) argued fell under this heading. In more recent years, this point has sometimes been framed as a rejection of methodological individualism (Lukes, 1977). In the cases I shall review, however, the difference is more one of interest and emphasis than simple opposition to studies in psychology and other disciplines that focus on the individual.

One important strand of research concerned with what Olick views as genuinely collective memory concerns the social formation of groups. From this perspective the group is a product, rather than a prerequisite of shared memory. This is a line of reasoning that is often traced to the writings of the sociologist Mannheim, especially his essay "The problem of generations" (Mannheim, 1951). There he argued for the need to follow a romantic-historical, as opposed to a positivist, approach to group membership. Specifically, he argued for the need to view a generation as subjectively constructed and defined rather than a cohort determined by objective dates. From this perspective, generations are "mental and spiritual units" (Mannheim, 1951: 289) that come into being because people share historical experience and memories.

Schuman and his colleagues have explored several implications of this line of reasoning (e.g., Schuman et al., 1997; Schuman and Scott, 1989). Instead of being a reflection of group membership, these investigators view collective memory as playing a role in the construction of the mental and spiritual units of generations. The emphasis is on how a generation comes into being as its political outlook is shaped by the events its members experience, especially during the critical period of young adulthood. Thus the greatest generation (Brokaw, 2001) was fundamentally shaped by events it experienced in World War II, the Vietnam generation is haunted (McPherson, 2002) by events it experienced in the 1960s and 1970s, and so forth.

An essential part of Schuman's argument is that "adolescence and early adulthood are a stage of life uniquely open to gaining knowledge about the larger world" (Schuman et al., 1997: 47). For example, Schuman et al. (1997) conducted a survey about knowledge of past events among Americans between the ages of 18 and 80 and documented that this knowledge is dependent on the point in the life course when an event is experienced. Their results generally support the claim that knowledge for events encountered in young adulthood tends to be more extensive than for events experienced before or after this critical period.

For example, Americans who were in their early twenties when the Tet Offensive of the Vietnam War occurred demonstrated greater knowledge about it a quarter century later than both younger and older people. The curvilinear pattern of knowledge involved in this case also emerged for other events such as the Holocaust and Watergate (Schuman et al., 1997). In a few instances, Schuman et al. did not find the results they predicted, something that serves as a reminder that generations are subjectively defined cohorts rather than objective periods. For example, Schuman et al. (1997) failed to find distinctive generational effects for some aspects of the Watergate scandal, namely, John Dean's role in it, and they interpreted this to "indicate that a purely mechanical approach to cohort effects on knowledge is inadequate" (Schuman et al., 1997: 74). What may be required for events to be salient for collective memory is that they are taken to be what Pennebaker and Banasik (1997) term important turning points for American self-views.

The appearance of the term knowledge throughout these discussions raises a question about why it, rather than the term memory should be invoked. In order to make the case that memory is indeed involved, one must recognize that two functionally differentiated types of knowledge are at issue in such discussions. On the one hand, there is knowledge that may be widely shared but has little relevance to identity or self-views. As reflected in the title *1066 and All That* (Sellar and Yeatman, 1931), much of what people might have been taught about the past

is not taken to be particularly relevant to their contemporary concerns.

In contrast, other aspects of knowledge about the past are central to understanding and defining who we are. Just as stories we live by (McAdams, 1993) are essential means for personal identity, certain narratives play an essential role in forming collectives such as nation-states. In these latter cases, it is not simply knowledge about the past that is involved; it is knowledge that is crucial to understanding and defining identity and creating self-views. In this connection, Zerubavel (2003) notes, "acquiring a group's memories and thereby identifying with its collective past is part of the process of acquiring any social identity, and familiarizing members with that past is a major part of communities' efforts to assimilate them" (Zerubavel, 2003: 3).

Assmann (1997) has discussed these issues under the heading of a distinction between history and memory. For him, the latter is vitally tied to contemporary discussions of identity. In its case, "The past is not simply 'received' by the present. The present is 'haunted' by the past and the past is modeled, invented, reinvented, and reconstructed by the present" (Assmann, 1997: 9).

In this respect, there are several points of possible contact between collective memory research and psychological studies of individual memory. For example, the centrality of identity in collective remembering suggests interesting parallels with the notions of a self-memory system as outlined by Conway and Williams (*See* Chapter 2.46). It also raises issues of how collective memory is related to constructs from psychology such as semantic and episodic memory. In many instances of collective remembering, the focus is clearly on an episode from the past, hence suggesting parallels with episodic memory. However, closer analysis usually reveals that some sort of more general category or abstract schema seems to be involved, suggesting parallels with semantic memory. As Balota and Coane (*See* Chapter 2.28) point out, however, the distinction between semantic and episodic memory is difficult to maintain in discussions of psychological processes, and trying to examine the psychological dimensions of collective remembering may further complicate this. It is also possible that research on grounding semantics in perceptual motor systems (*See* Chapter 2.28) may eventually provide insight into the psychological dimensions of collective remembering.

The major point to be kept in mind when engaging in such discussions is that knowledge about the past counts as collective remembering when it becomes crucial to the project of constructing group identity. A usable past is almost always crucial to such projects, and for this reason people become very attached to certain historical narratives, even going to far as to invent, reinvent, or reconstruct them to meet the needs of the present. Among other things, this points to the need to understand the emotional dimensions of collective remembering, perhaps harnessing some of the ideas of Barsalou (1999) about how knowledge is stored in perceptual symbol systems.

Claims about assimilating people into a mnemonic community (Zerubavel, 1997) beg the question of how this is done and when in the lifespan the effort might be most effective, and it is here that other fruitful connections among various traditions of memory studies can be forged. In this connection, there are obvious ties to be made between sociological studies by researchers such as Schuman et al. on critical periods in the formation of a generation and the notion of a reminiscence bump outlined in psychological studies of memory and the fact that the autobiographical memories retrieved are disproportionately from ages 15–25. Rubin et al. (1986) proposed this notion when analyzing the life span retrieval curve that has been observed in individuals above the age of 35. They collected evidence for this phenomenon by employing techniques such as presenting subjects with a word and asking them to provide the first autobiographical memory that comes to mind.

Rubin et al. (1998) report that such procedures repeatedly yield greater numbers of memories dating between the ages of 10 and 30 than for earlier or later periods in subjects' lives. Conway and Pleydall-Pearce (2000) similarly conclude that "the knowledge acquired during the reminiscence period is highly accessible and more accessible than knowledge outside this period" (Conway and Pleydall-Pearce, 2000: 19). Rubin et al. (1998) consider several explanations for why this is so and accept an account based on the novelty of experience and its effect on memory.

Building on the ideas of Erikson about identity development and on further empirical studies, Conway and Pleydell-Pearce provide another interpretation, namely that "the reminiscence bump reflects preferential retention of events from a period of consolidation of the self" (Conway and Pleydall-Pearce, 2000: 20). This involves forming long-term allegiances and friendships, developing a life story schema, and generating a life narrative. To the extent that these processes take place in the context of major

historical events such as war, economic depression, or assassinations, one can expect events to have a formative role in individuals' and generations' political outlook.

Combining ideas about the reminiscence bump with the analysis of how memory functions in the social formation of collectives points to the importance that young adults' experiences have on the political outlook of generations. This is a point that has been recognized by those who wish to control this experience. In this connection Mannheim noted, "it may sometimes happen that a feeling for the unity of a generation is consciously developed into a basis for the formation of concrete groups, as in the case of the modern German Youth Movement" (Mannheim, 1951: 288).

In general, studies of memory in the social construction of groups focus on how effective such memory is in the formation of collectives. This orientation has led sociologists such as Fine (2001) to write about how reputational entrepreneurs try to shape the way that deeds are remembered as part of an effort to enhance group pride and membership. This emphasis contrasts with research on socially framed memory, which tends to be more concerned with accuracy or inaccuracy. This does not mean that those concerned with memory in the social construction of groups have no interest in accuracy, but their primary emphasis remains on which memory or which aspect of memory will be most effective in creating group identity. For example, Novick (1999) has argued that collective memory for the Holocaust in America is to a large degree motivated by a desire to reproduce Jewish identity, but at the same time there obviously remains a deep commitment to accuracy in representing the past.

Renan (1990) recognized this in his famous lecture (delivered at the Sorbonne in 1882) 'What is a nation?' where he noted that collective memory, whose core role is to enhance national identity, stands in contrast to analytic studies of history, which aspire to be maximally accurate and complete. As suggested by Assmann's assertion that the present reconstructs the past in memory, Renan believed that "Forgetting, I would even go so far as to say historical error, is a crucial factor in the creation of a nation, which is why progress in historical studies often constitutes a danger for [the principle of] nationality" (Renan, 1990: 11). From this perspective, elites such as state authorities constantly try to shape what Renan called the daily plebiscite on national identity, and collective memory is one of their main tools for doing this.

Perhaps the most forceful formulation of this point can be found in *Nineteen Eighty-Four*, where George Orwell warned, "Who controls the past controls the future; who controls the present controls the past" (Orwell, 1949: 204). While seldom stated in such stark terms, all modern states make an effort to create and maintain collective remembering that will enhance identity and loyalty. This is crucial for what the anthropologist Anderson (1991) has termed imagined communities and what the political theorist Smith (2003) calls people making. Both authors are concerned with the massive institutional resources devoted to constructing group identity. Huntington (2004) takes Anderson's notion a step further in arguing that a nation is "more specifically a remembered community, a community with an imagined history, and it is defined by its historical memory of itself" (Huntington, 2004: 115).

An essential feature of remembered communities is that more than one account of the past competes for the role of being the officially recognized memory. Bodnar (1992) has addressed these issues from the perspective of what he formulates as public memory. This involves a dialectic between the official culture promulgated by state authorities and other elites, on the one hand, and the vernacular culture of everyday life, especially of the nonelites, on the other. For example, in constructing war memorials, official culture celebrates the triumphal vision of the unified nation, whereas vernacular culture often seeks to find a way to interpret events from a perspective of the private pain experienced by those who lost a friend or family member. Bodnar notes that by including the names of individual Americans on the monument, the Vietnam War Memorial in Washington, DC, breaks with earlier practices of celebrating the official cultural view while downplaying the vernacular perspective. In this case, vernacular cultural practices that commemorate private loss and pain have become as much a part of public memory as the official culture perspective that emphasizes the nation as a whole.

Bodnar has explored the dialectic between public and vernacular culture in many other settings as well. For example, he harnesses this conceptual opposition to provide insight into why groups highlight their local and ethnic identities while participating in July Fourth parades that celebrate the unifying vision of the United States. Such analyses address a crucial issue of collective memory in the social formation of groups. Namely, they provide insight into how competing accounts of the past engage in an ongoing

debate, thereby making collective remembering into something like an arena of ongoing contestation rather than a set body of received knowledge. Orwell's dictum reminds us that state authorities and other elites have a natural tendency to present the past as such received knowledge, but this is always open to challenge through processes such as those Bodnar outlines under the heading of vernacular culture.

2.48.3 Collective Memory as Semiotic Distribution

The third tradition of collective memory studies I shall consider provides yet another answer to the question: What makes collective memory collective? The starting point in this case is the notion of a distributed, as opposed to a strong, version of collective remembering (Wertsch, 2002). In this view, remembering is taken to be distributed in the sense that along with active individuals, it requires cultural tools such as written symbols or mnemotechnics (Yates, 1966). And what makes it collective is that members of a group share the same cultural tool kit (Bruner, 1990). All this does not mean that the tools somehow remember on their own, a claim that would amount to instrumental reductionism, but it does emphasize how extensively humans rely on semiotic means provided by their cultural, historical, and institutional contexts.

As an example of distributed memory at the individual level, consider the analysis of Hutchins (1995) of how a cockpit remembers its speed. By seeming to give cognitive instruments their own agency ("a *cockpit* remembers"), Hutchins emphasizes the importance that they can play in cognition and memory. In this particular case, he examines how a pilot can set and then check with recording devices in an airplane cockpit to keep track of information, and in the process he argues that any assignment of memory to the individual or to instrumentation alone is misguided. Instead, both human agents and the cultural tools they employ must be viewed as integral components of a memory system.

In most studies of semiotically distributed remembering, the emphasis is on how written or spoken language serves as a cultural tool. A major historical transformation in this regard came with what Donald (1991) calls the third transition in human cognitive evolution, one characterized by "the emergence of visual symbolism and external memory as major factors in cognitive architecture" (Donald, 1991: 17). The primary engine of change in this case was not within the individual, but external symbolic storage such as written texts and financial records. Donald stresses that these new forms of external symbolic storage have a transformational impact on psychological and neurological processes; they "impose search strategies, new storage strategies, new memory access routes, new options in both the control of an analysis of one's own thinking" (Donald, 1991: 19). As a contemporary example in the early twenty-first century consider the new skills and strategies that have emerged with the appearance of Google and other search engines on the Internet.

Approaching memory from the perspective of semiotic distribution raises the question of how the use of different linguistic tools gives rise to different forms of memory. Instead of being viewed as simply facilitating existing forms of memory, leaving them otherwise unchanged, such tools are assumed to shape remembering in fundamental ways. A further twist to this line of reasoning stems from the fact that the primary function of language is not to serve as a cognitive or memory tool. Instead, its primary function is communication, and the role it takes on as a tool for remembering is derivative in an important sense. Authors such as Middleton and Brown (2005) have made this point in their study of collective memory. There they argue that the language used to recount the past may depend as much on the need to be convincing or on other communicative goals as it depends on any inclination to be accurate.

A major focus in the study of how language affects remembering is narrative. Researchers from a variety of disciplines have found it useful to make a basic distinction between forms of memory that are mediated by narratives and those that are not. In the case of individual memory, for example, Pillemer (1998) distinguishes between imagistic and narrative forms of "personal event memories" (Pillemer, 1998: 7). Pillemer and White (1989) argue that imagistic memory is "present from birth and operational throughout life ... The memories are expressed through images, behaviors, or emotions" (Pillemer and White, 1989: 326). In contrast, the narrative memory system "emerges during the preschool years ... Event representations entering the higher-order system are actively thought about or mentally processed and thus are encoded in narrative form. ... Memories in the higher-order system can be accessed and recounted in response to social demands" (Pillemer and White, 1989: 326).

Pillemer formulated this distinction in order to analyze developmental issues such as childhood amnesia, where the concern is how imagistic memory is eventually supplemented by remembering mediated by narratives. This does not mean, however, that the former is thought to disappear. Instead, imagistic memory is assumed to continue to exist in adulthood, a claim reflected in Brown and Kulik's account of flashbulb memory, which they speculated "is not a narrative and not even in verbal form, but represented in other, perhaps imaginal ways" (Brown and Kulik, 1977: 85).

Other discussions in the research literature on individual memory focus on distinctions between implicit and explicit memory (Roediger, 1990; Schacter, 1996) or unaware and aware uses of memory (Jacoby, 1988). An essential property of implicit memory is that it is nonconscious (Tulving and Schacter, 1990), which contrasts with explicit memory involving episodic form, which, in turn, is usually taken to involve narrative. Such narrative form is taken to be essential in organizing information and making it available to consciousness. According to Schacter (1994), "a key function of the episodic system is to bind together perceptual with other kinds of information (e.g., semantic, contextual) and thereby allow subsequent recall or recognition of multiattribute events" (Schacter, 1994: 257).

The relationship between imagistic and narrative forms of remembering is often formulated in terms of translation. For example, Pillemer provides an alternative account of repressed memories in terms of a failure of translation (Pillemer, 1998: 133). From this perspective, it is a failure to translate imagistic forms of remembering into narratives that gives rise to what others have called repression. And in the quite distinct realm of historical research, the semiotician Lotman (1990) made an analogous claim.

> Even when the historian is an observer of the events described (examples of this rare occurrence are Herodotus and Julius Caesar), the observations still have to be mentally transformed into a verbal text, since the historian writes not of what was seen but a digest of what was seen in narrative form … The transformation of an event into a text involves, first, narrating it in the system of a particular language, i.e., subjecting it to a previously given structural organization. (Lotman, 1990: 221)

From a psychological perspective, one of the important implications of such translation is that it makes possible reflection and control, processes that take on particular importance when dealing with traumatic experience. In a discussion of overcoming traumatic events, for example, Harber and Pennebaker (1992) report that "victims must consciously confront the memories and emotions associated with their traumatic ordeals. This confrontation is best accomplished by translating the chaotic swirl of traumatic ideation and feelings into coherent language" (Haber and Pennebaker, 1992: 360).

As in the case of research on memory in individuals, narrative form provides the basis for distinguishing between different types of collective remembering. The Egyptologist and historian Assmann (2006), for example, distinguishes between cultural memory, under which he includes nonnarrative forms such as foods and landscapes, on the one hand, and national narratives, which impose "a coherent ordering of events along a strict narrative line serving as an intellectual and emotional backbone of national identity" (Assmann, 2006: 21), on the other. This latter form of representation brings along with it tendencies toward being "mono-perspective, ethnocentric, and narcissistic" (Assmann, 2006: 21). As is the case for narratives in general, national narratives are assumed to "grasp together" (Ricouer, 1985: 44) events, characters, and motives into a coherent representation of the past, much in the way that Schacter says episodic memory binds together information.

Research on individual and collective remembering is distinguished, however, by assumptions about the source of the narratives involved. Psychological studies of episodic memory typically assume that narrative organization is generated by the individual. There is little doubt that narrative cognition (Feldman and Kalmar, 1996) is widely used in the effort after meaning that shapes collective remembering as well, but it is typically viewed as involving narrative tools that are provided by the sociocultural context in which individuals function. Again, from this perspective, what makes collective memory collective is the fact these narrative tools are shared across the members of a group.

In this account, collective remembering harnesses existing narratives in the "tool kit" that is "already 'there,' deeply entrenched in culture and language" (Bruner, 1990: 11) to make sense of the past. Of course active agents are always involved and every use of these tools is unique, even creative in some way, but this performance is viewed as harnessing items in what MacIntyre (1984) calls a society's stock of stories. One implication is that Orwell's dictum could be restated as: He who controls the present,

controls 'narratives about' the past. He who controls 'narratives about' the past, controls the future.

Heated debates and memory wars provide striking illustrations of these issues. Such debates occur over commemorative monuments, holidays, museums, and history teaching. In the United States, these debates have been over how to represent the atomic bombing of Hiroshima and Nagasaki (Linenthal and Engelhardt, 1996); in India they reflect an ongoing struggle between secularists and religious parties over what narrative would be appropriate for textbooks (Thapar, 2003); and in China they may touch on Japan's collective amnesia (Chang, 1997) about the rape of Nanking in the 1930s.

In reality these concerns do not surface only in history wars; they are part of everyday life in the modern world. Forces of the everyday, unnoticed practices of banal nationalism (Billig, 1995) and the national narratives that are a part of it exist everywhere, and they sometimes come into sharp focus in encounters between collective memory communities. As an example of this, consider an interchange reported by Wertsch (2002) between an American adult and a Russian high school student (Sasha) in the late 1990s. During a visit to a history class in a high school in Moscow, the American adult posed a question to the class about the role of Soviet allies in World War II. The first reaction by Sasha and his classmates suggested that they viewed this as a sort of pedagogical – if not pedantic question to which everyone knows the answer. After making it clear that he took the exercise to be just that, Sasha replied:

> The United States made a lot of money from selling arms and other things to countries during the early years of the war, but it did not really contribute as an ally. In fact, along with Great Britain it refused to open a second front in 1942 and again in 1943. It was only after the U.S. and Britain began to think that the Soviet Union might win the war by itself and dominate post-war Europe that they became concerned enough to enter the war in earnest by opening a second front in 1944. (Wertsch, 2002: 4)

This account differs strikingly from what one finds in the United States – as well as many other places in the world. Indeed, Sasha's narrative might appear to American observers as an effort to be provocative, but in fact he produced it at time of relatively positive feelings toward the United States, and he and many, if not most Russians take what he said simply as a straightforward depiction of what occurred. Furthermore, like many Russians, he would undoubtedly have remained committed to this narrative in the face of what appears to others to be contradictory evidence.

An important fact about Sasha's account is that it is very unlikely that he arrived at it through independent research or the consideration of a range of alternatives. Instead, like most of us in such situations, he employed a standard narrative from the cultural tool kit provided by the textual tradition into which he had been socialized.

In such cases, speakers often fail to appreciate the power of narrative tools to shape what they are saying. Sasha, for example, demonstrated little awareness of the existence of the standard narrative he was using, let alone of how it might be contested. He said nothing like: "What we read in our history books is..." or "I saw in a movie that ..." or "I know that the U.S. has another account, but we believe ..." Instead, he presented his account as simply a description of what really happened, something that reveals a fundamental property of narratives in recounting the past: their transparency (Wertsch, 2002). It was as if he were looking through this narrative tool just as he would look through a clear pane of glass without recognizing that it separated him from the events being reported.

This anecdote about Sasha reflects a larger picture of Russian collective memory and how the narrative tools it employs differs from what can be found elsewhere. Consider, for example, an exercise I often conduct with American undergraduates in which they are to list the most important events of World War II. The procedure consistently yields the following most frequently mentioned items:

Attack on Pearl Harbor (December 7, 1941)
Battle of Midway (June 1942)
D-Day (June 6, 1944)
Battle of the Bulge (winter 1944–45)
Holocaust (throughout the war)
Atomic bombing of Hiroshima and Nagasaki (August 1945)

Results from surveys of Russians in Moscow as well as Novosibirsk in the late 1990s (Wertsch, 2002) provided a quite different list of most frequently chosen items:

German attack on USSR (June 22, 1941)
Battle of Moscow (winter 1941–42)
Battle of Stalingrad (winter 1942–43)
Battle of the Kursk salient (summer 1943)
Siege of Leningrad (1942–44)
Final Battle of Berlin (1945)

A striking fact about these two lists and the narratives they suggest is that there is no overlap. Many Russians know about the events on the American list, but they do not view them as central to the narrative of the war. For example, Russians are quite familiar with the episode called opening the second front in June of 1944. For them, this refers to something that was not only a second, but clearly a secondary front (there is no word for D-Day in Russian), and it is not considered a major event, let alone a turning point in World War II. Conversely, American students often know little about events typically listed by Russians. For example, the largest tank battle in history at the Kursk salient is something that has no resonance in American collective memory, but it is taken to be one of the turning points in the Russian narrative of World War II. Furthermore, it is at the center of scholarly accounts, including those of Western historians such as Overy (1997).

In contrast to such national differences in collective remembering, recent findings by Liu and colleagues (Liu et al., 2005) suggest some similarities. They report a high level of consensus when they asked subjects in six Western and six Asian countries to list the most important events and figures in history for the past 1000 years. The subjects across the groups shared a tendency to focus on the recent past and to include at the top of their list political events and wars, especially World War II. Such findings about similarities across groups led the authors of this study to conclude that "the degree of cross-cultural consensus suggests that hybridity across Eastern and Western cultures in the representation of knowledge may be underestimated" (Liu et al., 2005: 1).

In the end, these results may not contradict the picture of difference between mnemonic communities outlined in the Russian-American case because the nature of the events and the time frames involved are so different. It will only be with much more research of the sort conducted by Liu and colleagues that we will begin to gain clarity on these issues.

In the study of how narratives shape collective remembering, a useful distinction between specific narratives and schematic narrative templates (Wertsch, 2002) can be made. Specific narratives include information about concrete events, actors, times, and places. Sasha's account of the role of the Allies in World War II is an example. Schematic narrative templates, in contrast, are more abstract in nature. They are schematic in the sense outlined by Bartlett in his account of the schemas that shape remembering or by the folklorist Propp (1968) in his analysis of abstract functions in folktales. They are narrative in the sense that they are organized around basic narrative principles such as those outlined by Bruner (1990) and Ricouer (1985). And they are templates in the sense that they involve a generalized form from which several copies (i.e., specific narratives) can be generated.

Wertsch (2002) has outlined a basic Triumph over Alien Forces schematic narrative template that is often employed by Russians when talking about several episodes from their past. This is a general narrative template that is employed by people other than Russians, to be sure, but it plays a particularly important role as a national narrative in their case. Specific narratives that fit this pattern for Russians include accounts of the Mongol invasion of the thirteenth century, the Swedish invasion of Charles XII in the eighteenth century, the Napoleonic invasion of the early nineteenth century, the German attack in World War II, and even the reign of communism in the twentieth century.

The Triumph over Alien Forces schematic narrative template can be summarized as:

1. Russia is peaceful and not interfering with others.
2. Russia is viciously and wantonly attacked without provocation.
3. Russia almost loses everything in total defeat.
4. Through heroism and exceptionalism, and against all odds, Russia triumphs.

Emphasizing the importance of this basic narrative template in shaping Russians' interpretation of the past does not suggest that this interpretation is without foundation. Russia clearly has been the victim of numerous attacks in its history, and its people have undergone great suffering. But the effort after meaning in this case is shaped by the particularly Russian way the events are emplotted and contrasts with other interpretations. In particular, it has to do with how the meaning of actions and the motivation of those who carry them out are interpreted. For example, Estonians have long held that some of the actions that Russians interpret through the lens of the Triumph over Alien Forces are better understood in terms of Russia's longstanding, aggressive imperialism (Tulviste and Wertsch, 1994).

Partly because they are even more transparent than specific narratives, schematic narrative templates are often not recognized, let alone subject to reflection, criticism, and change. Evidence of their conservative nature can be found in the transition from the Soviet to post-Soviet official histories. On the surface, changes in

the official collective memory of World War II found in textbooks are striking. Many of the things routinely included in post-Soviet Russian textbooks in 2005 would have landed people in prison had they written them in the Soviet Union of 1970. For example, the Communist Party was routinely feted as the moving force of history in Soviet accounts, but post-Soviet history textbooks, as well as the popular press, stress that it was the masses of Russian people, not the party, that won World War II. Indeed, some of these post-Soviet accounts go out of their way to say it was 'despite' the party and only through the heroism of the Russian people that the country won the war (Wertsch, 2002).

Another form of evidence that points to the conservative nature of this narrative template in Russian national consciousness can be found in references to Hitler as a second Napoleon. This formulation has remained constant across the radical changes in Russian accounts, and of course it also provides a reminder that the narrative tools used by one collective can be quite distinct from those used in other places. Whereas this is a familiar and widely repeated expression for Russians, it is not something that most Americans would have heard repeatedly as they grew up.

A great deal remains to be studied when it comes to understanding the degree to which collective remembering does or does not change. The line of argument developed by Wertsch (2002) suggests that specific narratives may change fairly quickly, but at the level of schematic narrative templates, there is a high level of conservatism and resistance to change.

Schuman et al. (2005) have recently presented a more elaborated picture of this issue. They examined Americans' account of Columbus over the past few decades and draw an important distinction between what happens with elite revisionists, on the one hand, and popular beliefs, on the other. They report that elite culture's attempt to revise the narrative about this figure has had an impact on what is presented in forums such as textbooks, but it has not had the impact on popular beliefs that one might expect. The endurance of Columbus's reputation and the "inertia of tradition" (Schuman et al., 2005: 3) can be understood only by differentiating elite and popular beliefs and tracing their dynamics.

2.48.4 Conclusion

The three traditions of inquiry on collective memory that I have outlined provide different visions of what makes collective memory collective. In many cases, however, differences stem more from disciplinary isolation rather than conceptual contradiction. Little citation of literature across the traditions occurs, and in many cases they do not even seem to know of the others' existence.

The study of the social framing of collective remembering has been conducted largely by psychologists and is viewed by sociologists such as Olick (1999) as being such a distinct approach that it should be labeled collected rather than collective memory. The concern with collective memory in the construction of social groups has surfaced largely in sociology and political science and shows only occasional signs of contact with the psychological study of memory. And studies of collective memory as semiotic distribution have been conducted primarily in disciplines such as history, semiotics, and anthropology, often with little benefit from the findings of the other two traditions.

Despite the dearth of contact among these approaches, there are several obvious points of contact to be made in building a more comprehensive picture of collective memory. For example, obvious complementarities exist between sociological studies of the role of memory in the formation of generations and psychological studies of the reminiscence bump. Other points of contact may have even more potential. For example, claims about the role of memory in the social formation of groups often imply notions of narrative tools, and for this reason, statements about remembered communities and people-making are as much about national narratives as about political processes.

It remains useful at this point, however, to recognize the differences among traditions of inquiry in the field of collective memory studies since this suggests a conceptual map of ideas and methods. In the end, the justification for such a map will be its ability to generate new ideas and insights into collective memory phenomena that are widely noted, but little understood. The field is rich enough that it will require a diverse set of constructs and methods to address the issues. One of the key issues that will undoubtedly continue to shape the discussion, however, will be how these ideas and methods fit together into some sort of integrated whole.

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LEARNING AND MEMORY: A COMPREHENSIVE REFERENCE

Volume 3
MEMORY SYSTEMS

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3.01 Introduction and Overview

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In 2001, Neal Cohen and I reviewed the existing literature on the analysis of brain systems that support memory (Eichenbaum and Cohen, 2001) and reached two central conclusions. First, memory is a consequence of the fundamental plasticity of the brain. Accordingly, memory storage is intimately tied to ongoing information processing in the brain. Second, because the brain is organized into several functional systems, there are multiple forms of memory that have distinct psychological and information processing characteristics, composing multiple, functionally and anatomically distinct memory systems. According to this view, memory is not an entity stored in a localized warehouse, nor is it fully distributed in an interconnected network of the brain's neurons. Instead, memory should be conceived of as a fundamental property of brain systems, and the consequent changes in behavior and remembered information as a natural outcome of the brain's various processing activities. Thus, memory is an integral part of those ongoing information processing activities and is tied to the brain systems and structures that support those activities. Experience is reflected in changes in the operation of these systems in one manner or another. Indeed, the manner in which memory is expressed varies importantly and is entirely dependent on the functions of the brain system involved. While there is enormous consensus on this view, the number and nature of memory systems and their anatomical circuits are still only partially understood. Investigations aimed to characterize the functional organization of the brain's memory systems are still at an early stage. This volume should be considered a progress report on our understanding of the major memory systems of the brain.

3.01.1 Early Ideas About Multiple Forms of Memory

Modern ideas about multiple forms of memory foreshadowed current conceptions that have come from technologically driven scientific investigations. Indeed, the fundamental insights of the current era can be viewed as resurrecting basic theories proposed many years ago by thinkers who had profound insights that remain key to our modern understanding of learning and memory. The first elaborated proposal about multiple forms of memory may have come from Aristotle (c. 350 BC), who distinguished memory and reminiscence. He proposed that memory is an extension of the senses that incorporates the passage of time since the actual sensory experience. He envisioned a memory as a replicate of a perceived object on some sort of recording device and compared it to an impression on a plastic surface. Memory, then, is a passive reperception of the recorded object, independent of its original context. Aristotle also argued that the likeness of the memory to the original percept was quantitative and involved the knowledge of having perceived the item before. Aristotle contrasted this perceptually based memory with reminiscence, which, he asserted, involved the active replay of an entire experience, triggered by an initial memory of one event in that experience. This first memory would lead to a recovered sense of the situation and flow of events that compose the experience. Thus Aristotle viewed reminiscence as a process of recovering single items in succession. The difference Aristotle conceived between memory and reminiscence is recognized today in the distinction we make between familiarity and recollection. Chapters

by Voss and Paller (*See* Chapter 3.05), Nyberg (*See* Chapter 3.06), and Stern and Hasselmo (*See* Chapter 3.08) consider modern psychological and biological distinctions between familiarity and recollection.

Two thousand years after Aristotle, a less familiar philosopher using the pen name Maine de Biran (1804/1929) offered a prescient view of different forms of long-term memory. He began with the assumption that there is a simple and automatic mechanism for associating events that underlies three different forms of memory. The most complex form was called representative memory, which involves the ability to consciously relive and think about a prior experience. The other two forms of memory Maine de Biran identified, mechanical memory and sensitive memory, differed from representative memory in two important ways. First, neither of these forms of memory could access consciousness. Second, both of these unconscious forms of memory had rigid limits on their expression. That is, they could be expressed only under circumstances closely resembling the learning experience, and they could not be used to solve new problems. Specifically, mechanical memory involves learning a movement through repetition and improves the speed or coordination of an action through practice. Sensitive memory occurs during emotional experiences and involves recalling a feeling without memory for the circumstances of the emotional situation. In a chapter here (*See* Chapter 3.02), White presents a modern formulation of these three kinds of memory and reviews the evidence of distinct neural substrates for each. These ideas are further elaborated in many of the chapters in this volume, with aspects of Maine de Biran's representative memory discussed in chapters on declarative memory, aspects of his mechanical memory discussed in the chapter on procedural memory, and aspects of his sensitive memory discussed in chapters on emotional memory.

At the beginning of experimental psychology, William James (1890/1918) made two major distinctions about types of memory. James differentiated primary memory, the ability to hold and manipulate information in mind for a short period, from the permanent memory store he called secondary memory. The notion of a distinct mechanism and brain system for primary memory is maintained today in our characterizations of working memory, discussed in chapters by Buchsbaum and D'Esposito (*See* Chapter 3.13) and Ranganath and Blumenfeld (*See* Chapter 3.14). James also distinguished between secondary memory and a simple and automatic mechanism he called habit. James was guided by observations from biology that identified reflex pathways in the nervous system through which a stimulus (such as a pinch) automatically generates a specific motor response (withdrawal). Influenced by the descriptions of these reflex arcs, James suggested that nerve impulses in reflex paths more readily traverse paths previously taken. Thus, he wrote, habits form when reflex paths become well worn. Like Maine de Biran, James attributed great importance to habits as the building blocks of more complicated behaviors. James said that practiced behaviors and skills are mediated by sequentially linked discharges that "awaken each other in succession" through connected reflex paths. He held that this kind of chain reaction mediated the production of learned movement sequences. James distinguished active memory as a complex cognitive phenomenon from the acquisition of skills and from a sense of familiarity. He also emphasized the warmth and intimacy of recollection from the cold repetition of practiced movements and from the passive feeling of familiarity for a reexperienced stimulus or event. In addition, James characterized memory in terms of its structure as an elaborate network of associations, vastly richer and more complicated than a connected series of habits or a general sense of familiarity. Thus the underlying foundation of recall involves a complex set of systematic associations between particular memories. James' characterizations of memory and habit carry strong similarities with the current views on declarative and nondeclarative memory that are strongly dissociated in the phenomenology of amnesia discussed by Squire and Shrager (*See* Chapter 3.04).

The fundamental distinctions between familiarity and recollection, and between habits, emotional memories, and conscious forms of memory, were initially lost in early experimental psychologists' efforts to characterize memory as a single system. However, the distinctions were rediscovered and confirmed by modern cognitive and neuroscience research. This volume provides a survey of our current understanding of the different forms of memory and the brain systems that support them.

3.01.2 The Cognitive Neuroscience Revolution

The initial breakthrough in the rediscovery of multiple memory systems came from the study of patients with pervasive, global amnesia. Scoville and Milner (1957) described a case study that involves probably

the most famous neurological patient in the literature, H.M. This patient had the medial temporal lobe area removed to alleviate his severe epileptic attacks. H.M. consequently suffered what appeared to be a nearly complete loss of the ability to form new long-term memories. His impairment, tested over the last 50+ years, has been shown to extend to verbal and nonverbal memory and spatial and nonspatial memory and, indeed, seems to cut across all categories of learning materials. Yet a second line of discovery about global amnesia revealed a spared domain of learning capacity. Even from the outset, a few exceptions to the otherwise pervasive deficit were apparent. H.M. was able to learn new motor skills, and he showed a facilitation of perceptual identification resulting from prior exposure to objects or words (an effect that later came to be understood as reflective of a preserved priming) (*See* Chapter 3.12).

A further breakthrough came in 1980 when Cohen and Squire (Cohen and Squire, 1980) proposed that these exceptions to amnesia were indicative of a large domain of preserved learning capacities in amnesia. Their conclusion was based on the observation of complete preservation of the acquisition and retention of a perceptual skill (reading mirror-reversed words) in amnesic patients. These patients showed fully intact skilled performance yet were markedly impaired both in recognizing the particular words on which they trained and in recollecting their training experiences. These investigators were struck by the dissociation between the ability to benefit or otherwise have performance shaped by a series of training experiences, an ability that appeared fully normal in the amnesic patients, and the capacity to explicitly remember or consciously recollect those training experiences or their contents, which was markedly impaired in the patients. Cohen and Squire attributed the observed dissociation, together with the earlier findings of spared memory in amnesia, to the operation of distinct forms of memory, which they called procedural memory and declarative memory, respectively. These forms of memory were viewed as functionally distinct memory systems, one dedicated to the tuning and modification of networks that is expressed by implicit changes in perceptual, cognitive, or motor performance, and the other to the encoding, storage, and retrieval that supports explicit expression of memories for specific facts and events. Furthermore, these functionally distinct memory systems were tied to separate brain systems, with declarative memory seen as critically dependent on the medial temporal-lobe and midline diencephalic structures damaged in various amnesias. Procedural memory was seen as being mediated by various brain systems specialized for particular types of skilled performance.

The initial reports on H.M. spurred several efforts to model global amnesia in animals. As a part of those investigations, evidence for multiple forms of memory and theories of the nature of these forms of memory and the brain structures that support them emerged. In the mid- and late 1970s three separate theoretical themes developed, each espousing a multiple memory systems approach and each suggesting a selective role for the hippocampus in a distinct higher-order form of memory versus hippocampal-independent mechanisms for simpler forms of learning (Hirsh, 1974; O'Keefe and Nadel, 1978; Olton et al., 1979). One proposal was that the hippocampal system mediates cognitive mapping, whereas nonhippocampal systems support nonspatial learning and memory for responses and reward values of stimuli (O'Keefe and Nadel, 1978). O'Keefe and Nadel's analysis of a large literature on the effects of hippocampal lesions and the anatomy and physiology of the hippocampus supported their conclusion that the hippocampus plays a critical role in many forms of spatial learning and memory, and conversely, many forms of nonspatial learning and memory did not require hippocampal function. However, their view also went well beyond making a simple distinction between spatial and nonspatial learning modalities. Their proposal about spatial learning involved the acquisition of cognitive maps that corresponded roughly, if not topographically, to the salient features of the physical environment. They referred to the domain of memory supported by the hippocampus as a locale system characterized as maintaining a molar model of space in terms of relations among objects in the environment, as driven by curiosity rather than reinforcement of specific behaviors, and as capable of very rapid learning. In contrast they proposed that hippocampal-independent learning is supported by a taxon system characterized as mediating dispositions of specific stimuli into categories, as driven by reinforcement of approach and avoidance behaviors, and as involving slow and incremental behavioral adaptations.

An alternative line of study led Hirsh (1974) to propose that the hippocampus supports a capacity for contextual retrieval, the ability to utilize the context in which conditioned cues occur to retrieve the appropriate association. Hirsh's experiments showed that hippocampal damage affected learning to turn one direction or the other in a T-maze depending on

an imposed motivational context (hunger or thirst). The ambiguity in turn direction was, according to this account, resolved by a hippocampal-dependent mechanism that employed motivational state as a contextual cue for retrieving one of the possible responses. Conversely, Hirsh characterized the behavior of animals with hippocampal damage as habit prone, reflecting simple stimulus–response conditioning. Many experiments have subsequently shown that animals with hippocampal lesions are less influenced by contextual cues than are normal animals.

In 1979, Olton and colleagues proposed that the hippocampus is essential for working memory, and nonhippocampal systems support reference memory. Notably, the term working memory as used in this context differs in meaning from the same term used in characterizations of human memory (*See* Chapter 3.13). As characterized by Olton and colleagues, working memory involves memory for specific behavioral episodes and not information accrued over many experiences, very similar to the characterization of episodic memory (Olton, 1984). Olton and his colleagues provide substantial evidence that animals with hippocampus damage are severely impaired in radial maze tasks that involve memory for specific experiences but not memory for consistently rewarded maze locations or nonspatial stimuli. Thus Olton's distinction between working and reference memory shares much in common with the current distinction between episodic and semantic memory in humans, outlined in chapters by Nyberg (*See* Chapter 3.06) and Martin and Simmons (*See* Chapter 3.07).

These efforts focused on the hippocampus and on distinguishing the kind of memory in which that structure plays a critical role versus any other form of memory. Many other lines of study now have clarified the nature and brain pathways involved in other forms of memory. Our understanding of these pathways has grown from a diverse range of studies, some seeking to anatomically dissociate hippocampal-dependent versus hippocampal-independent forms of memory, and others arising from studies on plasticity in various functional systems of the brain. Several distinct lines of study have demonstrated key roles for the basal ganglia (*See* Chapters 3.17 and 3.18), cerebellum (*See* Chapters 3.19 and 3.20), and motor cortex (*See* Chapters 3.21 and 3.22) in different aspects of procedural learning, for the amygdala in emotional memory and modulation of memory (*See* Chapters 3.24 and 3.26), and for the cerebral cortex in priming (*See* Chapter 3.12), working memory (*See* Chapter 3.13), and semantic memory (*See* Chapter 3.07). A general, anatomically based framework for the major memory systems has emerged from many experiments that provide dissociations among the role of specific brain structures in different forms of memory, combined with the known anatomical pathways of the key structures. A taxonomy of some of the prominent memory pathways currently under investigation is provided in **Figure 1** (for a similar outline see Suzuki, 1996). In this scheme, the origin of each of the memory systems is the vast expanse of the cerebral cortex, focusing in particular on the highest stages of the several distinct sensory and motor processing hierarchies, the cortical association areas.

Some forms of learning and memory are accomplished mainly within the cerebral cortex. Perceptual learning (*See* Chapter 3.11), involving our capacity for familiarity and for categorizing and identifying stimuli, depends on modifications of the cortical areas mediating specific types of stimuli (visual, tactile, and the like) as well as semantic memory (*See* Chapter 3.07). The prefrontal cortex and its interactions with other cortical areas support conscious manipulation of information and working memory (*See* Chapters 3.13 and 3.14). In addition, the cerebral cortex provides major inputs to each of three main pathways of processing related to distinct memory functions. One major pathway is from virtually all cortical association areas to the hippocampus via the parahippocampal region (*See* Chapter 3.03). As introduced above, this pathway supports recognition memory (*See* Chapters 3.08 and 3.10) and declarative memory in humans (*See* Chapter 3.04) and their analogs in animals (*See* Chapter 3.09). The main output of hippocampal and parahippocampal processing is back to the same cortical areas that provided inputs to the hippocampus, and these cortical areas are viewed as the long-term repository of declarative memories.

In addition, motor learning is supported by areas of the cortex itself (*See* Chapters 3.21 and 3.22), and these areas send inputs to specific subcortical targets that are critical nodes in habit and skill learning. One of these pathways involves the striatum as a nodal stage in the association of sensory and motor cortical information with voluntary responses via the brainstem motor system (*See* Chapters 3.17 and 3.18). The putative involvement of this pathway in associating cortical representations to specific behavioral responses has led many to consider this system as specialized for habit or skill learning, two forms of procedural memory. An additional, parallel pathway that mediates different aspects of sensorimotor adaptations involves sensory and motor systems pathways

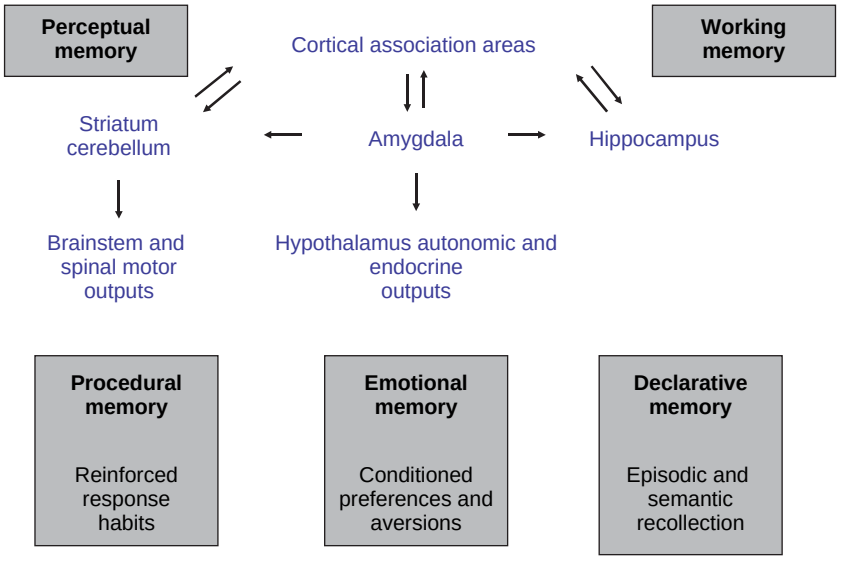

Figure 1 Anatomic pathways of memory systems. Systems for perceptual and working memory involve networks within the a cortical association areas also send major inputs into and are strongly influenced by other memory systems. The procedural memory system involves pathways through the striatum and cerebellum that connect directly with motor outputs. The emotional memory system involves pathways through the amygdala that connect directly with the hypothalamus to direct autonomic, motor, and endocrine outputs, and the amygdala influences the strength of memories in other systems. The declarative memory system involves bidirectional interactions between the hippocampus and cortical association areas.

through the cerebellum (*See* Chapters 3.19 and 3.20). Another major pathway involves the amygdala as a nodal stage in the association of exteroceptive sensory inputs to emotional outputs effected via the hypothalamic–pituitary axis and autonomic nervous system, as well as emotional influences over widespread brain areas (*See* Chapter 3.24). The putative involvement of this pathway in such processing functions has led many to consider this system specialized for emotional memory. This anatomical scheme can be a useful framework for understanding how the brain mediates the different memory systems discussed in succeeding chapters.

3.01.3 The Organization of This Volume

The chapters in this volume are arranged to reflect the organization of the major memory systems of the brain outlined above. We begin with an overview of memory systems by White (*See* Chapter 3.02), a pioneer in the anatomical dissociations of the forms of memory first characterized by Maine de Biran. According to White, these systems are best distinguished as a hippocampal-dependent form of memory that supports stimulus–stimulus associations, a striatum-dependent form of memory that supports stimulus–response associations, and an amygdala-dependent form of memory that supports stimulus–reinforcer associations. Succeeding chapters consider these and other memory systems and brain areas that influence memory separately.

3.01.4 The Declarative Memory System

Several chapters are devoted to different aspects of the cortical–hippocampal system that supports declarative memory. We begin with Burwell and Agster's overview of the anatomy of this system (*See* Chapter 3.03). Their survey indicates that the circuitry of this system is largely conserved across mammalian species, with the most prominent species differences derived from variations in cortical areas. Squire and Shrager then review the literature on amnesia associated with damage to this system in humans (*See* Chapter 3.04). This review reinforces and extends the original findings on the patient H.M., showing that damage to the hippocampus, and more so following additional damage to the surrounding cortical areas, results in a selective deficit in the ability to remember everyday facts and

events. The impairment involves both an anterograde component that involves loss of the ability to remember information acquired following medial temporal damage and a temporally limited retrograde component that involves the loss of memories for information acquired for some time prior to the damage. The combination of anterograde and temporally graded retrograde amnesia has led to the general view that the medial temporal lobe plays an essential role in the consolidation of memories from a labile state to a stable permanent form. Voss and Paller (*See* Chapter 3.05) review the literature from the approach of event-related potentials (ERPs) that sheds light on distinctions among different phases of memory (encoding and retrieval) and different types of memory (familiarity, recollection, perceptual and conceptual priming). These studies provide strong evidence of distinct memory processes generated by different brain network activities.

Declarative memory is composed of two subsystems, one for remembering everyday personal experiences, called episodic memory, and the other for storing and retrieving factual knowledge, called semantic memory. Nyberg (*See* Chapter 3.06) characterizes episodic memory as composed of several distinct processes that involve conscious control of information and associative processes that encode and retrieve unique experiences as our personal past. Correspondingly, episodic memory is supported by large network of areas in the cerebral cortex, particularly in the frontal and parietal areas, as well as in the temporal lobe. Stern and Hasselmo (*See* Chapter 3.08) focus on a fundamental function of episodic memory, our ability to recognize previously experienced stimuli. This capacity relies on the same system as other capacities of episodic memory and emphasizes the involvement of the cortical areas just outside the hippocampus. Martin and Simmons (*See* Chapter 3.07) then review our understanding of the brain's organization of semantic memory. They focus on the nature of semantic organization and consider the evidence on whether semantic information is stored as distinct categories of knowledge or as a distributed network of perceptual and motor attributes of concepts. The weight of the evidence indicates that semantic knowledge lies in a distributed fashion in which particular categories of knowledge are represented by distinct organizations within functionally specific areas of the cortex.

The development of animal models of amnesia and studies on the physiology of memory in animals have also contributed significantly to our understanding of declarative memory. Studies on animals have the inherent challenge of identifying the psychological processes that correspond to declarative memory in humans. At the same time, these studies are immensely valuable because they offer the ability to distinguish the functional contributions of specific brain structures and the ability to examine the nature of memory representation at the level of single neurons, the units of information processing that encode and retrieve memories. Alvarado and Bachevalier (*See* Chapter 3.09) describe the initial difficulties in the development of animal models of amnesia and the eventual successes in models of recognition memory, which have identified the distinct roles of the hippocampus and cortical areas surrounding the hippocampus. They also describe the critical role of the hippocampus in two aspects of episodic memory in humans, relational memory, the ability to associate and distinguish combinations of stimuli that compose distinct experiences, and spatial memory, the ability to remember where important events occurred. Brown and Eldrige (*See* Chapter 3.10) review the evidence on the nature of memory representations in the perirhinal cortex, a part of the cortex surrounding the hippocampus that is essential to recognition memory. Neuronal networks in the perirhinal cortex encode individual stimuli that we have previously experienced, as well as associations among items that support our ability for recognition memory.

3.01.5 The Cerebral Cortex and Memory

The cerebral cortex receives and processes the most complex aspects of perceptual and motor information and supports critical attentional and cognitive functions, all of which contribute to memory in a broad variety of ways. Indeed, as **Figure 1** illustrates, the cerebral cortex is both the source and recipient of information within virtually all the brain's memory systems. Therefore, a consideration of cortical plasticity and function is essential to understanding memory. Weinberger (*See* Chapter 3.11) begins this survey with an overview of studies on the plasticity in sensory cortical areas. While these studies began as a relatively accessible model system in which one can study how the cortex reorganizes well-controlled perceptual representations as they become significant, Weinberger argues that we have learned that

plasticity at even early stages of cortical processing supports associative and cognitive functions as well as perceptual memories.

The cerebral cortex is also critical to short-term memory processes. As Grill-Spector (*See* Chapter 3.12) tells us, one way in which the cortex contributes to short-term memory processing is by supporting an implicit form of memory called priming, an increase in the speed or fluency of perceptual processing as a result of prior experience with particular stimuli. The study of priming offers insights into how perceptual memory is intertwined with sensory processing, revealing the intimacy of sensory and memory functions of the cortex. The cerebral cortex is also the place where memories are held in mind during working memory. Buchsbaum and D'Esposito (*See* Chapter 3.13) review the literature on the neural basis of working memory, revealing that a large network of cortical areas maintains information briefly in short-term memory, or indefinitely during rehearsal and cognitive manipulation in working memory. The roles of sensory processing areas versus those of prefrontal areas involved in cognitive processes are varied, depending on the nature of current perceptual and cognitive demands, such that short-term forms of memory emerge from many types of processes and interactions among cortical areas. Ranganath and Blumenfield (*See* Chapter 3.14) review the extensive literature showing that the functions of the prefrontal cortex are not limited to working memory but extend to encoding and retrieval processes in long-term episodic memory as well, and that these roles may depend on different networks within the prefrontal region.

3.01.6 Procedural Learning and Memory

Procedural learning is generally conceived as the process by which we learn how to do a behavior independent of explicit memory for what we learned. Procedural memories are therefore expressed by an acquired bias or increased fluency of actions, very much the way Maine de Biran envisioned mechanical memory and William James described habit learning. The best known examples of procedural memory involve acquired habitual responses and skills. These are often embodied in choice behavior or complex motor sequences but can involve quite complex cognitive procedures as well. These characterizations of procedural learning and memory are reviewed by Knowlton and Moody (*See* Chapter 3.17), who focus on this memory system in humans, and Packard (*See* Chapter 3.18), who focuses on studies in animals. Both lines of study reveal the basal ganglia as a critical brain structure involved in procedural learning, along with pathways through the frontal cortex and other brain areas involved in the execution of behavioral actions.

Particular forms of procedural learning are studied within different brain systems. Prominent examples include studies that focus on the role of the cerebellum in motor reflex learning, including classical conditioning of the eyelid blink response, as reviewed by Poulos, Christian, and Thompson (*See* Chapter 3.19), and acquisition of the vestibulo-ocular reflex as reviewed by Cullen (*See* Chapter 3.20). Also, Nudo and Sanes (*See* Chapters 3.21 and 3.22) survey the literature on the role of the motor cortex in habit learning. Finally, Mooney, Prather, and Roberts (*See* Chapter 3.23) provide a synthesis of findings on an example of expert procedural learning, the acquisition of song in birds. This system shares with mammalian procedural learning systems a critical role of basal ganglia and provides a model for understanding how that system adapted for a specialized form of procedural learning.

3.01.7 Emotional Memory and Modulation of Memory

Emotion and memory intersect in two ways. One way involves a specialized memory system in which otherwise neutral stimuli acquire emotional significance, very much the way Maine de Biran described sensitive memory. Notably, like procedural memory, the acquisition and expression of emotional memories can occur independent of explicit memory for the circumstances in which the emotional significance of a particular stimulus was acquired. These characteristics of emotional memory are discussed by Maren (*See* Chapter 3.24), focusing on behavioral and neurophysiological studies in animal model paradigms, particularly with regard to forms of fear conditioning. These studies highlight the central role of the amygdala as a nodal area where neural responses to emotional stimuli are acquired and a main nucleus that generates the expression of learned emotional responses.

The other major way that emotion and memory intersect is that emotionally arousing events modulate the strength of memories, including declarative and procedural memories. McGaugh and Roozendaal (*See* Chapter 3.26) review the literature on the modulation

of memory by arousal, arguing that the effect of emotional arousal is mediated via hormonal signals that influence the amygdala, and that projections from the amygdala influence the strength of memory consolidation in other memory systems. Juraska and Rubinow (*See* Chapter 3.25) show that other hormone systems, including gonadal steroids as well as adrenal steroid hormones, modulate memory, and that both can have different effects depending on the age of the subject and duration of hormone exposure in humans and animals.

An additional major modulation of memory processing is generated by the ascending influences of lower brain areas. One of these influences arises from the basal forebrain, specifically the projections of the medial septum and diagonal band to hippocampal regions and from the nucleus basalis/substantia innominata to the cerebral cortex. These projections interact to influence distinct aspects of hippocampal and cortical memory processing. Sarter and Demeter (*See* Chapter 3.16) review the literature on noradrenergic and cholinergic ascending systems from the basal forebrain to the cortex that interact to gate attentional resources that are critical to detection, selection, and outcome processing of cues. Chiba and Quinn (*See* Chapter 3.15) focus specifically on the role of basal forebrain structures in memory performance. Finally, Gold (*See* Chapter 3.27) reviews progress in the discovery of chemicals that can enhance memory. The existing examples include hormones and neurotransmitters, as well as other signaling molecules that are involved in cellular plasticity mechanisms that influence memory consolidation or the arousal-mediated modulation of memory.

3.01.8 Aging and Memory

Aging has a particularly devastating effect on memory, both in its benign form of memory loss without well-identified pathology and in Alzheimer's disease. Daselaar and Cabeza (*See* Chapter 3.28) review the literature on aging and memory in humans, showing that aging is associated with a shift in cognitive strategies that underlie the characterizations of memory abilities lost and preserved in old individuals. Brickman and Buchsbaum (*See* Chapter 3.29) address the pathology of Alzheimer's disease and provide an update on the theories and mechanisms that underlie this prominent memory disorder of aging in comparison to mechanisms of normal, age-associated memory loss.

I find it just amazing that recent advances in the cognitive neuroscience of memory can be conceived largely as the rediscovery of the notion of multiple memory systems and the specific systems envisioned by the early thinkers. We should take heart in the legitimacy of this rediscovery: It is unlikely that the current taxonomies of memory are just driven by the technologies of our time; they rather must now also be coming from a thoughtful analysis of the ways experiences modify our cognition and behavior. In addition, the current findings from neuroscience have served to validate the early conceptions of how memory is organized. Yet, to truly understand the distinct operations that characterize each memory system, we still have a long way to go. Progress so far should be viewed as only an introduction to our understanding of the functional circuitries of the memory systems of the brain. There have been quite significant strides made in identifying the major memory systems and the various ways in which their processing is modulated by other brain systems, neurochemicals, and aging. Yet our understanding of these systems and modulatory influences is still in its infancy. Our aim as systems neuroscientists is to flesh out that preliminary understanding into the details of the functional organization of these memory systems.

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3.02 Multiple Memory Systems in the Brain: Cooperation and Competition

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3.02.1 Introduction

Both casual and scientific observation inform us that behavior changes with experience. This phenomenon is called learning. Its existence implies that information about experience is retained or stored in some way so it can influence future behavior. This is called memory. Learning can be observed. Memory cannot be observed but is inferred from learned behavior. Such inferences are quite common in daily life. One of their best-known applications is the inference that students have acquired certain specific memories from their performance on an examination.

Identification and description of the information stored as memory is a major subject of this chapter. Perhaps the earliest recorded example of such a description was associationism, the notion that neural representations of certain ideas and events are connected in some way. This inference was made from the observation that temporally contiguous ideas and events tend to evoke memories of each other, expressed as thoughts and behavior.

As early as 1804, Maine de Biran described how language reveals memory for several different kinds of information, including motor functions (speech), emotions and the situations that evoke them, and abstract associations among ideas and concepts (Maine de Biran, 1804). In his 1883 book, *Les Maladies de la Mémoire* (Diseases of Memory), Ribot described a series of patients with apparent damage to the cerebral cortex who had lost all personal, conscious memories and temporal information but retained normal memory for habits and skills such as handicrafts (Ribot, 1883). He suggested that personal and conscious memories are stored in the cortex, and that memories for skills and habits must be stored elsewhere in the brain. Together, these two early French authors described a basic version of the multiple memory systems idea: that different types of information are stored in different parts of the brain.

Other early evidence for localization of memory types came from observations of pathological states such as the amnesic syndromes named for Alzheimer (1987) and Korsakoff (Ljungberg, 1992), who suggested that damage to the cortex (including the hippocampus) and medial thalamus were involved in storing the memories their patients had lost.

This early evidence for the localization of memories for different kinds of information contributed to a controversial science called phrenology, the idea that the human personality could be studied by observing the shape of the head (Gall and Spurzheim, 1819). The basis of this claim was the hypothesis that individual differences in the development (i.e., size) of brain areas with different functions determines both personality and the shape of the skull that encloses them. Although the idea that skull shape is related to brain function was discredited, the concept of localization of function in the brain has become a basic principle of neuroscience. The multiple memory systems idea applies this principle to the processing and storage of different types of information.

3.02.2 Inferring Information Type from Learned Behavior

3.02.2.1 The Rigorous Study of Learning

The scientific study of memory requires a rigorous methodology for observing and recording learned behavior. The first such investigations are usually attributed to Ebbinghaus (1885). Ebbinghaus memorized lists of nonsense syllables (used because differences in the familiarity of words would have influenced learning and recall rates). He recorded the number of trials required to reach specific levels of performance and observed how this number was reduced each time the same list was rememorized (savings). By this experiment and many others, he showed that memory could be brought under experimental control and studied with precision, even though it could not be directly observed. Other researchers extended Ebbinghaus' original work (Anderson and Bower, 1979), and some began applying the principles he developed to the study of learning and memory in animals.

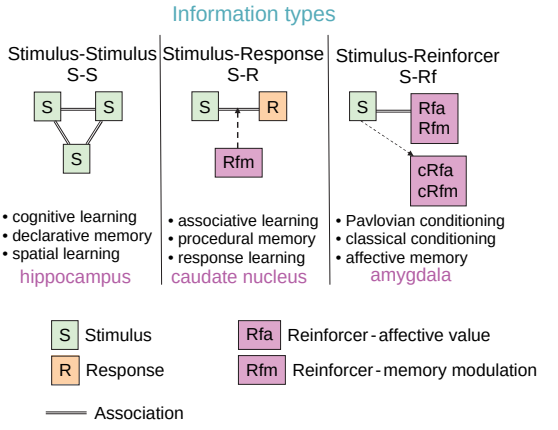


Figure 1 Information types. The headings of each column show the names of the three types of information, derived as explained in the text. Each type consists of associations in which three elements (stimuli, responses, reinforcers) are associated in different ways. The diagrams show how the elements are associated in each type of information. Below each diagram are common synonyms for each type of information used in the learning and memory literature. At the bottom of each column is the name of the brain structure central to the anatomical system thought to process each type of information.

3.02.2.2 Theories of Learning

During the first part of the twentieth century the behavioral investigation of learning in rats formed the basis of several major research programs which differed, sometimes radically, in the inferences they made from behavior about the kinds of information stored in memory (Hilgard and Bower, 1966). The concepts that emerged from this research (**Figure 1**) are useful starting points for describing the kinds of information stored as memory.

3.02.2.2.1 Stimulus-response (S-R) associations

The simplest theory, initially proposed by Thorndike (1898, 1911), was based on the observation that the probability of a stimulus evoking a response depends on the number of times the response has been made in the presence of the stimulus and followed by a reinforcer (e.g., food). Thorndike postulated that all learned behavior is the result of a series of associations or bonds between neural representations of stimuli and responses that have been strengthened, or 'stamped in' by a reinforcer. This makes the stimulus more likely to elicit the response.

The idea that behavior is based on stored stimulus-response, or S-R, associations became the foundation of an elaborate system developed by Hull (1943), whose theory predicted the probability of a response based on numerous factors, including the number of times the reinforced S-R pairing was experienced, the number of hours of food deprivation, the amount of reinforcer given, the properties of the reinforcer, how often the response had been made recently, and many others. One purpose of this complex specification was to explain behavior without considering any unobservable variables such as conscious knowledge of a situation or awareness of its emotional (or affective) content, which were considered inadmissible by the behaviorist approach to psychological investigation (Watson, 1912; Bergmann and Spence, 1964).

3.02.2.2.2 Stimulus-stimulus (S-S) associations

In direct contrast, Tolman (1932, 1948, 1949) argued that behavior is based on cognitive information rather than being controlled by specific response tendencies. One example of an observation leading to this inference was the partial reinforcement extinction effect (Skinner, 1938; Humphreys, 1939). One group of rats received reinforcement after every correct response they made in a learning task, another group received reinforcement after only half of their correct responses. When tested with no

reinforcement (extinction), the group that had been reinforced for half of its responses kept responding longer than the group that had been reinforced for all responses. This result is the opposite of what was predicted by Hull's S-R theory, which held that the strength of a response tendency is directly related to the number of times it has been reinforced. Tolman concluded that the rats' behavior was based on their knowledge of the situation and what events they expected. The rats in the 100% group expected reinforcement for every response, so a few unreinforced responses were enough for them to detect the change in conditions and stop responding. The rats in the 50% group did not expect reinforcement for every response, so more unreinforced responses were required before they stopped.

Tolman (1932) postulated that learning consists of acquiring information about the relationships among stimuli and events. This information constitutes 'knowledge' and is represented as a series of interlocking relationships among stimuli known as stimulus-stimulus (S-S) associations (**Figure 1**). S-S associations can represent spatial relationships, leading to the concept of the spatial map (see section 3.02.3.4), and temporal relationships, leading to expectancy (knowing what comes next in a sequence of events).

3.02.2.2.3 Stimulus-reinforcer (S-Rf) associations

The third type of information was described by Pavlov (1927). Pavlov was studying the internal secretions produced by food in hungry dogs. When the dogs were repeatedly exposed to the experimental situation, the secretions began to occur in the absence of food. Accordingly, Pavlov postulated that an association was formed between the food (the unconditioned stimulus, or US) and stimuli in the experimental situation (the conditioned stimulus, or CS). This made the CS capable of eliciting conditioned responses (CR) similar to those produced by the US.

USs are events that elicit responses without previous experience. These include food, water, sexual partners, and certain aversive events. The elicited unconditioned responses (URs) include approach or withdrawal and responses of the autonomic nervous system and certain brain structures and neurotransmitters. Neutral stimuli acquire CS properties when a US occurs in their presence. The issue of whether Pavlovian learning is the result of a CS-US or a CS-UR association is controversial (e.g., Donahoe and Vegas, 2004). Reflecting this controversy, the process is often described as a CS-US/UR learning. In the multiple memory systems context, this form of learning is called stimulus-reinforcer (S-Rf) learning (**Figure 1**), because it is restricted to responses that are elicited by reinforcers.

3.02.2.3 Reinforcers

The events called USs in Pavlovian theory are the same ones that Thorndike labeled reinforcers. The three theories include three different functions of the responses elicited by reinforcers:

1. Reinforcers elicit internal, unobservable responses that strengthen, or modulate, S-R and S-S (Packard and Teather, 1998; Packard and Cahill, 2001) and S-Rf (White and Carr, 1985; Holahan et al., 2006) associations when their occurrence is temporally contiguous with the acquisition of the association (Thorndike, 1933; Landauer, 1969; McGaugh and Herz, 1972; White, 1989b; White and Milner, 1992) (shown as Rfm in **Figure 1**).
2. Reinforcers elicit internal, unobservable responses that are perceived as positive or negative affect (Young and Christensen, 1962; Cabanac, 1971; White, 1989b; White and Milner, 1992; Burgdorf and Panksepp, 2006) (shown as Rfa in **Figure 1**). Humans and animals can learn about these affective states and how to obtain or avoid them in, a process called instrumental learning.
3. Reinforcers elicit observable orienting and approach or withdrawal responses. Normally, stimuli that elicit approach also elicit positive affect and are sometimes called rewards. Stimuli that elicit withdrawal and also elicit negative affect are described as aversive, or as punishments. Both rewarding and aversive events strengthen or modulate memory (Huston et al., 1977; White, 1989b; Holahan et al., 2006).

Because the learning theorists used rats in their experiments, they also had to use biologically relevant reinforcers to control behavior and the information being processed and stored during the experimental trials. Although it is not necessary to use such reinforcers with humans, who can follow instructions, feedback about correct and incorrect responses is also used and has many of the same functions as biological reinforcers in rats (see Thorndike, 1933). Both instructions and feedback control human behavior and information processing in experimental trials.

3.02.2.4 Information Types: Relationships among Elements

Each learning theory made different inferences from observed behavior about the kinds of information processed and stored during learning, but, as illustrated in **Figure 1**, in each case the information consists of different combinations of the same three elements: stimuli, responses, and reinforcers. The information types differ in the relationships among these elements. S-S, S-R, and S-Rf associations can all be thought of as different types of information created by experience, stored in the brain, and recalled to influence behavior. Large parts of this chapter describe evidence that these types of information are processed and stored in different parts of the brain.

3.02.3 Localization of Information Processing

3.02.3.1 Early Localization Attempts

S. I. Franz (1902) was among the first to apply systematic behavioral methodology to the study of brain areas involved in memory. He reported that cats could learn a series of complex responses to escape from a box and that large lesions of various parts of the cortex had only temporary effects on this learned behavior. He concluded that there was no evidence for localization of memory functions in the brain.

Franz's student, Karl Lashley, pursued these studies, testing rats on a variety of memory tasks with similar results (Beach et al., 1960). No part of the cortex seemed more important than any other – temporary deficits in learned behavior were usually quickly reversed with additional training. Only very large lesions including most of the cortex produced permanent, although generally still partial, deficits. Lashley concluded that the memory functions of any part of the cortex could substitute for any other part, a principle he called equipotentiality. Lashley's frustration at his inability to localize and understand memory is revealed by his summary statement in a 1950 article describing a lifetime of work on the problem:

> I sometimes feel, in reviewing the evidence on the memory trace, that the necessary conclusion is that learning is just not possible. (Lashley, 1950, p. 477)

Notwithstanding their methods, Franz's and Lashley's experiments failed to localize memory functions because they assumed that a single brain structure (the cerebral cortex) processed and stored all memories. It followed from this assumption that damaging the critical structure should eliminate all forms of memory. Although the idea that different parts of the brain process different kinds of memory had been suggested, Franz and Lashley did not make use of this idea, possibly because of behaviorist strictures on the use of such unobservable entities as information types.

3.02.3.2 HM and the Function of the Hippocampus

In the early 1950s new clinical evidence for the localization of different types of memory in the brain emerged. A patient with intractable epilepsy, known by his initials (HM), underwent a bilateral excision of a major portion of his temporal lobes (including much of the hippocampus) as a last-chance treatment, making him perhaps the most famous patient in modern neurology (Scoville and Milner, 1957). He was given a full psychological assessment by Brenda Milner, who reported (Milner and Penfield, 1955; Milner, 1958, 1959) that he was unable to recall personal experiences or other events from the previous 20 or so years (retrograde amnesia), and that he was also unable to remember any current experience for more than a few minutes (anterograde amnesia). HM also had difficulty finding his way around, even in familiar environments.

However, HM retained other forms of memory. He was able to learn a simple maze that required him to move a stylus through a series of left and right turns on a board (Corkin, 1968; Milner et al., 1968). He performed this task accurately, while at the same time claiming he had never seen it before. This showed that the memory deficit produced by temporal lobe resection was limited to information about HM's previous experiences. The deficit did not include the information that allowed him to learn the maze, which must have been dependent on some other part of the brain.

Although Milner and her coauthors carefully described their observation that the effects of bilateral hippocampal ablation are limited specifically to memory for new ongoing experience and spatial orientation, and that other kinds of memory, including verbal abilities, emotion, and skills and habits

remained normal, these distinctions were largely ignored at first.

3.02.3.2.1 Attempts to replicate HM's syndrome with animals

3.02.3.2.1.(i) Monkeys Orbach et al. (1960) proposed that a strong test of the temporal lobe memory hypothesis would be met only if temporal lobe damage impaired performance regardless of what behavioral task their monkeys were required to learn. These workers did find deficits on some memory tasks, but only with very large temporal lobe lesions.

The reasons for this (and other) failures to replicate HM's syndrome were revealed by Mahut and coworkers, who reported that monkeys with hippocampal (Mahut, 1971), fimbria-fornix, or entorhinal cortex (Mahut, 1972; Zola and Mahut, 1973) lesions were impaired on spatial tasks when no local stimuli were available to guide their behavior. In the same monkeys, nonspatial tasks were unaffected, and when small cue objects were added to spatial discrimination tasks, performance actually improved relative to that of normal controls (Killiany et al., 2005). These experiments showed that memory deficits are produced by hippocampal damage in monkeys, provided tasks requiring the use of the kind of information processed in the hippocampus are used (Mahut et al., 1981).

3.02.3.2.1.(ii) Rats A number of workers also tried to demonstrate the effects of hippocampal lesions on memory in rats. These studies had such limited success that several theories suggesting alternate functions for the rat hippocampus were proposed (Kimble and Kimble, 1965; Douglas and Pribram, 1966; Kimble, 1968; Isaacson and Kimble, 1972) and refuted (Nadel et al., 1975). These hypotheses did not completely reject memory-related functions but were primarily concerned with explaining the highly inconsistent effects of hippocampal lesions on the performance of various memory tasks. The inconsistency was the result of a failure to appreciate the specific type of information processed in the hippocampus and the consequent use of tasks that could be performed on the basis of some other kind of information processed in other parts of the brain.

3.02.3.3 Contextual Retrieval

In what is now considered a major conceptual breakthrough, Hirsh (1974) provided an explanation for the inconsistent effects of hippocampal lesions on memory tasks in rats. Hirsh pointed out that hippocampal lesions did not affect learning tasks that could be performed on the basis of a single S-R association, but they impaired performance of tasks that could not be correctly performed using this kind of information. He suggested that tasks impaired by hippocampal lesions involved acquisition of two or more S-R associations, and that the hippocampus mediated a process by which one of these was selected by the context. An example of a behavioral observation that led to the inference of this contextual retrieval process is illustrated in **Figure 2**. Rats were trained in a T-maze to turn left for food when hungry and right for water when thirsty (Hsiao and Isaacson, 1971). The choice point in the maze became a stimulus associated with two responses: the left and right turns. The deprivation state was the context that selected the appropriate S-R association. Normal rats learned to make the correct turn depending on their deprivation state. Rats with various impairments of the hippocampal system (Hirsh and Segal, 1971; Hsiao and Isaacson, 1971; Hirsh et al., 1978a,b) made one turn or the other, regardless of their deprivation state. They were unable to use the motivational context to select the correct response.

Another example is reversal learning. As shown in **Figure 3**, normal rats and rats with hippocampal lesions learned to turn left for food at similar rates. When the food was switched to the right arm of the maze, normal rats adjusted their behavior much more quickly than rats with hippocampus lesions. The normal rats were able to change the direction of their turn on the basis of new information about the location of the food obtained on the first few trials after the switch. According to Hirsh, the switch altered the context that selected the appropriate response. Hippocampectomized rats were unable to use this type of information. They had to extinguish the old S-R association and acquire a new one when the reinforcer was switched.

Hirsh's main contribution to the idea of localized memory functions based on the content of the memory was to explain it in terms of the existing animal learning literature, an area of research that was very well developed and had up to that time generally rejected or ignored this possibility. Although it took some time, "The hippocampus and contextual

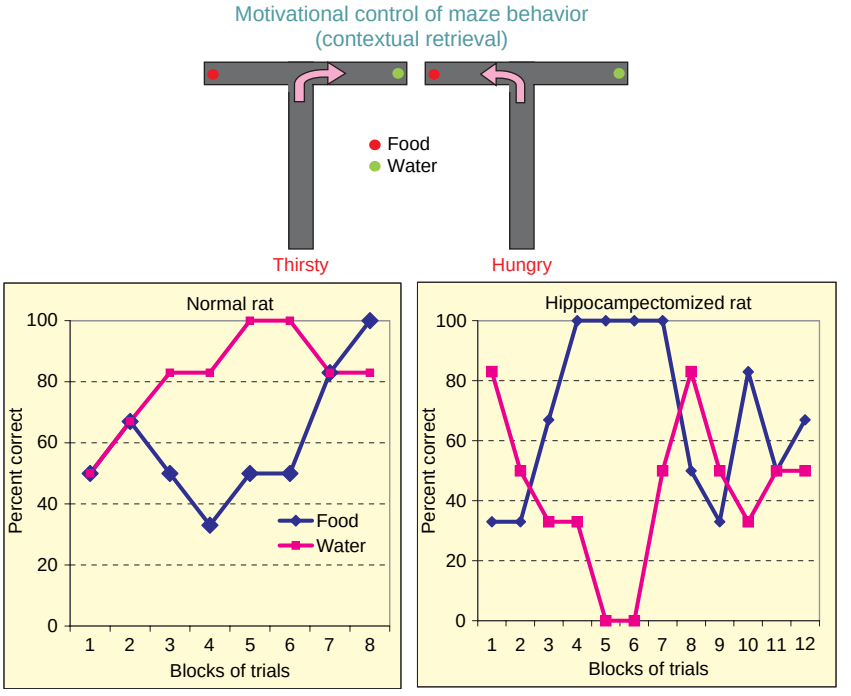


Figure 2 Motivational control of maze behavior (contextual retrieval). The figure illustrates an experiment in which rats were trained on a T-maze with food in one arm and water in the other. The rats were food or water deprived on alternate days. The graph on the left shows the behavior of a normal rat that learned to turn right for water on days when it was thirsty first (trial blocks 1–5). Between blocks 5 and 8 the rat also learned to turn left for food when hungry, while maintaining the correct response for water. This shows that the rat had learned to use its motivational state to perform the correct response at the choice point in the maze. The graph on the right shows the behavior of a hippocampectomized rat that began by learning to turn right for water. As the rat's performance on the days when it was hungry improved, its performance on thirsty days declined. After a few more trial blocks, the rat's performance when thirsty improved, whereas its performance when hungry declined. Several more such reversals were observed, suggesting that this rat was able to learn one of two simple S-R associations but was unable to use its motivational state to select between the two. Adapted from Hirsh R (1980) The hippocampus, conditional operations and cognition. *Physiol. Psychol.* 8: 175–182.

retrieval of information from memory: A theory" (Hirsh, 1974) has come to be regarded as a major turning point in research on memory.

3.02.3.4 Spatial Learning

In *The Hippocampus as a Cognitive Map*, O'Keefe and Nadel (1978) presented evidence that the hippocampus is the primary structure for representing and possibly storing spatial information. The two main lines of evidence for this idea were impairments in spatial learning produced by hippocampal lesions and the observation that the activity levels of certain hippocampal neurons increased whenever a rat was in a specific spatial location (O'Keefe and Dostrovsky, 1971; O'Keefe, 1976). This suggestion that the hippocampus contains information about the animal's position in space led O'Keefe and Nadel to postulate the spatial map, a neural representation of the relationships among constant features of an environment. The information in these representations can be described as consisting of a series of S-S associations. This relational information is purely descriptive, with no specific implications for behavior. O'Keefe and Nadel distinguished hippocampus-based spatial learning (also called allocentric) from taxon (or egocentric) learning, in which each environmental feature can evoke one specific response, as in the case of S-R learning. O'Keefe and Nadel did not speculate about the neural substrate for taxon learning.

The idea that the hippocampus processes information consisting of relationships among features of an environment has been extended and incorporated into the theory that the structure processes relational information of all kinds (Hirsh, 1980; Cohen and

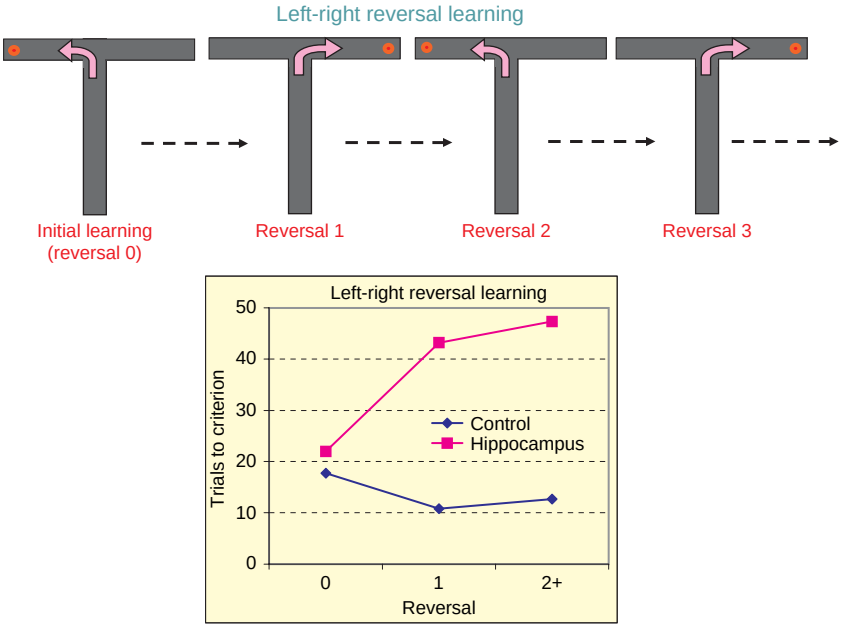


Figure 3 Effects of hippocampus lesions on reversal learning. Hungry rats learned to find food in a maze by turning left at the choice point. Both normal rats and rats with hippocampal lesions learned the initial response to criterion (five consecutive correct responses) equally well (Reversal 0). However, when the correct response was reversed, the hippocampectomized rats were severely impaired. The impairment continued on subsequent reversals. All rats were given a total of 100 trials, during which the control rats met the criterion and were reversed an average of 8.1 times. Hippocampectomized rats met the criterion and were reversed an average of 2.2 times. Adapted from Kimble DP and Kimble RJ (1965) Hippocampectomy and response preservation in the rat. *J. Comp. Physiol. Psychol.* 60: 474–476.

Eichenbaum, 1991; Eichenbaum and Cohen, 2001). This information, stored as S-S representations, can be used flexibly to produce behaviors appropriate to different circumstances. The theory that the information processed in the hippocampus is purely spatial, and the idea that it represents relationships of a more general nature including spatial have been debated (Wiener et al., 1989; Cohen and Eichenbaum, 1991; Nadel, 1991; Eichenbaum et al., 1992).

3.02.3.5 Memory and Habit

Mishkin et al. (1984) described an experiment in which monkeys were shown pairs of objects. Displacing one of them revealed a morsel of food. When shown the same two objects a short time later, displacing the other object revealed food. The monkeys were given a large number of trials over several days, with different pairs of objects on every trial. Normal monkeys learned this 'delayed nonmatching to sample' rule, but monkeys with hippocampal lesions were unable to do so – a deficit that is consistent with a general loss of memory for recent events. In contrast, when a large number of object pairs were presented repeatedly, normal and hippocampal monkeys learned to respond to the correct member of each pair equally well. Thus, hippocampal lesions impaired memory for novel objects seen for the first time but did not affect learning to make a consistent response to each pair of objects after repeated reinforced trials. Mishkin et al. described the latter behavior as a habit and speculated that the memory for this type of behavior might be mediated in the caudate nucleus.

3.02.3.6 Declarative versus Procedural Memory

Cohen and Squire (1980) trained human participants to read words in a mirror. Both normal participants and patients with amnesia resulting from medial temporal lobe dysfunction learned the mirror reading skill; they improved with practice and improved more for words that were repeated in each session than for words that were new. However, when tested shortly after a training trial, normal participants were able to remember most of the words they had just read; the amnesic patients were severely impaired.

Cohen and Squire concluded that memory for the words (declarative memory) requires a functional hippocampus (*See* Chapter 3.03), but that the mirror reading skill (procedural memory) must be dependent on some other part of the brain.

Declarative and procedural memory describe different kinds of remembered information, corresponding to S-S and S-R learning as described by Tolman and Thorndike, respectively, as well as to Mishkin et al.'s distinction between memory and habit, to Hirsh's descriptions of contextual memory versus S-R memory, and to O'Keefe and Nadel's concepts of the spatial map and taxon learning. These distinctions were all made by showing that hippocampal damage impairs performance on tasks requiring S-S information but has no effect on tasks that can be performed using S-R information. None of these distinctions includes evidence concerning the neural substrate of S-R information processing.

3.02.3.7 Double Dissociation of S-S and S-R Learning in Humans

Butters and coworkers (Martone et al., 1984; Butters et al., 1986; Heindel et al., 1989) found that patients with Korsakoff's syndrome, who are amnesic as a result of hemorrhagic lesions of medial thalamus and mammillary bodies, learned the mirror reading skill normally but were unable to recognize words they had recently read. In contrast, patients with Huntington's disease, in which neurons in the basal ganglia (including the caudate nucleus) degenerate, had the opposite pattern of disabilities: They were unable to acquire the mirror reading skill but recognized previously seen words normally.

This pattern of effects constitutes a double dissociation, in which subjects with impairments of one of two brain areas were compared on two memory tasks. Impaired function of each brain area affected only one of the two tasks, leaving performance on the other task intact. This led to the conclusion that each brain area was involved in processing the type of information required for the impaired task, but not the information required for the unimpaired task. As described in the previous section, recall of recently seen words requires declarative, or S-S, information; mirror reading is a skill that may require a complex of S-R associations.

Although there was no direct confirmation of the brain damage in these studies, they are significant because they provide evidence for the neural substrate that processes information that may include S-R information and dissociate it from S-S information processing. The studies point to the basal ganglia, specifically the caudate nucleus, as the location of S-R information processing. A number of experiments with rats are consistent with this conclusion about the caudate nucleus (Divac et al., 1967; Divac, 1968; Thompson et al., 1980; Chozick, 1983; Mitchell and Hall, 1987, 1988; Cook and Kesner, 1988; Packard and Knowlton, 2002). Phillips and Carr (1987) specifically proposed that S-R learning is mediated in the basal ganglia.

3.02.3.8 Dissociation of Three Information Types in Rats

Packard et al. (1989) used a double dissociation to compare the impairments in information processing capacity produced by lesions to the fimbria-fornix (a major input-output pathway of the hippocampus) and the caudate nucleus. This was extended by McDonald and White (1993) to include the amygdala in a triple dissociation of memory tasks. All three tasks were performed on an eight-arm radial maze (**Figure 4**) consisting of a center platform with eight arms radiating from it in a sunburst pattern about 1 m from the floor. Each task required the use of a different type of information.

3.02.3.8.1 Win-shift task – hippocampus-based S-S memory

The win-shift radial maze task (Olton and Samuelson, 1976; Olton and Papas, 1979) was used to examine S-S information processing. The maze was situated in a room with various extra-maze stimuli (or cues) that constituted a spatial environment. A small food pellet was placed at the end of each arm. Hungry rats were placed on the center platform and allowed to forage for food. Pellets consumed were not replaced, so to obtain the eight available pellets most efficiently the rat had to enter each arm once only. Second or more entries to arms were considered to be errors. Normal rats attained an average of less than one error per trial after five to seven daily trials.

To enter each arm once only, a rat must remember which arms it has entered as the trial proceeds (working memory). To do this, it must be able to discriminate the arms from each other. There is evidence that rats perform this discrimination by associating each arm with the environmental cues that are visible from it (O'Keefe and Nadel, 1978; Suzuki et al., 1980). Since all the individual cues are visible from several of the arms, no individual cue can

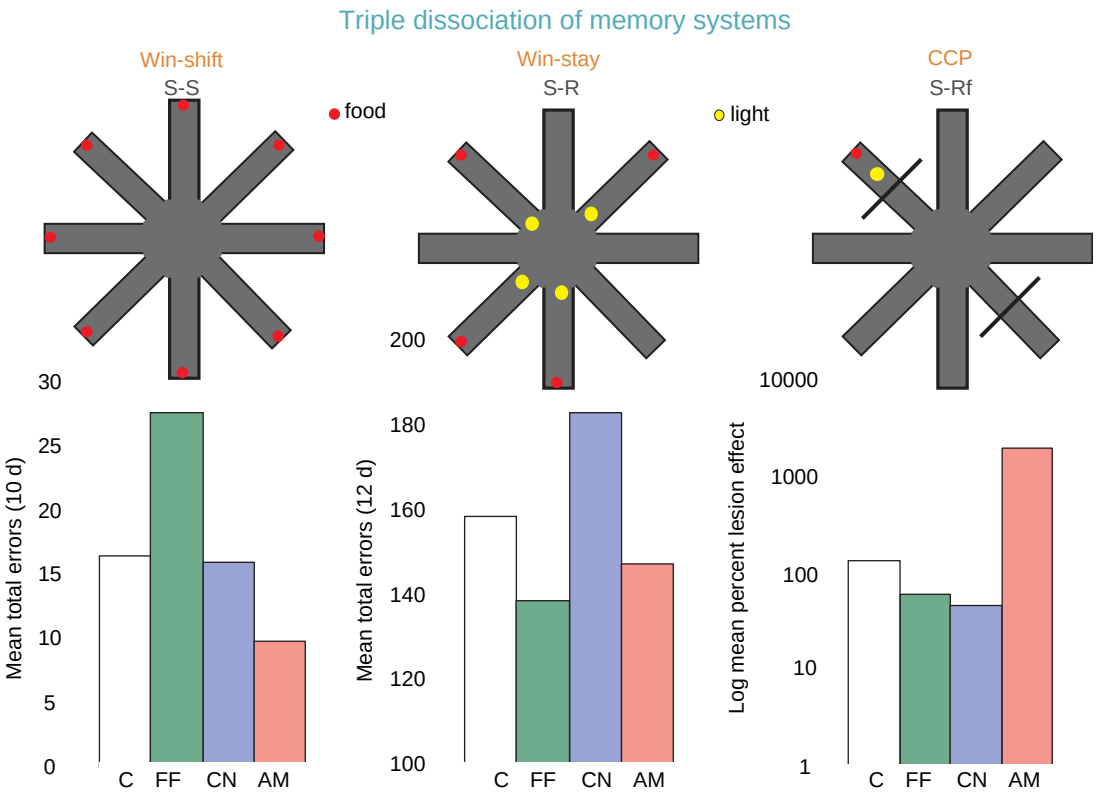


Figure 4 The triple dissociation. Three different tasks on the eight-arm radial maze are illustrated at the top. The type of information processing thought to be required to perform each task is indicated, corresponding to the information types described in **Figure 1**. As explained in the text, correct performance of the win-shift task requires rats to obtain the food pellet at the end of each arm without reentering arms. This requires spatial (or S-S) information processing. Performance of this task was impaired by lesions of fimbria-fornix (FF), but not by caudate nucleus (CN) or amygdala (AM) lesions. The win-stay task requires a simple association between a cue light and the arm entry response, reinforced by eating the food at the end of the lit arms. A different set of four arms is lit and baited on each daily trial. This is an instance of S-R information processing. Performance on this task was impaired by lesions of the caudate nucleus but not by lesions of fimbria-fornix or amygdala. The improved performance on this task produced by fimbria-fornix lesions is explained in **Figure 5**. In the conditioned cue preference (CCP) task, the rats acquire an S-Rf association between the light and the food reinforcer, causing the light to acquire conditioned stimulus properties. This results in a preference for the food-paired arm even when no food is present on the test trial. Performance on this task was impaired by lesions of the amygdala, but not by lesions of fimbria-fornix or caudate nucleus. Adapted from McDonald RJ and White NM (1993) A triple dissociation of memory systems: Hippocampus, amygdala and dorsal striatum. *Behav. Neurosci.* 107: 3–22.

identify any single arm. However, the arms can be identified by their unique locations within a spatial map of the environment. This requires relational or S-S information.

Good performance on this task requires continually updated information about the status of each arm as a trial proceeds. At the start of a trial all arm-identifying cue arrays indicate the location of a food pellet, but once an arm has been entered and the pellet consumed, the same array must indicate the absence of food. In this way information about the spatial environment is used flexibly to guide behavior according to changing conditions.

3.02.3.8.2 Win-stay task – caudate-based S-R memory

The win-stay task was used to study S-R information processing. The same maze was used, but the location of the food was indicated by a small light mounted at the entrance to each arm. When a hungry rat was placed on the center platform at the start of a trial, four of the arms were lit, and only those four arms contained a food pellet. The other arms were empty. Efficient performance required the rats to enter lit arms and avoid dark arms. The information required to produce this behavior is simple: When a rat approaches a lit arm (S), enters it (R), and consumes

the reinforcer, the S-R association is strengthened, increasing the probability that the stimulus will elicit the response again. No spatial information or working memory is required for the win-stay task.

3.02.3.8.3 Conditioned cue preference task – amygdala-based S-Rf memory

The conditioned cue preference (CCP) task was used to study S-Rf information processing. The radial maze was surrounded by curtains. Hungry rats were confined on an arm with a light and a supply of food and on alternate days were confined on a different, dark arm with no food. After several days of such trials the rats were placed on the center platform of the maze and given a choice between the two arms, neither of which contained food. Normal rats spent more time on the food-paired than on the unpaired arm.

Since the rats were exposed to the food while confined on a maze arm they could not have acquired any S-R associations or instrumental responses leading to their preference for the food-paired arm. The curtains surrounding the maze and confinement in one arm at a time severely limited the rats' ability to acquire a spatial map of the environment (Sutherland and Linggard, 1982; Sutherland and Dyck, 1984; Sutherland, 1985; White and Ouellet, 1997). Therefore, the preference was probably not a result of learned information about the spatial location of food (i.e., S-S learning). The alternative is that consuming the food (US) during the training trials elicited an internal rewarding response (UR). This caused the arm cues (light or dark) in the food-paired arm to acquire CS properties. On the test trial, the CS elicited a conditioned reward response, causing the rat to remain in the food-paired arm longer than in the no-food arm. This analysis explains the preference for the food-paired arm as the product of S-Rf information processing.

3.02.3.8.4 Dissociation by damaging brain structures

As shown in **Figure 4**, performance on each of the three tasks was impaired by only one of the three lesions. (Performance of the win-stay task was actually improved by fimbria-fornix lesions. This phenomenon is explained in **Figure 5**.) Since the three tasks had identical sensory and motor requirements, and the same reinforcer was used for all three, the differences in the effects of the lesions were attributed to differences in the kinds of information required to perform the tasks. The effects of the lesions imply that each lesioned structure was involved in processing only one of the three kinds of information.

None of these attributions was original to the triple dissociation experiments. It was already well known that lesions of the hippocampus and related structures impair spatial learning (Hirsh and Segal, 1971; O'Keefe et al., 1975; Olton et al., 1978; Olton, 1978; Morris et al., 1982), and that Pavlovian conditioning involving S-Rf information processing is impaired by amygdala lesions (Weiskrantz, 1956; Bagshaw and Benzies, 1968; Jones and Mishkin, 1972; Nachman and Ashe, 1974; Peinado-Manzano, 1988; Everitt et al., 1989, 1991; Hatfield and Gallagher, 1995; Davis, 1997; Fanselow and Gale, 2003). There were also numerous reports of learning impairments produced by caudate nucleus lesions, but the evidence that these involved S-R learning was not clear (e.g., Gross et al., 1965; Divac et al., 1967; Divac, 1968; Kirkby, 1969; Winocur and Mills, 1969; Mitchell and Hall, 1987, 1988). The contribution of the triple dissociation experiment was to define these various tasks in terms of the kinds of information required to perform them and to show that the processing of each type of information was dependent on a different part of the brain.

3.02.3.8.5 Dissociation by reinforcer devaluation

Reinforcer devaluation is a procedure in which a rewarding reinforcer is paired with an aversive event, reducing the positive affective value of the reinforcer (Young and Christensen, 1962; Dickinson et al., 1983). The prototype is the conditioned taste aversion (Garcia and Koelling, 1966; Nachman and Ashe, 1974; Yamamoto and Fujimoto, 1991), in which pairing consumption of a rewarding substance such as a sugar solution with injections of lithium chloride (LiCl) (which produces gastric illness) decreases or eliminates consumption of the solution.

Yin and Knowlton (2002) used the devaluation procedure in conjunction with the CCP task on the radial maze. After CCP training the rats ate some of the same food pellets in their home cages, followed by LiCl or saline (control) injections. When tested in their home cages the rats that received LiCl ate much less than the rats that received saline, demonstrating the reduced net reward value of the food. When given a preference test on the radial maze with no food present the rats that received saline preferred their food-paired arms, but the rats that received LiCl exhibited an aversion to their food-paired arms (see **Figure 6**). This finding

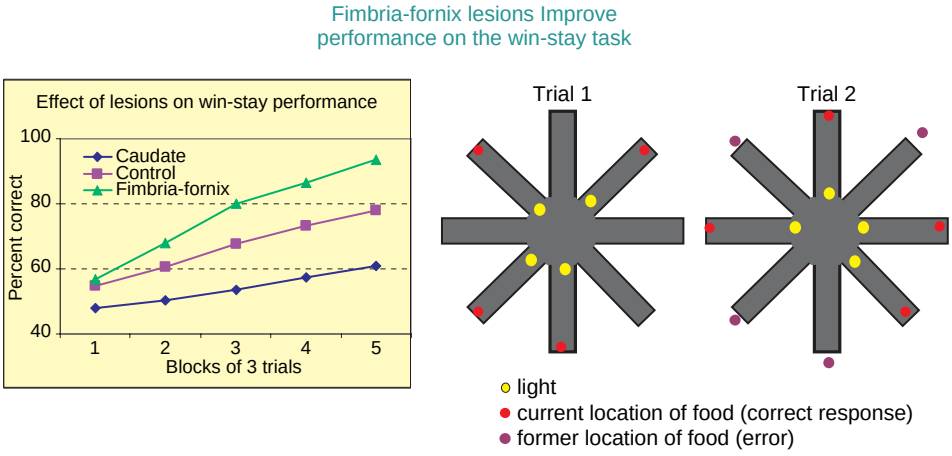


Figure 5 Fimbria-fornix lesions improve performance on the win-stay task. The graph shows the effects of lesions on performance of the win-stay task (see **Figure 4**). Compared with normal control rats, rats with caudate lesions performed poorly, suggesting that this structure is involved in processing the S-R information required for performing the task. In contrast, lesions of fimbria-fornix (part of the hippocampal system) improved win-stay performance compared with controls. The maze diagrams on the right explain this improved performance. They show two consecutive trials on the win-stay task. In both cases the arms containing food are indicated by lights at their entrances, but the food is located in different arms on the two trials. Caudate-based processing of information comprising the consistently reinforced S-R association between the lights and the arm entry response results in correct behavior on both trials. However, on Trial 1 the hippocampus system acquires information about the fixed cues in the spatial environment and about the location of food in relation to those cues. As shown in the figure, this information is incorrect on Trial 2, because a different set of four arms contains food. Therefore, any tendency to enter arms that previously contained food promoted by the hippocampal system would result in errors. Fimbria-fornix lesions eliminate this S-S information and the erroneous responses it promotes, resulting in improved performance of the S-R task. Because the information processing capacities of the two systems cause them to promote different behaviors in the same situation, the effect of fimbria-fornix lesions on win-stay performance reveals a competitive interaction between the behavioral effects of the two kinds of information processing. Adapted from Packard MG, Hirsh R, and White NM (1989) Differential effects of fornix and caudate nucleus lesions on two radial maze tasks: Evidence for multiple memory systems. *J. Neurosci.* 9: 1465–1472.

suggests that the preference for the food-paired arm was the result of a memory that involved the affective property of the reinforcer. This memory was positive in the saline-injected rats, leading to a preference for the food-paired arm. It was much less positive, even negative, in the LiCl-injected rats, leading to avoidance of the food-paired arm. This is consistent with the idea that the CCP is based on information comprising an S-Rf association.

In contrast to these findings, Sage and Knowlton (2000) found that the same devaluation procedure did not affect the performance of well-trained rats on the win-stay task, even though they stopped eating the food at the ends of the lit arms (**Figure 6**). This observation is consistent with the idea that performance on this task is unrelated to the affective value of the reinforcer, which acts simply to strengthen the caudate-based S-R association that produces the win-stay behavior.

Sage and Knowlton (2000) also found that rats trained on the win-shift task performed normally after devaluation (**Figure 6**). These rats did not completely reject the food while running the maze. Most of them ate the pellets in the first few arms entered and then stopped eating while continuing to perform correctly. This suggests that they did not spontaneously transfer the aversion from the home cage to the new situation but recalled it only after tasting the food in the new context. That this rejection of the food had little effect on the accuracy of their win-shift performance is consistent with the hypothesis that information involving the affective value of the food is not required for win-shift performance, which depends on information about the presence and absence of food in each arm as the trial progresses.

Although the devaluation data do not dissociate the neural substrates of information processing in the three tasks, they constitute evidence that S-S and S-R information can control behavior independently of the affective value of reinforcers in the situation. This contrasts with the evidence for the representation of these affective properties in S-Rf information processing.

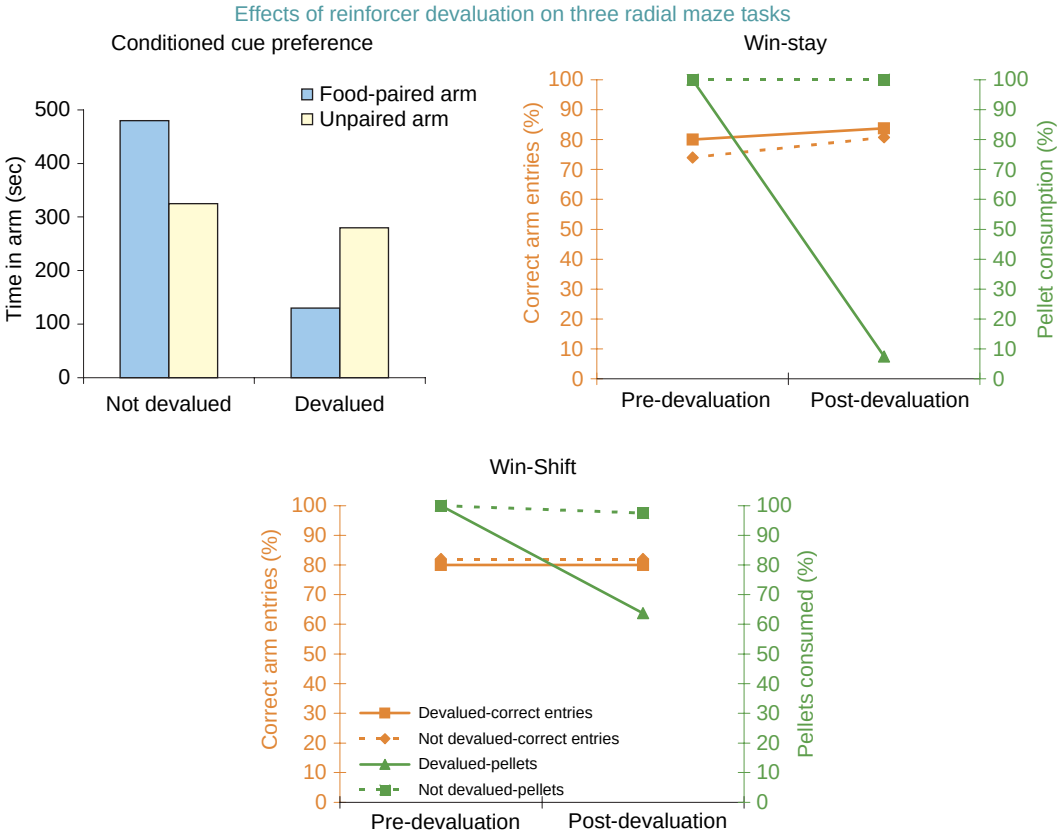


Figure 6 Effects of reinforcer devaluation on three radial maze tasks. *CCP:* The graph on the top left shows the amounts of time spent by rats in their food-paired and unpaired radial maze arms in a CCP test (see section 3.02.3.8.3). Rats that experienced reinforcer devaluation (explained in the text) exhibited an aversion to their food-paired arms. Rats that did not experience devaluation exhibited a normal conditioned preference for their food-paired arms. The effect of devaluation on the preference is evidence that the rats' behavior is controlled by information that includes a representation of the affective value of the reinforcer. *Win-stay:* The graph on the top right shows the percent correct responses and the percent of total food pellets available that were consumed before and after reinforcer devaluation. Before devaluation the rats ate all pellets available; after devaluation they ate very few. However, this did not affect the accuracy of their win-stay performance. This constitutes evidence that the affective value of the reinforcer is irrelevant to win-stay behavior and is consistent with the idea that the reinforcer acts to strengthen the S-R association that controls behavior in this situation. *Win-shift:* The graph on the bottom shows the percent correct responses and percent of total pellets consumed on the win-shift task before and after reinforcer devaluation. Performance was maintained after devaluation, whereas pellet consumption decreased. The pellets were not completely rejected, suggesting that a representation of the affective value of the reinforcer may have contributed to win-shift performance. However, the maintenance of accuracy shows that positive affect was not required for performance of this task, which can be produced by information about the presence and absence of food in the arms. Adapted from Sage JR and Knowlton BJ (2000) Effects of US devaluation on win-stay and win-shift radial maze performance in rats. *Behav. Neurosci.* 114: 295–306; Yin HH and Knowlton BJ (2002) Reinforcer devaluation abolishes conditioned cue preference: Evidence for stimulus-stimulus associations. *Behav. Neurosci.* 116: 174–177.

3.02.4 Information Processing Systems

This section describes a series of theoretical ideas that constitute a theory of multiple parallel memory systems in the brain. The ideas are suggested by the findings already described and will be useful for organizing and interpreting the literature describing dissociations among brain structures and processing of information types.

3.02.4.1 Systems Concept

Although the idea that different kinds of information are processed in different parts of the brain is suggested by the effects of damage to individual

brain areas, the fact that no individual brain area can perform any function on its own leads naturally to the idea that information is processed in neural systems or pathways that can, in principle, be defined anatomically. The two visual systems described by Ungerleider and Mishkin (1982; Mishkin et al., 1983) are an example of anatomically defined neural systems that process different kinds of information. Both systems originate in the striate and prestriate visual cortex, making these structures parts of both pathways. A ventral path connects to inferior temporal areas; damage to structures in this pathway impairs object recognition. The dorsal path connects to inferior parietal areas; damage to this pathway impairs learning the visual location of objects. Visual information originating in striate cortex is integrated with other information at stages along each pathway. In this way the visual information comes to represent spatial relationships in one system and object properties in the other.

A similar concept can be applied to the evidence for independent processing of different types of information that is the subject of this chapter (see **Figure 7**). Information originating in the external and internal sense organs is processed and represented in the thalamus and cerebral cortex. This information is distributed to several different neural pathways or systems. It flows through the systems as neural activity, undergoing processing and modification at stages along the way. This results in the differentiation of the information into a unique representation in each system. The outputs of the systems converge to influence behavior and thought.

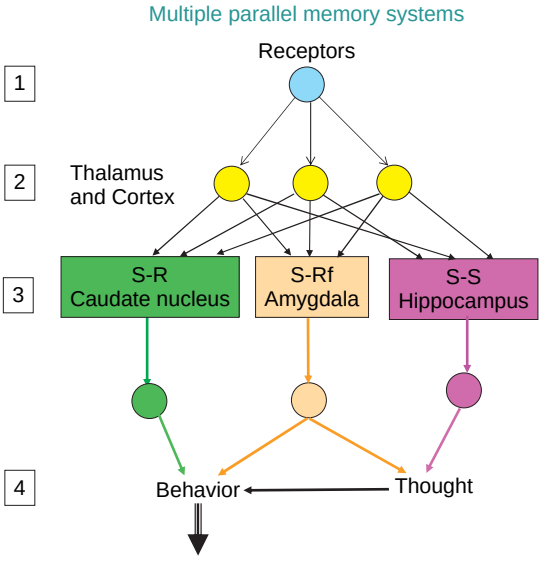


Figure 7 The multiple memory systems concept. 1. Patterns of neural activity originate in exteroceptors and interoceptors. 2. These patterns converge on thalamic and cortical areas, where they are processed and combined into activity patterns that represent the current external and internal environments. 3. These processed activity patterns are transmitted to subcortical areas, each of which is specialized to extract and represent a different kind of information contained in the activity patterns: the caudate nucleus represents reinforced stimulus-response (S-R) associations, the amygdala represents stimulus-reinforcer (S-Rf) relationships, and the hippocampus represents stimulus-stimulus (S-S) relationships. 4. The outputs of the systems converge on brain areas that mediate behavior and thought. See text for more information about the information types, how they are represented in the systems, and how the outputs of the systems interact.

3.02.4.1.1 Systems process incompatible information

Sherry and Shachter (1987) suggested that independent neural systems evolved to process fundamentally incompatible information. According to this idea, flexible memory for facts and unique situations and memory for stereotyped skills and habits are incompatible. This concept corresponds to the mutually exclusive involvement of the hippocampus and caudate nucleus in these types of memory. Although Sherry and Shachter did not discuss the amygdala, evidence from the triple dissociation experiment suggests that similar considerations may apply to the unique kind of information processed in that structure.

3.02.4.1.2 Systems are internally specialized

The information processing specialization of each system is determined at the level of its neuronal and synaptic microcircuitry. Although neural activity representing all of the elements reaches all systems, their internal specializations lead to differences in the ways they represent the relationships among the elements (**Figure 1**). There have been several attempts to describe how the internal structures of the hippocampus (Muller et al., 1996; Knierim et al., 1998; Taube, 1998; Mizumori and Leutgeb, 2001; Lever et al., 2002; Leutgeb et al., 2005; Sargolini et al., 2006), caudate nucleus (Centonze et al., 2001; Graybiel, 2001, 2004; Gurney et al., 2004), and amygdala (Pitkänen et al., 1997; Fendt and Fanselow, 1999;

Maren and Quirk, 2004; Maren, 2005; Schafe et al., 2005; Kim and Jung, 2006) process and store the information mediated in these structures.

3.02.4.1.3 Coherence: Some representations are better than others

Coherence is a hypothetical property of the representation of a learning situation in a neural system. The coherence of a representation reflects the degree to which all parts of the system are activated in a similar way when processing information that represents a particular situation. This is determined by the correspondence between the information content of the learning situation and the representational specialization of the system. The better the correspondence, the more coherent the representation. The coherence of the representation of a learning situation in a system is assumed to determine the degree to which the system influences behavior and/or thought when the representation is activated during recall.

The development of hippocampal place cells, which occurs during the first 10–30 min of exposure to a novel environment (O'Keefe and Dostrovsky, 1971; O'Keefe, 1976; Ferbinteanu and Shapiro, 2003; Kentros et al., 2003; Kennedy and Shapiro, 2004), may be an example of the creation of a coherent representation of a spatial map (S-S information) of the environment. Conversely, the same system is very slow to form a coherent representation of the S-R information content of a learning situation such as the win-stay task. This is suggested by the failure of rats with caudate lesions but an intact hippocampus to learn the win-stay task (see **Figure 4**). This and other evidence (see section 3.02.5) suggest that the caudate system acquires a coherent representation of S-R information more easily (in fewer trials) than the hippocampus system.

3.02.4.1.4 The learning-rate parameter

The coherence of the representation of a situation in a system usually improves with repeated exposure to the situation. One system may represent a situation coherently after only a single trial; another system may simultaneously form a representation of the same situation, but this may require many more trials to attain coherence. The amount of exposure required to form a coherent representation in a system is a learning-rate parameter. Differences in this parameter are important determinants of the interactions among the systems.

3.02.4.1.5 Cooperation and competition among systems

At any given point during training in any learning situation, each system has acquired a representation of the situation with some degree of coherence that determines the amplitude of its output. The fact that the systems all influence the behavior of the same animal (or person) means that these outputs must converge (**Figure 7**). The nature of the interactions among these outputs depends on the behavioral tendency promoted by the representation in each system and on the relative amplitudes of their outputs.

In some situations the outputs of the systems may promote the same behavior or different parts of a complex behavior required to perform a task. These are cooperative interactions. In other situations the outputs of the systems may promote different behaviors. This results in competitive interactions. The improvement in win-stay performance produced by fimbria-fornix lesions, explained in **Figure 5**, is thought to reflect such an interaction.

3.02.4.2 Information Processing and Memory

As we have seen, observation of learned behavior is a tool for studying the neural basis of information processing. The existence of a learned behavior also implies the existence of a memory for the information processed by each system. The definition of a system means that the information processed by the system must be stored somewhere within the system.

The neuroplastic processes that store representations of information processed by each system could be located in a discrete part or parts of each system, or they could be distributed throughout the systems. A single storage location (e.g., the cerebral cortex) could store and provide information to all systems. All of these possibilities may apply in various combinations at different stages of the learning process.

The idea that different kinds of information are processed in differently specialized parts of the brain means that, before the relationship between neuroplastic processes and the storage of information can be studied, it is critical to identify the type of information stored in each system. In the absence of this information, evidence for synaptic or neurophysiological changes correlated with the development of learned behavior cannot be interpreted. Accordingly, the evidence reviewed in this chapter does not pertain to memory storage

mechanisms. It addresses the prior issue of localizing the processing and storage of different kinds of information.

3.02.4.3 Dissociations of Memory Systems

The remaining sections of this chapter focus on evidence that dissociates the information processing properties of the three proposed memory systems. As in the review of the historical development of the idea of independent memory systems, the nature of the information being processed is inferred from the ability to perform a memory task of some kind, and the anatomical focus is on the hippocampus and fimbria-fornix, the caudate nucleus, and the amygdala. Evidence for independent information processing in the three structures, taken two at a time, is described. Evidence for competition among the systems is a major feature of the review.

The review is limited to studies in which parts of the brain are functionally disabled in various ways and to studies that measure relative levels of activation in brain areas (primarily imaging studies in humans). Space limitations do not permit a consideration of neurophysiological evidence for localized processing of information types (Mizumori et al., 2004; Davis et al., 2005; Gill and Mizumori, 2006), evidence obtained by manipulations of memory consolidation (Packard and White, 1991; Packard and McGaugh, 1992; Packard et al., 1994; Packard and Teather, 1997, 1998; Packard and Cahill, 2001), or evidence that each of the three systems appear to include subsystems that probably process subtypes of information.

3.02.5 S-S versus S-R Information Processing

3.02.5.1 Studies with Rats

3.02.5.1.1 Competition on the radial maze

As we have already seen, lesions of the fimbria-fornix impair win-shift (S-S) but not win-stay (S-R) learning, and lesions of the caudate nucleus impair win-stay but not win-shift learning (Packard et al., 1989; McDonald and White, 1993), a double dissociation. Furthermore, as described in **Figure 5**, fimbria-fornix lesions did not just impair win-stay learning, they actually improved it so that the performance of the lesioned rats was better than that of normal controls. This observation can be explained as the result of competition between the tendency to enter lit arms, represented as S-R information in the caudate system, with a tendency to forage for food in places where it had previously been available, represented as S-S information in the hippocampus system. Disabling the hippocampus-system with fimbria-fornix lesions eliminated these errors, resulting in improved win-stay performance.

Competition between caudate-based S-R learning and spatial learning has also been demonstrated with caudate lesions (Mitchell and Hall, 1988). Rats were trained to find food in a constant arm of a radial maze. To start each trial the rat was placed in the arm immediately to the right or the arm immediately to the left of the food arm. The food remained in the same spatial location, but either a right or a left turn was required to reach it. Rats with caudate lesions made fewer erroneous turns than normal rats, suggesting that in normal rats S-R associations between local maze cues and either the left or right turn response interfered with behavior based on information about the spatial location of the food. Caudate lesions eliminated S-R learning and the erroneous responses it produced.

3.02.5.1.2 Cross maze

The cross maze paradigm (Tolman et al., 1946; Blodgett and McCutchan, 1947), illustrated and explained in **Figure 8**, is a simple and elegant method for distinguishing between behavior produced by processing S-S and S-R information. Although the task was originally introduced as a demonstration of learned behavior that is not dependent on S-R information, it was soon shown that both spatial (S-S) and S-R information are acquired during training (Restle, 1957). Increasing the number of training trials favors behavior controlled by S-R information; increasing the availability of spatial stimuli favors behavior controlled by S-S information

3.02.5.1.2.(i) Competition on the cross maze Packard and McGaugh (1996) exploited the training trial effect on this task to dissociate the caudate and hippocampal memory systems. As shown in **Figure 9**, when tested after 8 days of training, most normal rats ran to the correct spatial location of the food, indicating spatial learning, but when tested again after 8 more days of training the same rats made the turn that had been reinforced during training, which led away from the food, indicating S-R based learning.

In other groups of rats either the hippocampus or the caudate nucleus was temporarily inactivated with lidocaine during the test trials. After 8 days of

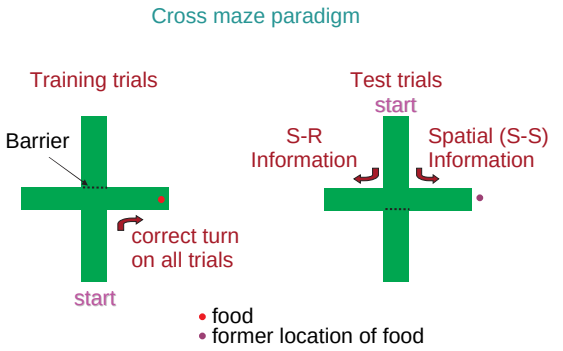


Figure 8 Use of the cross maze to distinguish between responding based on S-S and S-R information. The apparatus is a maze built in the shape of a cross. It is used with a barrier that makes it function as a T-maze. As illustrated on the left, during the training trials rats are placed into the start arm and allowed to explore until they find the food, which is available on every trial. This requires a right turn. After a number of training trials a test trial is given. As shown on the right, the barrier is moved and the rat is placed into the maze at the opposite end of the start arm. The direction of the turn the rat chooses to make on this trial indicates what kind of information was acquired during the training trials. A right turn (the response made during training), which leads away from the food location, indicates behavior controlled by an S-R association – a specific response to the choice point stimulus. A left turn, which leads toward the food location, indicates behavior controlled by an S-S association – spatial information about the location of the food.

training, the normal tendency to make the spatial response was eliminated by hippocampal inactivation, but caudate nucleus inactivation had no effect (**Figure 9**). This is consistent with other findings showing that processing spatial information requires a functional hippocampus but not a functional caudate nucleus.

After 16 days of training, the normal tendency to make the S-R response was eliminated by caudate nucleus inactivation, but hippocampal inactivation had no effect (**Figure 9**). These observations are consistent with others showing that processing S-R information requires a functional caudate nucleus but not a functional hippocampus. Taken together with the findings for 8 training days, they constitute a double dissociation of S-S and S-R information processing with respect to the hippocampus and caudate nucleus, respectively.

Perhaps the most interesting observation in this study focuses on what the rats did when an inactivation treatment impaired their normal behavior. After 8 days hippocampal inactivation resulted in a random choice of arms. This shows that no other information capable of influencing behavior had been acquired at that time.

After 16 days rats that underwent caudate inactivation made the spatial response. This shows that the hippocampus-based spatial information was not eliminated by the additional training that resulted in acquisition of caudate-based S-R information. The spatial information was still present and able to influence behavior when the caudate was inactivated.

The hippocampus system apparently acquired a coherent spatial representation of the location of the food in fewer trials than the caudate system required to acquire a coherent representation of the S-R association. Therefore, behavior was controlled by the hippocampal system after 8 days of training. When additional trials had strengthened the S-R representation sufficiently, the behavioral output of the caudate system assumed control of the rats' behavior. Inactivation of the caudate system allowed the output of the hippocampus system to reassume control. This shows that the behavioral outputs of the systems were in competition with each other.

These findings illustrate several important points. First, they show that S-R memory is inflexible, producing the same response to a stimulus regardless of conditions. In contrast, the S-S system is flexible, using sensory information at the choice point to produce an appropriate response. Second, they reveal a difference in the learning rate parameter: A coherent hippocampus-based representation of the spatial environment was acquired in fewer trials than are required for the formation of a coherent caudate-based representation of a reinforced S-R association. Finally, the pattern of effects shows that the outputs of the systems compete with each other for the control of behavior.

3.02.5.1.2.(ii) Dissociations in measures of neural function Using cross-maze training parameters that produced approximately equal numbers of rats that made the spatial and S-R responses on the test trial, Columbo et al. (2003) measured phosphorylated response element binding protein (pCREB) in the hippocampus and caudate nucleus 1 h after testing (as explained elsewhere, CREB has been linked to the formation of long-term memories). Increased levels of pCREB expression were found in the hippocampus but not the caudate nucleus of the rats that made the spatial response, and in the caudate but not the hippocampus of rats that made the S-R response. This double dissociation is consistent with the findings in the inactivation experiment.

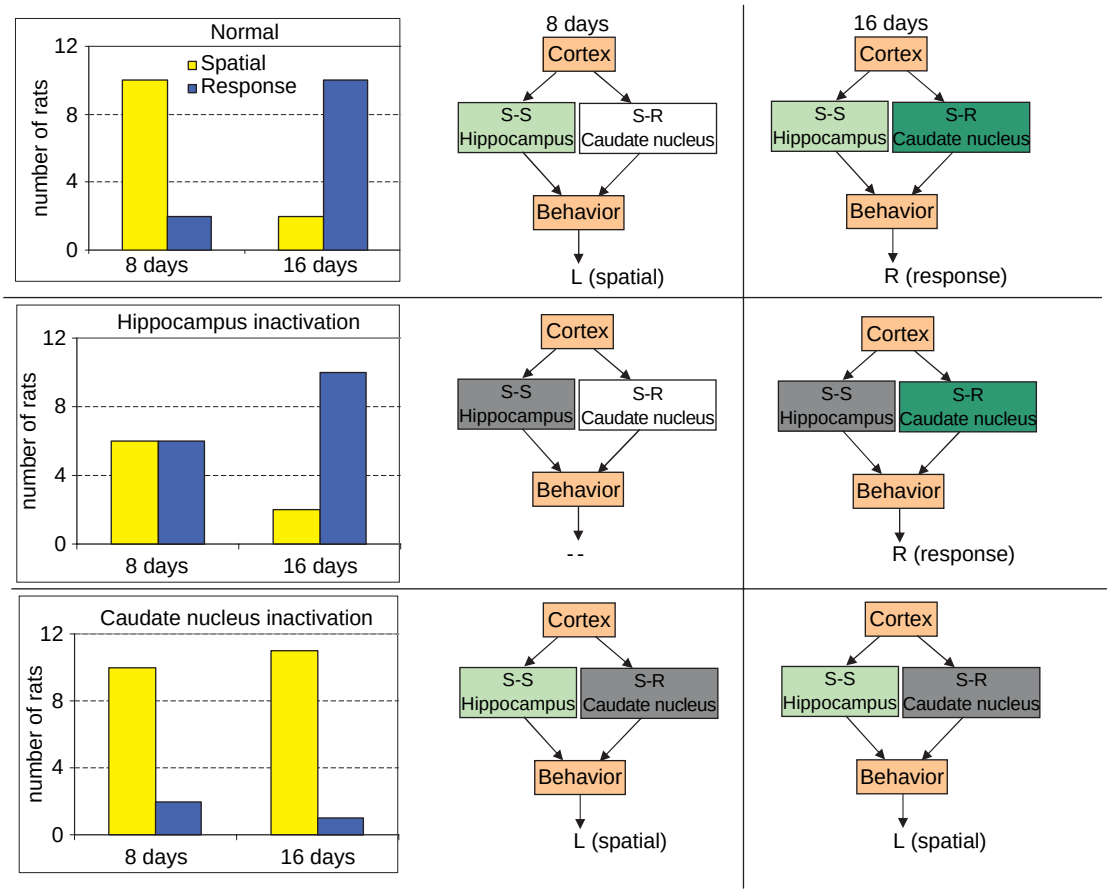


Figure 9 Dissociation and competition on the cross maze. *Top row:* normal rats. The graph shows the number of rats that turned in the direction of the food, indicating the use of spatial information, or away from the food, indicating use of S-R information on the test trials given after 8 or 16 days of training. After 8 days most rats turned toward the food as a result of hippocampus-based processing of spatial information (light green). At this time insufficient caudate-based S-R learning had occurred to influence the response (white). After 16 training days, S-R learning had become strong enough for the output of the caudate system (dark green) to compete with the hippocampus-based response and take control over the rats' behavior, causing them to make the response acquired during training, leading away from the food. *Middle row:* inactivation of the hippocampus during the test trials. After 8 days, hippocampal inactivation (grey) produced random responding, as predicted by the hypothesis that turning toward the food was the result of hippocampus-based spatial information processing. After 16 days, hippocampal inactivation (grey) did not affect the tendency to turn away from the food, consistent with the hypothesis that the hippocampus was not involved in this S-R type behavior. *Bottom row:* inactivation of the caudate nucleus during the test trials. After 8 days, caudate inactivation (grey) had no effect, as predicted by the idea that this structure was not involved in processing the spatial information that produced the turn toward the food. After 16 days, caudate inactivation (grey) resulted in turning toward the food, reversing the behavior of normal rats, which turned away from the food at this point (top row). This is predicted by the hypothesis that the caudate nucleus mediated the S-R information that produced the turn away from the food in the normal rats. It also shows that the hippocampus-based spatial information was still present and able to influence behavior, but that it was prevented from doing so in normal rats by competition from the output of the caudate system. Eliminating the caudate output allowed the spatial information to resume its influence, allowing the rats to turn toward the food. Data from Packard MG and McGaugh JL (1996) Inactivation of hippocampus or caudate nucleus with lidocaine differentially affects expression of place and response learning. *Neurobiol. Learn. Mem.* 65: 66–72.

Increased release of acetylcholine (ACh) is observed in certain brain structures while learning occurs (Ragozzino et al., 1996). Chang and Gold (2003) measured ACh release in the hippocampus and caudate nucleus while rats were being trained and tested on the cross maze. ACh release in the hippocampus increased early in training and remained high throughout, even after the rats had switched from spatial to S-R behavior. This is consistent with the finding in the inactivation

experiment that the hippocampal system continues to process information even when it is no longer reflected in behavior. ACh release in the caudoputamen remained low at first, increasing with repeated trials and reaching its peak in individual rats at the same time as they switched from spatial to S-R behavior. These observations are consistent with those from the inactivation experiment showing that the hippocampal system acquires a coherent spatial representation quickly, but that its influence on behavior is eliminated by competitive behaviors originating from the caudate system that acquires a coherent S-R representation more slowly.

3.02.5.1.3 Water maze

In this task (Morris, 1981) rats are placed into a large pool of opaque water and allowed to swim in search of a small platform submerged just below the surface so that no local cues, including visual cues, identify its location. The rats can escape from the water by climbing onto the platform. Rats learn to swim directly to the platform location regardless of their starting position in the pool, showing that their behavior does not depend on any specific response or sequence of responses, but on S-S information about its spatial location. If the platform is moved the rats have to relearn its location. This task is usually contrasted with learning to swim to a platform that protrudes just above the surface of the water so that it is visible to the rats. They also learn to swim directly to this platform, but this behavior is not affected when the platform is moved, showing that the rats are responding to a local cue, which could be a result of S-R memory. Morris and coworkers (1982) reported that lesions of the hippocampus impaired learning to swim to a hidden, but not to a visible, platform.

Using a slightly different version of this task Packard and McGaugh (1992) found that learning to discriminate between two identical stimuli on the basis of their spatial locations in a pool was impaired by fimbria-fornix but not by caudate nucleus lesions. Learning to discriminate between two different stimuli that were moved to new locations on each trial was impaired by caudate nucleus but not by fimbria-fornix lesions. This double dissociation between a task requiring spatial information and one that could be performed on the basis of an S-R association is further evidence for the differences in the kinds of information processed in the neural systems that include the hippocampus and the caudate nucleus.

3.02.5.1.3.(i) Competition in the water maze

McDonald and White (1994) trained rats to find a platform that remained in a constant location in the pool. It was visible on some trials and hidden on others (see **Figure 10**). Normal rats learned to swim to the platform in both conditions. As previously shown, rats with fimbria-fornix lesions swam to the platform normally when it was visible but were severely impaired at finding it when it was hidden because of their inability to process spatial information. Rats with caudate nucleus lesions learned to swim to the hidden platform normally, suggesting normal spatial information processing. Although S-R information processing may have been impaired in these rats, no deficit in their ability to locate the visible platform would be expected because the platform was in the same location on all trials. The spatial information that allowed the caudate-lesioned rats to locate the platform when it was hidden could serve the same purpose when the platform was visible.

To examine the possibility that S-R information processing was impaired in the caudate-lesioned rats, a final test trial was given. The platform was moved to a new location, where it was visible (see **Figure 10**). As shown in **Figure 11**, most of the rats with caudate lesions swam directly to the former location of the platform. When they failed to find it, they swam to the new location, where the platform was visible. This behavior suggests that these rats were impaired at processing S-R information, allowing the competing spatial information to control behavior. In contrast, all of the rats with fimbria-fornix lesions swam directly to the visible platform. This is consistent with an impairment in spatial (S-S) information processing, which allowed acquired S-R information to control behavior. The fact that these shifts in behavior were produced by lesions of fimbria-fornix and caudate nucleus is consistent with the hypothesis that these structures are critical for S-S and S-R information processing, respectively.

Figure 11 also shows that half of the rats in the normal control group swam directly to the visible platform, while the other half swam to its former location first. The behavior of these two subgroups of control rats on the hidden platform trials during training is shown in **Figure 10**. The rats whose behavior was controlled by spatial information on the final trial initially learned the location of the hidden platform much faster than the rats whose behavior was controlled by S-R information

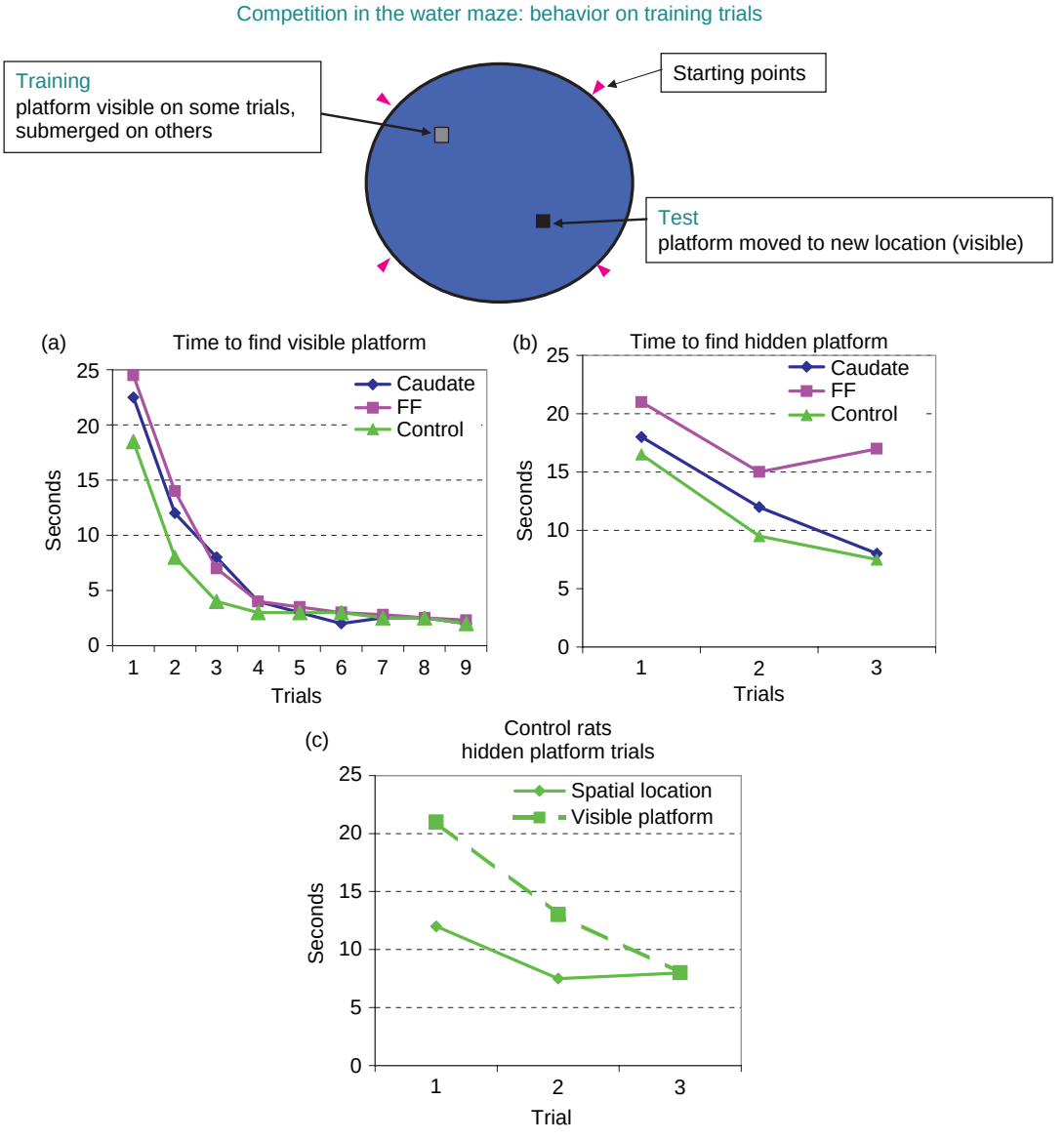


Figure 10 Competition in the water maze: training trials. The blue circle at the top of the figure represents a circular swimming pool about 1.5 m in diameter. Rats were placed into the pool once at each of the four starting points on each training trial. The platform was always in the same location; it was visible on some trials and submerged (invisible) on others. (a) Average time taken to swim to the platform over nine trials when it was visible. (b) Average time to locate the platform during three trials when it was hidden. Hidden platform trials were given after three, five, and nine visible platform trials. Note impairment in fimbria-fornix lesion group on the hidden but not the visible trials. (c) Two subgroups of the control group, which differed in the rate at which they learned to locate the hidden platform. The significance of the difference between these two groups is explained in **Figure 11** and in the text. Data from McDonald RJ and White NM (1994) Parallel information processing in the water maze: Evidence for independent memory systems involving dorsal striatum and hippocampus. *Behav. Neural Biol.* 61: 260–270.

on the final trial. This difference suggests the possibility of constitutional individual differences in the relative learning rates of the hippocampus and caudate systems (see also Colombo and Gallagher, 1998).

3.02.5.1.3.(ii) Involvement of synaptic functions Packard and Teather (1997) found that injections of AP5, an *N*-methyl-D-aspartate (NMDA) receptor antagonist (as described elsewhere, NMDA receptors have been implicated in the synaptic

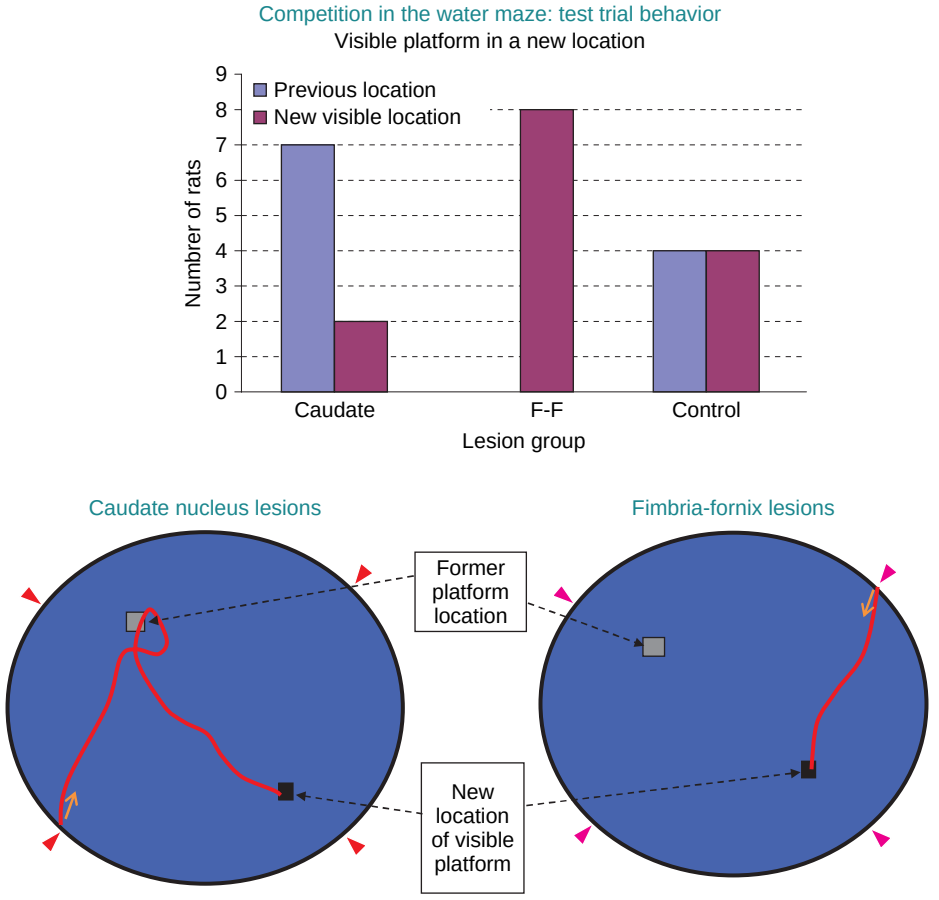


Figure 11 Competition in the water maze: test trial. On the test trial the rats were placed into the pool with the visible platform in a new location, so that spatial learning about the location of the platform competed with the S-R tendency to swim to the visible stimulus. The graph at the top shows that 7/9 rats with caudate nucleus lesions swam to the old spatial location of the platform first; when they failed to find it there, they swam to the visible platform. This is illustrated by the swim path (red line) of a rat with caudate nucleus lesions shown at the bottom left. In the presence of a visible platform, this behavior suggests impaired processing of S-R information, allowing spatial information to control the behavior of most rats in this group. In contrast, as shown in the graph and illustrated on the bottom right, 8/8 rats with fimbria-fornix lesions swam directly to the visible platform. This suggests impaired processing of spatial information, allowing S-R information to control the behavior of these rats. In the control group, four rats swam directly to the visible platform and four swam to its former location first. The behavior of these two subgroups of control rats during the initial hidden platform training trials is shown in **Figure 10**. Data from McDonald RJ and White NM (1994) Parallel information processing in the water maze: Evidence for independent memory systems involving dorsal striatum and hippocampus. *Behav. Neural Biol.* 61: 260–270.

events involved in memory storage), into the hippocampus impaired learning to find a hidden but not a visible platform, whereas injections into the caudate nucleus impaired learning to swim to a visible, but not a hidden, platform. These findings suggest the possibility that synaptic changes involved in the storage of S-S and S-R information may occur in the hippocampus and caudate nucleus, respectively.

In another demonstration Teather et al. (2005) counted cells expressing c-Fos and c-Jun (immediate early genes thought to be expressed in the presence of neural activity and synaptic changes involved in memory) after water maze training. In the hippocampus more cells expressing these products were counted following hidden platform training than visible platform training; in the caudate nucleus more expressive cells were found following visible than hidden platform training. These increases were also observed relative to control rats not trained on either task. A similar dissociation has recently been made using cytochrome oxydase as a measure of metabolic

activity in the hippocampus and caudate nucleus while rats learned to locate a platform using spatial or local cues (Miranda et al., 2006). Aside from replicating the dissociation between the behavioral tasks and the brain areas with different measures of brain function, these observations again suggest the possibility of synaptic activity related to memory storage in the appropriate brain areas when either S-S or S-R information is being processed.

3.02.5.1.4 Medial versus lateral caudate nucleus

The caudate nucleus has been described as part of the system that processes S-R information, but evidence shows that in rats, where the structure is often called the caudoputamen, this function is actually confined to its dorsolateral part (White, 1989a; Yin et al., 2004; Featherstone and McDonald, 2004a,b, 2005a,b). This portion of the caudoputamen in the rat may be homologous with the caudate nucleus in primates (Heimer et al., 1985).

There is also evidence that the dorsomedial part of the caudoputamen in the rat processes S-S information (possibly as it interacts with the dorsolateral caudoputamen in the selection of responses – see section 3.02.3.3). One set of experiments suggesting this possibility shows that fimbria-fornix and dorsomedial caudoputamen lesions have similar effects in the water maze (Devan et al., 1996, 1999). Holahan et al. (2005) found that blocking NMDA receptors in the dorsal hippocampus and dorsomedial caudoputamen had similar effects on the long-term retention of spatial information. Yin and Knowlton (2004) showed that lesions of the posterior dorsomedial caudoputamen, but not of the anterior or dorsolateral parts of the structure, facilitated reinforced S-R responding on the cross maze, an elimination of the competition effect similar to that produced by inactivation or lesions of the hippocampus system. These findings suggest that the dorsomedial caudoputamen in the rat might be considered part of the S-S information processing hippocampal system.

3.02.5.2 Studies with Humans

Evidence from studies with humans on the dissociation of hippocampus-based declarative (S-S) learning from caudate nucleus-based procedural (or S-R) learning parallel the rat findings quite closely (*See* Chapters 3.17, 3.18; Poldrack and Packard, 2003; Hartley and Burgess, 2005). A selection of this evidence is reviewed here.

3.02.5.2.1 Spatial learning

Iaria et al. (2003) created a virtual eight-arm radial maze on a computer screen and tested normal participants on the win-shift task originally developed for rats. A trial started with the participants at the center of the maze, from where they could look down each of the eight arms and see a virtual landscape of mountains, trees, rocks, and the sun surrounding the maze. They could freely control their movements through the maze with the computer keyboard. Goal objects were not visible from the platform but could be obtained by descending a virtual flight of stairs at the end of each arm. Participants were instructed to obtain all of these objects by entering as few arms as possible. Reentries were scored as errors. Some of the experiments were conducted with the subjects in an fMRI scanner, which is thought to provide indirect information about the relative levels of neural activity in different parts of the brain.

The performance of all participants improved over a series of trials. When asked how they had solved the task, they spontaneously sorted themselves into three groups. One group reported they had used extramaze landmarks, such as proximity to the sun and a tree for one arm and to the sun and a rock for the adjacent arm, to identify and avoid reentering arms. This constitutes a spatial strategy requiring the processing of relational S-S information. Participants using this strategy showed significantly increased activation in the hippocampus. Another group reported using a nonspatial strategy: counting arms in a constant direction from the first arm they saw or from a single landmark. Analysis of this behavior showed that it involved a consistent series of turns to enter each of the correct arms in order around the maze. As the participant returned to the platform from each arm (stimulus), the correct turn (response) was elicited, leading to the retrieval of a goal object (reinforcement). Accordingly, Iaria et al. (2003) concluded that this behavior was produced by processing S-R information. Participants using this strategy showed significantly increased activation in the caudate nucleus.

A third group of participants said they started with the spatial strategy but switched to a nonspatial strategy at some point during the session. Each of these participants started by exhibiting significantly more

activation in the hippocampus than in the caudate nucleus, but this relationship was reversed as training progressed and the strategy switch occurred. Throughout the experiment, participants who used the spatial strategy made more errors than those who used a nonspatial strategy. Errors for the group that switched strategies decreased as soon as they switched.

These findings show that two different kinds of information are processed in this task: hippocampus-based spatial (S-S) information and caudate-based nonspatial (S-R) information. This dissociation is similar to the one observed in rats. The findings for the group that switched strategies also corresponds to rat evidence showing that increased training produces a shift from behavior controlled by S-S to behavior controlled by S-R information, processed in the hippocampus and the caudate nucleus, respectively. The basis of the shift may be a difference in the rates at which the two systems acquire coherent representations of the information they process.

Findings similar to those of Iaria et al. (2003) about the relationship between hippocampal and caudate nucleus activation in humans measured in more naturalistic virtual environments have also been reported by Maguire and coworkers (Maguire et al., 1998; Hartley et al., 2003; Hartley and Burgess, 2005).

3.02.5.2.2 Probabilistic classification

In the probabilistic classification task, also known as the weather forecasting task (Knowlton et al., 1994, 1996), participants are presented with a set of distinct cues selected from a large group and asked whether the set predicts rain or shine. After they respond by pressing one of two keys on a keyboard, they are told that their response was either correct or incorrect (feedback). The feedback provided for a given response to each cue or combination of cues within a set is probabilistic. This means that consistent feedback is given on most trials, but on some trials the opposite feedback is given. Furthermore, the specific mapping between the cues and the feedback is quite complex. Although it is possible to perform the task using declarative information about the feedback given most often, this requires a large number of trials because of the probabilistic feedback and the complexity of the mapping. However, when the probability of consistent feedback for a given response to a set of cues is sufficiently high, and when the stimulus and the reinforced response have been repeated sufficiently often, acquisition of an S-R association that produces the correct response can occur.

Knowlton et al. (1994) found that a group of normal participants and a group of amnesic patients (with impaired hippocampal function) learned this task at the same rate until both groups attained a moderate level of performance (70% correct). This suggests that within this range, performance is not based on information processed by the hippocampal system. However, the amnesic patients did not improve beyond the 70% level, whereas the normal participants continued to improve, possibly because they acquired some declarative knowledge of the mapping between the cues and outcomes (*See* Chapter 3.04). This finding was subsequently replicated in a study that also reported that Parkinson's patients (with impaired caudate nucleus function) were unable to learn the weather forecasting task at all (Knowlton et al., 1996). When given a series of questions about the conditions of the experiment (declarative memory), the amnesiacs were unable to answer most of the questions, whereas the Parkinson's patients performed normally. These findings constitute a double dissociation between hippocampus and caudate nucleus function with respect to declarative memory for the experimental situation and probabilistic classification (S-R) learning, respectively.

Poldrack and coworkers (1999) studied the brain areas involved in probabilistic classification in normal participants using fMRI. During the early trials, increased activation was seen in the right caudate nucleus (and in the frontal and occipital cortex). Activity in the left hippocampus was suppressed. However, the hippocampus became more active as performance continued to improve and reached levels unattainable by amnesic patients in the Knowlton et al. (1994) study. These findings coincide with the neuropsychological data on the probabilistic classification task (see also Shohamy et al., 2004) and with the general distinction between the processing of procedural or S-R information by a neural system that includes the caudate nucleus, and the processing of declarative, or S-S, information by a hippocampus system.

These findings were replicated and extended by comparing two different versions of the weather forecasting task (Poldrack et al., 2001). One was the standard probabilistic classification task, in which participants are shown the stimulus, make a response, and receive feedback. As already shown, initial acquisition of this task appears to depend on information processed by the caudate system. In a new task the participants were shown the same sets of cues

together with the correct response for each set. In this paired associates version of the task, the participants did not make responses or receive feedback. Instead, they acquired information about the relationships among the stimuli and the weather they predicted. This type of task is known to depend on medial temporal lobe function (Warrington and Weiskrantz, 1982; Marchand et al., 2004). By the end of the training trials, the weather prediction performance of both groups was similar.

fMRI scanning showed that the reinforced S-R version of the task activated the caudate nucleus more than the hippocampus, whereas the paired associates version activated the hippocampus more than the caudate nucleus. Accordingly, these findings constitute a double dissociation of caudate-based processing of reinforced S-R information and hippocampus-based processing of S-S information consistent with other animal and human data described.

3.02.5.3 Summary: Competition and Coherence

As reviewed here and elsewhere (Poldrack and Packard, 2003), data from both animal and human studies strongly suggest that hippocampal function is implicated when the information required to solve a task can be characterized as consisting of S-S associations, and that caudate nucleus function is implicated when the task can be performed using information consisting of S-R associations. The evidence implicating these brain structures includes the effects of impaired function produced by trauma (including lesions made in the laboratory), disease processes, or chemical inactivation, and indices of increased functionality, including activation detected by fMRI, ACh release, and c-Fos expression.

Several instances of competition for control of behavior between the two systems in which these structures reside have been described. These findings are strong evidence for independent, parallel information processing functions in the systems.

The data from studies with normal animals and humans suggest that the kinds of information available in each learning situation – the task demands (Poldrack and Rodriguez, 2004) – determine which neural system controls behavior. This is consistent with the idea that the information processing specialization of each system determines the coherence of the representation it forms of a learning situation (see section 3.02.4.1.3). The system with the most coherent representation is the one that has the dominant effect on behavior. In the fMRI studies, increased hippocampal activation occurs when S-S information is being processed, and increased activation in the caudate nucleus occurs when S-R information is being processed. These relationships lead to the hypothesis that the level of activation revealed by the fMRI may be a reflection of the coherence of the representations of these information types in the systems.

In some studies an inverse relationship between activation of the hippocampus and caudate nucleus was observed, with depressed activation in the nondominant structure (Poldrack and Rodriguez, 2004). This may be a result of the relationships between the processing capacities of the structures and the information that activates them. Applying Sherry and Shachter's (1987) suggestion that these systems process incompatible information (see section 3.02.4.1) suggests the possibility that, when one of the two systems develops a coherent representation of a situation, the information available in that situation is incompatible with the specialized representational capacity of the other system. This may lead to an incoherent representation, reflected in decreased activation in the fMRI.

While not ruling out the possible existence of direct or indirect functional interactions, inhibitory or otherwise, between the hippocampus and caudate systems (Poldrack and Rodriguez, 2004), this suggestion shows that it is not necessary to postulate such direct influences to explain the data, which can be understood simply in terms of independent parallel systems that process incompatible information. According to this view, differences in the coherence of the representations formed in the systems determine which one wins the competition to control behavior.

3.02.6 S-S versus S-Rf Information Processing

The triple dissociation experiment (**Figure 4**) included a double dissociation between performance on the win-shift (impaired by fimbria-fornix lesions) and CCP (impaired by amygala lesions) tasks. This was interpreted as a double dissociation between the neural systems that process S-S and S-Rf information. Other studies with rats and humans support this interpretation.

3.02.6.1 Studies with Rats

3.02.6.1.1 CCP with spatial cues

During the CCP training trials in the triple dissociation experiment, the radial maze was surrounded by curtains, and the arms were differentiated by a light in one of them. Similar preferences are learned when the maze is open to the surrounding extramaze cues and the food-paired and no-food arms are on opposite sides of the maze (the separated arms CCP [sCCP]; see **Figure 12**). This preference is eliminated by amygdala but not by fimbria-fornix lesions (White and McDonald, 1993). When the two arms are adjacent to each other (the adjacent arms CCP [aCCP]) the preference is eliminated by hippocampal lesions, but not by amygdala lesions (Chai and White, 2004). This double dissociation is explained by differences in the nature of the extramaze cues visible from separated and adjacent maze arms.

In the sCCP situation, completely different sets of extramaze cues are visible from the ends of the two separated arms (**Figure 12**). The rats acquire a conditioned response to the environmental cues visible from the food-paired arm when they eat while confined on that arm during the training trials. They do not acquire a response to the cues visible from the unpaired arm while confined there, because no food is available. On the test day, the conditioned response causes the rats to approach and spend more time in the presence of the food-paired cues, resulting in a preference for that arm.

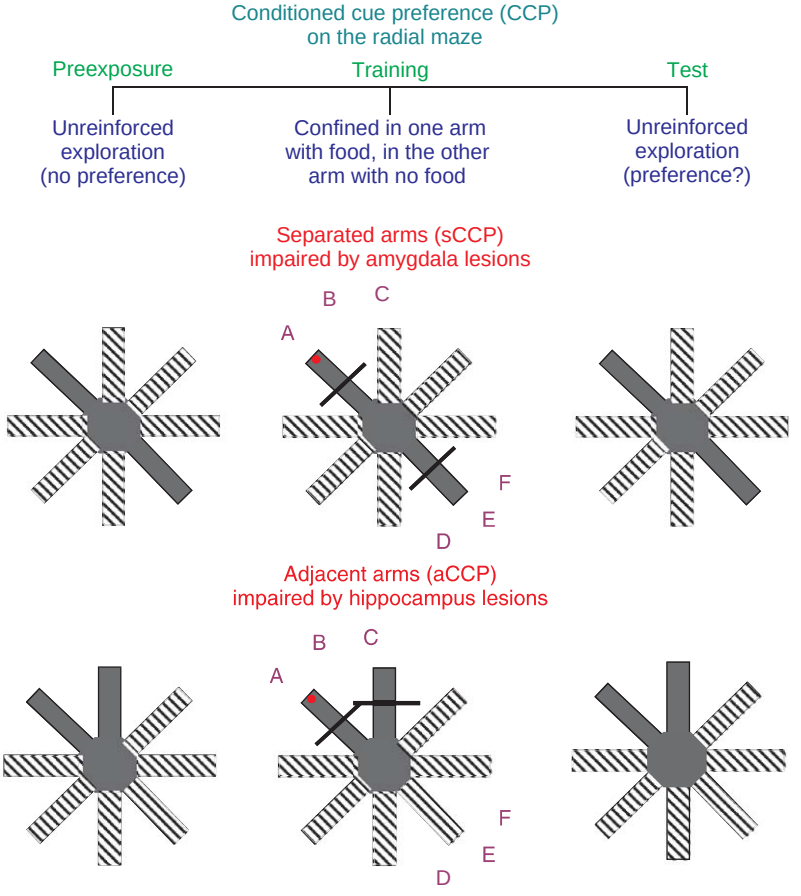


Figure 12 Conditioned cue preference with separate and adjacent radial maze arms. The three phases of the conditioned cue preference (CCP) paradigm (preexposure, training, and test) are shown at the top. The lower part of the figure shows the maze configurations for each phase in the separated and adjacent arms conditions. ABC and DEF are sets of environmental cues visible from the maze arms. In the separated arms CCP situation, completely different sets of cues are visible from the food-paired and no-food arms. During the training trials, a conditioned rewarding response is acquired, with ABC as the conditioned stimulus (CS). No response to DEF is learned. On the test trial, conditioned reward elicited by the CS causes the rat to enter and spend more time in the food-paired than in the unpaired arm. In the adjacent arms CCP situation, the same cues (ABC) are visible from both the food-paired and the unpaired arms. Discrimination between the two arms using these cues requires spatial learning.

This arm discrimination is produced by amygdala-based processing of S-Rf information.

In the aCCP situation, most of the same extramaze cues are visible from the ends of both arms (**Figure 12**). Discriminating between them requires learning about differences in the relationships of their locations to those cues. This is spatial learning, requiring hippocampus-based processing of S-S information. Therefore, the double dissociation between the effects of amygdala and hippocampus system lesions on the aCCP and sCCP tasks constitutes a dissociation between the processing of S-S and S-Rf information, respectively.

3.02.6.1.2 Cooperation and competition in adjacent arms CCP learning

Although the aCCP and sCCP paradigms differ in the relationship of the arms to be discriminated, all other aspects of the two procedures are identical. If a conditioned response to cues visible from the food-paired arm is acquired in the sCCP procedure, the same response should also be acquired in the aCCP procedure. However, since most of the same cues are visible from both arms in the aCCP procedure, this response should produce an equal tendency to enter both arms. This prediction has been confirmed (Chai and White, 2004).

The parallel occurrence of hippocampus-based spatial learning and amygdala-based S-Rf learning in the aCCP paradigm results in competition between the behavioral outputs of the two systems. Although a conditioned response to the food-paired cues is acquired during the training trials, spatial information about the layout of the maze cannot be acquired during these trials, because spatial learning in rats is severely attenuated when they are prevented from moving around in an environment (Sutherland, 1985; White and Ouellet, 1997; Terrazas et al., 2005). For this reason, rats do not learn the aCCP unless they are preexposed to the maze before the training trials (see **Figure 13**). During these sessions, the rats are allowed to move around freely on the maze with no food present. It has been known for some time that rats acquire spatial information during unreinforced exploration of a novel environment, a phenomenon called latent learning (Blodgett, 1929; Tolman and Honzik, 1930). As shown in **Figure 13**, normal rats require a minimum of three preexposure sessions to express the aCCP.

Interference with expression of this spatial information by the amygdala-based conditioned response is revealed by the finding that rats with amygdala lesions require only one preexposure session to learn the aCCP (**Figure 13**) (Chai and White, 2004). In normal rats, the amygdala-based tendency to enter both arms competes with the hippocampus-based tendency to discriminate between the arms. A minimum of three freely moving preexposures to the maze environment is required for the hippocampus system to acquire a sufficiently coherent representation of the spatial environment for its output to win the competition with the output from the amygdala system for control of behavior. When the competition is eliminated by amygdala lesions, a less coherent hippocampal representation (with, hence, less preexposure) is required to produce output that results in expression of the aCCP.

The competitive interaction between the amygdala and hippocampus systems in the aCCP task has been investigated in more detail (White and Gaskin, 2006). Both amygdala-based S-Rf learning and hippocampus-based S-S learning promote a tendency to enter the food-paired arm, a cooperative interaction. At the same time, the S-Rf learning promotes a tendency to enter the no-food arm, but this tendency is blocked by an interfering tendency resulting from S-S information about the lack of food in the arm, a competitive interaction. These cooperative and competing tendencies are illustrated in **Figure 13**.

3.02.6.1.3 Path integration versus visual cue conditioning

In addition to using visible spatial cues to navigate in space, rats also use internal cues based on proprioceptive stimuli generated by their movements for this purpose (Etienne et al., 1996; McNaughton et al., 1996). This form of information processing, called path integration, is hippocampus dependent (Smith, 1997; Whishaw et al., 1997, 2001; Whishaw, 1998). Ito et al. (2006) trained rats in an apparatus consisting of a triangular central platform with compartments attached to each of the three sides. Prior to each trial, the rats were exposed to a polarizing cue in the experimental room. The cue was removed before the trial started, so subsequent use of the orienting information it provided was a result of path integration. During the first phase, a flashing light together with a sucrose solution were presented in any of the three compartments, establishing the light as a conditioned cue. The rats acquired a tendency to approach the flashing light; rats with amygdala lesions required more trials than controls, but rats with hippocampal lesions required fewer trials than

controls to acquire this behavior. This suggests that the cue preference was a result of amygdala-based processing of S-Rf information and that information processed in the hippocampus of the normal rats interfered with the expression of this behavior. The interference could have been caused by the simultaneous acquisition of information about the location of the sucrose solution based on the polarizing cue. Because this information was irrelevant, its effect on behavior may have interfered with expression of the amygdala-based cue preference in the same way spatial information can interfere with win-stay performance, as described in **Figure 5**.

The rats were then given a series of trials in which flashing lights appeared in any of the three compartments, but sucrose delivery accompanied this cue only when it appeared in one of the compartments. The compartment in which sucrose was available remained constant on all trials. The rats were then given a compartment preference test in the absence of flashing lights and sucrose. The sham-operated rats and the rats with amygdala lesions showed a preference for the compartment in which they had received sucrose, but the rats with hippocampus lesions did not exhibit this preference. Because neither the conditioned cues nor visual spatial cues were available during this test, Ito et al. (2006) attributed the preference to information about the spatial location of the sucrose based on the polarizing cue, processed in the hippocampus system. This information would have been incidentally acquired during the cue-training procedure.

The results of the two tests taken together double dissociate the use of path integration based on the processing of spatial information derived from movement by the hippocampus and the processing of S-Rf information by the amygdala. The demonstration of interference with the expression of the amygdala-based information by hippocampus-based path integration is evidence for independent functioning of the two systems.

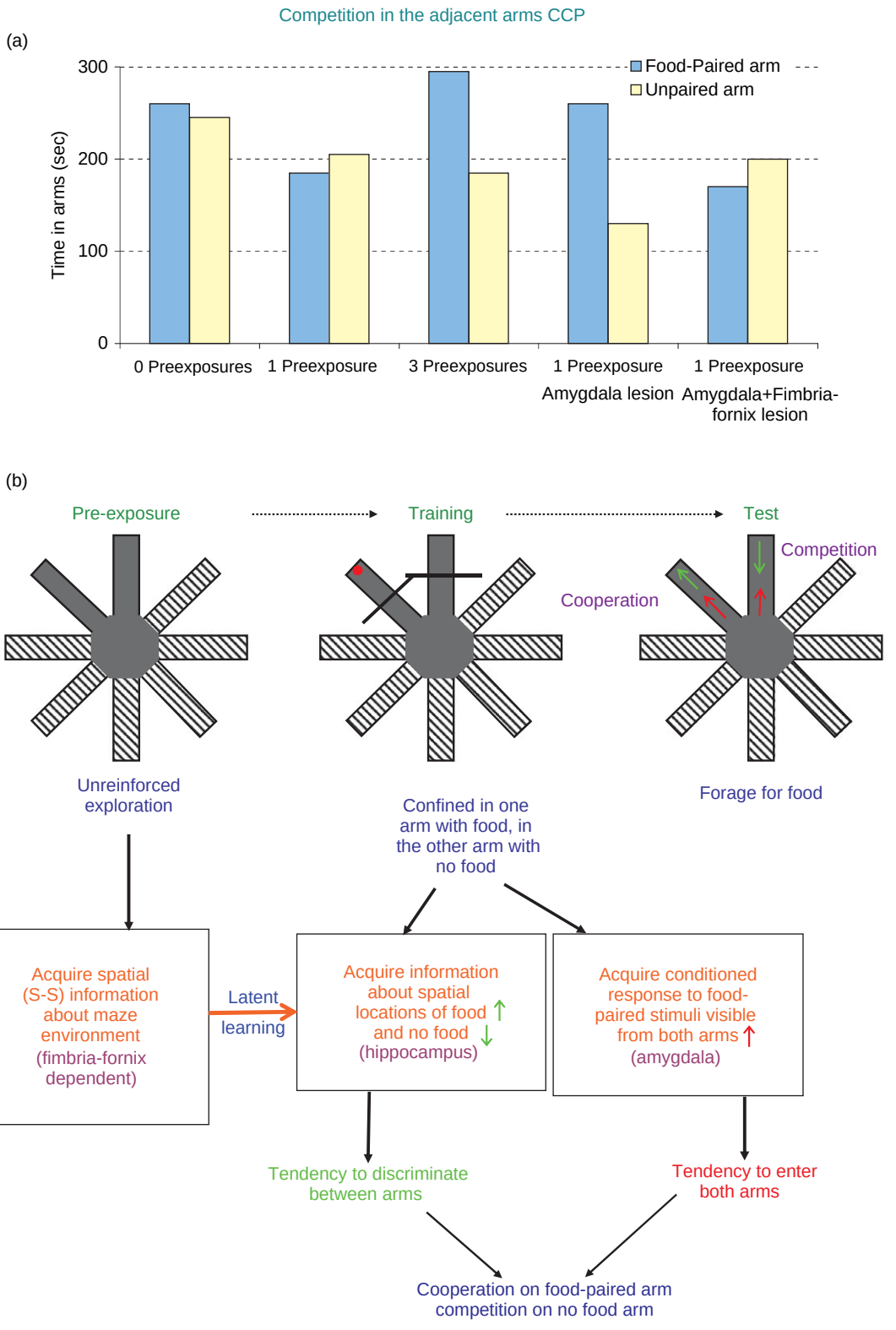
3.02.6.1.4 Fear conditioning

In these experiments, rats are placed into a test cage and given one or more foot shocks as the US. The shock evokes a constellation of URs including an aversive internal state (usually called fear) and skeletal responses, primarily consisting of jumping and running. Neutral stimuli become CSs with the capacity to elicit the internal state of fear without the skeletal responses. This conditioned internal state is thought to be reflected by freezing, a withdrawal response (LeDoux, 1993; Fanselow and Gale, 2003).

Two kinds of stimuli can serve as the CS. In context conditioning, the CS is the test cage itself (as well as the room in which it is located and every other feature of the situation). In cue conditioning, a discrete cue such as a tone or light immediately precedes the shock. The cue becomes a CS, but the context also becomes a CS in this situation (Phillips and LeDoux, 1992). Context conditioning requires a minimum amount of unreinforced preexposure to the context (Fanselow, 1990). This appears to be another instance of latent learning, as in the case of unreinforced preexposure in the appetitive aCCP situation.

Both lesions of the hippocampus (Kim and Fanselow, 1992; Phillips and LeDoux, 1992; Frankland et al., 1998) and lesions of the amygdala (Phillips and LeDoux, 1992; Gale et al., 2004) impair contextual fear conditioning, but only amygdala lesions impair conditioning to a discrete cue CS (Phillips and LeDoux, 1992; Gale et al., 2004). In multiple memory systems terms, the involvement of the amygdala but not the hippocampus when the CS is a discrete cue suggests that this form of fear conditioning is a result of amygdala-based processing of S-Rf information. When the CS is a context requiring preexposure for conditioning to occur, the involvement of the hippocampus suggests that conditioning requires hippocampus-based processing of S-S information. However, context conditioning also requires an intact amygdala, suggesting that S-Rf information is also required for context conditioning. These findings constitute a partial dissociation of S-S and S-Rf information processing by the hippocampus and amygdala in fear conditioning.

This pattern of effects differs from that for the relationship between hippocampus-based spatial learning with appetitive reinforcers and amygdala-based appetitive conditioned responding. However, the fear conditioning studies do not explicitly test multiple memory systems hypotheses, and the numerous differences in the experimental apparatus and procedures used in the two sets of studies make it difficult to conclude that the systems have different functions in appetitive and aversive learning. Examination of this issue requires experiments that directly compare appetitive and aversive learning in the same experimental conditions.


Competition in the adjacent arms CCP
(a)
Food-Paired arm
Unpaired arm
Time in arms (sec)
300
200
100
0
0 Preexposures
1 Preexposure
3 Preexposures
1 Preexposure
Amygdala lesion
1 Preexposure
Amygdala+Fimbria-fornix lesion
(b)
Pre-exposure
Training
Test
Competition
Cooperation
Unreinforced exploration
Confined in one arm with food, in the other arm with no food
Forage for food
Acquire spatial (S-S) information about maze environment (fimbria-fornix dependent)
Latent learning
Acquire information about spatial locations of food ↑ and no food ↓ (hippocampus)
Acquire conditioned response to food-paired stimuli visible from both arms ↑ (amygdala)
Tendency to discriminate between arms
Tendency to enter both arms
Cooperation on food-paired arm competition on no food arm

3.02.6.1.5 Skeletal conditioning

The Pavlovian conditioning paradigm has also been used to study at least two overt discrete skeletal responses directly elicited by a US: eye blink (Thompson and Krupa, 1994; Thompson, 2005) and leg flexion (Maschke et al., 2002; Dimitrova et al., 2003, 2004). Only the former will be discussed here. In eye-blink conditioning, a puff of air to the eye or a mild orbital shock (US) elicits a blink (UR). This response is similar to other URs because it is elicited by a reinforcer without previous experience.

In the delay conditioning procedure, the CS is presented first, the US follows after a brief delay, and the two stimuli terminate simultaneously. Using this procedure, eye-blink conditioning in rabbits and cats is impaired by lesions of specific parts of the cerebellum – cortex or nucleus interpositus (Skelton, 1988; Thompson and Krupa, 1994) – but not by lesions of the hippocampus (Christian and Thompson, 2003; Thompson, 2005). These findings suggest that S-Rf information involving a skeletal response (eye blink) is processed in the cerebellum.

In a slightly different procedure, the CS is presented and terminates. After an interval of 0.5–1.0 s, the US is presented. This is called trace conditioning because it is thought that a trace (or representation) of the CS is maintained during the CS-US interval, resulting in the formation of an S-S association between the trace and the US. Trace conditioning is impaired by lesions of the nucleus interpositus of the cerebellum in rabbits (Woodruff-Pak et al., 1985) and by lesions of the hippocampus in rats (Moyer et al., 1990; Thompson and Kim, 1996; Clark et al., 2002). These findings suggest that, in this paradigm, the cerebellum processes S-Rf information only, and the hippocampus processes both S-Rf and S-S information, a partial dissociation. This pattern corresponds to that for aversive fear conditioning with a contextual CS, but not to appetitive CCP learning.

3.02.6.2 Experiments with Humans

3.02.6.2.1 Conditioned preference

In an adaptation of the rat CCP task for humans (Johnsrude et al., 1999), normal participants were told that one of three black boxes on a computer screen contained a red ball. Touching a box caused it to open. If the box contained the red ball, the participant received a reward (a raisin or small candy). If the box contained a black ball, a mildly aversive tone sounded. The participants were instructed to count the number of times the red ball appeared in each of the three black boxes. In addition to a ball, each opened box also revealed one of six abstract designs in the background. All designs were presented an equal number of times. Two of them were paired with reward on 90% of the trials on which they appeared, two were paired on 50% of

Figure 13 Competition in the adjacent arms conditioned cue preference (aCCP) task. (a) Learning the aCCP. The top part of the figure shows the effects of preexposure on the separated arms CCP (sCCP) learning. The bars show the mean amounts of time the rats chose to spend in the maze arms during the 20-min test trial with no food present. Rats that received fewer than three sessions of unreinforced preexposure to the maze (see text for explanation) failed to learn the aCCP, but rats that received three sessions of unreinforced preexposure to the maze spent more time in their food-paired than in their unpaired arm, a conditioned cue preference. This suggests that in normal rats, three preexposure sessions permit the acquisition of sufficient spatial information to express an aCCP when combined with information about the locations of food and the absence of food acquired during the training trials. These hippocampus-based behaviors are shown as green arrows on the maze illustrating the test trial in (b). (b) Amygdala-based competition. As shown in (a), rats with amygdala lesions expressed a CCP after only one session of unreinforced preexposure to the maze (compared to a minimum of three for a normal rat), suggesting that amygdala-processed information competed with the hippocampus-based arm discrimination. As explained in the text, an amygdala-based conditioned response to ambiguous cues visible from both arms, acquired during the training trials, results in an equal tendency to enter both arms (red arrows). This results in cooperation between the two systems on the food-paired arm. Amygdala lesions eliminate the amygala-based tendency to enter the food-paired arm, but this does not eliminate the preference, because the hippocampus produces the same tendency. The amygdala-based tendency to enter the no-food arm competes with the hippocampus-based tendency to enter the food-paired arm instead of the no-food arm. Amygdala lesions eliminate the competition, allowing hippocampus-based spatial information to produce the CCP after only a single preexposure session. This is a case of amygdala-based processing of S-Rf information competing with a spatial discrimination resulting from hippocampus-based processing of S-S (spatial) information. Adapted from Chai S-C and White NM (2004) Effects of fimbria-fornix, hippocampus and amygdala lesions on discrimination between proximal locations. *Behav. Neurosci.* 118: 770–784; Gaskin S and White NM (2006) Cooperation and competition between the dorsal hippocampus and lateral amygdala in spatial discrimination learning. *Hippocampus* 16: 577–585.

the trials, and two on 10% of the trials. These designs were not mentioned in the instructions.

After the training trials participants were asked to say how many times the red ball had appeared in each box. All did very well on this task. They were then shown the abstract designs in randomly selected pairs with no balls present and asked to indicate which one they preferred. The 90% patterns were chosen significantly more often than the 10% patterns, a conditioned preference. In the final phase of the experiment the participants were shown their preferred patterns and asked why they preferred them. All participants attributed their preferences to properties of the patterns themselves; none mentioned any relationship between the patterns and reward. This was interpreted to mean that they had acquired a conditioned preference for the reward-paired patterns that did not depend on cognitive or declarative information.

Two groups of surgical patients were tested on this task (Johnsrude et al., 2000). Patients with unilateral resections of the amygdala did not acquire the preference for the reward-paired patterns, but performed accurately on the ball counting task. In contrast, patients with frontal lobe resections acquired normal pattern preferences but were severely impaired on ball counting. This double dissociation between amygdala-based processing of S-Rf information and S-S information requiring an intact frontal cortex corresponds to the dissociations between the processing of these same two kinds of information involving amygdala and the hippocampal systems in the rat.

3.02.6.2.2 Conditioned fear

Bechara and coworkers (1995) compared three patients, one with bilateral damage restricted to amygdala as a result of Urbach-Weithe disease, one with hippocampal lesions caused by anoxia secondary to cardiac arrests, and one with damage to both structures resulting from herpes simplex encephalitis. The patients were tested on a Pavlovian conditioning task in which the US was a loud horn and the UR was the electrodermal response produced by the startling noise. A series of color slides was shown during conditioning; one color coincided with the presentation of the US, making this discrete cue the CS. Shortly after testing on this task, the subjects were asked a series of questions about the experimental situation as a test of their declarative memory.

The patient with impaired amygdala function failed to acquire a conditioned electrodermal response but had excellent recall of the experimental situation. The patient with impaired hippocampal function acquired the conditioned electrodermal response but had poor memory for details of the experimental situation. The patient with damage to both areas performed poorly on both tasks. These findings dissociate amygdala-mediated processing of S-Rf information from hippocampus-mediated processing of S-S information in humans. This double dissociation corresponds to the dissociation found for appetitive spatial learning in rats and to the data for aversive conditioning in rats with a discrete cue CS.

3.02.6.2.3 Skeletal responses

As is the case for rats, in humans, eye-blink conditioning with the delay paradigm is impaired by lesions of the cerebellum (Daum et al., 1993), but not by lesions of the hippocampus (Daum et al., 1991; Gabrieli et al., 1995), and trace conditioning is impaired by lesions of the cerebellum (Gerwig et al., 2006) and the hippocampus (Thompson and Kim, 1996; Clark et al., 2002). This partial dissociation coincides with the data from studies of eye-blink conditioning in the rat.

A study measuring the magnetic flux of specific brain areas as an index of neural activity (magnetoencephalography) in humans (Kirsch et al., 2003) found that delay conditioning evoked only activation in the cerebellum, and that trace conditioning evoked only activation in the hippocampus. This suggests a more complete dissociation of processing the information types involved in trace and delay conditioning than the lesion data.

3.02.6.3 Summary

Several studies, in both rats and humans, consistent with the dissociation of hippocampus-based S-S and amygdala-based S-Rf information processing have been reviewed. These are fewer in number and are usually more difficult to demonstrate than dissociations between S-S and S-R processing. Furthermore, the available data on aversive conditioning in rats are not consistent with the notion of independent parallel processing of the information types required for contextual fear conditioning or for trace conditioning of the eye-blink response. Further experiments are required to determine whether these apparent differences in the relationships among the systems during appetitive and aversive conditioning are real.

3.02.7 S-Rf versus S-R Information Processing

In the triple dissociation experiment, the CCP task was impaired by lesions of the amygdala but not by lesions of the caudate nucleus; the win-stay task was impaired by lesions of the caudate nucleus but not by lesions of the amygdala. This double dissociation of S-Rf and S-R information processing has been replicated and extended.

Although S-Rf and S-R information both consist of stimulus-response relationships, these differ in important ways. Caudate-mediated S-R information comprises associations between representations of any response an individual can make and representations of contemporaneous stimuli. If the creation of these two representations is temporally contiguous with the occurrence of a reinforcer, the association between them is strengthened, but the reinforcer is not part of the information that constitutes the memory. Amygdala-mediated S-Rf information is limited to associations between responses (URs) that are elicited by reinforcers (USs), which become associated with contemporaneous stimuli (CSs). The responses are unrelated to any spontaneous behavior that may occur in the learning situation and, as already described, are largely unobservable internal changes in autonomic and hormonal function. The behavioral influence of these conditioned responses is often indirect. Observing their existence often requires some form of additional instrumental learning (Dickinson and Dawson, 1989; Dickinson, 1994; Dickinson and Balleine, 1994; Everitt et al., 2001).

In this section, only a single experiment dissociating amygdala-based S-Rf and caudate-based S-R information processing is described. The author is unaware of any other direct dissociations between these two systems with animals or of any parallel experiments with humans.

3.02.7.1 Win-Stay and CCP Learning

The S-R and S-Rf systems have been dissociated with a simultaneous comparison of the win-stay and CCP radial maze tasks. McDonald and Hong (2004) trained rats with lesions of the dorsolateral caudate nucleus or amygdala on the win-stay task (see **Figure 14**). After reaching a performance criterion, all rats were tested for CCP learning in the same apparatus. The rats were placed on the maze for 20 min with all arms open and no food present. The light used for win-stay training was on in four randomly selected arms; the other four arms were dark. Normal rats spent more time in the lit than in the dark arms, a CCP. Rats with caudate lesions were impaired on win-stay performance but exhibited a preference for the lit arms. Rats with amygdala lesions were normal on win-stay but failed to exhibit a CCP.

These findings dissociate the neural system that processes the S-R information required for win-stay performance (caudate nucleus) from the system that processes the S-Rf information required for the CCP (amygdala). The acquisition of a CCP by rats that were never explicitly trained in that task is a demonstration of the fact that the caudate and amygdala memory systems function simultaneously to acquire different kinds of information in the same learning situation. Since lesions of either structure affected performance of only one of the two tasks and did not improve performance of the other task, there is no evidence of a competitive interaction between the systems in this situation.

3.02.8 Summary and Some Outstanding Issues

The historical evidence, the triple dissociation on the radial maze, and the double dissociations in both rats and humans all point to the idea that different parts of the brain are involved in learning that requires memory for different kinds of information. The observations of cooperative, and especially competitive, interactions between systems are strong evidence that these parts of the brain, called systems, function independently and in parallel. This is the multiple parallel memory systems hypothesis, as summarized in **Figure 7**. The analysis in this chapter concludes that the hippocampus and related structures process S-S information, the caudate nucleus and connecting structures process S-R information, and a neural system that includes the amygdala processes S-Rf information. These information-processing specializations may result from differences in the internal microstructure of the systems.

The systems are not completely divergent. All of them receive input from the cortex, so the cortex could be considered a shared part of all systems. Points at which the outputs of the systems converge could also be shared parts. However, the evidence shows that there is clearly divergence among the systems, at least at the level of the hippocampus, caudate nucleus, and amygdala.

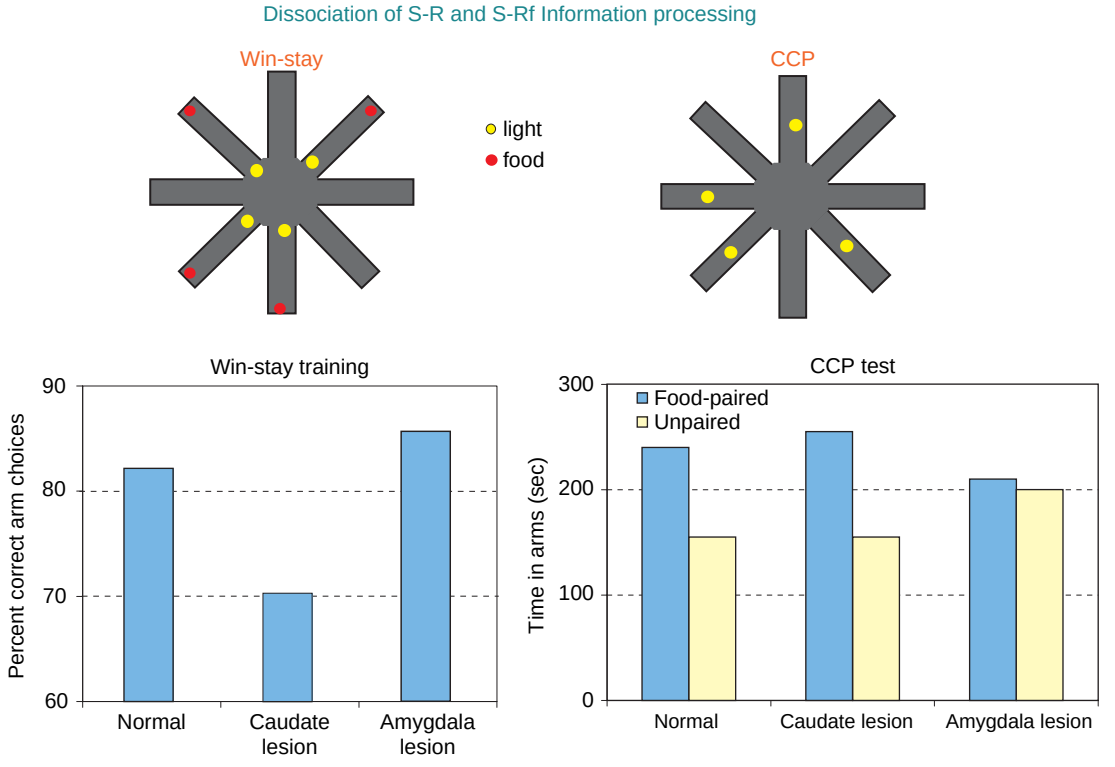


Figure 14 Dissociation of stimulus-response (S-R) and stimulus-reinforcer (S-Rf) information processing in the caudate nucleus and amygdala, respectively. The left side of the figure shows performance of three groups of rats trained on the win-stay radial maze task. Rats with amygdala lesions performed normally, but the performance of rats with caudate nucleus lesions was impaired. The graph shows the mean percent correct responses over the last 3 days of training for each group. In the second part of the experiment, shown on the right, the same rats were given a win-stay test. Four randomly selected arms were lit, and four arms remained dark. The rats were placed on the maze with no food available and allowed to move around freely. The graph shows that the normal rats and the rats with caudate lesions spent more time in the lit arms than in the dark arms, a conditioned cue preference (CCP). The rats with amygdala lesions did not exhibit this preference. This pattern of effects shows that rats can process and store amygdala-based S-Rf information (a conditioned approach response to the light CS) during win-stay training, even if their win-stay performance is very poor because of caudate lesions. The findings dissociate caudate-based processing of S-R information from amygdala-based processing of S-Rf information and are consistent with the hypothesis that the two systems function independently of each other. Adapted from McDonald RJ and Hong NS (2004) A dissociation of dorso-lateral striatum and amygdala function on the same stimulus-response habit task. *Neuroscience* 124: 507–513.

As information flows through the systems (**Figure 7**) it may produce temporary or permanent alterations resulting from the neuroplasticity of their internal structures. These alterations influence the processing of information that flows through the system on future occasions, altering the output of the system and the effect it has on thought and behavior. This is how experience alters behavior. The alterations themselves are memories (note that these neuroplastic changes influence neural activity representing information, but this does not mean the changes actually represent the information). The memories are therefore located in the systems. Although several parts of each system are known to have neuroplastic properties, there is little or no definitive evidence localizing specific memories to specific structures within a system.

Although all information flows through all systems, the specialization of each system means that each can represent only one type of information. For any given situation at any point in time, the representations in each structure will probably differ in coherence. Coherence is a function of two factors. First, the coherence of a representation is determined by the degree to which the information content of a learning situation coincides with the information-processing specialization of a system. When the degree of coincidence is high, most neural elements or microcircuits within the system will be activated

in the same way, forming an accurate representation of the situation. This kind of coherent representation produces output with a powerful influence on behavior. When there is a mismatch between the information content of a learning situation and the specialization of a system, it will form a less coherent, or even incoherent, representation of the information. Fewer of its neural elements may be activated, and not all may be activated in the same way. Less coherent representations produce output with weak or no influence on behavior.

The second factor influencing coherence is practice. Increased exposure to a situation (by increasing the number of training trials) increases the coherence of a representation, increasing its influence on behavior. Practice can increase the coherence of even a poor representation sufficiently for the output of the system to influence behavior. This may become apparent when a lesion impairs performance on a task that recovers after additional training.

The data also suggest that the systems have different learning rates. Specifically, the hippocampal system can apparently acquire information about a situation with very little experience (sometimes with a single exposure), but the caudate system requires numerous repeated exposures even to represent situations that correspond to its S-R specialization.

Because all systems receive all information, they all form some kind of coherent or incoherent representation of all situations. A given situation might produce coherent representations in two systems. Even though the representations consist of different information, the outputs of both systems might produce the same behavior. This would be cooperation between the systems. In this case, impairing the function of either system would have no apparent effect. Eliminating the behavior would require impairments of both systems.

The outputs of two systems with coherent representations of a situation might produce different behaviors. This would be a competitive interaction between the systems. Because the outputs may produce different behaviors, they interfere with each other. Impairing the function of either system would eliminate the interference and improve performance of the behavior produced by the intact system.

3.02.8.1 Some Outstanding Issues

Numerous issues requiring further investigation are suggested by the multiple parallel memory systems theory. This list is necessarily limited to a few of the most general ones.

1. A major question is the degree of genuine functional independence of the proposed systems within which memories are stored. The present summary has emphasized evidence for their independent cooperative or competitive influence on behavior, but possibilities for direct facilitatory or inhibitory actions of one system on another also require further consideration and investigation.

2. The idea that each proposed system is specialized to represent a different type of information, tentatively defined by the triple dissociation experiment, requires further investigation on two levels. First, it should be tested in more different behavioral learning situations that can be parsed into their elements: stimuli, responses, and reinforcers. As more situations are tested, the effects of disabling one or more systems should become increasingly predictable.

3. The idea that systems represent information can also be investigated at the level of the neural microstructure of each system. The theory suggests that experience alters neural systems, which in turn changes how they process the information that flows through them. The implications of this idea for understanding the functional contributions to memory of synaptic and other changes between and within neurons require further examination and definition. Within each system the study of these neuroplastic processes should be done in parallel with an examination of behavioral changes known to be produced by that system. Ultimately, it should be possible to specify how situational information reaches and flows through each system and exactly how this information changes the synaptic relationships in the system so that its processing of similar information on future occasions produces different output.

4. The postulated cooperative and competitive interactions among the outputs of the systems require further investigation on both the anatomical and functional levels of analysis. Specifically, where and how do the outputs of the systems interact?

5. Finally, several criticisms of the multiple memory system concept have been published (Gaffan, 1996, 2001, 2002; Wise, 1996). These complaints largely focus on behavioral evidence from experiments with nonhuman primates and point out that support for the hypothesis is lacking in these species. Experiments directly investigating the multiple memory systems hypothesis in monkeys would either support the theory or reveal its deficiencies.

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3.03 Anatomy of the Hippocampus and the Declarative Memory System

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3.03.1 Introduction

3.03.1.1 A Short History of the Anatomy of Declarative Memory

A half century ago, Scoville and Milner (1957) described profound memory loss following bilateral medial temporal lobe resection in the landmark patient HM. In the following years, scientists studying memory and the brain narrowed in on the hippocampus as the critical structure for everyday memory for facts and events. In the past two decades, however, we have come full circle: It is now apparent that the cortical areas surrounding the hippocampal formation also play critical roles in memory. Today, it is generally accepted that the hippocampal formation and the nearby parahippocampal region together are necessary for human declarative memory, but many questions remain concerning the functional diversity of structures within the so-called declarative memory system. To what extent can the function of hippocampal and parahippocampal substructures be dissociated? How discrete are such functions? How do these structures interact to permit encoding, storage, consolidation, and retrieval of representations of facts and events? What additional cognitive functions might be supported? Understanding the structure and connectivity of these regions is necessary for generating and testing sound hypotheses about the neurobiology of memory.

3.03.1.2 Overview of the Hippocampal System

3.03.1.2.1 Nomenclature

The structures that are the topic of this chapter have variously been termed the medial temporal lobe memory system (Squire and Zola-Morgan, 1991), the hippocampal memory system (Eichenbaum et al., 1994), and the hippocampal region (Witter and Amaral, 2004). The terms hippocampal region or hippocampal system have the advantage that the terminology translates effectively from humans to animal models of human memory, including rodents.

These regions are thought to support a type of memory that has been variously called episodic memory, declarative memory, or autobiographical memory. For research on memory using animal models, the terms episodic or episodic-like memory may be most appropriate.

The hippocampal system comprises the hippocampal formation and the parahippocampal region (**Figure 1**). The hippocampal formation includes the dentate gyrus, the hippocampus proper (fields CA1, CA2, and CA3), and the subiculum (**Figure 2**). The primary criterion for inclusion in the hippocampal formation is the trilaminar character of the structures. In addition, the included structures are connected by largely unilateral pathways beginning with the dentate gyrus granule cell input to the CA3 (**Figure 3**). CA3 pyramidal cells, in turn, provide a unidirectional input to the CA1. Finally, CA1 projects to the subiculum. Because corticocortical connections in the brain are overwhelmingly reciprocal, such a multisynaptic, unidirectional circuit is unique. In contrast, the entorhinal cortex projects to all portions of the hippocampal formation. The connectivity and laminar structure of the entorhinal cortex differentiate it from hippocampal formation structures. The dentate gyrus, hippocampus proper, and subiculum are therefore collectively referred to as the hippocampal formation (**Figure 3**, structures shown in yellow), and the entorhinal cortex is considered part of the parahippocampal region.

The parahippocampal region, also called the retrohippocampal region, includes the perirhinal, postrhinal (or parahippocampal), entorhinal, presubicular, and parasubicular cortices (**Figure 1**). The postrhinal cortex in the rodent brain is considered the

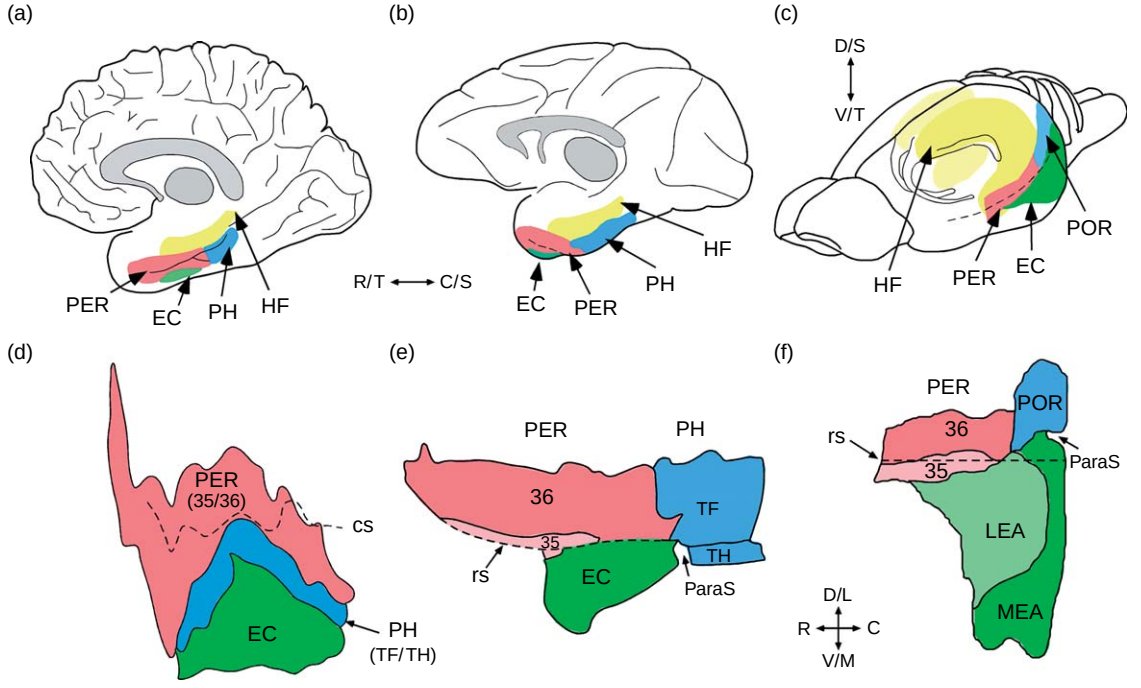

Figure 1 Comparative views of the hippocampal system for the human (left), monkey (middle), and rat (right). The upper panel shows the relevant structures in lateral views of the human brain (a), the monkey brain (b), and the rodent brain (c). The lower panel shows unfolded maps of the relevant cortical structures for the human brain (d), the monkey brain (e), and the rodent brain (f). Shown for the human and monkey brain are unfolded layer IV maps of the perirhinal (PER) areas 35 and 36, parahippocampal (PH) areas TF and TH, and entorhinal cortex (EC). Figures adapted from Burwell RD, Witter MP, and Amaral DG (1995) The perirhinal and postrhinal cortices of the rat: A review of the neuroanatomical literature and comparison with findings from the monkey brain. *Hippocampus* 5: 390–408; Insausti R, Tuñón T, Sobreviela T, Insausti AM, and Gonsalo LM (1995) The human entorhinal cortex: A cytoarchitectonic analysis. *J. Comp. Neurol.* 355: 171–198. Shown for the rodent brain are unfolded surface maps of the PER areas 35 and 36, the postrhinal cortex (POR), and the lateral and medial entorhinal areas (LEA and MEA). The rodent POR is the homolog of the primate PH (see text for details). In the monkey and the rat brain, the parasubiculum (ParaS) is interposed between the entorhinal and POR/PH (arrows). The pre- and parasubiculum, which are components of the parahippocampal region, are not shown (but see Figure 2). Abbreviations: cs, collateral sulcus; rs, rhinal sulcus; DG, dentate gyrus; D, dorsal; L, lateral; M, medial; ParaS, parasubiculum; PreS, presubiculum; S, septal; Sub, subiculum; T, temporal; V, ventral.

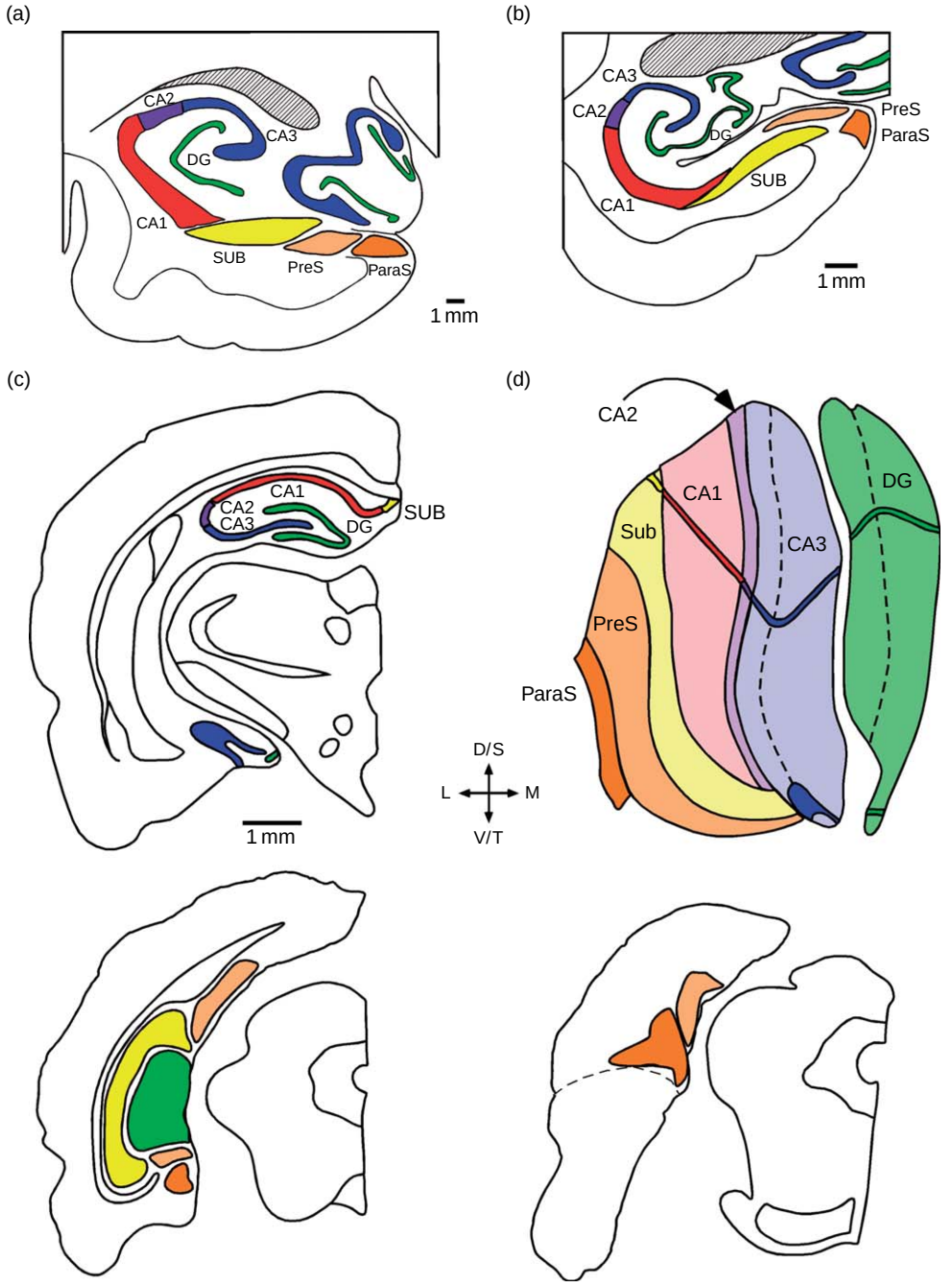

Figure 2 Comparative views of the hippocampal formation with the pre- and parasubiculum. (a) Coronal sections of the human brain (a), monkey brain (b), and rat brain (c) showing the cellular layers of the hippocampal formation structures: the dentate gyrus (green), CA3 (blue), CA2 (purple), CA1 (red), and the subiculum (yellow). (d) An unfolded map of the rodent hippocampal formation. Rodent schematics adapted from Burwell RD and Witter MP (2002) Basic anatomy of the parahippocampal region in monkeys and rats. In: Witter MP and Wouterlood FG (eds.) *The Parahippocampal Region, Organization and Role in Cognitive Functions.* London: Oxford University Press. The presubiculum (light orange) and parasubiculum (dark orange) are also shown at two rostrocaudal levels in panels (e) and (f). Also shown are perirhinal areas 36 and 35 (panels (c) and (e)), the lateral and medial entorhinal areas (LEA and MEA, panels (e) and (f)), and the postrhinal cortex (POR, panel (f)). Abbreviations: DG, dentate gyrus; D, dorsal; L, lateral; M, medial; ParaS, parasubiculum; PreS, presubiculum; S, septal; Sub, subiculum; T, temporal; V, ventral.

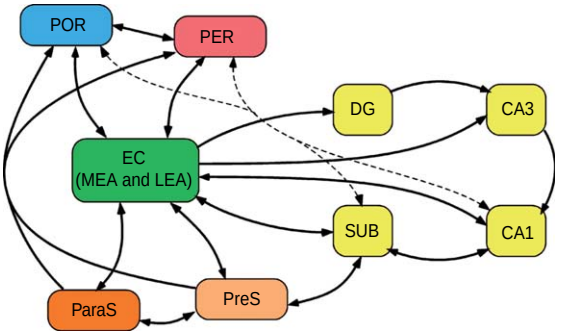

Figure 3 Simplified schematic of the hippocampal system. The schematic includes the hippocampal formation (structures in yellow) and the parahippocampal region (structures in red, blue, green, and orange). The hippocampal formation comprises three-layered structures characterized by largely unidirectional connections, whereas the parahippocampal region comprises six-layered cortices characterized by reciprocal connections. Note that the perirhinal and postrhinal cortices (PER and POR) have reciprocal connections with CA1 and the subiculum. Abbreviations: DG, dentate gyrus; EC, entorhinal cortex; LEA, lateral entorhinal area; MEA, medial entorhinal area; ParaS, parasubiculum; PER, perirhinal cortex; PH, parahippocampal cortex in the primate brain; POR, postrhinal cortex in the rodent brain; PreS, presubiculum; subiculum (SUB).

homolog of the parahippocampal cortex in the primate brain. The perirhinal and postrhinal cortices are the major recipients of cortical afferents, and they project heavily to entorhinal cortex. The entorhinal cortex and the pre- and parasubiculum also receive direct cortical inputs. The entorhinal cortex projects directly to all components of the hippocampal formation and all other components of the parahippocampal region (**Figure 3**). The entorhinal connections with CA1, the subiculum, and all other parahippocampal structures are reciprocal.

One practical problem in the comparative anatomy of these structures is the confusing use of the term parahippocampal. In the rodent brain, the term has only one use (i.e., in the phrase 'parahippocampal region'). In the human and monkey brains, the term is used in two additional ways. First is the parahippocampal cortex, a cortical region in the medial temporal lobe that is a component of the parahippocampal region (and is the homolog of the rodent postrhinal cortex). Second, the parahippocampal gyrus is the fold or gyrus that contains a large portion of the entorhinal, perirhinal, and parahippocampal cortices.

There are also discrepancies in the terminology for the perirhinal cortex. In Brodmann's (1909) nomenclature, which includes verbal and numeric terms, the verbal term for area 35 was perirhinal, and the verbal term for area 36 was ectorhinal. Although Brodmann defined area 36 as a very narrow strip of cortex that did not include the temporal pole, other classic studies, which reported more detailed cytoarchitectonic analyses of these regions, included the temporal pole in area 36 (von Economo, 1929; Von Bonin and Bailey, 1947). There was no designation in Brodmann's nomenclature for the caudally located region we now call the parahippocampal cortex (reviewed in Suzuki and Amaral, 2003b). Using a different nomenclature, von Economo and Koskinsas named the rostral perirhinal/ectorhinal region areas TG and TGa and the caudal (parahippocampal) region areas TF and TH. In modern terminology, the term perirhinal cortex was used to designate the combined areas 35 and 36 (Amaral et al., 1987) or 35a and 35b (Van Hoesen and Pandya, 1975). In the latter nomenclature, area 36 was termed TL.

Currently, the most commonly used nomenclature for memory research in the primate brain is perirhinal cortex comprising areas 35 and 36. Burwell and colleagues (Burwell et al., 1995; Burwell, 2001) adapted that nomenclature for use in the rodent brain. The term ectorhinal is no longer in use except in rodent brain atlases. Thus, within a comparative framework for experimental neuroscience, it seems reasonable to adhere to the nomenclature of perirhinal cortex as designating the combined areas 35 and 36 for both the rodent and primate brains.

3.03.1.2.2 Location of the hippocampal system structures

The focus of this chapter is the rat hippocampal system about which we have the most detailed anatomical information, but it is worth noting that there are surprising similarities and interesting differences between these structures in the rodent and the primate brains. The upper panel of **Figure 1** shows that the hippocampus is C-shaped and relatively larger in the rodent brain (**Figure 1(c)**). The dorsal or septal portion of the region is associated with the fimbria-fornix and the septal nuclei. The ventral or temporal portion of the structure is associated with the temporal cortices. The hippocampus is relatively smaller in the primate brain (**Figure 1(b)**). The structure is still shaped like a C, though shallower and rotated

about 90° clockwise, such that the opening is pointing upward. In the primate brain, the rostral hippocampus is associated with the temporal cortices, and the caudal hippocampus is associated with the septal nuclei. Accordingly, for cross-species comparisons, the best terminology for the long axis of the hippocampus is the term septotemporal.

In the human brain, the rhinal sulcus is relatively small and is associated with only the most rostral portion of the perirhinal cortex. The collateral sulcus forms the lateral border of the parahippocampal gyrus (**Figure 1(d)**). As in the monkey brain, the entorhinal, perirhinal, and parahippocampal cortices occupy the parahippocampal gyrus and the temporal pole. The perirhinal cortex occupies the temporal pole and continues caudally. The entorhinal cortex lies in the medial portion of the anterior parahippocampal gyrus and is bordered rostrally and laterally by the perirhinal cortex. The parahippocampal cortex forms the caudal border of the perirhinal cortex.

In the monkey brain, which is less gyrencephalic (smoother) than the human brain and more gyrencephalic than the rat brain, the rhinal sulcus is associated with the full extent of the perirhinal cortex. Area 35 is a narrow band of agranular cortex that occupies the fundus and the lateral bank of the rhinal sulcus. Area 36 is a larger strip of dysgranular cortex located lateral to area 35 and including the temporal pole (**Figure 1(b)** and **1(e)**). All but the most rostral part of the lateral border of area 36 is shared with area TE of inferotemporal cortex. The rostrolateral border is formed by the superior temporal gyrus. Suzuki and Amaral (2003a) extended the border of area 36 rostrally and septally to include the medial half of the temporal pole on cytoarchitectonic and connectional grounds. The monkey parahippocampal cortex, comprising areas TH and TF, is located caudal to the perirhinal and entorhinal cortices (**Figure 1(b)** and **1(e)**). Area TF is larger than area TH and is laterally adjacent to area TH. Area TH is a thin strip of largely agranular cortex medially adjacent to area TF. The parahippocampal cortex is bordered rostrally by the entorhinal and perirhinal cortices, laterally by TE, medially by the para- and presubiculum, and caudally by visual area V4.

In the rodent brain, the rhinal sulcus, or fissure as it is sometimes called, is the only prominent sulcus (**Figure 1(c)** and **1(f)**). It extends along the entire lateral surface of the brain, though it is quite shallow in its caudal extent. The region is bordered rostrally by the insular cortex. Insular cortex is classically defined as the region overlying the claustrum. The transition from insular cortex to the perirhinal cortex occurs when claustral cells underlying layer VI of the cortex are no longer visible. The perirhinal cortex comprises two cytoarchitectonically distinct strips of cortex, areas 35 and 36. Area 36 lies dorsally adjacent to area 35. The entorhinal cortex provides the ventral border of area 35. The dorsal border of area 36 is formed by secondary somatosensory cortex, rostrally, secondary auditory cortex at midrostrocaudal levels, and ventral temporal association cortex at caudal levels. The postrhinal cortex is located caudal to perirhinal cortex and provides the caudal border. It lies ventral to the ventral temporal area and dorsal to the medial entorhinal cortex (**Figure 2(f)**).

3.03.1.2.3 Cross-species comparisons: Human, monkey, and rodent

A comparative analysis of the unfolded maps of the human, monkey, and rat brains shows that the spatial relationships of the perirhinal, parahippocampal/postrhinal, and entorhinal cortices are similar (**Figure 1**). Aside from the obvious differences in scale, the relative size differences are also interesting. Studies in rats, monkeys, and humans suggest that the perirhinal cortex accounts for roughly 3% of the cortical surface area, suggesting that the region scales linearly with cortical surface area. Also, in all three species, the surface area of the perirhinal cortex is roughly twice that of the postrhinal/parahippocampal cortex. Therefore, postrhinal/parahippocampal cortex also appears to scale linearly with brain size. The relative size of the entorhinal cortex, however, differs dramatically across species. In the rat brain, its surface area is more than three times that of the perirhinal cortex, but in the primate brain, entorhinal cortex is considerably smaller than the perirhinal cortex.

The homology of the rodent postrhinal cortex with the primate parahippocampal cortex is based on the structural and connectional similarities. In rodents and primates, the region receives substantial input from visual associational, retrosplenial, and posterior parietal cortices. Subcortical connections are also similar. For example, the rat postrhinal cortex is strongly and reciprocally connected with the lateral posterior nucleus of the thalamus (LPO). Likewise, the monkey parahippocampal cortex is connected with the pulvinar, the homolog of the lateral preoptic area (LPO) in the rodent.

In human, monkey, and rat, the entorhinal cortex is a six-layered cortex characterized by a cell sparse layer (lamina dissecans) separating the deep and

superficial layers. The medial part of the entorhinal area is, structurally, more highly differentiated as compared to the lateral part, and the lamina dissecans is more evident. It should be noted that in the rat, the medial entorhinal area is more caudal and ventral, whereas the lateral entorhinal area is more rostral and dorsal (**Figure 1(c) and 1(f)**). In both rat and monkey, the intrinsic connectivity of the entorhinal cortex appears to be organized into intrinsic bands of interconnectivity that form discrete associational networks. In the rat, these bands of intrinsic connectivity project to different levels of the dentate gyrus, suggesting a functional topography. There is evidence that a similar topography exists for the monkey.

All hippocampal formation structures observed in the human brain are also present in the monkey and rat brains (**Figure 2**). The absolute size of the hippocampal formation is largest in the human brain and smallest in the rodent brain, though the structure is relatively larger in the rodent brain. As previously mentioned, the hippocampus is situated differently in different species. In the human and monkey brains, it is as if the hippocampal formation has swung down and forward, such that rostral hippocampus in the primate brain is comparable to ventral hippocampus in the rodent brain. Similarly, caudal hippocampus in the primate brain is comparable to dorsal hippocampus in the rodent brain. For ease of comparative analysis, it is most efficient to use the terms septal and temporal to describe the long axis because these terms can be applied similarly across all species. The septotemporal axis in the rodent hippocampus is equivalent to dorsoventral axis, and the septotemporal axis in the primate hippocampus is equivalent to the caudorostral axis.

3.03.2 The Parahippocampal Region

3.03.2.1 The Postrhinal Cortex

The postrhinal cortex is located near the caudal pole of the rat brain, caudal to the perirhinal cortex, dorsal to the rhinal sulcus and to the medial entorhinal area (**Figure 1(c)**). Usually the postrhinal cortex arises at the caudal limit of the angular bundle when subicular cells are no longer present in coronal sections (**Figure 4(a)**). At this level, postrhinal cortex is characterized by the presence of ectopic layer II cells at the perirhinal–postrhinal border near the ventral border with the medial entorhinal cortex (**Figure 4(b)**, arrow). Moving caudally, the postrhinal cortex rises dorsally above the caudal extension of the rhinal fissure and wraps obliquely around the

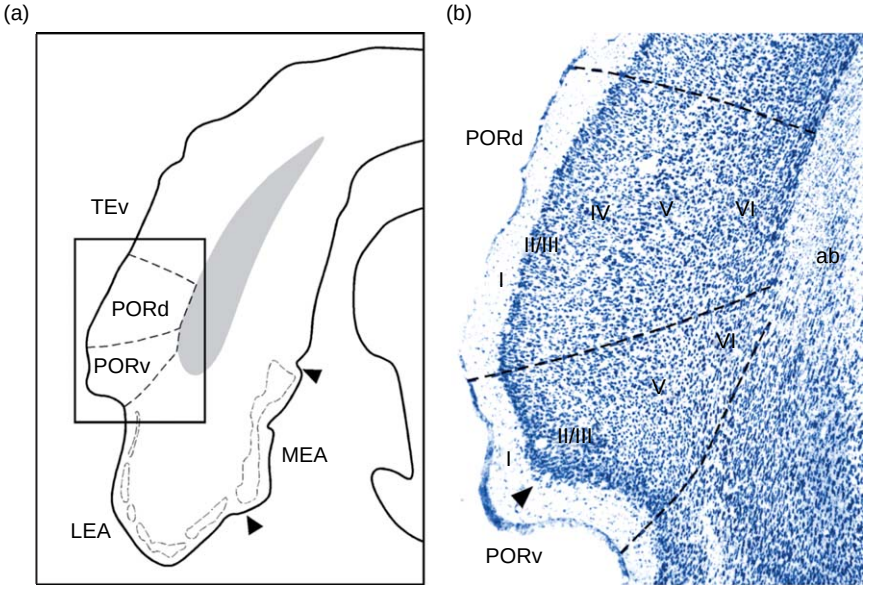

Figure 4 Location and photomicrograph of the postrhinal cortex (POR). (a) Drawing of a coronal section of the rat brain at the level of the rostral limit of the postrhinal cortex. (b) Nissl-stained coronal section showing the septal and temporal subregions of the POR (PORd and PORv, respectively). Layers are labeled I–VI. The septal subregion has a more differentiated laminar pattern. The ventral subregion is characterized by ectopic layer II cells (arrow) that appear near the rostral border with area 36. Abbreviations: ab, angular bundle.

caudal pole of the brain. Visual association cortex, which forms the dorsal border of postrhinal cortex, has a more differentiated laminar pattern and a broader layer IV. The precise location of the dorsal border is difficult to distinguish cytoarchitectonically. A convenient landmark, however, is provided by the parasubiculum. The dorsal border of the postrhinal cortex on the lateral surface tends to be at the same dorsoventral level as the parasubiculum on the medial surface. The medial entorhinal cortex borders the postrhinal cortex ventrally and is easily distinguished by the large layer II cells and distinct laminar look of the cortex.

The cell layers of the postrhinal cortex have a homogeneous look because the packing density of cells is similar across layers (**Figure 4(b)**). In coronal sections, there is a broadening of the deep layers, which is due to the conformation of the region at the caudal pole of the brain (**Figure 4**). In sagittal sections, however, layers II–III, V, and VI each occupy about one-third of the cortical depth. The region can be subdivided into dorsal and ventral subdivisions based on cytoarchitectonic features. In general, the dorsal subregion has a more organized and radial appearance. The primary difference between the two subdivisions is that the dorsal portion has a distinguishable granule cell layer IV. Another difference is that layer V of the dorsal subregion is slightly narrower than in the ventral subdivision.

Retrograde tract tracing studies show that three-quarters of postrhinal afferentation arise in neocortex. The remainder is roughly evenly divided between subcortical and hippocampal afferents. The neocortical connections of the postrhinal cortex distinguish it from the nearby perirhinal cortex, in that cortical input to the postrhinal cortex is strongly dominated by visual and visuospatial inputs. In terms of sensory input, the postrhinal cortex receives almost a third of its total input from visual associational regions. The strongest associational input arises in the posterior parietal cortex. Dorsal retrosplenial cortex also provides a strong projection. The input from frontal associational regions largely arises in ventrolateral orbital frontal cortex. A strong input arises in the caudal and ventral temporal area, which is itself strongly interconnected with visual association cortices. For the most part, all cortical connections are equally reciprocated. The exception is that the postrhinal cortex projects strongly to the perirhinal cortex, but the return projection is substantially weaker.

The subcortical afferents are dominated by the thalamic inputs, which arise predominantly in the lateral posterior nucleus of the thalamus. That projection is reciprocal. There is also input from the anteromedial dorsal thalamic group and the intralaminar nucleus of the thalamus. The input from the amygdala is very small and is mainly from the lateral and basolateral nuclei. The postrhinal cortex also projects back to the lateral and basolateral amygdala nuclei. The inputs from the septum are also relatively small and are dominated by the medial septum.

The postrhinal cortex projects strongly to the medial entorhinal cortex, particularly to the lateral band. The entorhinal projection is weakly reciprocal. Postrhinal cortex has strong reciprocal connections with the septal presubiculum and the parasubiculum. In addition to these parahippocampal connections, there are strong direct connections with the hippocampus. The postrhinal cortex projects directly to the septal CA1 and subiculum, and both projections are returned. Connections with the temporal hippocampus are modest.

3.03.2.2 The Perirhinal Cortex

The perirhinal cortex arises at the caudal limit of the insular cortex and can be distinguished from insular cortex by the absence of the underlying claustrum. It is bordered dorsally by temporal association regions, ventrally by piriform and entorhinal cortex, and caudally by the postrhinal cortex. For most of its rostrocaudal extent, the perirhinal cortex includes the fundus and both banks of the rhinal sulcus (**Figure 1(f)**). At its caudal limit, the region rises dorsal to the fundus. A signature feature of the perirhinal cortex in the rodent and monkey brains is the presence of large heart-shaped cells in deep layer V that appear in both area 36 and area 35 (**Figure 5**).

Perirhinal area 36 is located dorsal to the rhinal sulcus. Although the region has a more prominent laminar structure dorsally than ventrally, area 36 is generally described as dysgranular cortex. The dorsal border of area 36 is best discerned by characteristics of the granular cell layer, layer IV. Area 36 has a fairly rudimentary layer IV as compared to the discrete granular layer of the dorsally adjacent neocortical areas. Another feature of the region is the patchy layer II in which medium-sized cells are organized in clumps or patches. The organization of layer V cells into lines gives the region a radial look, especially dorsally. Layer VI has a bilaminar appearance in that the cells in the deep portion of the layer

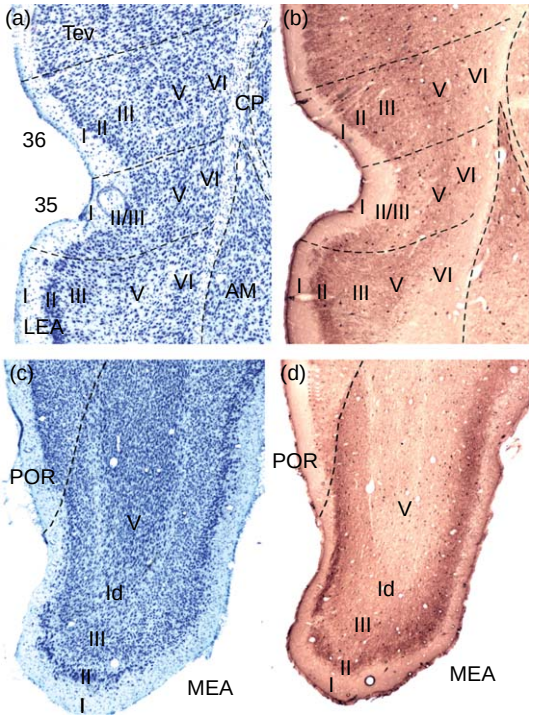

Figure 5 Photomicrographs of the perirhinal (PER), postrhinal (POR) and lateral and medial entorhinal cortices (LEA and MEA). (a) Nissl-stained coronal section showing PER areas 36 and 35 and the LEA. (b) Adjacent section stained for parvalbumin. Heavy staining of layer II in the LEA provides a useful marker for the PER-LEA border. (c) Nissl-stained coronal section showing MEA and POR. (d) Adjacent section stained for parvalbumin. Layers are labeled I–VI. Parvalbumin staining also differentiates the MEA-POR border. Other abbreviations: AM, amygdala; CP, caudate putamen.

are smaller, darker, and more densely packed than the cells in the superficial portion of the layer.

Area 35 is generally characterized by a broad layer I. Layers II and III tend to blend together (**Figure 5**). The region lacks a layer IV and is thus considered agranular cortex. Layer V of area 35 has a disorganized look as compared to the radial appearance in area 36. As in area 36, layer VI has a bilaminate appearance. A general characteristic of area 35 is the organization of its cells into an arcing formation that spans all layers. This feature is most evident below the rhinal sulcus. The entorhinal cortex forms most of the ventral border and can be distinguished from ventral area 35 by the medium to large darkly staining stellate cells of layer II and by the appearance of the lamina dissecans, a cell-sparse area between layers III and V.

The input to the perirhinal cortex is roughly evenly divided between cortical and subcortical structures. The perirhinal cortex receives input from nearly all unimodal and polymodal associational regions of neocortex, but there are subregional differences. For example, area 36 receives roughly equal input from olfactory, auditory, visual and visuospatial, and sensorimotor regions, whereas area 35 is dominated by olfactory input from piriform cortex. There are also subregional similarities and differences in polymodal association input. Area 36 receives the largest cortical input from temporal association regions followed by insular and frontal regions. In contrast, area 35 receives the larger input from insular cortex followed by temporal association and frontal regions. Of course, there is also a heavy intrinsic input from area 36. Areas 36 and 35 each receive only small inputs from posterior associational regions. As would be expected, these associational connections are largely reciprocal.

Perirhinal areas 36 and 35 are also differentiated by subcortical connections. The strongest subcortical connections of area 36 are with the amygdala. The afferent input arises largely in the lateral nucleus, but the basolateral and basomedial nuclei also provide substantial inputs. Substantial thalamic input arises largely in the dorsolateral group and in the reticular thalamic nucleus. In contrast, area 35 receives its strongest subcortical afferents from olfactory structures, primarily from the endopiriform nucleus, but also from the piriform transition area. Other substantial inputs arise in the amygdala, the midline and lateral thalamic groups, and the medial geniculate nucleus of the thalamus.

Like the postrhinal cortex, the perirhinal cortex projects strongly to the lateral entorhinal cortex. The projection arises in area 35 and terminates most heavily in the so-called lateral band of the entorhinal cortex (see following). The entorhinal projection is weakly reciprocated. Area 36 is weakly connected with hippocampal and subicular structures, although these connections may be functionally important. Area 35 receives input back from the septal presubiculum. The strongest projection back to area 35 arises in temporal CA1, but it also receives input from temporal subiculum and presubiculum. Other smaller inputs arise in septal CA1 and the parasubiculum.

3.03.2.3 Entorhinal Cortex

The entorhinal cortex is of considerable interest in memory research. Not only does it provide the major conduit for sensory information to the hippocampal

formation, but a number of recent discoveries also suggest that the region may make unique contributions to the processing of spatial information. The entorhinal cortex is a relatively large and complicated structure, and its connections are topographically organized. Thus, understanding the areal differences in entorhinal structure could provide insight into its role in memory.

In rats and other animals, the entorhinal cortex has been divided into two subdivisions roughly equivalent to modern definitions of the lateral and medial entorhinal areas (LEA and MEA, **Figure 1(f)**) (Brodmann, 1909; Krieg, 1946). The LEA (**Figure 5**, top) is perhaps most easily distinguished from the MEA (**Figure 5**, bottom) by differences in layer II. LEA has a very clumpy layer II as compared to the more homogeneous layer II of the MEA. The sparsely populated layer IV, also called the lamina dissecans, is considered a landmark feature of the entorhinal cortex, but there are subregional differences. In general, the LEA exhibits a less prominent lamina dissecans as compared to the MEA (compare **Figure 5**).

Some time ago, the monkey entorhinal cortex was further subdivided on the basis of structural and connectional criteria (Van Hoesen and Pandya, 1975; Amaral et al., 1987). The rat entorhinal cortex has now been subdivided into six fields according to similar criteria (**Figure 6(a)**) (Insausti et al., 1997). The LEA comprises four fields: the dorsal lateral entorhinal field (DLE), the dorsal intermediate entorhinal field (DIE), the amygdalo-entorhinal transitional field (AE), and the ventral intermediate entorhinal field (VIE). Each field has unique connectional and/or structural characteristics. The medial entorhinal area (MEA) is subdivided into a caudal field (CE) and a medial field (ME). Medially, the MEA is bordered by the parasubiculum. The MEA border with the parasubiculum is marked by a layer II that thickens into a characteristic club-shaped formation.

The intrinsic connections of the entorhinal cortex are organized in a rostrocaudal manner, such that the cells located in each of three bands of the entorhinal area are highly interconnected but do not project outside the band of origin (**Figure 6(b)**) (Dolorfo and Amaral, 1998). Interestingly, each band of intrinsic connectivity spans the MEA and LEA. An important recent discovery about these regions has to do with the relationship of these bands of intrinsic connectivity with the perforant pathway, the entorhinal projection to the dentate gyrus. Briefly, the lateral band projects to the septal half of the dentate gyrus, whereas the intermediate and medial bands project to the third and fourth septotemporal quarters, respectively (**Figure 6(c)**). This connectional topography suggests that functional diversity within the entorhinal cortex may be in register with functional diversity in the hippocampus.

The entorhinal cortex is strongly connected with other parahippocampal region structures. Perirhinal

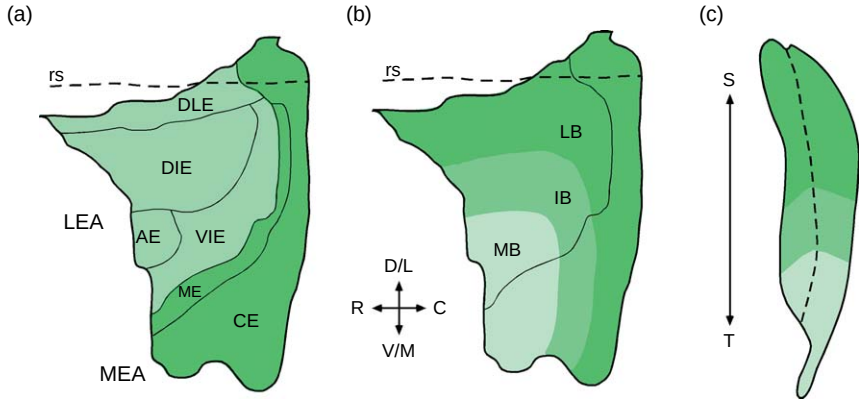

Figure 6 Unfolded maps of the entorhinal cortex and the target of the perforant pathway, the dentate gyrus. (a) Unfolded map of the rodent entorhinal cortex showing the LEA in light green and the MEA in dark green. Further parcellation of each subregion is noted by black lines (Insausti et al., 1997). (b) Unfolded map of the rodent entorhinal cortex showing the lateral (LB) in dark green, the intermediate band (IB) in medium green, and medial band (MB) in pale green. (c) The unfolded dentate gyrus, color coded to denote the terminations of the perforant pathway. The entorhinal LB projects to the septal half of the dentate gyrus, the IB projects to the third quarter, and the MB projects to the temporal quarter. Abbreviations: AE, amygdalo-entorhinal transitional field; CE, caudal entorhinal field; D, dorsal; DLE, dorsal lateral entorhinal field; DIE, dorsal intermediate entorhinal field; L, lateral; M, medial; ME, medial entorhinal field; S, septal; T, temporal; V, ventral.

input arises largely in area 35 and terminates preferentially to the lateral band of the LEA. The postrhinal input arises in all portions of the region and terminates primarily in the lateral band of the MEA. There is a heavy return projection to perirhinal cortex that arises in all layers and all portions of the entorhinal cortex, though the strongest projection arises in the lateral band. Strong inputs originate from the pre- and parasubiculum. The parasubiculum targets the entire entorhinal cortex, septal presubiculum projects more heavily to the MEA, and temporal presubiculum projects more heavily to the LEA. The entorhinal cortex provides modest reciprocal connections with the pre- and parasubiculum (Witter and Amaral, 2004).

The entorhinal cortex has neocortical connections, through weaker than perirhinal and postrhinal cortices. The LEA receives very strong input from the piriform and agranular insular cortices. Medial and orbital frontal regions provide a strong projection. Input from the cingulate, parietal, and occipital cortices is relatively weak. There is little differentiation across the lateral to medial bands. Piriform cortex also projects to the MEA, but the projection terminates in the lateral and intermediate bands. In contrast, the lateral band receives moderate to strong projections from frontal, cingulate, parietal, and occipital cortices. Projections to the medial frontal and olfactory structures tend to arise in the intermediate and medial bands. A very narrow strip of the entorhinal cortex that is positioned closest to the rhinal fissure gives rise to the major projections to other cortical areas, including the lateral frontal, temporal, parietal, cingulate, and occipital cortices.

The entorhinal cortex has widespread connections with subcortical structures, and it is possible that the subcortical afferents are as influential as the cortical afferents. Strong projections arise in claustrum, olfactory structures, the amygdala, and dorsal thalamus. The olfactory input arises in the endopiriform nucleus and the piriform transition area and is stronger to the LEA than the MEA. The dorsal thalamic input arises primarily in the midline thalamic nuclei and is stronger to MEA than to LEA. The LEA and MEA receive input from septal nuclei, though the inputs are relatively small. The amygdala input arises in all nuclei except the central nucleus and amygdalohippocampal area and is stronger to LEA than MEA. In addition, the entorhinal cortex projects to all amygdaloid structures except the nucleus of the lateral olfactory tract and the central nucleus (Pikkarainen and Pitkanen, 1999).

The entorhinal cortex projects to all hippocampal formation structures including the dentate gyrus, fields CA3, CA2, and CA1 of the hippocampus proper, and the subiculum (reviewed in Witter and Amaral, 2004). The entorhinal projections to the dentate gyrus, CA3, and CA2 originate in layer II of the entorhinal cortex. The terminations of the layer II projections exhibit a radial topography in that the LEA terminates in the outer DG molecular layer, whereas the MEA projects to the middle DG molecular layer (**Figure 7**). The projections to CA1 and the subiculum originate in layer III. The terminations of the layer III projections

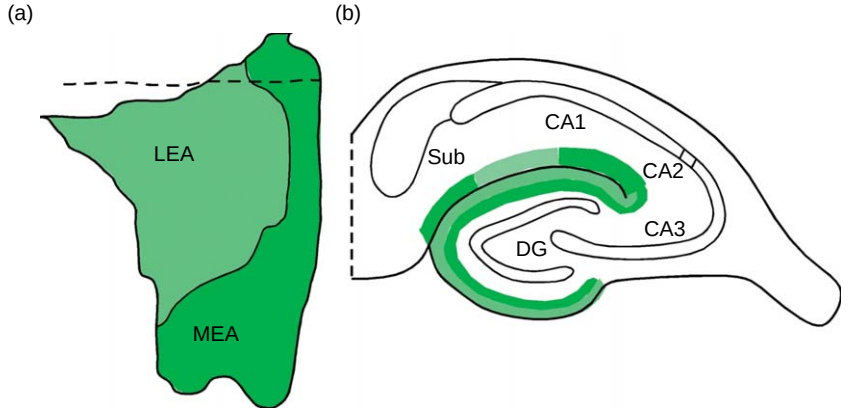

Figure 7 Illustration of the radial and transverse topography of the entorhinal projection to the hippocampal formation. (a) Unfolded map of the entorhinal cortex showing the LEA in light green and the MEA in dark green. (b) Line drawing of a hippocampal section perpendicular to the long axis. Adapted from Witter MP and Amaral DG (2004) Hippocampal Formation. In: Paxinos G (ed.) *The Rat Nervous System,* 3rd ed. San Diego, CA: Academic Press. The terminations of the LEA are in light green, and the terminations of the MEA are in dark green. The projection to the DG exhibits a radial topography, whereas the projection to CA1 and subiculum (sub) exhibits a transverse topography. See text for details.

exhibit a transverse topography such that the LEA projects to distal CA1 and proximal subiculum, and the MEA projects to the proximal CA1 and distal subiculum. The organization of the CA1 and subicular projections back to deep layers of the LEA and MEA roughly reciprocates the forward projections.

3.03.2.4 Presubiculum

The presubiculum is bordered dorsally by retrosplenial cortex, medially by the subiculum, and ventrolaterally by the parasubiculum (**Figures 2(e)**, **2(f)**, and **8(a)**). Areas 48 and 27 according to Brodmann (1909) are both included in the presubiculum. Area 48, the most dorsal extension of the presubiculum, is sometimes called the postsubiculum. Because the presubiculum and this dorsal component exhibit considerable cytoarchitectonic similarities, it may be more appropriate to designate the area collectively with a single term.

Layer II of the six-layered presubiculum is thick and contains small, densely packed, and darkly staining pyramidal cells (**Figure 8(b)**). Cells in layer III are even smaller, round, and also darkly staining. Whereas cells in layer II tend to form clusters, cells in layer III have a more homogeneous look. Layer III is separated from the deep layers by a narrow, sparsely populated gap that is continuous with the lamina dissecans of the parasubicular cortex. Deep to this cell-sparse gap are two layers. Layer V is very thin and contains pyramidal cells. Layer VI is slightly thicker and contains a mixture of cell types.

As it turns out, acetylcholinesterase (AChE) is an excellent marker for the presubiculum. Layer II stains moderately darkly (**Figure 8(c)**). Deep to layer II is a dark band that contains layers III, the cell-sparse gap, and layer V. Layer VI is moderately to lightly stained in AChE preparations. AChE is also a good marker for the parasubiculum.

The presubiculum has extensive associational, commissural, and hippocampal parahippocampal connections (Witter and Amaral, 2004). The septal and temporal parts of the presubiculum are highly interconnected. Connections with the contralateral presubiculum are also extensive, though commissural connectivity may be stronger ventrally than dorsally. The presubiculum provides a weak input to the dentate gyrus and all fields of the hippocampus proper. It is reciprocally connected with the subiculum. The presubiculum projects to superficial layers of the subiculum. The projection to the septal subiculum is moderately strong, and the temporally directed projection is relatively weak. The input from the subiculum terminates in layer I.

Regarding parahippocampal connectivity, the presubiculum projects to superficial layers of the

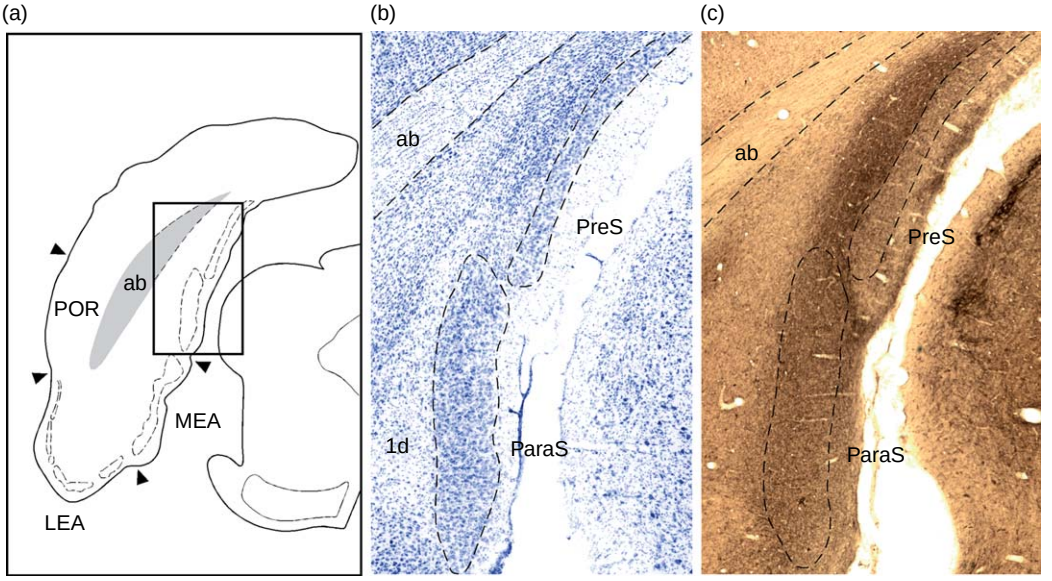

Figure 8 Photomicrographs of the presubiculum (PreS) and parasubiculum (ParaS). (a) Drawing of a coronal section of the rat brain at the level of the angular bundle (ab). The inset designates the areas shown in panels (b) and (c). (b) Nissl-stained coronal section showing the PreS and ParaS. Layer II is outlined for the PreS, and the combined layer II/III is outlined for ParaS. (c) Adjacent section stained for acetylcholinesterase (AChE). AChE provides an excellent marker for these regions.

parasubiculum, but the connections with the entorhinal cortex are by far the strongest. The presubicular–entorhinal projection is bilateral, largely directed to medial entorhinal cortex, and almost exclusively terminates in layer III. Septal presubiculum projects much more heavily to the MEA than the LEA, but temporal presubiculum projects heavily to both entorhinal divisions. Septal presubiculum also provides a moderately heavy input to the postrhinal cortex. The dorsal extension (Brodman's area 48, sometimes termed the postsubiculum) projects massively to postrhinal cortex. Temporal presubiculum projects heavily to the LEA and the MEA and moderately heavily to perirhinal areas 36 and 35. Septal presubiculum receives heavy input from postrhinal cortex and a moderately heavy input from the MEA portion of the lateral band. Temporal presubiculum receives a very heavy input from the MEA portion of the medial band, weak input from the LEA, and virtually nothing from the perirhinal and postrhinal cortices.

The heaviest neocortical input to the presubiculum arises in the granular retrosplenial cortex, but weaker inputs arise in prelimbic cortex, dorsomedial prefrontal areas, and the anterior cingulate cortex (Witter and Amaral, 2004). The primary subcortical inputs are from the dorsal thalamus, specifically, the anteroventral, the anterodorsal, and the laterodorsal nuclei. The presubiculum receives subcortical input from the thalamus, primarily the anterior thalamic nuclei including the anteroventral, anterodorsal, and laterodorsal nuclei. A massive return projection targets the same nuclei. There is also a strong cholinergic input arising from septal nuclei. Finally, the presubiculum has reciprocal connections with the mamillary nuclei of the hypothalamus.

3.03.2.5 The Parasubiculum

For most of its rostrocaudal extent, the parasubiculum is bordered by presubiculum dorsally and the medial entorhinal area ventrally (**Figure 2(e)** and **2(f)**). At more caudal levels, the parasubiculum is interposed between the postrhinal cortex and the medial entorhinal area. A broad, combined layer II/III contains large, densely packed, moderately darkly staining pyramidal cells. This layer is separated from the deep layers by a broad lamina dissecans. Layers V and VI can be distinguished from one another and tend to run continuously with deep layers of the medial entorhinal area. In AChE preparations, layers I and II/III are darkly stained (**Figure 8(c)**). The lamina dissecans and layer V are lightly stained, and layer V is moderately darkly stained.

The parasubiculum has associational connections that project dorsally and ventrally. The ventral projections are heavier and more extensive than the dorsal ones. Commissural projections terminate in layers I and III of the contralateral homotopic region.

The hippocampal input to the parasubiculum arises mainly in the subiculum and terminates in layer I and superficial layer II. There are also return projections to the hippocampal formation. The structure projects directly to the molecular layer of the dentate gyrus. This is especially interesting given that the parasubiculum receives strong inputs from anterior thalamic nuclei. As has been previously noted, the anterior thalamic projection to the parasubiculum provides a pathway by which the anterior thalamus can affect hippocampal processing of incoming information at very early stages.

Like the presubiculum, the parasubiculum exhibits substantial connections with other parahippocampal structures. The parasubiculum projects selectively to layer II of the entorhinal cortex. The entorhinal projection is much heavier to MEA than to the LEA. Interestingly, the parasubicular projection to POR is even heavier than that to the MEA. Parahippocampal inputs arise mainly from the MEA, with the medial band providing the heaviest return projection. There is also a modest presubicular input.

Extrinsic connections of the parasubiculum are few. The only neocortical afferents arise in retrosplenial cortex and visual cortex, and these inputs are quite weak. Other than the input from the anterior thalamus, the only other subcortical afferents arise in the amygdala from the lateral, basal, and accessory basal nuclei.

3.03.3 The Hippocampal Formation

The structures of the hippocampal formation are grouped together partly because of the sequential activation pattern that was identified several decades ago. The entorhinal cortex activates the dentate gyrus via the perforant pathway, the mossy fiber pathway from the denate gyrus activates CA3, and the CA3 Schaffer collaterals activate CA1. Some of the earliest and most famous studies of the structure of the nervous system were conducted by Ramón y Cajal, who used a technique developed by Camillo Golgi for darkly staining a small number of neurons

in the brain. Cajal's elegant studies and drawings, including the rodent hippocampus (**Figure 9**), provided the basis for the neuron doctrine.

Because of the complex architecture of the hippocampus, it is helpful to describe its structure in terms of three axes, the longitudinal, transverse, and radial axes. As previously discussed, we use the term septotemporal for the longitudinal axis of the hippocampus. Along the transverse axis, which is orthogonal to the long axis of the hippocampus, the dentate gyrus can be considered the proximal limit and transverse locations designated according to position relative to the dentate gyrus. Thus, the part of the CA3 lying in the V of the dentate gyrus is proximal CA3, and the part closest to CA1 is distal (**Figure 10**). Similarly, the part of CA1 closest to CA3 is proximal, and so on. Finally, the laminar structure is perpendicular to the radial axis. In this terminology, the molecular layers are superficial and the layers on the opposite side of the principle cell layers are deep.

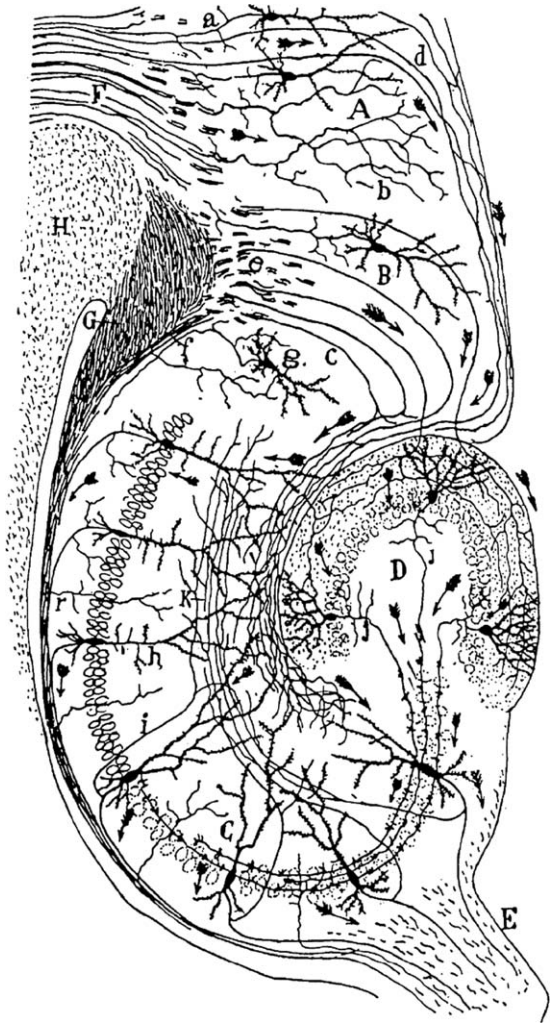

Figure 9 Drawing of the circuitry of the hippocampal formation by Ramón y Cajal (1909). Cajal proposed that the nervous system is made up of countless separate units, or nerve cells composed of dendrites, soma, and axons, each of which is a conductive device. He further proposed that information is received on the cell bodies and dendrites and conducted to distant locations through axons. Abbreviations: A, retrosplenial area; B, subiculum; C, Ammon's horn; D, dentate gyrus; E, fimbria; F, cingulum; G, angular bundle; H, corpus callosum; K, recurrent collaterals; a, axon entering the cingulum; b, cingulum fibers; c-e, perforant path fibers; g, subicular cell; h, CA1 pyramidal cells; i, Schaffer collaterals; collaterals of alvear fibers.

Based primarily on electrophysiological data and mapping of vasculature, Anderson and colleagues (1971) proposed that the hippocampal formation was organized in parallel lamellae stacked along the longitudinal axis. They further proposed that this lamellar organization would permit strips, or slabs, of the hippocampus to function as independent units. Although the lamellar hypothesis shaped research on the hippocampus for years to come and continues to influence modern concepts of hippocampal function, modern neuroanatomical research has revealed that the hippocampal projections are much more divergent than is suggested by the lamellar hypothesis. Indeed, the major hippocampal and dentate associational projections extend along the septotemporal axis as well as the transverse axis.

3.03.3.1 The Dentate Gyrus

The dentate gyrus is three-layered cortex whose principle cell layer is V shaped (**Figure 10**). The molecular layer lies outside the V, and the polymorphic layer lies inside the V. The beginning of the CA3 principle cell layer protrudes into the polymorphic area of the dentate gyrus. This conformation has generated some confusion over the border between CA3 and the dentate gyrus polymorphic layer, as well as the identity of these cells. In earlier nomenclatures, and occasionally in modern reports, the part of CA3 next to the dentate gyrus was sometimes called CA4. With modern techniques for defining connectional characteristics, however, it is now clear that those pyramidal cells belong to CA3.

The dentate granule cell layer contains small, very densely packed, oval cells that have a dark appearance in cell stains (**Figure 10(a)**). Each granule cell has a small number of primary dendrites (one to four) that are covered with spines. The dendrites

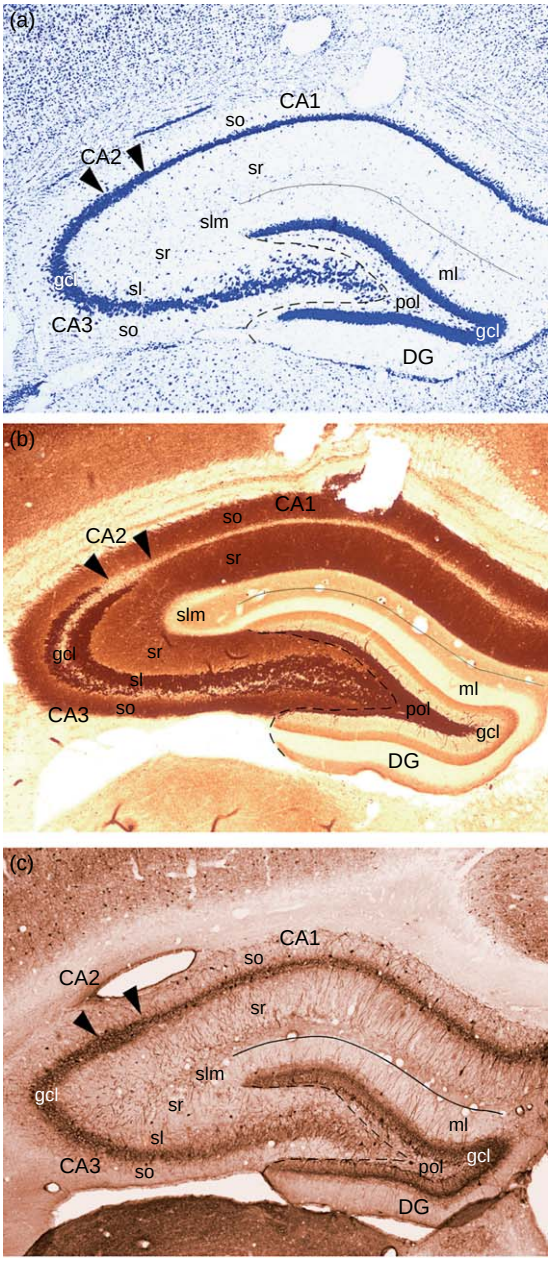

Figure 10 Photomicrographs of the hippocampal formation. (a) Nissl-stained coronal section showing the dentate gyrus (DG) and hippocampus proper, comprising fields CA3, CA2, and CA1. (b) Adjacent section stained for heavy metals using the Timm's method. (c) Parvalbumin-stained section showing the same regions. Layers of the DG are the outer molecular layer (ml), the granule cell layer (gcl), and the polymorphous layer (pol). The CA fields contain the outer stratum lacunosum-moleculare (slm), the stratum radiatum (sr), the gcl, and the stratum oriens (so). CA3 also contains the stratum lucidum (sl). The dashed line demarcates the border between the DG and CA3. The solid line shows the location of the hippocampal fissure and demarcates the border between DG and CA1.

extend into the molecular layer all the way to the hippocampal fissure. In the subgranular region, between the granule cell layer and the polymorphic layer, there are several cell types, most of which are immunoreactive for gamma-aminobutyric acid (GABA). A subset of these cells is also immunoreactive for the calcium-binding protein, parvalbumin (**Figure 10(c)**). The most prominent types are the basket cell and the axo-axonic, or chandelier, cell. The basket cell interneurons are quite large with a single, aspiny apical dendrite extending into the molecular layer and several basal dendrites extending into the polymorphic layer. Axo-axonic cells have a dendritic tree of radial branches extending through the molecular layer. The axon arborizes extensively in the granule cell layer.

The molecular layer contains mostly dendrites from cells of the granule and polymorphic layers. In material stained for heavy metals using the Timm's method, it is possible to visualize the three sublayers of the molecular layer (**Figure 10(b)**). The inner third of the molecular layer, next to the lightly colored granule cell layer, stains a reddish brown. The middle sublayer stains yellow, and the outer sublayer stains orange. Though the molecular layer mostly contains dendrites, there are a few cell types that stain for VIP, GABA, and parvalbumin. One interesting type of GABA-immunoreactive cell, with its soma in the molecular layer, has an axon restricted to the outer two-thirds of the molecular layer. Thus, its terminal field coincides with the perforant path terminations in the dentate molecular layer.

The polymorphic layer contains a number of cell types, but the best characterized is the mossy cell. These large multipolar cells are so named because of their large dendritic spines, the so-called thorny excrescences. The mossy fiber axons from the granule layer terminate on these spines. There are a number of interneurons with somata in the polymorphic layer that make inhibitory connections on the dentate granule cells. One such cell type is the hilar perforant-path (HIPP) associated cell, which has a dendritic tree in the polymorphic layer but extensive axonal arbor in the outer two-thirds of the molecular layer where the perforant path input terminates. Another type is the hilar commissural-associational pathway (HICAP) related cell, which innervates the inner molecular layer, the molecular layer perforant-path (MOPP) associated cell. The dendritic tree and axonal arbor of this cell occupy the outer two-thirds of the molecular layer, where the perforant path input terminates.

The entorhinal cortex provides the only cortical input to the dentate gyrus through the perforant pathway. There is substantial subcortical input from the septal nuclei, the hypothalamus, and the brain stem modulatory systems. The cholinergic input from the septal region originates in the medial septal nucleus and in the nucleus of the diagonal band of Broca. It is topographically organized such that cells in the medial septal areas tend to terminate septally, and cells in lateral septal structures terminate temporally. The projection terminates in the polymorphic layer. The primary hypothalamic input is from the supramamillary area and terminates in a narrow band of the molecular layer just superficial to the granule cell layer. This projection is probably excitatory and appears to target both granule cells and interneurons. The dentate gyrus receives input from each of the modulatory neurotransmitter systems in the brainstem. The noradrenergic input is from the pontine nucleus of the locus coeruleus and terminates in the polymorphic layer. The serotonergic input is from several of the raphe nuclei and also terminates in the polymorphic region. Minor dopaminergic inputs arise in the ventral tegmental area and the substantia nigra.

The only output of the dentate gyrus is the granule cell mossy fiber projection to CA3. Axon collaterals of each cell project to the full extent of the transverse axis. This is the one component of the hippocampal circuitry that does show a lamellar projection pattern. The fibers travel along or within the CA3 pyramidal layer, eventually reaching the stratum lucidum. In addition to the CA3 projection, the mossy fibers give rise to an associational connection consisting of about seven collaterals that terminate in the dentate gyrus polymorphic layer before entering CA3. The mossy fiber axons can be easily identified in Timm's preparations in which they stain very darkly (**Figure 10(b)**).

3.03.3.2 The Hippocampus Proper

The hippocampus proper consists of three fields. Proximal to distal from the dentate gyrus, they are the Ammon's horn fields CA3, CA2, and CA1 (**Figure 10**). Like the dentate gyrus, these fields are a three-layered cortex consisting of a principle layer located between cell-sparse layers. Deep to the principle cell layer is the stratum oriens. Superficial to the principle cell layer are the stratum radiatum and the stratum lacunosum-moleculare. CA3 has an additional thin layer, the stratum lucidum, which lies just superficial to the principle cell layer and deep to the stratum radiatum.

The principal cell layer consists, primarily, of pyramidal cells. The pyramidal cells in CA3 are larger and the layer thicker compared with CA1. The small and often-overlooked field CA2 contains larger pyramids, similar to CA3, but is similar to CA1 in other ways. For example, it lacks mossy fiber input. Each pyramidal cell has an apical dendritic arbor extending upward through the stratum radiatum and the stratum lacunosum-moleculare and a basal dendritic arbor extending into the stratum oriens. CA3 cells proximal to the dentate gyrus have smaller dendritic trees than the cells distal to the dentate gyrus, but overall, the dendritic arbors of cells in CA3 are larger than those in CA1. The dendritic arbors of pyramidal cells in CA2 are mixed, some with large arbors, similar to CA3, and some with small arbors, similar to CA1.

Interneurons undoubtedly play an important role in the regulation of local circuits in the hippocampus proper. Hippocampal interneurons differ in morphology, immunoreactivity, synaptic properties, laminar location, and connectivity. Hippocampal interneurons are GABAergic, but they may also be immunoreactive for somatostatin, neuropeptide Y, vasopressin, cholecystokinin, parvalbumin, calbindin, and calretinin. Most hippocampal interneurons have short axons, but there are also interneurons with long axons that project outside the hippocampal formation. We mention a few types here, but for a full discussion, see Freund and Buzsaki (1996).

One prominent interneuron type in the pyramidal cell layer is the chandelier or axo-axonic cell. The apical dendrites are radially oriented and span all superficial layers to the hippocampal fissure. The basal dendrites form a thick arbor in the stratum oriens. There is also a heterogeneous group of basket cells whose apical dendrites extend into the stratum moleculare and whose extensive basal dendrites span the entire depth of the stratum oriens. The axo-axonic cells and the basket cells are conveniently positioned to receive excitatory input from all afferents of the hippocampus proper.

Some hippocampal interneuron cell types have cell bodies in stratum oriens and innervate principal cell dendrites, similar to those described for the dentate gyrus. The oriens lacunosum-moleculare (O-LM) cells have a dense axonal arbor restricted to the stratum lacunosum moleculare. The dendritic tree is localized to layers that receive recurrent collaterals. There are many other interneuron

types including the bistratisfied, horizontal trilaminar, and radial trilaminar cells.

The CA3 pyramidal cell axons are highly collateralized and project to all CA fields both ipsilaterally and contralaterally. There is also a small collateral projection to the polymorphic layer of the dentate gyrus. CA3 does not, however, project to the subiculum, presubiculum, parasubiculum, or entorhinal cortex. The projection to CA1 is called the Schaffer collateral projection, the projections to CA3/CA2 are called the associational projections, and the projections to contralateral structures are called the commissural projections. The Schaffer collateral projection exhibits a topography such that proximal CA3 cells (closer to the dentate gyrus) tend to project to levels of CA1 that extend farther in the septal than temporal directions. Distal CA3 cells (farther from the dentate gyrus) tend to project farther in the temporal direction. In addition, projections of proximal CA3 cells tend to terminate more superficially in stratum radiatum, whereas distal CA3 cells tend to terminate more deeply in stratum radiatum and in stratum oriens. The associational projections exhibit complex transverse and radial topographies, but in general the CA3-CA3 associational projections terminate extensively along the septotemporal axis (Witter and Amaral, 2004). The pattern of the terminations of the commissural projections mirrors those of the associational projections.

Extrinsic connections of CA3 are not robust except for the substantial projection to the lateral septal nucleus. The major subcortical input to CA3 is cholinergic and arises in the medial septal nucleus and the nucleus of the diagonal band of Broca. There is a GABAergic component of the projection that terminates primarily on the GABAergic interneurons of the stratum oriens. Temporal CA3 receives a minor input from the amygdala basal nucleus, which terminates in the stratum oriens and the stratum radiatum. Inputs from piriform cortex have also been reported. A noradrenergic projection arises in the locus coeruleus, and a serontonergic input arises in the raphe nucleus.

Field CA2 can be differentiated from CA3 by the lack of mossy fiber input and the associated thorny excrescences (**Figure 10(b)**). The pyramidal cell layer also stains more intensely for parvalbumin (**Figure 10(c)**). The intrinsic projections are similar to those of CA3, although the topographies may not be the same. For example, like CA3, CA2 also provides a small collateral projection back to the dentate gyrus. Not much is known specifically about CA2 extrinsic connections, but available evidence suggests that the region is differentiated from CA3 by hypothalamic input from the supramamillary area.

Field CA1 exhibits only a weak associational/commissural connection, a feature that is in striking contrast to the robust associational network present in CA3. This difference has been interpreted as underlying some of the putative functional differences in CA3 and CA1. Other intrahippocampal connections, however, are extensive. CA1 interneurons project to CA3 and to the polymorphic layer of the dentate gyrus. The major projection from CA1, however, is to the subiculum, and that projection exhibits a strict topography. Distal CA1 projects to proximal subiculum, and proximal CA1 projects to distal subiculum. The mid-CA1 projection terminates in midproximodistal subiculum.

Field CA1 receives substantial cortical and subcortical input from extrahippocampal structures. Cortical input arrives from the perirhinal, postrhinal, and entorhinal cortices. Subcortical input to CA1 is grossly similar to the subcortical input to CA3 but differs in the details. The septal input is weaker and terminates in stratum oriens. The input from the amygdala is more substantial, especially to distal CA1. Amygdala input arises in the basal and accessory basal nuclei. There is a prominent input from the nucleus reuniens of the thalamus that terminates in the stratum lacunosum moleculare. Like CA3, CA1 receives weak noradrenergic input from the locus coeruleus and weak serotonergic input from the raphe nucleus. There is also a weak dopaminergic input.

Of the hippocampal CA fields, CA1 has the more robust extrinsic projections. The cortical projections include the perirhinal, postrhinal, entorhinal, retrosplenial cortices, preinfralimbic, and medial prefrontal cortex. In general, the septal half projects more heavily to postrhinal cortex, the medial entorhinal area, and retrosplenial cortex, whereas the temporal half of CA1 projects more heavily to perirhinal cortex, the lateral entorhinal area, and infralimbic cortex. Temporal levels also project to the anterior olfactory nucleus, the hypothalamus, nucleus accumbens, and the basal nucleus of the amygdala.

3.03.3.3 The Subiculum

The subiculum is widely considered the output structure of the hippocampal formation. In this way, it differs from its parahippocampal neighbors, the pre- and parasubiculum, which are considered to be

input structures. Like the CA fields of the hippocampus proper and the dentate gyrus, the subiculum is a three-layered cortex with a deep, polymorphic layer, a pyramidal cell layer containing the principle cells, and a molecular layer, which is continuous with the stratum lacunosum moleculare of field CA1. The subiculum can be distinguished from the proximally situated CA1 and the distally situated presubiculum by a principle cell layer that is more loosely packed. The border with CA1 is further demarcated by the widening of the middle layer of the subiculum.

The principle cell layer contains large pyramidal cells. The basal dendrites terminate in the deep part of the principle layer, and the apical dendrites extend into the molecular layer. The pyramidal cells are large and of uniform shape. Electrophysiological findings suggest that there are two populations of pyramids, though they cannot be distinguished morphologically. So-called regular spiking cells tend to be located superficially, and bursting cells tend to be located deep in the layer. Although both types are projection cells, it is possible that only the bursting cells project to the entorhinal cortex. Among the pyramids are numerous smaller cells, probably representing varied types of interneurons. Perforant pathway fibers contact GABAergic cells that stain for parvalbumin. Not much is known about subicular interneurons, but in general, the population of interneurons appears similar to that observed in field CA1 (Witter and Amaral, 2004).

The associational connections of the subiculum extend temporally from the point of origin and terminate in all layers. There is no commissural projection. There are also local associational connections confined to the pyramidal layer and the deepest part of the molecular layer. The available data suggests that the bursting pyramidal cells form a columnar network that is roughly interconnected.

Connections of the subiculum with other hippocampal structures is limited to input from CA1, which is massive. The projection exhibits a topography such that proximal CA1 projects to distal subiculum, midproximodistal CA1 projects to midsubiculum, and distal CA1 projects to proximal subiculum. The projection is not truly lamellar, however, as any part of CA1 projects to about one-third of the septotemporal extent of the subiculum.

The parahippocampal connections of the subiculum are more diverse, but the best-characterized projection is to deep layers of entorhinal cortex. Septal levels of the subiculum provide substantial input to the lateral and medial entorhinal cortices. Septal subicular input to the postrhinal cortex is equally strong, but input to perirhinal cortex, especially area 35, is modest. Temporal subiculum provides massive input to the entorhinal cortex and moderate input to perirhinal and postrhinal cortices. The subiculum also receives a substantial input from the entorhinal cortex. The entorhinal lateral band projects more strongly to septal subiculum, and the entorhinal intermediate and medial bands project more strongly to temporal subiculum. There is also modest input from the perirhinal and postrhinal cortices. Perirhinal cortex projects relatively more strongly to temporal subiculum, and the postrhinal cortex projects relatively more strongly to septal subiculum. The subiculum also projects heavily to the pre- and parasubicular cortices, though the return projections are modest (O'Mara et al., 2001).

The most prominent neocortical projections are to retrosplenial and prefrontal cortices. The distal and septal part of the subiculum projects to the ventral retrosplenial cortex. The presubiculum projections to frontal areas include the medial orbital, prelimbic, infralimbic, and anterior cingulate cortices. The retrosplenial projection is reciprocated, but available evidence suggests that the frontal projections are not.

The diverse subcortical projections target the septal complex, the amygdala, the nucleus accumbens, the hypothalamus, and the thalamus. All septotemporal levels of the subiculum project to the lateral septum, but the projection arises primarily in the proximal part. Septal input arises mainly in the nucleus of the diagonal band. The amygdala projection arises primarily in the temporal subiculum and targets the posterior and basolateral nuclei. The projection to the nucleus accumbens is topographic such that the proximal part of the septal subiculum projects to rostrolateral nucleus accumbens, and the proximal part of the temporal subiculum projects to the caudomedial nucleus accumbens. The hypothalamic projection also arises primarily in the temporal subiculum. It terminates in the medial preoptic area and the ventromedial, dorsomedial, and ventral premammillary nuclei. Finally, there are documented connections with thalamic nuclei, though the details are not well described. The proximal part of the septal subiculum projects to the anteromedial nucleus of the thalamus, but the distal part projects to the anterior thalamic complex. The latter projection is reciprocated. Temporal subiculum receives input from the nucleus reuniens. Available evidence suggests that the anteroventral nucleus of the thalamus also projects to the subiculum.

3.03.4 Conclusions

3.03.4.1 The Flow of Sensory Information through the Hippocampal System

The entorhinal cortex is widely recognized as the primary way station for sensory information on its way from the neocortex to the hippocampus. Much of the neocortical input arrives by way of the perirhinal and postrhinal cortices, but there are also direct neocortical inputs to the entorhinal cortex. The presubiculum and parasubiculum are also considered input structures for the hippocampal memory system. The distinct patterns of cortical afferentation to parahippocampal structures, the intrinsic connections, and the topography of the parahippocampal–hippocampal connections suggest that parahippocampal structures are involved in the preprocessing of sensory information provided to the hippocampus, and that there is functional diversity within the parahippocampal region. The view that parahippocampal structures have different functions is consistent with emerging evidence that there is also substantial functional diversity among hippocampal formation structures.

In Figure 11, we have attempted to schematize the flow of sensory information through the hippocampal memory system. Beginning with the input structures, perirhinal area 36 receives sensory input from visual, auditory, and somatosensory regions. Longitudinal intrinsic connections integrate across modalities before transmission to perirhinal area 35. This polymodal input to area 35 is joined by olfactory information and then passed on to entorhinal cortex, primarily the lateral band of the LEA. The postrhinal cortex receives visual and visuospatial input from posterior parietal, retrosplenial, and visual association regions, along with a small input from auditory association cortex. That information is integrated and transmitted to entorhinal cortex, primarily to the lateral band of the MEA. Presubiculum is targeted by the subiculum in a topographical manner such that septotemporal levels of the subiculum map onto septotemporal levels of the presubiculum. Subicular input is integrated with direct visuospatial input to the presubiculum, especially the septal component. That information is forwarded to the parasubiculum, the postrhinal cortex, and the MEA.

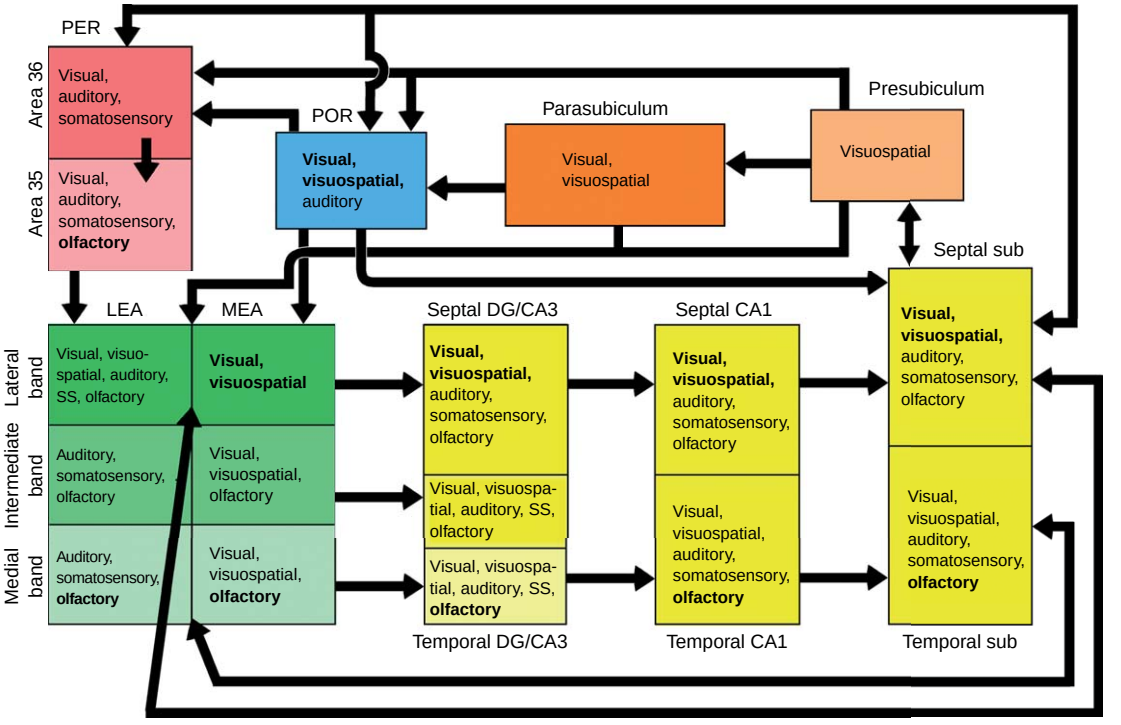

Figure 11 Diagram of the flow of sensory information through the hippocampal memory system. Black arrows indicate the primary pathways by which sensory information traverses the hippocampal memory system. Note that only the stronger connections are indicated and that emphasis is on the feedforward connections as opposed to the feedback connections. Abbreviations: CA, CA field; DG, dentate gyrus; LEA, lateral entorhinal area; MEA, medial entorhinal area; PER, perirhinal cortex; POR, postrhinal cortex; sub, subiculum.

The most septal component of the presubiculum, the area sometimes termed the postsubiculum, projects massively to the postrhinal cortex. Finally, the parasubiculum, which receives direct, but modest, visual and visuospatial input along with its subicular input, projects to postrhinal cortex and the MEA.

To summarize, information from all modalities reaches the full septotemporal extent of the hippocampus, but the degree of processing of different modalities is weighted differently along the septotemporal axis. Visual and visuospatial input is processed and elaborated along a pathway that includes the postrhinal cortex, lateral MEA, the septal hippocampal formation, septal presubiculum, parasubiculum, and then back to postrhinal cortex and lateral MEA. Olfactory information is less segregated but follows a pathway that includes the perirhinal cortex, medial LEA, and temporal hippocampal formation structures. Thus, there appears to be functional diversity in the processing of sensory information that is organized along the septotemporal axis; visual and visuospatial information is predominant in the septal hippocampus, and olfactory information is predominant in the temporal hippocampus.

3.03.4.2 The Comparative Anatomy of the Hippocampal System

As indicated earlier, all components of the parahippocampal region and the hippocampal formation are represented in both the rodent and the primate brain. Many of the connectional principles are also conserved. Taking into account the differences in brain size and sensory processing needs, cortical afferentation of the parahippocampal structures is similar across species. Additionally, the available evidence suggests that the architecture of the perforant pathway is similar in the primate and rodent brains. In the monkey and the rat, the perforant pathway projections to the dentate gyrus, CA3, and CA2 originate in layer II of the entorhinal cortex. Also in both, the projections to CA1 and the subiculum originate in layer III. In addition, the terminations of the projections originating in entorhinal layer III exhibit a transverse topography. The rostral entorhinal cortex in the monkey and the lateral entorhinal area in the rat project to the border of the CA1 and subiculum; the caudal entorhinal cortex in the monkey and the medial entorhinal area in the rat project to proximal CA1 and distal subiculum (Witter, 1986, 1993, Amaral, 1993). The intrinsic connections of the monkey entorhinal cortex also exhibit patterns similar to the lateral to medial bands of intrinsic connectivity observed in the rodent entorhinal cortex. Taken together, the evidence suggests that both the rat and monkey hippocampal memory systems are excellent models for the medial temporal lobe memory system in the human brain.

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3.04 Declarative Memory System: Amnesia

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3.04.1 Introduction

Memory is not a single entity but is composed of several separate systems (*See* Chapters 1.02, 1.04, 3.02; Squire, 1992; Schacter and Tulving, 1994). Long-term memory can be divided into several parallel memory systems. The major distinction is between declarative and nondeclarative memory. Declarative memory refers to conscious knowledge of facts and events. Nondeclarative memory refers to a collection of nonconscious knowledge systems that provide for the capacity of skill learning, habit formation, the phenomenon of priming, and certain other ways of interacting with the world. The terms 'explicit memory' and 'implicit memory' are sometimes used as well and have approximately the same meanings as declarative and nondeclarative memory, respectively.

The brain is organized such that declarative memory is a distinct and separate cognitive function, which can be studied in isolation from perception and other intellectual abilities. Significant information about how memory is organized has come from the study of patients with memory disorders (amnesia) and from animal models of amnesia (*See* Chapter 3.09). Amnesia (neurological amnesia and functional amnesia) refers to difficulty in learning new information or in remembering the past.

Neurological amnesia is characterized by a loss of declarative memory. It occurs following brain injury or disease that damages the medial temporal lobe or diencephalon. Neurological amnesia causes severe difficulty in learning new facts and events (anterograde amnesia). Patients with neurological amnesia also typically have some difficulty remembering facts and events that were acquired before the onset of amnesia (retrograde amnesia).

Functional amnesia is rarer than neurological amnesia and can occur as the result of an emotional trauma. It presents as a different pattern of anterograde and retrograde memory impairment than neurological amnesia. Functional amnesia is characterized by a profound retrograde amnesia with little or no anterograde amnesia. In some cases, patients fully recover. Functional amnesia is a psychiatric

disorder, and no particular brain structure or region is known to be damaged.

3.04.2 Etiology of Neurological Amnesia

Neurological amnesia results from a number of conditions including Alzheimer's disease or other dementing illnesses (*See* Chapter 3.29), temporal lobe surgery, chronic alcohol abuse, encephalitis, head injury, anoxia, ischemia, infarction, and the rupture and repair of an anterior communicating artery aneurism. The common factor in all of these conditions is the disruption of normal function in one of two areas of the brain – the medial aspects of the temporal lobe, and the diencephalic midline. Bilateral damage results in global amnesia, and unilateral damage results in material-specific amnesia. Specifically, left-sided damage affects memory for verbal material, while right-sided damage affects memory for nonverbal material (e.g., memory for faces and spatial layouts).

3.04.3 Anatomy

Well-studied cases of human amnesia and animal models of amnesia provide information about the neural connections and structures that are damaged. In humans, damage limited to the hippocampus itself is sufficient to cause moderately severe amnesia. For example, in one carefully studied case of amnesia (patient R.B.), the only significant damage was a bilateral lesion confined to the CA1 field of the hippocampus (Zola-Morgan et al., 1986). The severity of memory impairment is exacerbated by additional damage outside of the hippocampus. Thus, severe amnesia results when damage extends beyond CA1 to the rest of the hippocampus and to the adjacent cortex. (Animal studies later elucidated the critical anatomical components of this memory system; see following.) One well-studied case (H.M.) had surgery in 1953 to treat severe epilepsy. Most of the hippocampus and much of the surrounding medial temporal lobe cortices were removed bilaterally (the entorhinal cortex and most of the perirhinal cortex). Although the surgery was successful in reducing the frequency of H.M.'s seizures, it resulted in a severe and persistent amnesia.

It is also possible through structural magnetic resonance imaging (MRI) to detect and quantify the neuropathology in amnesic patients. Many patients with restricted hippocampal damage have an average reduction in hippocampal volume of about 40%. Two such patients whose brains were available for detailed, postmortem neurohistological analysis (patients L.M. and W.H.) proved to have lost virtually all the neurons in the CA fields of the hippocampus. These observations suggest that a reduction in hippocampal volume of approximately 40%, as estimated from MRI scans, likely indicates the near complete loss of hippocampal neurons (Rempel-Clower et al., 1996). The amnesic condition is associated with neuronal death and tissue collapse, but the tissue does not disappear altogether because fibers and glia remain. In patients with larger lesions of the medial temporal lobe, volume reduction as measured by MRI is more dramatic. For example, patient E.P., who became amnesic as a result of viral encephalitis, has damage to all of the perirhinal cortex, all of the entorhinal cortex, virtually all of the hippocampus, and most of the parahippocampal cortex (Stefanacci et al., 2000).

As questions about amnesia and the function of medial temporal lobe structures have become more specific, it has become vital to obtain detailed, quantitative information about the damage in the patients being studied. In addition, single-case studies are not nearly as useful as group studies involving well-characterized patients. In the case of patients with restricted hippocampal damage, one can calculate the volume of the hippocampus itself as a proportion of total intracranial volume. One can also calculate the volumes of the adjacent medial temporal lobe structures (the perirhinal, entorhinal, and parahippocampal cortices) in proportion to intracranial volume. Last, when there is extensive damage to the medial temporal lobe, it is important to calculate the volumes of lateral temporal cortex and other regions that might be affected. It is important to characterize patients in this way in order to address the kinds of questions now being pursued in studies of memory and the brain.

Functional MRI (fMRI) of healthy individuals who are engaged in learning and remembering allows one to ask what brain areas are associated with these memory processes. The same tasks that are administered to amnesic patients can also be administered to healthy individuals while their brain activation is measured. FMRI reveals activation during these tasks of learning and remembering in the same structures that, when damaged, cause amnesia.

To understand the anatomy of human amnesia, and ultimately the anatomy of normal memory, animal models of human amnesia have been established in the monkey (**Figure 1**) and in the rodent. An animal

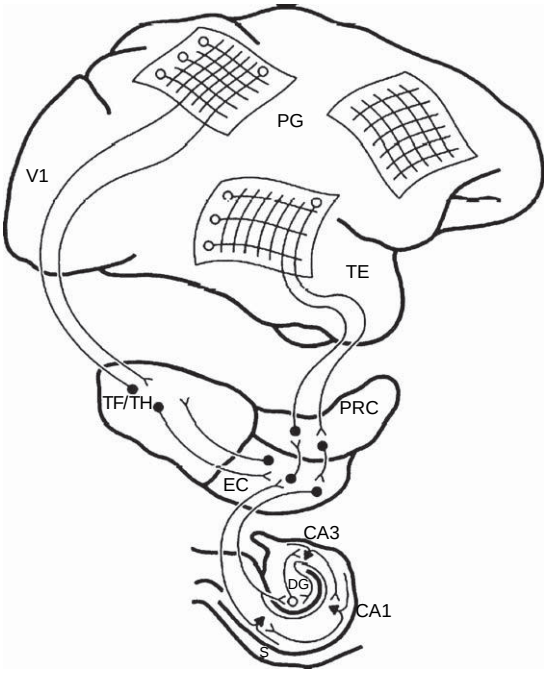

Figure 1 Schematic drawing of primate neocortex together with the structures and connections in the medial temporal region important for establishing long-term memory. The networks in the cortex show putative representations concerning visual object quality (in area TE) and object location (in area PG). If this disparate neural activity is to cohere into a stable, long-term memory, convergent activity must occur along projections from these regions to the medial temporal lobe. Projections from neocortex arrive initially at the parahippocampal gyrus (TF/TH) and perirhinal cortex (PRC) and then at entorhinal cortex (EC), the gateway to the hippocampus. Further processing of information occurs in the several stages of the hippocampus, first in the dentate gyrus (DG) and then in the CA3 and CA1 regions. The fully processed input eventually exits this circuit via the subiculum (S) and the entorhinal cortex, where widespread efferent projections return to neocortex. The hippocampus and adjacent structures are thought to support the stabilization of representations in distributed regions of neocortex (e.g., TE and PG) and to support the strengthening of connections between these regions. Subsequently, memory for a whole event (e.g., a memory that depends on representations in both TE and PG) can be revivified even when a partial cue is presented. Damage to the medial temporal lobe system causes anterograde and retrograde amnesia. The severity of the deficit increases as damage involves more components of the system. Once sufficient time has passed, the distributed representations in neocortex can operate independently of the medial temporal lobe. (This diagram is a simplification and does not show diencephalic structures involved in memory function.)

model of human amnesia was established in the monkey in the early 1980s (Mishkin, 1982; Squire and Zola-Morgan, 1983). Following lesions of the bilateral medial temporal lobe or diencephalon, memory impairment is exhibited on the same kinds of tasks of new learning ability that human amnesic patients fail. The same animals succeed at tasks of motor skill learning. They also do well at learning pattern discriminations, which share with motor skills the factors of incremental learning and repetition over many trials.

Systematic and cumulative work in monkeys, using the animal model, succeeded in identifying the system of structures in the medial temporal lobe essential for memory (Squire and Zola-Morgan, 1991). The important structures are the hippocampal region (hippocampus proper, dentate gyrus, and subicular complex) and adjacent, anatomically related structures (entorhinal cortex, perirhinal cortex, and parahippocampal cortex). The amygdala, although critical for aspects of emotional learning (Davis, 1994; LeDoux, 1996) and for the enhancement of declarative memory by emotion (Adolphs et al., 1997), is not a part of the declarative memory system itself. The consistency between the available neuroanatomical information from humans and the findings from monkeys have considerably illuminated the description of memory impairment and its anatomical basis. These lines of work have also made it possible to pursue parallel studies in simpler animals like rats and mice. As a result, one can now study memory in rodents and have some confidence that what one learns will be relevant to the human condition.

Anatomical connections from different parts of the neocortex enter the medial temporal lobe at different points (Suzuki and Amaral, 1994a, b), which raises the question of whether these medial temporal lobe structures play different roles in declarative memory. For example, visual association cortex projects more strongly to the perirhinal cortex than to the parahippocampal cortex, whereas the parietal cortex projects to the parahippocampal cortex but not to the perirhinal cortex. Further, the hippocampus lies at the end of the medial temporal lobe system and is a recipient of convergent projections from each of the structures that precedes it in the hierarchy. The possibility that different medial temporal lobe structures make different contributions to memory has been addressed in a number of studies in monkeys, comparing performance on memory tasks following damage to different components of the medial temporal lobe. This work has shown that the severity of memory impairment depends on the locus and extent of damage within the medial temporal lobe memory system. Damage limited to the hippocampal region causes significant memory impairment, but damage to the adjacent cortex increases the severity of memory impairment. It is also important to note that the discovery that larger medial temporal lobe lesions produce more severe amnesia than smaller lesions is compatible with the idea that structures within the medial temporal lobe might make qualitatively different contributions to memory function. Indeed, damage to the perirhinal cortex especially impairs object recognition, whereas damage to the parahippocampal cortex especially impairs spatial memory.

Another important brain area for memory is the diencephalon. The important structures include the mediodorsal thalamic nucleus, the anterior thalamic nucleus, the internal medullary lamina, the mamillary nuclei, and the mammillo-thalamic tract. Monkeys with medial thalamic lesions exhibit an amnesic disorder, and monkeys with mammillary nucleilesious exhibit a modest impairment. Because diencephalic amnesia resembles medial temporal lobe amnesia in the pattern of sparing and loss, these two regions likely form an anatomically linked, functional system.

3.04.4 The Nature of Amnesia

Amnesic individuals exhibit significant memory impairment. Yet, despite their memory deficit, patients have intact ability for some forms of new learning and memory (see section 3.04.5.2). Also, they have intact immediate memory and memory for a great deal of information from the past, especially childhood. In addition, patients with neurological amnesia can have intact intelligence test scores, intact language and social skills, and intact perceptual abilities. In fact, amnesic patients can appear quite normal on casual observation. It is only when one interrogates their capacity for new learning of conscious knowledge that the impairment becomes evident.

3.04.4.1 Impairment in Declarative Memory

It is important to appreciate that amnesic patients are not impaired at all kinds of long-term memory. The major distinction is between declarative and nondeclarative memory. Declarative memory is the kind of memory impaired in amnesia. Declarative memory refers to the capacity to remember the facts and events of everyday life. It is the kind of memory that is meant when the term 'memory' is used in ordinary language. Declarative memory can be brought to mind as a conscious recollection. Declarative memory provides a way to model the external world, and in this sense it is either true or false. The stored representations are flexible in that they are accessible to multiple response systems and can guide successful performance under a wide range of test conditions. Last, declarative memory is especially suited for rapid learning and for forming and maintaining associations between arbitrarily different kinds of material (e.g., learning to associate two different words).

3.04.4.2 Anterograde Amnesia

Amnesia is characterized especially by profound difficulty in new learning, and this impairment is referred to as anterograde amnesia. Amnesia can occur as part of a more global dementing disorder that includes other cognitive deficits such as impairments in language, attention, visuospatial abilities, and general intellectual capacity. However, when damage is restricted to the medial temporal lobe or midline diencephalon, amnesia can also occur in the absence of other cognitive deficits and without any change in personality or social skills. In this more circumscribed form of amnesia, patients have intact intellectual functions and intact perceptual functions, even on difficult tests that require the ability to discriminate between highly similar images containing overlapping features (Levy et al., 2005; Shrager et al., 2006). In some patients with memory impairment,

visual perceptual deficits have been described (Lee et al., 2005a,b). In these cases, damage might extend laterally, beyond the medial temporal lobe, and quantitative brain measurements are needed in order to understand what underlies these deficits. Thus, present data support the idea that declarative memory is separable from other brain functions.

Amnesic patients are impaired on tasks of new learning, regardless of whether memory is tested by free (unaided) recall, recognition (e.g., presenting an item and asking whether it was previously encountered or not), or cued recall (e.g., asking for recall of an item when a hint is provided). For instance, in a standard test of free recall, participants are read a short prose passage containing 21 segments. They are then asked to recall the passage immediately and after a 12-min delay. Amnesic patients with damage to the medial temporal lobe do well at immediate recall but are impaired at the delay, usually recalling zero segments (Squire and Shimamura, 1986). Amnesic patients are also impaired on recognition tests, where a list of words is presented and participants try to decide (yes or no) if each word had been presented in a recent study list (Squire and Shimamura, 1986). Last, in a cued recall task, individuals study a list of word pairs, such as ARMY–TABLE. During test, they are presented with one word from each pair (ARMY), and they are asked to recall the word that was paired with it (TABLE). Amnesic patients are impaired on this task as well.

The memory impairment in amnesia involves both difficulty in learning factual information (semantic memory) as well as difficulty in learning about specific episodes and events that occurred in a certain time and place (episodic memory). The term 'semantic memory' is often used to describe declarative memory for organized world knowledge (Tulving, 1983). In recalling this type of information, one need not remember any particular past event. One needs only to know about certain facts. Episodic memory, in contrast, is autobiographical memory for the events of one's life (Tulving, 1983). Unlike semantic memory, episodic memory stores spatial and temporal landmarks that identify the particular time and place when an event occurred. Both episodic memory and semantic memory are declarative.

Formal experiments have compared directly the ability to accomplish fact learning (or semantic memory ability) and event learning (or episodic memory ability). In one experiment, amnesic patients were taught new factual knowledge in the form of three-word sentences (e.g., MEDICINE cured HICCUP). Then, during testing, sentence fragments were presented with the instruction to complete each fragment with a word that had been studied (e.g., MEDICINE cured _____). The amnesic patients were similarly impaired on tests of fact memory (what word completed the sentence fragment) and on tests of event memory (what specific events occurred during the testing session) (Hamann and Squire, 1995). Taken together, the data favor the view that episodic memory and semantic memory are similarly impaired in amnesia (Squire and Zola, 1998). Semantic knowledge is thought to accumulate in cortical storage sites as a consequence of experience and with support from the medial temporal lobe. In contrast, episodic memory is thought to require these cortical sites and the medial temporal lobes to work together with the frontal lobes in order to store when and where a past experience occurred (Janowsky et al., 1989).

Last, the memory deficit in amnesia is global and encompasses all sensory modalities (e.g., visual, auditory, olfactory). That is, memory is impaired regardless of the kind of material that is presented and the sensory modality in which information is presented. For example, recognition memory of amnesic patients was assessed for line drawings of objects (visual), designs (visual), and odors (olfactory) (Levy et al., 2004). The patients were impaired on all three tasks, showing that their impairment spans the visual and olfactory domains. Formal experiments have also demonstrated recognition memory impairment for auditory stimuli.

3.04.4.3 Remembering versus Knowing and Recollection versus Familiarity

Remembering (R) is meant to refer to the circumstance when an item elicits a conscious recollection that includes information about the context in which the item was learned. Knowing (K) is meant to refer to a circumstance when an item appears familiar, but memory for the original learning context is not available (*See* Chapter 2.17; Tulving, 1985). Thorough studies of healthy individuals indicate that in practice, remembering and knowing responses are closely related to the strength of a memory and that items given K responses are often items for which information is also available about the original learning context (Wixted and Stretch, 2004). In any case, formal studies show that both R and K responses are impaired in amnesia. In one such experiment, amnesic patients and control participants were given a

recognition test 10 min after studying words. For each word, participants indicated whether they remembered it (R) or whether they knew that the word was presented but had no recollection about it (K). The patients were impaired for both R and K responses, and they performed like a control group that was tested after 1 week. That is, the patients were similarly impaired for R and K responses (Knowlton and Squire, 1995; also see Manns et al., 2003). Accordingly, the evidence suggests that remembering and knowing are two different expressions of declarative memory.

A distinction closely related to remembering and knowing is recollection and familiarity. Recollection involves remembering the contextual associations of the original learning experience, whereas familiarity does not require any recollection of the original experience. It has sometimes been proposed that recollection relies on the hippocampus, while familiarity can be supported by the adjacent cortex within the medial temporal lobe. In this view, patients with damage limited to the hippocampus should be selectively, or disproportionately, impaired at recollecting information and less impaired at recognizing material when it can be supported by familiarity.

An alternative view is that recognition decisions are based on a unidimensional strength-of-memory variable that combines estimates of recollection and familiarity. Thus, as in the case of remembering and knowing, a capacity for recollection is likely to be associated with strong memories and familiarity with weaker memories (Wixted, 2007). In one study, patients with hippocampal damage were impaired on a recognition memory test, where they gave confidence judgments (scale of 1–6). The results indicated that both processes, recollection and familiarity, were operative in the absence of the hippocampus (Wais et al., 2006). Last, electrophysiological recordings from patients being evaluated for epilepsy surgery found neurons in the hippocampus that responded to familiar images during a recognition test. These familiarity signals were present even when recollection failed (i.e., there was a familiarity signal in the hippocampus regardless of whether any recollection had occurred) (Rutishauser et al., 2006).

3.04.4.4 Retrograde Amnesia

In addition to impaired new learning, damage to the medial temporal lobe also impairs memories that were acquired before the onset of amnesia. This type of memory loss is referred to as retrograde amnesia. Retrograde amnesia is usually temporally graded. That is, information acquired in the distant past (remote memory) is spared relative to more recent memory (Ribot, 1881). The extent of retrograde amnesia can be relatively short and encompass only 1 or 2 years, or it can be more extensive and cover decades. Even then, memories for the facts and events of childhood and adolescence are typically intact. Indeed, severely amnesic patients can produce detailed autobiographical narratives of their early life. These memories were indistinguishable from the memories of healthy individuals with respect to the number of details, the duration of the narratives, and the number of prompts needed to begin a narrative (Bayley et al., 2003, 2005b).

The severity and extent of retrograde amnesia is determined by the locus and extent of damage. Patients with restricted hippocampal damage have limited retrograde amnesia covering a few years prior to the onset of amnesia. Patients with large medial temporal lobe damage have extensive retrograde amnesia covering decades. When damage occurs beyond the brain system that supports declarative memory, which can result from conditions such as encephalitis and head trauma, retrograde amnesia sometimes can be ungraded and extensive and include the facts and events of early life.

Because the study of human retrograde amnesia is based almost entirely on findings from retrospective tests, the clearest data about retrograde amnesia gradients come from studies using experimental animals, where the delay between initial learning and a lesion can be manipulated directly. Findings from such studies make three important points. First, temporal gradients of retrograde amnesia can occur within long-term memory. That is, retrograde amnesia does not reflect simply the vulnerability of a short-term memory that has not yet been converted into a long-term memory. Second, after a lesion, remote memory can be even better than recent memory. Third, lesions can spare old weak memories while disrupting strong recent ones, showing that it is the age of the memory that is critical.

These same points can be illustrated by a study of rabbits given trace eyeblink conditioning. Trace conditioning is a variant of classical conditioning in which the condition stimulus (CS), such as a tone, is presented and terminated, and then a short interval (e.g., 500 ms) is imposed before the presentation of the unconditioned stimulus (US). In normal rabbits, forgetting occurs gradually after training, and retention of the conditioned response is much poorer

30 days after training than after only 1 day. Nevertheless, complete aspiration of the hippocampus 1 day after training abolished the strong 1-day-old memory, whereas the same lesion made 30 days after training had no effect on the weaker 30-day-old memory (Kim et al., 1995).

The sparing of remote memory relative to more recent memory illustrates that the brain regions damaged in amnesia are not the permanent repositories of long-term memory. Instead, memories undergo a process of reorganization and consolidation after learning, during which time the neocortex becomes more important. During the process of consolidation, memories are vulnerable if there is damage to the medial temporal lobe or diencephalon. After sufficient time has passed, storage and retrieval of memory no longer require the participation of these brain structures. Memory is at that point supported by neocortex. The areas of neocortex important for long-term memory are thought to be the same regions that were initially involved in the processing and analysis of what was to be learned. Thus, the neocortex is always important, but the structures of the medial temporal lobe and diencephalon are also important during initial learning and during consolidation.

3.04.4.5 Spatial Memory

Since the discovery of hippocampal place cells in the rodent (O'Keefe and Dostrovsky, 1971), an influential idea has been that the hippocampus creates and uses spatial maps and that its predominant function is to support spatial memory (*See* Chapter 2.11; O'Keefe and Nadel, 1978). Discussions of amnesia have therefore focused especially on the status of spatial memory. It is clear that spatial memory is impaired in human amnesia. Amnesic patients are impaired on tests that assess their knowledge of the spatial layout of an environment, and they are also impaired when asked to navigate to a destination in a virtual environment (Maguire et al., 1996; Spiers et al., 2001). Similarly, the noted patient H.M. was impaired at recalling object locations (Smith, 1988). It is also clear, though, that amnesic patients are impaired on memory tests that have no obvious spatial component, such as recall or recognition of items (Squire and Shimamura, 1986). Furthermore, formal experiments that directly compared spatial and nonspatial memory in amnesic patients showed that the patients were similarly impaired on tests of spatial memory and tests of nonspatial memory. There was no special difficulty with the test of spatial memory (Cave and Squire, 1991).

As is the case with nonspatial memory, remote spatial knowledge is intact. One well-studied patient with large medial temporal lobe lesions and severe amnesia (E.P.) was able to mentally navigate his childhood neighborhood, use alternate and novel routes to describe how to travel from one place to another, and point correctly to locations in the neighborhood while imagining himself oriented at some other location (Teng and Squire, 1999). These findings show that the medial temporal lobe is not needed for the long-term storage of spatial knowledge and does not maintain a spatial layout of learned environments that is necessary for successful navigation. Accordingly, the available data support the view that the hippocampus and related medial temporal lobe structures are involved in learning new facts and events, both spatial and nonspatial. Further, these structures are not repositories of long-term memory, either spatial or nonspatial.

3.04.5 Spared Learning and Memory Abilities

It is a striking feature of amnesia that many kinds of learning and memory are spared. Memory is not a unitary faculty of the mind but is composed of many parts that depend on different brain systems. Amnesia impairs only long-term declarative memory and spares immediate and working memory, as well as nondeclarative memory. Immediate memory and working memory can be viewed as a collection of temporary memory capacities that operate shortly after material is presented. Nondeclarative memory refers to a heterogeneous collection of abilities, all of which afford the capacity to acquire knowledge nonconsciously. Nondeclarative memory includes motor skills, perceptual and cognitive skills, priming, adaptation-level effects, simple classical conditioning, habits, and phylogenetically early forms of experience-dependent behavior like habituation and sensitization. In these cases, memory is expressed through performance rather than recollection.

3.04.5.1 Immediate and Working Memory

Amnesic patients have intact immediate memory. Immediate memory refers to what can be held actively in mind beginning the moment that information is received. It is the information that forms the focus of

current attention and that occupies the current stream of thought. The capacity of immediate memory is quite limited. This type of memory is reflected, for example, in the ability to repeat back a short string of digits. Intact immediate memory explains why amnesic patients can carry on a conversation and appear quite normal to the casual observer. Indeed, if the amount of material to be remembered is not too large (e.g., a three-digit number), then patients can remember the material for minutes, or as long as they can hold it in mind by rehearsal. One would say in this case that the patients have carried the contents of immediate memory forward by engaging in explicit rehearsal. This rehearsal-based activity is referred to as working memory, and it is independent of the medial temporal lobe system (*See* Chapter 2.04). The difficulty for amnesic patients arises when an amount of information must be recalled that exceeds immediate memory capacity (typically, when a list of eight or more items must be remembered) or when information must be recalled after a distraction-filled interval or after a long delay. In these situations, when the capacity of working memory is exceeded, patients will remember fewer items than their healthy counterparts.

The intact capacity for immediate and working memory was well illustrated by patient H.M. when he was asked to remember the number 584. H.M. was left to himself for several minutes, and he was able to retain this information by working out mnemonic schemes and holding the information continuously in mind. Yet, only a minute or two later, after his attention had been directed to another task, he could not remember the number or any of his mnemonic schemes for holding the number in mind.

3.04.5.2 Nondeclarative Memory

Nondeclarative memory refers to a collective of nonconscious knowledge systems, but it is not itself a brain-systems construct. Rather, the term encompasses several different kinds of memory. Nondeclarative forms of memory have in common the feature that memory is nonconscious. Memory is expressed through performance and does not require reflection on the past or even the knowledge that memory is being influenced by past events.

The following examples illustrate that nondeclarative memory is distinct from declarative memory. It is spared in amnesia, and it operates outside of awareness. Nondeclarative forms of memory depend variously on the neostriatum, the amygdala, and the cerebellum and on processes intrinsic to neocortex (**Figure 2**).

3.04.5.2.1 Motor Skills and Perceptual Skills

One can learn how to ride a bicycle but be unable to describe what has been learned, at least not in the same sense that one might recall riding a bicycle on a particular day with a friend. This is because the learning of motor skills is largely nondeclarative, and amnesic

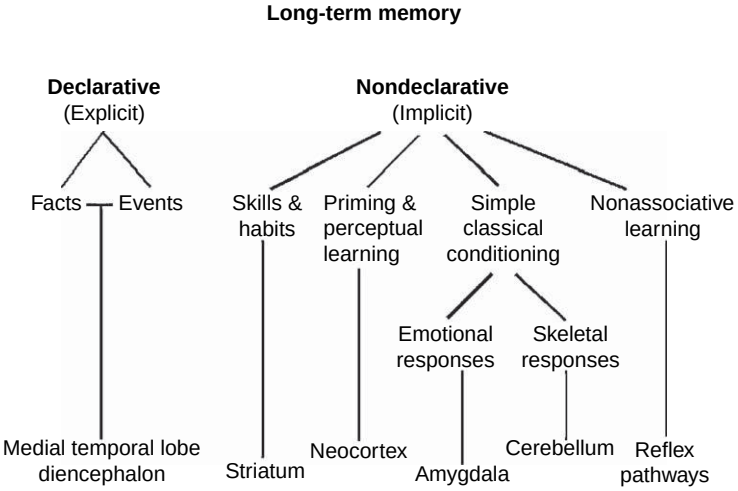

Figure 2 Classification of mammalian long-term memory systems. The taxonomy lists the brain structures thought to be especially important for each form of declarative and nondeclarative memory. In addition to the central role of the amygdala in emotional learning, it is also able to modulate the strength of both declarative and nondeclarative learning.

patients can learn these skills at a normal rate. In one experiment, amnesic patients and control participants performed a serial reaction-time task, in which they responded successively to a sequence of four illuminated spatial locations. The task was to press one of four keys as rapidly as possible as soon as the location above each key was illuminated. The amnesic patients learned the sequence, as did the normal participants, as measured by their decreased reaction times for pressing a key when it was illuminated. When the sequence was changed, the reaction times increased for both groups. Strikingly, the amnesic patients had little or no declarative knowledge of the sequence, though they had learned it normally (Reber and Squire, 1994).

Perceptual skills are also often intact in amnesic patients. These include such skills as reading mirror-reversed print and searching a display quickly to find a hidden letter. In formal experiments, amnesic patients acquired perceptual skills at the same rate as individuals with intact memory, even though the patients did not remember what items were encountered during the task and sometimes did not remember the task itself. For example, amnesic patients learned to read mirror-reversed words at a normal rate and then retained the skill for months. Yet, after they had finished the mirror-reading task, they could not remember the words that they had read, and in some cases they could not remember that they had ever practiced the mirror-reading skill on a previous occasion (Cohen and Squire, 1980).

3.04.5.2.2 Artificial Grammar Learning

Another kind of learning that is intact in amnesia is the learning of artificial grammars. In an artificial grammar–learning task, participants are presented with a series of letter strings that are generated according to a rule system that specifies what letter sequences are permissible. After viewing these letter strings, participants are told for the first time that the letter strings were formed according to a set of rules and that their task is to decide for a new set of letter strings whether each one is formed by the same set of rules as the set they had just studied. Even though individuals often report that they are simply guessing, they are able to classify new letter strings as grammatical or nongrammatical. Amnesic patients classify items as grammatical or nongrammatical as well as healthy individuals, despite being impaired at recognizing the letter strings that were used during the initial training (Knowlton et al., 1992; Knowlton and Squire, 1994, 1996).

3.04.5.2.3 Category Learning

In tasks involving category learning, participants are exposed to several exemplars of a single category. Then, participants try to classify new items according to whether they are or are not members of that category. In addition, participants identify the prototype, or central tendency of the category, as a member of the learned category more readily than the items used during training, even when the prototype itself was not presented. Amnesic patients are also able to classify items according to a learned category, despite a severe deficit in recognizing the items that were used to train the category (Knowlton and Squire, 1993). In one experiment, amnesic patients and control participants were shown a series of dot patterns formed by distorting a randomly generated pattern that was defined arbitrarily as the prototype of the category. Having seen a series of dot patterns, all of which were distortions of an underlying prototype, participants then were able to discriminate new dot patterns that belonged to the training category from other dot patterns that did not. Amnesic patients performed as well as control participants, even though the patients were severely impaired at recognizing which dot patterns had been presented for training. It is worth noting that it has been suggested that a model assuming a single memory system was able to account for the dissociation between categorization and recognition (Nosofsky and Zaki, 1998).

3.04.5.2.4 Priming

Priming refers to an improved ability to identify or produce a word or other stimulus as a result of its prior presentation (*See* Chapter 3.12). The first encounter with an item results in a representation of that item, and that representation then allows it to be processed more efficiently than items that were not encountered recently. For example, suppose that line drawings of a dog, hammer, and airplane are presented in succession, with the instruction to name each item as quickly as possible. Typically, about 800 ms are needed to produce each name aloud. If in a later test these same pictures are presented intermixed with new drawings, the new drawings will still require about 800 ms to name, but now the dog, hammer, and airplane are named about 100 ms more quickly. The improved naming time occurs independently of whether one remembers having seen the items earlier. Amnesic patients exhibit this effect at full strength, despite having poor memory of seeing the items earlier.

The dissociation between intact priming and impaired recognition memory in amnesic patients can be particularly compelling. One study investigated priming in patient E.P., who is so severely amnesic that he exhibits no detectable declarative memory (Hamann and Squire, 1997). E.P. sustained complete bilateral damage to the medial temporal lobe as the result of herpes simplex encephalitis. Two tests of priming were given. In one of the priming tests, word stem completion, participants were shown a short word list, which included, for example, the words MOTEL and ABSENT. Then, they were shown the fragments MOT___ and ABS___, with instructions to form the first word that comes to mind. Participants had a strong tendency (30–50%) to produce the words that were recently presented. (The probability was about 10% that participants would produce these words if they had not been presented recently.) In addition, two parallel tests of recognition memory for words were given: alternative forced-choice and yes–no recognition. E.P. performed entirely normally on the two priming tests but performed at chance (50%) on the recognition tests.

In another experiment, participants saw words slowly clearing from a mask. They tried to identify each word as quickly as possible and then make a recognition judgment (old/new) about whether the word had been presented in a preceding study phase. Amnesic patients exhibited intact fluency (the tendency to label those words that were identified more quickly as old) and intact priming, but their recognition was impaired (Conroy et al., 2005). These results support the idea that priming depends on brain structures independent of the medial temporal lobe, and they show that the combined effects of priming and fluency are not sufficient to increase recognition performance.

3.04.5.2.5 Adaptation-Level Effects

Adaptation-level effects refer to the finding that experience with one set of stimuli influences how a second set of stimuli is perceived (e.g., their heaviness or size). For example, experience with light-weighted objects subsequently causes other objects to be judged as heavier than they would be if the light-weighted objects had not been presented. Amnesic patients show this effect to the same degree as healthy individuals, even when they experience the first set of objects with one hand and then make judgments with the other hand. However, they have difficulty remembering their prior experience accurately (Benzing and Squire, 1989).

3.04.5.2.6 Classical Conditioning

Classical conditioning refers to the development of an association between a previously neutral stimulus (CS) and an unconditioned stimulus (US), and is a quintessential example of nondeclarative memory. One of the best-studied examples of classical conditioning in humans is delay conditioning of the eyeblink response. It is reflexive and automatic and depends solely on structures below the forebrain, including the cerebellum and associated brainstem circuitry (Thompson and Krupa, 1994). In a typical conditioning procedure, a tone repeatedly precedes a mild airpuff directed to the eye. After a number of pairings, the tone comes to elicit an eyeblink in anticipation of the airpuff. Amnesic patients acquire and retain the tone–airpuff association at the same rate as healthy individuals. In both groups, awareness of the temporal contingency between the tone and the airpuff is unrelated to successful conditioning.

In trace conditioning, a brief interval of 500–1000 ms is interposed between the CS and the US. This form of conditioning requires the hippocampus (McGlinchey-Berroth et al., 1997). Formal experiments suggest that trace conditioning is hippocampus dependent because it requires the acquisition and retention of conscious knowledge during the course of the conditioning session (Clark and Squire, 1998). Only those who became aware of the CS-US relationship acquired differential trace conditioning. There was a correlation between measures of awareness taken after the conditioning and trace conditioning performance itself, whereas there was no correlation between awareness and conditioning performance on a delay conditioning task.

3.04.5.2.7 Habit Learning

Habit learning refers to the gradual acquisition of associations between stimuli and responses, such as learning to make one choice rather than another. Habit learning depends on the neostriatum (basal ganglia). Many tasks can be acquired either declaratively, through memorization, or nondeclaratively as a habit. For example, healthy individuals will solve many trial-and-error learning tasks quickly by simply engaging declarative memory and memorizing which responses are correct. In this circumstance, amnesic patients are disadvantaged. However, tasks can also be constructed that defeat memorization

strategies, for example, by making the outcomes on each trial probabilistic. In such a case, amnesic patients and healthy individuals learn at the same gradual rate (Knowlton et al., 1992).

It is also true that severely amnesic patients who have no capacity for declarative memory can gradually acquire trial-and-error tasks, even when the task can be learned declaratively by healthy individuals. In this case they succeed by engaging habit memory. This situation is nicely illustrated by the eight-pair concurrent discrimination task, which requires individuals to learn the correct object in each of eight object pairs. Healthy individuals can learn all eight pairs in one or two test sessions. Severely amnesic patients acquire this same task over many weeks, even though at the start of each session they cannot describe the task, the instructions, or the objects. It is known that this task is acquired at a normal (slow) rate by monkeys with medial temporal lobe lesions, and that monkeys with lesions of the neostriatum (basal ganglia) are impaired. Thus, humans appear to have a robust capacity for habit learning that operates outside of awareness and independently of the medial temporal lobe structures that are damaged in amnesia (Bayley et al., 2005a).

3.04.6 Functional Amnesia

Functional amnesia, also known as dissociative amnesia, is a dissociative psychiatric disorder that involves alterations in consciousness and identity. Although no particular brain structure or brain system is implicated in functional amnesia, the cause of the disorder must be due to abnormal brain function of some kind. Its presentation varies considerably from individual to individual, but in most cases functional amnesia is preceded by physical or emotional trauma and occurs in association with some prior psychiatric history. Often, the patient is admitted to the hospital in a confused or frightened state. Memory for the past is lost, especially autobiographical memory and even personal identity (*See* Chapter 2.46). Semantic or factual information about the world is often preserved, though factual information about the patient's life may be unavailable. Despite profound impairment in the ability to recall information about the past, the ability to learn new information is usually intact. The disorder often clears, and the lost memories return. Sometimes, the disorder lasts longer, and sizable pieces of the past remain unavailable.

3.04.7 Summary

The study of amnesia has helped to understand the nature of memory disorders and has led to a better understanding of the neurological foundations of memory. Experimental studies in patients, neuroimaging studies of healthy volunteers, and related studies in experimental animals continue to reveal insights about what memory is and how it is organized in the brain. As more is learned about the neuroscience of memory, and about how memory works, more opportunities will arise for achieving better diagnosis, treatment, and prevention of diseases and disorders that affect memory.

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3.05 Neural Substrates of Remembering – Electroencephalographic Studies

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3.05.1 Introduction

3.05.1.1 Memory Subtypes

Remembering does not refer to a unitary ability. Rather, remembering can be fractionated into a set of component processes that are expressed in different combinations under different circumstances. Analyses of memory in healthy individuals and in patients with memory impairments have revealed a set of distinct memory functions that can be assessed using different memory tests. Various theoretical schemes have been used to categorize the memory phenomena measured in these tests, emphasizing either behavioral, cognitive/representational, neural, or subjective criteria (*See* Chapter 3.02 for an overview). Taxonomies of memory have thus helped to guide research into fundamental questions about memory. Beyond taxonomies, however, we must seek a comprehensive understanding of memory by describing the component processes in both cognitive and neural terms, by clarifying the relationships between cognitive and neural descriptions, and by showing how neurocognitive processes produce memory behavior and associated conscious experiences.

Amnesic patients have specific impairments in declarative memory, the ability to remember facts and events from the past, as assessed in recall and recognition tests. In contrast, other categories of memory phenomena, as listed in **Table 1**, are not impaired in amnesia (*See* Chapters 2.33, 3.04, and 3.12). Expressions of declarative memory tend to coincide with the potential for making the metamemory judgment that memory is being expressed – the awareness of remembering. For these reasons, declarative memory is usually regarded as fundamentally distinct from other expressions of memory.

Information can also be held in awareness for an extended period of time, while rehearsed and/or manipulated. Nonetheless, our emphasis here is on memory phenomena that take place when information that was initially encoded is later brought back to mind after a delay, which is what William James (1890: 648) termed secondary memory. (For a summary of research on primary memory or working memory, *See* Chapter 3.13.)

The neural substrates of remembering can be examined in healthy human volunteers using a variety of noninvasive neuroimaging techniques. In

Table 1 A memory taxonomy based on findings in amnesic patients

Type of memory	*Behavioral outcome*	*Findings in patients with amnesia*
Declarative memory	Recall and recognition of episodes and facts (i.e., episodic memory and semantic memory)	Impaired storage, causing deficits in new learning and in remembering information acquired prior to onset of amnesia
Immediate memory	Information available while kept in mind by continuous rehearsal (e.g., verbal working memory)	Preserved if performance is not supported in part by retrieving declarative memories for the rehearsed information
Nondeclarative memory (a large category that includes nonassociative learning, classical conditioning, category learning, habit learning, as well as the following)		Generally preserved, but with some notable exceptions
Perceptual priming	Speeded or more accurate responses in a priming test, based on item-specific or perceptual representations	Preserved if performance is not contaminated by declarative memory
Conceptual priming	Speeded or more accurate responses in a priming test, based on association-specific or conceptual representations	Preserved in some cases, but further investigation is required, particularly across stimulus domains
Skills	Behaviors that improve gradually with practice, including cognitive skills (e.g., reading mirror-reversed text) and motor skills	Preserved when skill acquisition is accomplished without reliance on declarative memory (i.e., not most skills learned outside laboratory circumstances)

Adapted from Paller KA (2004) Electrical signals of memory and of the awareness of remembering. *Curr. Dir. Psychol. Sci.* 13: 49–55, with permission.

particular, recordings of event-related potentials (ERPs) have been used to monitor the activity of the brain during memory tasks. These experiments have made significant headway in identifying neurocognitive processes that are responsible for memory and in specifying processes engaged in association with different memory feats.

Our goal in this chapter is to examine how ERP research has shed light on various processes. We emphasize memory processes that contribute to declarative memory, but we also include the related memory phenomena of priming (see the section titled 'Using ERPs to contrast memory subtypes'), given that dissociations between priming and declarative memory have provided important clues about why declarative memory is distinctive.

3.05.1.2 The ERP Technique

Neurons in the human brain generate electric fields that vary moment to moment. When these electric fields are sampled via recording electrodes connected to an amplifier system, the resultant electroencephalographic record – the EEG – shows voltage changes over time and can provide indications of the functioning of networks of neurons as cognitive processing unfolds. EEG recordings from electrodes placed on the scalp are used in a variety of clinical and research contexts.

To examine EEG activity associated with stimulus processing, signal-averaging methods applied to EEG recordings can be used to produce ERPs. Whereas ongoing EEG signals can vary in magnitude on the order of tens of microvolts over a few seconds, ERPs can be extracted so as to observe signals much smaller than 1 μV. When a set of EEG responses that are time-locked to a particular class of stimuli is averaged in this way, EEG signals unrelated to stimulus processing tend to decrease because they do not occur at a consistent time relative to the time of stimulus onset. ERPs thus reveal electrical signals produced during the course of stimulus processing, to the extent that such signals are not averaged out due to temporal variability. Signal averaging can be performed with respect to any class of repeated event occurring at a known time. ERPs elicited by stimulus events are viewed as a time series of voltage changes following stimulus onset, which is conventionally referred to as

'time 0.' ERP waveforms thus consist of a series of positive and negative deflections, the timing and waveshape of which vary with the nature of the stimuli and the neural operations performed in response to the stimuli. Some ERPs, such as the brainstem auditory evoked potentials produced during the first 10 ms after a click, have very small amplitudes and require hundreds of stimulus repetitions to obtain a signal-to-noise ratio sufficiently high to permit reliable quantification. Other ERPs, such as some associated with cognitive processing, can be several microvolts in amplitude and occur over a time interval of several hundreds of milliseconds. Typically, averaging over 30–100 stimulus events is required to observe reliable effects of experimental variables in psychological experiments, depending on the amplitude and reliability of the EEG signals in question and on the presence of other EEG signals and EEG artifacts of various sorts (for further methodological details, see Rugg and Coles, 1995; Luck, 2005).

ERPs can be most readily measured by examining the positive and negative deflections (peaks and troughs) that occur at various poststimulus time points. However, the entire waveform is most likely composed of the summation of neural activity from many distinct sets of neurons in the service of many different functions. Ideally, a complex ERP waveform would be decomposed into a series of ERP components, each bearing a unique and systematic relationship to a unitary neurocognitive function. In practice, however, the component structure of an ERP waveform is difficult to discern. Given this strict definition that requires a component to be identified with a unique neurocognitive function, it would be unwise to accept the assumption that each deflection corresponds to a particular component. There may be some cases when a hypothetical component may be adequately measured by examining a deflection. In other cases, deflections are based on the summation of multiple components, which themselves are unknown, such that the amplitude and latency of the composite peak does not provide a valid characterization of any of the individual components. Accordingly, the identification and measurement of specific ERP components can be problematic.

ERP waveforms can nonetheless be quantified in several different ways. Putative components can be identified based on a combination of factors, including latency, polarity, amplitude, distribution, and most importantly, relationships to experimental parameters. ERPs can also be quantified for specific latency intervals without making *a priori* assumptions about the components that might be present, but with an emphasis instead on differences between experimental conditions. When this approach is followed, the experimental manipulations play a critical role in focusing the analysis on neurocognitive functions that can be manipulated across conditions. Valid conclusions can be drawn based on such analyses, given that some conclusions are orthogonal to the challenge of determining whether specific aspects of the ERP display a convincing correspondence with ERP components that have been described previously. Although component identification can be informative, it may not be feasible in memory paradigms when a large number of components overlap with each other in the same time range. Indeed, when subjects engage a wide variety of cognitive transactions over an extended time interval, as is likely the case in many of the interesting paradigms cognitive neuroscientists choose to study, it can be misleading to assume that only a very small number of components have been produced. When a large number of ERP components occur simultaneously, specific ERP components cannot always be isolated from one another and separately characterized.

Accordingly, many ERP investigations in cognitive neuroscience no longer exemplify the strict component-centered approach, wherein a chief experimental goal was to understand an ERP component per se. Instead, difference-centered approaches have become prevalent, whereby experimental variables are manipulated based on theory-driven goals concerning specific neurocognitive functions. This shift from a focus on known ERP components to a focus on the thorough understanding of relationships between cognitive operations and neural events has facilitated a greater dialogue between ERP experimenters and those working with different methodologies. In the context of memory research, bringing together a variety of methods in cognitive neuroscience has been responsible for significant progress.

3.05.1.3 Characterizing ERPs

In ERP investigations of human memory functions, a central analysis question in any experiment is often to determine whether two ERPs differ reliably from one another. In other words, the experimenter may ask whether two or more hypothetical psychological processes are associated with reliably different electrical signals. Experimental contrasts are often based on comparing two conditions distinguished by a task manipulation, stimulus factors, response factors, or

some combination. A difference between ERPs can then be described and displayed (**Figure 1**).

An ERP difference may be statistically significant over a certain time interval. At any given latency, the difference can have a positive or negative polarity. Across a recording epoch, amplitude will vary with a particular wave shape. When an ERP difference between conditions is characterized in this manner, it may appear to correspond to a systematic enhancement of one ERP deflection over a discrete time interval, or it may appear to encompass a different time interval with a unique wave shape.

Another important facet of an ERP difference is the distribution of the potential field across multiple electrode locations on the scalp. This topographic information can help investigators make inferences regarding the responsible neural generators. Such inferences involve many assumptions. Models of electric currents and volume conduction of the head can be used to estimate the scalp topography that would be produced by activity at a certain location in the brain. Despite straightforward procedures for solving this so-called forward problem, the inverse problem of determining the brain sources based only on the scalp topography is not soluble, because many different configurations of intracranial generators can produce the same field on the scalp. Various ERP source-modeling procedures can nevertheless be used when considering the anatomical location of the sources of ERPs, although drawbacks of inferring brain sources based on scalp recordings have been heavily debated (McCarthy and Wood, 1985; Kutas and Dale, 1997; Urbach and Kutas, 2002; Wilding, 2006). When theorizing about ERP sources in the brain, it is therefore extremely helpful to bring multiple sources of evidence to bear on understanding the relevant brain structures or systems.

3.05.1.4 Advantages and Disadvantages of Using ERPs in the Study of Human Memory

ERP methods are noninvasive and relatively inexpensive compared to other neuroimaging methods. As just described, some limited information about relevant neural sources can be extracted from ERP distributions on the scalp. In most circumstances, other methods are preferable for precise neuroanatomical information. ERP waveforms comprise a time-series of voltages between a scalp electrode location and a reference electrode location, so both locations are relevant for determining ERP characteristics. Here we will emphasize results from

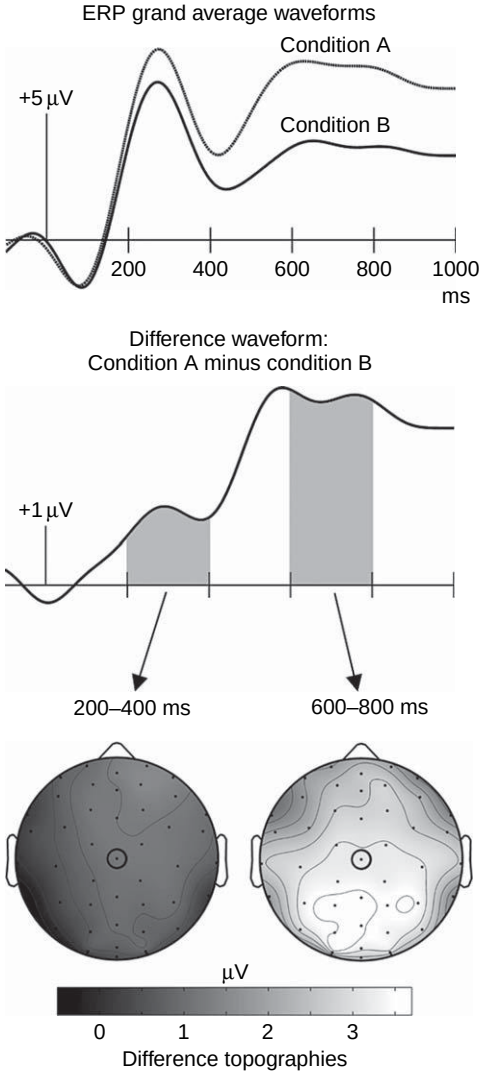

Figure 1 Visualizing ERPs. Waveforms averaged across experimental subjects for two experimental conditions, A and B, are considered at a single electrode (top). These waveforms are plotted with time shown on the x-axis going from left to right, and with voltage on the y-axis. Here, positive potentials are plotted in the upward direction, although some investigators plot amplitudes in the opposite manner. The time course of the difference in amplitude between these conditions can be visualized as a difference wave (middle), or by averaging over latency intervals of interest (gray shading) for many scalp locations and generating images of the voltage differences across the scalp (bottom). These topographic maps schematically represent the distribution of ERP differences between conditions A and B for the two latency intervals. The head is viewed from above, approximated by a circular shape, with anterior scalp regions toward the top. Black dots indicate the locations of recording electrodes. The black circle identifies the electrode location used for the waveforms shown. These images can thus demonstrate both temporal and spatial characteristics of an ERP effect.

recordings using a mastoid or average-mastoid reference, because this recording method is used in a majority of the relevant memory studies. Like any reference that can be used during recording or digitally created afterward, the mastoid reference is not inactive, as potentials generated near this location influence observed ERP effects in accordance with how currents are conducted through tissue to electrode locations.

Like other neuroimaging methods, ERPs do not provide a full view of neural activity. Rather, the EEG is sensitive to activity produced by a restricted set of neurons. These neurons are ones activated synchronously such that extracellular fields produced by their activity can summate. These fields must thus be generated by sets of neurons that are oriented together in such a way to produce electrical fields that will conduct to distant locations where recording electrodes are placed. Much neural activity may be electrically silent at the scalp, in the sense that the EEG may not include signals from some of the active neurons engaged in relevant processing.

A key advantage of ERP methods is that they provide measures of neural activity with very high temporal resolution. The superior temporal resolution of ERPs makes them well suited to examinations of neural events responsible for human memory, which can potentially be monitored by ERPs on a millisecond-by-millisecond basis. Critical memory processes often unfold within the first second after exposure to a stimulus, and ERPs can allow these processes to be resolved in real time and with randomized trial orders. Delivering trials in a predictable manner or blocking experimental trials, as required in positron emission tomography (PET) studies, can severely limit the range of memory phenomena amenable to examination. Extended intertrial intervals can also be undesirable to the extent that such procedures do not adequately constrain the timing of relevant cognitive events. In general, when cognitive events can be tightly time-locked to stimulus presentation and temporal blurring across trials decreased, ERP findings have better signal-to-noise ratios and arguably are most useful. Although randomized event-related designs with short intertrial intervals (Burock et al., 1998) are feasible with functional magnetic resonance imaging (fMRI), ERP signals may be better for isolating brief neurocognitive events or, especially, for defining a series of neurocognitive events that occur closely together in time.

Although ERPs provide temporal resolution that is unsurpassed by that of any other technique in cognitive neuroscience, other neuroimaging modalities that also provide high temporal resolution, including magnetoencephalography (MEG) and monitoring near-infrared optical signals. Such methods hold promise for substantial contributions to the memory literature in the future and may also provide further neuroanatomical insights. Additional information can also be extracted from MEG or EEG signals by conducting analyses in the frequency domain. For example, stimulus events produce reliable EEG oscillations that may reveal insights into neural activity that are complementary to those available from analyses in the time domain. Oscillations that occur in phase with stimulus onset are generally apparent in EEG records and are thus called evoked rhythms. Other oscillations can be time-locked to an event but be out of phase from trial to trial; these are called induced rhythms. A small but steadily increasing body of literature concerns memory-related cyclic EEG and MEG activity (e.g., Klimesch et al., 2001, 2006; Düzel et al., 2003). Prospects for combining ERP methods with frequency-domain analyses of EEG and MEG activity thus hold great promise.

3.05.2 ERPs and Memory Encoding

Experiments examining long-term memory generally employ an encoding phase, during which subjects attempt to commit items to memory, followed after some delay by a test phase, during which the success of memory storage and retrieval is evaluated. ERP measures can be collected both at encoding and at test, informing accounts of the relevant neural processing required during these stages. Given that declarative memories change over time, it will ultimately be important to examine relevant processing that can take place at various times between initial encoding and later retrieval. Less work has been devoted to this challenge.

The focus of most ERP studies of memory has been on explicit memory for episodes. Some studies have examined autobiographical memories formed outside the laboratory, or memories for general semantic knowledge learning over many years. The most commonly used paradigms concern memory for artificial events in a laboratory setting, such as viewing specific words or images. These studies are advantageous because the circumstances of acquisition can be

carefully monitored and controlled. As laboratory studies move closer to accurately simulating real-life memory experiences, it is possible that the artificial nature of this research will become less problematic in placing limitations on interpretations.

3.05.2.1 The Dm Approach

One way to isolate brain events responsible for successful encoding is to acquire ERP responses during encoding and sort trials based on subsequent memory performance (**Figure 2**). This general method was first reported with ERPs by Sandquist and colleagues (1980) and can be traced back to earlier work using skin-conductance methods. Indeed, many sorts of neural signals can be used in subsequent-memory analyses. The term Dm has been used to refer to neurophysiological difference measures found by sorting trials on the basis of subsequent memory performance (Paller et al., 1987). This term provides a convenient way to refer to the various phenomena that can be demonstrated using these methods (e.g., Dm for free recall, Dm for recognition, Dm for pure familiarity) and also avoids prejudging whether the differences reflect variations in a known ERP component. Subsequent-memory analyses can be conducted with different encoding requirements, different types of memory tests, and different retention intervals – and all of these parameters may influence the results. Dm potentials observed with different task, stimulus, and subject parameters can presumably index various neurocognitive operations to the extent that these operations partially determine what information will later be remembered.

Observing reliable Dm effects generally requires that multiple criteria are met, including some inter-item variability in encoding strength such that a sufficient number of items are subsequently remembered and subsequently forgotten. Logically, Dm effects will be greater when ERPs index a larger difference between mean responses in these two conditions. Dm analyses can thus gain power when confidence or other graded measures of retrieval success are considered. Ideally, a substantial polarization will be present in successful versus nonsuccessful encoding operations. Furthermore, temporal dynamics must also be suitable, such that processing that influences later memory performance is well time-locked to stimuli presented for encoding.

In many Dm studies, positive potentials maximal over parietal regions were found to be greater for later-remembered items than for later-forgotten items at approximately 400–800 ms. These effects have sometimes been attributed to ERP components such as P300 or the late positive complex (e.g., Karis et al., 1984; Fabiani et al., 1986). In the past two decades, Dm has been observed in many paradigms, including tests of recall and recognition (reviewed in Wagner et al., 1999; Paller and Wagner, 2002). A favored cognitive hypothesis about these Dm findings is that they reflect superior elaborative encoding for items later remembered. Semantic elaboration is well known to be effective for producing strong episodic memories, and deeper semantic elaboration

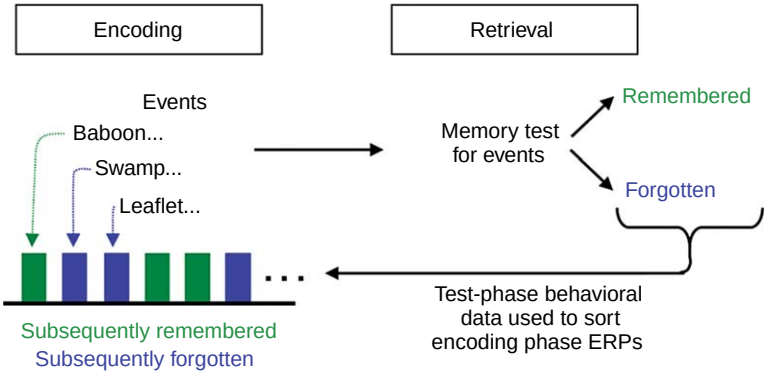

Figure 2 Schematic of the Dm or subsequent-memory methodology. During an encoding phase, neuroimaging measures are recorded while subjects attempt to remember stimuli. During the subsequent retrieval phase, memory tests for these stimuli are administered. Performance on these tests is used to classify study-phase stimuli and corresponding neural measures into two categories, subsequently remembered and subsequently forgotten. A comparison between neural correlates of these two conditions thus yields neurophysiological *d*ifferences computed on the basis of subsequent *m*emory performance, *Dm.* Figure adapted from Paller KA and Wagner AD (2002) Observing the transformation of experience into memory. *Trends Cogn. Sci.* 6: 93–102, with permission.

often corresponds with larger late posterior potentials. The nature of Dm effects due to elaboration can differ depending on the nature of the elaborative processes, however, in that frontal slow waves rather than late parietal-maximum positive potentials have been observed when subjects attempt to remember lists of unrelated words by generating novel associations to create relationships among items (Fabiani et al., 1990; Friedman, 1990). Creating novel associations likely requires a larger contribution from working memory processes supported by frontal cortex compared to elaborating on the inherent meaning of a single word.

In a recent study with faces, results revealed distinct Dm effects depending on the type of memory retrieval possible during the subsequent memory test (Yovel and Paller, 2004). As shown in **Figure 3**, a robust Dm that was bilaterally symmetric at posterior scalp locations was found to predict later recollection (when subjects could recall information previously associated with the face), whereas a smaller right-sided Dm predicted later familiarity (when subjects recognized the face but could retrieve no additional information).

Based on another type of Dm demonstrated in a few studies with words, it appears that left-frontal potentials starting at approximately 500 ms can be sensitive to the amount of information bound into a memory trace at encoding (reviewed in Friedman and Johnson, 2000). The magnitude of these potentials

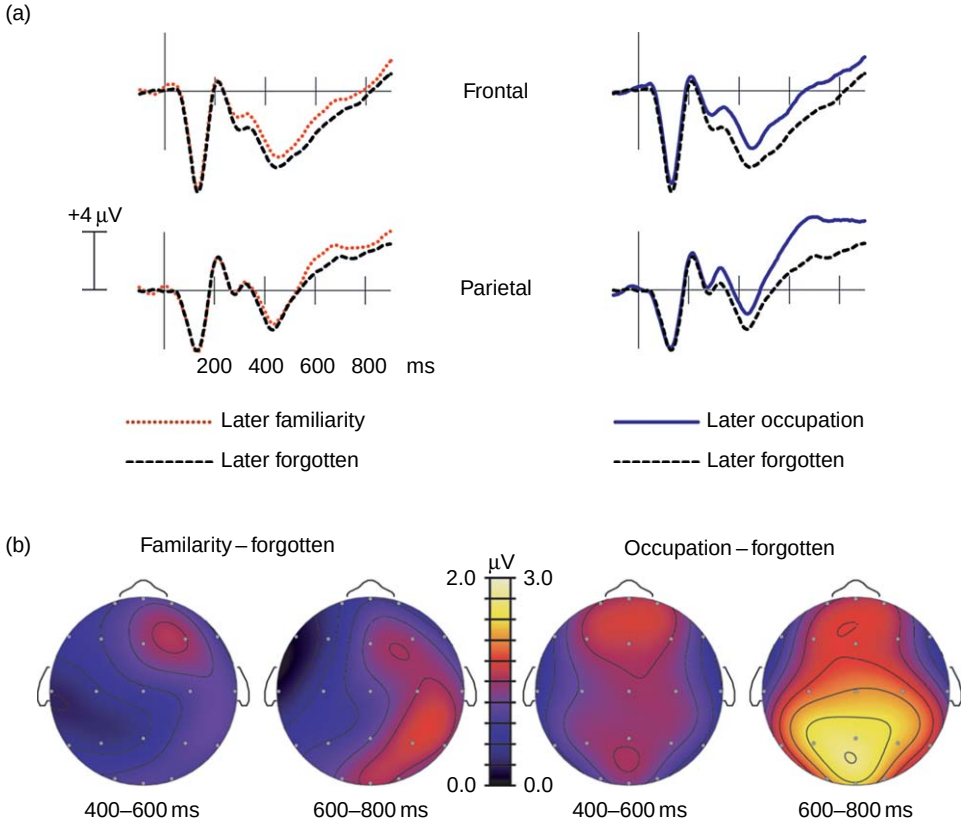

Figure 3 Representative Dm effects for two categories of memory. Two different memory experiences can be assessed after people learn to associate novel faces with randomly assigned occupations. Yovel and Paller (2004) categorized trials according to whether faces were forgotten or remembered with reference to the face alone with no contextual retrieval (familiarity) or remembered with retrieval of both the face and the paired occupation (recollection). ERPs were recorded when subjects studied these face/occupation pairs, and relatively more positive ERPs were found to predict later memory. These Dm effects are displayed at two representative electrodes in (a), and the scalp topography of these effects appears in (b). Scalp maps are of the head viewed from above, with anterior oriented toward the top. Comparing the two effects, Dm for recollection exhibited a bilateral topography with larger amplitudes spanning a longer time interval, whereas Dm for familiarity was smaller, briefer, and restricted to right posterior locations. Figure adapted from Yovel G and Paller KA (2004) The neural basis of the butcher-on-the-bus phenomenon: When a face seems familiar but is not remembered. *Neuroimage* 21: 789–800, with permission.

may be proportional to the amount of information remembered subsequently. One hypothesis is that these sustained positive potentials reflect the operation of strategic encoding processes.

In addition to stimulus-evoked neural processing that leads to later remembering, tonic brain activity that precedes the onset of a stimulus has also been linked to the efficacy of memory encoding. For example, in a study reported by Otten and colleagues (2006), ERPs over the front of the head were more negative for subsequently remembered versus subsequently forgotten items in the interval preceding stimulus onset by several hundred milliseconds. These electrophysiological findings have an important implication for investigations of encoding using other methods such as fMRI; anticipatory as well as stimulus-locked neural activity could be blurred together due to the poor temporal resolution of fMRI. Procedures are thus needed to separate fMRI correlates of these dissociable cognitive events. Although anticipatory Dm activity has not been extensively investigated, one compelling hypothesis is that it reflects working memory control operations, given that such operations can lead to better encoding of a declarative memory.

Gonsalves and Paller (2000b) showed that ERPs at encoding can reflect mistaken memories as well as accurate ones. ERPs were found to reflect the vivid visual imagery when subjects visualized the referent of a word, and this activity was found to be relevant to false remembering. Trials were categorized according to whether or not subjects subsequently claimed (erroneously) to have seen a picture of the visualized object, revealing that these posterior ERPs were predictive of whether or not a false memory for that item would happen in the test phase. ERPs thus revealed encoding activity that was partially responsible for the later mistake. In contrast, large and more widespread ERPs were predictive of accurate memory for viewed pictures.

On the whole, Dm effects may index a group of encoding operations that lead to superior memory, including detailed perceptual analysis, rote rehearsal, semantic elaboration, mental imagery, and so on. Further research is needed to clarify how Dm varies as a function of stimulus materials and type of memory test, and to precisely specify the relationship between these electrical measures and specific encoding operations. A fruitful approach for future neuroimaging studies, for example, would be to directly manipulate hypothetical mnemonic processes to determine the relative contribution of different operations to Dm effects. The usefulness of this approach for advancing explanations of memory formation can be exemplified by a recent fMRI investigation (Reber et al., 2002). Subjects were instructed to intentionally remember some words and intentionally forget others. By virtue of this directed-forgetting manipulation, fMRI correlates of the differential intention to remember were identified in addition to standard Dm effects based on retrieval success. fMRI Dm effects commonly include increased activity in both inferior prefrontal cortex and the medial temporal lobe (MTL). Although encoding condition and the probability of successful subsequent memory retrieval were correlated in this study, prefrontal activity was preferentially associated with encoding effort, whereas MTL activity was preferentially associated with success. Results thus identified the specific contribution to encoding of mnemonic operations guided by the intent to remember a word and supported by left inferior prefrontal cortex. In this way, future studies could examine the gamut of effective encoding operations in order to use Dm analyses to dissect the neurocognitive processes that support memory formation.

3.05.2.2 Intracranial Dm Effects

Electrodes implanted into the brains of patients who are candidates for surgery to remove an epileptic focus provide a special opportunity to combine the real-time temporal resolution of the ERP technique with superior spatial localization. This approach has limitations, however, in that activity can only be sampled from a limited number of brain regions, as electrodes are placed only where required for clinical purposes. In addition, generalizability can be questioned because recordings are made from a small number of individuals who have typically taken antiseizure medication for many years to try to control abnormal electrical activity in the brain. Results can nonetheless be used to inform theoretical accounts of the neural basis of memory formation.

In an ERP study reported by Fernandez and colleagues (2002), recordings were made from two areas within the MTL: the hippocampus and an adjacent cortical region near the rhinal sulcus. Based on research with amnesic patients and with nonhuman animals, the hippocampus, together with adjacent parahippocampal, entorhinal, and perirhinal cortical regions, has been considered as essential circuitry that is critical for the formation of declarative memories, in conjunction with widespread neocortical

regions that are ultimately responsible for memory storage (*See* Chapter 3.03; Squire et al., 2004). Potentials recorded from the rhinal region (entorhinal and/or perirhinal cortex) and hippocampus predicted subsequent free recall of visual words. The rhinal Dm peaked approximately 400–500 ms after word onset and was thought to be associated with the extent to which words were processed semantically. The hippocampal Dm, in contrast, started later and was taken to reflect the successful binding of multiple features into memory following semantic analysis.

These results support the notion that distinct MTL regions perform unique mnemonic operations. Other intracranial ERP studies have demonstrated a range of phenomena that may also reflect important memory functions (Heit et al., 1988; Grunwald et al., 1998; Allison et al., 1999; Guillem et al., 1999; Paller and McCarthy, 2002; Trautner et al., 2004; Engel et al., 2005; Viskontas et al., 2006). For example, a complex pattern of rhinal-hippocampal synchronization and desynchronization was found via frequency-domain analyses of the same intracranial data (Fell et al., 2001). EEG synchronization in various other brain locations has also been observed to correlate with subsequent memory performance (Sederberg et al., 2003, 2006). Furthermore, single-unit firing patterns in the hippocampus have been shown to vary as a function of subsequent memory performance (Cameron et al., 2001). Further studies are required to replicate and extend these various findings in order to elaborate on the information processing steps that are performed by neurons in each MTL region, as well as to explore the temporal dynamics of interactions across multiple brain regions. ERP measures of memory formation, in combination with findings from these other methods, will be very important for delineating the distinct contributions to memory formation dependent on different brain processes and regions.

3.05.3 ERPs and Memory Retrieval

Whereas veridical memory performance is only possible if some information was initially encoded, the nature of memory is also a function of events taking place at the time of retrieval. Furthermore, the most interesting distinctions between types of memory (e.g., declarative memory and priming) and between memory processes (e.g., recollection and familiarity) are largely realized at retrieval. This is the time when one can engage in the conscious experience of remembering that can approximate reliving a past event.

3.05.3.1 Identifying Correlates of Recognition

In most ERP studies of memory retrieval, electrophysiological responses are recorded while recognition is tested. In a recognition test for episodes studied during an encoding phase, subjects must discriminate old (repeated) items from novel items. ERP correlates of episodic memory (sometimes termed episodic memory effects or old/new effects) are commonly identified by contrasting ERP responses elicited by old items to those elicited by new items. Much effort in ERP studies of memory has been devoted to attempting to elucidate the specific memory processes that give rise to old/new effects.

Measures of neural activity obtained when people remember episodes are often interpreted in light of theories that posit two distinct recognition processes: recollection and familiarity (*See* Chapters 2.17, 2.23). Recollection involves the recognition that an event has occurred in the past along with the retrieval of specifics regarding the prior occurrence, thereby guiding the conscious experience of remembering. In contrast, familiarity denotes recognition of prior occurrence that remains unsubstantiated by retrieval of any specific detail. Familiarity can lead to a feeling of knowing in the absence of the ability to bring to mind any additional information. Recollection and familiarity are connected with the concepts of source memory and item memory, in that source memory concerns the spatiotemporal context and various other features that can support recollection, whereas familiarity for a stimulus might be driven by retrieval limited to item memory.

An experimental procedure known as the remember/know paradigm has been employed extensively in attempts to identify neural correlates of recollection and familiarity. Subjects are cued to introspectively classify their recognition of old stimuli as 'remember' if specific study-phase detail is simultaneously brought to mind or as 'know' if no such detail is retrieved. The remember/know response categories have been taken as generic indices of recollection and familiarity, respectively. Under some experimental conditions, however, remember/know responses may correspond to varying degrees of memory strength instead of qualitatively different memory processes (Eldridge et al., 2002). In addition, it is possible that results

obtained from this procedure are highly influenced by nonmnemonic variables, such as the capacity to introspect accurately. Great care is thus needed in applying this method. Confidence in results can be increased with convergent support from multiple methods, such as with memory judgments based on source information.

Indeed, a useful approach to separating ERP components related to recollection and familiarity is to test memory for specific source information based on the experimental context at encoding. For example, subjects may encode words spoken by either a male or female voice and later be tested with visual words and asked to recall the original gender. In general, correct source retrieval can be used to indicate recollection. Incorrect source retrieval with correct recognition, however, is not always a good indicator of familiarity, given that recollection may be supported by retrieval of information other than the specific source information in question.

Distinct ERP effects have been linked to three types of mnemonic processes associated with memory retrieval. These findings are outlined in the following three sections.

3.05.3.2 Recollection and Source Memory

The most consistently reported finding in recognition studies is that ERP amplitudes to old items are greater than those to new items from approximately 400–800 ms over much of the scalp. These effects typically show a maximal difference over midline or left parietal scalp locations. The amplitude of these differences generally increases with increasing memory strength based on various behavioral indices (e.g., recognition confidence). Compared to recognized items (hits), both old items that are forgotten (misses) and new items that are correctly identified (correct rejections) tend to elicit smaller ERPs.

Early investigations of these old/new ERP effects endorsed a variety of hypotheses concerning their functional significance, including associations with memory strength (Johnson et al., 1985), relative familiarity (Rugg, 1990), contextual retrieval (Smith and Halgren, 1989), and processes that do not contribute to recognition judgments (Rugg and Nagy, 1989). Despite this lack of consensus about the meaning of these ERPs during these years, a common assumption was that the effects included modulation of two ERP components: N400 potentials and P300 potentials (e.g., Halgren and Smith, 1987).

An early study that convincingly associated ERPs with recollection utilized a levels-of-processing manipulation at study (Paller and Kutas, 1992). Behavioral results showed that this manipulation influenced recall and recognition performance, with superior memory following semantic encoding that required visual imagery compared to encoding that focused attention on letter information. In contrast, the same level of priming (**Table 1**) was observed on an implicit memory test of word identification for both encoding tasks. ERPs recorded during the implicit memory test were compared between the two conditions defined by the task assigned at encoding, and corresponding differences were interpreted as ERP correlates of recollection. This ERP difference based on encoding task began at a latency of 500 ms and was only present for words that were successfully identified in the test phase. Unlike typical old/new ERP effects, this effect could not be attributed to differences associated with priming because priming was matched between the encoding conditions. Furthermore, behavioral evidence obtained at debriefing showed that subjects noticed that words from the encoding phase appeared during the word-identification test, even though this was irrelevant to their task. In other words, subjects were cognizant of specific contextual information with respect to some of the words in the word-identification test that repeated from earlier in the experiment. The authors thus inferred that incidental recollection took place during the test phase, particularly when word meaning had been encoded deeply, and that ERPs were sensitive to the differential processing associated with recollection.

Further studies using the same strategy in experimental design have substantiated the association between ERPs and recollection and extended the results to the use of other encoding tasks and memory tests (Paller et al., 1995), words presented in the auditory modality (Gonsalves and Paller, 2000a), and other types of stimuli such as faces (Paller et al., 1999). These late parietal ERPs can thus be taken as signals of the successful retrieval of episodic memories linked with conscious remembering (reviewed in Friedman and Johnson, 2000; Paller, 2000).

Results from remember/know as well as source-memory paradigms have also been used to associate late parietal ERPs with recollection. Late parietal ERP amplitudes are often found to be greater for remember compared to know responses and know responses compared to new trials. Importantly, there have not been convincing demonstrations that

the distribution of late parietal ERPs differ between remember and know responses, indicating that these ERPs may index a neurocognitive operation that differs only quantitatively between recollection and familiarity conditions (e.g., Smith, 1993). Similarly, correct source judgments elicit greater late parietal amplitudes compared to incorrect source judgments, and incorrect source judgments greater than new trials, without qualitative differences in scalp distribution (e.g., Wilding and Rugg, 1996; Trott et al., 1999).

In addition to corroborating the connection between recollection and late posterior potentials, many experiments have targeted ERP correlates of source and item memory in order to understand contextual memory in its own right. Experimental contexts have included: speaker gender (Senkfor and Van Petten, 1998); performed, watched, or imagined actions (Senkfor et al., 2002); background figures (Guo et al., 2006); spatial location (Van Petten et al., 2000); and stimulus color (Cycowicz et al., 2001), among others.

Results from memory-disordered patients have also confirmed associations between successful episodic retrieval and late positive ERPs. Amnesic patients exhibited impaired conscious recognition as well as reduced or absent late positive amplitudes (e.g., Olichney et al., 2000, 2006). In addition, administration of benzodiazepine drugs to healthy subjects prior to encoding created a temporary state of amnesia, and following this manipulation, both recollection and late parietal potentials were severely disrupted (e.g., Curran et al., 2006a). Taken together, evidence taken from a variety of experimental paradigms thus converges on the conclusion that recollection is a distinct expression of memory that reliably occurs with a particular ERP signature (**Figure 4**).

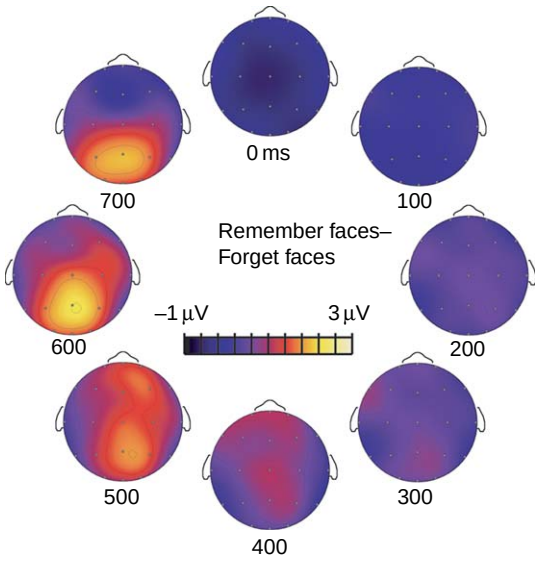

Figure 4 Late positive potentials index conscious recollection. Posterior, positive potentials from 300 to 800 ms after the presentation of faces that were previously encoded with the instruction to remember (Remember faces) were greater than for faces that were encoded with the instruction to forget (Forget faces). At encoding, each Remember face was presented with a short biographical vignette. Subjects were highly accurate at recollecting this information when cued by the corresponding face at test. Behavioral results showed that recognition was better for Remember faces than for Forget faces, whereas priming did not differ. Thus, these late positive potentials index recollective processing uncontaminated by priming. Data are from Paller KA, Bozic VS, Ranganath C, Grabowecky M, and Yamada S (1999) Brain waves following remembered faces index conscious recollection. *Brain Res. Cogn. Brain Res.* 7: 519–531.

3.05.3.3 Postretrieval Processing

Another memory phenomenon may be indexed by positive potentials at prefrontal scalp locations beginning approximately 500 ms after stimulus onset and extending for up to several seconds. These potentials often display a right-sided distribution. They do not index retrieval success, in that they tend to be similar for both successful and unsuccessful retrieval attempts. Instead, these frontal potentials are thought to index effortful retrieval processing, manipulation of working-memory contents, and/or postdecisional mnemonic processing such as further evaluation.

The functional significance of these late frontal potentials was difficult to decipher, partly due to the absence of a direct connection with retrieval success. Experimental manipulations of retrieval demands, however, were useful for clarifying the cognitive operations indexed by these potentials. In one study, images of common objects were encoded, and these objects were presented again at test in either the identical format or perceptually altered (Ranganath and Paller, 1999). Subjects performed one of two tests that differed in the demands placed on effortful retrieval of perceptual detail. In the highly demanding test, subjects responded 'old' only to objects in the identical format, whereas in the less-demanding test, objects were to be endorsed as old regardless of any format alterations. Late frontal potentials were larger in the highly demanding test than in the

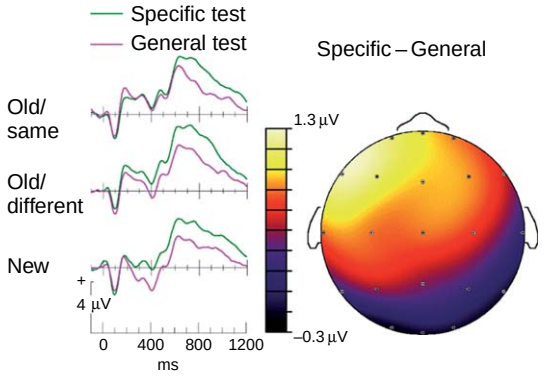

Figure 5 Late frontal potentials associated with retrieval processing. ERPs to drawings of common objects were compared between a highly demanding (specific) recognition test and a less-demanding (general) recognition test. Waveforms (left) showed a relative positivity for the specific test compared to the general test for all three stimulus classes in the experiment: objects that were perceptually identical to one in the study phase (old/same), objects that were perceptually altered from one in the study phase (old/different), and entirely novel object pictures. The corresponding ERP topography was computed over all conditions for the latency interval from 500 to 1200 ms. These late frontal potentials were maximal over the left anterior scalp, but in other experiments have also been found to be bilaterally symmetric or maximal over right anterior scalp. Figure adapted from Ranganath C and Paller KA (1999) Frontal brain potentials during recognition are modulated by requirements to retrieve perceptual detail. *Neuron* 22: 605–613, with permission from Elsevier.

less-demanding test for both categories of old stimuli as well as for new stimuli (**Figure 5**). These potentials thus appeared to track retrieval effort as manipulated across these two recognition tests. Subsequent investigations have confirmed this interpretation (Ranganath et al., 2000; Ranganath and Paller, 2000; Leynes, 2002), supporting the view that late frontal potentials track strategic processing that accompanies retrieval, a process likely mediated by prefrontal cortex. Hemispheric loci of late frontal potentials can also vary as a function of retrieval demands, with a right-to-left shift accompanying an increase in the complexity of the retrieved information (Johnson et al., 1997; Nolde et al., 1998).

3.05.3.4 Recognition with Pure Familiarity

Attempts to associate the memory experience of familiarity with specific ERPs have been most controversial. Results from many studies have been taken to indicate that familiarity is generically indexed by negative potentials peaking approximately 400 ms poststimulus, an N400-like potential with reduced amplitudes (i.e., more positive ERPs) for old compared to new items, especially at anterior locations. Beginning with the work of Düzel and colleagues (1997), many researchers have proposed that the frontal N400 old/new effect indexes recognition with familiarity, in contradistinction to recollection (reviewed in Curran et al., 2006b). Whereas late posterior potentials are greater in circumstances in which recollection is greater (for example, following semantically deep vs. semantically shallow encoding tasks), the frontal N400 old/new effect is generally found to be insensitive to such manipulations (e.g., Rugg et al., 1998a,b). In addition, frontal N400 potentials have been associated with phenomenological familiarity as indexed by the remember/know paradigm (e.g., Düzel et al., 1997; Woodruff et al., 2006) and have been found not to scale with the amount of recollection during tests of source memory (e.g., Wilding, 2000). In sum, frontal N400 potentials are sensitive to recognition success but not to manipulations that effect recollection, and thus are widely described in the extant ERP literature as a general correlate of familiarity.

On the other hand, there is reason to doubt this generalization about frontal N400 potentials, because the majority of findings used to argue in favor of this association are indirect (see following and Paller et al., 2007, for a review). Specifically, the logic of interpretation has generally been that frontal N400 potentials reflect recognition memory and do not behave as a neural correlate of recollection, and so the inference made has been that they therefore index familiarity. A direct challenge to this fragile interpretation arose with the identification of intact N400 repetition effects in amnesic patients (Olichney et al., 2000). A reasonable generalization is that amnesia disrupts familiarity, in the sense that a patient with severe amnesia does not behave as if people and various objects they encounter feel familiar. This generalization also stands on sound empirical footing (Knowlton and Squire, 1995; Yonelinas et al., 1998). Thus, one might expect patients with amnesia to exhibit reduced N400 old/new effects if these effects indeed index familiarity. Olichney and colleagues (2000) thus suggested that frontal N400 potentials might instead reflect the operation of conceptual implicit memory processes that can be engaged incidentally during recognition testing. In general, special steps are necessary to isolate the contribution of separate but potentially co-occurring

memory phenomena to neural correlates of recognition memory, as discussed in detail in the next section.

Several studies have examined the phenomenon of familiarity without recollection using faces, as in the classic example described by Mandler (1980) as the butcher-on-the-bus phenomenon. The butcher's face may seem extremely familiar yet not be identified when seen in an unusual context, such as on the bus, whereas in the butcher's shop, familiarity is more likely to occur together with memory for additional episodic and semantic information that uniquely identifies the specific person. Yovel and Paller (2004) used a variation of the remember/know paradigm to segregate trials for separate analyses of recollection and familiarity. Recognition with familiarity, compared to correct rejections of new faces, co-occurred with late positive ERPs that were maximal at midline parietal locations; recognition with recollection co-occurred with late positive ERPs that were much larger in amplitude, spanned a longer time interval, and showed a slightly more anterior topography. MacKenzie and Donaldson (2007) conducted a similar study and found statistically significant topographic differences; familiarity was associated with late posterior ERPs and recollection with larger and more anterior ERPs. However, a third study (Curran and Hancock, 2007) used a more heterogeneous mixture of faces (i.e., faces with different racial and ethnic features) and failed to replicate this pattern, instead attributing an N400 effect to familiarity for faces. Of course, it is plausible that characteristics of the people shown could influence the extent to which repeated faces engage conceptual priming, although this idea deserves further study. In sum, most studies of familiarity experiences during face processing associated familiarity with late posterior ERPs, not with the earlier frontal N400 potentials described in prior studies that used words or nameable objects. Further studies are needed to determine whether this divergence can be explained by showing that familiarity entails different neural events for verbal versus facial stimuli, that heterogeneity of face stimuli plays a crucial role, or whether alternative interpretations of frontal N400 potentials are viable.

Results from a limited number of studies can be taken as tentative evidence that familiarity may be indexed by potentials occurring earlier than frontal N400s (Tsivilis et al., 2001; Curran and Dien, 2003; Duarte et al., 2004; Friedman, 2004; Diana et al., 2005). These potentials occur between 100 and 300 ms, but otherwise closely resemble FN400 potentials – frontal ERPs to old items are more positive than to new items. Like FN400 potentials, these earlier frontal potentials have been associated with familiarity based on indirect evidence: the effect is present in association with phenomenological reports of familiarity and does not scale with recollection. More evidence is needed to determine if these potentials indeed index familiarity as opposed to other potentially co-occurring memory phenomena, such as various forms of priming and the initiation of memory search (e.g., Diana et al., 2005).

3.05.4 Using ERPs to Contrast Memory Subtypes

Memory performance undoubtedly reflects the operation of a variety of neural systems (*See* Chapter 3.02). Multiple memory systems or processes make variable contributions to performance on different mnemonic tasks. ERP investigations are especially well-suited for identifying the occurrence of these variable contributions and thereby disentangling the operation of distinct memory components. Indeed, we must first come to understand these separate components before we can work out how their interactions ultimately produce memory abilities.

Here we will highlight one distinction that has been particularly amenable to investigation with ERPs, that between explicit memory and forms of implicit memory known collectively as priming. In an explicit memory test, specific reference is made to remembering information learned earlier. In an implicit test of memory, in contrast, no reference is made to learning episodes, but rather, memory is demonstrated via a change in performance in a certain task due to a prior event that may or may not be consciously remembered. Contrasts between these two broad categories of memory phenomena have been very prominent in memory research over the past two decades (*See* Chapters 3.12, 2.33). Performance on explicit memory tests is typically disrupted in cases of amnesia, as described earlier. On the other hand, many types of implicit memory have been shown to be preserved in amnesia. Priming is a form of implicit memory that is indexed behaviorally as faster or more accurate responses on specialized priming tests, independent of conscious memory for study episodes. The most common types of priming tests are used to measure perceptual priming (also called item-specific implicit memory).

These behavioral effects are thought to reflect facilitated or more fluent perceptual processing of the physical features of repeated items, distinct from accessing a memory for the full episode in which the item occurred. A different set of mechanisms may be responsible for some types of priming (i.e., conceptual priming, novel-information priming, new-association priming, association-specific priming, or cross-domain priming), and in some of these cases priming may not be preserved in amnesia, although this is a topic currently under active investigation.

When memory tests are given to healthy individuals, performance may be guided by explicit memory, implicit memory, or by some combination. In this sense, memory tests may not be 'process-pure.' In addition to acknowledging that behavioral measures in memory tests can reflect multiple memory processes, it is important to note that neural measures such as ERPs are liable to be influenced by multiple memory processes as well. Moreover, neural measures can reflect memory processes whether or not those processes influence behavioral performance. In either implicit or explicit memory tests, ERPs can reflect neurocognitive processes responsible for both types of memory. Experimental manipulations that selectively influence the operation of distinct components of memory are thus essential. Other-wise, ERP or other neuroimaging results cannot be unequivocally associated with one type of memory versus another.

3.05.4.1 Direct Comparisons between Recollection and Perceptual Priming

Isolating neural correlates of perceptual priming uncontaminated by those of conscious remembering is problematic because of the difficulty of preventing subjects from recalling prior episodes during priming tests. Similarly, the automatic processing that supports perceptual priming may occur during recognition tests, ven if behavioral measures of priming are not obtained, and this processing can potentially be reflected in neural measures accompanying recognition.

In order to isolate ERP correlates of perceptual priming, Paller and colleagues (2003) used a condition in which faces were encoded only to a minimal extent such that priming occurred in the absence of recognition. Subjects viewed each face for 100 ms at a central location while simultaneously a yellow cross was shown unpredictably in one of the four quadrants 1.8° from fixation. While maintaining central fixation, subjects attempted to discriminate between two subtly different types of yellow crosses, and further stimulus processing was disrupted via backward masking. On a subsequent test, recognition of these minimally processed faces was not significantly better than chance. Perceptual priming for these faces, however, was observed behaviorally on two implicit memory tests. The logic of this design was thus that ERPs elicited by these faces could conceivably reflect neural events responsible for perceptual priming, whereas contributions from recognition processes would be negligible. Faces in another condition were presented for a longer duration, without disruptive perifoveal visual discriminations or backward masking, and were recognized at above-chance levels. These two conditions thus provided a direct comparison between ERPs associated with conscious memory for faces and ERPs associated with perceptual priming. Recognition-related neural correlates included late positive potentials (**Figure 6(a)**), closely resembling responses previously associated with face-cued recollection (Paller et al., 1999; Paller, 2000), whereas perceptual priming was associated with a relative ERP negativity over anterior recording electrodes from approximately 200–400 ms after face onset (**Figure 6(b)**). Spatiotemporally distinct ERPs of opposite polarities were thus associated with conscious remembering versus perceptual priming. This pattern of neuroimaging findings complements neuroanatomical dissociations identified in amnesic patients; the results imply that implicit access to memory is supported by processing within a network of brain regions that is qualitatively distinct from that supporting conscious access to memory.

Evidence for the independence of implicit and explicit memory can also be derived from contrasts between neural correlates of encoding responsible for later perceptual priming versus recollection. Schott and colleagues (Schott et al., 2002) used deep/semantic versus shallow/nonsemantic encoding conditions, followed by an ingenious two-stage procedure to assess memory. Three-letter word stems were presented in an explicit memory test (i.e., cued recall), but subjects were encouraged to guess if they could not remember a studied word so that priming might also occur. After each stem was completed, subjects indicated using strict criteria whether they recognized the word from the encoding phase. Trials were categorized as showing priming if the subject produced the word at the completion stage but failed to endorse it as an old word (i.e., priming-without-

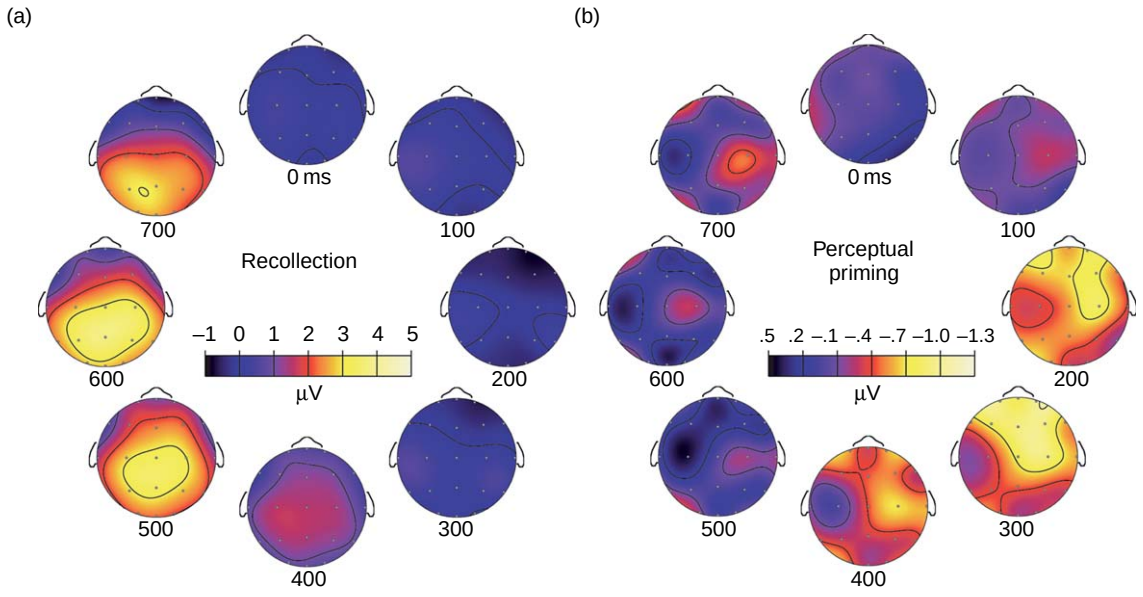

Figure 6 ERP correlates of recollection and perceptual priming. The ERP difference between recollected faces and new faces is displayed in (a). The difference between primed but not remembered faces and new faces is displayed in (b). Differences are averaged over 100-ms intervals starting at the latency indicated underneath each topographic map. Light colors indicate positive difference potentials in (a) and negative difference potentials in (b). Figure adapted from Paller KA, Hutson CA, Miller BB, and Boehm SG (2003) Neural manifestations of memory with and without awareness. *Neuron* 38: 507–516, with permission from Elsevier.

recognition). Trials were categorized as remembered when the correct response was made at both stages and as forgotten if not produced at the completion stage. Subsequent-memory analyses thus revealed a Dm for priming that took the form of a relative ERP negativity over central and fronto-central locations approximately 200–400 ms after word onset (resembling ERP correlates of perceptual priming identified during memory testing, e.g., Paller et al., 2003). Furthermore, Dm for priming was distinct from ERP differences between deep versus shallow encoding as well as from Dm for recognition, which both included relatively positive potentials at later intervals with different topographies. Collectively, these results (along with those from a follow-up study using fMRI, Schott et al., 2006) constitute critical first steps in characterizing the neurocognitive relationship between expressions of explicit memory and expressions of perceptual implicit memory.

3.05.4.2 Identification of Neural Correlates of Conceptual Priming

Another form of priming known as conceptual priming can occur whenever meaningful stimuli are repeated. Behavioral measures of conceptual priming are similar to those of perceptual priming in that they can occur in the absence of awareness of remembering and typically take the form of faster or more accurate responses to a specific stimulus. These alterations of behavioral responses are thought to reflect facilitated processing of stimulus meaning, and they potentially support some of the short-term mnemonic operations that are preserved in amnesia, such as language comprehension.

Because the neural processing that supports conceptual priming can occur whenever meaningful stimuli are repeated, regardless of whether a behavioral test of conceptual priming is provided, it is possible that neural activity associated with conceptual priming occurs incidentally during tests of recognition memory for meaningful stimuli. As reviewed above, frontal N400 potentials at retrieval have been postulated to index the explicit memory capability termed familiarity. The finding that similar potentials are intact in amnesic patients (Olichney et al., 2000), however, raised the possibility that frontal N400s instead reflect a form of memory that is not disrupted in amnesia. Olichney and colleagues (2000) proposed that residual conceptual priming in amnesic patients could be reflected by frontal N400 potentials. It is thus possible that frontal N400

potentials do not index familiarity but instead reflect conceptual priming that occurs concurrently with explicit memory (Paller et al., 2007). Further work is needed to disentangle these two memory functions.

One recent study directly examined this issue by using celebrity faces to elicit neural correlates of conceptual priming and explicit memory (Voss and Paller, 2006). Conceptual priming was manipulated by presenting associated biographical information along with a subset of celebrity faces. Later, electrophysiological recordings were obtained while subjects rapidly discriminated celebrity faces from other faces. Evidence for conceptual priming consisted of faster and more accurate responses to the subset of faces previously presented with biographical information. A baseline was provided by a counterbalanced set of celebrity faces that were previously presented without corresponding biographical information. Electrophysiological responses were obtained during the discrimination test and were characterized according to both conceptual priming and ratings of explicit memory for celebrities obtained in the last phase of the experiment. Conceptual priming was strongly associated with frontal N400 potentials (**Figure 7**), whereas explicit memory was related to late positive potentials at posterior locations.

These results attest to the likelihood that neural activity related to conceptual implicit memory is commonly produced in memory experiments designed to monitor explicit memory. Therefore, this contamination of neural signals is also possible in studies using other methods to measure brain activity, such as fMRI. Furthermore, the hypothesis that frontal N400 potentials are unique neural signatures of familiarity must be called into question, because it might partially (or entirely) reflect the operation of implicit memory. Much work will be needed to accurately elucidate the neural substrates of these memory processes, but doing so is critical for understanding the neural substrates of familiarity and of priming. This approach further highlights the necessity of employing experimental manipulations and multiple behavioral measures that can allow for valid associations between neuroimaging measures and memory functions, such that this information can be used to build an accurate characterization of the brain processes that support human memory performance.

3.05.5 Future Contributions of ERP Studies to Memory Research

Much progress has been made in identifying component processes of human memory capabilities and characterizing corresponding neural substrates of memory. However, there is much more to be learned so as to demystify the cognitive, biological, and phenomenological facets of memory. Given that a memory cue can unleash such a rapid flood of relevant neural events that precipitate remembering, ERP techniques have an important role to play in this endeavor.

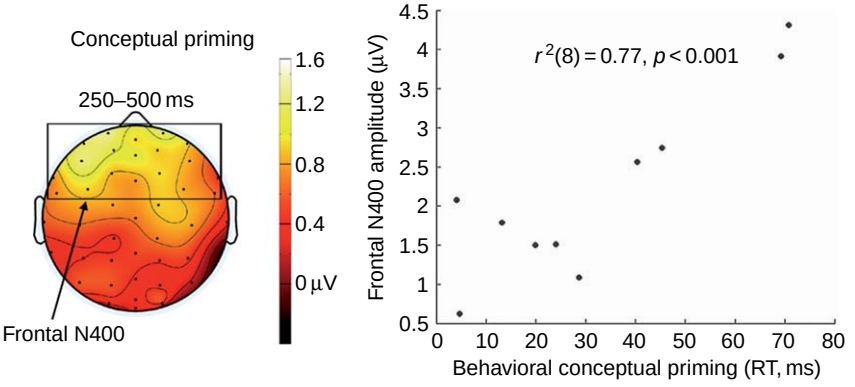

Figure 7 Neural correlates of conceptual priming include frontal N400 potentials. The ERP contrast between famous faces based on whether corresponding conceptual information was primed in an earlier phase of the experiment is shown topographically for the latency interval from 250 to 500 ms (left). The frontal N400 effect is indicated. The magnitude of these potentials (quantified in each subject at the electrode exhibiting the greatest frontal N400 conceptual priming effect) was correlated across subjects with the magnitude of conceptual priming indexed behaviorally (right). RT, reaction time. Data are from Voss JL and Paller KA (2006) Fluent conceptual processing and explicit memory for faces are electrophysiologically distinct. *J. Neurosci.* 26: 926–933.

One theoretical keystone involves identifying factors that make implicit and explicit expressions of memory unique. ERPs have been extremely useful in this regard and thus have a continuing role to play in conjunction with other methods of cognitive neuroscience. In addition to elucidating differences between conscious and nonconscious memory expressions, a deeper understanding of this issue will allow us to characterize how these distinct forms of memory interact in a variety of situations to drive memory performance. Whereas our understanding of such interactions has hitherto derived primarily from behavioral evidence obtained from healthy individuals and memory-impaired patients, physiological data are also needed. Valid descriptions of the neural bases and neural dynamics of explicit and implicit forms of memory are essential for understanding their functional relationships.

The detailed theoretical account of memory that can be achieved in this manner will ultimately be important for maximizing our memory capabilities in everyday life. Among the possible practical applications of memory research, possibilities for memory rehabilitation are critical in neurological and psychiatric diseases that affect memory and during the course of healthy aging. Much of human experience revolves around bringing to mind events from the past. Explorations of remembering via electrical recordings of brain activity may thus lead to a better understanding of ourselves.

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3.06 Structural Basis of Episodic Memory

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3.06.1 Introduction

In 1972 Endel Tulving introduced the concept of episodic memory (Tulving, 1972). Over the years the definition of episodic memory has been revised several times. A recent definition states that:

> Episodic memory is a recently evolved, late-developing, and early-deteriorating past-oriented memory system, more vulnerable than other memory systems to neuronal dysfunction, and probably unique to humans. It makes possible mental time travel through subjective time, from the present to the past, thus allowing one to reexperience, through autonoetic awareness, one's own previous experiences. Its operations require, but go beyond the semantic memory system [*See* Chapter 2.28]. Retrieving information from episodic memory (remembering or conscious recollection) is contingent on the establishment of a specific mental set, dubbed 'episodic retrieval mode.' Episodic memory is subserved by a widely distributed network of cortical and subcortical brain regions that overlaps with but also extends beyond the networks subserving other memory systems. (Tulving, 2002, p. 5)

Numerous studies of nonhuman species have aimed at examining aspects of episodic memory, and much has been learned from such studies (Squire and Knowlton, 1995; Morris and Frey, 1997; Eichenbaum, 2000). Some studies, in particular, have gone a long way toward meeting core aspects of the definition of episodic memory (e.g., Clayton and Dickinson, 1998). It nevertheless remains controversial whether experimental protocols in nonhuman species really tap episodic memory or are rather probing semantic (factual, knowledge) memory (Murray, 1996). Indeed, many memory tasks that seemingly tax episodic memory can in fact be solved by means of the semantic memory system. For example, learning and memory of paired associates are typically seen as episodic memory tasks, but if learning takes place over multiple sessions, retrieval may well be based on semantic rather than episodic memory. In that case, retrieval would not be characterized in terms of remembering or conscious recollection, but rather as knowing. In short, according to a strict definition, if and only if retrieval can be defined as involving reexperiencing through autonoetic awareness previous experiences, then one can claim to have taxed episodic memory (*See* Chapter 2.26). A comprehensive discussion of

issues related to the phylogenetic history of episodic memory and whether or not it is unique to humans goes beyond the scope of this chapter.

The crucial role of autonoetic awareness for episodic memory, relative to other memory systems, has been related to the slow ontological development of episodic memory (Wheeler et al., 1997). That age-related memory decline appears earlier for episodic than for semantic memory (Nyberg et al., 2003) may have to do with its putative reliance on a multitude of brain regions, some of which show age-associated changes (Hedden and Gabrieli, 2004). Similarly, that many brain regions seem to be engaged during retrieval likely accounts for why episodic memory is more vulnerable than other memory systems to various forms of neuronal dysfunction (Tulving, 2002). The main purpose of this chapter is to provide an empirical overview of brain regions that have been found to be engaged by episodic memory. To set the stage for this review, a brief discussion of component processes of episodic memory will follow next.

3.06.2 Episodic Memory Is Not a Unitary Construct: Component Processes

The current definition of episodic memory puts focus on retrieval of information, with a special emphasis on 'retrieval mode.' Similarly, in this chapter, the focus will be on episodic memory retrieval. Needless to say, though, retrieval is critically dependent on encoding/learning/acquisition of information (henceforth encoding) and the subsequent consolidation of such information.

Encoding is a complex construct. It can be guided by intention, such as when one meets a person and attempts to encode her/his name. It can also be an incidental process and occur as a by-product of other activities. The latter type may be the more common form of encoding, such as when one is attending a dinner party. Afterward one will be able to remember much of what happened at the party, who said what, and so on. This is so despite the fact that most people do not sit at dinner parties intentionally trying to memorize. It is not clear what determines what will be encoded into memory, but how attention-capturing or novel an event is seems to be one deciding factor (Ranganath and Rainer, 2004; Nyberg, 2005). How novel events are experienced is determined by previous experiences; hence encoding and retrieval operations are closely integrated. A neurobiological model for such integration has been proposed (Fernandez and Tendolkar, 2006).

To be stored over time, encoded information must undergo a consolidation process. This takes place at different macroscopic and time scales (Frankland and Bontempi, 2005). Fast morphological changes are needed for initial stabilization of memories and include restructuring of existing synaptic connections and growth of new ones. At the systems level, consolidation refers to a gradual reorganization of brain regions that support memory. Traditionally, it has been assumed that, once consolidated, a memory will remain stable and be relatively immune to insults. More recent research has challenged this view by showing that memory retrieval (i.e., reactivation of consolidated memories) can induce changes at the neural level (Nadel and Land, 2000; Frankland and Bontempi, 2005). These findings are in good agreement with psychological theories that memories are dynamic and subject to change (Schacter et al., 1998).

The main component process of episodic memory is retrieval. Retrieval, as the other processes, is best seen as an umbrella term for a set of subprocesses. In the definition of episodic memory given above, one subprocess was mentioned: retrieval mode. In his theory on the elements of episodic memory, Tulving (1983, 1984) argued that two necessary conditions must be met for retrieval to occur. One condition is that the system must be in the retrieval mode, and the other that an appropriate retrieval cue must be present. Retrieval mode has more precisely been described as a neurocognitve set that serves to hold a segment of one's personal past in focal attention and treats incoming information as retrieval cues for events in the past (Lepage et al., 2000). In an everyday situation, this mode might be instantiated by a question about what one did during the past vacation. Retrieval cues may then be self-generated or induced by additional questions. If information actually is retrieved from memory, which is believed to result from interactions between cues and stored information ('ecphory'), an additional element of retrieval is induced: remembering or conscious recollection. If information is not retrieved, strategic search processes may be elicited, and for both successful and unsuccessful retrieval attempts some form of monitoring processes is needed. In short, retrieval of episodic memories is most likely the result of several interacting subprocesses, and in the subsequent discussion of brain regions underlying episodic retrieval, when possible, specific retrieval processes will be considered. Functional brain

imaging studies are critical in this regard, as this technique allows component processes to be studied separately (e.g., it is possible to first examine how the brain is activated during encoding and subsequently during retrieval). It should be stressed that most if not all of the regions that will be discussed are not uniquely activated during episodic retrieval. The issue of relations among memory systems will be discussed later on in the chapter.

3.06.3 Episodic Memory – Brain Structures

3.06.3.1 The Temporal Lobe

3.06.3.1.1 The hippocampus and medial temporal cortex

It is natural to begin the review of brain structures implicated in episodic memory by discussing the hippocampus. Following the seminal paper by Scoville and Milner (1957), numerous papers have tried to determine the functional role of the hippocampus. It has been shown that lesions to the hippocampus impair episodic and semantic (declarative) memory (*See* Chapter 3.04) but leave other nondeclarative systems intact (Cohen and Squire, 1980). Further studies have examined the functional role of specific hippocampal subregions as well as nearby areas in the medial-temporal cortex (Squire and Knowlton, 1995; Eichenbaum, 2000). By now, some authors refer to multiple temporal lobe systems, such as the perirhinal versus hippocampal systems (Brown and Aggleton, 2001). Of these, the hippocampal system is more closely linked to episodic memory 'proper' by being related to remembering rather than knowing and to recollection of episodes (Brown and Aggleton, 2001), whereas perirhinal cortex seems more related to semantic memory processes (Murray and Bussey, 1999).

3.06.3.1.2 Lateral temporal cortex

Regions of lateral temporal cortex also subserve mnemonic functions. Perirhinal cortex extends from medial to lateral temporal cortex (Murray and Bussey, 1999), where it borders ventrolateral temporal regions of the ventral visual pathway. The activity in ventral temporal regions is modulated by stimulus familiarity (Desimone et al., 1995) and formed associations between visual stimuli (Fujimichi et al., 2004) and may thus be seen as critical for aspects of semantic memory. Interactions of lateral temporal and medial temporal regions may underlie episodic memory (Eichenbaum, 2004), with additional top-down contributions from frontal regions (Persson et al., 2002; see also the section titled 'The frontal lobe').

3.06.3.1.3 The amygdala

The amygdala is a key brain region for various forms of emotion, notably fear (LeDoux, 1994). The amygdala is not part of the core medial temporal memory system but has extensive connections with medial temporal as well as several other cortical and subcortical regions. Through such connections the amygdala seems able to emotionally influence various forms of cognition, including episodic memory (Phelps, 2004; LaBar and Cabeza, 2006). In episodic memory one such influence is the enhanced memory for emotionally arousing information. Such enhancement may in part reflect encoding and consolidation processes, but amygdala activation has specifically been related to recollecting emotional stimuli (Sharot et al., 2004) and to the successful retrieval of 1-year-old emotional memories (Dolcos et al., 2005). Thus, in interaction with other brain regions and neuromodulatory systems, the amygdala serves to emotionally flavor recollected episodic information (Markowitsch, 1995).

3.06.3.2 The Frontal Lobe

Lesions to the frontal lobes do not generally lead to amnesia, but frontal regions have direct or indirect connections with many cortical and subcortical areas, and a role for frontal cortex in various forms of learning and memory has been recognized for quite some time (*See* Chapter 3.14; Luria, 1973; Stuss and Benson, 1984). Of main concern here is that episodic memory impairment has been linked to frontal lobe damage (Wheeler et al., 1995). Frontal patients tend to be impaired on episodic recognition as well as recall tasks, with a more pronounced impairment for recall. That a deficit is seen on recognition tasks suggests that the frontal lobes contribute to the encoding of information, while the stronger reduction for frontal patients on recall tests indicates that frontal regions make a distinct contribution to retrieval processes. As noted earlier, functional brain imaging allows separate study of brain systems activated during the encoding and retrieval stages, and the results from such studies support a role for frontal regions in both encoding and retrieval of episodic information (Tulving et al., 1994; Nyberg et al., 1996a).

Frontal lobe contributions to episodic memory have been referred to as working-with-memory

(Moscovitch, 1992), or processes that control and optimize memory encoding and retrieval rather than storage *per se* (Fletcher and Henson, 2001). Indeed, the degree of activity in frontal regions during incidental encoding predicts subsequent episodic memory performance (Wagner et al., 1998). Several processes, mediated by frontal regions, may contribute to successful memory encoding, including generation, maintenance, and selection/organization of information (Fletcher and Henson, 2001).

At retrieval, frontal activity can reflect a number of processes as well. Retrieval mode, as was mentioned in the definition of episodic memory and in the earlier discussion of component processes of episodic memory, has been linked to several distinct frontal lobe regions, notably in the right hemisphere (Lepage et al., 2000). A defining feature of retrieval-mode type of activations is that they should be present in conditions of high as well as low levels of recollection (Lepage et al., 2000; **Figure 1**), whereas other processes and corresponding frontal activations depend on the level of retrieval success (Fletcher and Henson, 2001; Rugg, 2004). If a retrieval attempt fails, such as when one cannot remember the name of a person, additional search processes may be initiated. If the retrieval attempt succeeds, it may be necessary to verify that the retrieved information is correct. And for both these outcomes, failure and success, there is a need for

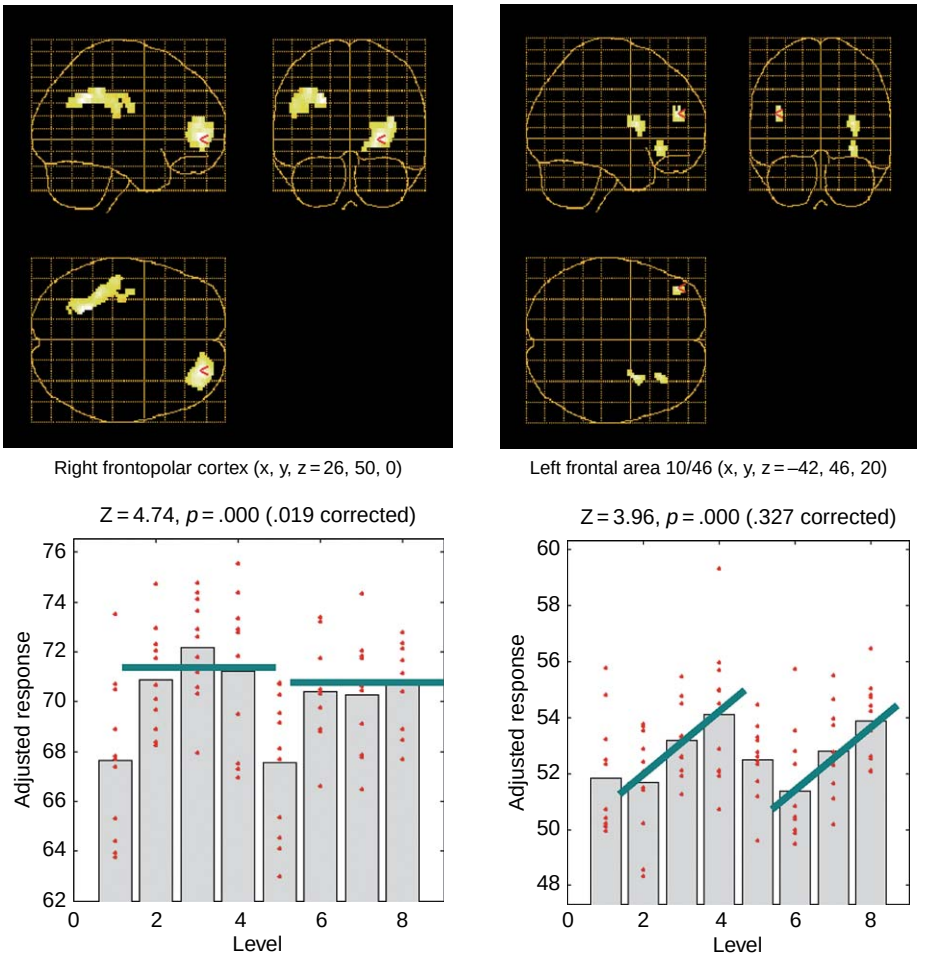

Figure 1 The left panel shows increased activity in right frontal cortex during episodic memory. The bar graph shows that right frontal cortex is associated with increased activity during verbal retrieval conditions of low, intermediate, and high target density (bars 2–4) relative to verbal encoding (bar 1). A similar pattern is seen for picture retrieval (bars 6–8) relative to picture encoding (bar 5). This pattern relates right prefrontal cortex to retrieval mode. By contrast, activity in a region of left frontal cortex (right panel) was modulated by retrieval success, as illustrated by the bar graph. Adapted from Nyberg L, Persson J, Habib R, et al. (2000) Large scale neurocognitive networks underlying episodic memory. *J. Cogn. Neurosci.* 12: 163–173, with permission.

monitoring the fate of the retrieval attempt. Specific regions of the frontal cortex seem to be involved in such component processes of episodic retrieval (Nolde et al., 1998; Cabeza and Nyberg, 2000; Buckner and Wheeler, 2001; Fletcher and Henson, 2001).

Episodic memory is typically classified as an explicit form of memory (Squire and Knowlton, 1995), and if explicit is defined in terms of intentional retrieval processes (Schacter et al., 1989), the recruitment of frontal regions critically contributes to explicit memory. More generally, frontal contributions to episodic memory are closely related to the concept of cognitive control (Wagner et al., 2004). For example, frontal activity is greater when participants have to identify target items among equally familiar distracter items than when they can base their response on stimulus familiarity alone (Persson et al., 2002; **Figure 2**), thereby relating increased levels of cognitive control to increased frontal activity (see also Fujimichi et al., 2004).

3.06.3.3 The Parietal Lobe

The parietal cortex is typically discussed in relation to various forms of attention. In a review of brain-imaging studies, it was found that parietal regions are often activated both in studies of selective attention and episodic memory retrieval (Cabeza and Nyberg, 2000). This observation suggests that, at least in part, parietal influences to episodic memory reflect attentional processes. However, activity in parietal regions is frequently modulated by level of retrieval success (Cabeza and Nyberg, 2000), indicating that parietal regions may indeed subserve mnemonic processes. This notion is further supported by findings that lateral and medial parietal regions show higher activity when old compared to when new stimuli are presented, and individuals correctly recognize the old information and correctly reject the new information (old/new effects; Wagner et al., 2005). Furthermore, the degree of activity seems to be modulated by recollective experience.

There are likely anatomical connections between regions of the parietal lobe and the medial-temporal lobe system that underlie various mnemonic processes (Wagner et al. 2005). Candidate processes that could account for the old/new parietal effect include attention to internal representations and the active representation of retrieved information. A related account of parietal (and frontal) activations was provided in a study of cross-domain similarities in brain activation patterns (Naghavi and Nyberg, 2005). Based on similarities in activation patterns for attention, working memory, episodic memory retrieval, and conscious perception (**Figure 3**), it was suggested that parietofrontal activity may reflect integration among multimodal distributed representations. Further research is needed to determine whether this activation pattern generalizes beyond the visual stimulus modality.

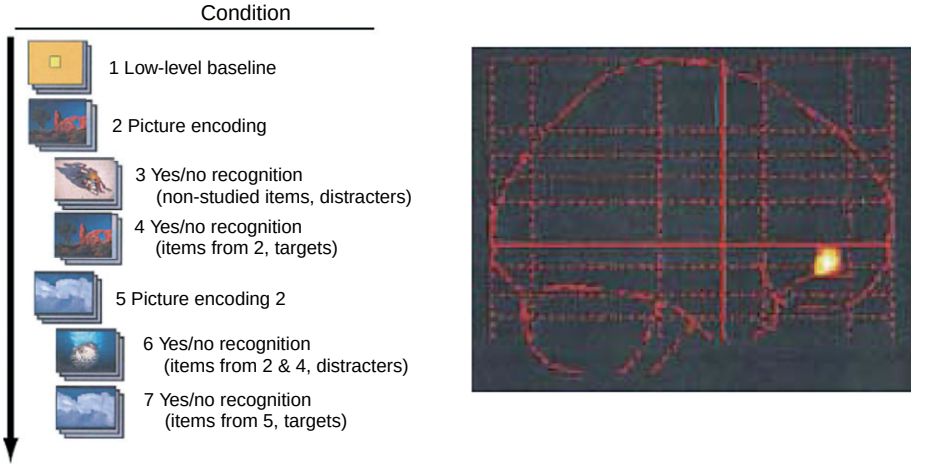

Figure 2 The left panel outlines the design of the experiment. By comparing recognition of old versus new pictures (condition 4 vs. 3) and recognition of old targets with equally familiar distracter items (condition 7 vs. 6), the demands on cognitive control could be varied (higher in the second than in the first comparison). Higher levels of cognitive control were associated with increased activity in left frontal cortex (right panel). Adapted from Persson J, Habib R, and Nyberg L (2002) Decreased activity in inferotemporal cortex during explicit memory: Dissociating priming, novelty detection, and recognition. *NeuroReport* 13: 1–5, with permission.

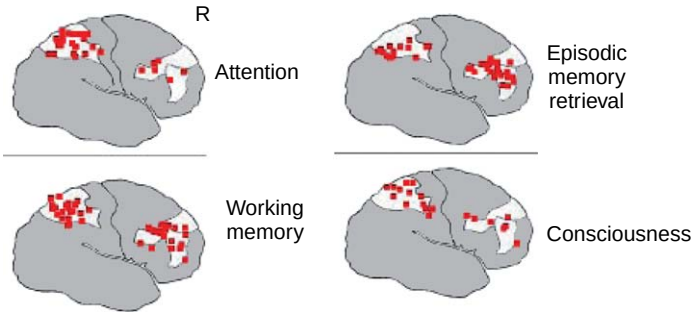

Figure 3 Overlapping patterns of frontoparietal activity in brain-imaging studies of attention, episodic retrieval, working memory, and visual consciousness. Adapted from Naghavi HR and Nyberg L (2005) Common fronto-parietal activity in attention, memory, and consciousness: Shared demands on integration? *Conscious. Cogn.* 14: 390–425, with permission.

3.06.3.4 Other Structures

3.06.3.4.1 Modality-specific cortical areas

In the discussion of regions of lateral temporal cortex it was mentioned that specific areas have been found to be engaged during episodic retrieval of visual information. This seems to be a general pattern that extends to other cortical regions and kinds of episodic information, such as parietal cortex for spatial information, occipital cortex for visual information, and motor cortex of the frontal lobe for information about activities (Buckner and Wheeler, 2001; Nyberg, 2002). The sites of retrieval-related activity overlap with how the brain was activated during initial perception/encoding. For example, regions of the parietal cortex that are engaged during spatial perception are subsequently reactivated during episodic retrieval of spatial information (Persson and Nyberg, 2000; **Figure 4**).

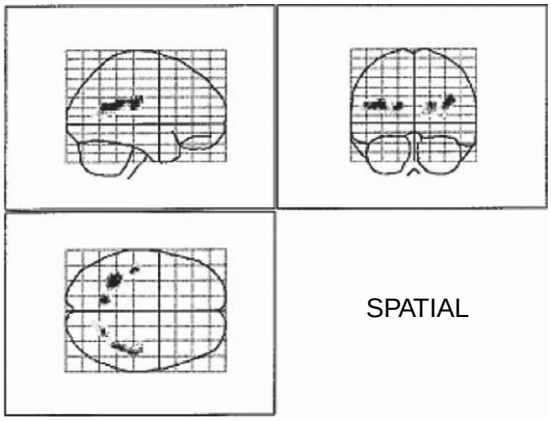

Figure 4 Areas of parietal cortex that were activated during both encoding and retrieval of spatial information. Adapted from Persson J and Nyberg L (2000) Conjunction analysis of cortical activations common to encoding and retrieval. *Microsc. Res. Tech.* 51: 39–44, with permission of Wiley-Liss, Inc., a subsidiary of John Wiley & Sons, Inc.

As suggested by theoretical models (Damasio, 1989), sites where overlapping activity is observed during encoding and retrieval may identify distributed sites of memory storage (Nyberg, 2002). Thus, the involvement of modality-specific regions during episodic memory may relate to 'ecphory' (i.e., an interaction between retrieval cues and stored information that results in actual retrieval of information). The hippocampus has been assigned an important role in integrating the distributed regions that jointly define a stored episodic memory (Teyler and DiScenna, 1986; Alvarez and Squire, 1994; McClelland et al., 1995; Nadel and Moscovitch, 1997).

3.06.3.4.2 Thalamus and the mammillary bodies

Damage to the medial portions of the diencephalon, notably the dorsomedial nucleus of the thalamus and the mammillary bodies, can cause diencephalic amnesia (Butters and Stuss, 1989). Butters and Stuss remarked that diencephalic amnesia may also result from damage to fiber tracts that connect the diencephalon with medial temporal lobe regions (a disconnection syndrome; see also Warrington and Weiskrantz, 1982). More recently, Vann and Aggleton (2004) provided support for the latter perspective by arguing that the mammillary bodies contribute to mnemonic processing through their projections to temporal regions.

It has been stressed that from the perspective of behavioral criteria there is striking similarity between diencephalic amnesia and medial temporal lobe amnesia, presumably due to close anatomical connections (Squire and Knowlton, 1995). For

example, both groups display similar forgetting rates. Therefore, the diencephalon may be seen as belonging to the same functional system as the medial temporal lobe complex (Squire and Knowlton, 1995).

3.06.3.4.3 The basal ganglia

The basal ganglia are part of the motor system. Patients with basal ganglia disorders, such as Huntington's or Parkinson's disease, show various forms of motor deficits. Critically, patients with basal ganglia deficits can exhibit cognitive deficits as well, including episodic memory impairment (Knowlton, 2002). The nature of episodic deficits is reminiscent of that seen in patients with frontal lobe damage, such as relatively greater impairment on more demanding tests of recall than on more supported recognition tests (Wheeler et al., 1995; cf. the section titled 'The frontal lobe'). This overlap in results patterns after lesions to the basal ganglia and the frontal lobe can be attributable to strong interconnections of the basal ganglia with most regions of the frontal lobe, via the limbic and the associative network (Yin and Knowlton, 2006).

3.06.3.4.4 The cerebellum

A final region to be mentioned is the cerebellum. This region is strongly associated with the coordination of movement. However, the cerebellum is not only connected with motor cortical areas but also with asserted cognitive regions (**Figure 5**), and the cerebellum has been argued to contribute to cognitive processing (Schmahmann, 1996). Increased activity in the cerebellum has specifically been observed during conscious episodic memory, and it was proposed that the cerebellum interacts with the cerebrum in a corticocerebellar network that initiates and monitors the conscious retrieval of episodic memories (Andreasen et al., 1999). Furthermore, deficient episodic memory in schizophrenia may, in part, stem from altered cerebellar activity (Achim and Lepage, 2005). Collectively, these findings indicate that the cerebellum should be considered a part of the brain network that subserves episodic memory.

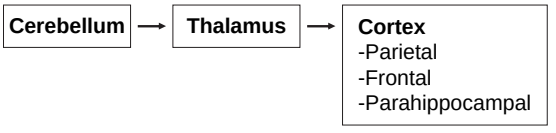

Figure 5 Schematic view of connections of the cerebellum with cortical areas that have been associated with mnemonic processes.

3.06.4 Relation Between Episodic Memory and Other Systems

Traditionally, most research activities have used the dissociation logic to define what is unique for various memory systems (Nyberg and Tulving, 1996). More recently, overlap in activity has begun to attract increased interest (Cabeza and Nyberg, 2003). In a multivariate analysis of brain-imaging data associated with measures of episodic memory, semantic memory, working memory, as well as nonmemory control tasks, similarities between episodic and semantic memory were observed (**Figure 6**; Nyberg et al., 2002). Commonly recruited regions for both episodic and semantic memory were located in left frontal cortex and right cerebellum (*See* Chapter 3.07). In addition, similarities were noted among measures of episodic and working memory, including right frontal and medial and lateral parietal cortex (**Figure 6**; Nyberg et al., 2002). These observations of overlap are in line with several other recent findings (Wagner, 1999; Cabeza and Nyberg, 2003) and also in keeping with the current definition of episodic memory, stating that episodic memory is subserved by a network of brain regions that overlaps with the networks that subserve other memory systems (Tulving, 2002). Assessment of regional overlap across a range of tasks can help to constrain the functional contribution of brain regions and define core cognitive component processes (Nyberg, 2006).

A controversial issue is whether some brain regions are specifically recruited by episodic memory tasks. This relates to the suggestion that the network subserving episodic memory extends beyond those subserving other systems (Tulving, 2002). The hippocampus, as was noted earlier, holds a special role in theories on the structural basis of episodic memory and has been referred to as a 'bottleneck structure,' as hippocampal damage leads to massive and enduring impairment of episodic memory (Markowitsch, 1995). The critical role of the hippocampus is closely related to its importance for conscious awareness (Squire et al., 2004), the core defining feature of episodic memory (Tulving, 2002). However, the performance on tasks that are *not* accompanied by awareness has been found to be impaired by hippocampal lesions (Chun and Phelps, 1999), indicating that hippocampal activation in and of itself is not a definite signature of episodic conscious memory (Eichenbaum, 1999; Squire et al., 2004). Instead, what may underlie the type of

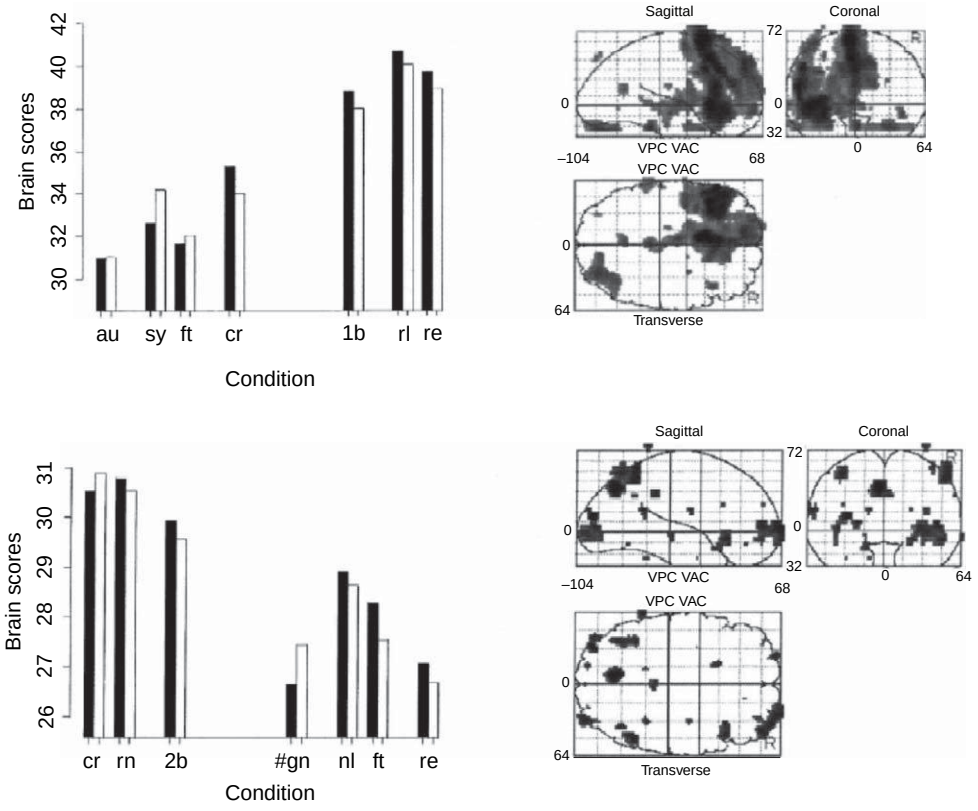

Figure 6 The top left panel shows how a multivariate analysis grouped tests of episodic (au, autobiographical memory; cr, cued recall) and semantic (sy, synonym generation; ft, fact retrieval) memory, and the top right panel shows brain regions that were jointly activated by episodic and semantic test. The lower panels show corresponding data for a grouping of tests of episodic (rn, recognition) and working (2b, 2-back test) memory. 1b, 1-back test; rl, read low demands (same word repeated); re, read; gn, random-number generation; nl, living/non-living categorization; VPC, VAC, posterior and anterior commissures. Adapted from Nyberg L, Petersson KM, Cabeza R, Forkstam C, and Ingvar M (2002) Brain imaging of human memory systems: Between-systems similarities and within-system differences. *Cogn. Brain Res.* 13: 281–292, with permission.

conscious awareness that signifies episodic memory is interactions among the hippocampus and other brain regions (Eichenbaum, 1999). In addition to the hippocampus, certain frontal regions have been suggested to have a special status in episodic memory by underlying the mental time travel component of episodic memory (Tulving, 2002). In particular, much discussion has concerned the role of anterior frontal cortex, corresponding to Brodmann's area 10, in particular in the right hemisphere. Right area 10 is frequently activated during episodic memory retrieval, but also by other tasks such as complex tests of working memory (MacLeod et al., 1998; Cabeza and Nyberg, 2000). Thus, as for the hippocampus, the evidence does not support a selective role of right area 10 in episodic memory (Ramnani and Owen, 2004), but it still remains a viable hypothesis that the frontopolar cortex, through interactions with other regions, critically contributes an episodic component to past and ongoing behavior (Koechlin et al., 2003). The crucial importance of considering network interactions is discussed further in the following section.

3.06.5 Interactions Among Episodic Memory Structures

It has been argued that all aspects of memory retrieval, including episodic retrieval, can be viewed as the activation of a network memory, i.e., increased firing of the neuronal ensembles that define the network (Fuster, 1997). In the review of episodic memory

brain structures, connections among several regions were highlighted, including:

- connections within temporal cortex (1) among different medial temporal regions; (2) between hippocampus and amygdala; and (3) among medial and lateral temporal regions;
- connections between medial-temporal and (1) parietal cortex; (2) modality-specific cortices; and (3) diencephalon;
- connections between frontal regions and cortical and subcortical areas such as (1) temporal cortex; (2) basal ganglia; and (3) cerebellum.

Although anatomical pathways have been identified for these connections, and although many of these regions have been found to be coactivated in brain-imaging studies of episodic memory, relatively little is still known about actual interregional interactions during task performance. This is so despite the fact that a network perspective has been emphasized for many years by several authors. One prominent example is the suggestion that amnesia might be seen as a disconnection syndrome in which frontal and temporal regions are functionally separated following lesions in thalamus and the mammillary bodies (Warrington and Weiskrantz, 1982). Most likely, the reason for the poor state of knowledge when it comes to regional interactions is methodological – it is difficult to measure and quantify such interactions. However, several lines of research substantiate the critical role of interactions (Simons and Spiers, 2003).

One source of evidence is animal studies that convincingly have shown that disruption of critical pathways has a profound impact on task performance (Hasegawa et al., 1998; Tomita et al., 1999; Gaffan et al., 2002). The findings of these studies substantiate the importance of frontotemporal interactions during memory retrieval. Computational models have also provided important information about regional interactions. This includes information about interactions among medial-temporal subregions (Norman and O'Reilly, 2003), interactions among hippocampus and modality-specific regions (Alvarez and Squire, 1994; McClelland et al., 1995; Norman and O'Reilly, 2003), and interactions between the basal ganglia and the frontal cortex (Atallah et al., 2004).

One further source of evidence comes from functional brain-imaging studies in humans, in which various connectivity approaches to data analysis are used (Büchel and Friston, 1997; McIntosh, 2000; Simons and Spiers, 2003). With these analytical tools, brain-imaging data can be used to quantify interactions and compare the strength of interregional connections between experimental conditions (Simons and Spiers, 2003, **Figure 7**). To date, the number of published imaging studies on episodic memory in which some form of connectivity analysis was used is quite low. However, important contributions have been made (Köhler et al., 1998; Büchel et al., 1999; Krause et al., 2000), and continued methods development (Lee et al., 2006) should pave the way for forthcoming contributions. Prefrontal-medial temporal interactions, in particular, should be critical to examine further for component processes of episodic memory retrieval (Simons and Spiers, 2003). Such examination should include variability in the degree to which a network is activated – both with

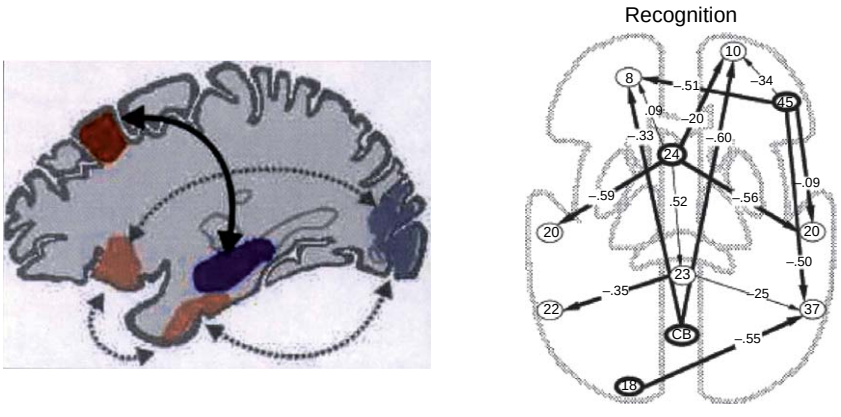

Figure 7 Illustration of connectivity approach to the analysis of functional brain imaging data. After having identified a set of regions and their connections (anatomical model; left), structural equation modeling can be used to estimate functional influences among regions of the network (right). Adapted from Nyberg L, McIntosh AR, Cabeza R, et al. (1996b) Network analysis of positron emission tomography regional cerebral blood flow data: Ensemble inhibition during episodic memory retrieval. *J. Neurosci.* 16: 3753–3759, with permission.

regard to the activation degree of its constituent elements at a given time and to variability in network activity over repeated ignitions (Fuster, 1997).

3.06.6 Variableness in the Structural Basis of Episodic Memory

The notion of a structural basis of episodic memory may give the impression that a static, fixed constellation of brain regions is recruited during retrieval. However, there is much evidence that the structural basis is quite variable or dynamic. One source of variation comes from the type of retrieval test that is used. As has been noted, less supported tests (e.g., free recall) generally tax some brain regions to a greater extent than more supported tests (e.g., recognition), such as frontal cortex, basal ganglia, and cerebellum. Also, there is some evidence that partly different brain regions are recruited during retrieval of information acquired in the laboratory compared to retrieval of real-life episodes (autobiographical memory). Depending on the testing conditions, autobiographical memory may be more similar in terms of its neural basis to semantic than episodic memory (Nyberg et al., 2002), or may more strongly activate the hippocampus and recollective processes than laboratory tests (Cabeza et al., 2004). Similarly, hippocampal activation has been found to be contingent on whether or not retrieval is accompanied by conscious recollection, such that hippocampal activity is specifically associated with recollective experience (remembering vs. knowing; Eldridge et al., 2000).

Another source of variation relates to practice or experience. There is converging evidence from several studies that network activity is strongly influenced by practice on a task, such that the degree to which different network elements are activated tends to decrease as a function of practice (Chein and Schneider, 2005). Regions that consistently show practice-related reductions are located in frontal and parietal cortices, and such reductions may reflect attenuated demand on supervisory control functions (Chein and Schneider, 2005). A related effect is that brain regions are recruited during retrieval in a time-dependent manner depending on the stage of memory consolidation (Maviel et al., 2004). Furthermore, the episodic memory system and related brain regions may be recruited during the initial stages of learning, whereas other systems and brain regions come into play at later stages of learning (Poldrack et al., 2001). All of these kinds of time- or practice-related effects will serve to induce variability in the brain structures that are engaged during episodic retrieval.

Yet another source of variation, which has not been extensively studied, is individual variability. Such variability may reflect genetically induced differences in personality and cognitive processes as well as between-person differences in real-life experiences (Fossella and Posner, 2004). The idiosyncratic nature of episodic memory may make it especially prone to show individual differences in the associated memory network (Fuster, 1997), and particularly so for network components that reflect memory storage. Future studies are called for that examine the brain bases of various forms of variability (MacDonald et al., 2006).

3.06.7 Summary and Future Directions

In summary, the main goal of the present chapter was to discuss the structural basis of episodic memory. We are still in the early stages of exploration, and many sources of variability must be taken into account, but a network of episodic-retrieval structures can tentatively be outlined (**Figure 8**; Damasio, 1989; Markowitsch, 1995; Fuster 1997). The activation of this network could start with a question, such as 'What did you do last Saturday evening?' This question would serve as a cue eliciting episodic retrieval mode and corresponding frontal activity. Signals from the frontal cortex, reflecting active retrieval, would then reach regions of the temporal lobe via subcortical pathways and trigger neuronal ensembles in the hippocampus that store sparse representations of past episodes. These ensembles would in turn activate specific neocortical sites, and in the case of emotional information, the amygdala, to reactivate memory traces. Feedback projections to the hippocampus would underlie recollective experience. The parietal cortex may represent retrieved information such that it is accessible for conscious processing, including monitoring and verification processes mediated by the frontal lobe. Additional search processes, if necessary, are subserved by the frontal cortex along with the basal ganglia and the cerebellum. This may initiate another memory network loop, as indicated by the dashed line in **Figure 8** from stage 6 to stage 1. Alternatively,

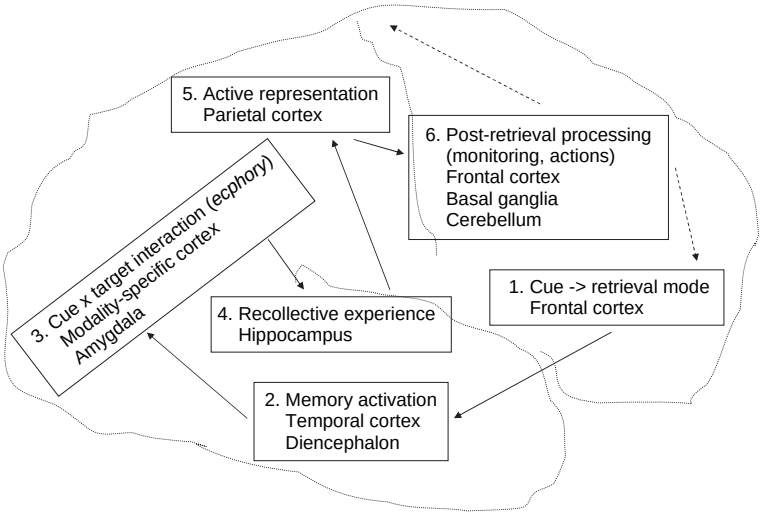

Figure 8 Schematic diagram of the episodic retrieval network. Both regions and component processes of retrieval are indicated. Arrows denote connections and information flow.

based on the output from the retrieval network, some form of action might be taken (represented in Figure 8 by the dashed line from stage 1 toward the motor cortex).

Obviously, future studies are needed to revise and refine this tentative episodic-retrieval network. Important issues to take into account include the following: (1) anatomical pathways – an interesting possibility is that *in vivo* studies of white matter connections can be used to study brain networks (Bürgel et al., 2006); (2) neuromodulation – the functioning of memory networks is modulated by various neurotransmitters such as dopamine (Bäckman and Farde, 2001; Meyer-Lindenberg et al., 2005; Schott et al., 2006); (3) temporal information – electromagnetic brain imaging techniques (Rugg, 2004) and advanced functional magnetic resonance imaging paradigms (Donaldson, 2004) can yield unique information about the timing and temporal profile of retrieval processes such as retrieval mode (Marklund et al., 2007); and (4) concepts – conceptual analysis is a crucial determinant of progress in defining the structural basis of episodic memory (Tulving, 2000).

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3.07 Structural Basis of Semantic Memory

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3.07.1 Introduction

Semantic memory refers to a major division of long-term memory that includes knowledge of facts, events, ideas, and concepts. Thus, semantic memory covers a vast cognitive terrain, ranging from information about historical and scientific facts, to details of public events and mathematical equations, to the information that allows us to identify objects and understand the meaning of words. This chapter focuses on our current understanding of how semantic memories, especially object concepts, are represented in the brain. As we discuss later, ideas about the neural systems underpinning conceptual knowledge have a long history in behavioral neurology and neuropsychology dating back at least to the late nineteenth century. In recent times, however, the idea of semantic memory as a distinct memory system began in 1972 with Endel Tulving's distinction between semantic and episodic memory (Tulving, 1972). Although the notion of episodic memory has undergone considerable evolution since that original formulation (for a brief history see Tulving, 2002), it remains helpful to describe the properties of semantic memory in relation to episodic memory. In current formulations, episodic memory can be thought of as synonymous with autobiographical memory. Episodic memory is the system that allows us to remember (consciously recollect) past experiences (Tulving, 2002) and perhaps may also be critical for imagining and/or simulating future events (Hassabis et al., 2007; Schacter and Addis, 2007). Semantic memories, in contrast, are devoid of information about personal experience. Unlike episodic memories, semantic memories lack information about the context of

learning, including situational properties like time and place, and personal dimensions like how we felt at the time the event was experienced. Remembering that you had cereal and toast for breakfast, that you read the newspaper, and that you had a slight headache is dependent on episodic memory. Knowing and, indeed, being able to visually recognize objects like cereal, toast, and newspaper, as well as understanding the words you are now reading, is dependent on semantic memory. In relation to episodic memory, semantic memory is considered to be both a phylogenically and an ontologically older system. In fact, rather than arising as an independent evolutionary development, it is commonly assumed that episodic memory emerged as an add-on or embellishment to semantic memory (Tulving, 2002). Although many animals, especially mammals and birds, acquire information about the world, they are assumed to lack the neural machinery to consciously recollect detailed episodes of their past. Finally, although retrieval of semantic memory often requires explicit, conscious mediation, the organization of semantic memory can also be revealed via implicit tasks such as semantic priming (e.g., Neely, 1991).

The idea that our semantic and episodic memories were dependent on a distinct neural substrate was perhaps first proposed by the American neurologist J.M. Nielsen (1958). As Nielsen noted, amnesia came in two types. One type, which he termed temporal amnesia, was defined by a loss of memory for personal experiences. The other type, which he termed categorical amnesia, was defined by a loss of acquired facts. Nielsen further noted that there were different varieties of categorical amnesias, including amnesias for animate objects and amnesias for inanimate objects (Nielsen, 1946, 1958), presaging a distinction that is prominently highlighted later in this chapter. Nielsen also maintained that the temporal (episodic) and categorical (semantic) amnesias could occur in isolation, thereby noting that their respective neural substrates might be at least partially independent (Nielsen, 1958). Indeed, studies of patients with conceptual deficits have provided some support for Nielsen's claim (Hodges and Graham, 2001; Simons et al., 2002). However, before discussing those patients, we first discuss studies of semantic memory in patients with profound amnesias resulting from damage to the medial temporal lobes. These studies have provided evidence that medial temporal lobe structures play a critical role in acquiring and retrieving both semantic and episodic memories.

3.07.2 Semantic Memory and the Medial Temporal Lobe Memory System

Studies of patients with impaired episodic memory resulting from damage to the medial temporal lobes have established three broadly agreed-on facts about the functional neuroanatomy of semantic memory. First, like episodic memory, acquisition of semantic memories is dependent on medial temporal lobe structures, including the hippocampal region (CA fields, dentate gyrus, and subiculum) and surrounding neocortex (parahippocampal, entorhinal, and perirhinal cortices). Damage to these structures results in deficient acquisition of new information about vocabulary and famous individuals (e.g., Gabrieli et al., 1988; Hamann and Squire, 1995; Verfaellie et al., 2000, patient SS) and public events (Manns et al., 2003), and the extent of this deficit is roughly equivalent to the deficit for acquiring personal information about day-to-day occurrences.

However, despite broad agreement that acquiring semantic memories requires medial temporal lobe structures, there is disagreement concerning the role of the hippocampal region. One position holds that the hippocampus is necessary for acquiring semantic information (for discussion, see Squire and Zola, 1998). In contrast, others have argued that acquisition of semantic memories can be accomplished by the surrounding neocortical structures alone; participation of the hippocampus is not necessary (for discussion, see Mishkin et al., 1998). Recent studies seem to favor the hippocampal position by showing that carefully selected patients with damage limited to the hippocampus are impaired in learning semantic information about public events (Manns et al., 2003). One potentially important caveat to this claim comes from studies of individuals who have sustained damage to the hippocampus at birth or during early childhood (Vargha-Khadem et al., 1997). These cases of developmental amnesia have disproportionately better semantic than episodic memories, suggesting that the hippocampus may not be necessary for acquiring semantic information. For example, cases of developmental delay resulting from hippocampal damage, although failing to provide accurate descriptions of their daily activities (episodic memory), were able to acquire normal language and social skills, keep up with their schoolwork, and perform in the average range on standard measures of vocabulary and general knowledge (Vargha-Khadem et al., 1997). These findings

pose a clear challenge to the standard hippocampal model of declarative memory. Although discussion of this important issue is outside the purview of this chapter, it is certain that a reconciliation of this issue will depend on detailed and direct comparison of adult onset and developmental amnesias with regard to the extent of medial temporal lobe damage and its behavioral consequences.

The second major finding established by studies of amnesic patients is that the medial temporal lobe structures have a time-limited role in the retrieval of semantic memories, which is, in turn, presumably related to a prolonged consolidation process (Squire and Alvarez,1995; but see Moscovitch et al., 2005, for a critique of prolonged consolidation and a reappraisal of the role of the hippocampus in memory retrieval). Evidence in favor of the claim of a time-limited role for the hippocampal region in retrieving semantic memories comes from studies assessing the status of information acquired prior versus after the amnesia onset. Such studies have revealed that, for example, public event knowledge is temporally graded, with increasing accuracy for events further in time from the onset of the amnesia (Kapur and Brooks, 1999; Manns et al., 2003). The length of this temporally graded amnesia, however, can be surprisingly long and probably varies as a function of type of information tested and testing method. For example, in the Manns et al. study, the temporal gradient for news events lasted from 10 to 15 years when evaluated by a test of recall but less than 5 years when evaluated by a recognition test (Manns et al., 2003).

Studies of object and word knowledge are also consistent with the claim that the hippocampus has a time-limited role in retrieving semantic memories. Conceptual information about the meaning of objects and words known to be acquired decades prior to amnesia onset remains intact as assessed by both explicit and implicit tasks. Patients with damage to the hippocampal region are unimpaired on tests of object naming, object property verification, and object category sorting (e.g., Schmolck et al., 2002) and show normal semantic priming (Cave and Squire, 1992).

The third major finding established by studies of amnesic patients is that semantic memories of all types are stored in the cerebral cortex. Most importantly for our present concerns, impaired knowledge about objects and their associated properties acquired prior to amnesia onset is not related to medial temporal lobe damage but rather to the extent of damage to cortex outside this region (e.g., Levy et al., 2004).

3.07.3 Cortical Lesions and the Breakdown of Semantic Memory

Studies of semantic memory in amnesia have concentrated largely on measures of public event knowledge. The reason for this is that these tasks allow memory performance to be assessed for events known to have occurred either prior to or after amnesia onset. These measures also allow performance to be evaluated for events that occurred at different times prior to amnesia onset to determine whether the memory impairment shows a temporal gradient – a critical issue for evaluating theories of memory consolidation (Moscovitch et al., 2005). However, because these patients have either no or, more commonly, limited damage to regions outside the medial temporal lobes, they are not informative about how semantic information is organized in the cerebral cortex. To address this issue, investigators have turned to patients with relatively focal lesions compromising different cortical areas. In contrast to the studies of amnesic patients, these studies have focused predominantly on measures designed to probe knowledge of object concepts.

3.07.3.1 Object Concepts

An object concept refers to the representation (i.e., information stored in memory) of an object category (a class of objects in the external world) (Murphy, 2002). The primary function of a concept is to allow us to quickly draw inferences about an object's properties. That is, identifying an object as, for example, a 'hammer' means that we know that this is an object that is used to pound nails, so we do not have to rediscover this property each time the object is encountered (see Murphy, 2002, for an extensive review of cognitive studies of concepts).

A major feature of object concepts is that they are hierarchically organized, with the broadest knowledge represented at the superordinate level, more specific knowledge at an intermediary level commonly referred to as the 'basic level,' and the most specific information at the subordinate level (Rosch, 1978). For example, 'dog' is a basic-level category that belongs to the superordinate categories 'animal' and 'living things' and has subordinate categories such as 'poodle' and 'collie.' As established by Eleanor Rosch and colleagues in the 1970s, the basic level has a privileged status (Rosch et al., 1976; Rosch, 1978). It is the level used nearly

exclusively to name objects (e.g., 'dog' rather than 'poodle'). It is also the level at which we are fastest to verify category membership (i.e., we are faster to verify that a picture is a dog than that it is an animal or a poodle). It is also the level at which subordinate category members share the most properties (e.g., collies and poodles have similar shapes and patterns of movement). Finally, the basic level is the easiest level at which to form a mental image (you can easily imagine an elephant but not an animal). As discussed next, studies of patients with cortical damage have documented the neurobiological reality of this hierarchical scheme and the central role of the basic level for representing objects in the human brain.

3.07.3.2 Semantic Dementia and the General Disorders of Semantic Memory

Several neurological conditions can result in a relatively global or general disorder of conceptual knowledge. These disorders are considered general in the sense that they cut across multiple category boundaries; they are not category specific. Many of these patients suffer from a progressive neurological disorder of unknown etiology referred to as semantic dementia (SD) (Snowden et al., 1989; Hodges et al., 1992). General disorders of semantic memory are also prominent in patients with Alzheimer's disease (who typically have a greater episodic memory impairment than SD patients) (Martin and Fedio, 1983) and can also occur following left hemisphere stroke, prominently involving the left temporal lobe (e.g., Hart and Gordon, 1990).

The defining characteristics of semantic dementia, initially described by Elizabeth Warrington in the mid-1970s, are relatively isolated deficits on measures designed to probe knowledge of objects and their associated properties (Warrington, 1975). These deficits include impaired object naming (with errors typically consisting of semantic errors – retrieving the name of another basic level object from the same category, or retrieving a superordinate category name), impaired generation of the names of objects within a superordinate category (i.e., semantic category fluency), and an inability to retrieve information about object properties – including sensory-based information (shape, color) and functional information (motor-based properties related to the object's customary use – but may include other kinds of information not directly related to sensory or motor properties) (Warrington, 1975; Martin and Fedio, 1983; Patterson and Hodges, 1995). In contrast to modality-specific agnosias, the impairment is not limited to stimuli presented in a single modality like vision but, rather, extends to all tasks probing object knowledge regardless of stimulus presentation modality (visual, auditory, tactile) or format (words, pictures). In agreement with studies of the psychological nature of concepts, the semantic deficit reveals a hierarchical structure. Broad levels of knowledge are often preserved, whereas specific information is impaired. Thus, these patients can sort objects into superordinate categories, having, for example, no difficulty indicating which are animals, which are tools, which are foods, and the like (Warrington, 1975; Martin and Fedio, 1983). Their primary difficulty manifests as a problem distinguishing among the basic level objects as revealed by impaired performance on measures of naming and object property knowledge. For example, when confronted with a picture of a specific basic level object like 'camel,' these patients often produce the name of another object from the same conceptual category (e.g., 'goat') or a superordinate term ('animal') (Warrington, 1975; Martin and Fedio, 1983).

Recent studies have expanded our understanding of SD in two important ways: one related to location of neuropathology, the other to functional characteristics of the disorder. The initial neuropathological and imaging studies of SD indicated prominent atrophy of the temporal lobes, especially to the anterolateral sector of the left temporal lobe, including the temporal polar cortex, the inferior and middle temporal gyri, and the most anterior extent of the fusiform gyrus (Hodges and Patterson, 1996). However, recent advances in neuroimaging that allow for direct and detailed comparison of brain morphology in SD patients relative to healthy control subjects have shown that the atrophy extends more posteriorally along the temporal lobe than previously appreciated (Mummery et al., 2000; Gorno-Tempini et al., 2004; Williams et al., 2005). In fact, the amount of atrophy in ventral occipitotemporal cortex, including the posterior portion of the fusiform gyrus, has been reported to be as strongly related to the semantic impairment in SD as is atrophy in the most anterior regions of the temporal lobes (Williams et al., 2005).

The other major advance in our understanding of SD is that it is not as global a conceptual disorder as initially thought. Rather, certain domains of knowledge may be preserved, and the pattern of impaired and preserved knowledge appears to be related to the locus of pathology. Specifically, left-sided atrophy seems to impair information about all object categories except person-specific knowledge

(i.e., information about famous people), which in turn is associated with involvement of the right anterior temporal lobes (Thompson et al., 2004). Also relatively spared is knowledge of number and mathematical concepts (Cappelletti et al., 2005), a domain strongly associated with left posterior parietal cortex (Dehaene et al., 2003).

3.07.3.3 Category-Specific Disorders of Semantic Memory

Although case reports of relatively circumscribed knowledge disorders date back over 100 years, the modern era of the study of category-specific disorders began in the early 1980s with the seminal reports of Warrington and colleagues (Warrington and Shallice, 1984; Warrington and McCarthy, 1987). Category-specific disorders have the same functional characteristics as SD, except that the impairment is largely limited to members of a single superordinate object category. For example, a patient with a category-specific disorder for 'animals' will have greater difficulty naming and retrieving information about members of this superordinate category relative to members of other superordinate categories (e.g., tools, furniture, flowers). Similar to patients with SD, patients with category-specific disorders have difficulty distinguishing among basic level objects (e.g., between dog, cat, horse), thereby suggesting a loss or degradation of information that uniquely distinguishes members of the superordinate category (e.g., four-legged animals) (for recent collection of papers on these patients see Martin and Caramazza, 2003).

A variety of category-specific disorders have been reported such as relatively circumscribed deficits for knowing about fruits and vegetables (Hart et al., 1985; Crutch and Warrington, 2003). However, consistent with Nielsen's clinical observations (Nielsen 1946, 1958), most common have been reports of patients with relatively greater knowledge deficits for animate entities – especially animals, than for a variety of inanimate object categories. Although less common, other patients show the opposite dissociation: a greater impairment for inanimate manmade objects – including common tools – than for animals and other living things (for extensive review of the clinical literature, see Capitani et al., 2003).

3.07.3.3.1 Models of category-specific disorders

Two major theoretical positions have been advanced to explain these disorders. Following the explanation posited by Warrington for her initial cases, most current investigators assume that category-specific deficits are a direct consequence of an object property-based organization of conceptual knowledge, an idea that was prominent in the writings of Karl Wernicke, Sigmund Freud, and other behavioral neurologists during the late nineteenth and early twentieth centuries. The central idea is that object knowledge is organized in the brain by sensory (e.g., form, motion, color, smell, taste) and motor properties associated with the object's use (Martin et al., 2000), and in some models other functional/verbally mediated properties such as where an object is typically found (for discussion of sensory/functional models, see Forde and Humphreys, 1999). In this property-based view, category-specific semantic disorders occur when a lesion disrupts information about a particular property or set of properties critical for defining and for distinguishing among category members. Thus, damage to regions that store information about object form, and form-related properties like color and texture, will produce a disorder for animals. This is because visual appearance is assumed to be a critical property for defining animals and because the distinction between different animals is assumed to be heavily dependent on knowing about subtle differences in their visual forms. A critical prediction of sensory-/motor-based models is that the lesion should affect knowledge of all object categories with this characteristic, not only animals. In a similar fashion, damage to regions that store information about how an object is used should produce a category-specific disorder for tools and all other categories of objects defined by how they are manipulated. Cognitive studies with normal individuals on the relationship between and among object features and attributes show broad consistency with the known patterns of category-specific disorders, thus providing additional evidence in support of property-based models (Cree and McRae, 2003).

The alternative to these property-based theories is the domain-specific view championed most recently by Alfonso Caramazza and colleagues (Caramazza and Shelton, 1998; Caramazza and Mahon, 2003). On this account, our evolutionary history provides the major constraint on the organization of conceptual knowledge in the brain. Specifically, the theory proposes that selection pressures have resulted in dedicated neural machinery for solving, quickly and efficiently, computationally complex survival problems. Likely candidate domains offered are animals, conspecifics, plant life, and possibly tools (for a detailed discussion

of these models, see Caramazza, 1998). Property-based and category-based accounts are not mutually exclusive. For example, it is certainly possible that concepts are organized by domains of knowledge, implemented in the brain by large-scale property-based systems (Mahon and Caramazza, 2003). Much of the functional neuroimaging evidence to be discussed later is consistent with this view.

3.07.3.3.2 Functional neuroanatomy of category-specific disorders

There is considerable variability in the location of lesions associated with category-specific disorders for animate and inanimate entities. Nevertheless, some general tendencies can be observed. In particular, category-specific knowledge disorders for animals are disproportionately associated with damage to the temporal lobes (Gainotti, 2000). The most common etiology is herpes simplex encephalitis, a viral condition with a predilection for attacking anteromedial and inferior (ventral) temporal cortices (Adams et al., 1997). Category-specific knowledge disorders for animals also have been reported following focal, ischemic lesions to the more posterior regions of ventral temporal cortex, including the fusiform gyrus (Vandenbulcke et al., 2006). In contrast, category-specific knowledge disorders for tools and their associated actions have been most commonly associated with focal damage to lateral frontal and parietal cortices of the left hemisphere (Tranel et al., 1997; Gainotti, 2000). However, it is important to stress that the lesions in patients presenting with category-specific knowledge disorders are often large and show considerable variability in their location from one patient to another (Capitani et al., 2003). As a result, these cases have been relatively uninformative for questions concerning the organization of object memories in cerebral cortex. In contrast, recent functional neuroimaging studies of the intact human brain have begun to shed some light on this thorny issue.

3.07.4 The Organization of Conceptual Knowledge: Neuroimaging Evidence

3.07.4.1 Neuroimaging of Semantic Memory

For nearly two decades cognitive neuroscientists have used positron emission tomography (PET) and functional magnetic resonance imaging (fMRI) to explore the functional neuroanatomy of semantic memory. Although the particular methods, experimental paradigms, and stimuli vary widely, the general tack taken in most studies has been to compare brain activity when subjects engage in tasks requiring the encoding or retrieval of conceptual information (e.g., Is this object a man-made artifact?) versus the activity associated with equally difficult nonconceptual processing (e.g., Does this object's name contain the letter b?), using the same stimuli. The neuroanatomical claims made by these studies are further strengthened when subsequent research observes activity in the same brain regions using stimuli that are conceptually related (e.g., judging whether objects are artifacts) but physically different (e.g., seeing photographs vs. hearing sounds vs. reading names of artifacts). Such findings demonstrate that the regions are responding to the stimuli's conceptual content rather than their physical characteristics. As we will see, however, this does not mean that objects' physical properties are unimportant to their neural representations. On the contrary, using a variety of concepts and object categories, it has been well documented that object concepts are represented in the brain as distributed property circuits, whereby the information most relevant to real-world interactions with an object is stored in the same sensorimotor regions active when that information was acquired.

Studies comparing conceptual to nonconceptual processing consistently identify three brain regions – the left ventrolateral prefrontal cortex (VLPFC) and the ventral and lateral regions of the temporal lobes. A large functional neuroimaging literature demonstrates that the VLPFC serves as a control center for semantic memory, guiding retrieval and postretrieval selection of concept property information stored in other brain regions (Bookheimer, 2002; Martin, 2001; Thompson-Schill, 2003). These functional neuroimaging findings are consistent with neuropsychological findings with patients who, subsequent to left inferior frontal lesions, exhibit word retrieval difficulties while retaining conceptual knowledge for those same words (Baldo and Shimamura, 1998; Thompson-Schill et al., 1998). Information about this region's role in semantic memory has been augmented by recent functional neuroimaging studies that find distinct mechanisms within the VLPFC for information retrieval and selection among competing alternatives (Badre et al., 2005). Although claims for dissociable retrieval and selection subregions in VLPFC remain controversial, there is wide agreement that this region's primary role is to control and manipulate information stored elsewhere (Gold et al., 2005, 2006).

3.07.4.2 Object Concepts as Sensorimotor Property Circuits

In addition to the VLPFC, a significant body of research demonstrates that various property regions located in or near perceptual cortex store information about object concepts. In particular, these studies find that the posterior ventral and lateral temporal lobes are particularly important for storing information about object concepts (Martin and Chao, 2001; Thompson-Schill, 2003; Martin, 2007). We will see that important clues about how knowledge is represented in the human brain come from how information is organized within these regions.

As described earlier, cases of category-specific deficits point toward a central role for property information in the organization of semantic memory. Early functional neuroimaging research using PET imaging supported these lesion study findings. Using a property production task in which subjects were required to generate a word describing a specific property of a visually presented object, Martin and colleagues (Martin et al., 1995; also see Chao and Martin, 1999; Wiggs et al., 1999) demonstrated that producing color-associate words (e.g., saying 'yellow' in response to an achromatic picture of a pencil or to its written name) elicited activity in the fusiform gyrus just anterior to regions activated when subjects passively viewed color stimuli. In contrast, producing action word associates (e.g., saying 'write' in response to a pencil) elicited activity in premotor cortex as well as a region of the left posterior middle temporal gyrus (pMTG) just anterior to primary visual motion-selective cortex MT/V5. Similar effects have now been observed for other property modalities as well, with sound, touch, and taste properties activating the corresponding auditory, somatosensory, and gustatory cortical regions (Kellenbach et al., 2001; Goldberg et al., 2006).

Functional neuroimaging findings demonstrating that retrieving object property information activated regions near perceptual and motor cortex are highly suggestive of the sensorimotor hypotheses generated in the literature on category-specific disorders. More recently, however, strong evidence for these accounts has come from fMRI studies demonstrating direct overlaps in the neural bases of knowledge, perception, and action. For example, Simmons et al. (in press) demonstrated a direct overlap in the neural bases of color perception and color knowledge retrieval. Using an attention-demanding task requiring fine-grain discriminations among color hues, they first mapped the brain regions underlying color perception. Next, in separate scanning runs, they presented subjects with a verbal property verification task in which they indicated whether color or motor property words could be true of a concept word. Using the color perception task as a functional localizer, they observed that the most color-responsive region in the perception task, located in the left fusiform gyrus, was also activated for color knowledge retrieval relative to retrieving motor knowledge (**Figure 1**). Evidence for direct overlaps between knowledge retrieval and sensorimotor

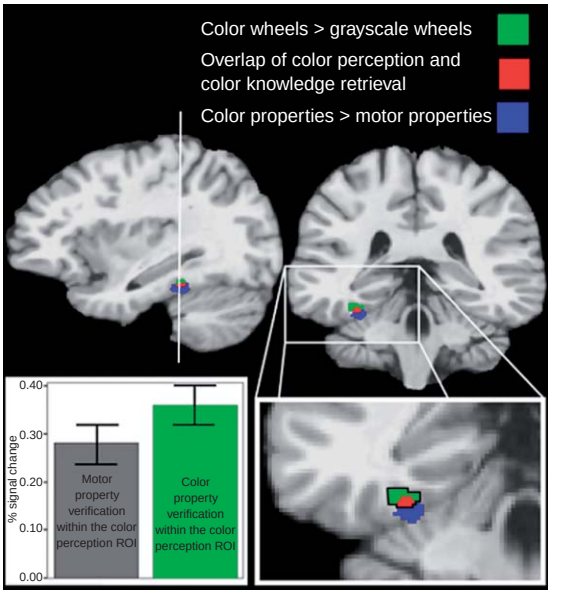

Figure 1 Overlap in perceptual and conceptual color processing. On top, the figure depicts sagittal and coronal sections from the N27 template brain warped to Talairach space (template available in AFNI). The functional overlays represent Talairach-normalized group data from the random effects analysis. Green patches indicate regions where activity was greater for processing color than grayscale wheels in the color perception task ($p < 0.0001$). Blue patches indicate regions where activity was greater for verifying color properties than motor properties in the knowledge retrieval task ($p < 0.01$ with a cluster size of at least 108 mm^3). The red patch in the left fusiform gyrus indicates the region of overlap between the two tasks. The inset bar graph demonstrates that within the left fusiform ROI, where color perception produced a greater response than grayscale perception (in other words, within the combined green and red patches), the average BOLD response to color property words in the property verification task was greater than the response for motor property words ($p = 0.006$). The *y*-axis indicates percent signal change relative to signal baseline, with error bars representing ± 1 standard error of the subject means. Adapted from Simmons WK, Ramjee V, Beauchamp MS, McRae K, Martin K, Martin A, and Barsalou LW (in press) A common neural substrate for perceiving and knowing about color. *Neuropsycholgia*.

property systems are not limited to color properties. For example, Pulvermuller and colleagues have demonstrated that simply reading words referring to actions performed with a particular body part (e.g., lick, kick, pick) activated the corresponding motor cortex (e.g., face, foot, and hand representations, respectively) (Hauk et al., 2004).

Together, these findings support claims that knowledge about a particular object property, such as its color or the actions associated with it, resides in the same sensorimotor regions that are active when that information is experienced in the external world. If this is correct, then object categories should have predictable neural representations based on their multimodal property profiles. This appears to be the case. For example, the most salient properties of appetizing foods are how they look, how they taste, and how rewarding they are to eat. Using fMRI, Simmons et al. (2005) demonstrated that viewing images of appetizing foods (e.g., cookies, pizza) while performing a low-level picture repetition detection task was enough to elicit bilateral activity in ventral occipitotemporal regions tuned to represent object form information (e.g., how objects look). Consistent with the sensorimotor account of knowledge representation, the researchers also observed activity in regions of the right insula/operculum (primary gustatory cortex) and left orbitofrontal cortex (OFC) (secondary gustatory cortex) activated in prior fMRI studies when subjects received tastants orally in the scanner (**Figure 2**).

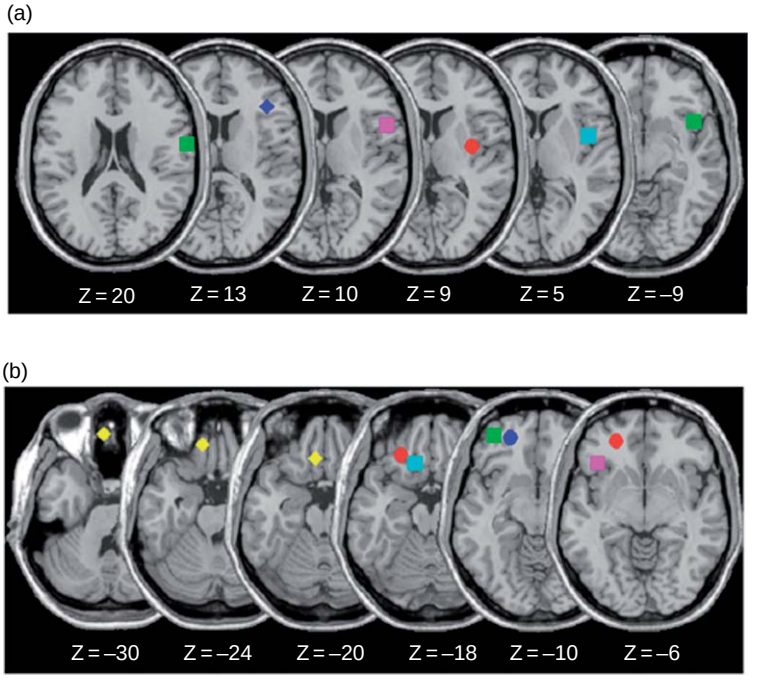

Figure 2 (a) Locations of peak right hemisphere insula/operculum activations reported in taste perception studies. (b) Locations of peak left orbitofrontal cortex (OFC) activations across various tasks. The green squares in the insula/operculum at $Z = 20$ and $Z = -9$ represent peak activations observed when participants taste sucrose, whereas the green square in the lateral OFC at $Z = -10$ is the peak activation in the area observed to respond to the combination of gustatory and olfactory stimuli, and thus it is a likely candidate for being the center of flavor representation (de Araujo et al., 2003b). The blue diamond in the insula/operculum at $Z = 13$ indicates an area of common activation when participants tasted either glucose or salt (O'Doherty et al., 2001b). The pink squares in the insula/operculum at $Z = 10$ and in the OFC at $Z = -6$ indicate the peak activations observed when participants taste umami (de Araujo et al., 2003a). The aqua squares in the insula/operculum at $Z = 5$ and in the OFC at $Z = -18$ represent peak activations when participants tasted glucose (Francis et al., 1999). Yellow diamonds in the inferior medial OFC represent peak activations observed when participants receive abstract rewards (O'Doherty et al., 2001a). The blue circle in the OFC at $Z = -10$ represents peak activation observed when participants verify the taste properties of concepts using strictly linguistic stimuli (Simmons, Pecher, Hamann, Zeelenberg, and Barsalou, Poster presented at the Annual Meeting of the Cognitive Neuroscience Society, New York, NY, April 2003). Finally, the red circles in the insula/operculum at $Z = 9$ and in the OFC at $Z = -18$ and $Z = -6$ indicate the activation peaks observed in the present study when participants viewed food pictures. When necessary, coordinates reported in other studies were converted from Talairach to MNI space. Adapted from Simmons WK, Martin A, and Barsalou LW (2005) Pictures of appetizing foods activate gustatory cortices for taste and reward. *Cereb. Cortex* 15: 1602–1608.

These findings demonstrate that information about object-associated properties is stored and represented across numerous property regions in the brain, rather than in a single unitary semantic memory storehouse. In addition, the property regions that compose a concept's neural representation overlap with the brain regions mediating that property's perception. Findings to this effect provide strong evidence that at least some aspect of object knowledge is maintained in a modality-specific, perceptual format (Barsalou, 1999). This stands in stark contrast to accounts describing human knowledge solely in terms of amodal, propositional, and linguistic formats that bear arbitrary relationships to the perceptual experiences through which the information was acquired (Fodor, 1975; Pylyshyn, 1984; Kintsch, 1998).

3.07.4.3 Object Categories in the Brain

The vast majority of studies examining the neural bases of object concept knowledge have presented subjects with photographs of exemplars from various object classes. Given that a large body of monkey neurophysiology and human neuroimaging evidence indicates that the occipitotemporal cortex plays a central role in object perception (Grill-Spector, 2003; Grill-Spector and Malach, 2004), it is unsurprising that in these studies the different object categories invariably activate this region. This does not imply, however, that the ventral occipitotemporal cortex is an undifferentiated object-processing system. Rather, comparisons between classes of objects demonstrate that local regions within the ventral occipitotemporal cortex are particularly responsive to some categories relative to others. Perhaps the most well-known category-responsive brain region is the fusiform face area (FFA), which responds reliably and selectively to face stimuli (Kanwisher et al., 1997; see Kanwisher and Yovel, 2006, for review). Other frequently studied object categories include environmental scenes (places), which reliably activate a region in parahippocampal cortex (Aguirre et al., 1998; Epstein and Kanwisher, 1998), as well as animals and tools, each activating lateral and medial fusiform cortex, respectively (Chao et al., 1999; see Martin and Chao, 2001, and Martin, 2007 for reviews).

The topographic relations among these category-responsive ventral temporal regions are a topic of much research interest. Clearly, distinct categories are associated with activation peaks in particular regions. fMRI pattern analysis techniques, however, have demonstrated that they are also associated with distinct neural signatures across large swaths of ventral occipitotemporal cortex (Haxby et al., 2001; Spiridon and Kanwisher, 2002; Cox and Savoy, 2003). For now, it remains an open question as to how central these nonpeak areas are in the cognitive representation of concepts from any particular category (Haxby et al., 2001; Spiridon and Kanwisher, 2002; Reddy and Kanwisher, 2006). In contrast, however, to this debate about the distributedness of concept representation within a single brain region, human functional neuroimaging evidence leaves little room for debate as to whether conceptual information is distributed across brain regions. Conceptual knowledge is unequivocally distributed throughout the brain, and some of the best evidence to this effect comes from the study of two broad classes of knowledge: animate entities and tools.

3.07.4.4 Two Case Studies in Category Representation: Animate Entities and Tools

Motivated by category-specific deficits for animals and tools reported in the neuropsychological literature, many functional neuroimaging studies have focused on defining the neural substrate underlying knowledge of animate entities and small, manipulable artifacts such as tools. As we will see, these studies have shown that tasks involving animate objects (i.e., people and animals) are associated with activity in the distributed neural circuit engaged while perceiving animate entities' most salient properties, namely, what they look like and how they move. For example, Chao et al. (1999) demonstrated that naming pictures or reading words denoting animal concepts activated lateral regions in the fusiform gyrus (located along the ventral surface of the temporal lobes and including the FFA), as well as the posterior extent of the superior temporal sulcus (pSTS), located laterally along the temporal lobe (**Figure 3**). In contrast, these authors demonstrated that performing these tasks with manipulable artifacts (e.g., tools) activates a distributed neural circuit underlying not only what these objects look like and how they move but also their function-associated motor properties, including medial regions in the fusiform gyrus, the pMTG, and in a later study, posterior parietal and ventral premotor regions (Chao and Martin, 2000).

The fusiform region activated by animal and tool stimuli is part of a larger object-form processing stream stretching along the ventral surfaces of the

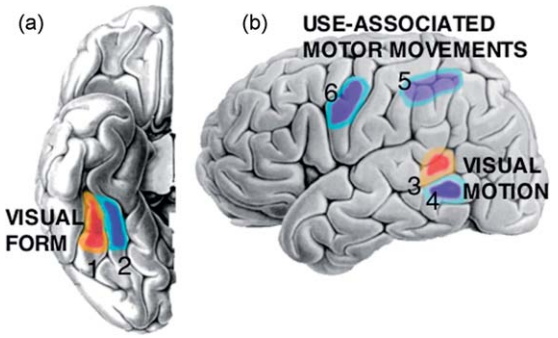

Figure 3 Schematic illustration of regions exhibiting category-related activity for animate entities such as animals and people (red) and manipulable artifacts such as tools (blue). (a) Ventral view of the right hemisphere showing relative location of regions assumed to represent visual form and form-related properties like color and texture of animate entities (1. lateral region of the fusiform gyrus, including, but not limited to, the fusiform face area) and tools; (2. medial region of the fusiform gyrus). (b) Lateral view of the left hemisphere showing relative location of regions assumed to represent biological motion typical of animate entities (3. pSTS) and rigid motion vectors typical of tools (4. pMTG). Also shown are the relative locations of the posterior parietal (5. typically centered on the intraparietal sulcus) and ventral premotor (6.) regions of the left hemisphere assumed to represent information about the motor movements associated with using tools.

occipital and temporal lobes (Ungerleider and Mishkin, 1982). Within this so-called ventral visual stream the form features of visual inputs are processed in a hierarchically organized manner, with more anterior regions representing higher-order information about what objects look like (Riesenhuber and Poggio, 1999; Grill-Spector and Malach, 2004). In contrast, the lateral temporal regions activated by animal and tool stimuli are located immediately anterior to the much-studied visual motion area V5/MT (Watson et al., 1993).

Although the location of these category-responsive regions relative to form and motion processing areas was suggestive as to their functional significance, the clearest evidence for the specific functions played by these two regions in conceptual processing *per se* comes from the work of Beauchamp and colleagues. In a series of studies, subjects were shown static and moving stimuli of humans (photographs and video clips of people performing actions such as jumping, walking, and sitting) and manipulable objects (e.g., photographs and video clips of tools such as hammers, saws, and scissors, moving in characteristic ways) (Beauchamp et al., 2002, 2003). As expected, the two classes of stimuli activated distinct regions in the ventral and lateral temporal lobes (**Figure 4**). Beauchamp et al. (2002) observed that in the fusiform gyrus, lateral regions responded more strongly to depictions of humans, and medial regions responded more to manipulable objects. Importantly, however, both regions responded equally to their preferred stimuli, regardless of whether those stimuli were static or dynamic. In a subsequent study, Beauchamp et al. (2003) observed that the lateral and medial fusiform responded much more to videos of humans and tools, respectively, than they did to point-light displays of humans and tools that lacked the form and color features of the video stimuli, but which maintained their motion vectors. Taking these two sets of findings together, we can infer that the lateral and medial fusiform regions are not modulated by motion but, rather, respond to the form features characterizing object concepts from their preferred categories – form features that are present in both static and dynamic depictions of an object.

Unlike the ventral temporal cortex, lateral temporal regions were more responsive to dynamic than static stimuli, with the pSTS and pMTG exhibiting strong category selectivity. The pSTS responded more strongly to dynamic depictions of human actions than to tool motion. This finding is consistent with monkey neurophysiology and human fMRI studies demonstrating that this region is particularly tuned to flexible, fully articulated motion vectors that characterize biological motion (Oram and Perrett, 1994; Puce et al., 1998; Grossman and Blake, 2001; Pelphrey et al., 2005). In contrast, relative to human actions, the pMTG responded more strongly to the rigid, unarticulated motion vectors characterizing dynamic depictions of tool motions. Thus, in the same way that activity in ventral temporal cortex differentiates along category boundaries, presumably due to different visual form characteristics for animate objects and manipulable artifacts, so activity in lateral temporal cortex similarly differentiates the distinctive motion properties of the two categories.

As reviewed earlier, behavioral (Cree and McRae, 2003) and imaging (see Martin, 2007) evidence demonstrate that an object concept's property profile (e.g., its form, motion, taste, sound) predicts the conglomeration of sensorimotor regions underlying that object concept's storage and representation in the brain. In light of this, it should come as no surprise that in addition to the temporal regions representing their form and motion properties, tasks involving manipulable artifacts also recruit posterior parietal and ventral premotor regions supporting the representation of

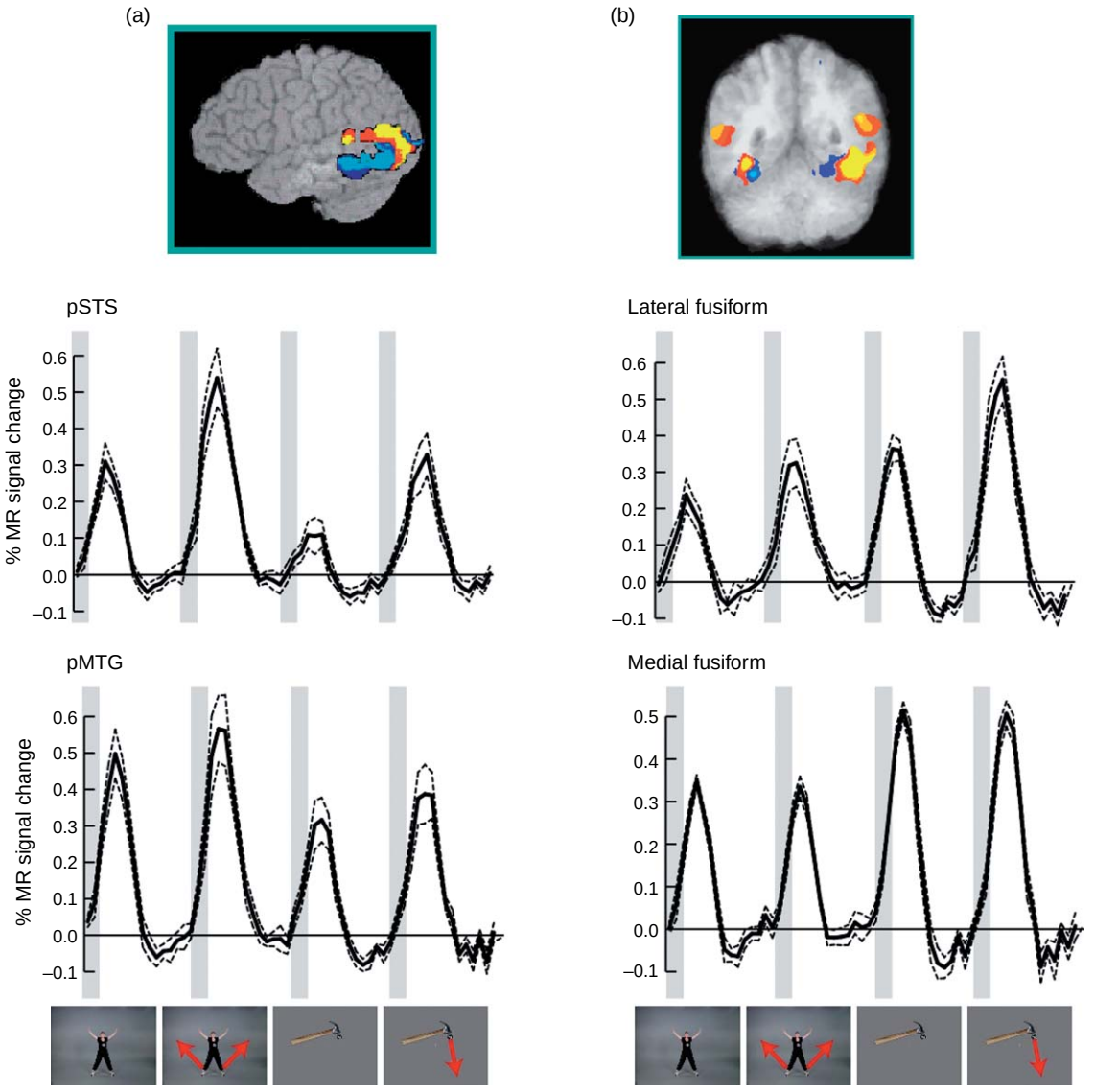

Figure 4 (a) Lateral view of the left hemisphere showing MTG regions that are more responsive when subjects identify static and moving images of tools than people (blue), and pSTS regions that are more responsive for identifying people than tools (yellow). (b) Coronal section illustrating medial fusiform regions that are more responsive when subjects identify static and moving images of tools than people (blue), and the lateral fusiform region that is more active for identifying people (yellow). Below each brain are group-averaged bold response functions depicting activity for static and moving images in each region shown in (a) and (b). The lateral cortical areas in (a) exhibit category and motion effects, where as ventral areas depicted in (b) exhibit only category effects. Vertical gray bars indicate stimulus presentation periods. Dashed lines indicate ±1 SEM. Adapted from Beauchamp MS, Lee KE, Haxby JV, and Martin A (2002) Parallel visual motion processing streams for manipulable objects and human movements. *Neuron* 34: 149–159.

object-associated actions (Chao and Martin, 2000). This finding is consistent with monkey neurophysiology evidence demonstrating that neurons in the ventral premotor and parietal cortices respond when monkeys grasp objects, as well as when they merely see objects they have previously manipulated (Jeannerod et al., 1995; Rizzolatti and Fadiga, 1998).

3.07.4.5 Category-Related Activations in Property Regions Are the Bases of Conceptual Representations of Objects

Processing various object categories elicits activity in sensorimotor property regions. But how do we know that the activity in property regions represents

conceptual information? Three findings in particular from the literature on animate and manipulable artifact object concepts strengthen the case that property regions are involved in conceptual-level processing.

3.07.4.5.1 Reason #1 to think that property regions are involved in conceptual-level processing: Activity in category regions transcends stimulus features

For both animate and manipulable artifact categories, substantial evidence demonstrates that activity in the categories' property regions is not stimulus specific. For example, differential responses are observed in the lateral fusiform to animate entities in response to pictures and written names of animals (Chao et al., 1999; Okada et al., 2000; Price et al., 2003; Devlin et al., 2005; Rogers et al., 2005; Wheatley et al., 2005; Mechelli et al., 2006), human voices (von Kriegstein et al., 2005), and when simply imagining faces (O'Craven and Kanwisher, 2000). In addition, lateral fusiform activity is also observed in response to stimuli depicting point-light displays of human bodies in motion (Grossman and Blake, 2001, 2002; Beauchamp et al., 2003; Peelen et al., 2006) and degraded and abstract visual stimuli such as human-like stick figures (Peelen and Downing, 2005). These findings are important because they demonstrate that this region is responsive to representations of animate entities, even after most form and color information has been stripped from the stimuli.

Perhaps most significantly, lateral fusiform activity has even been observed when participants view abstract representations of social situations depicted in interactions among simple geometric shapes (Heider and Simmel, 1944). For example, the lateral fusiform gyrus responds to animations suggesting social interactions such as hide-and-seek (Schultz et al., 2003), mocking and bluffing (Castelli et al., 2000, 2002), and sharing (Martin and Weisberg, 2003).

Similarly, differential responses in the medial fusiform to manipulable objects are observed in response to both pictures and written names of tools (Chao et al., 1999, 2002; Whatmough et al., 2002; Devlin et al., 2005; Mechelli et al., 2006), the spoken names of tools (Noppeney et al., 2006), and point-light displays depicting tools in motion (Beauchamp et al., 2003). Medial fusiform activity has even been observed when participants view simple geometric shapes that move and interact in ways that suggest mechanical interactions such as a bowling ball knocking down pins or billiards (Martin and Weisberg, 2003). Clearly, activation in these category-related property areas is not due to particular stimulus features but, rather, appears to be related to high-level conceptual representations.

3.07.4.5.2 Reason #2 to think that property regions are involved in conceptual-level processing: Activations in property areas occur as property inferences

Further evidence that property regions for animate and manipulable objects are involved in conceptual processing comes from studies in which property inferences manifest as activations in property areas; in other words, when a response occurs within a property area even though that particular property is not, in fact, present in the stimulus. For example, perceiving pictures of animals or people or reading animal names activates the region of the pSTS sensitive to biological motion, even when the stimuli presented are static photographs (Chao and Martin, 1999; Beauchamp et al., 2002). Similarly, perceiving static pictures of tools or reading tool names activates the region of the pMTG known to represent nonbiological motion (Chao and Martin, 1999; Beauchamp et al., 2002; Chao et al., 2002; Phillips et al., 2002; Kellenbach et al., 2003; Creem-Regehr and Lee, 2005; Devlin et al., 2005; Kable et al., 2005; Tranel et al., 2005a, b; Mechelli et al., 2006; Noppeney et al., 2006). In addition to the motion property inferences, however, motor property inferences are also observed in response to tool photographs, with subjects exhibiting activations in premotor cortex, even though they are not physically manipulating the tools (Chao and Martin, 2000; Chao et al., 2002; Creem-Regehr and Lee, 2005; Kan et al., 2006).

These findings are in the same vein as the observations described earlier when subjects viewed appetizing foods. Upon viewing pictures of appetizing foods, activations were observed in insula/operculum and OFC regions known to represent the tastes and taste rewards of foods, even though subjects were not receiving any gustatory stimulation (Simmons et al., 2005).

The ability to make inferences about an entity's properties is at the very core of what most cognitive scientists call conceptual knowledge. Across various categories, human subjects frequently exhibit activations in property regions that correspond to salient object concept information, often when that information is not present in the immediate stimulus. Given

its great utility, and likely survival value, we might expect that the ability to infer properties would be preserved across primate species, and recent evidence demonstrates that it is.

In a study demonstrating evolutionary continuity in the neural mechanisms for representing conceptual information, Gil-da-Costa et al. (2004) presented both species-specific calls and nonbiological sounds to awake rhesus macaques undergoing PET imaging. Although both the species-specific calls and nonbiological sounds were attended by activity in auditory cortex, the conspecific calls also elicited activation in area TE/TEO, the presumed monkey homologue of human fusiform gyrus, and in the STS (**Figure 5**). Note that these ventral and lateral temporal activations in visual form and motion property regions occurred to auditory stimuli. As with humans, when monkeys process information about animate entities, in this case other monkeys, activation occurs across a distributed network of property regions to represent those entities' salient features, namely, what they look like and how they move, even when those properties are not immediately present in the stimulus.

3.07.4.5.3 Reason #3 to think that property regions are involved in conceptual-level processing: Retrieving information from memory depends on reactivating property regions engaged while learning that information

A recent finding using fMRI brain state classification provides yet more evidence that property regions are involved in conceptual processing *per se*, rather than simply responding to features present in experimental stimuli. Polyn et al. (2005) demonstrated that

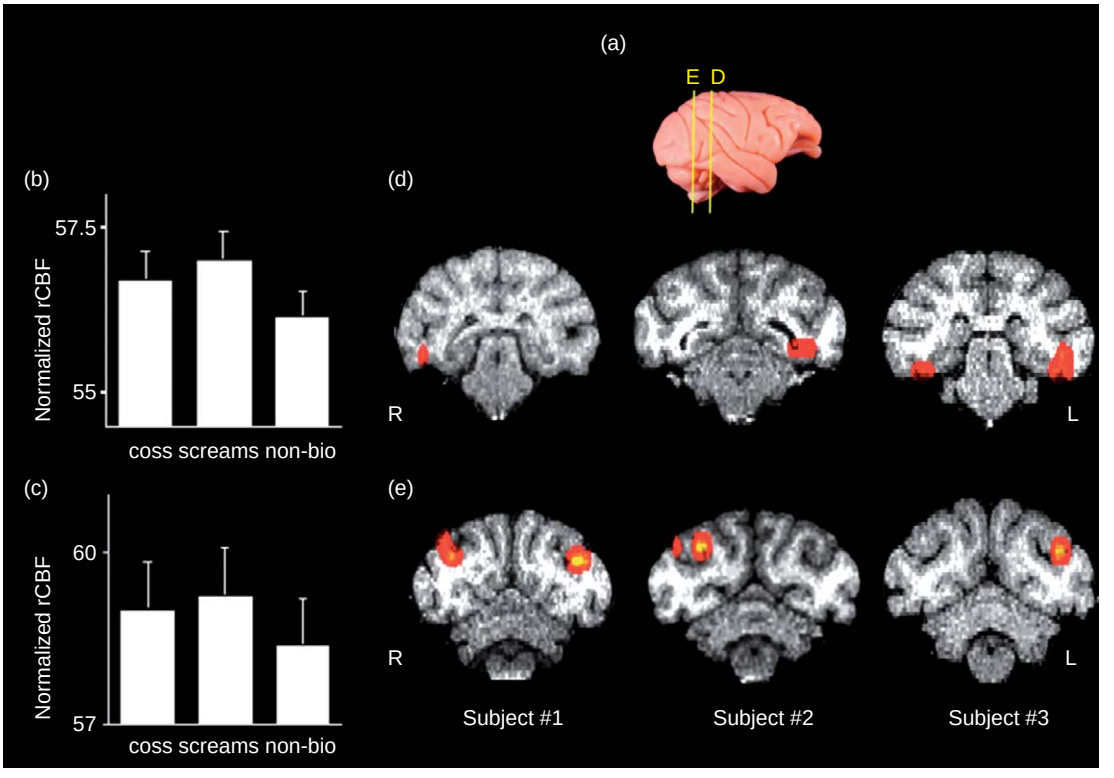

Figure 5 Activation of visual cortical areas in response to species-specific vocalizations in the rhesus macaque. (a) Lateral view of a rhesus monkey brain demonstrating the approximate locations of the coronal sections in (d) and (e). Both (b) and (c) show the mean (± SEM) normalized rCBF for the activations in TE/TEO and MT/MST/STS, respectively. In both regions, coos and screams exhibited reliably greater activation than nonbiological sounds. The coronal slices in (d) and (e) illustrate regions in each monkey that were more responsive to conspecific vocalizations (coos and screams) than to nonbiological sounds. Adapted from Gil-da-Costa R, Braun A, Lopes M, et al. (2004) Toward an evolutionary perspective on conceptual representation: Species-specific calls activate visual and affective processing systems in the macaque. *Proc. Natl. Acad. Sci. USA* 101: 17516–17521.

machine-learning algorithms are capable of detecting activity across the property regions underlying faces, places, and manipulable objects immediately prior to the free recall of information about each of these categories. During the classifier training phase of the study, subjects learned associations between labels and photographs of famous people, places, and common manipulable objects while undergoing fMRI. Later, while still undergoing fMRI, subjects were instructed to recall the items they had learned during the training phase. Brain state classifiers that were trained on data collected during encoding were able to detect distinct patterns of category-related activity that occurred several seconds prior to recall. Importantly, lateral fusiform activity served as the best predictor of famous face recall, left pMTG and parietal cortex activity best predicted manipulable object recall, and parahippocampal activity best predicted recall of places. By demonstrating that retrieving an item from memory depends on reactivating the pattern of activity in property regions that occurred during learning, this finding further establishes the centrality of property-specific information systems in the memory encoding, storage, and retrieval of conceptual information.

3.07.4.6 Learning about Objects by Building Property Circuits

The close correspondence between brain regions underlying perception and action with an object, and its representation in memory, suggests that conceptual property circuits develop out of experience with objects. A small but growing body of literature demonstrates that this is indeed the case. For example, Weisberg et al. (2007) asked subjects to perform a simple visual matching task on photographs of novel objects while undergoing fMRI. After scanning, the subjects were then given extensive training interacting with the objects, each of which was designed to perform a specific tool-like function. After training, the subjects were once again scanned while performing the visual matching task. Comparing the data from the two imaging sessions revealed that physical experience using the objects in a tool-like manner led to significant changes in the objects' neural representation. Whereas the novel objects elicited only diffuse ventral temporal activation in the first scan session, ventral temporal activity after training was largely restricted to the medial aspect of the fusiform gyrus, the same region previously implicated in representing the visual shape or form of tools. Similarly, new activations emerged after training in other regions observed in studies of tool knowledge, namely, the left pMTG (nonbiological motion) and left intraparietal sulcus and premotor cortex (physical manipulation).

Learning effects have also been observed for animate entities. As described earlier, viewing point-light displays of human forms in motion elicits activity in lateral fusiform and pSTS. Grossman et al. (2004) trained subjects to perceive human forms in point-light displays embedded within visual noise. After training, the subjects were not only better at indicating when a human form was present in a noisy visual display, but they also exhibited greater fusiform and pSTS activity in response to detecting those forms. Interestingly, the amount of activity in both regions was positively correlated with a subject's behavioral performance.

Yet further evidence for the development of property circuits with learning comes from James and Gauthier (2003), who demonstrated that property circuits can develop even through verbal learning. Prior to scanning, subjects learned verbally presented facts about families of novel animate-like entities called greebles. For example, subjects were trained that a particular family of greebles were associated with an auditory property (e.g., roars or squeaks), whereas other types of greebles had action properties (e.g., hops or jumps). After training, subjects underwent fMRI while performing a visual matching task that did not require retrieval of the learned associations. James and Gauthier found that viewing greebles associated with auditory properties produced activity in auditory cortex (as defined by an auditory functional localizer) and viewing greebles associated with action properties produced activity in the biological motion-sensitive region of the pSTS (as localized by moving point-light displays).

James and Gauthier's findings are important for at least two reasons. First, along with the findings of Weisberg et al. (2007), they illustrate how experience with category exemplars leads to the development of property circuits, which can later activate as property inferences. In both studies, simply seeing a particular object from the training set elicited activation in either premotor (for tools) or auditory and motion-sensitive cortex (for greebles), even though that information was unnecessary for successfully performing the task and not present in the stimuli. Second, it illustrates that this process can occur even when experience is verbally mediated. This finding is important precisely because so much of

our knowledge is acquired verbally, rather than through direct sensorimotor experience with objects and their properties.

3.07.5 Summary

The neuropsychological and functional neuroimaging findings presented here tell us much about the organization of conceptual knowledge in the brain. Surveying these studies, it appears that information about any particular object concept is distributed across a discrete network of cortical regions, rather than being represented in a single brain region. In addition, the particular neural circuit for a given object concept includes property regions that are most commonly engaged during perceptual experience with, or functional use of, the object, as demonstrated by training studies that documented the development of property circuits as subjects gained experience with an object.

One reason for suspecting that these property regions are not strictly perceptual but, rather, support conceptual representations is that some property regions activate automatically when an object is identified, regardless of whether their respective properties are immediately present in the stimulus. As such, these activations constitute property inferences about the object. At present, the extant findings suggest that for any given object, properties such as form, motion, and function-associated motor actions are particularly likely to be retrieved automatically. As such, these three property types may form a core set of information that is necessary and sufficient for representing object concepts in memory. There is good reason to believe, however, that future research using a wider array of object categories will reveal important roles for other property types as well (e.g., taste properties may be particularly important for food concepts). Finally, a close physical proximity exists between the neural systems underlying perception and knowledge representation for objects. Indeed, for color and motor properties, the neural bases underlying perceptual and conceptual property representation may partially overlap. Taken together, these findings strongly support so-called embodied cognition accounts of knowledge representation, which claim that conceptual property information is stored in the perceptual and motor systems active when that property information is learned.

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3.08 The Neurobiological Basis of Recognition Memory

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3.08.1 Introduction to the Concept of Recognition Memory

Recognition memory refers to the capacity to accurately identify a particular item or stimulus as having been previously encountered. An everyday example is our ability to recognize a person we have met once as someone that we have seen before. This ability to accurately recognize a person or an item requires that an individual encode the person or stimulus into memory at the time of encoding. After a delay, the subject must be able to then subsequently discriminate between a novel or new stimulus and a previously encountered stimulus. In the example in which a subject recognizes a person as someone whom they have met before, the subject would first need to have accurately encoded the person's face at an earlier time, and then at a later time would need to be able to accurately identify the person or select the person from a group of other subjects that the individual had not previously met. In research on memory function, recognition is often tested by having subjects distinguish between previously presented stimuli and stimuli that were not previously presented (termed lures). In the preceding example, we encounter novel and familiar stimuli all the time. Sometimes we are able to recognize with certainty a person or object: "That is my dog's vet" or "That is my coat." Other times the face or object looks familiar, but one is less certain about where the person or object was encountered before; "That person looks familiar, but I can't think of their name," or "I have seen that hat before, but I don't know who it belongs to."

Until the 1960s, memory was described as being a solitary process (Tulving, 2001), but in research over the last 50 years, memory researchers have moved toward describing memory as being comprised of multiple memory systems (Tulving, 1972; Eichenbaum and Cohen, 2001; Tulving, 2001). Within the framework of recognition memory, in the 1970s, Atkinson and Juola described two processes as being important for recognition (Atkinson and Juola, 1974). They proposed that an initial fast familiarity process was followed by a secondary slower process necessary for retrieving specific detailed information. In the recognition example given above, if you pass someone on the street, you may initially recognize that person as being someone who is familiar to you. This initial fast familiarity signal is then followed by a slower secondary search process in which you are able to retrieve detailed information about who that person is, their name, their relationship to you, and where you last saw them. We can all recall episodes in which this retrieval attempt is successful, and we remember the person's name and their relationship to us. In other cases, our retrieval attempt is unsuccessful, and while we still have our initial reaction of knowing that the person is familiar, we cannot recollect their name or recall where you last met this person.

In 1983, Endel Tulving suggested that long-term recognition memory could be could be divided into two separate components. He termed these components recollection (or remembering) and familiarity (or knowing). In keeping with the example given above, when you meet someone in the street, you can recognize that you simply know the individual, or you can clearly remember the individual encountered. The recollection process is proposed to involve the retrieval of the prior episodic experience, that is, you remember the contextual details associated with the last time you saw that individual. In contrast, familiarity is thought to represent a process that

does not rely on any explicit remembering of the details of the spatial or temporal context of the prior event, that is, you recognize the person as familiar but cannot place where or when you met them.

In addition to dissociating the processes of recollection and familiarity, another useful distinction that memory researchers make is the distinction between the processes of recognition and recall. Both recognition and recall require that stimuli be encoded, stored, and retrieved from memory. However, the process of recall places a much greater demand on the retrieval system, whereas recognition tests can assess whether information was encoded while placing less demand on memory retrieval systems by simply requiring that the subject distinguish previously learned information from new information. One analogy is that recognition memory tests resemble multiple-choice exams, where the answer is present but must be identified from false lures, whereas a recall test resembles a fill-in-the-blank exam, where the subject must retrieve the learned information from long-term memory in order to generate the answer. When memory declines, such as during aging and in diseases such as Alzheimer's disease, recall performance declines earlier and faster than recognition performance. A person may therefore be unable to recall someone's name, but if they are provided with multiple choices, they can recognize the correct one.

Recall can be divided into two types, free recall and cued recall. Both free recall and cued recall involve the retrieval or recall of a previously presented stimulus without the stimulus being presented during the test period. In a free-recall task, stimuli are presented during the sample period, and during the test period the subject is requested to generate as many stimuli as possible. Usually answers are provided verbally. In a cued recall test, the experimenter provides a cue, such as "name the animals that were on the list," that assists the subject in recalling the stimuli. In one specific type of cued recall task, known as a paired associates test, stimulus pairs are presented during the sample period; during the test period only a single stimulus in a pair is presented, and the subject must generate the previously paired stimulus. Free recall and cued recall can only be performed for stimuli that can be generated by the subject and therefore usually require the use of verbal stimuli in humans. Because recognition can be tested without requiring the subject to generate the previously presented stimulus, it proves very important in neuroscience studies of memory function in animals.

In the following sections, work on the neuroscience of recognition memory in humans and in animals is described in greater detail. First, an overview of recognition memory tests is provided, followed by sections describing lesion studies of recognition memory in humans and animals, electrophysiological studies of recognition memory in animals, imaging studies of recognition memory in humans, and computational studies of recognition. The human and animal studies indicate an important role for medial temporal lobe structures in the encoding of stimuli for subsequent recognition.

3.08.2 Experimental Tests of Recognition Memory

Recognition memory has been studied extensively in human subjects using a number of different paradigms. In one classic recognition memory task, the experimenter first presents subjects with a series of stimuli to be learned. After a variable delay period, the experimenter then presents the subject with a list of previously presented stimuli mixed with new ones (lures). The subject simply responds yes or no to each stimulus. This recognition paradigm is known as a simple yes–no recognition test. In another common recognition test, a single stimulus or set of stimuli are presented during a sample period, followed by a delay period of different durations, after which recognition is tested by presenting both a previously viewed sample stimulus and a novel lure at the same time during the test period. The subject must generate a response indicating which stimuli (target stimuli) were previously presented and which stimuli (lures) were not previously presented. This method is known as a forced-choice recognition memory test. The simple yes–no recognition test cannot be used effectively with patients with memory disorders, as they will often respond no to all stimuli. The method is not often used because of this response bias. The forced choice recognition memory test is less sensitive to response bias and can more sensitively assess recognition memory impairments, and measurements of response bias can be generated. There are a number of variations of these two basic behavioral designs that have been created and are described here.

More recent studies have elaborated on the simple yes or no response to more accurately characterize the two processes that combine to support recognition

memory, recollection, and familiarity. Both recollection and familiarity would generate a yes response in a yes–no or forced-choice recognition memory paradigm. By modifying the task, the experimenter can distinguish between recollective and familiarity responses. The distinction between a strong feeling of recollection and a weaker sense of familiarity is sometimes referred to as the remember versus know distinction. In experimental tests of these processes, a subject may be requested to identify whether they have a distinct recollection of the event of observing the stimulus (recollection) or whether they only have a general feeling of familiarity about the stimulus (familiarity). They may also be asked to retrieve information about contextual information related to the stimulus. Alternatively, in another twist on the yes–no recognition test, subjects may be requested to use an arbitrary scale to provide information about the strength of their memory or their level of confidence in remembering an item or event. Some behavioral and imaging studies have used a simple 3-point scale, corresponding to high-confidence yes (recollection), low-confidence yes (familiarity), and new (for the lures). Others have used 5- or 6-point scales ranging from a high-confidence yes (identify target) response (numerical value 5 or 6) to a high-confidence no (reject lure) response (numerical value 1). Studies of behavioral confidence rankings have been used to support a two-process theory of recognition memory in work by Yonelinas (2001). This work uses signal detection theory-based receiver-operator-characteristics (ROC) curves to analyze the nature of recognition memory. This work used the distribution of proportions of responses to argue that recognition memory includes a graded mechanism of familiarity recognition and a high-threshold process of recollection-based recognition. The Yonelinas model assumes that previously presented items give rise to a range of confidence ratings based on their familiarity, whereas new items (lures) give rise to some familiarity but not as much as the previously presented items. In contrast, when recollection is triggered for an individual item, the recollection process is assumed to provide sufficient confidence for subjects to respond with certainty in the highest yes category (allowing the responses to cross a high-threshold criterion). In the dual-process model, familiarity is a variable phenomenon, and recollection is an all or nothing response. There have been studies that suggest distinct medial temporal lobe regions support recollection and familiarity-based recognition. However, the studies remain controversial. Some researchers support the idea that the hippocampus is required for recollection, whereas parahippocampal structures support familiarity; however, other data suggest that recollection and familiarity are part of a unified single process, supported by the same neuroanatomical structures within the medial temporal lobe. Similar controversy surrounds efforts to make distinctions between regions supporting encoding during the sample period versus separate regions mediating recognition during the testing period.

Another common recognition memory task is known as the delayed match-to-sample task, or DMS task. The DMS task involves presentation of a single sample stimulus followed by a delay period, followed by a pair of test stimuli, where one stimulus matches the presentation stimulus and one does not match the sample stimulus. The subject must select the stimulus that matches the sample stimulus. In a variant of this task known as a delayed non-match-to-sample or DNMS task, the subject (human or animal) must select the nonmatching novel stimulus. A further variation involves continuous presentation of stimuli, in which the subject must respond on the basis of whether the current stimulus matches the stimulus presented n trials previously (an N-back task), which can be extended to longer intertrial intervals. Since it typically uses very short delays, the N-back task is considered to be a working memory task.

The DMS or DNMS task designs have been used most extensively in studies of the neural basis of recognition memory in animals. In these paradigms, the animal encounters the sample stimulus and, after a delay period, is presented with a pair of stimuli and must respond to either the match (target) or nonmatch (lure). Alternatively, the sample stimulus could be followed sequentially by single lure stimuli (during which the animal withholds its response) until the target stimulus appears and the animal must generate a response. Performance of these tasks requires recognition memory, but the process of recognition could be interpreted as involving either short-term active maintenance of the stimulus (working memory) or long-term synaptic encoding of the stimulus, depending on the length of the delay.

Other paradigms used in animals include presentation of a longer list of sample stimuli, which could include a series of visual stimuli presented to monkeys, or a set of odor stimuli presented to rats. After a delay period, the animals are then tested with a forced-choice design, in which the animal encounters pairs of stimuli, each of which includes one sample

stimulus and one lure stimulus. In rats, recognition memory is commonly tested using the non-match-to-sample paradigm, to capitalize on the natural tendency of rats to explore novel stimuli more extensively than familiar ones. The rat can be presented with a sample stimulus, and subsequently when presented with an identical sample stimulus and a novel lure, it will spend more time exploring the novel lure.

3.08.3 Lesion Studies of Recognition Memory

There is extensive lesion evidence supporting the idea that the medial temporal lobe structures, including the hippocampus and neighboring parahippocampal regions, are critical for encoding information into long-term memory. Research on the neuroanatomical structures critical for memory function focused on the medial temporal lobe, following the case of patient HM, first described by Scoville and Milner (Scoville and Milner, 1957) and studied extensively in further work by Milner and Corkin (Corkin, 1984). Patient HM showed a striking impairment of episodic memory encoding following bilateral removal of his medial temporal lobes in an attempt to control epileptic seizures. Initial descriptions of patient HM's impairment focused on his almost total loss of free recall and severe impairment of cued recall performance. Similarly, other hippocampal amnesic patients show strong impairments of free recall and cued recall. Hippocampal amnesics also show moderate impairment on tests of recognition memory (Manns et al., 2003), and the amount of impairment appears to be associated with the magnitude of damage to the parahippocampal structures including the entorhinal cortex, perirhinal cortex, and parahippocampal gyrus (Rempel-Clower et al., 1996).

Lesion studies in humans have also addressed the question of whether the processes of recollection and familiarity are subserved by different regions within the medial temporal lobes. However, the results continue to be controversial. Researchers including Yonelinas have argued that hippocampal lesions cause a greater impairment of recollection-based recognition than familiarity-based recognition (Yonelinas et al., 2001, 2005). On the other hand, studies by Manns and Squire have demonstrated impairments of both recollection- and familiarity-based recognition associated with medial temporal lesions (Manns et al., 2003).

In primates, lesions of medial temporal lobe structures cause impairments on the recognition of individual trial unique stimuli in a range of different studies on delayed matching function (Gaffan, 1974; Zola-Morgan and Squire, 1985, 1989, 1993; Alvarez et al., 1994; Leonard et al., 1995). Selective hippocampal lesions alone cause impairments in DNMS tasks (Zola-Morgan and Squire, 1985), but the impairment is much more severe if lesions include damage to surrounding cortical areas such as the perirhinal cortex and parahippocampal gyrus (Meunier et al., 1993, 1996; Zola-Morgan et al., 1993). Entorhinal and perirhinal cortex ablations also impair performance in DMS tasks (Gaffan and Murray, 1992). The impairments in these studies appear at longer delays (e.g., 1 min, 10 min) but not short delays (0.5, 1, or 3 s) (Gaffan and Murray, 1992; Alvarez et al., 1994), consistent with the notion that entorhinal cortex and perirhinal cortex are important for recognition memory function that is distinct from short-term active maintenance for working memory.

Impairments of performance in object- or odor-recognition tasks have also been shown with inactivation or lesions of the perirhinal cortex in rats (Otto and Eichenbaum, 1992; Ennaceur et al., 1996; Bussey et al., 2000). These studies show that object recognition is not impaired with lesions of the fornix or the orbitofrontal cortex, again suggesting an important role of the perirhinal cortex beyond its interactions with the hippocampal formation. The tasks designed for human studies of recollection and familiarity have been modified for odor recognition to examine these processes in rats (Fortin et al., 2004). The yes–no response used in human visual recognition memory was altered to a delayed nonmatching paradigm for odor recognition, in which a rat was exposed to a set of sample odors and then subsequently had to respond to individual test odors presented in cups filled with sand. If the odor did not match a previous sample odor (new), the rat could dig in the cup for a food reward. If the odor did match a previous sample odor (old), the rat would return to the back of the experimental chamber to dig in a separate cup for food reward. In these studies, the signal detection approach used in humans was applied to rats by manipulating the selectivity criterion for recognition responses from conservative to liberal. This was achieved by changing the relative cost and benefit of a positive recognition response by changing the

amount of reward given for an old response and by increasing the height of the cup containing the test odor. In this paradigm, rats show a threshold response for a conservative criterion, demonstrating a significant number of hits even when the false alarms are near zero. This resembles the threshold response proposed to result from the use of recollection-based memory in humans. Lesions of the hippocampal formation in rats appear to selectively remove this recollection-based response property, causing the response properties to resemble those proposed to indicate use of familiarity-based recognition alone.

Pharmacological manipulations can also affect recognition memory function, indicating a role for cellular neuromodulatory effects in this process. Systemic injections of muscarinic cholinergic antagonists has been shown to impair performance on recognition memory tasks at longer delays in monkeys, while sparing performance at 0-s delay; this occurs with both the antagonists scopolamine (Bartus and Johnson, 1976) and atropine (Penetar and McDonough, 1983). Injections of scopolamine appear to impair the encoding of new stimuli for recognition but not retrieval of stimuli learned before scopolamine injection (Aigner and Mishkin, 1986; Aigner et al., 1991; Tang et al., 1997). Scopolamine also impairs recognition memory in humans (Sherman et al., 2003; Schon et al., 2005) but has a stronger effect on free recall and cued recall (Atri et al., 2004). Systemic injections of scopolamine also impair arm choice behavior in an eight-arm radial maze in rats when there is a delay between the individual choices (Bolhuis et al., 1988). The locus of the cholinergic effect has been tested with localized injection of cholinergic antagonists in monkeys. Tang et al. (1997) demonstrated that infusion of scopolamine into perirhinal cortex before encoding impairs performance on a recognition memory task, whereas infusion into adjacent structures did not cause impaired performance. Microdialysis shows a 41% increase in acetylcholine levels in perirhinal cortex during encoding in this visual recognition task (Tang and Aigner, 1996). Selective impairments of the encoding of stimuli for subsequent recognition have also been induced with manipulations that remove the cholinergic innervation of cortical structures. Recent experiments used the selective agent IgG 192 saporin to lesion the cholinergic innervation of the entorhinal cortex in rats (McGaughy et al., 2005). These selective lesions caused a significant and selective impairment of DNMS function for novel odors (McGaughy et al., 2003, 2005). Similarly, DMS function for novel visual stimuli was also impaired by selective lesions of entorhinal cholinergic innervation in monkeys (Turchi et al., 2005).

3.08.4 Electrophysiological Studies of Recognition Memory

As noted, most studies of memory function in animals use tasks that test recognition memory, since it is not feasible to have animals perform recall tasks. Thus, most studies of electrophysiological activity associated with memory function have been conducted during short- or long-term recognition memory paradigms. In both monkeys and rats, unit recording studies analyze the generation of action potentials by individual neurons during performance of such tasks. The activity associated with recognition can be evaluated by comparing the response to a previously presented target stimulus during the test period with the response to a nonpresented lure or by comparing the response to the target stimulus with the response to the same stimulus during the sample period. A number of studies have shown changes in neural activity for a previously presented target stimulus in comparison to nonpresented lures. Many studies suggest that recognition memory for an item involves a reduction of neurophysiological activity, not more activity, as shown by recordings of single-neuron spiking activity during performance of a serial recognition task by monkeys (Brown et al., 1987; Wilson et al., 1990; Riches et al., 1991; Fahy et al., 1993). In those experiments, monkeys see a series of novel stimuli, with occasional repeat presentations of familiar stimuli. The familiar stimuli cause a reduced level of spiking activity in single neurons recorded from parahippocampal cortices (Brown et al., 1987; Wilson et al., 1990; Riches et al., 1991). A similar reduction of neuronal spiking activity to familiar stimuli was observed in recordings in the prefrontal cortex, inferotemporal cortex, and entorhinal cortex of monkeys during repeated presentation of stimuli in a DNMS task (Miller et al., 1991, 1993, 1996; Suzuki et al., 1997). Miller demonstrated that repetition suppression occurs even for repeated distractor stimuli presented during a delay period, whereas enhancement of the target test stimulus relative to the sample stimulus (match enhancement) only occurs for the target stimulus and not for distractor stimuli (Miller et al., 1993, 1996). Increased responses to matching stimuli were

shown in the anterior thalamus in studies of a serial recognition task (Rolls et al., 1982). Computational modeling indicates that the delay activity and match enhancement over short periods could be due to intrinsic currents, but the longer-term match suppression effects have been modeled as dependent upon synaptic long-term depression (Sohal and Hasselmo, 2000; Bogacz and Brown, 2003).

The process of recognition could be supported either by synaptic modification or by active maintenance of information during the delay period. In support of active maintenance, a number of unit recording studies during DMS tasks (Fuster, 1973; Fuster and Jervey, 1982; Fuster, 1995, 2000) have demonstrated that some neurons activated by the sample stimulus will maintain their activity during the delay period until the test stimulus is presented. This delay period activity could reflect active maintenance of the stimulus for performance of the recognition task. Much of this research has focused on the prefrontal cortex (Fuster, 1973, 1995; Miller et al., 1996; Fuster, 2000), but delay activity has also been shown for neurons in the inferotemporal cortex (Fuster and Jervey, 1982; Miller and Desimone, 1994) and in parahippocampal regions including the entorhinal cortex (Suzuki et al., 1997). The prefrontal cortex activity shows greater resistance to the effect of distractors, whereas the medial temporal delay activity is more likely to be terminated by a distractor (Miller et al., 1996). Persistent activity has also been shown for entorhinal neurons in the rat during the delay period of a continuous DNMS task using odors (Young et al., 1997). Neurons in the entorhinal cortex of the rat also show increases or decreases of activity associated with match or mismatch between sample and test (i.e., match enhancement or match suppression). The persistent activity could arise from intrinsic mechanisms within individual neurons or from excitatory feedback between neurons in a population. For example, network simulations demonstrate how the patterns of unit firing during DMS and DNMS tasks could arise from cholinergic activation of intrinsic persistent spiking mechanisms (Fransén et al., 2002; Hasselmo and Stern, 2006). However, most models of persistent spiking activity have focused on the hypothesis that persistent spiking activity during delay periods occurs due to attractor dynamics caused by excitatory recurrent synapses (Amit, 1988; Lansner and Fransen, 1992; Amit and Brunel, 1997; Lisman et al., 1998). This type of persistent spiking activity could contribute to the increase in cerebral blood flow measures seen during the delay period of DMS tasks or two-back tasks in human subjects.

The role of cholinergic receptors in recognition memory function described above could be due to involvement in either persistent spiking for active maintenance or synaptic depression for repetition suppression. These mechanisms may coexist in single neurons of perirhinal cortex. In the rat, the perirhinal cortex shows reduced levels of activity in response to repeated stimuli, and this effect has been proposed to arise from cholinergic activation of long-term depression (Warburton et al., 2003), because scopolamine blocked the induction of synaptic long-term depression in that study. However, scopolamine did not block repetition suppression in a study in monkeys, though it did cause a behavioral impairment (Miller and Desimone, 1993). This latter result suggests that cholinergic modulation may also play a role in persistent activity or match enhancement mechanisms important for recognition memory.

3.08.5 Functional Imaging Studies of Recognition Memory

Despite considerable evidence from human and animal lesion studies regarding the importance of the hippocampus and medial temporal lobes for encoding and recognition, early imaging studies using positron emission tomography (PET) primarily identified changes in activity in cortical structures outside of the medial temporal lobe, most notably within the prefrontal cortex (for review, see Schacter and Wagner, 1999). The tasks used in these earlier studies focused on priming and on recognition memory for verbal stimuli learned prior to scanning. Using functional magnetic resonance imaging (fMRI) and a task design that focused on examining functional changes during the encoding period, as opposed to examining the recognition period, Stern and colleagues (Stern et al., 1996) identified robust activation of the hippocampus and parahippocampal structures when subjects viewed sets of novel visual items with the goal of being able to identify them later. This simple block-designed paradigm contrasting blocks of stimuli that were novel with blocks of repeating stimuli provided clear evidence that the hippocampus and neighboring parahippocampal regions were necessary for encoding complex visual stimuli into memory. With the development of event-related fMRI methods (Rosen et al., 1998), it became possible to examine fMRI activity

during individual trials as opposed to blocks of stimuli. Researchers designed studies to focus on how the event-related fMRI activity during presentation of single stimuli during the encoding period would correlate with recognition performance for those individual stimuli during a subsequent recognition memory task. Two of the earliest event-related studies (Brewer et al., 1998; Wagner et al., 1998) demonstrated a subsequent memory effect, in which increases in fMRI activity in medial temporal lobe structures were noted for stimuli that were remembered with high confidence, but not for stimuli that were subsequently forgotten. Since the first block-designed studies (Stern et al., 1996; Gabrieli et al., 1997), these event-related studies have examined subsequent memory effects for individual words and pictures (Brewer et al., 1998; Wagner et al., 1998; Henson et al., 1999; Kirchhoff et al., 2000).

More recently, functional imaging studies have extended these subsequent memory studies to address the hypothesis that recollection and familiarity are dependent on different regions within the medial temporal cortex. Based on animal studies (see Brown and Aggleton, 2001, for review) and anatomical differences between the hippocampus and parahippocampal regions, neuroimaging researchers have examined the hypothesis that the hippocampus is critical for the process of recollection, whereas the parahippocampal gyrus is sufficient and necessary for supporting familiarity-based recognition. Ranganath et al. (2003) conducted an event-related fMRI study in which activity at the time of encoding within the rhinal cortex supported familiarity-based recognition, whereas encoding related activity within the hippocampus and posterior parahippocampal cortex predicted recollection. Proponents of this account suggest that the parahippocampal regions are associated with successful item recognition, whereas hippocampal activity is necessary for successful source memory, which includes information about contextual details. It should be noted that while there are several studies supporting the idea that separate neuroanatomical regions support recollection and familiarity (Ranganath et al., 2003; Daselaar et al., 2006), the issue remains controversial, as there is also research supporting the idea that the hippocampus and parahippocampal regions support both recollection and familiarity (Manns et al., 2003; Squire et al., 2004), supporting the idea that the strength of recognition varies along a single continuum as opposed to the dual-process model (Wixted and Stretch, 2004).

In addition to activity within medial temporal lobe regions, functional neuroimaging studies have provided data that support the idea that encoding and subsequent recognition require widespread activity outside the medial temporal lobe, including areas within the ventrolateral prefrontal cortex and parietal lobe (Schacter et al., 1997; Kirchhoff et al., 2000; Yonelinas et al., 2005). Current research is ongoing to determine whether this prefrontal and parietal activity is related to the successful retrieval of information from memory or is attributable to control mechanisms driving recollection attempts (Kahn et al., 2004).

In addition to the long-term recognition studies described above, another area of research has examined the recognition of stimuli that were observed only a short time previously. In neuroimaging research, these studies have primarily employed working memory N-back tasks as well as DMS tasks with short delays. It should be noted that at a cellular level, these tasks most likely require active maintenance rather than modification of synapses. At a systems level, the N-back task and other working memory tasks typically cause extensive activity in prefrontal cortex and parietal cortex, which increases when the memory load is increased by increasing the value of N to require matching with a stimulus presented two or three stimuli previously. When familiar stimuli are used in a delayed matching task, it does not cause significant medial temporal lobe activity, but when novel stimuli are used, it causes a significant increase in medial temporal lobe activity (Stern et al., 2001). This could reflect a greater role for medial temporal lobe structures in active maintenance and encoding of novel stimuli (Ranganath and D'Esposito, 2001; Stern et al., 2001). Recently, Schon et al. (2004) demonstrated that delay-related activity in the medial temporal lobe during a DMS task with novel stimuli correlated with subsequent recognition memory for the stimuli.

Primate neurophysiological studies have suggested that repetition suppression is involved in recognition memory function (Miller and Desimone, 1994; Suzuki et al., 1997; Brown and Aggleton, 2001). However, these previous studies were not able to answer the question of whether the reduction in spiking activity correlated with the parametric graded strength of recognition memory performance. A recent article by Gonsalves et al. (2005) provides crucial data to answer this question. This study demonstrated a correlation between reduced activity (as measured using fMRI and MEG) and higher confidence recognition

of stimuli. This study builds on extensive cognitive research using the remember versus know recognition memory paradigm, described earlier. Stimuli given the highest confidence recognition rating (recollected–R-hit) showed the least activity in the perirhinal and parahippocampal cortices, while stimuli showed somewhat more activity when the subject was less confident and merely had a feeling of knowing the stimulus (K-hit). Even greater activity was seen for the incorrect responses (miss) and correct rejections (CR). These data clearly demonstrated that the reduction of activity for familiar stimuli is correlated with stronger recognition of the stimuli as measured behaviorally. This study provides a crucial extension of previous neuroimaging studies showing a reduction in activation associated with novel stimuli becoming familiar. The earliest demonstration of changes in medial temporal lobe fMRI activity during a memory task showed less activity during repeated presentation of a single stimulus versus substantial activity during sequential presentation of different novel stimuli being encoded for subsequent recognition (Stern et al., 1996), and studies have demonstrated that the repetition reduction occurs for both visual and verbal stimuli and that greater activity for novel stimuli in hippocampal and parahippocampal regions is associated with the encoding of information into long-term memory (Brewer et al., 1998; Kirchhoff et al., 2000; van Turennout et al., 2003). Though these and other studies have shown correlations between increased medial temporal lobe activity during encoding and subsequent performance on recognition tasks, and others have shown reduced activity associated with recognition of old stimuli (Weis et al., 2004), the study by Gonsalves et al. (2005) evaluated correlations between reduced activity during recognition testing and the concurrent graded levels of performance on recognition tasks.

3.08.6 Computational Modeling of Recognition Memory

The processes involved in recognition memory have been modeled on a number of different levels. Many modeling studies have focused on the mechanisms of active maintenance, in which persistent spiking activity of a population of neurons during the delay period is proposed to form the basis for retaining information about the stimulus presented during the sample stimulus. Most models of active maintenance focus on the role of excitatory recurrent connections within cortical structures such as the prefrontal cortex (Amit, 1988; Lansner and Fransen, 1992; Amit and Brunel, 1997; Lisman et al., 1998). These excitatory recurrent connections can cause a population activated by the sample stimulus to continue to reactivate its component neurons, allowing the population to maintain activity for the full delay period and retaining the information until the test period, when a comparison can be made. In contrast, other recent models of active maintenance focus on intrinsic single-cell mechanisms, in which a single neuron can maintain persistent spiking activity without requiring excitatory synaptic feedback between neurons (Fransén et al., 2002; Hasselmo and Stern, 2006). This mechanism would be particularly appropriate for novel stimuli for which a representation has not previously been formed (Hasselmo and Stern, 2006). The persistent spiking of single cortical neurons stemming from intrinsic properties can persist for many minutes, allowing this mechanism to contribute directly to performance of DMS tasks with delays of several minutes.

For recognition over longer periods, it is not possible to maintain persistent spiking representing all previously viewed stimuli. In this case, some representation of stimuli based on changes in synaptic connections between neurons would be more appropriate. Computational models have demonstrated how presentation of a sample stimulus could induce Hebbian synaptic potentiation, in which synapses are strengthened dependent upon presynaptic activity coupled with postsynaptic activity (Hasselmo and Schnell, 1994; Hasselmo and Wyble, 1997; Sohal and Hasselmo, 2000). This synaptic modification results in greater activity of neurons during the test period if they were previously active during the sample period (match enhancement). In addition to nonselective match enhancement, recognition can also be modeled as the capacity to retrieve a context given a specific item (Hasselmo and Wyble, 1997). In contrast, match suppression or repetition suppression can be obtained by competitive long-term depression of synapses for nonactivated neurons (Sohal and Hasselmo, 2000). Alternately, researchers have proposed that repetition suppression might arise from Hebbian long-term depression, which provides a mechanism for recognition memory with higher capacity than Hebbian long-term potentiation (Bogacz and Brown, 2003). The above processes focus on responses to the item alone and could be seen as reflecting familiarity processes. The separate

theoretical processes of recollection- versus familiarity-based recognition have been modeled as different processes in different structures (Norman and O'Reilly, 2003). In contrast to familiarity, which could involve an increase or decrease in activity of the neurons representing the sample stimulus, recollection may involve a test item reactivating a full representation of contextual features associated with a specific episode (Hasselmo and Wyble, 1997; Norman and O'Reilly, 2003). This recollection process could involve more complex neuroanatomical circuits required for reactivation of the temporal or spatial context of the item being recognized.

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3.09 Animal Models of Amnesia

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3.09.1 Introduction

Our understanding of the nature of the human amnesic syndrome has expanded enormously in the last 60 years. This increased knowledge stems not only from the detailed description of memory disorders in patients with more circumscribed brain lesions and from more recent neuroimaging studies in both patients with memory disorders and normal subjects, but also from a growing number of animal models of different species. These animal models have been used to refine our understanding of the specific brain regions involved in human amnesic syndromes and the critical memory processes mediated by each region. Because the field is so broad and covers a number of brain systems whose contribution to memory is certain, but less well understood (e.g., diencephalic structures), this chapter will focus on research centered on the medial temporal lobe structures and their participation in memory processes. After a brief review of the early work in humans leading to the description of the temporal lobe amnesia syndrome, we will discuss the importance of developing animal models for the study of this syndrome. We then describe several classes of behavioral paradigm and their analogs that have been used in both rats and monkeys to more specifically determine the memory processes mediated by different medial temporal lobe structures, and their recent use to study both human amnesic patients and normal human subjects will be discussed. Finally, we will briefly review some of the conclusions derived from the use of animal models of amnesia and how they have contributed to our understanding of the neural substrates of both human and animal memory.

3.09.2 The Neural Substrates of Memory: Earlier Studies

The concept that memory can be localized in the brain dates back at least several hundred years when Descartes proposed that experience left traces in the

brain which could then be retrieved through the actions of the pineal gland (Descartes, 1662/1972). However, it wasn't until the beginning of the twentieth century that systematic searches for these traces were made. In a decades-long search to discover where in the brain memory traces, or 'engrams,' resided, Karl Lashley trained rats on mazes and then systematically disconnecting cortical areas using knife cuts, or ablating areas of cortical tissue, to assess their effects on performance. In spite of his efforts, a specific locus where memory resided could not be identified. The ability of knife cuts or ablations to affect memory was directly related to the amount of tissue damaged. In 1950, he published the results of 30 years of research in his landmark paper, 'In search of the engram,' in which he concluded that:

> It is not possible to demonstrate the isolated localization of a memory trace anywhere within the nervous system. Limited regions may be essential for learning or retention of a particular activity, but within such regions the parts are functionally equivalent. The engram is represented throughout the region. (Lashley, 1950: 478)

These conclusions were summarized in two principles of brain function: (1) principle of mass action, which states memory is distributed throughout the brain (no centralized loci), and (2) the principle of equipotentiality, which proposes that if one area of the brain is damaged, another is able to assume the functions of the damaged region. These two principles explained why only large lesions produced deficits and why recovery of function was possible from discrete lesions. Thus, it appeared that the theory of localized memory traces had been laid to rest. At about this same time, quite different results were discovered in the brain mapping work of neurologist Wilder Penfield. In preparing epileptic patients for removal of epileptic foci, he would stimulate areas of cortex to pinpoint the critical tissue. During some of these stimulations deep in the temporal lobe, he discovered that the patient would suddenly describe detailed memories depending upon the location of the stimulation. Furthermore, stimulating the same locus elicited the same memory (Penfield, 1958), and mild memory impairments were observed in some patients with unilateral temporal lobe resections. However, the landmark case that to this day drives the study of memory and the brain was that of patient H. M.

This patient was treated for intractable epilepsy by the bilateral removal of the temporal lobes, which included parts of the amygdala, hippocampus, and subjacent temporal cortex (See **Figure 1**). The result of this surgery was a profound anterograde amnesia that persists to this day, and a somewhat milder retrograde amnesia for events closer to the time of his operation, although childhood memories were intact (Scoville and Milner, 1957). What was

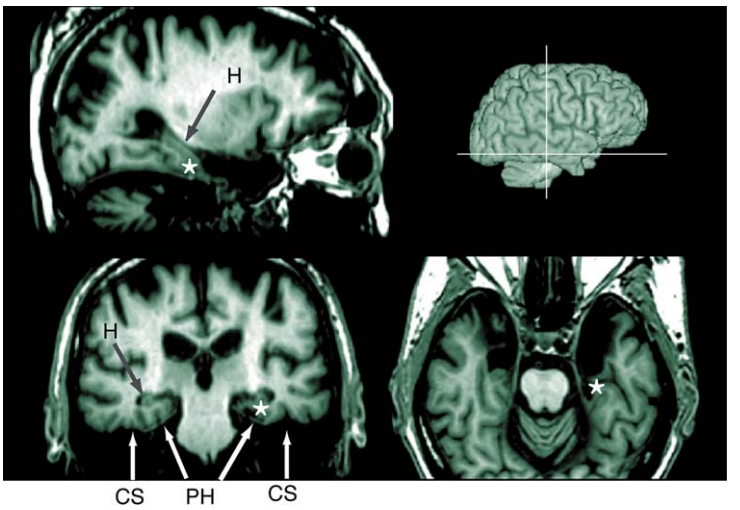

Figure 1 Multiplanar views of 18 averaged T1-weighted magnetic resonance imaging volumes showing preserved structures in H. M.'s left medial temporal lobe (MTL). The asterisk marks the intersection of the three viewing planes, just caudal to the left MTL resection, seen best in the transaxial view. Top left, sagittal view; bottom left, coronal view; bottom right, transaxial view; top right, surface rendering showing locations of transaxial and coronal planes. Abbreviations: CS, collateral sulcus; H, hippocampus; PH, parahippocampal gyrus. Reprinted from Corkin S (2002) What's new with the amnesic patient H. M.? *Nat. Rev. Neurosci.* 3(2):153–160, with permission from Macmillan Magazines Ltd.

particularly interesting about this patient was that, in spite of this devastating memory impairment, some learning capabilities survived (Corkin, 1968). The evidence from H. M. and other patients with milder impairments seemed to point to the hippocampus as the structure commonly damaged in patients with memory impairments (Penfield and Milner, 1958). Several important points came from this research: (1) the hippocampus and possibly other structures in the human medial temporal lobe (MTL) was required to encode some new memories, but some memory functions remained intact after MTL damage; (2) because some past memories survived, the hippocampus or related MTL structures were not the permanent storage site of long-term memory; and (3) to the extent it was involved in retrieval, this role must be time limited as well. The study of H. M. and other amnesic patients in the subsequent years revealed much about the neural basis of human memory. As is discussed elsewhere in these volumes, the idea of multiple memory systems, some of which are spared even in global amnesics, came directly from patients like H. M. What was not immediately forthcoming was similar evidence from the animal literature.

In spite of the effects of MTL damage on human memory, damage to the hippocampus in animals initially appeared to have very limited (Douglas, 1967; Weiskrantz and Warrington, 1975) and somewhat specific effects in rat or monkey memory (e.g., Mahut, 1971; Olton, 1977). For some time it appeared that there was no comparison even between humans and monkeys, in spite of the high degree of anatomical homology between human and nonhuman primates, and even rodents, with respect to the medial temporal lobe structures (see **Figure 2**). However, in 1975, Mishkin and Delacour described a modification to the matching-to-sample memory task developed by Gaffan (1974) that could be readily learned in monkeys and that was closer to tests of human recognition memory, known as trial unique, delayed *non*matching-to-sample (**Figure 3**). Earlier versions of this task (Gaffan, 1974) used small stimulus pools and were very difficult for monkeys to learn and served more as tests of *recency* rather than recognition. To better approximate the recognition tasks used in humans, Mishkin and Delacour (1975) used large pools of stimuli, creating a 'trial-unique' version of the task. That is, rather than one which required the subject to remember which stimulus had been seen most recently (i.e., recency memory), the new version required the subject to indicate which stimulus was novel (presumably known because the other stimulus was remembered, i.e., recognition memory). Combining this task with damage to the medial temporal lobe similar to that sustained by H. M. yielded at last an impairment in monkeys apparently equivalent to the memory deficits suffered by human amnesic patients. Mishkin (1978) demonstrated for the first time that, whereas amygdalectomy or hippocampectomy alone produced mild impairments at best, combined lesions, effectively replicating H. M.'s damage, not only produce a severe impairment on relearning the task postoperatively, but even once relearned, memory for an individual stimulus rapidly decayed across brief delays (**Figure 4**). Continued work in human amnesic patients further characterized the nature of the deficit as being limited to particular long-term memory processes, termed 'declarative' or 'episodic/semantic' or 'explicit' memory (Cohen and Squire, 1981; Cohen, 1984; Tulving, 1972), and those processes that are spared in human amnesics termed, for example, 'nondeclarative' or 'implicit' memory were also shown to be spared in the operated monkeys (Zola-Morgan and Squire, 1984). The pattern of impairment and sparing of memory functions in both humans and monkeys with medial temporal lobe damage led to the idea that there are multiple memory systems in the brain, which has been a major guiding force in the research of the last 30 years. This research has vastly increased our knowledge about the neural bases of memory and generated model systems in monkeys, rodents, and at the cellular level in invertebrates.

3.09.3 Animal Models

3.09.3.1 Why Animal Models?

From the outset, the goal in the creation of models of human amnesia has been to better understand the neural circuits that contribute to human memory. The underlying assumption, which is supported by detailed anatomical studies, is that there is conservation of structure and function among mammalian systems, that is, the organization of the infrahuman brain is sufficiently similar to that of the human brain to permit such comparisons. In spite of great differences in size and complexity, the basic organizational principles in the brain apply up the evolutionary scale (see **Figure 2**). The benefit is that animal studies can provide a degree of experimental control that

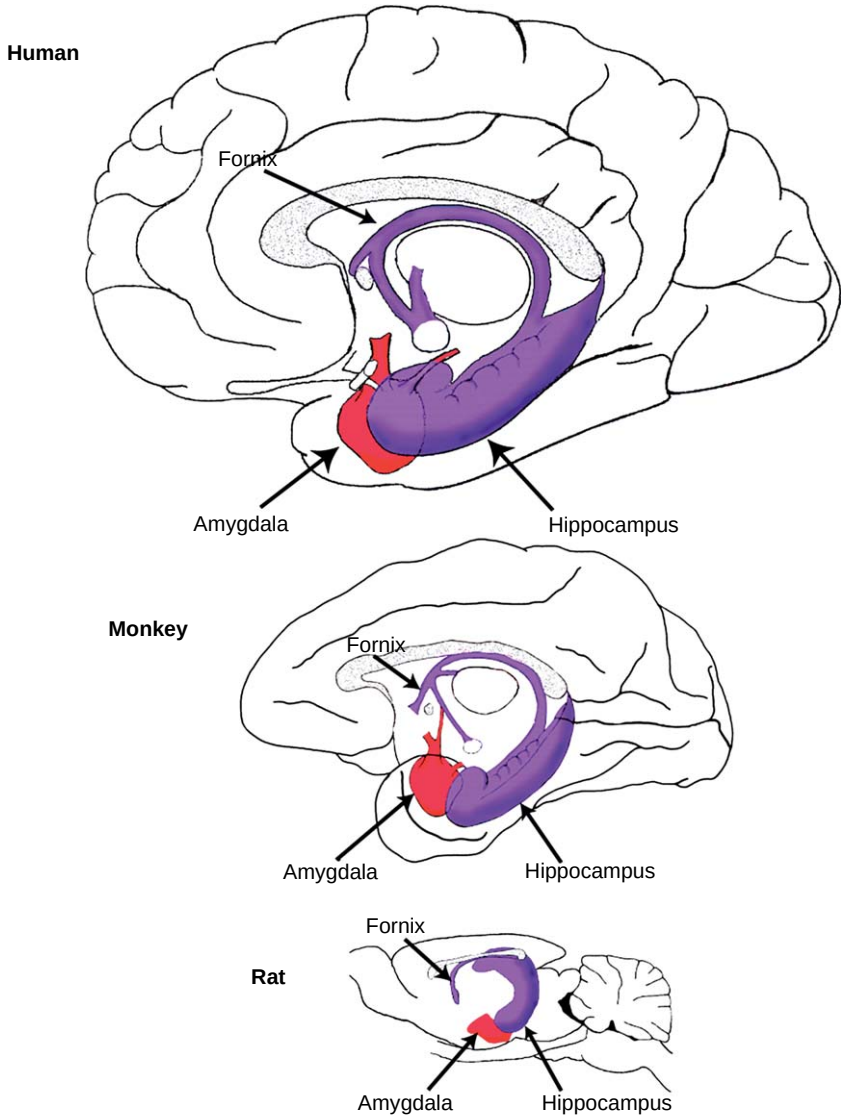

Figure 2 Midsagittal view of the human, monkey, and rat brains illustrating some components of the medial temporal lobe important for memory function: the amygdaloid complex, hippocampus, and fornix. Relative sizes are not to scale. *See also* Chapter 3.03.

is almost impossible to achieve with human studies. With very few exceptions, the neural damage associated with human amnesia has been fairly extensive, and not always determinable. Furthermore, though physical damage can be detected and mapped out, it is not always clear whether 'spared' tissue is functioning normally (a caveat for all lesion studies). The methods available to animal researchers improve selectivity in the assessment and manipulation of brain function, which allows not only for the revelation of the individual contributions of brain structures to memory, but also for the assessment of how different circuits interact with each other in the service of normal memory. As described earlier, initial studies suggested that only when damage approximated that sustained by H. M. was a clear memory impairment observed in monkeys. Although smaller lesions do impact memory, either with greater specificity (e.g., memory for objects vs. memory for space) or degree (e.g., only at long delays), these restricted lesions do not produce dense amnesia in animal or human subjects. Nevertheless, the investigation of how individual structures contribute to memory will better enable the understanding of amnesic symptoms and, in the case of organic causes of amnesia, the development of treatments.

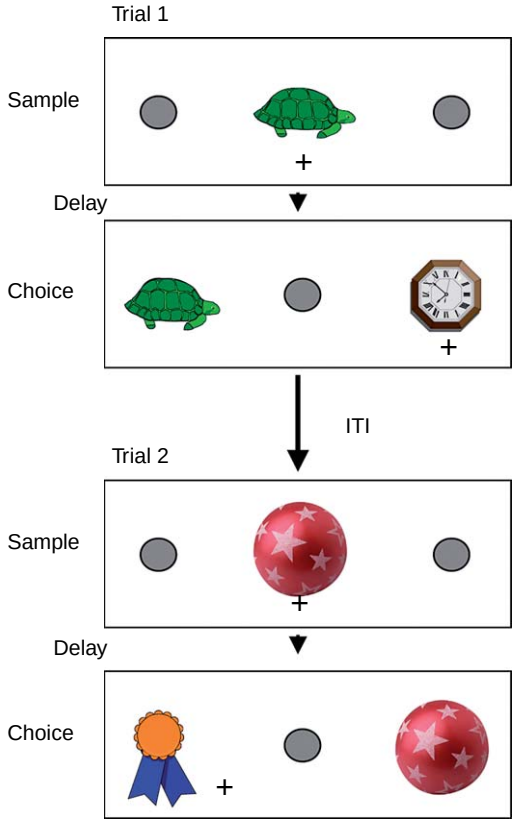

Figure 3 Delayed nonmatching-to-sample (DNMS) task. Trial 1: The monkey is presented with a sample object covering a food reward (+) in the central well of the testing tray. After a delay, during which the test tray is obscured, the sample object and a novel choice object are presented simultaneously covering the lateral wells, but the novel object is now rewarded (i.e., nonmatch-to-sample). After a brief intertrial interval (ITI), the next trial begins. Trial 2: For this and all subsequent trials, a new pair of objects serves as the sample and choice (i.e., trial unique).

3.09.3.2 What Is Being Modeled?

The focus thus far has been on the history of human amnesia resulting from damage to the MTL. It should be noted, however, that there is a wide literature for human Korsakoff patients, whose prolonged alcoholism has produced damage to the diencephalic structures of the brain, as well as anterograde declarative amnesia (*See* Chapter 3.04; Squire, 1982; Squire et al., 1990). Although animal studies have demonstrated a role for the diencephalic structures in memory (e.g., Aggleton and Brown, 1999; Vann and Aggleton, 2004), the research has been more extensive for structures in the MTL, and so we will focus on research in these structures of the brain in the remainder of this chapter. Nevertheless, the question must be raised: what exactly is it that is being modeled? Human memory has been defined as comprising two main systems: a declarative, or explicit system, containing rich memory representations that can be recalled at will, and a nondeclarative, or implicit system such as skill learning (for which there is not always a particular recollection of having acquired the skill). Whereas such divisions have a clear basis and intuitive appeal when applied to humans, how are we to ask a nonverbal animal to 'declare' what is in its memory store? Thus in animal models, there is an ongoing debate as to how best to define the animal equivalents of declarative and nondeclarative memory systems (*See* Chapter 1.04).

In humans, it can be verified that someone has a memory of an event, because they can recall and describe the event in detail at a later time. It is not possible to measure recall in the same way in animals; however, we can use several means to determine whether an animal can demonstrate that it has experienced an event and remembers it on some level. In this instance we infer memory by observing a change in the animal's behavior. To this end, there are some clear consistencies in the human amnesic syndrome that may be taken as core deficits that can be assessed with animal models. These include global (i.e., multiple modality) anterograde amnesia for facts and events, in the presence of intact skill learning or conditioning; spared memory for remote events, but possible temporally graded retrograde amnesia for information closer to the onset of amnesia; and intact short-term memory, with a forgetting curve that is steeper in amnesic patients than in normal subjects. Thus, although we cannot determine whether an animal can *recall* a specific memory, we can determine whether an animal can *recognize* a previously seen memorandum and use that information to guide behavior. We can then assess whether animals forget newly learned information more rapidly as a result of particular brain damage than controls. Finally, we can determine whether only information learned in a specific context (i.e., episode) is forgotten, or if the processes that allow encoding of context are somehow impaired.

In addition to the development of specific tests of animal memory, a number of theories of memory formation have been developed that are, to a greater or lesser degree, tied to specific neural structures. These 'memory systems' are reviewed elsewhere in these volumes, so discussion will be limited to pointing out where different theories make specific

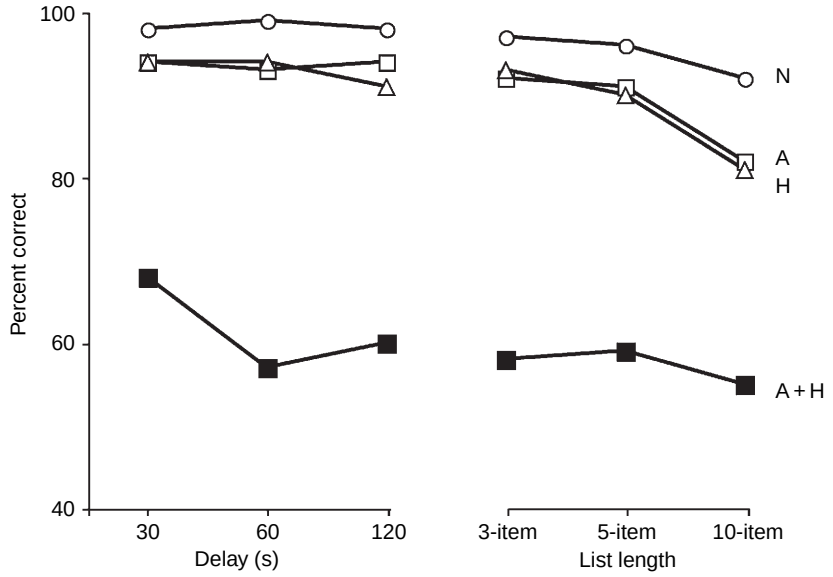

Figure 4 Performance of monkeys with damage to the medial temporal lobe on DNMS, when delays are increased from 30 to 120 s or the number of to-be-remembered objects is increased from three to ten. N, unoperated controls; A, animals with aspiration lesions of the amygdala; H, animals with aspiration lesions of the hippocampal formation; A + H, animals with combined amygdala and hippocampal lesions. Adapted from Mishkin M (1978) Memory in monkeys severely impaired by combined but not by separate removal of amygdala and hippocampus. *Nature* 273(5660): 297–298, with permission from Macmillan Journals Ltd.

predictions about a given task and to group the discussion of particular memory tasks into theoretical categories. In particular, we will focus on tasks requiring recognition memory, conjunctive/relational memory, or spatial memory. This is a loose distinction, as recognition memory tasks, for example, can be used to assess spatial or relational information and vice versa.

3.09.4 Recognition Memory

Two paradigms in particular have been used to assess recognition memory in rodents and monkeys: the delayed nonmatching-to-sample task (DNMS) and the visual paired comparison (VPC) task (also known as preferential looking, or spontaneous recognition). Like some tests of human recognition or recall, each task assesses whether subjects demonstrate that a given stimulus has been previously seen (judgment of prior occurrence; Brown, 1996). However, substantial differences in the task requirements may alter both the demands made on memory processes and, potentially, the specific brain regions necessary for successful performance of each. In particular, these tasks may be seen as testing memory for 'facts' as well as the duration of the memory trace, but with slight parametric modification.

3.09.4.1 Delayed Nonmatch to Sample

This task was the first to successfully demonstrate a deficit in recognition memory consequent to the same neural damage that produces human amnesia. The task is simple in that monkeys learn to displace junk objects to obtain a hidden food reward. However, food is only located under objects that have not been seen recently. That is, animals are trained to associate novelty with reward. In the basic paradigm, the monkey is seated in a sound-attenuated chamber (Wisconsin General Testing Apparatus, or WGTA) behind an opaque screen (see **Figure 3**). When the screen is raised, the monkey views a testing tray containing three equidistant food wells, which can be covered with junk objects, hiding either a baited or an empty well. Training takes place in two phases for each trial: sample and choice. During the sample phase, a single object covers the central food well, and when displaced, a food reward can be retrieved. The screen is then lowered and the now-familiar sample object is moved to cover a lateral well (empty), while a novel object covers the opposite lateral well (baited). After a brief period, typically

ranging from 10 s to 10 min, the screen is raised, and the monkey must choose one of the objects. If the animal remembers the sample object and correctly applies the nonmatching rule 'choose the unfamiliar item,' then the novel item is chosen and the food reward can be retrieved. When the animal reliably masters the rule governing the task, memory can be further manipulated by changing the length of the delay between the sample and choice phases, by changing the number of items to be remembered (i.e., list learning), or by changing the nature of the information to be remembered (i.e., stimulus location, or nonvisual modalities, such as tactual or olfactory).

The early work by Mishkin and others on this task confirmed that large lesions to the MTL dramatically impaired performance (Mahut et al., 1982; Mishkin, 1978; Squire and Zola-Morgan, 1983) while sparing performance on tasks such as visual discrimination learning in which monkeys learn a set of 20 concurrent discrimination problems that are presented only once per day, thus with 24-h delays (Malamut et al., 1984). These results seemed to match the spared and impaired performance capabilities of amnesic patients such as H. M., who could not retain new information for more than a few seconds without active rehearsal, but could improve over many trials to perform tasks such as mirror drawing or the incomplete figures task (Gollin, 1960; see Milner, 2005). However, as a measure of *hippocampal* memory function, the results from the DNMS task have been called into question.

Using more selective lesion techniques, it has become possible to parse out the individual contributions of MTL structures to recognition memory. For example, Zola-Morgan et al. (1989) showed that the addition of cortical-sparing amygdala damage to animals with hippocampal damage did not exacerbate their memory impairment, suggesting that Mishkin's (1978) initial findings were due in part to the cortical damage sustained by the animals with combined lesions. Indeed, further studies demonstrated that damage to the subjacent perirhinal and entorhinal cortices, removed along with the hippocampus and amygdala in the initial Mishkin studies, devastates performance of this task in monkeys (**Figure 5**; Zola-Morgan et al., 1989; Meunier et al., 1993, 1996). However, damage to these cortical areas (collectively referred to as 'rhinal' cortex) that spares the hippocampus and amygdala produced as large an impairment as the combined lesion (Murray and Mishkin, 1998; Baxter and Murray, 2001).

The use of neurotoxic lesions of the hippocampus has not entirely solved the question. Murray and Mishkin (1998) showed that monkeys with selective neurotoxic damage to the hippocampus and amygdala showed normal DNMS performance out to 2-min delays. Nor were they impaired on lists of 40 items, which were then tested in reverse order, resulting in delays ranging from 30 s to 40 min. Zola and colleagues (2000) found that regardless of lesion method, hippocampal damage impaired DNMS performance at delays of 10 min and beyond (see also Beason-Held et al., 1999, for similar findings). However, a meta-analysis of the results across several laboratories suggested that there was a negative correlation, even within studies, with the amount of damage on performance of the task (Baxter and Murray, 2001; see Zola and Squire, 2001, for reply). By contrast, one study has suggested a positive correlation between lesion size and memory deficit at delays of 10 min (Nemanic et al., 2004). Indeed, those results suggested that the lack of impairment in monkeys with hippocampal damage might be due in part to alternate performance strategies that allow the animal to bridge the longest delays. When a distraction is inserted into the delay period (opening the screen at short delays, having the animal perform a motor task at longer delays, or removing it from the apparatus during 10-min delays) an impairment is revealed in animals with selective neurotoxic hippocampal damage at the longest delay, which correlates positively with the amount of hippocampal damage (Nemanic et al., 2004). However, in this same study, damage to the perirhinal cortex produced an even greater impairment on performance of DNMS, with or without distraction, even after short delays.

Taken in context with what we now know of the anatomy of the region, it is clear that the perirhinal cortex is important for object memory, in particular for object identity (Ungerleider and Mishkin, 1982), and that damage limited to this region produces a severe impairment in visual recognition memory (Suzuki et al., 1993; Eacott et al., 1994; Buffalo et al., 1999). Indeed, it is currently being argued that the perirhinal cortex may be important for visual perception as well as memory (e.g., Murray and Bussey, 1999; Buckley et al., 2001; but see also Hampton, 2005, for a different interpretation of the data). Hippocampal damage, by contrast, leaves short-term memory intact on this task, which is maintained both by the temporal cortical region and also by active mechanisms when necessary, possibly controlled by the prefrontal cortex.

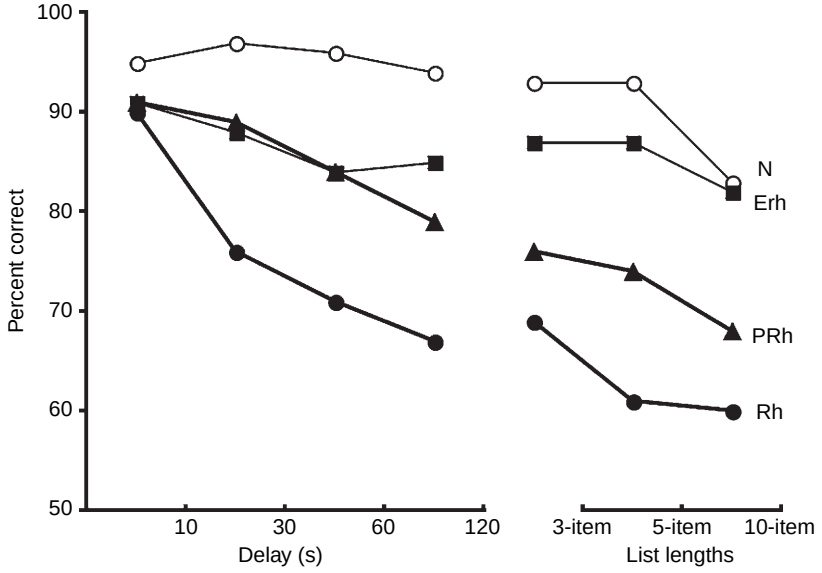

Figure 5 Effects of entorhinal (Erh), perirhinal (PRh), and rhinal (Rh; Erh + PRh) lesions on DNMS performance as compared to unoperated (N) animals. Adapted from Meunier M, Bachevalier J, Mishkin M, and Murray EA (1993) Effects on visual recognition of combined and separate ablations of the entorhinal and perirhinal cortex in rhesus monkeys. *J. Neurosci.* 13: 5418–5432, with permission from the Society for Neuroscience.

Several rodent versions of the DNMS task for objects have been developed (e.g., Aggleton, 1985; Kesner et al., 1993; Mumby and Pinel, 1994; Rothblat and Hayes, 1987); however, results from rodent studies have also questioned the view that the hippocampus is necessary for object memory. As recently reviewed by Mumby (2001), the majority of studies assessing the effects of hippocampal damage on DNMS or delayed matching-to-sample (DMS) have shown no effect on recognition memory in rodents, except in instances where cortical damage was also present (e.g., Clark et al., 2001) or in those studies where hippocampal damage was produced by forebrain ischemia. The hippocampus is extremely sensitive to ischemia, and as has been shown in humans, ischemic events produce quite profound memory deficits in monkeys (Zola-Morgan et al., 1986; Rempel-Clower et al., 1996). However, although ischemia was thought to have damaged only a single hippocampal cell field (CA1) in human patients (Squire and Zola, 1996), there is evidence to suggest that the damage is more widespread, and particularly detectable through the use of specific histological methods (Bachevalier and Meunier, 1996). Furthermore, although ischemic damage in human amnesic patients impaired performance on a DNMS-like task, thus seemingly providing a direct link between the hippocampus and recognition memory, later work in rats by Mumby and colleagues (1996) showed that ischemia-related memory impairments were likely due to either extra-hippocampal damage or to abnormal discharges in the remaining cortical tissue. They showed that producing ischemia, followed by removal of the entire hippocampus, actually spared learning on DNMS. Thus, at best, the effect of hippocampal damage on DNMS for objects is mild and restricted to long delays and lists.

Finally, it is worth noting recent developments in the nature of recognition memory. It has been suggested that two processes support recognition memory performance: recollection and familiarity. These two processes are in play when an animal or human must determine whether or not a given item was previously seen (see Aggleton and Brown, 1999, for review). In the absence of strong recollection, familiarity processes will determine whether they recognize the item or not. These two processes can be measured by the use of receiver operating characteristic (ROC) (see **Figure 6**) curves in which the shape of the curve determines the degree to which recollection or familiarity contributes to performance (Yonelinas, 1994, 1997). For normal memory, the shape of the ROC curve reflects the contribution of both recollection and familiarity, showing an asymmetrical and curvilinear shape (**Figure 6(a)**). It has been proposed that the familiarity component has a symmetrical and curvilinear shape, whereas the recollection component is

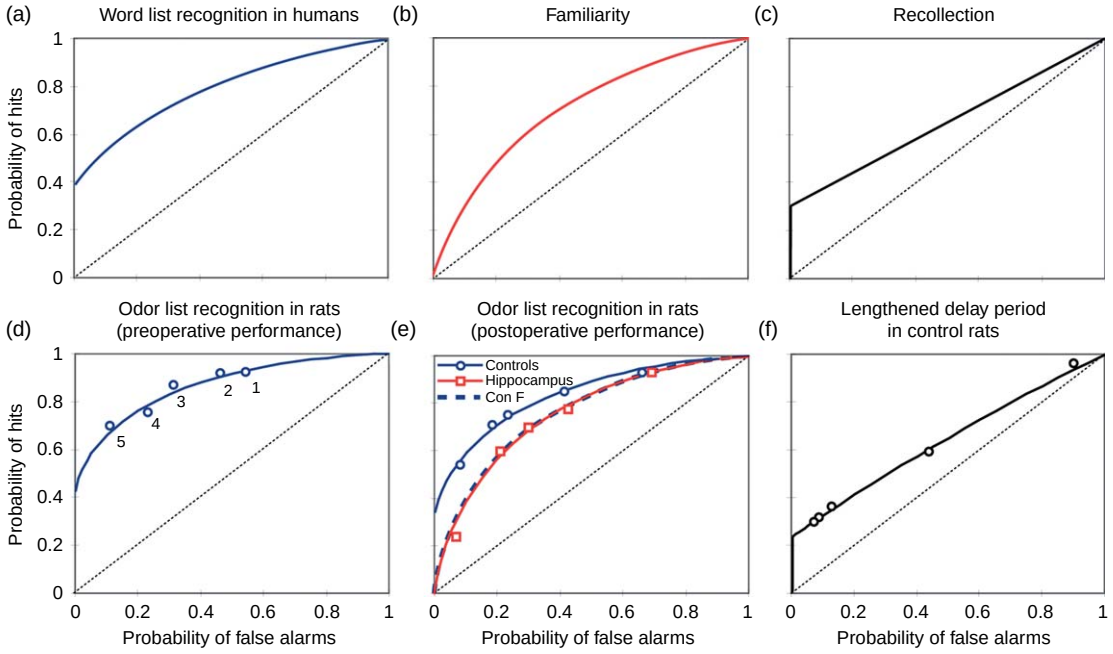

Figure 6 Example of receiver operator characteristic (ROC) curves for normal human performance (a) and the portions of the curves contributing to (b) familiarity and (c) recollection. (d) Performance of normal rats on odor discrimination tasks. (e) Performance of normals vs. hippocampectomized rats illustrating characteristic familiarity curve in the hippocampal group. (f) Performance of control rats after 75-min delay, illustrating the recollection component of the curve. From Fortin NJ, Wright SP, and Eichenbaum H (2004) Recollection-like memory retrieval in rats is dependent on the hippocampus. *Nature* 431(7005): 188–191; reprinted with permission from Macmillan Publishers Ltd.

asymmetrical and linear. In an odor recognition task, Fortin et al. (2004) showed that rats demonstrated both the asymmetrical and curvilinear components of the ROC curve by manipulating the rat's 'bias' level. By changing the amount of reward and the effort required to retrieve it, rats shifted their response criterion to more or less strict levels, shifting their tendency to correctly identify previously encountered odors ('hits') or incorrectly identify new odors as old ('false-alarm'), suggesting that both recollection and familiarity play roles in normal rodent recognition memory performance (**Figure 6(d)**). By contrast, selective hippocampal damage produced symmetrical and curvilinear ROC curves (**Figure 6(e)**), indicating that only familiarity was contributing to their performance (Fortin et al., 2004). Finally, after very long delays, the ROC curve for control rats was asymmetrical and linear, suggesting that only recollection contributed to performance after long delays (**Figure 6(f)**). Thus, these results suggest that the hippocampal formation in rodents is necessary for recall, but not recognition. Although these results remain to be replicated, studies in humans with selective hippocampal damage also show similar shifts in the ROC characteristics (e.g., Yonelinas et al., 1998; Aggleton et al., 2005; Cipilotti et al., 2006), although there is active debate about the interpretation of the results (e.g., Wixted and Squire, 2004; Wais et al., 2006).

3.09.4.2 Visual Paired Comparison/ Spontaneous Recognition

Similar to DNMS, the VPC task utilizes a familiarization phase and a choice phase, but the task demands are quite different. This task takes advantage of monkeys' natural preference for looking at novel things in their environment, and in contrast to the DNMS, it in fact requires no specific learning and no forced choices between two objects to obtain rewards. For VPC, monkeys passively view a visual stimulus, typically a black/white image, and are allowed to look at it for a sufficient period to show habituation (i.e., they cease visual exploration). This familiarization period may range from 15 to 30 s of looking time. At this point, the image disappears, and after some delay period (as brief as 1 s) the image reappears on the screen, side by side with a novel image. Monkeys, rats, and humans

naturally prefer to look at (explore) the stimulus they have not yet seen (novelty preference); thus we infer that they remember the familiar stimulus. Given that the subject is not actively performing a task, it should not be surprising that performance levels on VPC are much lower than on DNMS, where animals are trained to a 90% correct criterion. With VPC, typical novelty preference is in the range of 65–70% preference for novelty, but this effect is reliable. Unlike the DNMS task, VPC has proven to be very sensitive to hippocampal damage at delays as short as 1 min (Pascalis and Bachevalier, 1999; Zola et al., 2000; Clark et al., 2001; Nemanic et al, 2004). Similar to DNMS, however, damage to the temporal cortex produces impairments at shorter delays (~30 s for parahippocampal gyrus, and ~10 s for perirhinal cortex). Thus, though the sensitivity may be greater in detecting recognition memory deficits in the VPC task, the contributions of at least three regions of the MTL maintain a similar relationship to each other; that is, perirhinal contributes in the initial encoding and short-term retention of visual stimuli, whereas parahippocampal area TH/TF and hippocampus are required for longer retention. Similar to the DNMS task, the role of the hippocampus and area TH/TF in VPC becomes more prominent when arrays or locations of objects are used as stimuli (see **Figure 7**), making the task more spatial in nature, but the role of perirhinal cortex is evident in all object conditions, regardless of their spatial relations (Nemanic and Bachevalier, 2002).

Studies in rodents initially employed what was termed the spontaneous recognition paradigm (Ennaceur and Delacour, 1988). In this task, rodents are allowed to examine a junk object using vision, olfaction, and touch during a sample phase, and then after some delay during which the object is not present, an identical old object and a novel one are placed equidistant from the rat. Rats preferentially spend time exploring the novel object. Variants of the task have been used to explore recognition of single items, locations, or item configurations as well as the effects of specific neural damage on performance. Initial studies failed to find effects of fornix damage on object recognition, but did find an effect on recognition for location (Ennaceur and Aggleton, 1994; Ennaceur et al., 1997). By contrast, using methodology similar to the monkey visual VPC experiments, Clark and colleagues showed deficits as early as 1 min in rodent visual preference (Clark et al., 2000) after neurotoxic damage to the hippocampus. Later studies have also been equivocal. Spared novelty preference has been reported in rats with selective hippocampal damage at delays of 48 h (Forwood et al., 2005; Winters et al., 2004) and recently as long as 3 weeks (Mumby et al., 2005), although the latter study used five daily familiarization sessions. By contrast, damage to the peri-postrhinal region

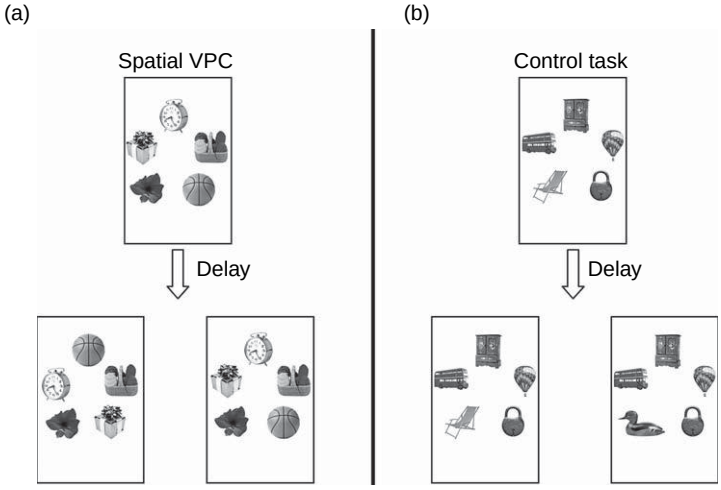

Figure 7 Spatial variant of the visual paired comparison (VPC) task using arrays of objects. (a) Spatial VPC: Five objects are presented in a visual array. Following a brief delay, the sample array and a novel arrangement (in which three objects are repositioned) are presented to the monkey. (b) Control task: A similar spatial array is presented in the sample phase, but the novel array has one object replaced with a new one.

showed impaired novelty preference (Ennaceur et al., 1996; Winters et al., 2004). It is possible that this difference between rodent and monkey results in part from the methods used. With the exception of the Clark et al. (2000) study, most rodent paradigms allow the rat to physically explore the object, using any or all of three sensory systems: vision, touch, smell. The primate studies were limited to visual recognition (see Mumby, 2001).

In sum, these two measures of recognition memory show very different sensitivity to selective damage to hippocampus or temporal cortex. As noted, however, in both tasks, each region contributes to recognition memory in the same relative manner, but the absolute delay for which impairment occurred differs for each region damaged.

3.09.4.3 Performance of Human Amnesics on Animal Tests of Recognition

Human amnesia has, by and large, been tested with numerous tasks that are quite different from those used with animals (*See* Chapter 3.04). Thus differences in the nature or extent of impairments between animal models and the human impairment may be due to task differences, to lesion differences, or to evolutionary forces, in addition to the lack of language capabilities. Thus, it is important to test humans on animal memory tasks both to validate the behavioral assays and to better understand specific impairments in human patients. Regarding the lesion differences, the recent reports of patients with selective damage to individual components of the MTL provides an opportunity to test specific memory functions in humans with similarly selective damage and to compare the results with those from other species, and eventually may inform the assessment of human patients.

Tests of human amnesic patients on animal tests of recognition, while relatively few, are largely in agreement with the animal findings when large temporal lobe lesions were used. For example, Squire et al. (1988) reported that a group of patients whose presumed damage was either diencephalic (Korsakoff's patients) or hippocampal (ischemic/anoxic patients) were severely impaired on the DNMS task, similar to the initial monkey studies (Mishkin, 1978). Similarly, Reed and Squire (1999) reported marked impairments on DNMS in patients with damage to the hippocampal formation. Unlike the monkey studies, however, they were also impaired on a concurrent discrimination task that is routinely spared in monkey studies and that presumably is a model of procedural memory. Based on the finding that performance levels on the task were directly related to declarative knowledge of the solution, Squire et al. (1988) suggested that, unlike monkeys, humans normally learn this task declaratively. By contrast, patients with damage restricted to the hippocampus have shown spared performance even on DMS at delays up to 30 s (Holdstock et al., 2000a), which follows more closely the results in monkeys and rodents and suggests that the impairment seen in the early studies of Squire may have resulted from damage to the adjacent MTL structures (*See* Chapter 3.08).

Similarly, recognition memory impairments have been demonstrated on the VPC task, and as in animals, these impairments are often larger than those obtained from matching or nonmatching tasks. For example, McKee and Squire (1993) reported novelty preference in human amnesic patients when the delays were short (0.5 s) but not when delays were extended to 2 min or 2 h. Direct comparisons of VPC and DMS performance in human amnesic patient Y. R., who became amnesic after a possible ischemic infarct, resulting in reduced hippocampal volume and no other obvious pathology as measured by magnetic resonance imaging (MRI) (Holdstock et al., 2000b). This patient had previously shown impaired recall, but intact recognition on standard human memory tests (Holdstock et al., 2000b). She also demonstrated severe impairments in memory on VPC (Pascalis et al., 2004), demonstrating preference for novelty at the 0-s delay only. At the longer delays of 5 and 10 s, her performance fell to chance. By contrast, as described above, her performance was as good as controls on the DMS task with delays up to 30 s (Holdstock et al., 2000a). Unfortunately, she was not tested at longer delays on DMS, but the reported results map onto those reported in rats and monkeys in that hippocampal damage impairs VPC performance at much shorter delays than on DNMS (Clark et al., 2000; Zola et al., 2000; Nemanic et al., 2004). As for the animal studies, it is possible that the difference between VPC and DMS/DNMS performance is related to the forced choice required by the matching tasks that may encourage the subjects to rely on familiarity in the absence of recall to support good performance.

3.09.5 Conjunctive/Relational Memory

As is described in detail elsewhere in these volumes (*See* Chapter 1.04), a number of memory systems have been proposed to uniquely account for the

types of deficits observed in animals with selective hippocampal damage. Within the animal literature, this discussion has often centered around theories proposing that the hippocampal formation is predisposed toward spatial or contextual memory processing, and those which contend that this is too narrow a view and does not account for deficits on nonspatial memory tasks except by invoking 'context.' Two of these viewpoints, the conjunctive account (Sutherland and Rudy, 1989; O'Reilly and Rudy 2001) and the relational account (Eichenbaum et al., 1989), have generated much attention in the search for hippocampal function. Both of these viewpoints address the way in which the hippocampus forms associations among individual elements in the environment, with Rudy, Sutherland, and O'Reilly taking the conjunctive viewpoint and Eichenbaum and colleagues taking the relational viewpoint. The distinctions between these accounts will be addressed in detail in the relevant chapters of this book; for the purposes of this chapter, we will focus on two tasks that are representative of the two viewpoints. These two tasks have been selected because they have been tested in a variety of species, including humans, and have provided evidence for nonspatial deficits in animals and humans with hippocampal or MTL damage.

3.09.5.1 Transverse Patterning

The transverse patterning task was first described by Spence (1952) as an example of a task that cannot be solved on the basis of individual stimulus representations. It is formed of three concurrent discrimination problems that are presented as follows: A+ versus B–, B+ versus C−, and C+ versus A−, where A, B, and C are individual stimuli, and + or − indicates the rewarded or unrewarded member of the pair, respectively. Because the individual elements (A, B, and C) are inconsistently associated with reward, a representation of the three conjunctions <AB>, <BC>, and <CA> allows for the disambiguation of the individual elements. By contrast, it has also been suggested that this task requires a relational solution, in that the subject must learn that A, B, and C stand in unique relationships to each other (Dusek and Eichenbaum, 1998).

Using a swim-maze version of the Lashley jump stand, Alvarado and Rudy (1992) trained normal rats on a visual discrimination version of the transverse patterning task. By adding problems incrementally, they were able to determine that learning two problems at once (e.g., A+ vs. B− and B+ vs. C−) produced configural learning in normal rats, in spite of the possible elemental solution (pick A, avoid C) the animals could have used. However, there is a clear point in training beyond which configural representations are needed, which is the addition of the third discrimination (C+ vs. A−). It is at this point that hippocampal damage was predicted to impair performance, a prediction that has been empirically proven in further rodent studies using neurotoxic lesions of the hippocampal formation (Alvarado and Rudy, 1995a,b). Rats with hippocampal lesions were able to perform the task only when an elemental solution was possible (i.e., when two problems had to be simultaneously resolved), but not when the third was added. Furthermore, they were unimpaired at learning three elemental discriminations (A+ vs. B−, C+ vs. D−, and E+ vs. F−). In another swim version of the task, Driscoll et al. (2005) replicated Alvarado and Rudy's (1995a,b) basic findings, but further found a retrograde impairment for *both* elemental and configural information learned presurgically. Thus, the elemental impairment was in the retrograde direction only (i.e., remembering previously learned elemental problems was impaired, but learning new elemental problems was intact). The impairment for conjunctive information, however, was both anterograde and retrograde (Driscoll et al., 2005).

In a related study, Dusek and Eichenbaum (1998) used an olfactory digging version of the task in which three scented sand-filled cups served as stimuli, and a food reward was buried in the correct cup. Using a slightly different training paradigm, they found that animals with fornix transection or damage to the temporal cortex were impaired when stimulus presentation was random, although they could manage to perform when the problems were presented in blocks. Similarly, these authors showed that NR1-knockout mice, which have been genetically modified such that they have no *N*-methyl-D-aspartate (NMDA) receptors in hippocampal cell field CA1, were also impaired on the odor version of transverse patterning, but were unimpaired at learning a set of concurrent odor discriminations (Rondi-Reig et al., 2001).

More recently, monkeys with neonatal damage to the hippocampus and subjacent cortex (but not those with damage to the amygdala and subjacent cortex; Alvarado et al., 2002) and adult macaques with neurotoxic hippocampal damage (Alvarado and Bachevalier, 2005b) were impaired in an object

version of the transverse patterning task. These operated animals were unimpaired in performing a linear sequence of discriminations A+ versus B−, B+ versus C−, C+ versus X−, confirming that the impairment was only for the use of conjunctive representations.

By contrast, neither fornix transection nor selective hippocampal damage was able to impair a touch screen version of the task in either rats or monkeys (Bussey et al., 1998; Brasted et al., 2003; Saksida et al., 2007). The reason for the differences between the findings on the effects of hippocampal damage on the transverse patterning task are not yet clear, but methodological differences between the studies could be responsible. For example, it is noteworthy that, in the touch screen version of the transverse patterning task, control rats and monkeys required more trials to learn the task and reached lower performance levels (performing at 60–70% correct after several thousand trials when the three problems are intermixed) as compared to control rats trained on the swim or digging versions of the task and monkeys in an object version of the task (performing at 90% correct within 90–300 trials for rats and 300–500 trials for monkeys). It will be informative to assess in future studies whether the different versions of the task encourage the use of specific strategies that determine the success or failure of control subjects. Such information will likely reveal the types of memory processes engaged for task performance and have implications for the degree to which the hippocampus or cortical structures are preferentially engaged.

3.09.5.2 Transitive Inference

This task specifically tests predictions from the relational memory view of hippocampal function. In particular, it assesses a proposed property of declarative memory that allows for the inferential or flexible use of memories in new situations (Cohen, 1984; Eichenbaum, 1992). The logical structure of the task takes the form of A+ versus B−, B+ versus C−, C+ versus D−, and D+ versus E− (Dusek and Eichenbaum, 1997). Note that, with the exception of the endpoints A and E, all other stimuli are equally often rewarded or nonrewarded. It has been suggested that humans and animals form an orderly series of representations, such that A > B > C > D > E. The crucial test of this hypothesis is a novel test between B and D. Although these two stimuli have never before been paired together, if the subject has learned a set of hierarchical relationships, it should be able to *infer* that B is greater than D (i.e., if John is taller than Bill and Bill is taller than Peter, who is taller, John or Peter?). Eichenbaum and colleagues have suggested that the hippocampus allows for the formation of these orderly representations, and importantly, that these relational representations permit the flexible use of memory, so that in novel situations, animals and humans can respond correctly. In support of this hypothesis, they have shown that, whereas some 88% of normal animals correctly choose B over D on the probe, animals with hippocampal damage were at chance (Dusek and Eichenbaum, 1997; but see Frank et al., 2003; van Elzakker et al., 2003, for alternative interpretations).

3.09.5.3 Role of the Temporal Cortex

Finally, it should be noted that a number of recent studies have implicated the perirhinal, entorhinal, or parahippocampal cortices in conjunctive or relational tasks. For example, damage to the perirhinal cortex (Alvarado and Bachevalier, 2005a; Saksida et al., 2007) or parahippocampal areas TH/TF in monkeys (Alvarado and Bachevalier, 2005a) impaired performance on transverse patterning, but not elemental discriminations. Similarly, combined perirhinal-entorhinal lesions in rodents impaired transverse patterning performance (Dusek and Eichenbaum, 1998). Variants of purely configural tasks, such as the biconditional discrimination (e.g., AB+, CD+, AD−, CB−), have been shown to be sensitive to perirhinal damage but not fornix damage (Whishaw and Tomie, 1995; Buckley and Gaffan, 1998; Bussey et al., 1998) or hippocampal damage (Saunders and Weiskrantz, 1989; Killiany et al., 2005; Saksida et al., 2006). Finally, monkeys with entorhinal lesions (Buckmaster et al., 2004) and rodents with combined perirhinal and entorhinal lesions (Dusek and Eichenbaum, 1997) are impaired on transitive inference. The fact that perirhinal and parahippocampal lesions do impair performance on several relational tasks certainly suggests a role for the cortex in the use or formation of configural or relational cues (e.g., Eichenbaum et al., 1994; O'Reilly and Rudy, 2001; Bussey and Saksida, 2002; Saksida et al., 2007), but the precise nature of this role is yet to be determined.

3.09.5.4 Performance of Human Amnesics on Animal Tests of Conjunctive/Relational Memory

The use of conjunctive and relational tasks to tap human memory processes has flourished in the last few years, in particular in the service of functional neuroimaging studies. With respect to configural tasks, such as transverse patterning, recent reports have confirmed hippocampal activation while control subjects perform the tasks (Hanlon et al., 2003; Astur and Constable, 2004) and impaired performance in schizophrenic patients who also showed abnormal hippocampal activity while performing the task (Hanlon et al., 2005). By contrast, these same patients were unimpaired and showed normal hippocampal activation when performing the uniquely human version of this task (i.e., the children's Rock-Paper-Scissors hand game). This game, however, can be solved verbally as well as on the basis of the inherent properties of the stimuli (e.g., Rock crushes, Paper wraps, Scissors cut), which can be compared at the time of presentation without further reference to the other discriminations. Thus, it provides an interesting control mechanism for studies of transverse patterning in humans. Importantly, these same patients were also unimpaired in elemental discriminations or a feature-neutral task that have also been shown in animals not to depend upon an intact hippocampus (Gallagher and Holland, 1992; Alvarado and Rudy, 1995a,b). Activation in the temporal cortical areas, though present, did not differ between controls and patients.

Several behavioral studies of human amnesic patients also reported deficits on transverse patterning. Rickard and Grafman (1998) compared performance of four patients with ischemic, anoxic, or hypoglycemic (insulin overdose)-related amnesia on transverse patterning and elemental discriminations. Similar to animal results, these patients performed like controls on elemental discriminations and two problems of transverse patterning. Only when all three problems were trained and a configural solution was required did their performance drop. This report contrasts somewhat with another by Reed and Squire (1999) who not only found impaired performance on transverse patterning in human amnesic patients, but also reported that these patients were impaired on six-pair elemental, concurrent discriminations as well, which suggested that amnesic patients had a more general deficit in solving multiple discrimination problems, of which transverse patterning was a particularly difficult example. However, as suggested by Rickard et al. (2006), when comparing performance *per problem*, those patients required the same number of trials to criterion whether solving three or six elemental discriminations, but required twice as many to perform the transverse patterning problem (Rickard et al., 2006). This hypothesis was confirmed with additional amnesic subjects; however, because their damage was not quantified, it remains to be answered whether damage to the hippocampus or temporal cortical areas played a greater role in the observed impairments. One indication of specific hippocampal involvement is provided by Driscoll and colleagues (2003), who showed that aged human subjects who were impaired on transverse patterning (but not elemental discriminations) showed correspondingly reduced hippocampal volumes (Driscoll et al., 2003).

In a study that supports both the relational and conjunctive accounts, Davachi and Wagner (2002) asked human subjects to memorize triplets for which, at the time of encoding, they were required to either simply repeat, or process elaborately by mentally ordering them along the lines of subjective desirability. This elaborate processing was thought to encourage encoding of the relationships between items, or the conjunction of the members as a unique triplet. Not surprisingly, elaborate processing improved memory of the triplets over simple repetition. Furthermore, elaborate processing was associated with greater hippocampal activity (which also correlated with memory), whereas item-based processing increased activity in the entorhinal and parahippocampal cortices (Davachi and Wagner, 2002).

Human studies of transitivity have also suggested a role for the hippocampus in relational learning tasks. Performance of relational memory tasks is associated with changes in hippocampal activation in humans (Heckers et al., 2004; Preston et al., 2004; Titone et al., 2004), and in the anterior hippocampus in particular for humans performing the transitive inference task (Heckers et al., 2004; Preston et al., 2004). By contrast, spatial relational memory in humans activates posterior hippocampus (e.g., Pihlajamaki et al., 2004), and studies in primates have suggested that damage to the posterior hippocampus was particularly correlated with impairment on transverse patterning (Alvarado and Bachevalier, 2005b). It is possible that these discrepancies reflect differences in task demands, encoding versus retrieval

effects, or even enhanced anterior signals in the human studies as a result of novelty detection for the new pairs during the test phase in humans.

3.09.6 Spatial Memory

The initial research into the role of the hippocampus in spatial memory was conducted for the most part in rodents. In the years following the first description of H. M.'s amnesia, work in the rodent fared little better than that in the monkey with respect to finding a link between the hippocampus and memory. The discovery of hippocampal place cells by O'Keefe and Dostrovsky (1976) firmly linked the hippocampus with spatial mapping. In 1978, O'Keefe and Nadel published their landmark work that laid the foundation for studies of the hippocampus in spatial memory. In their book (O'Keefe and Nadel, 1978) they suggested that the unique contribution of hippocampus to memory was the formation and storage of cognitive maps (*See* Chapter 1.04). In the following decades, lesion and electrophysiological studies have clearly shown the key role played by the hippocampus in place learning. Indeed, the litmus test for functional damage to the hippocampal formation has been whether or not spatial memory is impaired. Damage to the hippocampus proper, in the absence of extrahippocampal damage, impairs performance on a variety of spatial tasks, be they contextual, navigational, or locational, although disconnection of the hippocampus from its diencephalic targets via fornix transection has given less clear results.

3.09.6.1 Radial Arm Maze

Originally developed by Olton and Samuelson (1976), the radial arm maze was a departure from previous maze tasks, as it was designed to test whether the hippocampus was required for working memory, which in this case refers to memory of information that changes, or is updated from trial to trial, as opposed to reference memory, which refers to information that is consistent over trials. In this case, the information was whether the rat had recently foraged for food in a specific location. This task differed from most maze tasks, which were more concerned with some aspect of navigation. While the radial arm maze does require animals to discriminate locations, the routes are predefined. In its original form, eight arms projected out radially from a central location. For each trial, a food reward was placed at the end of each arm; then the rats were placed onto the central platform of the maze and allowed to enter the different arms repeatedly until all the food was retrieved. Thus, rats had to learn not to reenter arms already visited on a given trial to achieve the optimum foraging strategy. A reentry counts as an error. This version of the task contrasted with a reference memory version in which four of the arms were consistently baited. In this version, both working and reference memory errors are possible, in the first case by reentering an arm that was already visited on that trial, and in the case of reference memory, by entering a never-baited arm (Olton et al., 1979). The addition of a delay imposed after a certain number of choices permits the assessment of retrograde and anterograde working memory as the remaining choices are completed. Reentry of an arm visited prior to the delay constitutes a retrograde error, whereas repeatedly entering an arm not visited prior to the delay constitutes an anterograde error (Olton, 1983).

Rats with lesions to the hippocampal formation are quite impaired on the spatial working memory variants of the task (Olton et al., 1979; Olton and Papas, 1979; Olton, 1983) and somewhat on the spatial reference memory version (Jarrard, 1983). Pretraining prior to receiving hippocampal lesions selectively disrupts working memory, but not reference memory. If a delay is imposed, more retrograde working memory errors than anterograde working memory errors will be generated (Olton et al., 1979; Olton, 1983).

3.09.6.2 Morris Water Maze

In contrast to the radial maze, which measures working memory for location, the swim task developed by Morris (1981) is generally a reference memory task (though one can test working memory) that tests spatial navigation based on extramaze cues. In this task, the rat swims in a large round tank full of opaque water. Submerged somewhere in the tank is a platform onto which the rat can climb to escape from the water. On each trial, the rat is randomly released into the water at one of four cardinal starting points. The rat then swims until it locates the platform, or is guided to it by the experimenter if it fails to find it. To solve this task, the rat must form a spatial representation of the cues in the room, and the position of the invisible platform in relationship to those cues, and on each trial find the quickest trajectory to navigate to the platform. Normal rats learn this task quickly, learning to navigate to the platform in a few seconds and taking fairly accurate direct headings in order to get there. The final performance test entails the removal of the

platform and the release of the rat from a novel starting position. Normal rats quickly orient then take a heading toward the platform location. Upon reaching the spot, they concentrate their search over where the platform had been. Hippocampal damage produces a severe impairment in rats' ability to locate the hidden platform (Morris et al., 1982). For these operated animals, the swim path may be concentrated toward the side of the maze that contained the platform, but with a less concentrated search pattern and fewer platform location crossings, or adopting a strategy, i.e., swimming at a certain distance from the wall, which will eventually bring them in contact with the platform. On the probe trials, these operated rats circle the tank many times, crossing all potential platform locations.

3.09.6.3 Spatial Tasks in Monkeys

Unlike the various spatial navigation studies conducted in rodents, spatial memory in monkeys has more frequently used the DNMS paradigm to test for nonmatching to location on the test tray, or to test for recognition of a previously seen item in the location where it was previously viewed (object-in-place). The results of these studies have been equivocal, with some reporting impairment after hippocampal damage on place recognition (Angeli et al, 1993; Málková et al., 1995; Beason-Held et al., 1999; Alvarado and Bachevalier, 2005b), object-in-place/scene (Parkinson et al., 1988; Doré et al., 1998), or spatial scene learning (Murray et al., 1998), whereas others have found spared performance on place or object-in-place memory (Murray and Mishkin, 1998; Málková and Mishkin, 2003) and have suggested that the temporal cortical areas may be equally or more important in monkey spatial learning (Murray et al., 1998; Málková and Mishkin, 2003; Alvarado and Bachevalier, 2005a). One large difference between these nonhuman primate studies and the rodent studies discussed earlier is the lack of movement through space to reach a goal and the potential for the use of egocentric information to solve the tasks.

Recently, however, several foraging studies have been published in which freely moving monkeys forage for food in large enclosures akin to the radial or Morris mazes. For example, Hampton and colleagues (2004) trained monkeys on a match-to-location task that took place in an open room containing three visually identical foraging containers placed in three different locations. Although hippocampal monkeys were impaired on a match-to-location task as compared to controls, both groups of monkeys were impaired when they reentered the room from the opposite side, suggesting that neither group was consistently using allocentric cues to locate the correct site, nor were cues in the room manipulated to assess which ones were controlling behavior (Hampton et al., 2004). By contrast, Lavenex et al. (2006) used a large hexagonal foraging board placed in an open room that contained 18 foraging sites (**Figure 8**). Identical local cues (colored cups) distinguished sets of foraging sites that were either potentially baited or never baited. The tendency to use egocentric strategies was controlled by using four different entry points to the foraging array. Controls and monkeys with hippocampal damage were able to forage correctly based on local cues. In addition, control monkeys also demonstrated the ability to use spatial relations to solve the task when the local cues were removed, but hippocampal-damaged animals were impaired in this condition. Thus, consistent with studies in rodents, the primate hippocampus is required for the allocentric use of environmental cues.

3.09.6.4 Performance of Human Amnesics on Animal Tests of Spatial Memory

Consistent with reports in rats, and now monkeys, reports of human amnesic patients have indicated impaired recent or anterograde spatial memory, although remote spatial memory may be preserved. For example, H. M. never learned the location of his home at the time of his surgery, although he could navigate to his previous home (Milner, 1966) and could draw a map of the layout of his present home (Corkin, 2002). Similarly, patient E. P., with extensive MTL damage due to herpes encephalitis (Teng and Squire, 1999), and K. C., with MTL damage in addition to widespread damage due to closed-head injury (Rosenbaum et al., 2000), both demonstrated remote, but not recent, topographic knowledge of neighborhoods lived in prior to the onset of amnesia. By contrast, neither demonstrated acquisition of new spatial knowledge. Similarly, Astur and colleagues (2002) trained patients with unilateral temporal lobe resection in a virtual reality version of the Morris water maze. Regardless of the side of resection, and in a manner similar to that reported in rodent studies, these patients showed longer latencies to locate the platform and failed to prefer the platform quadrant on probe trials (Astur et al, 2002). These results contrast with those reported by Bohbot and colleagues

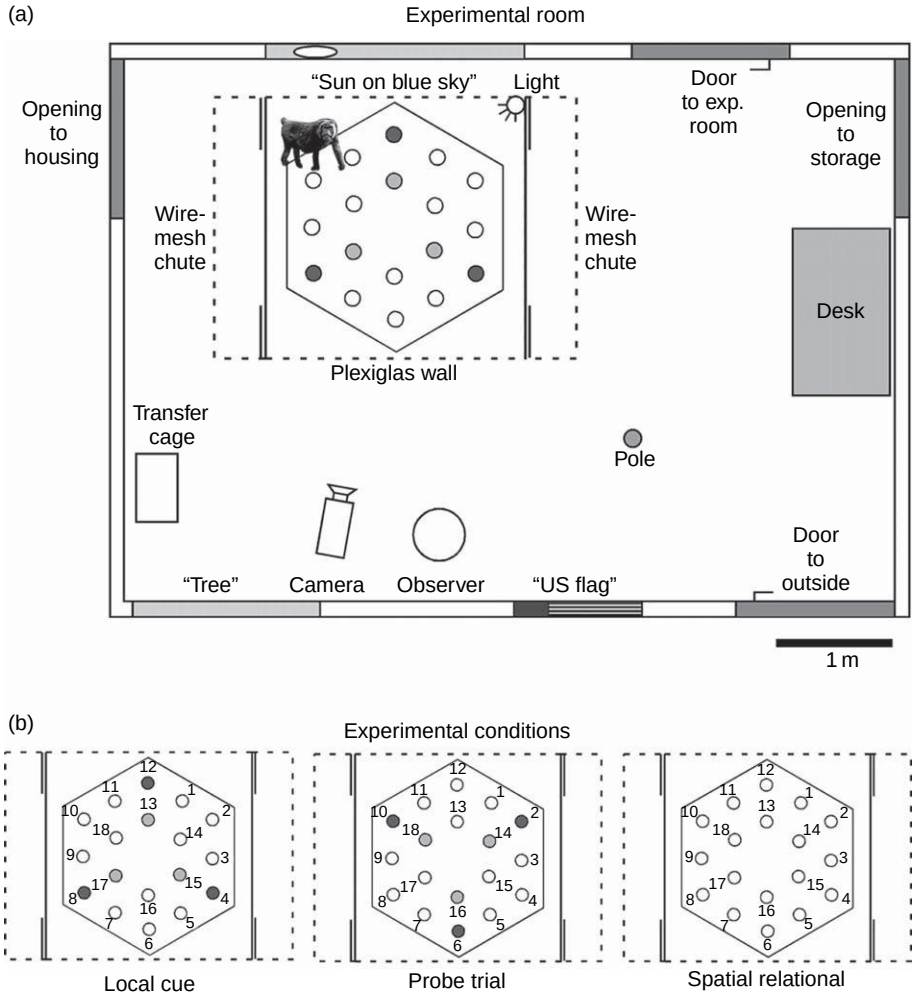

Figure 8 Open field spatial foraging task for monkeys. (a) Animals foraged under plastic cups for food in several of 18 possibly baited locations. (b) Food could be indicated by local cues (red or blue cup color) or by distal cues alone (all beige cups). Reproduced from Lavenex PB, Amaral DG, and Lavenex P (2006) Hippocampal lesion prevents spatial relational learning in adult macaque monkeys. *J. Neurosci.* 26(17): 4546–4558, with permission from the Society for Neuroscience.

(1998), who showed spared performance in patients given one-trial learning to locate a hidden sensor in the floor of a large room, but impaired performance when required to relocate the sensor after 30 min (Bohbot et al., 1998, 2002). Procedural differences among the studies may clarify the differences, but in general, the results from the task closest to the rodent version of the Morris maze were quite similar in rats and humans. Finally, a case of developmental amnesia with restricted damage to the hippocampal formation associated with impaired episodic, but some spared semantic, memory also demonstrated impairment in recognizing object locations when tested from a viewpoint different from that used during training (King et al., 2002).

3.09.7 Episodic Memory

The deficit in autobiographical, or episodic, memory has been a hallmark of human amnesia and a point of contention between human and animal researchers. As defined by Tulving (1983), a requirement of episodic memory is that it be available to conscious recollection, which makes it difficult to assess in nonverbal species. However, Tulving also suggested that episodes can be distinguished by the storage of temporal and spatial relations between events. Using this definition, Clayton and Dickinson (1998) suggested that animals do demonstrate episodic-like memory by encoding 'what,' 'when,' and 'where' specific experiences occur. Thus, scrub-jays do indeed

express knowledge of what, when, and where with respect to food caching (*See* Chapter 1.23). Episodic-like memory has also been reported in rats (Clayton and Griffiths, 2002; Bird et al, 2003; Eacott et al., 2005; Babb and Crystal, 2006) and apes (Schwartz and Evans, 2001), and monkeys have demonstrated 'what' and 'where' knowledge (but not 'when') within the same paradigm (Hampton et al., 2005). Although no studies have assessed the effects of hippocampal lesions of these episodic-like memory tasks, to the degree that they have a spatial or contextual component, damage to the hippocampus would be predicted to have an effect. However, other evidence of how the hippocampus might contribute to episodic-like memory comes from electrophysiological studies which have shown differential firing patterns of hippocampal place cells depending upon context (see Smith and Mizumori, 2006, for review) or differentiating where the animal has been and where it has to go next (Frank et al., 2000; Wood et al., 2000; Ferbinteanu and Shapiro, 2003) or differentiating sequences of actions (see de Hoz and Wood, 2006, for review), and these correlates of behavior may provide better insight into the workings of episodic-like memory than lesion studies (*See* Chapter 1.21).

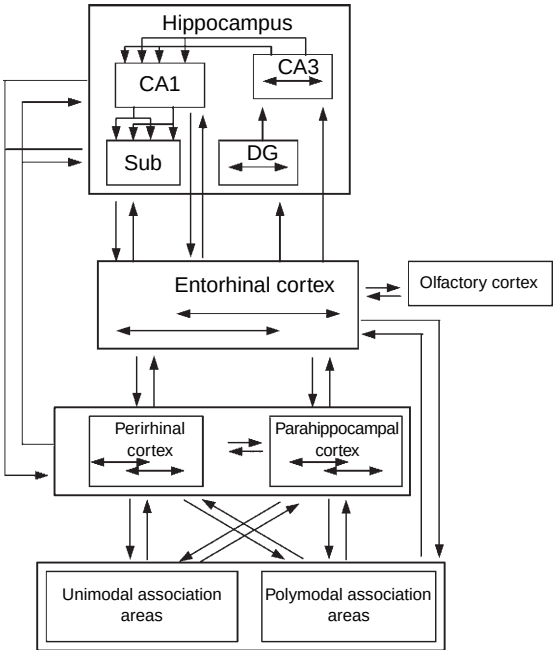

Figure 9 Suggested hierarchical organization of medial temporal lobe structures contributing to memory functions. CA, hippocampal cell field; DG, dentate gyrus; Sub, subiculum. Adapted from Lavenex P and Amaral DG (2000) Hippocampal-neocortical interaction: A hierarchy of associativity. *Hippocampus* 10(4): 420–430, with permission from Wiley-Liss.

3.09.8 What We Have Learned from Animal Models

A particularly important contribution of the animal modeling research is a richer appreciation of how the MTL structures contribute individually to memory. Anatomical and physiological evidence from animal studies certainly suggests that there is a hierarchy of function within the MTL, with increasing convergence of information as one proceeds from the neocortex toward the hippocampus (**Figure 9**; e.g., Mishkin et al., 1998; Lavenex and Amaral, 2000; *See* Chapter 3.03). Thus, projections from association cortex to the perirhinal and parahippocampal cortices are a first level of integration; projections from these areas to the entorhinal cortex are a second level of integration, and finally projections to the hippocampus from all three areas provide a final level of integration (Lavenex and Amaral, 2000). However, as reviewed, the animal models also suggest that those individual MTL structures likely contribute to normal behavioral processes in addition to simply passing sensory information to the hippocampal formation. Indeed, not only have the animal models suggested that the cortical areas are functionally heterogeneous, but there is increasing evidence to suggest that the hippocampus may also demonstrate a degree of heterogeneity.

Anatomically, connections between the hippocampus and its afferents suggest that visuospatial information projects primarily to the dorsal hippocampus in rats and the posterior hippocampus in monkeys, whereas visual information about object identity reaches the ventral or anterior hippocampus, respectively (e.g., Witter et al., 2000; Burwell et al., 2002; Lavenex et al., 2006). Thus, damage to the hippocampal formation may have different consequences for memory function depending upon where the damage occurs. For example, rats with lesions of the dorsal hippocampus were impaired in spatial learning as measured by the Morris water maze task (Duva et al., 1997), the radial arm maze (Ferbinteanu and McDonald, 2001; Lee and Kesner, 2003; Pothuizen et al., 2004), or T-maze (Hock and Bunsey, 1998). By contrast, rats with ventral lesions, with the dorsal half intact, were able to successfully learn the water maze task (Moser et al., 1993, 1995; Moser and Moser, 1998), suggesting that spatial information processing takes place predominantly in the dorsal hippocampus.

Similar findings have been reported in primates, although the distinction in the primate is between anterior and posterior hippocampus (Witter et al., 1989; van Groen and Wyss, 1990; Witter, 1993). For example, Jackson (1984) showed that damage to the middle hippocampus, but not the anterior or posterior hippocampus, impaired acquisition of matching-to-sample. One study recorded differential patterns of activity in the hippocampus of monkeys performing either a spatial or a nonspatial delayed matching-to-sample task and revealed greater spatial delay-dependent activity in cells within the posterior than the anterior hippocampus, whereas nonspatial delay-dependent activity was found along the entire length of the hippocampus (Colombo et al., 1998). Although not specifically discussed in this context, recent findings by Rolls et al. show a gradient from anterior to posterior of object, place, and object-place cells recorded from the hippocampus of awake macaques (Rolls et al., 2005).

Some research in humans also supports a heterogeneous view of temporal lobe function. For example, damage to the right temporal lobe in patients is associated with deficits in detecting changes in the spatial location of objects (Smith and Milner, 1981, 1989; Pigott and Milner, 1993), in maze learning (Milner, 1965), and in spatial memory (Abrahams et al., 1997), whereas those with left temporal lobe damage were not. Functional MRI studies have shown that activation in the hippocampus and posterior parahippocampal cortex versus perirhinal cortex during encoding differentially predicts successful source or item recognition, respectively (Davachi et al., 2003). Similarly, encoding activity in these same regions predicts recollection (hippocampus and posterior parahippocampal gyrus) or familiarity (rhinal cortex) in humans (Ranganath et al., 2004). Activation of the right hippocampus was shown to occur when control subjects used spatial landmarks to navigate within a virtual environment during early training (Iarai et al., 2003). Human amnesic patients with predominantly hippocampal or medial temporal cortical damage were differentially impaired on tasks adapted from the nonhuman primate literature such that hippocampal damage was associated with impairments on scenes (Graham et al., 2006; Lee et al., 2006), but spared performance on object memory and feature ambiguity (Barense et al., 2005), whereas damage to the cortical areas was associated with deficits in object memory or perception (Lee et al., 2006; Barense et al., 2005, respectively).

With respect to the anterior/posterior distinction in hippocampal functions, the posterior hippocampus appears to be more involved in spatial/episodic memory processing than the anterior hippocampus in humans (Hartley et al., 2003; Pihlajämaki et al., 2004; Ranganath et al., 2004). Furthermore, the distinction between the left and right hippocampus in spatial memory processing has received additional support from case studies in humans. Finally, human imaging studies have suggested an anterior/posterior distinction with respect to encoding versus retrieval processes in hippocampus (Lepage et al., 1998; Schacter and Wagner, 1999).

There is also evidence of behaviorally relevant circuits between hippocampal subfields and cortex. For example, Kesner and colleagues found a double dissociation between the dentate gyrus and CA1 with respect to spatial or temporal pattern separation (Gilbert et al., 2001), and between perforant path and dentate gyrus inputs to CA3 on retrieval versus encoding of spatial memory (Lee and Kesner, 2004). Finally, evidence for the functionality of the direct Erh-to-CA1 pathway was provided by a 2-deoxyglucose analysis of hippocampal activity while adult monkeys were performing a DNMS task (Sybirska et al. 2000). Performance of this task activates CA1, subiculum, and entorhinal cortex, but not dentate gyrus or CA3.

3.09.9 Summary

We began by highlighting the key deficits in human amnesia and then by describing some of the behavioral tasks being used to tap into these memory deficits. In spite of initial difficulties that delayed the development of animal models, the choice of appropriate tasks provided the means to assess memory processes in animals similar to those impaired in human amnesic patients. However, the animal literature quickly diverged from modeling human amnesia to modeling the function of the individual brain structures, whose collective damage were the putative cause of human amnesia. Consequently, each particular animal paradigm only models a subset of the observed impairments in human amnesic patients (e.g., spatial, relational, recognition, encoding, retrieval, etc.). Nevertheless, given the increasing reports of patients with more selective memory deficits and lesions (e.g., Baddeley et al., 2001; Aggleton et al., 2005; Barbeau et al., 2005), almost as restricted as those used in animals, the fields of animal and human memory have now begun to draw from each other in ways that promise to advance our understanding of human amnesia. Testing human amnesic patients using the animal paradigms has

strengthened the validity of several models and will further our understanding of how the brain contributes to normal memory, and of how memory-impaired animal and human subjects express that impairment. By mapping out the contributions of individual brain regions to learning and memory, we may produce even better assays of the milder impairment seen in these patients. Lastly, the use of functional imaging in humans will illuminate which areas of the brain are working together to solve a given task, and thus point the way for new studies in animals using either electrophysiological measures or imaging to further our understanding of memory processes at the systems level (i.e., the entire network of structures that support memory processes) as well as at the level of individual structures.

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3.10 Perirhinal Cortex: Neural Representations

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3.10.1 Introduction

3.10.1.1 Scope of Chapter

Perirhinal cortex is importantly involved in a number of different memory functions. For elements of recognition memory, paired associate learning, and reward sequence learning, there is evidence of the direct involvement of perirhinal neurons in the learning process per se. However, perirhinal cortex also has perceptual functions, and through these functions, it can also be indirectly involved in other learning processes.

This chapter primarily concerns what is known of neural representations in perirhinal cortex and how these may relate to its roles in learning and memory. After some background anatomical information, a summary is given of the results of ablation experiments that indicate the functions of perirhinal cortex. These functions include roles in perception, particularly for object-related information, and in memory, notably but not exclusively, recognition memory and paired associate learning. A brief overview of recent attempts to find synaptic plastic substrates for the memory functions of perirhinal cortex is also included. The chapter does not include the results of human brain imaging except by passing mention where of particular pertinence.

3.10.1.2 Location of Perirhinal Cortex

In the last 20 years, perirhinal cortex (**Figure 1**) has received increasing scientific attention because of its important roles in perception and learning. Perirhinal cortex is located in the anterior and medial portion of the inferior aspect of the medial temporal lobe of primates. While in the past there has been considerable variation in the definition of the

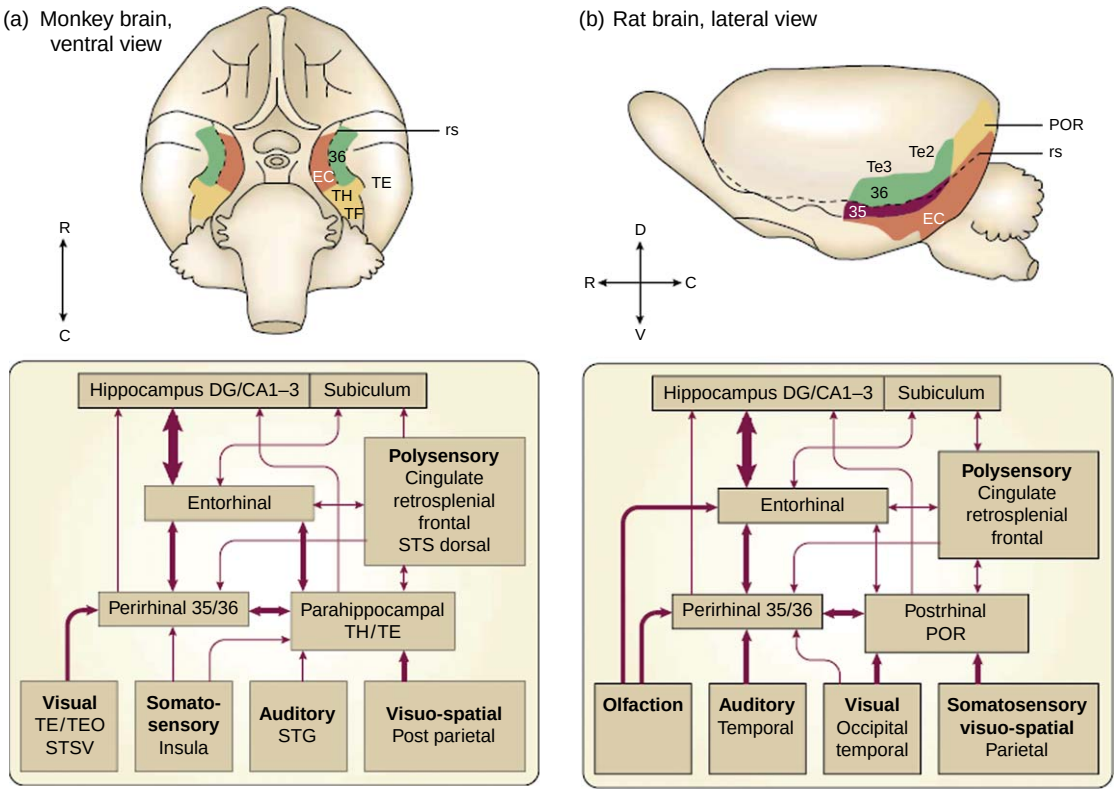

Figure 1 Position and some major connections of the perirhinal and neighboring cortices in the monkey (a) and rat (b). Routes are shown by which sensory information may reach perirhinal cortex and the connections from perirhinal cortex to the hippocampus, again in the monkey and the rat. The arrow thickness indicates the relative size of the projections. Perirhinal cortex: areas 35 and 36; dentate gyrus, DG; entorhinal cortex, EC; postrhinal cortex, POR; parahippocampal cortex, TH and TH; rhinal sulcus, rs; superior temporal gyrus, STG; superior temporal sulcus, STS; subicular complex, SUBIC; visual association cortex, TE and Te2. Reproduced with permission from Brown MW and Aggleton JP (2001) Recognition memory: what are the roles of the perirhinal cortex and hippocampus? *Nat. Rev. Neurosci.* 2: 51–61. As adapted from Witter MP, Groenewegen HJ, Lopes da Silva FH, and Lohman AHM (1989) Functional organization of the extrinsic and intrinsic circuitry of the parahippocampal region *Progr. Neurobiol.* 33: 161–253.

boundaries of perirhinal cortex (Suzuki and Amaral, 2003), there is now broad agreement that in the monkey it extends immediately lateral to the full extent of the rhinal sulcus and includes cortex corresponding to both areas 35 and 36 of Brodmann. In the rat, perirhinal cortex (areas 35 and 36) is located on either side of the caudal part of the rhinal sulcus. The precise extent of the corresponding region in the human brain remains uncertain.

3.10.1.3 Overview of Connections

Perirhinal cortex is highly interconnected with many other brain regions (for reviews, see Suzuki and Amaral, 1994; Burwell et al., 1995; Suzuki, 1996; Witter et al., 2000). It receives highly processed sensory information of all modalities and provides an important route for transferring information to and from the hippocampus (**Figure 1**). Its position at the top of the sensory processing hierarchy and at the gateway to the limbic system means that it is ideally placed to play important roles in sensory perception and memory. Once a stimulus may be identified, it is appropriate to be able to process aspects of its past history and associations.

In the monkey, the most prominent input arises from inferior temporal cortex (unimodal visual areas TE and TEO), although it also receives input from auditory, olfactory, and somatosensory association areas of cortex. Further substantial inputs arise from polymodal association areas including prefrontal and cingulate cortex, entorhinal cortex (and thereby, indirectly from the hippocampus), parahippocampal cortex (areas TH and TF), and cortex of the dorsal bank of the superior temporal sulcus. Perirhinal outputs largely mirror its inputs, with

strong connections to entorhinal cortex (again, therefore, indirectly connecting to the hippocampus), the parahippocampal gyrus, prefrontal cortex, and unimodal and polymodal sensory association areas. Perirhinal cortex also has reciprocal connections with the amygdala and thalamus (mediodorsal and midline nuclei) and sends outputs to the tail of the caudate nucleus and ventral putamen. It additionally receives input from brainstem monaminergic cell groups and cholinergic input from the basal forebrain. A parallel, similarly widespread pattern of connectivity is found in the rat.

Perirhinal cortex is juxtallocortex, cortex of a type that is transitional between the neocortex and the archicortex and paleocortex of the hippocampal and entorhinal regions. Unlike neocortex, area 35 lacks an inner granular layer (layer 4), and this layer is very narrow in area 36. Again, in contrast to neocortex, perirhinal cortex does not have an obviously columnar organization.

3.10.1.4 Overview of Functions

There is a very large body of evidence indicating that perirhinal cortex plays a central role in certain aspects of recognition memory. The evidence is most clear for familiarity discrimination for single items (Brown and Xiang, 1998; Brown and Aggleton, 2001). There is also much evidence of a role in perceptual functions (Buckley and Gaffan, 2006), though this role is not universally accepted (Squire et al., 2004; Hampton, 2005). Additionally, perirhinal cortex is involved in other types of memory linked to learned associations made with items (notably including reinforcement learning and paired associate learning). More detailed discussions of the findings of ablation studies may be found in several reviews (Murray and Bussey, 1999; Brown and Aggleton, 2001; Murray and Richmond, 2001; Murray and Wise, 2004; Squire et al., 2004; Eacott and Gaffan, 2005).

Overall, ablation studies provide strong evidence for a role for perirhinal cortex in object identification (Buckley and Gaffan, 2006), though some findings have challenged this role (Squire et al., 2004; Hampton, 2005). Monkeys with perirhinal lesions have been shown to be impaired in a series of tasks designed to test object identification. Thus there is impairment in perceptual oddity tasks in which the odd one of a set of images must be chosen, tasks requiring object identification from different views or partial views of the same object (Buckley et al., 2001; Buckley and Gaffan, 2006), and tasks in which an object only previously experienced by touch must be selected using vision (Goulet and Murray, 2001). Additionally, monkeys with perirhinal lesions are particularly impaired on tasks requiring discrimination between visual stimuli with a high level of feature ambiguity (Bussey et al., 2005). It has been argued that, where perceptual deficits have not been found, the tasks may have been solved using discrimination based on individual features rather than whole objects as entities in themselves. Where test has been made, the evidence indicates that it is possible to dissociate the mnemonic and perceptual functions of perirhinal cortex (Buckley and Gaffan, 1998b, 2006; Saksida et al., 2006).

The ability to recognize the novelty or familiarity of individual items is an important aspect of recognition memory. Such recognition memory relies not only on identification (perception) but also on judgment of prior occurrence (memory). The involvement of perirhinal cortex in the judgment of prior occurrence for individual items is widely agreed. Although in one view it forms part of a unified medial temporal lobe memory system (Squire et al., 2004), an alternative view is that its role is differentiable from that of the hippocampus (the hippocampus being concerned with recollective, complex associational and spatial aspects of recognition memory, while perirhinal cortex is concerned with single-item familiarity discrimination and simple associations) (*See* Chapters 2.23, 3.08; Brown and Aggleton, 2001). The pattern of impairments after perirhinal cortex lesions has been doubly dissociated from neighboring areas such as the middle temporal gyrus, which corresponds to the dorsal part of visual area TE, the hippocampus, and the amygdala. Thus, whereas lesions of the middle temporal gyrus impair color discrimination but spare recognition memory, lesions of perirhinal cortex impair object recognition memory but not color discrimination (Buckley et al., 1997). Further, in both monkeys and rats, hippocampal or fornix lesions lead to marked impairment in spatial tasks with relatively mild or no effect upon recognition memory, whereas perirhinal cortex lesions result in a reversed pattern of deficits (Gaffan, 1994). Amygdala lesions impair food preference learning but not delayed matching to sample with infrequently repeated stimuli, whereas perirhinal lesions result in the opposite pattern of impairment (Gaffan, 1994). These double dissociations demonstrate that perirhinal cortex is necessary for visual recognition memory even when functionally isolated from structures to which it is connected both in the ventral visual processing stream and in

the medial temporal lobe. In addition to the above results for visual tasks, perirhinal lesions impair tactile recognition memory in monkeys (Goulet and Murray, 2001) and olfactory recognition memory in rats (Petrulis and Eichenbaum, 2003), though auditory recognition memory seems to be spared (Kowalska et al., 2001; Fritz et al., 2005).

Ablation studies also indicate that perirhinal cortex is necessary for making certain (though not all) associations involving objects (Murray and Bussey, 1999; Murray and Richmond, 2001). Such work has shown that perirhinal lesions impair tasks involving associations of objects with other objects (Murray et al., 1993; Higuchi and Miyashita, 1996; Buckley and Gaffan, 1998a), odors (Petrulis and Eichenbaum, 2003), tastes (Parker and Gaffan, 1998), and tactile information (Goulet and Murray, 2001). Such associations may also be abstract. Monkeys with perirhinal lesions are impaired in a task where they must respond to a sequence of discriminations to obtain reward; the results suggest a role of perirhinal cortex in forming associations between objects and position in a series, or proximity to reward in a sequence (Liu et al., 2000). Additionally, perirhinal lesions in rats produce impairments in conditioned avoidance tasks, notably contextual fear conditioning. The impairment relates to processing of the conditioned stimulus rather than the spatial context of the task (Corodimas and LeDoux, 1995).

3.10.2 Neural Responses Related to Judgment of Prior Occurrence

The responses of a subset of neurons in perirhinal and neighboring cortex signal information concerning the previous occurrence of infrequently encountered stimuli (Brown et al., 1987; Brown and Xiang, 1998). Most strikingly, responses of such neurons are strong for stimuli that have not been seen previously and weak once these stimuli appear again. Such responses will here be termed repetition sensitive and have characteristics necessary for making judgments of prior occurrence. They have been related to the recency and familiarity discrimination aspects of recognition memory. These response changes have also been called stimulus-specific adaptation (Ringo, 1996) and response suppression (Desimone, 1996); however, these phrases carry implications concerning the underlying mechanisms or functionality of the changes that cannot currently be justified. In particular, to date no evidence has been found that the response reductions (decrements) are dependent upon an active suppressive or inhibitory mechanism. Moreover, the term 'response suppression' is used in relation to attentional processes; the use of the same term for a different process involved in familiarity discrimination is inappropriate. It is important to note that as perirhinal neurons with repetition-sensitive responses typically respond only weakly to stimuli that have been presented multiple times, they are likely to form a different subset of perirhinal neurons to those whose responses are discussed in sections 3.10.3 and 3.10.4.

Most information on these responses comes from monkeys and visual stimulation. The information in sections 3.10.2.1, 3.10.2.2 and 3.10.2.3 is based on this information. Those sections are followed by comments concerning generalizations across modalities and species. The characteristics of these neuronal responses is presented here.

3.10.2.1 Response Characteristics

Up to 25% of perirhinal neurons have a reduced response to familiar compared with novel visual stimuli, representing about 60% of the visually responsive neurons (**Figure 2**) (Riches et al., 1991; Li et al., 1993; Miller et al., 1993; Sobotka and Ringo, 1993; Xiang and Brown, 1998). Thus, collectively, such perirhinal neurons give a strong signal on the occurrence of a novel stimulus but a weak signal when a stimulus is familiar. Note that such a response change divides perirhinal neurons into two classes: those that have repetition-sensitive responses signaling information concerning even a single prior occurrence of a stimulus, and the remainder, which do not. The overall tendency to response reduction on repetition is sufficiently large that it is readily seen in population measures of neuronal activity from this cortex. Except under specific circumstances (see section 3.10.2.2), response increases with stimulus repetition occur at a level below that expected by chance (Miller and Desimone, 1994; Brown and Xiang, 1998). Response reductions with repetition have been described for three-dimensional objects and two-dimensional pictures of objects, faces, patterns, and scenes. Repetition-sensitive responses also encode information concerning physical features of the stimuli, just as do responses that are not repetition sensitive. Thus, for example, some neurons respond only to novel faces and not novel objects, or only to novel stimuli of a certain color (Riches et al., 1991; Fahy et al., 1993). Similar response changes are found in

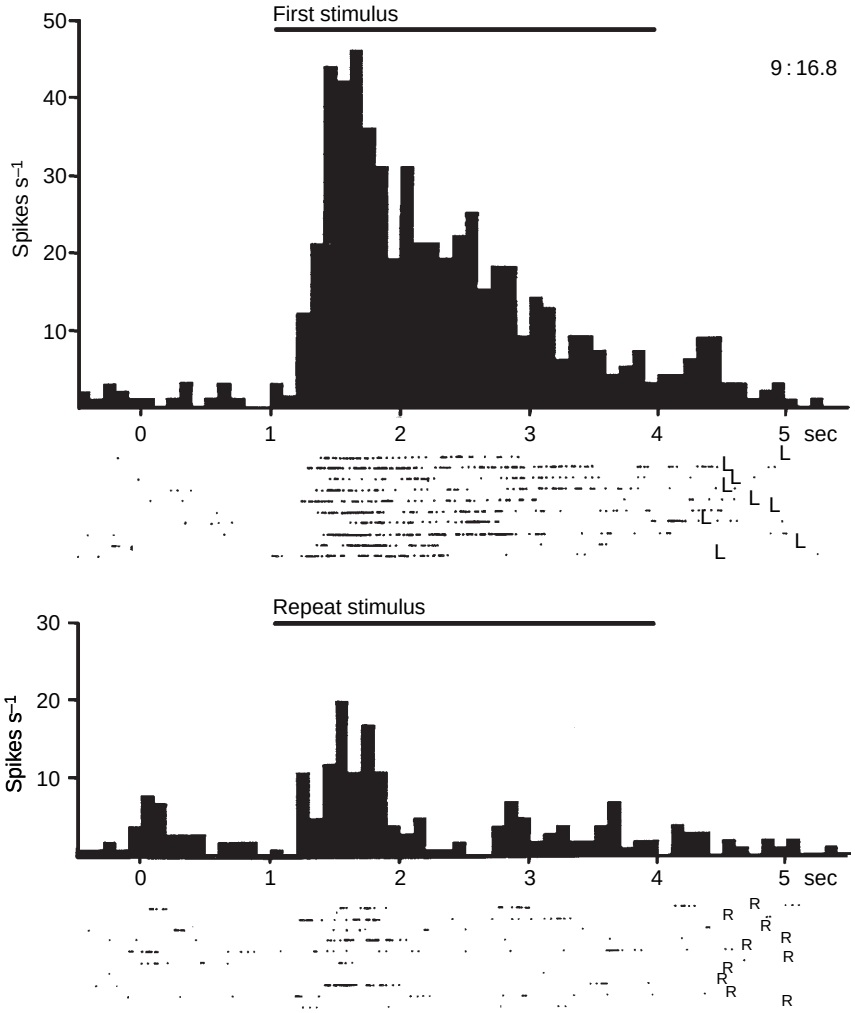

Figure 2 Example of repetition-sensitive monkey perirhinal neuronal response. Responses are stronger to first (upper peristimulus histogram and rasters) than repeat (lower peristimulus histogram and rasters) appearances of ten initially novel pictures. One stimulus was shown (bar above histogram) on each trial of a serial recognition memory task. To be rewarded, the monkey had to press left (L) for first and right (R) for repeat appearances of the stimulus. The first and repeat trials have been grouped in this display but were intermixed when presented. Controls (not shown) established that the difference in activity was not a result of the different behavioral responses. Note also that the neuron does not respond equally strongly to the first presentations of the ten different pictures; that is, its activity carries information about stimulus features as well as prior occurrence. Reproduced with permission from Brown MW (1990) Why does the cortex have a hippocampus? In: Gabriel M and Moore J (eds.) *Learning and Computational Neuroscience: Foundations of Adaptive Networks,* pp. 233–282. Cambridge, MA: MIT Press.

perirhinal cortex (areas 35 and 36), adjacent visual association cortex (anterior TE), and neighboring lateral entorhinal cortex (Brown and Xiang, 1998).

In experiments where both novel and highly familiar stimuli have been repeated, it has been possible to show that there is more than one pattern of response change on stimulus repetition (Fahy et al., 1993; Li et al., 1993; Zhu et al., 1995; Xiang and Brown, 1998). Under the conditions of the original experiments, these response types were termed 'novelty,' 'recency,' and 'familiarity' because that was the type of information signaled (Xiang and Brown, 1998). Novelty responses were strong to first presentations of novel stimuli and weak when these stimuli were repeated or already familiar stimuli were shown; the responses gave out a novelty signal. Recency responses were strong for novel stimuli and for highly familiar stimuli that had not been

seen for some time (e.g., 24 h) but weak when either type of stimulus had been seen recently; accordingly, such response changes contained information about the recency of presentation of stimuli. In contrast, familiarity responses were strong for novel stimuli even when they were seen for a second time after a brief interval (e.g., <5 min) but weak for highly familiar stimuli even if they had not been seen for a long time (e.g., 24 h): accordingly, such response changes contained information about the relative familiarity of stimuli. Importantly, these different patterns of response change indicated that information about recency of occurrence could be encoded separably from that about relative familiarity (Fahy et al., 1993; Xiang and Brown, 1998). Collectively, perirhinal neurons signaled information about both familiarity and recency. Further study of the properties of the response changes indicated that the term 'familiarity response' was potentially misleading. A better description is that they are slow-change responses, because such responses are reduced when an initially novel stimulus is shown for a second time if the interval between its presentations is sufficiently long (e.g., >10 min) (Xiang and Brown, 1998). They are not reduced if the interval is brief, and multiple repetitions of a stimulus in a short time do not result in reduction of the response. In contrast, the novelty and recency responses are reduced even when an initially novel stimulus is repeated after an interval of <1 s; correspondingly, such responses are fast change (**Figure 3**). This new designation does not alter the fact that more than one type of information is being signaled and that there must be more than one type of underlying synaptic change (see also section 3.10.5).

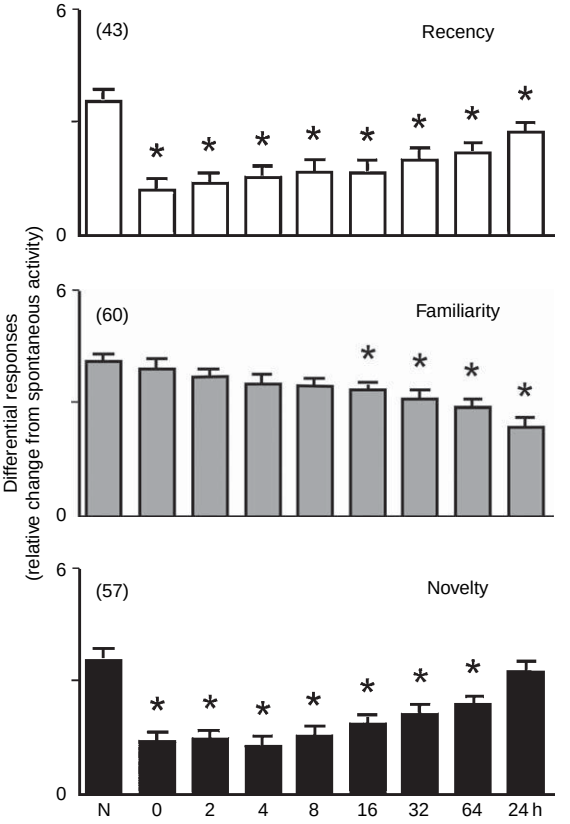

Figure 3 Population neuronal memory spans. Mean population responses (averaged within neurons and then across neurons) are shown for first presentations (N) and second presentations after different intervals (numbers of intervening trials in a serial recognition memory task or 24 h) for recency, familiarity, and novelty neurons in monkey anterior inferior temporal cortex. For these intervals, memory across the population increased with time elapsed for familiarity neurons but decreased for novelty and recency neurons. The decrement developed rapidly (<10 s) for recency and novelty neurons but took more than about 5 min to become significant for the population of familiarity neurons. $*p < .05$, difference in mean response to second presentations compared with first presentations. Reproduced with permission and adapted from Xiang JZ and Brown MW (1998) Differential neuronal encoding of novelty, familiarity, and recency in regions of the anterior temporal lobe. *Neuropharmacology* 37: 657–676.

3.10.2.2 Evidence of Relationship to Recognition Memory

The findings of a large number of ablation studies in both rats and monkeys are in agreement that lesions involving perirhinal cortex cause major impairment of recognition memory tasks that can be solved using familiarity discrimination for single items (see, for reviews, Murray and Bussey, 1999; Brown and Aggleton, 2001; Squire et al., 2004; Eacott and Gaffan, 2005). Perirhinal cortex is thus necessary for the solution of such tasks. Repetition-sensitive perirhinal responses provide sufficient information to solve such tasks (Brown and Xiang, 1998). *First,* they signal the information concerning stimulus novelty or familiarity and recency of occurrence. *Second,* the responses are changed by a single occurrence of a new stimulus. They therefore evidence single-exposure learning, as does animal and human recognition memory. *Third,* the system appears to have a very large capacity. Even after a monkey has been shown many hundreds of stimuli, the neurons still respond on the basis of whether or not an individual stimulus has been seen before. The responses are in this way

highly stimulus selective. This property also has an important corollary, namely, that the neurons do not respond weakly to a repeated stimulus because of response fatigue; they will again respond strongly to a subsequent new stimulus. *Fourth,* the response changes show access to information held in long-term memory. In particular, the response to the second appearance of a stimulus is reduced even when many other stimuli have been seen and a long time has elapsed since the stimulus was first seen. For many such perirhinal neurons (~70%, compared with ~45% in anterior TE), responses are reduced even when a stimulus has not been seen for 24 h (**Figure 3**). Neuronal memory spans (the length of time over which reduced responses are still found) of a few days have also been reported, but longer delays have not been tested. These properties establish that the underlying mechanism differs from that of simple habituation produced by a monotonously repeated stimulus. Moreover, the change is not a simple analogue of adaptation to prolonged sensory stimulation. Although the fast change responses might be related to priming mechanisms, evidence from human lesion and psychological studies makes this unlikely (*See* Chapters 2.23, 3.12; Brown and Xiang, 1998). Slow change responses do not have the characteristics necessary to explain priming. It should be noted that for many neurons, response reductions fade with the passage of time and that this provides information within the network concerning how recently a stimulus was presented. In addition, response reductions with stimulus repetition are found both when stimuli are presented within a recognition memory task, so that the animal must use their relative familiarity to gain reward, and when they are presented without the animal being trained in familiarity discrimination. The response changes are therefore automatic and endogenous and are not solely induced by training.

Three other types of perirhinal neuronal response change may also be observed during delayed matching to sample tasks in which judgments about the prior occurrence of a target stimulus have to be made in a choice phase following a delay. These other response changes are discussed in section 3.10.4. They signal information of importance to the solution of particular types of delayed matching to sample tasks but do not have the properties necessary to explain the behavioral features of general familiarity discrimination for infrequently repeated individual visual stimuli (Brown and Xiang, 1998; Brown and Aggleton, 2001). First, when only one stimulus need be held in mind at a time and a decision related to its prior occurrence made within a short period (seconds rather than many minutes), increased firing (delay activity) is found between the initial stimulus presentation and the subsequent presentation of the choice (match or nonmatch) stimulus (Fuster and Jervey, 1981; Miyashita and Chang, 1988; Riches et al., 1991; Miller et al., 1993). Such delay activity is not observed when more than one stimulus must be held in mind and the interval before the decision is long (Xiang and Brown, 1998), so that delay activity cannot provide the substrate for general familiarity discrimination. Second, if a monkey is trained to distinguish between a repeated unrewarded and a repeated rewarded stimulus, some perirhinal neurons show an increase in response to the repeated rewarded stimulus (Miller and Desimone, 1994); however, such increases have only been found when there is only a single repeated target stimulus and after training on the specific task. Thus, again, the mechanism appears to be specific to a particular type of task. Third, in delayed matching tasks using frequently repeating stimuli, many perirhinal neurons respond differently to the choice stimulus on match and nonmatch trials. However, it has been shown for many neurons (notably those outside perirhinal cortex in, e.g., the hippocampus) that these match/nonmatch differences are the same for novel as for familiar stimuli (Riches et al., 1991); hence, such differences do not signal the novelty or familiarity of a stimulus. Match/nonmatch differences therefore cannot provide a substrate for general familiarity discrimination, though they may provide information important to judging recency of occurrence of the stimulus.

One other aspect of perirhinal neuronal activity must also play an important role in familiarity discrimination: the correlated firing of action potentials of neurons within local and distal networks. There is still relatively little known about such interactions or the makeup of such networks in perirhinal cortex. Nevertheless, it has been established that, when the activity of synchronously recorded pairs of perirhinal neurons is studied, the firing of one neuron is quite commonly correlated with that of the other (Brown and Xiang, 1998; Erickson et al., 2000). So far, the evidence from such pairs indicates that synchronized firing, when the two neurons fire at approximately the same time, carries less information about the relative familiarity of stimuli than does the firing rate of the neurons.

3.10.2.3 Evidence for the Local Generation of the Response Changes

Neuronal response changes with the repetition of infrequently repeated stimuli are found in other areas than perirhinal and its neighboring cortex. For example, such changes are found in parts of prefrontal cortex and the striatum, and a small number (~1%) are found in the hippocampus (Brown and Xiang, 1998). (Note that neuronal response changes with frequently repeating stimuli are found in even more regions, including the many that demonstrate habituation.) Nevertheless, there is good evidence that response changes with the necessary properties to underlie the familiarity discrimination component of visual recognition memory are initially generated in perirhinal cortex, though possibly also in neighboring anterior inferior temporal cortex.

More posteriorly, and earlier in the visual processing pathway, repetition-sensitive response changes have memory spans that last at most for only a minute or so and are disrupted by one or two intervening stimuli. Such changes cannot explain those found in perirhinal cortex (Brown and Xiang, 1998). Therefore, perirhinal response changes are not merely passive reflections of responses earlier in the visual pathway. There is also evidence that the changes are not passive reflections of those occurring in subsequent processing areas. This evidence comes from the very fast latencies of the response changes found in monkey perirhinal cortex and neighboring area TE. In the fastest cases, the change between the response to a novel and that to a familiar stimulus occurs within milliseconds (measurements are accurate to only 10–20 ms) of the response to the visual stimulus itself (70–80 ms) (Miller et al., 1993; Xiang and Brown, 1998). Accordingly, there is no time for feedback from areas further on in the processing hierarchy. Indeed, where such latencies have been measured in the hippocampus, prefrontal cortex, and striatum, they are significantly longer than in perirhinal cortex (Brown and Xiang, 1998; Xiang and Brown, 2004). The conclusion is that at least the initial response change must be first generated in perirhinal cortex (and/or anterior TE) (Brown and Xiang, 1998). This conclusion receives support from experiments in which drugs have been infused locally into rat perirhinal cortex. Local infusions of different glutamate receptor antagonists block acquisition of longer- or shorter-term recognition memory, and no other brain region is able to substitute for perirhinal cortex for either type of delay period (see also section 3.10.5; Barker et al., 2006b).

The recording experiments where repetition-sensitive response changes have been found have employed many controls to establish that the changes are not explicable by behavioral or motivational changes, including changes in alertness, eye movements, and pupil diameter (see, for review, Brown and Xiang, 1998). The speed of the response changes further establishes that they are not artifactually generated, as they occur too soon to be explicable as secondary to ocular or attentional changes. It also establishes that these neurons provide a very rapid signal indicating the presence of a novel stimulus. It has been argued that the evolutionary advantage conveyed by the capacity to attend early to novelty provides a reason for the existence of such a network (Brown and Aggleton, 2001).

Thus, the available evidence strongly indicates that the occurrence of stimuli is registered (acquired) in perirhinal cortex. Moreover, if rat perirhinal cortex is reversibly inactivated (by a localized infusion of the AMPA/kainate glutamate receptor antagonist CNQX), retrieval of stimulus familiarity is blocked (Winters and Bussey, 2005a); the animals treat familiar stimuli as if they were novel. Hence, perirhinal cortex is either the information store or is necessary for access to the store. As modeling (see section 3.10.5.4) indicates that perirhinal cortex has ample storage capacity to allow familiarity discrimination, there is no good theoretical reason for the store being elsewhere. Indeed, in support of this contention, perirhinal lesions in rats produce retrograde amnesia for the prior occurrence of objects made familiar 4 weeks before the lesion (Mumby et al., 2002).

3.10.2.4 Generalization across Modalities and Species

Similar neuronal response changes on stimulus repetition have been described in both monkey (Brown and Xiang, 1998) and rat (Zhu et al., 1995) perirhinal cortex. Moreover, recent functional magnetic resonance imaging (fMRI) studies have also indicated that signals in the analogous human cortical region are also weaker for familiar than for novel stimuli (*See* Chapters 2.23, 3.08; Henson et al., 2003; Weis et al., 2004; Gonsalves et al., 2005; Henson et al., 2005; Fernandez and Tendolkar, 2006; Montaldi et al., 2006). Thus there is a strong indication that the same type of repetition-sensitive response reductions are likely to occur generally across mammalian species.

Such response changes have been largely studied using visual stimuli because of the availability of such

stimuli in the very large numbers necessary for such experiments. As the connections of perirhinal cortex indicate that it should be a multimodal area, it might be expected that similar results will be found whichever modality is used. Indeed, findings in the rat for olfactory stimuli are consistent with those for visual stimuli (Young et al., 1997). Neuronal activity measurements have not been made with somatosensory stimuli, though perirhinal lesions impair tactile familiarity discrimination (Suzuki et al., 1993). However, lesion experiments suggest that perirhinal cortex may not be necessary for auditory familiarity discrimination (Kowalska et al., 2001; Fritz et al., 2005). When neuronal activity is imaged using Fos (which has proved to be a useful marker for imaging activity changes related to visual recognition memory), activity is higher for novel than familiar sounds in auditory association cortex (as it is for visual stimuli in visual association cortex) adjacent to perirhinal cortex, but not in perirhinal cortex itself (Wan et al., 2001). The implication is either that another region performs the role of perirhinal cortex for auditory stimuli or that there is a difference in the nature/complexity of the auditory and visual stimuli being employed. The latter suggestion relates to the proposed role of perirhinal cortex in the perception of objects and the question as to whether the particular auditory stimuli employed are processed as objects; if they are not, this could explain the lack of differential activation of perirhinal cortex by the novel and familiar sounds (Wan et al., 2001).

3.10.3 Neuronal Responses Related to Paired Associate Learning

3.10.3.1 Correlates of the Sequential Pairing of Stimuli

Both recording and lesion experiments in monkeys have established the importance of perirhinal cortex for visual paired associate learning (see, for review, Miyashita, 2004). In the task used, the animal learns across many trials to associate arbitrarily assigned pairs of complex visual stimuli so that, after a delay following an initial (cue) stimulus presentation, the animal will select (e.g., by touching) the other stimulus of the pair (its paired associate). Two neural substrates of this form of multitrial associative learning have been identified in perirhinal cortex, namely (1) stimulus-specific delay activity (see section 3.10.4.2) and (2) the occurrence of neurons with strong responses to both of the stimuli that have been paired (Miyashita and Chang, 1988; Sakai and Miyashita, 1991; Naya et al., 2001; 2003; see also, Erickson and Desimone, 1999). Such paired responsiveness occurs more frequently than expected by chance (see **Figure 4**). Thus the learning results in single neurons that can code for both of a pair of stimuli, that is, information from both stimuli has come to converge on individual perirhinal neurons. Such 'pair-encoding' neurons occur far less frequently

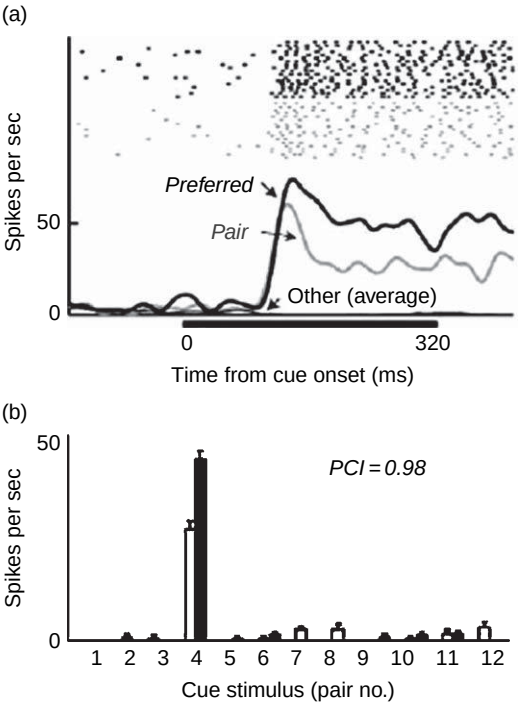

Figure 4 Example stimulus-selective neuronal response for learned paired associate stimuli. An example monkey perirhinal neuron that was strongly activated by both members of a particular pair of associated stimuli. In contrast, its responses were negligible when presented with members of any of the 11 other stimulus pairs. (a) Raster display and peristimulus time histogram for trials where the preferred stimulus (preferred, thick black) or its paired associate (pair, thick grey) were presented as the cue stimulus. The thin black line denotes the averaged responses in the trials where other stimuli were used as the cue (other). The horizontal gray bar indicates the cue presentation period. Note the continuing (delay) activity for the preferred stimuli after the cue stimulus is turned off. (b) Mean (+ SEM) discharge rates during the cue period (60–320 ms from the cue onset) for each of 12 learned pairs of stimuli. The responses to both members of pair 4 are much greater than the response to any member of the other 11 paired associates. Reproduced and adapted from Naya Y, Yoshida M, and Miyashita Y (2003) Forward processing of long-term associative memory in monkey inferotemporal cortex. *J. Neurosci.* 23: 2861–2871, with permission from Society for Neuroscience.

in TE than in Area 36 (5% cf. 33%). Thus perirhinal neurons can learn to encode new sensory constructs or contingencies, in this case the association between two stimuli.

Although sensory information is fed forward from TE to perirhinal cortex, memory retrieval is fed back from perirhinal cortex to TE (Naya et al., 2001; 2003). **Figure 5** illustrates the firing rate of a perirhinal neuron and that of a neuron from area TE in response to the stimulus to which they both respond preferentially. Memory-retrieval signals appear first in perirhinal cortex, after which TE neurons are gradually recruited to represent the sought target. For this paired associate learning, there is evidence that memory acquisition, storage, and retrieval are all perirhinal-dependent processes. Neurotoxic lesions involving the perirhinal and entorhinal cortices impair behavior and disrupt neuronal activity in TE related to paired coding (but not to individual stimuli) for paired associates learned either pre- or postoperatively (Higuchi and Miyashita, 1996; Miyashita et al., 1998).

It has been established that the perirhinal changes involve and are dependent upon the immediate early gene Zif268 (Tokuyama et al., 2002). Anatomical tracing indicates that paired associate learning is associated with changes in the pattern of axonal connectivity between TE and perirhinal cortex (Yoshida et al., 2003). Such alterations in the functional architecture of perirhinal cortex may facilitate the reactivation of newly formed object representations and, moreover, may form a neural basis for the process of memory retrieval (Miyashita, 2004).

3.10.3.2 Correlates of the Simultaneous Pairing of Stimuli

In contrast to findings for paired stimuli presented sequentially, when pairs of stimuli are shown simultaneously, inferior temporal (including perirhinal) neuronal responses to each of the individual stimuli appear to be unchanged even after many such pairings (Sobotka and Ringo, 1993; Erickson et al., 2000). However, responses to the stimuli shown as a pair are slowly changed by learning. In such experiments, the animal learns to respond (saccade) to whichever one of the two stimuli has been previously shown as a cue (Erickson et al., 2000). After training, when both stimuli are displayed simultaneously, the neuronal response is enhanced if the animal must saccade to the stimulus to which the neuron responds better but is reduced when the neuron must respond to the stimulus to which it responds more weakly. Thus the cortical representation of the pair appears to have become more differentiated. This differential responding develops across trials in which the two stimuli are paired when the pairing is repeated many times. The alteration of the representation of the pair

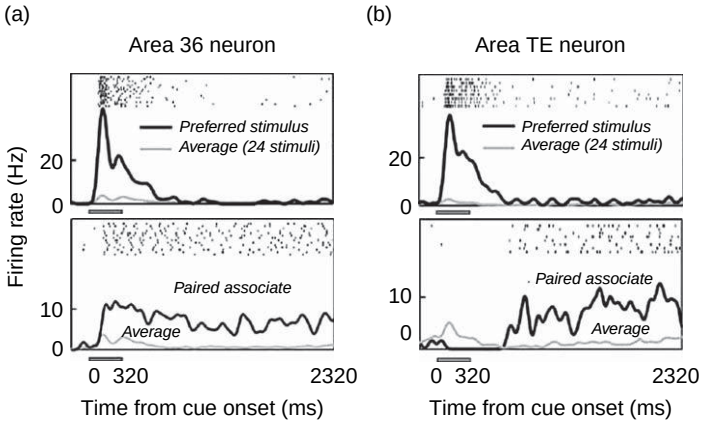

Figure 5 Neuronal activity during paired associate memory retrieval for a neuron in monkey: (a) perirhinal cortex (A36) and (b) area TE. For each neuron are shown raster displays and peristimulus time histograms for trials where the preferred stimulus (upper panel) or its paired associate (lower panel) was presented as the cue stimulus. The black lines indicate responses to the preferred cue stimulus (upper panel) or its paired associate (lower panel); the grey lines indicate mean responses to all 24 stimuli. Note that for the paired associate of the preferred stimulus (lower panel), the delay activity in perirhinal cortex is high from the time that stimulus presentation ends, whereas there is a delay before such activity appears in TE. The delay activity in TE is dependent upon a signal fed back from perirhinal cortex. Reproduced with permission and modified from Naya Y, Yoshida M, and Miyashita Y (2001) Backward spreading of memory retrieval signal in primate temporal cortex. *Science* 291: 661–664.

of stimuli in the cortex is presumably as a result of the altered effectiveness of interconnections within the cortex (Erickson et al., 2000). Moreover, measured across a range of stimuli, two neighboring perirhinal neurons tend to respond more similarly if the stimuli are familiar (presented many times on the previous day) than if they are relatively novel (not seen before the day of recording). This finding suggests that, as a result of repeated experience, neuronal responsiveness changes so that neighboring perirhinal neurons develop similar stimulus preferences (Erickson et al., 2000). It has not been established to what extent the learning occurs within perirhinal cortex itself.

3.10.4 Neural Responses Related to Stimulus Identification and Linked Associational Functions

3.10.4.1 Stimulus Identification

Visual information processing is largely divided between two pathways: a dorsal visual stream – primarily associated with spatial perception and sensorimotor output – and a ventral visual stream – primarily associated with object identification (Mishkin and Ungerleider, 1982). The latter pathway runs in the posterior–anterior direction through successive processing areas from primary visual cortex, via area V4, to inferior temporal cortex, including area TE, which supplies strong projections to perirhinal cortex. Electrophysiological evidence indicates that neurons in the anterior regions of the ventral visual stream code more complex visual representations than do neurons in the more caudal regions (Tanaka, 1996). Perirhinal cortex can be considered to be at the top of the hierarchy of processing regions (Felleman and Van Essen, 1991) and has been suggested to be necessary for the perception of objects as entities, as opposed to the identification of their individual features (Murray and Wise, 2004; Bussey et al., 2005). It is to be noted that the high-level perceptual categorization ascribed to perirhinal cortex is likely to be learned, presumably mainly during development. Thus, any such high-level discriminative properties of perirhinal neurons will represent the results of learned feature extraction and hence will be dependent upon long-term memory. Insofar as any such learning involves synapses on perirhinal neurons, these synapses must therefore be capable of long-term storage of information. To date there is only one published study of such potential perirhinal learning (Jo et al., 2006). However, as is discussed below, the responsiveness of perirhinal neurons to particular sensory stimuli can be changed as a result of training in the adult.

Most studies of neuronal response properties concerning stimulus identification in the anterior temporal lobe have concerned visual stimuli and inferior temporal cortex. While it is clear that some such studies have included perirhinal neurons, the sensory response properties of perirhinal neurons have rarely been distinguished from those of other anterior inferior temporal neurons. (Past uncertainties in the boundaries of perirhinal cortex have not assisted in making such distinctions.) Indeed, only a few distinctions have been described between the responses of neurons in anterior TE and in perirhinal cortex, and these chiefly relate to associative or mnemonic properties (specifically, neuronal responses in relation to judgment of prior occurrence, paired associate learning, and reward associations).

In conscious monkeys, typically more than half of recorded perirhinal neurons are reported as being visually responsive (Riches et al., 1991; Li et al., 1993; Miller et al., 1993; Sobotka and Ringo, 1993). Where reported, more than half of these visually responsive neurons respond to the majority of complex stimuli tested, though by no means with equal vigor to every stimulus. Nevertheless, some perirhinal neurons respond only to restricted classes of stimuli, such as faces or stimuli of a particular color (Riches et al., 1991; Fahy et al., 1993). Accordingly, the responses of perirhinal neurons signal information about the physical features of visual stimuli. It has been reported that a higher proportion of neurons are stimulus selective in perirhinal cortex than area TE (Brown et al., 1996). Unfortunately, in spite of the suggestion that perirhinal cortex is critical to object identification, no recordings examining this issue have included perirhinal cortex, and in particular, no recording studies have investigated whether learning to categorize stimuli or form an object percept involves changes at perirhinal synapses.

As in TE, when single stimuli are viewed, perirhinal receptive fields are large and may encompass a region extending into both visual hemifields (Desimone and Gross, 1979). There has been little study of perirhinal responses in relation to stimulus constancy, but again evidence indicates that it is similar to that found in TE, with similar responses being found when stimuli are shown across different positions or at different sizes (Lueschow et al., 1994). Although some tendency for clustering of similar perirhinal responses has been reported (Fahy et al., 1993;

Nakamura et al., 1994; Erickson et al., 2000), there is not strong anatomical or physiological evidence that perirhinal cortex has a columnar organization. Studies of neuronal responses in rats indicate that the responses of perirhinal neurons encode stimulus-specific visual (Zhu et al., 1995) and olfactory information (Young et al., 1997) but do not encode detailed spatial information (Burwell et al., 1998; Zironi et al., 2001).

Anatomical connectivity indicates that perirhinal cortex is a polymodal area, yet there is still no published major study of polymodal perirhinal responses (though, commonly, perirhinal neuronal activity in both monkeys and rats is found to vary with all aspects of the behavioral task during which recordings are made and not merely with the presentation of sensory stimuli). Again, there is no reported study of the establishment of a cross-modal perirhinal response through learning.

3.10.4.2 Correlates of Attention and Short-Term Memory

Two types of neuronal activity that occur in many brain regions in relation to attentional and short-term memory tasks are also found in perirhinal cortex, namely, response suppression and delay activity.

When monkeys are trained to select a target stimulus presented amongst nontarget stimuli, neural responses to the behaviorally irrelevant stimuli are suppressed, whereas the response to the behaviorally relevant target stimulus remains unchanged (Desimone, 1996); see **Figure 6**. This change in responsiveness reflects a neural mechanism encoding the learned salience of a given stimulus. The change may involve both bottom-up and top-down mechanisms (Pessoa et al., 2002), but the precise location of the learning remains to be determined. Any involvement of perirhinal neurons that is distinctive rather than typical of other sensory processing regions remains to be established.

Delay activity is found during the interval between stimulus presentations in short-term or working memory tasks such as delayed matching to sample (Fuster and Jervey, 1981; Miyashita and Chang, 1988; Riches et al., 1991; Sakai and Miyashita, 1991; Miller et al., 1993). In such tasks a stimulus is presented in an acquisition phase, and after a possibly variable but relatively brief (<1 min) delay interval, there follows a choice phase in which a decision must be made on the basis of the stimulus presented in the acquisition phase. For many perirhinal neurons, the activity in the delay period carries information concerning the stimulus presented during the acquisition phase, that is, it is stimulus specific. Such activity maintains a representation of that stimulus online and may be a correlate

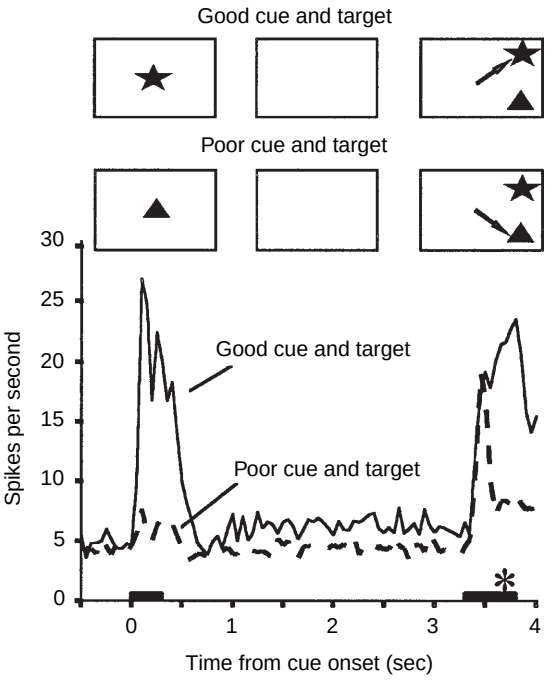

Figure 6 Differential delay activity and response suppression. For each neuron, one stimulus that evoked a strong response and one that evoked a weak response were chosen. Either stimulus was presented (bar on time scale) as the cue, and after a delay, the cue stimulus had to be selected (by making a saccadic eye movement to it) from a display of both stimuli in the choice phase. Thus, whichever stimulus was the cue, the display during the choice phase was of both stimuli. The activity was averaged for each neuron for trials using the good and poor stimuli and then across neurons to produce a population response. Shown is the population response for 22 monkey inferior temporal (including perirhinal) neurons for the good cues and targets (solid line) and the poor cues and targets (dashed line). Note that the delay activity occurring between the end of the cue stimulus presentation and the appearance of the choice stimuli is greater for the good than for the poor stimuli. Also note that the response to the display of both stimuli during the choice phase is rapidly suppressed when the poor stimulus is the target. Thus, across the population of neurons only those that respond strongly to the target are strongly active during the delay and the choice phases. The responses of other neurons are rapidly (~200 ms) suppressed in the choice phase. *Start of saccadic eye movement (NB later than start of suppression). Reproduced with permission from Desimone R (1996) Neural mechanisms for visual memory and their role in attention. *Proc. Natl. Acad. Sci. USA* 93: 13494–13499.

of the stimulus being 'held in mind' (Desimone, 1996; Naya et al., 1996). In certain cases the response of the neuron to the stimulus may then be enhanced when it is repeated on match trials (Miller and Desimone, 1994). Again, the activity change shows that perirhinal activity is modulated by attentive and mnemonic factors, but it has not been established that the delay activity is generated by learning mechanisms within perirhinal cortex itself, rather than being a reflection of activity generated elsewhere.

3.10.4.3 Correlates of Long-Term Memory and Learned Associations of Frequently Repeated Stimuli

A number of different types of long-term change have been reported in the responses of perirhinal neurons trained on different tasks where stimuli are seen many times and associated with reward. The findings emphasize the plasticity of perirhinal responses in relation to different processing demands. This plasticity and adaptation to specific learning situations may explain what are at first apparent inconsistencies in some reported findings. Information is now given about perirhinal neuronal responses related to four examples of such long-term changes and learned associations that are concerned with frequently presented stimuli.

First, the response change that has been most often reported is found in delayed matching tasks using small stimulus sets where the stimuli are frequently repeated (Gross et al., 1972; Riches et al., 1991; Nakamura and Kubota, 1995, 1996; Young et al., 1997). In such experiments the response to a stimulus changes (becoming variously either stronger or weaker for different neurons) according to whether it occurs as the choice stimulus on a match or mismatch trial. Such response changes establish that perirhinal neuronal responses can be determined by more than the physical characteristics of the presented stimulus: The responses can also reflect the behavioral context in which the stimulus is presented. In one description, such responses therefore contain information about learned associations of the stimulus and hence may be said to convey information concerning the behavioral meaning of the stimulus. In another description, the responses contain information about the recent occurrence or nonoccurrence of the stimulus and hence are related to temporal, rather than any more abstract, context, and therefore to judgment of prior occurrence. The same types of response change are found both in area TE and the hippocampus, so that they are not particular to perirhinal cortex (Gross et al., 1972; Mikami and Kubota, 1980; Riches et al., 1991; Otto and Eichenbaum, 1992). Once more, there is no evidence establishing that such changes are being generated in perirhinal cortex rather than passively reflecting changes generated elsewhere.

Second, sequence learning provides stronger evidence that perirhinal neurons can respond on the basis of the behavioral meaning of a stimulus (its abstract associations) rather than solely according to the physical characteristics of the stimulus (Murray and Richmond, 2001). Consider findings when a monkey has been trained in a task in which different-length sequences of stimulus presentations occur before the availability of reward (Liu and Richmond, 2000). The animal learns that certain stimuli signal the position in the sequence (e.g., first or last). Certain perirhinal neurons respond according to the position in the sequence leading to reward that is signaled by a stimulus, rather than to the physical attributes of the particular stimulus (Liu and Richmond, 2000). This type of responding to the implication of a stimulus rather than its physical characteristics was not observed for neurons in TE. Recent results have indicated that the development of such perirhinal responses can be blocked by local infusion of an oligonucleotide that interrupts dopaminergic transmission (Liu et al., 2004). Therefore, for such responses there is evidence that the learning (acquisition) is dependent upon perirhinal cortex, consistent with the results of ablation (Liu et al., 2000).

Third, after extensive training, perirhinal neuronal responses can be strongly modulated by the learned reward value of a stimulus (Mogami and Tanaka, 2006). Indeed, perirhinal responses are more strongly modulated than are responses in TE (Mogami and Tanaka, 2006). Again, while this establishes that learned reward-related information is present in perirhinal responses, previous ablation studies have indicated that perirhinal cortex is only necessary for stimulus–reward learning where the task requires discrimination of stimuli that is made difficult by feature ambiguity (Buckley et al., 2001; Bussey et al., 2005). Accordingly, this information might not be essential to task performance. Indeed, the modulation of the neuronal responses according to whether the stimulus signals reward or no reward has a mean latency that is >90 ms later than the onset of the visual response. Such a latency difference allows the modulation to be

transmitted from elsewhere rather than being generated locally (Mogami and Tanaka, 2006).

Fourth, when the same stimuli are used repeatedly for tens or hundreds of trials and the animal must attend to these stimuli to gain reward, the responses to these highly familiar stimuli show a gradual increase in magnitude (Holscher et al., 2003). This finding indicates that perirhinal neuronal representations of stimuli are plastic even in the adult. They adapt to experience, presumably to optimize the processing of behaviorally important information. Once more, although the changed activity reflects learning, the site of the learning remains to be established, and similar types of change with extensive training have been seen in other cortical areas (Buonomano and Merzenich, 1998; Kobatake et al., 1998).

In summary, after prolonged training perirhinal neuronal responses reflect task contingencies, signaling information potentially useful to task solution that is additional to the sensory properties of the stimuli. Such information may concern reward or more abstract associations of the stimuli and may involve tuning of the sensory responses. So far, in relation to the changes discussed in this section, there is published evidence suggesting that perirhinal cortex is the site of the learning-related change only in the case of sequence learning.

3.10.5 Synaptic Plasticity and Modeling of Perirhinal Neuronal Response Changes

The preceding sections describe several different types of perirhinal neuronal response changes related to learning. It is possible to list these under the following headings: (1) feature extraction (perceptual categorization), (2) familiarity/recency discrimination (potentially several different mechanisms), (3) paired associate learning, and (4) reward association learning. Yet other changes, thought to be related to attentional and/or short-term memory mechanisms, have also been described, such as delay activity and response suppression. It is already clear that there will also be developmental changes. The interrelationships and independencies of these different response changes and their underlying synaptic plastic mechanisms are largely unknown, but in the list above are at least eight types of activity change, all of which are observable in the activity of perirhinal neurons under different conditions. The changes observed are dependent on the particular conditions of the experiment and on the particular perirhinal neuron under study. Not all perirhinal neurons show all types of changes, though some may show more than one; again, information here is limited.

Perirhinal cortex provides a promising region for the investigation of neural substrates of memory. In recent years, increasing efforts have been made to relate perirhinal neuronal response changes to underlying synaptic plastic changes. As indicated above, this work has variously encompassed paired associate learning, reward learning, developmental changes, and familiarity discrimination. It has also involved the study of synaptic plasticity mechanisms in slices of perirhinal cortex.

3.10.5.1 Perirhinal Plasticity Studied in Brain Slices

In vitro studies have established that both long-term potentiation (LTP) and long-term depression (LTD) can be evoked in perirhinal cortex by suitable stimulation. The induction of both LTP and LTD are dependent on *N*-methyl-D-aspartate (NMDA) glutamate receptors in perirhinal cortex (Ziakopoulos et al., 1999; Cho et al., 2000) and elsewhere (Bliss and Collingridge, 1993). However, perirhinal LTD has been shown to have an unusual dependency on activation of metabotropic glutamate receptors (Cho et al., 2000). Moreover, in adult perirhinal cortex, LTP is dependent on activation of NMDA receptors containing NR2A subunits, whereas LTD (and depotentiation) is dependent on NMDA receptors containing NR2B subunits (Massey et al., 2004). Again, as in other regions (Kemp et al., 2000), there are differences between the adult and immature cortex in glutamate NMDA receptor composition and LTD induction mechanisms. Indeed, recently it has been shown that visual experience triggers such a change between immature and adult perirhinal LTD induction mechanisms (Jo et al., 2006).

3.10.5.2 Signaling Mechanisms Related to Paired Associate and Reward Sequence Learning

As mentioned above, studies of paired associate learning in the monkey have established that underlying perirhinal changes involve and are dependent upon the immediate early gene Zif268 (Tokuyama et al., 2002). Similarly, reward sequence learning in the monkey involves dopaminergic mechanisms, as

perirhinal neuronal response changes are blocked by local infusion of an oligonucleotide that down-regulates expression of D2 dopaminergic receptors (Liu et al., 2004). Interestingly, antagonism of NMDA receptors did not prevent development of the response changes in reward sequence learning.

3.10.5.3 Signaling Mechanisms Related to Recognition Memory

Several studies have been carried out looking at potential substrates of familiarity discrimination in the rat. This work has established that recognition memory is impaired following local infusions into perirhinal cortex of the muscarinic receptor antagonist scopolamine (Warburton et al., 2003; Winters and Bussey, 2005b) or selective antagonists of AMPA, NMDA, kainate, and metabotropic glutamate receptors (Winters and Bussey, 2005a; Barker et al., 2006a,b). When AMPA receptors are blocked, both acquisition and retrieval are impaired. Otherwise, all the studied drug actions are on acquisition and not retrieval. Interestingly, blockade of NMDA and metabotropic glutamate receptors produces recognition memory impairment only when the memory has to be held across a long (24-h) and not shorter (20-min) delay. In contrast, and most unusually, kainate receptor antagonism impairs recognition memory after a 20-min but not after a 24-h delay. These results parallel the evidence provided by perirhinal neuronal responses that there must be more than one substrate underlying familiarity discrimination (Fahy et al., 1993; Xiang and Brown, 1998). The indication is that there must be a rapidly induced shorter-term process that is dependent on kainate receptor activation and one or more longer-term processes more slowly induced and dependent on NMDA and/or metabotropic glutamate receptor activation (Barker et al., 2006b). In addition, viral transduction of perirhinal cortex has shown that phosphorylation of CREB (cyclic AMP responsive element binding protein) is necessary for both perirhinal LTP and long-term (24-h) recognition memory (Warburton et al., 2005), a dependency that parallels findings elsewhere (e.g., hippocampus).

3.10.5.4 Theoretical Models and Plasticity Mechanisms

There are now several theoretical neuronal models both of networks designed to extract features and thereby achieve stimulus categorizations leading to perception and of networks designed to achieve familiarity discrimination (*See* Chapter 3.08; Sohal and Hasselmo, 2000; Bogacz et al., 2001; Bogacz and Brown, 2003; Norman and O'Reilly, 2003). Such models have demonstrated that the relatively small number of neurons in perirhinal cortex (~0.1% of the total number of cortical neurons) potentially have the storage capacity to explain human visual recognition memory. Under the optimized conditions of such models, the occurrence can be stored, and hence the familiarity can be judged, of $\sim 10^9$ stimuli; that is, the capacity is sufficient to record an encounter with a new stimulus every several seconds for the whole of a human lifetime (Bogacz and Brown, 2003).

None of these models yet uses specific details of perirhinal circuitry (about which there is still relatively little information). Most of the models have employed synaptic enhancement (i.e., LTP-like mechanisms) as their primary synaptic plasticity mechanism. One way of achieving the reduced neuronal responses for familiar compared with novel stimuli in such models is by increasing inhibition, but experimental evidence of such increased inhibition is lacking. Recently, it has been argued that models that use separate (though interlinked) networks for feature extraction and familiarity discrimination are more economical (Bogacz and Brown, 2003). The idea of separate networks is consistent with the observation that perirhinal neurons have responses that are either repetition sensitive or not, rather than some mixture of the two. Moreover, the calculations indicate that the use of synaptic weakening as the primary mechanism is considerably more efficient than synaptic enhancement (Bogacz and Brown, 2003). Heuristically, this is because when features repeat across different stimuli, synaptic enhancement leads to certain synapses being successively strengthened, so that the neurons with these synapses come to fire selectively strongly for these features; that is, they signal the presence of such features. In contrast, synaptic weakening has the opposite effect; it emphasizes what is not common rather than what is common across stimuli – but this is what is required for novelty detection. Hence neuronal modeling has provided an explanation for why perirhinal responses are weaker for familiar than novel stimuli. Under this hypothesis the synapses that are most strongly activated by a novel stimulus are weakened; when the stimulus occurs again, that is, when it is familiar, the weakened synapses will give rise to a weaker response.

3.10.6 Summary

The position of perirhinal cortex at the top of the hierarchy of sensory processing areas and at the gateway to the limbic system means that it is ideally placed to play important roles in sensory perception and memory. Ablation evidence indicates that perirhinal cortex is indeed involved in both mnemonic and perceptual (stimulus categorization) functions. The results of recording studies establish that the responses of perirhinal cortical neurons carry information about complex stimuli and relate to stimulus identity, but they have yet to provide evidence for the categorization of objects as entities (as suggested by certain lesion studies). Recording studies have described perirhinal response changes indicating information processing related to short-term memory and multiple types of long-term memory – including recognition memory, paired associate learning, and stimulus–reward association learning. Perirhinal cortex is important in acquisition, storage, and retrieval of information related to such long-term memory. A variety of different neuronal response changes have been related to these different types of memory, and studies of their underlying synaptic plastic mechanisms are underway.

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3.11 Cortical Plasticity in Associative Learning and Memory

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3.11.1 Introduction

Cortical plasticity concerns the totality of nontransient changes in the structure and function of the cerebral cortex at the levels of hemispheres, lobes, systems, circuits, cells, and molecules. Restricted to the domain of learning and memory, its scope remains colossal. This chapter focuses on a central aspect of learning and memory – associative learning and memory and neurophysiological plasticity at the

levels of systems and cells in the primary sensory cortical fields: auditory (A1), somatosensory (SI), and visual (V1). For an earlier related account with a somewhat difference emphasis, see Edeline (1999). Space limitations preclude broad coverage of priming and related phenomena in humans (Schacter et al., 2004; Grill-Spector et al., 2006) and of cellular and molecular mechanisms of associative plasticity (Buonomano and Merzenich, 1998; Palmer et al., 1998; Rauschecker, 1999; Barth, 2002; Diamond et al., 2003; Edeline, 2003; Metherate and Hsieh, 2004; Weinberger, 2004b).

3.11.1.1 Rationale

Why focus on primary sensory cortices (PSCs)? There are several reasons. First, the primary sensory cortices are the last regions of the cerebral cortex to be included into the field of the neurobiology of learning and memory. Of course, there can be found no formal, and indeed only rarely a semiexplicit, denial of their importance in learning and memory. But PSCs have been largely ignored, particularly in the case of associative learning.

Second, general conceptions about cortical organization for learning and memory should be informed by the established, but still underappreciated, realization that primary sensory fields are not merely sensory analyzers. Rather, their functions, as related to intrinsic operations and integration of influences from the thalamus, other cortical areas, and neuromodulatory systems, render them as important nodes in the acquisition, representation, storage, and ultimate use of information in thought and action.

Third, beyond associative and mnemonic processes, PSCs are involved in a wide variety of functions that are not strictly sensory, including attention, motivation, the encoding of behavioral significance, motor acts, and higher cognitive functions such as learning strategy, expectancy and preparatory set, cross-modal integration, category learning, and concept formation. The emerging, broadened view of PSCs challenges normative conceptions of the structural/functional organization of the cerebral cortex as largely hierarchically sensory, associational, and motor and calls for a new cortical schema.

Fourth, but perhaps of paramount importance, is the issue of the specificity of plasticity. The substrates of memory must contain information that is sufficiently detailed to subserve cognitive processes and behaviors that are dependent on that memory. It is insufficient to merely identify structures that are involved in memory because involvement is only the first step. Without knowing the type of information that such involved structures acquire and hold, one cannot conclude that they actually do hold the type and level of detail (i.e., the contents) of memory that comprise the storage of experience.

Primary sensory cortices provide a convenient entry point into the search for specificity, largely because they contain systematic representations of their respective sensory epithelia. Of course, primary sensory cortices are not the sole sites of memories but, rather, are parts of probably widely distributed networks consisting of complex feedforward and feedback connections. But the study of PSCs contributes a powerful set of experimental tools from the field of sensory neurophysiology. Their application provides a level of specificity of plasticity beyond what can be obtained with standard approaches to the neurophysiology of learning. Furthermore, they permit the assessment of specificity for any learning task. Moreover, when appropriately employed, the use of sensory neurophysiological approaches yields posttraining determination of the specificity of plasticity that is exempt from the usually unavoidable influences of experimental extinction. In so doing, consolidation and the course of long-term retention can be studied without fear of contamination from repeated measures.

3.11.1.2 Neurophysiological Plasticity in Associative Learning and Memory

As used here, neurophysiological plasticity refers simply to any learning-related changes in the activity of neurons, regardless of the method of recording: electroencephalogram (EEG), evoked potentials, unit discharges, metabolic activity, and so forth. As generally understood, the minimum duration of change that is considered plasticity is on the order of minutes, to distinguish it from purely sensory responses that may last many seconds. This article concentrates on behaviorally validated cases of learning and memory rather than demonstrations of plasticity that are alleged to constitute learning. Conflating neural plasticity with memory is experimentally confusing and conceptually fallacious. Although learning and memory, which are behavioral-level constructs, undoubtedly are caused by neural plasticity, equating the two constitutes a category error (Ryle, 1963).

The recording of neural activity in learning and memory provides correlates of these processes and the behaviors that experimenters use to infer learning and

memory. Such correlates can gain importance for inferences about causality if they are predictive of later behavioral assessments of learning and memory. For example, the amount of conditioned stimulus (CS)-elicited gamma band activity in the EEG of A1 during training predicts the amount of specificity of behavioral memory tested 24 h later (Weinberger et al., 2006). Nonetheless, such predictive correlates cannot establish that the neural plasticity so identified is either necessary or sufficient for the learning or memory in question. Their major advantage is in identifying brain structures and systems that develop learning-based neural plasticity. In so doing, the use of neural correlates is perhaps the only way to find out what the brain is doing while it is doing it. Indeed, the discipline of sensory physiology is based essentially on neural correlates, specifically, correlations between a given stimulus and sensory system responses (Kiang, 1955). Such sensory physiology correlations have been used to understand processes such as developmental plasticity in PSCs (Weisel and Hubel, 1963).

The study of PSCs in learning and memory is more reliant on neurophysiological approaches than investigation of other structures, such as the amygdala and hippocampus, because lesions entail unique difficulties of interpretation. Because, as will be documented in the section titled 'Specificity of associative plasticity in primary sensory cortices,' PSCs develop associative plasticity during learning, their responses to sensory stimuli are governed not only by the physical parameters (e.g., sound frequency, locus of touch on the body surface) but also by the acquired behavioral relevance of those stimuli. Therefore, if lesions of a PSC disrupt learning, then resultant deficits could have been caused either by impairment of sensory/perceptual processing or by destruction of information stored in the removed tissue or both. One approach to circumvent this problem is to apply a treatment to a primary sensory cortex (e.g., stimulate, inactivate) after the sensory stimulus has ended but before a behavior dependent on the storage of that information is required (Harris et al., 2002).Investigations of plasticity in learning and memory in the primary sensory cortices vary greatly with the type of learning studied. Thus, associative learning, including classical and instrumental conditioning, has been investigated most extensively in the primary auditory cortex. Priming and perceptual learning have been most intensively examined in the visual cortex. The somatosensory cortex is becoming increasingly studied, particularly using the vibrissal system of animals, which has the advantage of a one-to-one correspondence between each whisker and its anatomically distinct recipient primary somatosensory cortex. This chapter reflects these characteristics of the literature.

3.11.2 What about Perceptual Learning?

As stated in the introduction, this chapter concerns associative learning. Where does that leave perceptual learning? Perceptual learning refers to an increase in sensory acuity within a stimulus dimension, usually as a result of increasingly difficult discrimination training (Kellman, 2002). Experiments of perceptual learning in humans typically involve thousands of trials over many days. For example, a recent study of human frequency discrimination learning involved 4000–5000 trials (Irvine et al., 2000), whereas a pitch discrimination experiment used more than 10 000 trials (Demany and Semal, 2002). Even when a single training session is employed, the number of stimulus trials is typically large. A study of learning of melodic patterns used 1200 different stimuli (Tervaniemi et al., 2001). Animal neurophysiological studies of perceptual learning typically employ very extensive discrimination training for each subject. For example, an investigation of primary visual cortex used ~130 000–325 000 trials (Schoups et al., 2001), and an experiment on inferotemporal cortical visual processing employed ~750 000–1 150 000 trials (Logothetis et al., 1995). There are reports of perceptual learning in considerably fewer trials under specific circumstances (Hawkey et al., 2004), but even so, specific plasticity in associative learning can develop in only five trials (Edeline et al., 1993).

Aside from the rate of learning, why make a distinction between perceptual learning and associative learning? One might expect that if any type of learning is characteristic of primary sensory cortices, then it must be perceptual learning. In fact, the existence of perceptual learning is often thought to subsume the category of associative learning, so that all learning-induced plasticity in PSCs is regarded as perceptual learning.

The distinction is actually critical. One might simply ask, "After a bout of perceptual learning, what is changed in a primary sensory cortex?" The answer would seem to be, "After perceptual learning, the machinery of the cortex has been altered to enable greater acuity. The sensory cortical machine now analyzes the same physical stimuli differently

(e.g., at a finer grain)." This effect certainly constitutes a type of learning by any definition. But interestingly, the study of perceptual learning does not include perceptual memory. This situation probably reflects the fact that investigators of perceptual learning are more concerned with sensory/perceptual processes than with learning and memory, and in any event, subjects do not actually remember the specific contents of their experience, that is, the particular stimuli or stimulus values given during certain of their multitude of trials. Thus, although perceptual learning alters the gateway to memory, increased acuity by itself is not necessary for memory, as the term is normally understood (i.e., as the contents of experience). However, the extant level of acuity can determine the precision with which the information is analyzed and may then be encoded and stored.

Consideration of the stages of training in perceptual learning indicates that, actually, it may be considered a subclass of associative learning. Subjects must first learn an association between a sensory stimulus and a reinforcer (simple classical conditioning) and, second, must learn an association between a specified response and a reinforcer contingent on whether a sensory stimulus is a CS+ (rewarded) or CS− (nonrewarded) (i.e., discriminative instrumental conditioning). After that, the discriminations simply become increasingly difficult. Because basic associative learning and its correlated cortical plasticity develop first (often in a few trials), understanding their substrates may help elucidate mechanisms of perceptual learning. Given this consideration and space limitations, perceptual learning is not reviewed. However, some brief comments will help to place it in perspective.

A general summary, necessarily imperfect, is that PSC plasticity is often (but not always) found after perceptual learning and in many instances correlates well with the type and amount of behavioral perceptual improvement, thus providing a potential explanation of the behavioral effects. For example, monkeys trained to discriminate between frequencies of vibration on a digit develop increased temporal precision of evoked discharges in S1 (i.e., decreased variance in the representation of each stimulus cycle) that could account for behaviorally measured frequency discrimination performance (correlation = 0.98) (Recanzone et al., 1992).

In addition, studies of perceptual learning can illuminate properties of PSC neurons because the extensive training allows for training the same subjects on multiple tasks. In a particularly noteworthy case, Gilbert and colleagues trained monkeys to perform two tasks using a single visual array consisting of five line segments: a central line flanked by two parallel lines at either end and either side. Subjects were trained to switch between a line bisection and a vernier task. The tuning properties of neurons in primary visual cortex (V1) changed depending on the task. Most importantly, an information theoretic analysis revealed that neurons "carried more information about a stimulus attribute when the animals were performing a task related to that attribute" (Li et al., 2004: 651). The authors suggested that V1 as a whole is an adaptive processing unit that performs different computations depending on the current problem to be solved (i.e., the behavioral context) (Li et al., 2004). These neuronal characteristics are certainly in line with the associative specificity obtained even in simple classical conditioning studies, as reviewed later.

A major issue concerns whether or not PSCs or higher cortical areas are responsible for perceptual improvement (Fahle, 2004). For example, in the case of successful frequency discrimination, the primary auditory cortex exhibits either extensive plasticity (Recanzone et al., 1993), a lack of plasticity (Brown et al., 2004), or partial plasticity (Witte and Kipke, 2005). There is no *a priori* reason why substrates of all perceptual learning should involve PSCs. It is sufficient for our present purposes to note that there is compelling evidence that some perceptual learning is tightly linked to the development of neuronal plasticity in primary sensory cortices. It seems likely that an adequate understanding of the neural bases of perceptual learning will require determination of precisely what has been learned in each study (e.g., absolute vs. relative discriminations). Interested readers should consult available reviews (Goldstone et al., 1997; Gilbert et al., 2001; Calford, 2002; Pleger et al., 2003; Ghose, 2004; Fahle, 2005; Skrandies, 2006). However, there remains a pressing need for an integration of conceptions and findings in the still separate disciplines of associative learning/memory and perceptual learning.

3.11.3 The Enduring Influence of Sensorimotor Conceptions of Cortical Organization, or Campbell's Ghost

Neurophysiological plasticity in PSCs is interesting both in its own right and as a case study in the intersection of sensory neurophysiology and the neurobiology of learning and memory, two fields that had developed

separately and with little crossover until the latter part of the last century. This is particularly remarkable because these are the two disciplines in neuroscience whose subject matter deeply involves the 'fate' of environmental stimuli in the brain. The former has traditionally been concerned with the coding and representation of the physical parameters of sensory stimuli. The latter has focused on how a previously neutral stimulus comes to influence cognition and behavior through learning. Furthermore, this topic provides a clear example of how assumptions constrained thought and experiment for most of the twentieth century. This is not merely of historical interest because the problem is still present.

Attempts to understand sensory cortex (as well as other brain systems and structures) began in the nineteenth century within the framework of a sensorimotor conception of the nervous system. Some of the first structural–functional relationships discovered concerned the spinal cord: The dorsal roots are sensory, and the ventral roots are motor. These seminal findings are attributed jointly to the separate but largely simultaneous studies of Charles Bell in England and Francois Magendie in France, roughly in the period 1812–1840 (Fearing, 1970). Although Bell can be given primacy for establishing the major function of the ventral roots, historical analysis has revealed that Magendie discovered the sensory function of the dorsal roots (as well as independently showing ventral root function), notwithstanding Bell's subsequent falsification of the record (Cranefield, 1974). After the discoveries of Magendie and Bell, much of the research program for the rest of the century concerned the extent to which the entire neuraxis was organized on sensorimotor principles (Young, 1970). The last 30 years of the nineteenth century witnessed the discovery of the motor cortex by Fritsch and Hitzig and the approximate delineation of sensory cortices based on modality specific sensory deficits following cortical ablations (Ferrier, 1886).

Still, sensory and motor areas did not comprise the entire neocortex. Could an overarching principle of cortical organization be discovered? In 1901 Flechsig, a neuroembryologist, reported that axons in different parts of the human cortex became myelinated at different times (Flechsig, 1901). Sensory and motor cortices exhibited myelination at birth, whereas other areas could require as long as 1 postnatal month to myelinate. Flechsig's observations of fibers in the internal capsule led him to the erroneous conclusion that only the sensory and motor cortices had subcortical connections, the association areas were thought to receive inputs only from other cortical regions. In short, Flechsig's schema was that the cortex consisted of sensorimotor zones that were connected to the thalamus and brainstem and were functional at birth, and association cortices that were connected only to other cortical regions and was not functional until well after birth. The late Irving Diamond pointed out that as association cortical areas myelinate later, this sequence of myelination "is just what would be expected if an infant sees sensory qualities such as color and brightness before these impressions are associated with another to form the perception of objects." (Diamond, 1979: 5). Thus, Flechsig had provided an anatomical basis for the distinction between lower (i.e., sensorimotor) and higher psychological functions.

It remained only to specify in greater detail, and perhaps with a more authoritative voice, the nature of these lower and higher functions. This was supplied by impressive cytoarchitectonic studies. In 1905 AW Campbell (Campbell, 1905) published a landmark monograph entitled *Histological Studies on the Localization of Cerebral Function*. Campbell asserted structural–functional relationships on the grounds of cytoarchitecture. While perhaps not the first worker to use the terms sensory and psychic cortex, his influence has been profound. For example, Campbell labeled the region now identified as V1 'visual sensory,' and called regions nearby (e.g., areas 17 and 18) 'visual psychic.' Similarly, the region now known as A1 was termed 'auditory sensory,' whereas adjacent areas, in modern parlance, auditory belt areas (Kaas and Hackett, 2000), were auditory psychic. In this, Campbell intended to make a clear distinction between cortical regions he considered to be purely sensory from those he believed concerned the understanding of the meaning of stimuli. His implicit assumption was one of strictly hierarchical cortical functional architecture, with what are now referred to as cognitive functions dependent on input from sensory structures (i.e., PSCs). Association cortex has come to denote both some modality-specific regions (e.g., auditory association) and cortical territory that lies between modality-dominated cortex, generally posterior to the central sulcus.

This conceptual schema of sensory-association-motor cortex as the basis for understanding cortical function has strong resonance today. Irving Diamond held that Campbell's monograph was instrumental in removing learning and memory from primary sensory cortices (Diamond, 1985). Although a less-sequential,

more parallel thalamocortical structural organization, championed by Diamond, is generally acknowledged, its influence on beliefs about cortical functional organization seems minimal.

Within the field of learning and memory, Pavlov's theory of conditioned reflexes provided authoritative support for the distinction between sensory fields that analyze stimuli and association fields, in which learning allegedly occurs. Pavlov's proposed physiological mechanism, that learning was due to spreading ripples of excitation from the cortical sensory fields of the CS and the unconditioned stimulus (US) that met in intervening association cortex (Pavlov, 1927), has long been superseded by action potentials. But the fundamental assumed functional architecture has not changed. The standard schema based on sensory-to-association-to-motor cortex fails to the extent that primary sensory cortices are involved in associative learning and memory. We now turn to a consideration of the relevant literature.

3.11.4 Overview of Cortical Plasticity in Associative Learning: 1935–1984

This section provides a brief summary of the 50-year period (1935–1984) during which PSC plasticity in associative learning was discovered and well characterized for training trials. The unspoken assumption was that recording during training trials would be sufficient to reveal the neural bases of learning. The following section provides a more detailed account of contemporary approaches that reveal the degree of specificity of associative PSC plasticity.

Near the dawn of electroencephalography, a serendipitous discovery launched neurophysiological studies of cortical plasticity in learning/memory. The alpha rhythm, an oscillation of 8–12 Hz in the human EEG prominent from occipital (i.e., visual cortical) leads, was known to be consistently blocked by visual stimulation. (The actual effect was a shift to higher-frequency, lower-amplitude waves (i.e., EEG desynchronization or activation). Durup and Fessard were investigating alpha blocking when they found that it occurred shortly before they turned on the flashing light intended to disrupt alpha waves (Durup and Fessard, 1935). Upon further consideration of this paradoxical result, they realized that the blocking was caused by the click sound made by the shutter of their camera, which they activated immediately preceding the repeated presentation of flashes, so that they could photograph alpha blocking displayed on the screen of their oscilloscope. Durup and Fessard had inadvertently been conducting a classical conditioning experiment, with the CS being the click and the US being the flashing light. This stimulus–stimulus (S–S) pairing produced conditioned blocking of the alpha, the first demonstration of learning-induced cortical plasticity. The signal importance of this finding was immediately recognized, and other laboratories quickly replicated and expanded the finding of conditioned alpha blocking (Morrell, 1961). Thus, perhaps ironically, primary visual cortex was the first discovered site of learning-induced cortical plasticity.

Studies of cortical plasticity, much of them involving further EEG studies, expanded greatly after the end of World War II and were extended to nonhuman animals (hereafter animals). Emphasis was directed to understand classical conditioning and the major focus was to find the locus of closure, that is, the place(s) in the cortex of convergence of input from the CS and US. The dominant finding, across species and modalities and types of sensory stimuli, was that EEG changes followed a particular trajectory according to the stage of learning. First, relaxed subjects exhibited widespread cortical activation to the US. Second, continued pairing of the CS and US rapidly produced the first signs of associative learning, consisting of widespread activation that was now elicited by the previously ineffective CS. Third, as learning progressed, the cortical domain of conditioned EEG desynchronization shrank, becoming confined largely to the PSCs of the CS and US. Various controls were used to establish that conditioned EEG activation was genuine, including the use of unpaired or randomly presented CSs and USs in control groups or the use of discrimination paradigms in which a CS+ was paired with the US, whereas a CS− was not paired (Gluck and Rowland, 1959; Rowland and Gluck, 1960). The CS+ developed the ability to produce EEG activation while the CS− failed to do so (reviewed in John, 1961; Morrell, 1961; Thomas, 1962; Galeano, 1963).

That the CS developed the ability to elicit EEG plasticity in its own primary sensory cortex was a critical demonstration that PSCs were involved in learning and memory. However, this fact received little recognition. To the best of my knowledge, there has been no historical analysis of these studies in the context of the extant *Zeitgeist*. My guess is that this finding was overshadowed by the disappointment at the apparent failure to find the locus of closure. According to Pavlov and the dominant thinking at

that time, the CS and US should have converged in association areas. But conditioned EEG effects were not uniquely found in association cortex and in fact soon disappeared from there during learning.

Experiments on sensory-evoked potentials overlapped with studies of the EEG and learning and ultimately gained dominance. In an influential early study, Robert Galambos and his colleagues performed a seminal experiment in which cats were classically conditioned by pairing an auditory (click) conditioned stimulus with a puff of air (US) to the face (Galambos et al., 1956). Learning was validated by the development of behavioral conditioned responses. Evoked potentials in the primary auditory cortex elicited by the CS became larger during conditioning. This study also addressed the critical issue of stimulus control. To show that inadvertent changes in CS intensity (level) were not responsible, the authors also tested subjects under neuromuscular blockade, maintaining stimulus constancy at the periphery while eliminating putative contractions of the middle ear muscles. Interestingly, the authors failed to include a nonassociative control, such as a group that received the CS and US randomly. However, subsequent investigations confirmed and extended this basic finding, showing that facilitation of response to the CS in A1 was associative (Marsh et al., 1961; Majkowski and Sobieszek, 1975).

During the period from the late 1950s until the 1980s, an extensive literature also documented associative learning effects in V1 both in classical and instrumental conditioning in a variety of species. The dominant finding was facilitation of responses to visual conditioned stimuli (John and Killam, 1959, 1960; Saunders, 1971; Peck and Lindsley, 1972; Buresova and Bures, 1973; Suzuki et al., 1974; Sasaki and Yoshii, 1984a,b). During this period of active research, even evidence of evoked potential correlates of memory retrieval in V1 was reported (John et al., 1973; Bartlett et al., 1975; John et al., 1975). Additionally, the associative plasticity of V1 was emphasized by demonstrations that an experimenter-selected component of evoked responses could be operantly conditioned to change amplitude, contingent on the receipt of rewarding intracranial stimulation in the rat (Hetzler et al., 1977) and reward of liver and milk in the cat (Rudell, 1977). In contrast, the use of somatosensory conditioned stimuli was rarely, if ever, employed during this period.

Not surprisingly, as techniques were further developed for recording from behaving animals, research was extended to multiple-unit and even single-unit studies of plasticity. Much of the evoked potential and unit research used sounds as CSs because of convenience. Numerous laboratories demonstrated that learning-related increases in the amplitude of CS-elicited evoked potentials and unit discharges in the primary auditory cortex were indeed associative. For example, multiple unit discharges in A1 increase to a sound (CS+) paired with shock, decrease in response to a nonreinforced sound (CS−), and exhibit reversal when the CS+ and CS− are reversed. Moreover, the CSs come to elicit responses in the PSC of the unconditioned stimulus (i.e., S1), although with less specificity with respect to reinforcement contingency than in A1 (Oleson et al., 1975). For a general review, see Weinberger and Diamond (1987).

However, PSC plasticity in learning was generally ignored both within the neurobiology of learning and memory and also in the discipline of sensory neurophysiology. Several factors may have contributed to this state of affairs. For example, within learning and memory, the discovery of patient HM understandably focused attention on structures that appeared to be essential for memory, such as the hippocampus. In animal conditioning, attention was drawn to model systems, such as conditioned eyeblink and fear conditioning. Second, there was no conceptual framework within which to incorporate the findings of associative plasticity in sensory cortices, particularly as dominant assumptions of brain function restricted sensory cortices to the status of stimulus analyzers. Third, workers in learning and memory had no vested interest in sensory systems and therefore were not concerned that the research initiated by Galambos and colleagues disproved the view that sensory systems encoded only sensory parameters, not psychological parameters, such as the acquired behavioral significance of a stimulus. The ghost of A.W. Campbell might have smiled.

Neglect from sensory neurophysiologists might seen particularly unexpected because PSC plasticity has major implications. Thus, that learning modifies sensory cortical responses to physically constant stimuli implies that such neural responses are inherently ambiguous. For example, an increase in stimulus-elicited response magnitude might be due to an increase in stimulus intensity, an increase in its behavioral significance, or both. But it seems likely that sensory neurophysiology paid little heed to the learning findings both because of the dominance of the Campbellian formulation and the impoverished stimulus set used by behavioral neurophysiologists (myself included). Studies of associative learning typically

employ a small number of different stimuli. For example, standard conditioning studies use a single acoustic CS and discrimination studies use only two, a CS+ and a CS−. However, the concept of the receptive field is fundamental to sensory physiology. No sensory physiologist would attempt to describe the response properties of a cell with such a limited stimulus set.

But even within the neurophysiology of learning, a certain disquiet was growing. The EEG and evoked potential findings seemed fairly easy to interpret as either increased excitability or increased response to a behaviorally important CS. But single-unit studies invariability reported not simply increased discharges to the CS but also many instances of decreased discharges, even within the same study (Gasanov and Galashina, 1976; Woody et al., 1976; Weinberger et al., 1984b; Dumenko and Sachenko, 1979a,b). Although such cortical plasticity was shown to be associative, the findings of opposite sign made little functional sense. Thus, although recording in PSCs during training had provided foundational information, this approach appeared to be yielding diminishing returns after 50 years of use.

New questions were being asked. Whereas most prior studies had documented cortical plasticity during various phases of learning and in different structures and diverse tasks, a critical aspect of the functional significance of plasticity had not been adequately addressed. Missing was information concerning the specificity of cortical plasticity. As noted in the introduction, knowledge of the specificity of plasticity transcends the detection of a structure or group of neurons as being involved in learning and memory. Rather, knowledge of the specific changes in neural activity (i.e., not merely the fact of plasticity but also the substance of plasticity) provides insights into the type of information being processed and its role in learning and memory.

3.11.5 Specificity of Associative Plasticity in Primary Sensory Cortices: The Overarching Importance of Experimental Design

3.11.5.1 Introduction

What may reasonably be termed the contemporary era began in the 1980s with the use of a new experimental paradigm. It involved two major departures from prior research, in which neurophysiological recordings had been obtained principally during training trials. First, the new approach obtained neuronal data either after training in between-groups designs (Gonzalez-Lima and Scheich, 1984, 1986) or before and after training for within-subjects designs (Diamond and Weinberger, 1986; Weinberger et al., 1984a). Assessing learning after training was not unprecedented, as it had been standard in purely behavioral studies of learning and memory. It just hadn't been much applied to the neurophysiology of learning and memory.

Second, the new paradigm combined basic methods from sensory neurophysiology to determine the effects of learning on neuronal tuning. This latter method required the presentation of many different stimuli (e.g., acoustic frequencies) to obtain receptive fields and assess potential receptive field plasticity. Obviously, there was nothing new about this approach in neurophysiology. Neither was this method novel in learning and memory. In fact, training with one (or a few) stimuli but later assessing learning with many stimulus values had been done starting with Pavlov; it is the method of obtaining stimulus generalization gradients in learning. Apparently, it had not been applied to neurophysiological studies of learning and memory.

Before proceeding directly to a review of major findings in the study of specificity in PSCs, it will prove helpful to take a short detour to consider why recording during training is insufficient to determine the specificity of plasticity and some other critical factors about the new experimental approaches.

3.11.5.2 Cortical Plasticity during Training and Its Limitations

The standard approach to neural correlates of learning consists of obtaining neurophysiological recordings during training trials (the DUR design; see the section titled 'Primary auditory cortex (A1) habituation'). This very logical approach, almost universally used since the inception of research in the 1930s, is not restricted to the study of cortical plasticity but is applied to all brain structures (John, 1961; Thompson et al., 1972). However, obtaining recordings during training trials has at least two major limitations: (1) they can be influenced by state factors, and (2) they do not permit assessment of the degree of specificity of plasticity.

3.11.5.3 State Factors

First, nonlearning factors are invariably present during training, due in part to the presence of positive or negative reinforcement. These factors include, but are not limited to, changes in attention, arousal state, motivational level, and motor performance. For example, arousal level and motivational state alter evoked potentials and unit discharges in the auditory cortex (Murata and Kameda, 1963; Teas and Kiang, 1964; Wickelgren, 1968; Molnar et al., 1988). Moreover, the degree of influence of performance factors can vary during training trials. It may be high early in training, when subjects have not yet solved whatever problem confronts them, and then later decrease as solutions are found and as performance improves.

It must be emphasized that cortical plasticity obtained during training trials is usually associative. That factors such as motivational state are operative in no way weakens the case for associativity, given that the plasticity in question does not develop under nonassociative circumstances, such as random CS–US presentation. However, the associative plasticity may be influenced by state and similar factors, so that it is difficult to obtain pure associative effects.

Rescorla has emphasized the dangers of relying on behavioral data obtained during training to infer the strength of learning and those aspects of an experience that enter into memory. Rather, these attributes are best determined by appropriate posttraining assessments of behavior (Rescorla, 1985, 1988). This counsel is equally applicable to neurophysiological plasticity that develops during training trials. Although such plasticity may constitute adequate evidence that associative learning has a neural correlate (given controls for sensitization and pseudoconditioning), the form and magnitude of that correlate are not necessarily a reflection of associative processes alone.

3.11.5.4 Specificity of Plasticity

Second, and probably of even greater importance, recordings during training do not permit determination of the overall specificity of plasticity, because training protocols necessarily employ a limited number of stimuli along a dimension. For example, subjects may be trained with a visual stimulus, such as a vertical line followed by food reinforcement. If neurons in the primary visual cortex develop increased responses to that stimulus during training, then one can conclude that V1 cells develop associative plasticity during the task. (Of course, this assumes a control group that did not develop the plasticity when the vertical line and food were presented randomly, or equivalent control.) However, one cannot determine from such data alone if this cortical plasticity reflects changes in the processing and representation of line orientation *per se*. To resolve this issue, it would be necessary to interrogate the visual cortex with lines of many orientations after training. This could be done in a posttraining extinction session, as is routinely done when investigators obtain behavioral stimulus generalization gradients. As seen later, it is quite easy to obtain such information about the overall specificity of cortical plasticity in the absence of complications endemic to the use of extinction training (i.e., new learning that inhibits the prior learning).

3.11.5.5 Unified Experimental Designs

Both the potential problems of performance factors and the determination of specificity of cortical plasticity can be overcome by expanding the experimental designs to include assessment of plasticity by sensory neurophysiological approaches (unified designs). We describe the basic Pre-Post design and the Post design approaches (**Figure 1**).

3.11.5.5.1 The Pre-Post training trials design

The Pre-Post design allows the experimenter to determine how PSC information processing changes as a result of learning. This design involves a minimum of three stages: (1) pretraining recording, (2) actual training or other designated controlled experience, and (3) posttraining recording. The Pre and Post periods should present many sensory stimuli of interest (e.g., different frequencies of tone bursts, deflection of different whiskers, locations of targets in different visual quadrants). This contrasts with the standard presentation of a stimulus during a discrete training trial. The effects of the experiential treatment, whether habituation, classical conditioning, instrumental discrimination learning, or any other task, are determined by comparing the Post data with the Pre data. If the Pre and Post data are not statistically different, then one can conclude that the experience has had no effect on the processing or representation of the particular information under study. Conversely, significant differences between

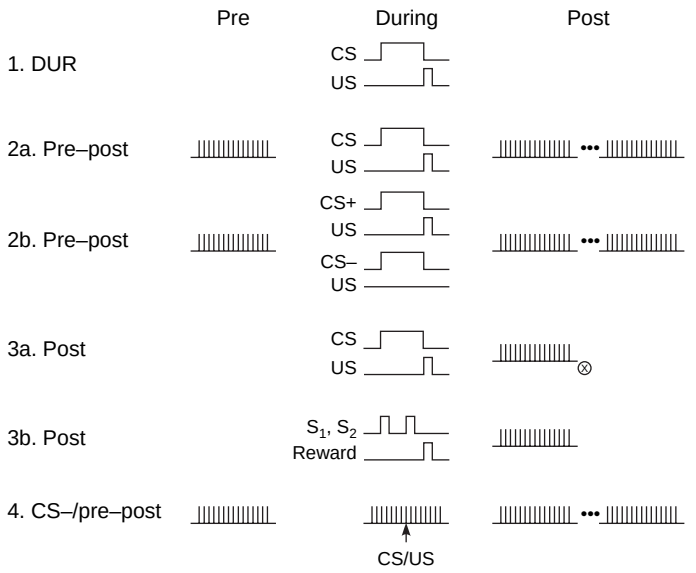

Figure 1 Schematic summary of experimental designs employed in the neurophysiological study of learning and the auditory cortex. Depicted are four basic designs (1–4) and their treatments during three experimental periods, Pre (before training), During (during training), and Post (after training). The DUR design is traditional, involving recording during training trials. Pre-Post designs 2a and 2b illustrate the fact that any training paradigm can be used. 2a shows single-tone conditioning, and 2b illustrates two-tone discrimination conditioning. Designs 3a and 3b also illustrate the fact that any training paradigms can be used with a Post design. 3a illustrates the case of single-tone conditioning, whereas 3b shows an example of two-tone instrumental training, in which reward is contingent on the correct response (i.e., one response if the two tones – S1 and S2 – are the same and another response if they are different) (responses not shown). The repeated vertical lines represent presentation of tone bursts. The dotted lines in the Post period for designs 2 and 4 indicate that additional Post periods can be used to determine long-term retention. In 3a, the x in the Post period signifies sacrifice of the animal for 2-deoxyglucose analysis following repeated presentation of a conditioned stimulus stone. In design 4, the conditioned stimulus (CS)/unconditioned stimulus (US) denotes that one of the frequencies in a series of tone bursts is designated as the CS and is paired with shock; the serial order of tones is random from one sequence to another. The problems with this design are that the behavioral state is likely to be different between the Post and Pre periods, and extinction is likely to occur in the Post periods (see text). Illustrations are not to scale.

the two periods can be attributed to the intervening experience (**Figure 1(2a), (2b)**).

It is essential that the test stimulus presentations be identical during the Pre and Post periods. Moreover, these data should be obtained in identical experimental settings to eliminate any confounds due to changes in the local environment context. No reinforcement is present during these Pre and Post tests, as that would defeat the goal of avoiding performance factors. However, despite the absence of a reinforcer, extinction learning does not develop during Post training assessment (see the section titled 'The importance of context').

It is also important to perform the training in a separate experimental setting, such as a different room with salient differences in visual and other nonauditory cues (see next section). Of course, it is important that the subjects be in the same state during the Pre and Post tests. This can be accomplished by amply habituating them to the testing (Pre and Post) situation, and this can be objectively assessed by recording heart rate or other physiological measures. Typically, heart rate is high when an animal is placed in a novel situation, but such tachycardia habituates fairly rapidly.

The Pre-Post design permits assessment of specificity while avoiding confounding performance factors that are present during training trials. Another advantage is that posttraining tests can be presented at desired intervals of hours to months, permitting detection of neural consolidation (i.e., increase in effect over time without additional training) and long-term retention or forgetting. This design also is very flexible because any desired training task can be used, yet the assessment of receptive field plasticity can be identical. This permits a direct comparison of the effects of learning on sensory representation for as many types of learning as may be of interest.

3.11.5.5.2 The importance of context: Elimination of performance factors and extinction

At least two issues are raised by this design. First, how does it eliminate or control for performance factors? Second, how does it avoid experimental extinction? (See also Diamond and Weinberger, 1989; Weinberger, 1998.)

Performance factors are reduced, if not eliminated, by minimizing similarities between the training context and the testing circumstances. The purpose of this maneuver is to reduce or eliminate any generalization from the training environment to the testing environment. For example, if an animal receives food or shock during training, it will also associate the location (context) of the training with these reinforcers. If tested in the same place, its arousal level and expectations could be affected.

Perhaps the most salient difference is the absence of a reinforcer in the Post period, which also reduces and can even eliminate changes in state (see the section titled 'The Post training trials design (POST)'). The Pre-Post design also permits sensory stimuli to be presented with several parameters that differ from the training session. Thus, training can consist of standard, discrete conditioning trials with standard parameters (e.g., an individual CS tone with a duration of 5s, a long intertrial interval of 1–2 min, and a stimulus level that is well above threshold (80 db SPL)). In contrast, determination of receptive fields can be accomplished with completely different parameters randomly presenting many tones to cover the frequency spectrum (e.g., 24 tones at quarter-octave intervals, 100 ms duration, intertone intervals of 400 ms, at stimulus levels of 0–80 dB SPL to cover the audible range). In short, the acoustic context of RF determination can be, and indeed must be, different from that during conditioning trials.

This difference in context has proven to be sufficient to eliminate any behavioral or arousal response to the CS frequency when it is embedded as a brief tone in a series of test tones. Objective measures indicate that subjects do not regard the CS frequency as a conditioned stimulus during determination of receptive fields (Diamond and Weinberger, 1989). In addition to the lack of performance confounds, elimination of potential effects of arousal to the CS frequency or any other tone can be accomplished by training subjects while they are awake (of course) but obtaining receptive fields while they are under general anesthesia (Weinberger et al., 1993).

Responses to the training stimulus during discrete trial presentation can, of course, be recorded, as long as the likely confound of performance factors is kept in mind. In such cases, it is beneficial to compare plasticity to a CS tone during training trials with plasticity in receptive fields as seen after training. Such a study revealed that there was little correspondence between changes to the CS during training with responses to that same frequency when it was presented as one of a series of rapidly presented frequencies in the Post period. In many cases, the sign of change was opposite (e.g., a decrement in response to the CS tone but a specific increase in response to that frequency during RF determination) when tuning might shift toward or to the frequency of the conditioned stimulus (Diamond and Weinberger, 1989).

The problem of experimental extinction is also eliminated by the Pre-Post design. As subjects do not regard the presentation of the frequency used as a CS as an actual CS, they neither respond to it nor extinguish during posttraining determination of receptive fields (Diamond and Weinberger, 1989).

A negative case illustrates the importance of appreciating the unified design (Ohl and Scheich, 1996, 1997, 2005). The authors failed to appreciate the difference between plasticity observed during training trials from plasticity observed in Post-training assessments. They used a tone-shock discrimination protocol in which a single CS+ tone was randomly intermixed with 11–30 different CS− (no shock) frequencies in a single training session. The same frequencies were presented before, during, and after training without break, so the presence of an occasional shock during training provided the only information that training was underway (**Figure 1(4)**). Relative to pretraining, the posttraining period exhibited a specific decrease in response to the CS frequency in A1. Unfortunately, no behavioral data were obtained, so there was no substantiation for the assumption that the animals had learned this unique, difficult discrimination. However, if learning had occurred, then the posttraining period would have constituted a period of extinction, due to the elimination of shock. Thus, the posttraining period undoubtedly differed from the pretraining period in the subjects' state of arousal and fear due to the anticipation of shock, and in the new extinction learning that shock was no longer forthcoming. Therefore, the findings might document a decrease in response to a CS frequency due to experimental extinction. The authors' claim that the behavioral importance of the CS is represented by a specific decrease in response seems unsupported.

3.11.5.5.3 The Post training trials design (POST)

The POST design has been used extensively in sensory neuroscience to determine the effects of peripheral sensory denervation or central insult (stroke) on the organization of primary auditory (Robertson and Irvine, 1989; Mount et al., 1991; Kaltenbach et al., 1992; Harrison et al., 1993), somatosensory (Kelahan et al., 1981; Rasmusson, 1982; Wall et al., 1986; Calford and Tweedale, 1988), and visual (Weisel and Hubel, 1963; Rauschecker et al., 1987; Heinen and Skavenski, 1991; Pettet et al., 1992) cortices.

Review of resultant plasticity is beyond the scope of this chapter, but a general conclusion is that peripheral denervation produces an expanded cortical representation of intact neighbors on the receptor epithelia: in the cochlea, on the body surface, and in the retina. However, these conclusions are necessarily oversimplifications, and the original sources should be consulted. What these sorts of studies do demonstrate is that adult PSCs are capable of considerable reorganization as a function of experience. Whether such experiences constitute genuine learning is unknown. Learning may well develop in animals and humans who find themselves with deficits in peripheral sensory capabilities. Of interest, prior learning can be necessary for adjustment or recalibration of a sensory system following peripheral impairment. Ferrets can relearn to localize sounds after reversible blocking of one ear, but only if they had been trained previously to use the relevant spatial cues (Kacelnik et al., 2006).

The considerations necessitating the Pre-Post design, and the advantages thereof, also apply to the POST design. Simply put, this design consists of recording only after the designated learning experience. Because of the absence of pretraining data, at least two groups are needed, one that receives the learning experience and a control group that either is not trained or preferably undergoes a different learning protocol (e.g., sensitization). The POST design has often been used when data can be obtained only once from a subject (e.g., in detailed terminal studies of cortical organization) (**Figure 1(3a), (3b)**).

3.11.6 Specificity of Associative Plasticity: Contemporary Approaches

The following sections review associative plasticity in the primary auditory, somatosensory, and visual cortices since 1985. The coverage is extensive but not exhaustive, as the goal is merely to establish PSC plasticity in learning and memory.

3.11.6.1 Primary Auditory Cortex (A1)

3.11.6.1.1 Habituation

Studies of habituation that used the DUR design had documented that repeated presentation of sounds results in a progressive response decrement in A1 (Marsh and Worden, 1964; Wickelgren, 1968). Specificity of habituation with control for state of arousal was first studied (before 1985) using a preliminary version of the Pre-Post design (Westenberg and Weinberger, 1976; Westenberg et al., 1976). Evoked potential was recorded in A1 of the waking cat. Two frequencies (A and B) were presented as alternating brief tone bursts (prehabituation). In the next stage, one tone (A) was presented repeatedly. Finally, the posttest was performed; the tones were again presented in an alternating pattern identical to the prehabituation phase. Average evoked potentials for each tone were determined separately for the pre- and posthabituation periods and compared. Because the Pre and Post tones alternated, the average responses were obtained for both A and B when the subjects were in the same state; hence, any differences between responses to the tones could be attributed to the effects of the intervening repeated stimulation with one frequency. The Post responses to the repeated tone (A) were significantly smaller than the Pre responses to this tone, but there was no difference for the nonrepeated (B) tone. Counterbalancing using repetitive presentation of B also yielded frequency specific decrements. These findings demonstrate that repeated acoustic stimulation produces frequency specific habituation.

The Pre-Post design has been expanded to determine the entire frequency receptive field (RF, tuning curves) of auditory cortical neurons in waking guinea pigs (Condon and Weinberger, 1991). After researchers determined the tuning of unit clusters and ensured their stability, subjects received single tone pips at the rate of 1.25 Hz for 5–7 min. Habituation produced a decreased response that was specific to the frequency that had been repeatedly presented; frequencies 0.125 octaves from the habituated frequency exhibited little or no response decrement. Consolidation, in the form of continued increased development of specific decrements, was often observed for periods as long as an hour. This attribute links frequency-specific habituation to other forms of memory.

3.11.6.1.2 Conditioning

In this section we consider studies of the specificity of plasticity in A1 induced by associative learning. Research to date has focused on classical and instrumental conditioning, with the implication that investigation of these heavily studied forms of learning is an appropriate entry point into the issue of specificity.

Gonzalez-Lima and Scheich (1984, 1986) investigated the specificity of plasticity by determining the effects of conditioning on the uptake of 2-deoxyglucose (2-DG) after training. As this necessitated a terminal treatment of the subjects, the authors used a between-groups POST design. (The use of metabolic measures permits investigation of specificity without using many different stimuli when the locus of stimulus representation in a PSC map is known.) Gerbils received tone paired with strong aversive electrical stimulation of the mesencephalic reticular formation or a control treatment (e.g., CS–US unpaired, CS alone, US alone). The paired group alone developed the behavioral index of learning, conditioned bradycardia. All groups received continual presentation of the CS alone during an injection of 2-DG in a posttraining session. As this treatment may involve some experimental extinction, the associative findings might be considered somewhat weakened. Nonetheless, analysis of patterns of 2-DG uptake in A1 revealed a CS-specific increase in metabolic activity for the cortical area that represented the CS frequency. The absence of similar effects in the other groups showed that the CS-specific plasticity was associative.

The first use of the Pre-Post design with receptive field (RF) analysis involved single units in two nonprimary auditory fields, secondary (AII) and ventral ectosylvian (VE) cortices (Diamond and Weinberger, 1986, 1989; Weinberger et al., 1984a). Cats were trained in a single, brief (20–45 trials) session of tone-shock pairing. Behavioral learning was validated by the formation of the pupillary dilation conditioned response. CS-specific plasticity was found in the paired group, but not when tone and shock were unpaired. Some cells developed a CS-specific increase, whereas others developed a CS-specific decrease. Extinction produced loss of the RF plasticity. The findings received little notice, probably because these auditory fields were not well understood.

Similar studies were then undertaken in A1 of the guinea pig with behavioral validation of associative learning (e.g., conditioned bradycardia). Following determination of frequency receptive fields, the frequency to be used as the CS was then selected to not be the best frequency (BF, peak of the tuning curve) to determine whether conditioning caused shifts of tuning toward the CS frequency. Animals then received a single session (30–45 trials) of tone paired with shock. A comparison of posttraining with pretraining RFs revealed a dominance of CS-specific increased responses. Moreover, responses to the pretraining BF and other frequencies tended to decrease. These opposing changes were often sufficiently large to produce frank shifts of tuning toward, and even to, the frequency of the CS, which could become the new BF (Bakin and Weinberger, 1990) (**Figure 2(a)**). RF plasticity was found to be associative, as it required stimulus pairing; sensitization training produced only a general increase in response to all frequencies across the RF (Bakin and Weinberger, 1990; Bakin et al., 1992).

CS-specific increased responses in RFs also can develop when tuning curves are complex and even nonexistent. **Figure 2(b)** shows an example of a pretraining double-peaked frequency RF. The CS frequency was selected to be in the valley between the peaks. Posttraining, the maximum change was an increase in response at the CS frequency. **Figure 2(c)** illustrates a case in which there was no response to any frequency before conditioning. Nonetheless, postconditioning observations revealed a clear excitatory response to the previously ineffective CS frequency, alone.

Several other attributes of RF plasticity make it an attractive candidate for a process that operates in normal concert with sensory coding processes to subserve the storage of behaviorally relevant auditory information. First, RF plasticity is highly specific to the CS frequency; responses to frequencies a small fraction of an octave away are attenuated. Second, it exhibits generality across different types of training (e.g., instrumental avoidance conditioning) (Bakin et al., 1996), two-tone classical discrimination training (i.e., increased responses to the CS+ frequency but decreased responses to the CS−, BF, and other frequencies) (Edeline and Weinberger, 1993; Edeline et al., 1990), and discriminative instrumental avoidance conditioning (Bakin et al., 1996). Third, RF plasticity develops very rapidly, after only five training trials, as rapidly as the first behavioral (e.g., cardiac) signs of association (Edeline et al., 1993). Fourth, RF plasticity exhibits long-term retention, enduring for the longest periods tested, up to 8 weeks after a single 30-trial conditioning session

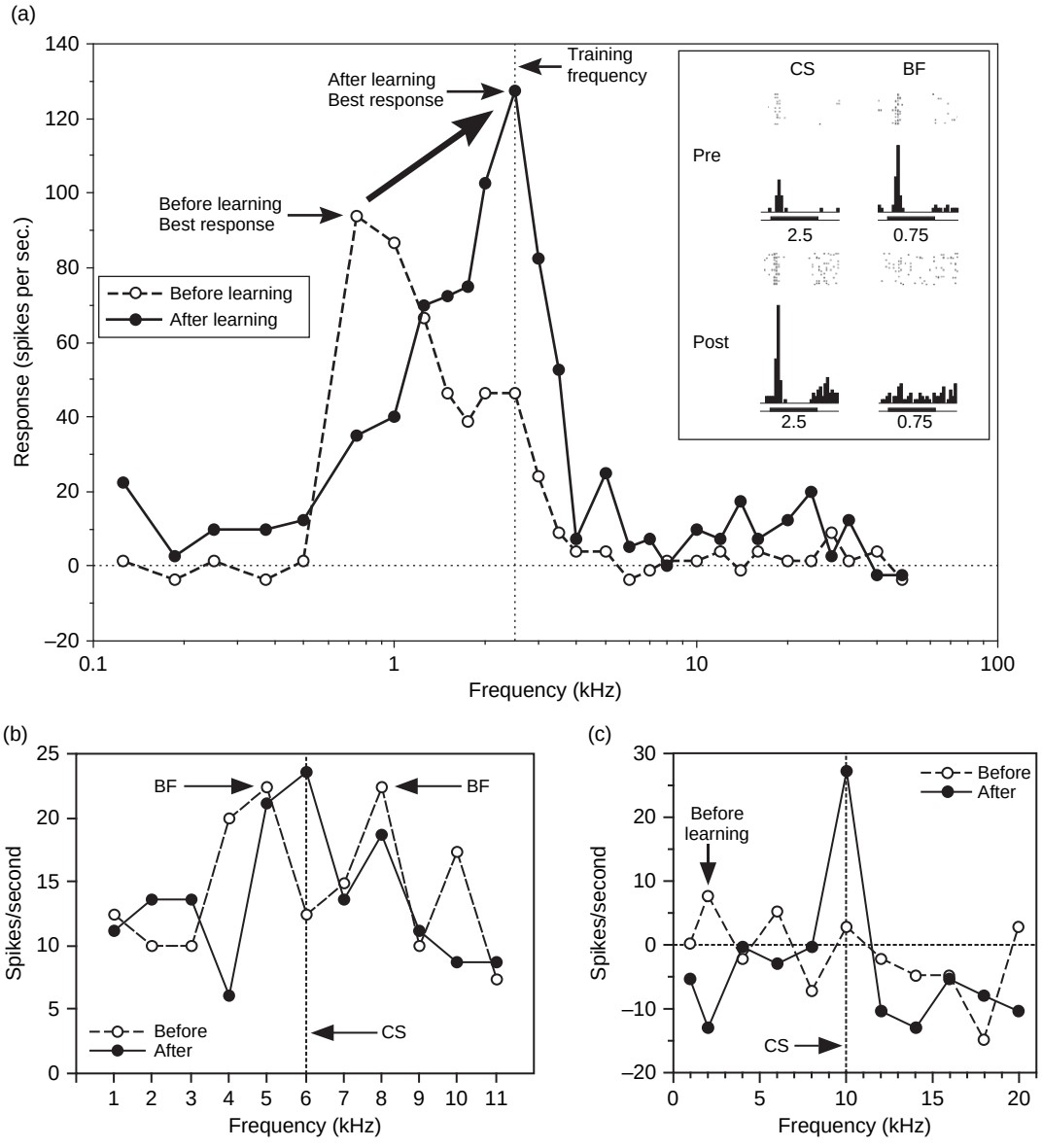

Figure 2 Classical conditioning produces conditioned stimulus (CS)-specific facilitation and tuning shifts. (a) An example of a complete shift of frequency tuning of a single cell in A1 of the guinea pig, from a pretraining best frequency (BF) of 0.75 kHz to the CS frequency of 2.5 kHz after 30 trials of conditioning. Inset shows pre- and posttraining poststimulus time histograms (PSTHs) for the pretraining BF and the CS frequencies. (b) Double-peaked tuning, with pretraining BFs at 5.0 and 8.0 kHz. The CS was selected to be 6.0 kHz, a low point. After conditioning (30 trials), responses to the CS frequency increased to become the peak of tuning. (c) A cell that exhibited minimal or no response to tones before tuning developed tuning specifically to the CS frequency after conditioning (30 trials).

(Weinberger et al., 1993). Fifth, RF plasticity exhibits consolidation (i.e., continues to develop increased responses to the frequency of the CS *vs.* decreased responses to other frequencies in the absence of further training over hours) (Edeline and Weinberger, 1993) and days (Weinberger et al., 1993; Galvan and Weinberger, 2002).

CS-specific associative tuning shifts develop in the primary auditory cortex of diverse taxa. Thus, studies of fear conditioning (tone, shock) in the big brown bat demonstrate not only that learning induces tuning shifts but that these can develop with small absolute differences in frequency in these echolocating animals (Gao and Suga, 1998, 2000; Ji et al., 2001; Ji and Suga,

2003; Suga and Ma, 2003). Nonassociative controls did not develop tuning shifts. In these studies, leg flexion was used to validate associative learning, but the use of a fixed (30-s) intertrial interval does not rule out possible effects of temporal conditioning.

Kisley and Gerstein questioned whether tuning shifts are caused by learning because they observed tuning changes over days in the absence of training (Kisley and Gerstein, 1999). This concern was rather curious because spontaneous shifts had previously been eliminated as tuning shifts are directional (toward the CS), associative, and discriminative, that is, toward the CS+, not the CS– (Bakin and Weinberger, 1990; Bakin et al., 1992; Edeline and Weinberger, 1993).

The authors next studied classical conditioning, pairing a tone with intracranial self-stimulation (Olds, 1962). In so doing, they extended inquiry to another species, the rat, and another type of motivation, reward (Kisley and Gerstein, 2001). Animals underwent a single 30-trial session of classical conditioning. Subjects were studied under light ketamine anesthesia throughout, rather than in an undrugged waking state (see also Edeline, 1990). Behavioral evidence of conditioning to the tone was nonetheless obtained. The authors did find that conditioning produced CS-specific plasticity, including shifts of tuning toward or to the frequency of the CS. Also in agreement with prior studies, this RF plasticity was associative because it required CS–US pairing. The learning effects were above and beyond any spontaneous changes, which were indexed by reduced day-to-day correlation coefficients actually between entire tuning curves. However, the authors did not actually measure tuning by tracking the best frequency over days. The reduced correlations reflect the fact that responses to all frequencies within a tuning curve received equal weighting in the statistical analysis. Thus, decreased correlations might well be caused by loss of weak responses to frequencies distant from the BF (i.e., at the lower and upper limits of the tuning curves). The stability of tuning has been established over periods of 2–4 weeks, during which time there were no drifts of the best frequency (Galvan et al., 2001).

As RF plasticity is not an artifact of spontaneous changes in tuning, neither is it an artifact of state. As noted earlier, whereas animals exhibit arousal and related responses to sustained (e.g., 2–5 s) CS frequencies during training trials, they do not exhibit any behavioral responses to the frequency of the CS when it is presented as one of a number of rapidly presented, brief (e.g., 200-ms) sequential tone pips during RF determination (Diamond and Weinberger, 1989). Moreover, animals trained in the waking state exhibit RF plasticity when tested under deep general anesthesia (Lennartz and Weinberger, 1992b; Weinberger et al., 1993).

Learning induced tuning plasticity is not limited to animals. The same paradigm of classical conditioning (tone paired with a mildly noxious stimulus) produces concordant CS-specific associative changes in the primary auditory cortex of humans (Molchan et al., 1994; Schreurs et al., 1997; Morris et al., 1998).

In summary, RF plasticity has major characteristics of associative memory. It is not only associative but is also highly specific, discriminative, rapidly acquired, retained at least for many weeks, develops consolidation over hours and days, and exhibits generality across training tasks, types of motivation, and species. Thus, RF plasticity in the auditory cortex reflects the learned importance of experiences. **Figure 3** summarizes changes in tuning for conditioning, sensitization, and habituation.

The associative specificity of tuning for acoustic frequency raises the issue of whether the observed effects indicate a special type of adaptation for frequency or are exemplars of a general neural strategy for the processing, representation, and storage of experience. Recent studies have addressed this issue by determining the effects of associative processes on parameters other than acoustic frequency.

Bao and colleagues (2004) trained rats in a sound maze in which food reward was contingent on successful navigation using only auditory cues. In this task, the repetition rate of noise pulses increased as the distance between the rat and target location decreased. After subjects had learned this maze, the responses of neurons in A1 were investigated in a terminal posttraining session. A1 cells exhibited enhanced responses to high-rate noise pulses and stronger phase-locking of responses to the stimuli. The effects were due to learning because controls that had received identical sound stimulation, but were given free access to food, failed to exhibit such plasticity of temporal processing and, in fact, were not different from naive subjects. Thus, learning produced a shift in tuning to high repetition rates (i.e., the stimulus feature that was most closely associated with procurement of food).

The plasticity of sound intensity (level) processing has been investigated in A1 (Polley et al., 2004b). Rats were trained to move to a place in a small arena at which sound levels to ongoing sound bursts

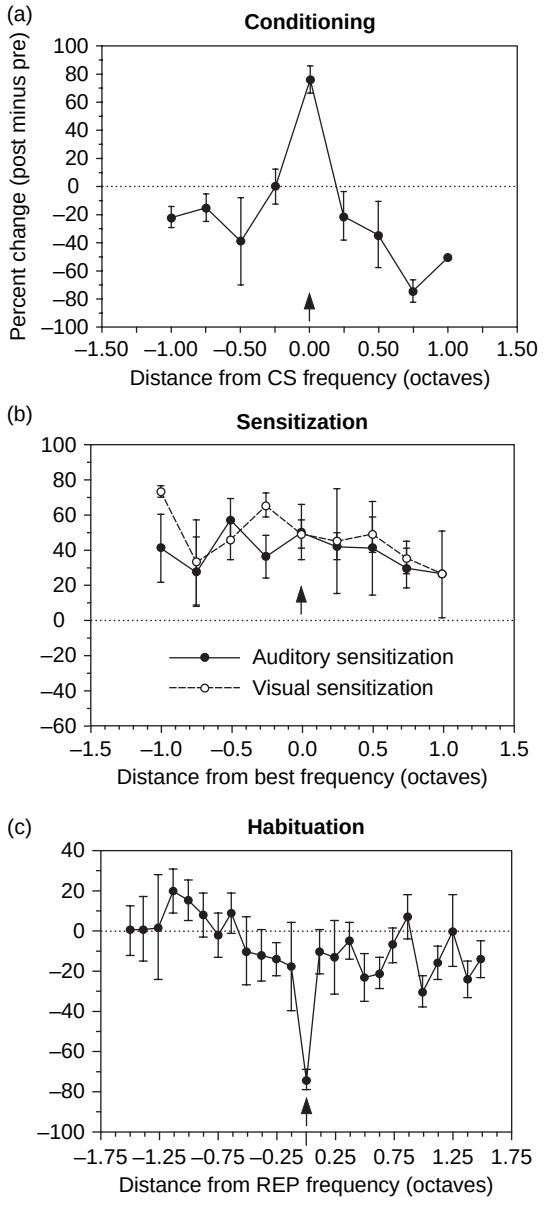

Figure 3 Summary of the effects of conditioning, sensitization, and habituation on frequency receptive fields in the primary auditory cortex of the guinea pig. Data are normalized to octave distance from (a) the conditioned stimulus (CS) frequency, (b) the presensitization best frequency, or (c) the repeated frequency. Note that conditioning produces a CS-specific increased response, whereas sensitization (tone–shock or light–shock unpaired) produces general increases across the spectrum. Habituation produces frequency-specific decreased response.

became maximal. They were guided by having stimulus levels increase as the subject moved closer to the otherwise unidentified locus, and contrary, levels were reduced as the rats moved farther away. Yoked controls received the same acoustic experience, which was not linked to their behavior. In trained animals only, A1 responses became selective to more restricted ranges of sound intensities. The findings indicated that associative processes can selectively modify the representation of stimulus magnitude.

3.11.6.2 Primary Somatosensory Cortex (S1)

Stimulation (deflection) of the mystacial vibrissae of the rat has often been the conditioned stimulus of choice in the study of associative plasticity in S1, specifically in the posteromedial barrel subfield (PMBSF, hereafter whisker barrel field) (Welker, 1976; Simons, 1978). Early studies simply paired the deflection of a vibrissa that elicited little response at a particular barrel recording site with that of a subsequent deflection of a whisker that did elicit an excitatory response; a typical CS–US interval was 500 ms. In many cases, pairing produced an increased response to the CS (Delacour et al., 1987, 1990a,b). When a whisker is stimulated alone continuously, the response in S1 declines, probably due to habituation, whereas subsequent pairing with shock enhances response amplitude (Kublik, 2004). Such studies were not concerned with the specificity of plasticity and included neither nonassociative controls nor behavioral evidence of learning. However, the findings attested to the malleability of the vibrissal system in the adult, waking animal.

The specificity of plasticity, with nonassociative controls, has been studied in this system. The findings appear to be generally concordant with studies of auditory cortex. That is, tactile stimuli that gain behavioral significance as signals for reinforcement appear to gain cortical territory. For example, pairing the deflection of a row of mystacial vibrissae (CS) with a tail shock (US) in mice produces behavioral evidence of conditioning (e.g., postural changes and/or CS-elicited bradycardia). The following day, S1 was analyzed using with 2-DG mapping of functional activity. Conditioning sessions produced an increase of the functional representation in somatosensory cortex of the specific CS row of the whiskers stimulated during the training. The effect is associative, as it did not develop in pseudoconditioned mice or in animals that received only the CS. When training was discontinued, the enlargement of vibrissal representation returned to baseline. This loss of

specific associative plasticity could be accelerated by extinction training (CS alone) (Siucinska and Kossut, 1996).

As in the case of the auditory cortex, CS-specific increased representation is not limited to aversive reinforcement. Mice received pairing of whisker stimulation with a drop of sweet water. Cortical representations of rows of whiskers were mapped by 2-DG autoradiography after 3 days or 2 months of training. No quantified behavior was obtained, although the authors noted increased activity to the CS. In any event, conditioning resulted in enlargement of the cortical representation of vibrissae comprising the CS, compared with the contralateral representation of a row of whiskers that were not stimulated. Interestingly, the duration of training affects laminar plasticity. Three sessions increased the width of the 2-DG activity in supra (II/III) and infragranular (V/VI) layers, whereas the effects of 2 months of training were confined to layer IV. The changes were not observed in animals that received whisker stimulation alone or unpaired stimuli (Siucinska and Kossut, 2004).

Recently, Disterhoft and colleagues have used the well-studied conditioned eyeblink response to study associative changes in the representation of vibrissae in S1 (Galvez et al., 2006). Rabbits received deflection of a single row of whiskers (CS), followed by corneal air puff after a 500-ms trace period. Trace conditioning is known to require an intact hippocampus in contrast to delay conditioning (i.e., no intervening trace period) (Solomon et al., 1986; see also Beylin et al., 2001; Bangasser et al., 2006). The subjects developed conditioned responses over a period of 7–9 days, in contrast to pseudoconditioned rabbits. Barrels were identified by cytochrome oxidase staining and confirmed by single-unit electrophysiology. The authors found a specific expansion in the representation of the CS row of whiskers, compared both to the control animals and to the nontrained hemisphere, showing specific associative plasticity.

Matthew Diamond and coworkers have argued that, "if memories are stored within topographically organized sensory cortical areas, then access to those memories should be topographically distributed" (Harris et al., 2001b: 316). In one study, rats were trained on a gap-crossing task using the only whisker that had not be trimmed. After successful learning, the trained whisker was clipped and a prosthetic whisker attached to a different whisker stub. Transfer was tested by the rate of new learning, which was inversely related to the distance between the original and new whisker. Electrophysiological recordings revealed that the degree of overlap between cortical response patterns of two whiskers could account for the degree of transfer ($r = 0.98$) (Harris et al., 1999).

They also trained humans to use one fingertip to discriminate between two stimuli in the submodalities of vibration, punctate pressure, and roughness. Tests of posttraining generalization (transfer) to digits on the trained and untrained hands revealed no transfer for vibration but positive transfer for pressure and roughness discrimination as a function of topographic distance from the trained fingertip. The authors concluded that "tactile learning is organized within a somatotopic framework" (Harris et al., 2001a: 1056). Interestingly, Pavlov had predicted the existence of somatotopic cortical organization on the basis of similar training and generalization tests in dogs (Pavlov, 1927).

3.11.6.3 Primary Visual Cortex (V1)

In contrast to the primary auditory and somatosensory cortices, there appear to be no studies of the specificity of associative processes in the primary visual cortex. This is curious in light of the previous extensive investigation of V1 in classical and instrumental conditioning in animals during the period of 1935–1984. As noted earlier, most subsequent studies of V1 have focused on perceptual learning. Recent reports of classical conditioning in humans may indicate a resurrection of interest in associative learning. Functional magnetic resonance imaging (fMRI) has been used to investigate Pavlovian delay fear conditioning, using a blinking red light as the CS and shock as the US (Knight et al., 1999). Paired subjects exhibited a larger amount of active tissue in V1 compared to controls that received light and shock unpaired. However, the individual group effects were a decrease in response in the controls and no change in the paired group, suggesting that associative processes prevented habituation in this case. The absence of a behavioral measure of learning limits interpretations as well.

A further study of both delay and trace (10 s) conditioning has been more revealing. Using a discrimination protocol in which the three conditioned stimuli (CS+, CS+ trace, and CS−) were different colored shapes, the authors found significant behavioral learning and discrimination (development of conditioned galvanic skin responses, GSR) for both delay and trace protocols. Functional MRI responses

were larger to the CS+ versus CS− in delay conditioning and also larger to the CS+ trace versus the CS− in trace conditioning.

Perhaps similar studies will be conducted in animals using a stimulus dimension (e.g., line tilt) that could determine receptive field plasticity and thus reveal the extent of specificity of associative processes in V1. Given the earlier EEG and evoked potential studies, as well as the recent fMRI experiments, there is reason to expect that, like A1 and S1, V1 is specifically involved in associative learning and memory.

3.11.7 Do Lesions of PSCs Impair or Prevent Learning and Memory?

A frequently asked question (which I paraphrase) is, "As associative plasticity develops in PSCs, shouldn't lesions of PSCs impair or prevent conditioning? If not, why be interested in plasticity that would appear to be epiphenomenal?" The answer to the first question is based on studies of A1, for which the relevant findings are known. The simple answer is "No." It has long been known that simple (i.e., single-tone, nondiscriminative) classical conditioned responses can develop after ablation of A1 alone or as part of extensive cortical destruction (DiCara et al., 1970; Berntson et al., 1983; Romanski and LeDoux, 1992).

However, these questions are based on certain assumptions, which include (1) neurophysiological studies of conditioning seek stimulus–response (S-R) circuitry, (2) the memory trace formed is localized, (3) neurophysiological plasticity that develops is the substrate of the CR, and (4) destruction of the site of such plasticity should disrupt the CR (see Ohl and Scheich, 2004, vs. Weinberger, 2004a).

These assumptions may be relevant to learning a single, specific S-R relationship, such as learning to produce a conditioned eyeblink to a tone paired with air puff (Christian and Thompson, 2005). However, they do not apply to the vast majority of associative learning, which involves stimulus–stimulus (CS–US) links. First, classical conditioning transcends stimulus–response associations. Even S-R conditioning involves prior stimulus–stimulus (CS–US) associations. Moreover, other associations are invariably formed, such as between a stimulus and the location or general context within which learning occurs. Second, CS–US associations are known to develop rapidly, in a few trials, preceding CS–CR associations (Lavond et al., 1984), and therefore are likely be involved in the development of S–R links (Schlosberg, 1937; Mowrer, 1947; Konorski, 1967; Lennartz and Weinberger, 1992a). Third, multiple behavioral CRs develop during rapid CS–US learning (e.g., changes in heart rate, respiration, blood pressure, pupillary diameter, skin resistance, behavioral freezing), any one of which can be used to verify that a CS–US association has developed (of course, given appropriate nonassociative controls). Fourth, although its components may be localized, the total memory for a given event is almost certainly distributed across the cortex and other brain regions, including relevant sensory systems. Fifth, although circuitry for an association can be completely subcortical, the cerebral cortex also can store information in parallel with subcortical systems. Sixth, the cerebral cortex has access to a much greater range of information than the subcortex. It undoubtedly stores information that can be used in a highly flexible manner to subserve adaptive behaviors in an unknown future. For example, although simple auditory conditioning is not destroyed by lesions of A1, as soon as two-tone discrimination is demanded, A1 is required (Teich et al., 1988). A1 is also obligatory to achieve experimental extinction (Teich et al., 1989).

Thus, in response to the question posed at the outset of this section, PSCs can be profitably studied to determine the cortical fate of stimuli that enter into associations. In short, such studies of neurophysiological plasticity can be directed to issues of the representation of stimuli and the transformations, which they may develop during learning, without any reference to particular behaviors that index the establishment of stimulus–stimulus associations.

3.11.8 Memory and Retrieval in Primary Sensory Cortices

As primary sensory cortices develop highly specific representational plasticity in learning, they may be expected to exhibit memory. One such case has already been mentioned, that of a 2-month retention of CS-specific tuning shifts in A1 (Weinberger et al., 1993). Further, memory codes in PSCs have been proposed. Memory codes specify the relationship between an experience and the nature of its neural storage (e.g., the memory code for the acquired behavioral importance of a stimulus might be the number of cells that become tuned to that stimulus)

(Weinberger, 2001). We now summarize additional evidence of mnemonic processes in A1, S1, and V1.

3.11.8.1 Working and Reference Memory

If PSCs store at least some contents of memory, then they should hold that information for a period of time following presentation of a stimulus. There is evidence that the primary somatosensory cortex is involved in working memory. Monkeys trained in a haptic delayed matching to sample (DMTS) task (two different objects), with an 18-s delay period, exhibit sustained changes in activity of neurons in S1 (Koch and Fuster, 1989). Similar effects were also found in a haptic DMTS study in which objects were identical in shape but differed in surface features (Zhou and Fuster, 1996). Patterns of spike trains, not merely changes in unit firing, also are correlates of working memory in S1 (Bodner et al., 1998; Bodner et al., 2005).

Disruption of working memory in S1 also has been found. Humans were trained to discriminate the frequencies of two vibrations presented at an interval of 1500 ms. A pulse of transcranial magnetic stimulation (TMS) applied to the contralateral S1 early in the retention period of the first vibratory stimulus (600 ms or less) impaired discrimination performance. There was no effect when TMS was applied to the ipsilateral S1. The authors concluded that S1 serves as a temporary site of information storage (Harris et al., 2002).

Sakurai's laboratory has documented neural correlates of working memory and also long-term storage (reference memory) in A1. Rats were trained in a continuous nonmatching-to-sample working memory task to remember whether or not the current tone frequency was the same or different from the preceding tone. About 20% of single units in A1 (and the medial geniculate as well) developed sustained differential activity during the delay period after exposure to the sample tone, leading the authors to conclude that the thalamocortical auditory system retains auditory information in working memory (Sakurai, 1990). The task was then modified so that either working memory (WM) or reference memory (RM) could be tested in alternating fashion within a single session (Sakurai, 1992). Unit recordings were obtained from hippocampus CA1, CA3, dentate gyrus, and A1. Only the auditory cortex showed differential responses to the physical characteristics of the stimuli, low and high tones, respectively, in both WM and RM tasks. This itself is not surprising. Cells in the hippocampal formation exhibited changes in firing related to either WM or RM but not both. In contrast, neurons in A1 could exhibit increased activity for both the WM and RM tasks, indicating the flexible involvement of A1 in both working and reference memory (Sakurai, 1994). Further research using cross-correlations between pairs of neurons to detect cell assemblies revealed that most correlated pairs in the hippocampal formation occurred during WM, whereas correlated cells in A1 could participate equally in WM and RM (Sakurai, 1998). The results highlight the flexibility of the same cells in PSCs to participate in multiple functional networks.

3.11.8.2 Procedural Memory

There is at least one report that links PSC neural plasticity to procedural memory. Rabbits trained in eyeblink conditioning (tone/corneal air puff) developed tone-elicited neural responses in primary somatosensory cortex together with conditioned eyeblink responses. However on trials of CR failure, there was also an absence of S1 responses to the acoustic CS. It might be thought that both omissions were due to a failure to process the CS, perhaps in the lower auditory system. However, on such failure trials, the unconditioned response was still modulated by the CS, as indexed by more vigorous unconditioned responses, compared to unconditioned response–alone trials. Another explanation might be that the response in S1 represented sensory feedback from CRs, but its latency preceded CRs. The authors concluded that the response of S1 to the acoustic CS reflects the efferent copy of the memory of the response that was elicited by the CS from the cerebellum, which they view as a procedural memory trace (Wikgren et al., 2003).

3.11.8.3 Imagery

If cortical networks involved in memory storage and retrieval include PSCs, then they should reveal themselves in the absence of relevant sensory stimulation. That is, neural activation should occur when the prior sensory experiences are recalled. Although probably less widely accepted than some other approaches, studies of imagery in humans support such involvement. Imagery often is considered to be a higher cognitive function, but it seems most relevant to retrieval in the present context. Bearing in

mind caveats concerning precise localization and the need to validate the presumptive imagery behaviorally, there is evidence for the involvement of PSCs. For example, imaging studies have detected activation of V1 after subjects studied novel visual stimuli while they had to make decisions about visual details with their eyes closed. Further, the extent of activation of V1 was proportional to the size of the stimulus feature being remembered, supporting the view that imagery was taking place within the retinotopic map (Kosslyn et al., 1995, 1999).

Somatosensory imagery effects also have been reported. S1 was imaged in humans during the tapping of a finger. When subjects later imagined tapping the finger, the same region of S1 was activated (Porro et al., 1996). One cannot rule out undetected kinesthetic stimuli or subthreshold motor contributions to these findings, however. Studies of musical imagery are less subject to such problems. For example, imagery for musical timbre activates the primary auditory cortex with some right-side asymmetry, also present for timbre perception (Halpern et al., 2004; see also Halpern and Zatorre, 1999; Halpern, 2001; Zatorre and Halpern, 2005).

3.11.9 Nonsensory and Higher Cognitive Functions

In addition to associative processes, PSCs are also involved in nonsensory and higher cognitive processes. These are summarized alphabetically.

3.11.9.1 Attention

Attention has long been known to modulate sensory cortices. Although many effects are seen in higher areas, PSCs are also subject to selective attention, such as auditory (Alho, 1992), somatosensory (Johansen-Berg and Lloyd, 2000), and visual (Sengpiel and Hubener, 1999). However until recently, studies of attention had not been able to demonstrate the extreme specificity of rapid attentive modulation in PSCs. Fritz, Shamma, and colleagues devised a clever and sensitive method of obtaining spectrotemporal receptive fields (STRFs) while ferrets waited to detect a previously learned tone to avoid shock. They found that attention modulates primary auditory cortex by facilitating responses to the target frequency while suppressing responses to other frequencies (Fritz et al., 2003). When trained in both frequency detection and frequency discrimination tasks, the reinforced and target frequencies were enhanced, as might be expected, but because the target during tone detection (CS+) could be the nonreinforced (CS−) frequency during discrimination, the authors were able to show that responses to the same physical stimulus could be facilitated or suppressed depending on the task (Fritz et al., 2005b). In a third study, ferrets learned both tone detection and gap detection tasks. As expected, tone detection had the same target-specific enhancement. Additionally, during gap detection, the STRF was changed along the temporal dimension, specifically, the temporal dynamics of discharge were sharpened (Fritz et al., 2005a). In all the studies, the effects could last for hours in some cases, suggesting an involvement in memory as well as in selective attention. Overall, the findings demonstrate that attention has a strong influence on A1. At least some of this plasticity is due to top-down processes because switching tasks, and therefore the significance of a particular frequency, differentially affects responses to the same physical stimulus. The findings also highlight the ability of the same cells to participate in multiple adaptive networks. Of course, RF shifts in learning could be the result of top-down, bottom-up, or a combination of these influences.

3.11.9.2 Category Learning and Concept Formation

Perceptual category formation involves grouping sensory stimuli by abstract relationships based on some aspect of similar physical attributes. Ohl et al. (2001) trained rats to form the categories of rising and falling frequency modulation of tones (i.e., independent of their absolute frequencies). They detected category learning by a sudden change in learning strategy. Recordings from A1 revealed that the transition to category formation was correlated with the emergence of patterns of stimulus representation in the EEG in which frequency-modulated tones are distinguished into the categories of rising and falling modulation. The authors believe that the electrographic plasticity reflects the abstraction that defines these categories (Ohl et al., 2001). Categorical effects have also been observed in monkeys trained for more than 2 years to classify steps in acoustic frequency (Selezneva et al., 2006). The authors found that phasic activity of cells in A1 indicates the direction of frequency steps, whereas slow changes in activity are tied to procedures involved in solving the task.

A recent related experiment extends the involvement of auditory cortex to humans. Subjects were required to classify sound on the basis of either the direction of change (rising or falling frequency modulation) or the duration of stimulation (short or long). fMRI revealed activation in the right auditory cortex for categorization by direction of change, whereas categorization by duration of stimulation activated the left auditory cortex (Brechmann and Scheich, 2005). The hemispheric specializations for the two types of stimulus parameters are consistent with prior studies of the human auditory cortex, but the linkage of the findings to categorization, rather than to the physical parameters of the stimuli *per se,* demonstrates that the human auditory cortex is involved in higher cognitive processes.

3.11.9.3 Cross-Modality Effects

Associative involvement of PSCs is also evident in cross-modal effects, consisting of a stimulus in one sensory modality being able to elicit responses in the PSC of another sensory modality. Indeed, cross-modality effects are so numerous that their significance is generally overlooked. They began with the EEG studies of conditioning. For example, the development of the ability of the click of a camera shutter to block alpha waves in the human occipital cortex when it preceded a flashing light US is evidence for cross-modal effects, although in this serendipitous seminal study, the authors could not have detected actual auditory evoked responses in visual cortex (Durup and Fessard, 1935). In animal studies of conditioning, the ability of the CS to elicit EEG activation in the PSC of the US is similarly an example of cross-modality effects. Beyond EEG findings, direct studies of evoked sensory responses consistently documented the development of CS-elicited responses in the PSC of the US (Oleson et al., 1975).

Recent studies reinforce these earlier findings. For example, studies in which primates are cued to perform a tactile discrimination by an auditory or visual stimulus found the development of responses to the auditory (Zhou and Fuster, 2004) and visual cues (Zhou and Fuster, 1997, 2000), respectively, in the primary somatosensory cortex. The reverse relationships also exist. For example, monkeys were trained to perform a complex auditory discrimination. Following presentation of a cue light, they could initiate a sequence of tones by pressing a bar. Many neurons in A1 developed responses elicited by the bar press, of course, prior to the presentation of the sounds. And given the prevalence of cross-modality neural responses, one would predict that presentation of the cue light also would elicit responses in A1, and this also was observed (Brosch et al., 2004).

Similar effects are reported in A1 of humans. Several studies have linked the primary auditory cortex of humans to speech in the absence of sound. Thus, the presentation of visual stimuli associated with language sounds, whether the sight of a letter or of silent speech, elicits neural activity in the primary auditory cortex (Sams et al., 1991; van Atteveldt et al., 2004; Pekkola et al., 2005; Ruytjens et al., 2006). This cross-modal effect might derive from earlier associative learning, as the sight of a face, especially the lips, during speech is highly correlated with hearing the emitted speech. Some effects are not limited to putative associations. Visual stimuli can be transformed into a phonological code. Apparently, the left primary auditory cortex is activated during such recoding (Suchan et al., 2006).

A recent anatomical study in the gerbil may provide an anatomical basis for some cross-modality effects. The authors found a surprisingly large number of inputs to A1 from nonauditory regions of both the cortex and the thalamus. They argue against the view that primary sensory cortices are unimodal (Budinger et al., 2006).

3.11.9.4 Expectancy, Preparatory Set

Subjects previously trained on a task form expectancies or preparatory sets that are based on the probability that a certain stimulus or event is likely to occur. Although generally regarded as a higher cognitive function, they are manifest in PSCs. In one such case, rats were trained in a visual reaction time task with a very brief (10-ms) warning tone, 1.4 s preceding the light stimulus. A subset of single neurons in A1 developed a significant sustained increase in discharge rate during the warning period that did not occur when the same warning stimulus was given by itself. The authors suggested that this activity constitutes a substrate of preparatory set (Shinba et al., 1995). A phasic increase in arousal might have been responsible for increased cellular activity, as preparatory set and expectancy often involves increased arousal. Direct measures of arousal level and recordings taken in arousing situations outside the task would help resolve this issue.

A more detailed study by Villa and coworkers provides compelling evidence for an expectancy function in A1 (Villa et al., 1998). They obtained

simultaneous single-unit spike trains while rats performed a complex cognitive task, specifically a two-choice task (Go/NoGo) with a two-component (pitch and location) auditory stimulus lasting 500 ms. They observed that functional interactions (cross-correlations) are dynamically modified in the waiting period preceding the onset of auditory stimulation. Further, they found spatiotemporal firing patterns both within and across spike trains several seconds before the actual stimulus delivery. These patterns have a very precise repetition of spike discharges separated by long intervals (up to several hundreds of milliseconds) in the absence of a change in mean rate. The authors suggested that network activity in A1 reflects "participation of recurrent neuronal networks in processes anticipating the expected sensory input" (Villa et al., 1998: 269).

3.11.9.5 Motivation, Behavioral Importance

Several studies have found plasticity in PSCs based on motivational level or the acquired behavioral importance of sensory stimuli. In a complex appetitive instrumental task, rats were trained in three phases, and the amount of c-Fos expression was determined in different subgroups at the end of each phase: (1) tone–food association, (2) two-tone discrimination, and (3) two-tone discrimination contingent on location of the sound source. Auditory stimuli were bursts of complex sounds lasting 500 ms. Compared to various control groups, successful animals exhibited no difference in phase 1 but had significantly greater cFos activity in primary auditory cortex during the next two phases. No subcortical auditory structures (cochlea through medial geniculate) differed from controls (Carretta et al., 1999). The authors concluded that auditory cortex is involved in the coding of stimulus significance. Of note, their failure to find an effect after simple tone–food association suggests that not all associative learning involves PSC plasticity. Moreover, it is clear that more complex learning (i.e., discrimination problems) is more likely to engage a PSC.

Although the previous study neither manipulated the level of motivation nor determined specificity of plasticity, a recent experiment addressed these issues directly. Rats were trained to bar press for water contingent on the presence of a tone (6.0 kHz), each at different levels of water deprivation. Terminal mapping of A1 revealed a specific increase in area of representation of the CS frequency in the tonotopic map. More importantly, the amount of area was directly proportional to the level of correct performance, itself controlled by the level of motivation for water (**Figure 4**) (Rutkowski and Weinberger, 2005). These findings were predicted by the memory code hypothesis that the level of behavioral significance of a stimulus is encoded by the increase in the number of cells that become tuned to that stimulus (Weinberger, 2001).

The primary visual cortex has been studied during associative learning only rarely during the past 30 years. However, V1 has now been linked to reward processes. Rats wearing goggles that provided controlled stimulation to either eye were trained to associate whole-field illumination with water reward. Many neurons in V1 that originally responded only to the visual stimuli developed discharges that accurately predicted the timing of the reward (Shuler and Bear, 2006).

There is evidence of motivational effects in the barrel field of S1. Rats were studied under conditions of (1) searching for an object with their vibrissae for a food reward, (2) whisking in the air for the goal of returning to the home cage, or (3) whisking in the absence of reward (Ganguly and Kleinfeld, 2004). The amount of phase-locking of neurons in S1 was greatly increased when whisking resulted in reward compared to whisking without reward.

3.11.9.6 Learning Strategy

The strategies used to solve problems are significant factors in human cognition. However, the role of learning strategy has been neglected in studies of learning and brain plasticity. A recent study reveals that learning strategy can be decisive in the development of specific plasticity in the primary auditory cortex. Two groups of animals were trained to solve the same problem, achieve the same asymptotic level of performance, and exhibit the same degree of learning about absolute frequency, but accomplish these goals using two different learning strategies. Specifically, adult male rats were trained to bar-press for water contingent on the presence of a 5.0-kHz tone. A bar-press response made during the silent intertrial interval resulted in a time out (i.e., 3–7 s lengthening of time to the next trial) signaled by a flashing cue light. This apparently simple problem can be solved two ways: 'bar-press during tone presence' ('tone duration strategy') or 'bar-press from tone onset until receiving the error signal' ('onset-error strategy'). However, these alternatives cannot

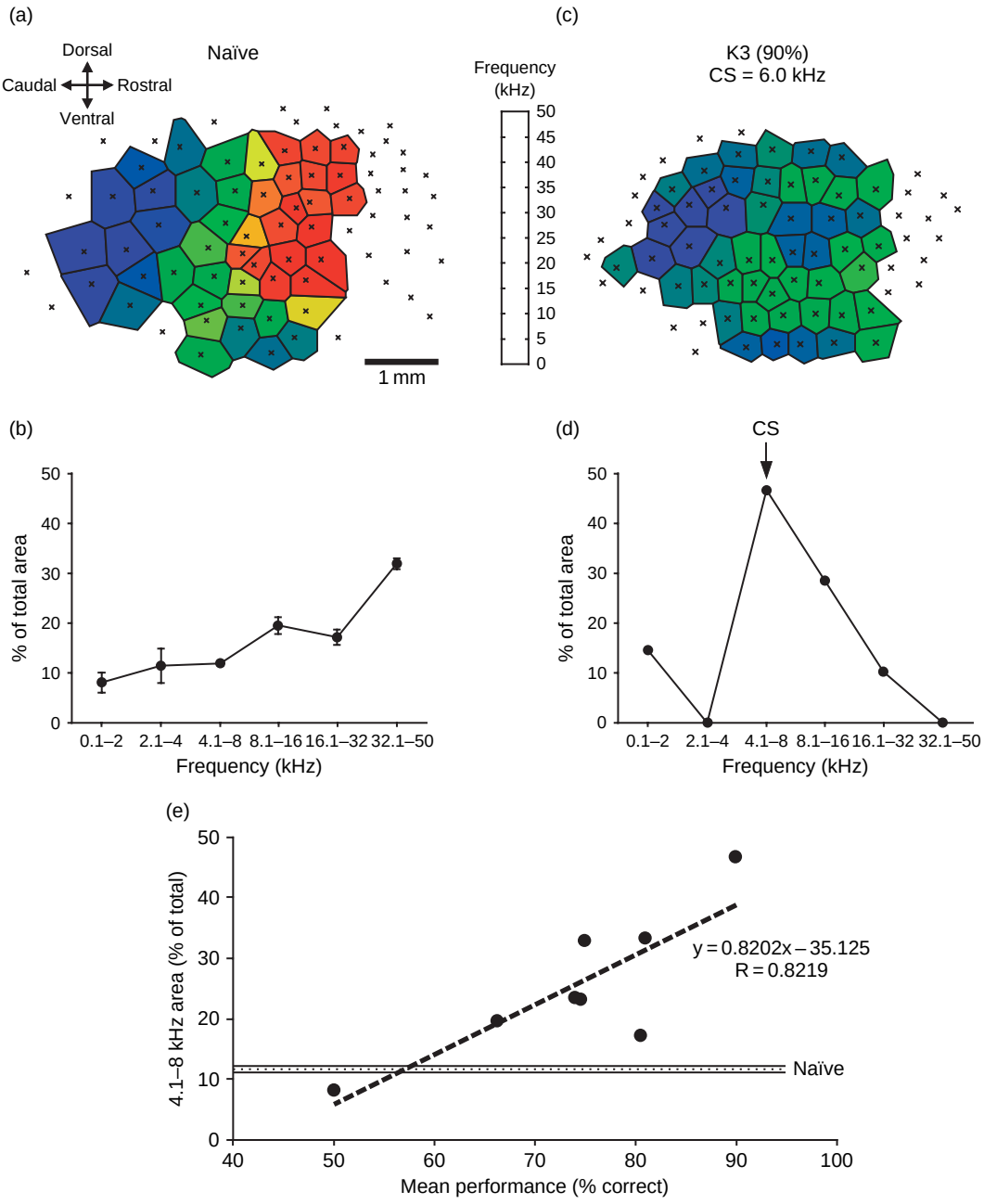

Figure 4 Motivational effects on area of frequency representation in tonotopic map of A1. Trained rats received water reward for bar-presses in the presence of a 6.0-kHz tone. (a). The tonotopic maps of a naive rat. (b). Quantification of the percent of total area for each six octave bands for the map above. (c). The tonotopic map of a subject that attained over 90% correct performance. (d). The corresponding quantification. Note that the band containing the CS frequency is much greater (~50% vs. ~10% in the naive). (e). Evidence of a memory code for the acquired behavioral importance of sound. Level of tone importance was controlled by the motivation for water (amount of water deprivation). Asymptotic performance was significantly correlated with motivation level. The area of representation of the frequency band containing the 6.0-kHz tone signal increases as a direct function of the level of behavioral importance of the tone, as operationally indexed by the level of correct performance. For details, see Rutkowski RG, and Weinberger NM (2005) Encoding of learned importance of sound by magnitude of representational area in primary auditory cortex. *Proc. Natl. Acad. Sci. USA* 102: 12664–13669.

be distinguished because error signals are always given for responses made starting immediately after tone offset. Therefore, although one group was trained in the standard manner, a second group was given a 2-s grace period starting at tone offset, during which bar-presses did not produce the error signal (or water reward). Evidence for the 'onset-error strategy' would be continued bar-pressing after tone-offset during the grace period.

Both groups achieved the same high levels of correct performance, and both groups revealed equivalent learning of absolute frequency during training. Analysis of behavior showed that the second group did indeed use the 'onset-error strategy.' Posttraining 'mapping' of the auditory cortex revealed that this group alone developed specific plasticity. Threshold was decreased ~10 dB, and tuning bandwidth was narrowed by ~0.7 octaves. Moreover, this plasticity was restricted to the frequency band of the 5.0-kHz tone cue. The findings show both that auditory learning can develop without plasticity in threshold or bandwidth and, perhaps more important, that learning strategy – not learning performance – can determine cortical plasticity (Berlau and Weinberger, 2008).

3.11.9.7 Motor Processes

It might seem most unlikely that primary sensory cortices could be intimately linked to motor functions, but there is clear evidence of motor involvement. Within A1, patterns of neuronal activity constituted predictive neural correlates of later motor behavior. Rats were trained in a Go/NoGo task to distinguish combinations of two frequency-modulated sounds (based on low- [3.0 kHz] or high- [12.0 kHz] base frequencies) and two speaker locations (left or right). They were rewarded for correct Go and NoGo behavior (e.g., Go to low tone on left, NoGo to high tones simultaneously from left and right). Single-unit discharges were recorded simultaneously from up to 15 neurons while animals waited for the auditory cues. Using a pattern-detection algorithm, the authors detected 235 reliable spatiotemporal patterns of activity, 55% of which were significantly related to the type of response that the animal made several seconds later. Patterns predictive of Go and NoGo behaviors were about equal in number. Note that the predictive correlates were not elicited by the acoustic stimuli, which were delivered after the waiting period (by definition). Of particular interest, mean discharge rates did not vary, emphasizing the importance of the analysis of temporal parameters of cellular discharge. The patterns in A1 might be parts of motor programs that are engaged in auditory tasks (Villa et al., 1999).

3.11.10 Concerning the Direction of Plasticity

Heretofore, we have not focused on the direction or sign of changes in the magnitude of PSC plasticity. (Plasticity of temporal aspects of cellular discharge may be equally important but has not yet been studied extensively in PSCs during learning; see Edeline, 1999.) The dominant, almost exclusive, finding in conditioning has been that stimuli that gain behavioral importance become facilitated, as indexed by the fact that they come to elicit increased magnitude of response. (Of course, memory in habituation is indexed by a decrease in response magnitude.) Larger responses could be advantageous by increasing the signal-to-noise ratio when the task at hand is stimulus detection. In many cases, increased response involves CS-specific tuning shifts and increased area of representation, indicative of the recruitment of more cells to the CS or other important stimulus. As noted previously, the amount of increased area of representation is directly proportional to the level of behavioral importance of a tone (Rutkowski and Weinberger, 2005). More cells could also increase the probability of attention to stimuli whose behavioral significance has increased due to learning. The strength of memory might be an increasing function of the number of cells that preferentially involved the sensory experience. The favored processing of important stimuli by more cells could also increase the probability of complete processing in an unknown future, including one of neuropathology. In the latter case, more cells could serve the function of a safety net. Thus, increased response magnitude would seem to make good functional sense. If associative processes produced decreased responses or reduced the number of cells tuned to an important stimulus, in the limit to zero, then the stimulus in question would seem to decrease, or lose, its ability to be stored and to guide current and future behavior.

But increased magnitude of response is undoubtedly only part of the complete story of PSC plasticity. Observations from studies involving naturalistic environmental situations indicate that specific decreased responses are the likely outcome of at least some

experiences (for review, see Frostig, 2006). For example, many formulations of PSC plasticity are based on a competition model, in which stimuli that produce more afferent input win (i.e., elicit larger responses and/or gain control of more cells). This outcome is prevalent in the whisker barrel field of S1 for surviving whiskers when others are clipped (Armstrong-James et al., 1994; Diamond et al., 1993). However, when the vibrissae are not altered, the representation of a whisker is smaller during active exploratory whisking than during quiet wakefulness (Fanselow and Nicolelis, 1999). Spared whiskers exhibit reduced representation when rats are permitted to explore for brief periods outside their home cages (Polley et al., 1999), and the same occurs for the intact vibrissal system when animals live for weeks in a naturalistic habitat, including tunneling, which makes increased demands on the use of whiskers (Polley et al., 2004a). Behavioral arousal, expected during active whisking, produces reduced representation coupled with increased synchronization of firing, resulting in sharper receptive fields that could subserve increased discrimination (Castro-Alamancos, 2004).

These studies did not involve explicit associative learning. However, one cannot discount the possibility of associative learning during naturalistic whisking. That is, an episode of whisking involves numerous instances of stimulus–stimulus relationships (e.g., successive tactile stimuli constituting within-modality sensory preconditioning). However, naturalistic situations are not optimal for determining fine-grain stimulus relationships. The trade-offs between increased ecological relevance in field or fieldlike studies and decreased manipulation of stimulus relationships are well known.

Exploratory whisking may also be related to .-perceptual learning, as animals have had uncountable trials of experience, discriminating objects by use of their vibrissae. The possibility that decreased responses reflect mechanisms underlying perceptual learning is supported by a recent study of frequency discrimination in A1 of the cat. Decreased responses were found for frequencies between the CS+ and CS−, during extended tonal perceptual learning. The authors suggest that such plasticity may facilitate the classification of stimuli with different behavioral outcomes by reducing similarity between reinforced and nonreinforced stimuli (Witte and Kipke, 2005). This finding contrasts with rapid two-tone discriminative learning (i.e., a single training session), in which responses to the CS+ are increased while responses to the CS−, pretraining BF, and other frequencies are reduced, the effects becoming even more pronounced during a subsequent silent period of consolidation (Edeline and Weinberger, 1993).

In summary, rapid associative learning appears to be dominated at present by findings of increased responses to behaviorally important stimuli. In contrast, extensive discrimination learning, particularly formal learning of increasingly difficult discriminations (i.e., perceptual learning), may involve reduced response magnitude with concomitant increased precision of receptive fields. However, a comprehensive understanding of the sign of plasticity must await further research of the relationships between the direction of PSC plasticity and its functional and behavioral significance.

3.11.11 Implications

The primary auditory, somatosensory, and visual cortices develop associative plasticity during behaviorally validated learning. They exhibit evidence of the storage of memory, including procedural, working, and reference memory, the latter validated for an indefinite period of at least months after training. Recent studies have revealed a high degree of specificity of plasticity in A1 and S1 (appropriate studies of V1 have not been conducted), including a remodeling of cortical processing that emphasizes responses to signal stimuli of acquired behavioral importance and indicative of the storage of discrete details of experience. Moreover, A1, S1, and V1 are clearly involved in a number of higher processes including attention, expectancy/preparatory set, category learning/concept formation, cross-modality interactions, imagery, learning strategy, motivation, and the degree of acquired behavioral importance of stimuli and motor actions.

This range of involved functions amply documents the extensive engagement of PSCs in adaptive cognitive and behavioral processes that far transcend their presumptive normative sensory functions. Space permits brief consideration of four implications.

First, primary sensory cortex, as commonly functionally understood, does not exist. There is probably no region of the cerebral cortex that is devoted only to the analysis of sensory stimuli; certainly, A1, S1, and V1 are not so limited. It follows that the responses of all neurons in these PSCs are almost certainly affected both by nonsensory processes (such as the acquired meaning of a stimulus) and the physical parameters of modality-specific stimuli as commonly studied in sensory neurophysiology. The caveat is that the cell type

that generates each type of neural response cannot yet be known, a task that may require many years to complete. So it is possible that some types of PSC cells respond only to the physical parameters of stimuli but so far have escaped detection.

Second, the developing concept that information is stored where it is processed, which attributes mnemonic functions to PSCs (as well as myriad other cerebral structures) may be correct but highly incomplete. For example, concept and category formation deal with abstract properties of stimuli, not merely their root physical parameters. Therefore, PSCs appear to serve supra-stimulus functions.

Third, and a related point, because the degree of plasticity reflects the level of acquired behavioral importance (Rutkowski and Weinberger, 2005), motivational information that is presumably not assessed in PSCs is nonetheless represented therein (see also Shuler and Bear, 2006). Therefore, PSCs appear to serve extrastimulus functions.

Fourth, normative concepts of cortical organization need to be revised. Because of specific, associative plasticity in PSCs, they cannot be distinguished from other cortical regions on the basis of sensory versus associative functions. Campbell was wrong, and all theories based on such a distinction are obsolete. Formulation of new, testable organizational principles is perhaps the major task facing the integrative neurobiology of the cerebral cortex.

3.11.12 Toward a New Functional Architecture of the Cerebral Cortex

Kuhn's analysis of scientific revolutions holds that extant but outmoded paradigms are not discarded until a new paradigm accounts both for old facts and newer, inharmonious findings (Kuhn, 1970). The issue of new principles for the functional organization of the cerebral cortex is far beyond the capabilities of this chapter. However, as its major point has been to show that primary sensory cortices are involved much more broadly in learning, memory, cognition, and adaptive behavior than generally recognized, one approach to thinking anew about cortical organization is to consider PSCs from a different point of view. With that in mind, I suggest the following.

First, the sensory specialization of each PSC must be retained. Regardless of cross-modality influences, V1 is specialized for vision, S1 for somatic sensation, and A1 for audition. But if they are not purely sensory, then what are they?

Second, I propose that PSCs deal with their modality-specific information in the context of solving whatever problem, challenge, or opportunity faces the organism. We may regard them as continually assembling components to form situational functional systems (see also Anokhin, 1974).

Thus, PSCs are reconceptualized as having three types of function: (1) modality-specific sensory, (2) learning/memory, and (3) broadly integrative with respect to modality-dominated problem solving.

For example, if a specific motor act is required with respect to acoustic stimulation, then the auditory cortex integrates (unknown aspects of) motor function with the analysis, acquisition, and storage of information about the relevant sound. The result would not be confined to auditory information but, rather, auditory information combined with relevant motor and spatial information for the situation in question. This may explain why selective lesions of frequency bands within the tonotopic map of A1 in the cat produce selective impairment of locomotion to the source of the corresponding sound frequency (Jenkins and Merzenich, 1984).

In no case would A1, S1, or V1 execute the requisite behavioral act. Nor would they assess or determine the motivational state or determine the nature goal object, such as food, water, or opportunity for sex. Nonetheless, they would be major sites in which the relevant information is brought together. PSCs would not be the only sensory cortical sites because belt and higher sensory regions would be involved as well. In fact, the manner in which all PSCs and their related higher fields work together, with both feedforward and feedback interactions, remains a central problem for cortical function.

As animals face many problems, including gathering information without the need for immediate effector action (overt behavior), it follows that their PSCs must be able both to store a multiplicity of relevant modality-specific information while also being able to rapidly organize and mobilize the appropriate information as environmental situations, challenges, and opportunities appear – often rapidly. The available data do indicate that the same PSC cells not only participate in multiple behaviorally relevant networks but also can rapidly switch mode as the task changes (Sakurai, 1994, 1998; Fritz et al., 2005a,b; see also Gilbert et al., 2001).

We currently have little information about multifunctional aspects of PSCs because the issue has seldom been broached and so few appropriate experiments have been conducted. Fortunately, their

involvement in learning and memory is now on such solid ground that it can provide a foundation for further inquiry about other cognitive processes. Although learning-induced plasticity is now widely accepted, it is often contrasted with much greater plasticity during development, the adult animal being regarded as retaining some developmental plasticity. However, instead of regarding associative plasticity as an add-on to sensory processing, the default position probably should be that PSCs store information as a normal part of their functions.

Clearly, this liberalized view of PSCs is preliminary and really little more than an outline of an idea. However, even in its nascent state, it offers an alternative to the traditional sensory role of primary auditory, somatosensory, and visual cortices. If it does nothing more than provoke thought and reaction, it will have served a beneficial purpose. However, I believe that although further characterization of PSCs is needed, it is now time to seek the functional principles governing the involvement of A1, S1, and V1 in learning, memory, and a multiplicity of nonsensory processes.

We may currently be as explorers in a strange land, approaching a barely discernible, yet clearly existing, mountain range. At present, details cannot be seen, and perhaps different peaks can barely be distinguished, but by continuing the journey, the detailed topography will be discovered eventually, as well as the inner geology. Perhaps we truly have only a dim view of the functional principles of the cerebral cortex. The next steps should be revealing, but they require a willingness to travel from the comfort of the conceptually comfortable to the extreme discomfort of a landscape fraught with unknown and perhaps strange hazards.

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3.12 Visual Priming

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3.12.1 Introduction

A fundamental property of the brain that distinguishes it from artificially constructed computational devices is its ability to continuously update its functional properties based on prior experience. This property, plasticity, is apparent in many forms of learning and memory in humans. One important manifestation of plasticity in the brain is priming: the behavioral phenomenon of improved processing of a stimulus following prior experience. Priming typically manifests as increased accuracy and/or faster speed in making judgments on a stimulus that has been previously encountered (**Figure 1**). It is thought to reflect an implicit form of memory and learning, as it does not involve explicit memory of the prior experience.

This chapter is concerned with visual priming (priming related to presentation of visual stimuli) and the neural correlates underlying this phenomena. Visual priming is one of the most ubiquitous manifestations of priming and has been extensively studied in many levels from the behavioral level to the neural level in both humans and animals. Thus, visual priming is an excellent model to study plasticity in the visual system and its relation to object perception. Studying the neural mechanisms of visual priming is important because it enables understanding the neural bases of cortical representations as well as the mechanisms involved in rapid implicit learning. In particular, recent interest for understanding priming and its neural correlates has been heightened as an increasing number of scientists use priming methods to characterize representations in the human brain.

This chapter is organized into three main sections: it begins with a review of the behavioral aspects of visual priming, then examines neuroimaging experiments of the neural correlates of priming, and concludes with a theoretical overview of three models that have been recently suggested for explaining the neural bases of priming.

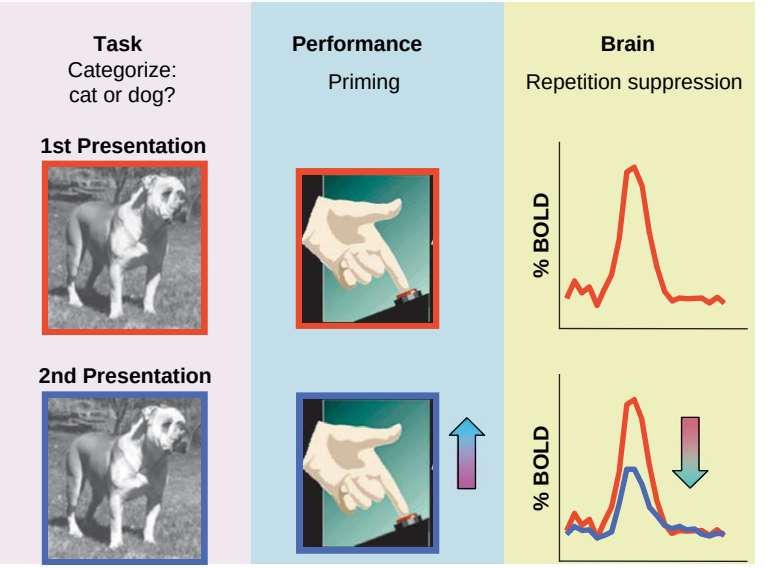

Figure 1 Schematic illustration of priming and repetition suppression/adaptation. *Left:* subjects are asked to perform a task, for example, classify an object. *Middle:* performance is measured on the first presentation and subsequent presentations of the same stimulus. During repeated presentations (e.g., the second presentation), performance improves (i.e., priming), as indicated by the arrow. Typically, accuracy increases and response time decreases. *Right:* Brain activity in object-selective cortex measured during the same experimental conditions shows lower BOLD responses for repeated presentations (blue) of the object compared to the initial presentation (red).

3.12.2 Behavioral Aspects of Visual Priming

In a typical priming experiment, subjects are shown an initial stimulus (prime) and are required to make a decision (e.g., categorize the stimulus; **Figure 1**) or produce a response (generate a word) on a subsequent stimulus (test) that is identical or related to the initial stimulus (e.g., the same object in different views, or a new object that is related perceptually, conceptually, or semantically to the prime). The priming effect (i.e., improvement in performance) is largest when the repeated stimulus is identical to the initial stimulus (prime). In some behavioral paradigms of priming, many intervening stimuli occur between the test and the prime. However, in other paradigms, the test immediately follows the prime. One particular striking aspect of priming is that it can be manifested after a single exposure to an object and is preserved in timescales ranging from seconds to even an year (Cave, 1997).

The level of priming is modulated by several factors such as the number of stimulus repetitions, the number of intervening stimuli, and the time between repeats (**Figure 2(a)**). The magnitude of response time (RT) priming increases with the number of stimulus repetitions both in short timescales (seconds/minutes) and in longer timescales (days and weeks), and this advantage remains over week-long delays compared to single exposures of stimuli (Brown et al., 1996). Similarly, RT priming is largest when there are no intervening stimuli between the prime and the test stimulus and when the temporal interval between them is shortest (**Figure 2(a)**). Thus, immediate repetitions produce a larger priming effect compared to when repetitions occur after several minutes or days (van Turennout et al., 2000; Sayres and Grill-Spector, 2006). Interestingly, the graded nature of priming is reliable even in patients who are unable to remember the stimuli or judge the frequency of these stimuli in explicit tests (Wiggs et al., 1997).

Another important aspect of visual priming is its specificity, because visual priming has been used as an experimental tool to infer the characteristics of object representations. Visual priming is preserved even when the appearance of objects changes across repetitions. Visual priming is invariant to changes in object size (Cooper et al., 1992; Fiser and Biederman, 1995), position (Cooper et al., 1992), color (Cave et al., 1996), symmetry (Fiser and Biederman, 2001), and to some degree the viewing angle of the object (Biederman and Bar, 1999; Biederman, 2000). However, a recent experiment suggests that there

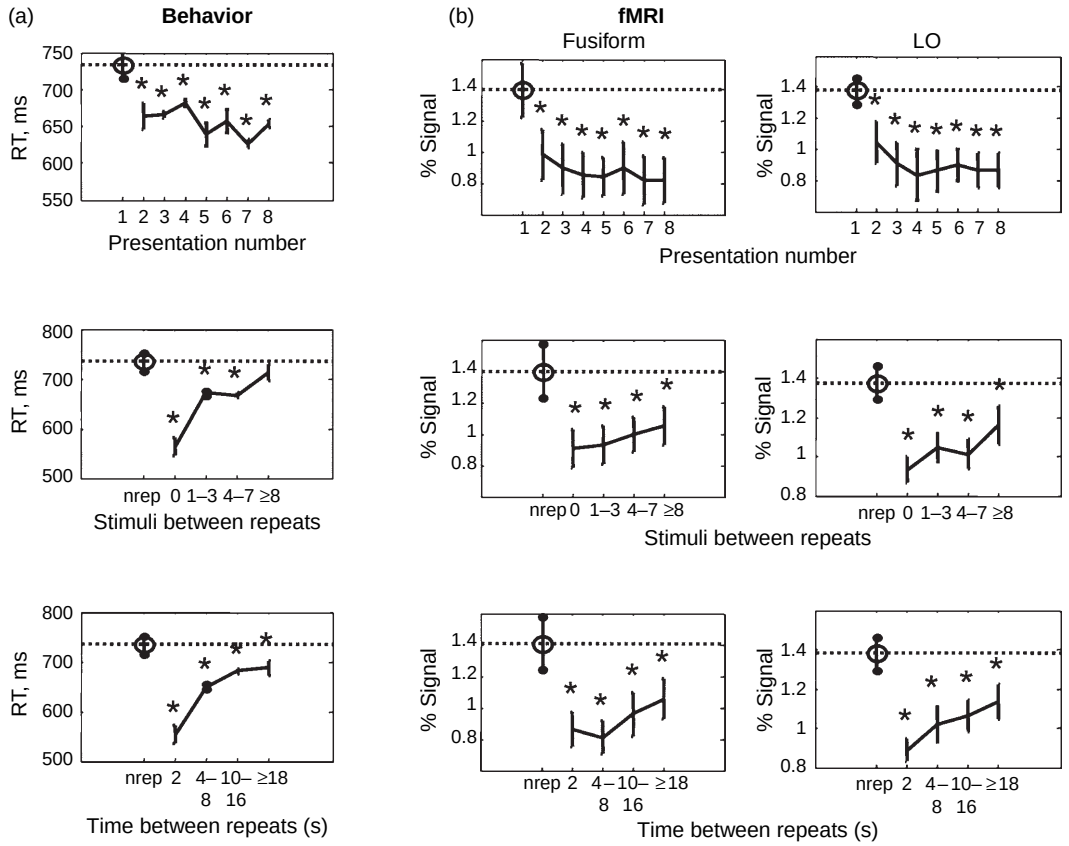

Figure 2 Effect of repetition parameters on priming and repetition suppression in object-selective cortex as measured with functional magnetic resonance imaging (fMRI). (a) Mean response time. (b) BOLD response amplitude for object-selective regions in the fusiform gyrus (middle) and lateral occipitus (LO) (right). Error bars indicate SEM across eight subjects. BOLD responses are averaged across hemispheres. Asterisks indicate significantly lower than first presentation ($p < .05$). Dashed line: response to the first presentation. *Top:* sorting by presentation number. *Middle:* sorting by intervening stimuli between repeats. *Bottom:* sorting by time between repeats. Adapted from Sayres R and Grill-Spector K (2006) Object-selective cortex exhibits performance-independent repetition suppression. *J. Neurophysiol.* 95: 995–1007.

may be an interaction between the effects of shape and location on visual priming (Newell et al., 2005). Further, visual priming is diminished but still preserved for new exemplars from the same category (e.g., upright piano vs. grand piano). Experiments in which subjects viewed stimuli that were presented either in the right or left visual field suggest differential priming effects across the left and right hemispheres; priming effects show higher specificity when stimuli are presented to the left visual field, as they do not generalize across object rotation and exemplars of a category, whereas priming effects generalize across exemplars and rotation when stimuli are presented to the right visual field (Marsolek, 1995; Burgund and Marsolek, 2000; Koutstaal et al., 2001; Vuilleumier et al., 2002; Simons et al., 2003). These experiments have led to suggestions that object representations in the right hemisphere are more specific than left-hemisphere representations, which may be more abstract in nature (Marsolek, 1995; Burgund and Marsolek, 2000; Koutstaal et al., 2001; Vuilleumier et al., 2002; Simons et al., 2003).

Several lines of research suggest that visual priming is an implicit form of learning and memory. A particularly important finding is that priming occurs in amnesic patients even though they are unaware of prior exposure to the primed stimulus (Tulving et al., 1991; Hamann and Squire, 1997) and they are significantly impaired on explicit tests (such as recognition memory, recall, and recollection of contextual information) on the same stimuli (Hamann and Squire, 1997).

Another striking aspect of priming is subliminal priming (Dehaene et al., 1998; Bar and Biederman,

1999; Naccache and Dehaene, 2001), that is, priming without awareness of the content of the priming stimulus. For example, Bar and colleagues (Bar and Biederman, 1999) showed subjects briefly presented stimuli (average 47 ms) that were masked. Subjects' naming performance on these stimuli was low (~14%). However, when the same stimuli were presented for the second time, naming performance on primed stimuli significantly increased (to about 35%). Subliminal priming may show higher specificity than suprathreshold priming, as it generalizes only to objects presented in the same hemifield. Therefore, Bar and colleagues have suggested that subliminal priming may be mediated by neural mechanisms distinct from suprathreshold priming. Other priming experiments of briefly presented masked stimuli show that the magnitude of priming is larger for the specific items that were primed compared with other exemplars of the category (Furmanski and Engel, 2000; Grill-Spector et al., 2000), and that priming effects increase across days and repeated exposures (Grill-Spector et al., 2000) and generalize across object size (Furmanski and Engel, 2000). Further, experiments of subliminal priming of words show generalization of priming effects across fonts and letter size (Naccache and Dehaene, 2001).

Overall, evidence from amnesic patients and subliminal priming experiments suggests that awareness may not be necessary for priming. These experiments lead to the prevailing theory that posits that priming reflects an implicit form of memory that is distinct from explicit memory and that relies on distinct neural and cognitive mechanisms.

3.12.3 Neural Correlates of Priming

Many studies have investigated the neural correlates of priming in humans using functional magnetic resonance imaging (fMRI), electroencephalography (EEG), and magnetoencephalography (MEG). Under experimental situations similar to behavioral paradigms of visual priming, the most robust and consistent finding with fMRI is reduced brain activations to repeated presentations of a stimulus relative to the initial presentation of that stimulus (**Figure 3**). This reduction has been referred to as repetition suppression (RS), fMRI-adaptation (Sobotka and Ringo, 1994; Ringo, 1996; Grill-Spector and Malach, 2001), mnemonic filtering (Miller et al., 1993), repetition suppression (Desimone, 1996), decremental responses (Brown and Xiang, 1998), and neural priming (Maccotta and Buckner, 2004). We use RS to refer to decreased neural responses following stimulus repetition. However, it remains mysterious how reduced cortical responses provide for improved performance. Further, although the most ubiquitous cortical phenomenon related to stimulus repetition is reduced responses, in some cases there is evidence for increased responses with stimulus repetition or repetition enhancement (RE) (Dolan et al., 1997; George et al., 1999; Grill-Spector et al., 2000; Henson et al., 2000; James et al., 2000; Kourtzi et al., 2005; James and Gauthier, 2006; Turk-Browne et al., 2006). We consider both phenomena and their relation to priming in turn.

3.12.3.1 Repetition Suppression

When stimuli are repeated, as in typical priming paradigms, neural activity is usually reduced. This neural repetition effect has been reported at multiple spatial scales, from the level of individual cortical neurons in monkeys (Li et al., 1993; Miller and Desimone, 1994; Sobotka and Ringo, 1996) to the level of hemodynamic changes (measuring the pooled activation of millions of neurons) in humans, using functional imaging such as fMRI (Buckner et al., 1995; Demb et al., 1995; Stern et al., 1996; Grill-Spector et al., 1999; Henson et al., 2000; Jiang et al., 2000; Naccache and Dehaene, 2001). Repetition-related reductions also occur at multiple temporal scales, both in their longevity – from milliseconds (Sobotka and Ringo, 1996) to minutes (Henson et al., 2000) and days (van Turennout et al., 2000) – and in the latency of their expression (Dale et al., 2000; Henson et al., 2004). Therefore, RS is a robust phenomenon that occurs across many timescales, in multiple brain regions, and across an impressively large number of experimental conditions.

In experiments when subjects view repeated presentations of objects and scenes, there is robust and reproducible RS as measured with fMRI also referred to as fMRI-adaptation (for reviews, see Grill-Spector and Malach, 2001; Kourtzi and Grill-Spector, 2005; Grill-Spector et al., 2006a). RS/fMRI-adaptation typically occurs in object-selective cortex (**Figure 3**) including the lateral occipital complex (LOC – consisting of regions overlapping the lateral occipital sulcus, inferior occipital gyrus, and occipitotemporal sulcus), as well as more ventral regions including the fusiform gyrus (Fusiform) and the parahippocampal

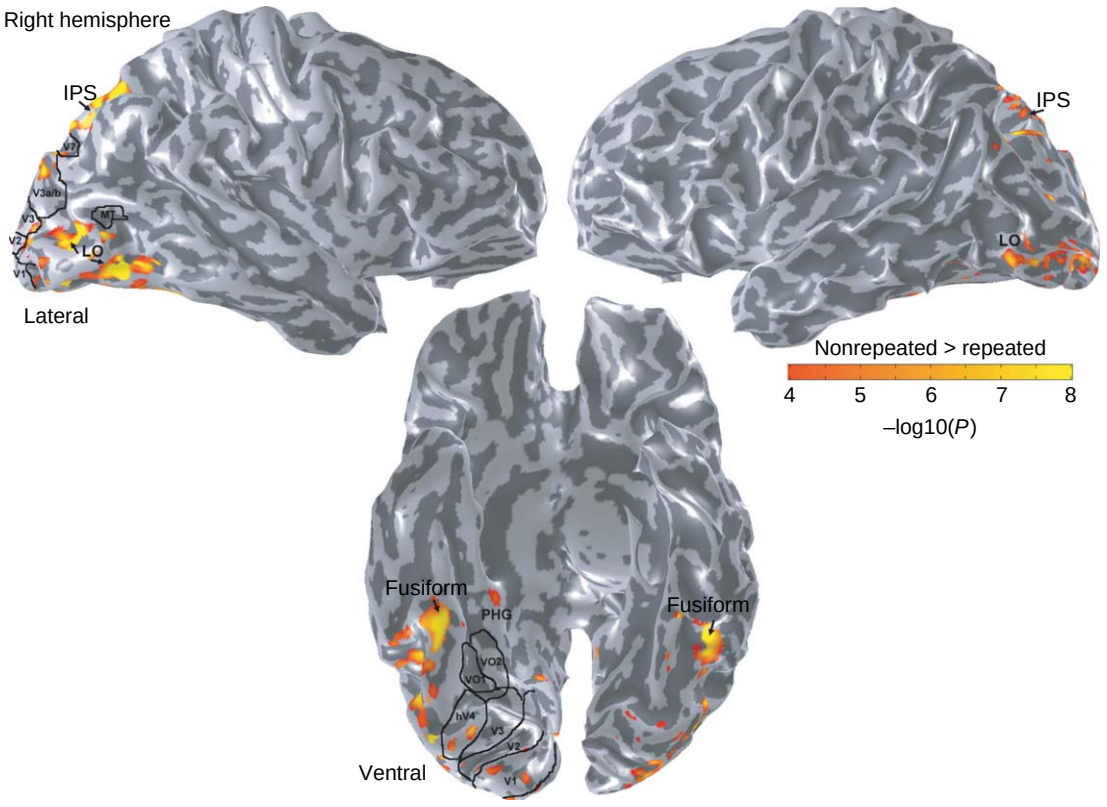

Figure 3 Occipitotemporal regions that show reduced responses to repeated versus nonrepeated stimuli ($p < 10^{-4}$, voxel level uncorrected). Color bar indicates statistical significance. Data are shown for a representative subject on her partially inflated brain. Dark gray regions indicate sulci, and lighter gray regions indicate gyrii. Retinotopic visual areas (delineated by black lines) were defined from independent retinotopic scans of polar angle and eccentricity and are shown for simplicity only on the right hemisphere. Abbreviations: PHG, parahippocampal gyrus; IPS, intraparietal sulcus.

gyrus (PHG). RS also occurs in dorsal regions (**Figure 3**), including regions lateral to and partially overlapping V3a and regions in the posterior bank of the intraparietal sulcus (IPS). Other regions that show RS to repeated presentation of object and scene images include medial temporal cortex (Stern et al., 1996) and frontal cortex (Wagner et al., 1997; Buckner and Koutstaal, 1998; van Turennout et al., 2003).

RS is not an all-or-nothing phenomenon: The magnitude of RS in object-selective cortex increases with repetition number and with fewer intervening stimuli between repetitions (**Figure 2(b)**). Therefore, the magnitude RS in block-design fMRI experiments is typically larger than during event-related fMRI experiments in which many intervening stimuli occur between repetitions of the same image (**Figure 4**). A recent study (Ganel et al., 2006) suggests that RS to immediate repetitions of identical stimuli is more prominent in object-selective regions of the LOC and fusiform cortex, whereas RS effects for stimuli that had been presented several minutes previously and occur after many intervening stimuli are more prominent in more anterior and medial regions of the temporal lobe. Further, they suggest that effects of immediate repetition and long-lagged repetition with intervening stimuli are largely additive (except for the left fusiform gyrus).

Repetition suppression in high-level visual areas has been associated with visual priming (Schacter and Buckner, 1998; Wiggs and Martin, 1998) because both phenomena occur under the same experimental conditions (**Figure 1**). However, it is mysterious why reduced cortical responses provide for improved behavioral performance. Notably, RS measured by fMRI may be related to other factors (unrelated to priming), such as repetition effects independent of behavioral improvements (Sayres and Grill-Spector, 2006), attentional differences between conditions (Yi and Chun,

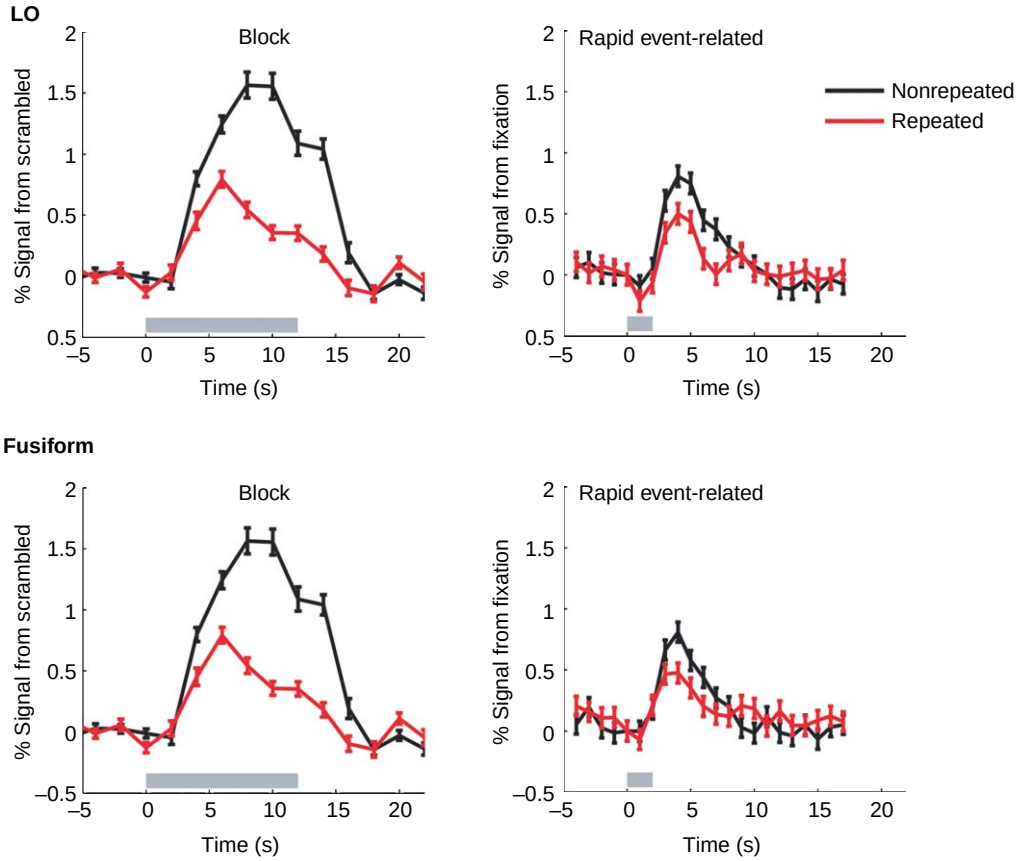

Figure 4 Repetition suppression in object-selective cortex: time course data. Data are shown for one representative subject. Object-selective cortex regions were defined as regions that showed higher activation for animals than scrambled animals with $p < 10^{-3}$ at the voxel level. *Black:* first presentation of the stimulus; Red: Repeated presentations of the same stimulus. In the block design fMRI experiment, stimuli were repeated up to 12 times within a block. In the rapid event-related fMRI experiment, stimuli were repeated up to eight times across a 4-min and 38-s experiment. Horizontal gray bar: duration in which stimulus was presented. Error bars indicate SEM across trials for this subject. Adapted from Grill-Spector K, Henson R, and Martin A (2006) Repetition and the brain: neural models of stimulus-specific effects. *Trends Cogn. Sci.* 10: 14–23.

2005; Yi et al., 2006), and/or learning of a response mapping between a stimulus and a cognitive decision (Dobbins et al., 2004; Schacter et al., 2004). Conversely, behavioral effects, such as visual priming, may be a consequence of activity in multiple cortical regions. Thus, RS in specific cortical regions may not relate directly to the behavioral changes associated with priming.

While keeping these caveats in mind, the section titled 'Investigations of the relation between RS and priming' describes several experiments that investigated the relation between priming and RS, and the section titled 'Neural models of repetition suppression and priming' lays out three models of the neural bases of priming, providing hypotheses for the relation between reduced cortical responses to repeated stimuli and improved performance – namely, visual priming.

3.12.3.1.1 Characterizing neural representations using repetition suppression

In addition to examining the relation between RS and priming, many neuroimaging experiments use RS to probe the functional properties of neural populations. This tool has been termed fMRI-adaptation (Grill-Spector and Malach, 2001) and also the priming method (Naccache and Dehaene, 2001; Vuilleumier et al., 2002). In the basic paradigm used in fMRI experiments, one first measures the basic RS (or fMRI-adaptation) effect induced by repetitions of identical stimuli. This is done by measuring the level of RS or adaptation to repeated presentations of identical stimuli relative to the response of nonrepeated stimuli (**Figure 5** – identical). Subjects are also presented with repeated stimuli that vary along

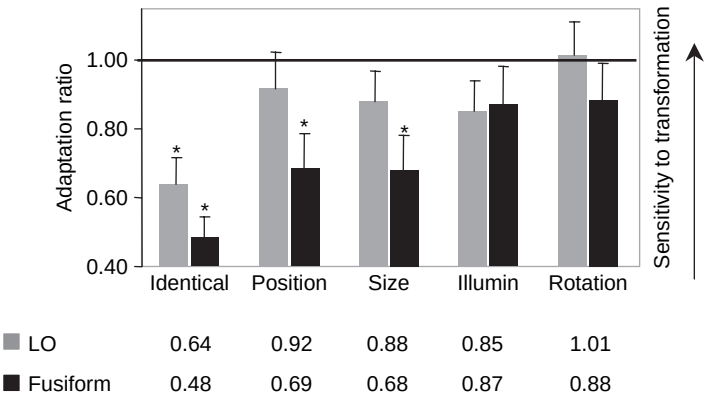

Figure 5 Using fMRI-adaptation to measure sensitivity to face transformations. To examine the level of adaptation to face transformations, we measured the adaptation ratio: repeated/nonrepeated. Repeated reflects blocks in which the same face was repeated, and nonrepeated reflects blocks that included nonrepeated presentations of different male faces in the same view, illumination, size, and position. An adaptation ratio of 1 indicates no adaptation (solid line); ratios that are significantly less than 1 are indicated by asterisks. Identical: repetitions of identical images of the same face; Position: repetitions of the same face in different retinal positions (~6° around fixation); Size: repetitions of the same face in different sizes (~threefold size change); Illumin: repetition of the same face illuminated from different directions; Rotation: Same face at different rotations around the vertical. Gray bars: lateral occipitus; Black bars: Fusiform; Error bars indicate SEM across 14 subjects. Numbers indicate adaptation ratios for each of the conditions. Data from Grill-Spector K and Malach R (2001) fMR-adaptation: A tool for studying the functional properties of human cortical neurons. *Acta Psychol. (Amst.)* 107: 293–321.

some dimension (e.g., the same object, but different sizes). The hypothesis tested is whether the underlying neural representation is sensitive or not to change along this dimension. If the underlying neural representation is insensitive to the change in the stimulus, then neurons will show a reduced response to repeated transformed versions of the object, and the fMRI signal will be reduced (i.e., fMRI-adaptation will be observed) similar to the reduction produced by repetitions of identical stimuli. Alternatively, if the neurons are sensitive to the change, the level of the fMRI signal will be similar to the initial level, and no RS/adaptation will be measured.

An example of using fMRI-adaptation to characterize neural representations is shown in **Figure 5**. In these experiments, subjects were shown either repeated presentations of the same image of a face (identical) or images of the same individual that varied in size (up to threefold changes in size), position (~6° around fovea), illumination (five different illuminations), viewpoint (rotation around the vertical, −90° to 90°). Activations to repeated versions of the same face were compared to nonrepeated presentations of faces of different individuals that were taken under the same viewing conditions (e.g., same size, position, illumination, and view). We found differential effects of fMRI-adaptation across object-selective cortex: LO regions show fMRI-adaptation for repetitions of identical images of objects but no fMRI-adaptation when the object varied in position, size, illumination, or viewing angle. In contrast, more ventral regions along the fusiform and occipitotemporal sulcus showed fMRI-adaptation for changes in object position and size but no fMRI-adaptation for different illuminations or rotations of the same object. These experiments provide evidence for differential sensitivity to object transformations across the human ventral stream.

fMRI-adaptation has been widely used by researchers to examine sensitivity in object-selective cortex to object size and position (Grill-Spector et al., 1999; Vuilleumier et al., 2002), viewpoint (Grill-Spector et al., 1999; James et al., 2002; Vuilleumier et al., 2002; Epstein et al., 2003), object format (Kourtzi and Kanwisher, 2000), perceived shape (Kourtzi and Kanwisher, 2001), contrast (Avidan et al., 2002; Murray and He, 2006), contour completion (Kourtzi et al., 2003), and face representation (Grill-Spector et al., 1999; Andrews and Ewbank, 2004; Winston et al., 2004; Eger et al., 2005; Loffler et al., 2005; Rotshtein et al., 2005; Jiang et al., 2006). fMRI-adaptation has also been used to probe higher-level conceptual representations using object pictures (Koutstaal et al., 2001) and words (Wheatley et al., 2005). Overall, these studies have documented that RS in occipitotemporal cortex is not limited to the identical image but also occurs, albeit to a lesser

extent, to transformed versions of the same object, to different exemplars that share the same name (e.g., two different umbrellas), and even to different words that are conceptually related (Wheatley et al., 2005).

3.12.3.2 Repetition Enhancement

Although the most ubiquitous cortical phenomenon is repetition suppression, there is evidence also that some aspects of visual priming are related to repetition enhancement (RE) (Dolan et al., 1997; George et al., 1999; Grill-Spector et al., 2000; Henson et al., 2000; James et al., 2000; James and Gauthier, 2006; Kourtzi et al., 2005; Turk-Browne et al., 2006). RE effects have been reported for improved recognition of repeated degraded stimuli (compared to performance on their initial presentation). RE was observed when repeated exposure to subthreshold, briefly presented objects led to better recognition (Grill-Spector et al., 2000) (**Figure 6**), when repeated exposure to unfamiliar shapes made them familiar (Henson et al., 2000), when inverted contrast faces (that were initially unrecognizable) were primed with positive contrast faces and became recognizable (George et al., 1999), and when observers learned to detect low-salience shapes in noisy backgrounds (Kourtzi et al., 2005; Turk-Browne et al., 2006).

One possibility is that RE and repetition suppression reflect dissociable forms of visual priming (Henson et al., 2000; Gruber and Muller, 2005); thus, repetition suppression may reflect suprathreshold priming, and RE may reflect subliminal priming (see also Kourtzi et al., 2005). These findings suggest that learning of unfamiliar or degraded stimuli is mediated by increased neural activity across high-level visual areas as new representations are formed for these previously unseen or unfamiliar stimuli. In contrast, learning of prominent suprathreshold (and/or familiar) stimuli is mediated by the sharpening of existing representations, leading to sparser coding of objects. An alternative account suggests that there is only one underlying mechanism, but it produces differential signals below and above recognition threshold (James et al., 2000; James and Gauthier, 2006). James and colleagues (James and Gauthier, 2006) proposed an accumulation model for recognition, in which recognition occurs when sufficient evidence for identifying an object has accumulated (*See* Chapter 2.23). Accumulation predicts a faster rise of activity

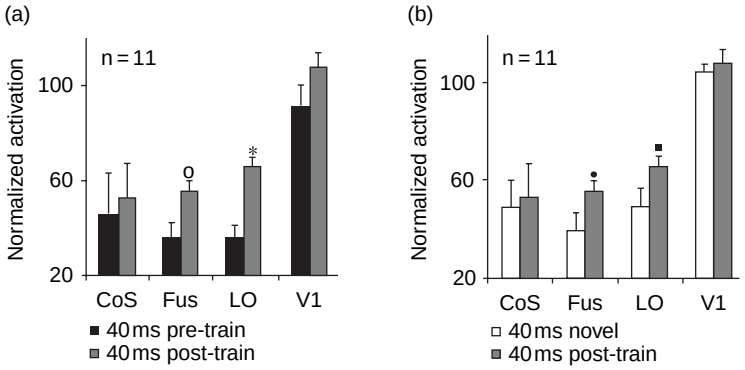

Figure 6 Repetition enhancement in object-selective cortex. Subjects were first scanned in the pretrained session when they viewed briefly presented images for 40 ms followed by a 460-ms mask. Subjects then viewed these masked images during five training sessions, and then they were scanned again in a second posttraining scan in which they viewed the same briefly presented stimuli (posttrain) and another set of novel masked images shown for the same duration (40 ms – novel). Initial recognition performance (40 ms pretrain) was about 25% and posttraining about 60%. (a) Normalized BOLD signal elicited by identical images viewed for 40 ms during pretraining and posttraining scans. Activations are plotted as percentage of activation to object stimuli shown for 120 ms followed by a mask for 380 ms. Recognition of 120 ms stimuli was close to ceiling. *X*-axis, brain area; *Y*-axis, normalized signal compared to activation elicited by stimuli presented for 120 ms. Dark bars: pretraining; Gray bars: identical stimuli posttraining. Error bars: SEM. Asterisks ($p < .001$) and circles ($p < .03$): significantly larger signal posttraining versus pretraining. (b) Normalized BOLD signal in the posttraining scan for two sets of images (trained and novel) shown for 40 ms. White bar: novel images; Gray bars: trained images; Error bars: SEM. Square ($p < .01$) and circle ($p < .05$): significantly stronger signal for trained images compared to novel images. Abbreviations: CoS: collateral sulcus; Fus: Fusiform gyrus; LO: lateral occipital. Adapted from Grill-Spector K, Kushnir T, Hendler T, and Malach R (2000) The dynamics of object-selective activation correlate with recognition performance in humans. *Nat. Neurosci.* 3: 837–843.

for primed compared to unprimed stimuli, as the evidence accumulates faster for primed stimuli. Another consideration for interpreting RE effects is that, close to perpetual threshold, recognition performance on trials in which subjects recognize objects (hits) is correlated with a higher signal than trials in which subjects fail to recognize objects (misses) (Bar et al., 2001; Grill-Spector, 2003; Grill-Spector et al., 2004). Thus, RE associated with improved recognition of previously unrecognized stimuli could reflect a higher hit rate for repeated stimuli that is not related to priming per se.

Although RE has been suggested to reflect a distinct form of priming than RS, to date there is no parametric study that systematically varied factors that affect RE and visual priming to test whether RE and visual priming are quantitatively linked.

3.12.3.3 Repetition Effects Measured with EEG and MEG

Repetition effects have also been studied by measuring changes in the electrical (EEG) or magnetic (MEG) field, usually recorded above the scalp. These effects reflect changes in the amplitude and/or synchrony of local field potentials (LFPs) caused by transmembrane currents in large numbers of neurons.

Most EEG studies examine event-related potentials (ERPs), which reflect changes in electrical potential during the few hundred milliseconds following stimulus onset, averaged across trials. The earliest object repetition effects are typically observed approximately 200 ms after stimulus onset (Eimer, 2000; Doniger et al., 2001; Schendan and Kutas, 2003; Henson et al., 2004; Schweinberger et al., 2004; Gruber and Muller, 2005), but some experiments show earlier repetition effects 150–170 ms after stimulus onset. Campanella, Henson, and colleagues (Campanella et al., 2000; Henson et al., 2004) found repetition effects when the same view of an object was repeated, as early as 160–190 ms when there were no intervening objects; with one or more intervening objects, repetition effects only emerged from approximately 200 ms onward.

Other EEG studies concentrate on changes in the power of electrical or magnetic oscillations that are induced by stimulus repetition (high-frequency oscillations are not observed in ERPs if they are not phase-locked across trials). Some studies report decreased high-frequency (>40 Hz) power around 220–350 ms for repetition of familiar objects across lags of one or two intervening objects (Gruber and Muller, 2005). Such changes in power in certain frequency bands have been shown to correlate with the blood-oxygen-level-dependent (BOLD) changes measured by fMRI (Brookes et al., 2005; Sayres and Grill-Spector, 2006).

3.12.3.4 Investigations of the Relation between RS and Priming

One useful approach used to examine the relation between priming and RS is to parametrically manipulate factors that influence the level of priming and/or RS and to examine whether the modulation of priming and RS effects covary or follow distinct profiles. Researchers have shown that many factors modulate the level of priming and also the level of RS. These include the number of stimulus repetitions (Henson et al., 2003; Sayres and Grill-Spector, 2006), frequency of repetition (Sayres and Grill-Spector, 2006), duration of stimulus presentation (Zago et al., 2005; Sayres and Grill-Spector, 2006), stimulus contrast (Avidan et al., 2002), and amount of noise added to the stimulus (Turk-Browne et al., 2006), as well as high-level cognitive factors such as attention (Eger et al., 2004; Murray and Wojciulik, 2004; Yi and Chun, 2005; Yi et al., 2006), relevance to the task (Henson et al., 2002b; but see Jiang et al., 2000), familiarity (Henson et al., 2000), and emotion (Ishai et al., 2004, 2006).

Recently, researchers have used this approach to examine the correlation between RS and priming (Dobbins et al., 2004; Maccotta and Buckner, 2004; Zago et al., 2005; Sayres and Grill-Spector, 2006). Results of these experiments are mixed. Some experiments suggest that some factors modulate the level of priming and RS in a similar way, suggesting a quantitative relation between priming and RS (Maccotta and Buckner, 2004; Zago et al., 2005). However, other factors may affect priming, but not RS (or RS but not priming), suggesting that RS in specific occipito-temporal regions may not be a direct neural correlate of visual priming (Henson et al., 2003; Dobbins et al., 2004; Sayres and Grill-Spector, 2006).

3.12.3.4.1 Evidence for a correlation between priming and repetition suppression

Several recent studies have reported a correlation between the level of response time priming and RS in prefrontal cortex (PFC) (Dobbins et al., 2004; Lustig and Buckner, 2004; Maccotta and Buckner, 2004). Lusting and Buckner (2004) report across-subject correlation between the level of

priming during a meaning-based word classification task and PFC activity across young adults, older adults, and adults with initial signs of Alzheimer's disease (Lustig and Buckner, 2004). Similarly, Maccota and Buckner (2004) showed that the level of priming as a function of number repetitions of a word and the level of RS in PFC was correlated across subjects. Dobbins and colleagues (Dobbins et al., 2004) also reported a positive within-subject correlation between response time priming induced by repeated presentations of visually presented objects and RS in PFC. In the same study they reported a negative within-subject correlation between priming and RS in the left fusiform cortex.

Zago and colleagues (Zago et al., 2005) showed that both visual priming and RS vary as a function of the initial exposure of the stimulus, which varied between 40 and 1900 ms. The largest priming and RS effects were observed for 250-ms exposure durations. Further, the average level of priming across subjects is correlated with the average level of RS in occipito-temporal object-selective regions. However, Zago and colleagues do not report within-subject correlations between visual priming and RS.

Together, these studies suggest that under some circumstances there is a correlation between priming and RS. The most consistently reported cortical region in which activation is associated with priming is PFC. Further research is necessary to examine the generality of these results to additional stimulus manipulations to understand whether these effects are modality specific and to investigate more comprehensively whether the correlation between priming and RS can be found within individual subjects, as responses and brain activations are likely to vary across individuals.

3.12.3.4.2 Evidence for dissociable effects of performance and repetition on the level of repetition suppression

Priming effects can be reduced when the responses to a stimulus are changed across repetitions. A recent study examined whether changes in priming effects and RS effects were dependent on the particular response/judgment made about the stimulus. Dobbins and colleagues (Dobbins et al., 2004) measured priming and RS effects as a function of repetition number under two experimental conditions – when the same judgment was made on the stimulus (indicate whether an object is larger than a shoebox, for both initial and repeated presentations) and when different judgments were made (initially subjects judged whether the item was larger than a shoebox, and when the item was repeated they were asked if it was smaller than a shoebox). Priming effects were larger when the question was identical in the initial and repeated conditions. However, priming was observed even when the judgment differed. RS in PFC was correlated with priming effects in both experimental conditions. In contrast, RS in fusiform cortex was observed when items were repeated and the judgment was identical but was abolished when the judgment changed. These data suggest that RS in fusiform cortex was related to the ability of the subject to use prior responses during repeated trials, rather than reflecting a priming effect.

Another consideration when relating BOLD responses to performance is that several factors may contribute to BOLD responses measured in a specific brain region, and performance is likely to be an outcome of activation across several brain regions. Therefore, while RS and priming occur in the same experimental situations, the two phenomena may not coincide under all experimental conditions. For example, RS may be driven by shorter RT (e.g., shorter RT may produce lower BOLD responses) but not repetition. This alternative predicts lower BOLD responses for trials with shorter RT regardless of whether these trials contain stimuli shown for the first time or contain stimuli that have been seen previously. Alternatively, repetition may produce lower responses independent of performance changes. This alternative suggests that repeated trials will correspond to trials with lower BOLD response, even if there is no change in performance – that is, a memory component to RS that is independent of performance.

Another question is whether RS and visual priming reflect changes in neural activity during the recognition process, or whether they reflect changes after recognition has occurred. Under many experimental conditions the stimulus is presented for longer periods than the minimal time necessary for recognition (Grill-Spector and Kanwisher, 2005) (~67–120 ms), yet both performance and BOLD signals are measured after recognition has occurred.

In a recent study (Sayres and Grill-Spector, 2006), we examined both factors: Is RS in object-selective cortex correlated with response time priming or repetition independent of performance changes? Second, do RS and priming occur during or after recognition? To quantitatively examine the relation between priming and RS, we first measured the

relation between priming and RS in object-selective cortex as we parametrically varied repetition parameters (number of repetitions, number of intervening stimuli, time between repeats). We then measured whether there are independent contributions of RT and repetition to RS effects. Second, to assess whether RS and priming occurred during or after recognition, we compared RS and priming effects for long exposure durations (1750 ms) and for short exposures (67–101 ms). Exposure durations were set for each subject to be the minimal duration for 85% accuracy performance on a classification task (67–101 ms for our subjects). Stimuli were presented briefly and then masked for the remainder of a 2-s trial. These brief presentations allowed us to tap into repetition effects during recognition.

We found that both RT priming and RS occurred for both long and short exposures, and these effects were modulated by repetition parameters (**Figure 2**). The level of RS also varied with stimulus duration, as the magnitude of RS was lower for short compared to long exposure durations. In contrast, the magnitude of RT priming was not significantly different across these exposure durations. We did find an improvement in accuracy for short exposures, but not long exposures (perhaps due to a ceiling effect), but this did not depend on repetition parameters.

Importantly, we found that when exposure durations were long (1750 ms), there was significant correlation between RS in object-selective cortex and RT priming for some repetition parameters (stimuli between repeats, time between repeats) but not others (number of repeats). When exposures were short (67–101 ms), we observed significant priming and significant RS, but they were not correlated. Thus, both priming and RS can occur under the same experimental conditions, but they do not always covary. These data suggest that RS in object-selective cortex may not reflect improved RT performance observed during priming.

Finally, we examined whether there are separable contributions of response time and repetition to RS by sorting our data into repeated and nonrepeated conditions. For each condition, we ranked each subject's trials according to response time and grouped the trials into four equally sized bins according to RT. Responses to repeated trials were lower than nonrepeated trials even when response times were equated between conditions (**Figure 7**). Importantly, for both long and short image durations and all object-selective regions of interest (ROIs), we found a significant effect of repetition independent of response time. In contrast, we did not find significant effects of response time independent of repetition. There was a weak, statistically significant effect present in LO and only for stimuli that were presented for 1750 ms. Finally, we found no significant interaction between repetition and response times. Taken together, these data reveal that RS in object-selective cortex reflects stimulus-specific repetition, even when performance is matched between repeated and nonrepeated objects and when stimuli are presented close to the minimum time required for recognition. This suggests that there is a

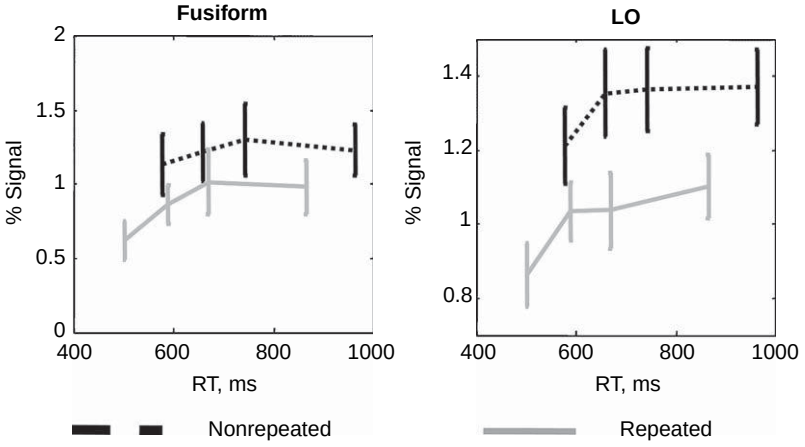

Figure 7 Separable contributions of repetition and response-time. Data were sorted first into repeated (solid gray) and nonrepeated (dashed black) correct trials and then grouped into four response time bins for each subject. The first response time bin represents fastest quartile of correct trials for each subject. Error bars indicate SEM across seven subjects. Adapted from Sayres R and Grill-Spector K (2006) Object-selective cortex exhibits performance-independent repetition suppression. *J. Neurophysiol.* 95: 995–1007.

performance-independent component to RS in object-selective cortex that may be an implicit form of memory.

Overall, our experiments show that both priming and RS effects depend on repetition parameters. However, different factors have dissociable effects on priming and RS. These experiments underscore the importance of conducting future experiments using parametric designs, systematically varying factors that modulate priming and RS to quantitatively measure the relation between these phenomena.

3.12.4 Neural Models of Repetition Suppression and Priming

Experiments have provided important insights about the characteristics of priming, RS, RE, and their relation. However, the neural mechanisms underlying visual priming and RS are yet unknown.

Three models have been suggested for the neural mechanisms underlying repetition suppression that may explain priming effects (Grill-Spector et al., 2006a) (**Figure 8**): (1) the Fatigue model, whereby the amplitude of firing of stimulus-responsive neurons decreases (Miller and Desimone, 1994; Grill-Spector and Malach, 2001), (2) the Sharpening model, whereby fewer neurons respond (Li et al., 1993; Desimone, 1996; Wiggs and Martin, 1998), and (3) the Facilitation model, whereby the latency (James and Gauthier, 2006) and/or duration of neural activity is shortened (Sobotka and Ringo, 1996; Henson and Rugg, 2003). An important consideration to keep in mind is how each of these models may account for visual priming.

3.12.4.1 Fatigue Model

According to this model, all neurons initially responsive to a stimulus show a proportionally equivalent reduction in their response to repeated presentations of the same stimulus. As a consequence the mean population firing rate declines, but there are no changes in the pattern of relative responses across

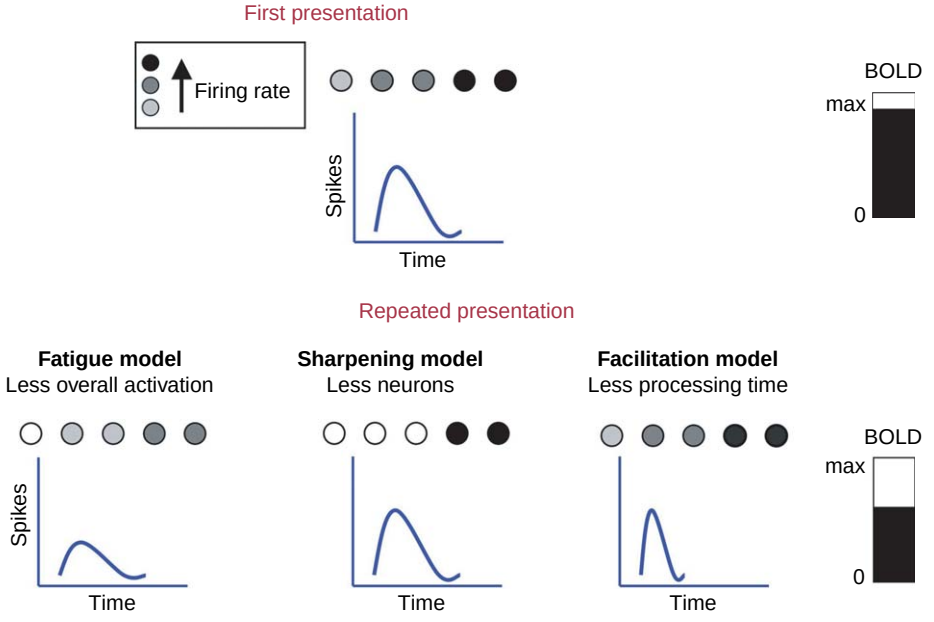

Figure 8 Models for repetition suppression. The top panel indicates neural responses in a putative brain region to the initial presentation of a stimulus. The bottom panels indicate responses in this region to repeated stimuli as posited by each of the three models. *Left:* Neural responses. Circles indicate the peak spiking rate of neurons in this region (darker circles indicate higher spiking rates). Blue time courses illustrate the spiking rate as a function of time for the most responsive neurons (indicated by black circles). *Right:* BOLD response in this region of cortex. Since the BOLD signal integrates neuronal activity over time, all of these models predict reduced BOLD responses for repeated stimuli, but due to different reasons: Fatigue: lower firing rates across the entire time window; Sharpening: fewer neurons respond, but those which remain active show similar spiking as for the first presentation; Facilitation: shorter duration of neural processing, but the peak firing is similar to the initial presentation. Adapted from Grill-Spector K, Henson R, and Martin A (2006) Repetition and the brain: neural models of stimulus-specific effects. *Trends Cogn. Sci.* 10: 14–23.

neurons or in the temporal window in which neurons are responding. One mechanism for fatigue may be firing-rate adaptation, in which the reduction in a neuron's firing rate is proportional to its initial response (Li et al., 1993; Avidan et al., 2002) (similar to a gain mechanism; Carandini and Ferster, 1997). However, this mechanism does not explain the specificity of RS, that is, why the neuron's response is reduced to some stimuli, yet resumes high firing rates to other stimuli. An alternative mechanism is reduced synaptic efficacy of specific synapses from connected neurons (synaptic depression). In this manner, only specific patterns of presynaptic input to the neuron (which are stimulus dependent) reduce its firing rate. This type of mechanism has been implicated in early visual cortex and usually occurs with prolonged repetitive stimulation.

One prediction from this model is that the amount of RS will be greater in neurons that respond optimally to a stimulus than in other neurons. As a result, the sensitivity of the system to stimuli that are different from the repeating stimulus is increased, thereby providing a mechanism for 'novelty detection.' Reducing the firing rate may also help prevent saturation of the neural response function by increasing its dynamic range. Another advantage hypothesized for such a mechanism is that it reduces redundancies in the neural code, which increases the efficiency of information encoding (Muller et al., 1999).

However, it is not immediately clear how reduced firing rates can account for increased speed and accuracy of processing repeated stimuli (in addition to increased sensitivity to novel stimuli), as arises in priming. One explanation is provided in a computational model by Gotts (2002), in which a reduction in the mean and variance of firing rates allows for greater synchrony of neural responses. Since greater synchrony of presynaptic input is believed to be more effective in triggering a postsynaptic response (Fries et al., 2001), this would imply more rapid transmission of information throughout the network, resulting in faster responses (priming). A key prediction of this model is that synchrony can increase while stimulus-specific firing rates decrease. While there is evidence in support of this possibility (Stopfer and Laurent, 1999), others have argued for the opposite effect (Chawla et al., 1999). An increase in synchrony may also be difficult to reconcile with observations of reduced oscillatory power following stimulus repetition (Gruber and Muller, 2005), though it is possible that decreased amplitude of local field potentials outweighs the increased synchrony of those potentials.

3.12.4.2 Sharpening Model

Desimone (1996) and Wiggs and Martin (1998) have suggested that repetition results in a sparser representation of stimuli across cortex. According to this model, some, but not all, neurons that initially responded to a stimulus will show RS to subsequent presentation of that stimulus. Thus, repetition-related changes are viewed primarily as a learning process, in which representations (e.g., tuning curves) are 'sharpened,' and as a consequence, the distributed representation becomes sparser, resulting in fewer responsive neurons in total (**Figure 8**). An important difference between the Sharpening and Fatigue models is that for Sharpening, many of the neurons that are optimally tuned to the repeating stimulus show little or no response reduction, rather than exhibit the greatest response reduction, as in the Fatigue model.

Sparser representations clearly have adaptive value in terms of a reduced metabolic cost. Also, because the representation becomes sharper (tuning curves become narrower), the neurons become more sensitive to change. Sparser representations may also allow for more efficient or faster processing, though this depends on the manner in which their information is read out by downstream neurons. Because the Sharpening model suggests a changed and improved representation for repeated stimuli, this model has been widely used to explain priming (Wiggs and Martin, 1998; Henson and Rugg, 2003; Zago et al., 2005). However, a recent study suggests that RS in object-selective cortex may reflect response learning and implies that object representations do not necessarily reorganize as a consequence of repetition (Dobbins et al., 2004).

The mechanism underlying the formation of sparser representations is unknown but could reflect inhibition from lateral connections between neurons within a population. For example, Norman and O'Reilly (2003) used a competitive Hebbian learning rule to simulate the sharpening of representations with repetition (within medial temporal cortex). Initially, many neurons respond weakly to a distributed input pattern representing the stimulus. Through competitive interactions, the neurons with the strongest initial response get 'stronger' and inhibit the 'weaker' neurons. Thus, some neurons show increased firing rates following repetition, whereas

others show decreased firing rates. By assuming that the number of 'strong' units is less than the number of 'weak' units, the population response decreases with repetition because there are more neurons showing reduced activity than showing increased activity. If information only from those neurons with high firing rates is 'read out' by downstream neurons, their increased firing rate following repetition (despite the global decrease) could explain the faster processing of repeated stimuli.

3.12.4.3 Facilitation Model

At its simplest, this model predicts that repetition causes faster processing of stimuli, that is, shorter latencies or shorter durations of neural firing, and thereby may explain faster response times observed during priming. One example is the 'accumulation' model of James and Gauthier (2006), in which stimulus information is accrued faster following repetition. Given that the hemodynamic signal measured by fMRI integrates over several seconds of neural activity, a shorter duration of activity results in a decreased amplitude of the fMRI signal. A shorter duration of neural activity is also consistent with earlier peaking of the fMRI response (Henson et al., 2002a) and might explain why decreases in firing rate can appear to arise after the initial visual response (Ringo, 1996): The neurons initially fire robustly to both first and repeated presentations, but this firing stops sooner for repeated presentations.

An extension of the Facilitation model assumes that the cause of this faster processing is synaptic potentiation between neurons following an initial stimulus presentation, and that this potentiation can occur at many levels in the processing stream. As a consequence, information flows through the stream more rapidly, and hence processing of a repeated stimulus occurs faster. In terms of attractor neural network models, synaptic potentiation might be viewed as deepening the basin of attraction, resulting in a shorter time for the network to settle on a stable pattern corresponding to identification of the stimulus. An example of such a dynamical network model is sketched by Friston (2005). The key idea behind this model is that the firing rate of the long-range excitatory (output) neurons in a population codes 'prediction error' (Rao and Ballard, 1999), which is the difference between bottom-up input ('evidence') and top-down input ('prediction'). The dynamics of the network are such that prediction error decreases over time after stimulus onset, and synaptic changes serve to accelerate this decrease when the stimulus is repeated (i.e., repetition improves prediction).

This emphasis on recurrent activity between many levels of the processing stream is consistent with the spatiotemporal pattern of repetition effects emerging from MEG/EEG data. Moreover, if interregional interactions require an initial volley of activity through the network (Sugase et al., 1999), this model could further explain the relatively late onset of long-lag repetition effects recorded with EEG/MEG. However, such a model would not explain why much earlier repetition effects have been observed in some neurons (e.g., 75–100 ms, which is thought to be too early for feedback; Xiang and Brown, 1998), and further, this model does not necessarily predict decreases in the peak firing rate of individual neurons.

Each of the above models would clearly benefit from further elaboration, including instantiation as detailed computational models. It is possible that different models may apply in different brain regions and under different experimental conditions (e.g., different paradigms/tasks). Nevertheless, specific neural mechanisms matter, because the interpretation and design of experiments depend on the nature of the underlying neural model. For example, models differ as to whether RS reflects quantitative or qualitative changes in representations. One important possibility is that there are multiple models that vary in their relevance across space, time, and task, which may parallel the multiplicity of potential neural/synaptic mechanisms. Finally, it is yet unknown whether the same or different mechanisms operate in different brain regions.

3.12.4.4 Distinguishing the Neural Models

There are three main directions in which these models can be distinguished: (1) examining the relationship between RS and stimulus selectivity, (2) examining the effect of repetition on the tuning of cortical responses along a stimulus dimension, and (3) examining the temporal window in which processing occurs for new and repeated stimuli.

3.12.4.4.1 Examining the relationship between RS and stimulus selectivity

The models differ in their predictions on whether RS is strongest for the preferred stimulus or for nonpreferred stimuli. The Sharpening model predicts that neurons showing little or no RS to a repeated

stimulus are highly selective for that stimulus. In contrast, both the Fatigue and Facilitation models predict that RS is proportional to the initial response. Thus, neurons that respond optimally for a stimulus should show the largest suppression. These hypotheses can be tested with single-cell recording.

3.12.4.4.2 Examining the effect of repetition on neural tuning

Another way to distinguish the models would be to find a single dimension (e.g., motion, orientation) along which stimuli differ and examine the effect of repetition on the tuning curves of different neurons along that dimension. The models differ in their prediction of how repetition will change the shape of neuronal tuning. According to the Fatigue model, repetition reduces the response in proportion to the initial response, but the tuning width does not change. Most likely, the reduction will be maximal for tuning curves centered on the location of the repeating stimulus along the stimulus dimension, and lesser for tuning curves centered farther away. This is consistent with adaptation of V1 neurons to orientation (Dragoi et al., 2002), spatial frequency (Movshon and Lennie, 1979), and motion direction (Kohn and Movshon, 2004). In contrast, according to the Sharpening model, repetition sharpens tuning curves. This is consistent with studies of learning-related changes in IT cortex and V4 (Baker et al., 2002; Sigala and Logothetis, 2002; Rainer et al., 2004). Finally, the Facilitation model does not suggest any particular effect on tuning curves. Indeed, even a widening of the curves might be possible if repetition enlarged the attractor basin in an attractor network model.

3.12.4.4.3 Examining the temporal window of processing for new and repeated stimuli

The models may also be distinguished in the temporal domain. In particular, the Facilitation model suggests that the latency and/or duration of the response to repeated items will be shorter than to first presentations. The Fatigue and Sharpening models do not suggest a difference in the temporal processing window for repeated stimuli. The latency and duration of processing might be examined via single-cell recordings and/or EEG/MEG techniques.

3.12.5 Conclusions and Directions for Future Research

Visual priming is one of the most studied cognitive processes, as it is a window to understanding the underlying representations and mechanisms of rapid implicit learning and memory in the human brain. Progress has been made using priming to infer the nature of representations in different cortical regions or as a marker for increased processing efficiency, without a complete understanding of its neural basis. Nevertheless, specific neural mechanisms matter, because interpretation of experimental data depends on the nature of the underlying neural mechanisms.

Clearly, many questions remain regarding the neural basis of visual priming. For example, how do the Fatigue and Sharpening models account for improved performance during priming? Do different mechanisms occur in different timescales (e.g., immediate priming vs. priming with many intervening stimuli)? Do fundamentally different mechanisms underlie RE and RS? How does the specificity of priming correlate with particular representations in different visual regions? Are there different mechanisms underlying subliminal and suprathreshold visual priming?

Notably, any empirical data relevant to the models presented here is likely to depend on other factors, such as the lag between repetitions. One of the central outstanding questions is whether different models apply at different timescales. One possibility is that the mechanisms related to the Fatigue model operate during immediate repetitions of a stimulus within a few hundred milliseconds and reflect transient stimulus-specific effects that onset rapidly, whereas the effects of repetition across many intervening stimuli may be more consistent with the Sharpening or Facilitation models and reflect long-term learning that leads to changes in the spatial pattern of stimulus-selective responses and/or dynamics of those responses. Also models differ as to whether priming is associated with quantitative or qualitative changes in cortical representations. One possibility is that there are multiple mechanisms that vary across space, time, and task. Finally, it is yet unknown whether the same or different mechanisms operate in different brain regions.

Progress will be aided by integrating data using a combination of behavioral and neuroimaging methods such as fMRI and EEG/MEG (provided important differences between these measurements

are kept in mind), linking between electrophysiology data in animals and neuroimaging data in humans, and improvements in the spatial resolution of fMRI (Beauchamp et al., 2004; Schwarzlose et al., 2005; Grill-Spector et al., 2006b). Future experiments will yield important empirical data that will validate (or refute) current theoretical predictions. Understanding the neural bases of priming will be critical for understanding whether priming reflects quantitative or qualitative changes in the brain and will allow a fundamental understanding of implicit learning and memory in the adult brain.

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3.13 Short-Term and Working Memory Systems

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3.13.1 Introduction

Humans and other animals with elaborately evolved sensory systems are prodigious consumers of information: each successive moment in an ever-changing environment nets a vast informational catch – a rich and teeming mélange of sights, sounds, smells, and sensations. Everything that is caught by the senses, however, is not kept; and that which is kept may not be kept for long. Indeed, the portion of experience that survives the immediate moment is but a small part of the overall sensory input. With regard to memory storage, then, the brain is not a pack rat, but a judicious and discerning collector of the most important pieces of experience. A good collector of experience, however, is also a good speculator: The most important information to store in memory is that which is most likely to be relevant at some time in the future. Of course, a large amount of information that might be important in the next few seconds is very unlikely to be of any importance in a day, a month, or a year. It might be stated more generally that to a large degree the relevance of information is time-bounded – sense-data collected and registered in the present is far more likely to be useful in a few seconds than it is to be in a few minutes. It would seem, then, that the temporary relevance of information demands the existence of a temporary storage system – a kind of memory that is capable of holding onto the sense-data of the 'just past' in an easily accessible form, while allowing older information to discreetly expire.

The existence of this kind of 'short-term memory' has been well established over the past century through the detailed study of human performance on tasks designed to examine the limits, properties, and underlying structure of human memory. Moreover, in recent years, much has been learned about the neurobiological basis of short-term memory through the study of brain-damaged patients, the effect of cortical ablations on animal behavior, electrophysiological recordings from single cells in the nonhuman primate, and regional brain activity as measured by modern functional neuroimaging tools such as positron emission tomography (PET) and functional magnetic resonance imaging (fMRI). In this chapter, we examine how the psychological concept of short-term memory (STM) has, through a variety of neuroscientific investigations, been validated as a biological reality.

3.13.2 Evidence for the Existence of Short-Term Memory

An important scientific tenet, often referred to as the principle of parsimony, dictates that when two competing theories are put forth to explain a phenomenon,

and neither one is clearly superior to the other in its explanatory power, then the simpler of the two is to be preferred. The simplest conceivable theory of memory is that it is a unitary mental faculty; that all memories, no matter how recent or how remote, are made possible by a single functional system. This idea also appears to be the way in which people naturally conceive of memory, inasmuch as the single term 'memory' has been sufficiently expressive and precise to refer to the act of remembering in all its everyday variety. But soon after psychologists began to characterize memory in terms of human performance, a strong case emerged for the existence of different kinds of memory involving separate systems for the storage of old and new experiences.

In the mid-1960s, evidence began to accumulate in support of the view that separate functional systems underlie memory for recent and memory for more distant events. A particularly robust finding came from studies of free recall in which it was demonstrated that when subjects are presented a list of words and asked to repeat as many as possible in any order, performance is best for the first few items (the primacy effect) and for the last few items (the recency effect) – a pattern of accuracy that when plotted as a function of serial position (see **Figure 1**) appears U-shaped (Waugh and Norman, 1965; Glanzer and Cunitz, 1966). When a brief filled retention period is interposed between stimulus presentation and recall, however, performance on early items is relatively unaffected, but the recency effect disappears (Postman and Phillips, 1965; Glanzer and Cunitz, 1966). These findings suggest that in the immediate recall condition the last few items of a list are recalled best because they remain accessible in a short-term store, whereas early items are more permanently represented (and thus unaffected by the insertion of a filled delay) in a long-term store. This idea that memory, as a functional system, contains both short- and long-term stores is exemplified by the two-store memory model of Atkinson and Shiffrin (1968). In this prototype psychological memory model, comprising a short-term store (STS) and long-term store (LTS), information enters the system through the STS, where it is encoded and enriched, before being passed on to the LTS for permanent storage (**Figure 2**). Although the idea that short-term storage is a necessary prerequisite for entry into the LTS has not held up, the two-store model of Atkinson and Shiffrin crystallized the very idea of memory as a divisible, dichotomous system and provided the conceptual framework for the interpretation of patterns of memory deficits observed in patients with brain damage.

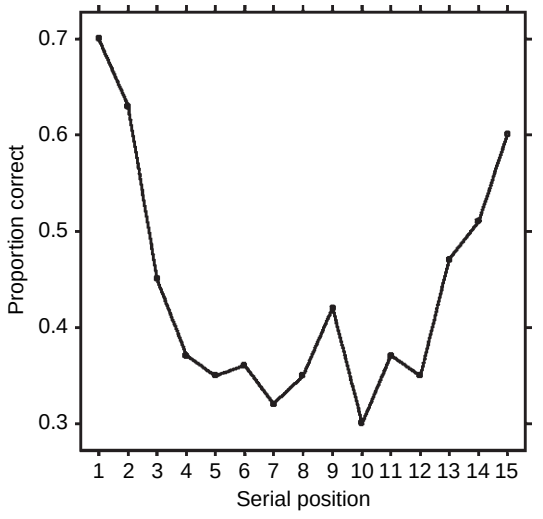


Figure 1 Plot of recall accuracy as a function of serial position in a test of free recall. Primacy and recency effects are evident in the U-shaped pattern of the curve. Adapted from Glanzer M and Cunitz A-R (1966) Two storage mechanisms in free recall. *J. Verbal Learn. Verbal Behav.* 5: 351–360; used with permission.

3.13.2.1 Evidence from Neurology and Neuropsychology

Perhaps the most compelling evidence for the existence of two memory stores comes from case studies of persons with focal brain lesions. In the early 1950s an astonishing, if tragic, discovery was made. A surgical procedure for the treatment of intractable epilepsy that involved bilateral removal of the medial temporal lobe in patient H. M. resulted in a catastrophic impairment in his ability to form new long-term memories, though, remarkably, his STM was left intact (Scoville and Milner, 1957). Thus, H. M., although perfectly capable of repeating back a string of digits – the classic test of STM – was unable to permanently store new facts and events. In the following decade, when Warrington and Shallice (Warrington and Shallice, 1969; Shallice and Warrington, 1970) reported a number of case studies of patients with temporoparietal lesions who had dramatically impaired STM for numbers and words coupled with a preserved ability to learn supra-span (e.g., greater than 10 items) word lists with repeated study, the case for a separation between STM and long-term memory (LTM) was immeasurably strengthened. It is important to emphasize that

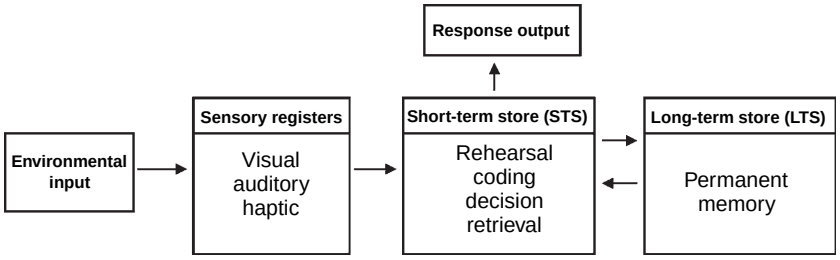


Figure 2 An information-flow diagram of Atkinson and Shiffrin's (1968) memory model. Information arriving to senses enters a short-term store (STS), where it can be maintained temporarily before entering a long-term store (LTS).

the STM deficits exhibited by such patients were, in the purest cases (Shallice and Warrington, 1977), not accompanied by any obvious deficits in ordinary language comprehension and production. Thus, for instance, patient J. B. was able to carry on conversations normally and to speak fluently without abnormal pauses, errors, or other symptoms of aphasia; in short, the 'language faculty,' considered to encompass the processes necessary for the online comprehension and production of meaningful speech, need not be disturbed even in the presence of a nearly complete eradication of verbal STM (Shallice and Butterworth, 1977). This established an important dissociation between the STM syndrome and the aphasic syndromes – a class of neurological disorders that specifically affect language ability – and argued, again, for a dedicated system in the brain for the temporary storage of information.

In summary, the discovery of 'short-term memory patients,' as they were to be called in the neuropsychological investigations of Warrington, Shallice, and others, provided a kind of evidential death blow to extant single-store hypotheses of memory (e.g., Melton, 1963), insofar as it established a double dissociation both in brain localization (LTM – medial temporal lobe, verbal STM – temporoparietal cortex) and patterns of performance, between short- and long-term memory systems. In addition, the STM disorder could be clearly distinguished, at the behavioral level at least, from the major language disorders such as Broca's and Wernicke's aphasia.

3.13.2.2 From Short-Term Memory to Working Memory

STM had, until the landmark work of Baddeley and colleagues (Baddeley and Hitch, 1974; Baddeley, 1986), typically been viewed as a more or less passive and amorphous medium for the brief storage of information derived from the senses. Questions tended to focus on the principles governing the mnemonic 'life cycle' of an item in memory – that is, why and at what rate are items forgotten? What is the role of passive decay? What is the role of interference, both proactive and retroactive, in forgetting? What is the route from STM to LTM, and what are the factors that influence this process? These questions, though of fundamental importance to understanding how memory works, tended to emphasize the general mechanisms – the procedures and principles of memory – rather than the underlying functional architecture of the system. What was missing from this line of research was the recognition that the contents of STM are not physical elements governed by certain lawful and inexorable processes of decay and interference, but rather dynamic representations of a fluid cognition, capable of being maintained, transformed, and manipulated by active, executive processes of higher control. Thus, for instance, two of the most important variables in studies of STM before the emergence of the Working Memory model were time (e.g., between stimulus presentation and recall) and serial order (e.g., of a list of items), both of which variables are defined by the inherent structure of the environmental input. (The term working memory has taken on the general meaning in much of psychology and neuroscience as active maintenance or manipulation of information held in memory, independent of the specific model of Baddeley and colleagues to which we refer with initial capital letters as 'Working Memory' or 'the Working Memory model.') In more recent years, at least as great an emphasis has been placed on variables that reflect an ability or attribute of the subject, for instance, his or her rate of articulation (Hulme et al., 1999), memory capacity (Cowan, 2001), or degree of inhibitory control (Hasher et al., 1999). Interest in these 'internal variables' is a recognition of the fact that what is 'in memory' at a moment in time is defined to various degrees by the structure of the input (e.g., time, serial order), the passive properties

of the storage medium (e.g., rate of decay, interference susceptibility), and the active processes of control that continually monitor and operate on the contents of memory. It is this last ingredient that puts the 'work' into working memory; it makes explicit the active and transformative character of mental processes and acknowledges that the content of memory need not mirror the structure and arrangement of environmental input, but rather may reflect the intentions, plans, and goals of the conscious organism.

With that introduction in mind, let us now give a brief overview of the Working Memory model of Baddeley and colleagues (Baddeley and Hitch, 1974; Baddeley, 1986). Whereas contemporary models of STM tended to emphasize storage buffers as the receptacles for information arriving from the senses, Baddeley and Hitch (1974) focused on rehearsal processes, that is, strategic mechanisms for the maintenance of items in memory. Thus, for example, when one is trying to keep a telephone or license plate number 'in mind,' a common strategy is to repeatedly rehearse, either subvocally or out loud, the contents of the numeric or alphanumeric sequence. Research had shown that in tests of serial recall, when subjects are prevented from engaging in covert rehearsal during a delay period that is inserted between stimulus presentation and recall, overall performance is dramatically impaired (Baddeley et al., 1975). In the case of verbal material, then, it was clear that in many ways the ability to keep words in memory depended in large part on articulatory processes. This insight was central to the development of the verbal component of Working Memory, the 'phonological loop' (see the section titled 'The phonological loop'), and led to a broader conceptualization of STM that seeks not only to explain how and why information enters and exits awareness, but rather how resources are marshaled in a strategic effort to capture and maintain the objects of memory in the focus of attention.

The central tenets of the Working Memory model are as follows: (1) It is a limited capacity system; at any moment in time, there is only a finite amount of information directly available for processing in memory. (2) The specialized subsystems devoted to the representation of information of a particular type, for instance, verbal or visuospatial, are structurally independent of one another; the integrity of information represented in one domain is protected from the interfering effects of information that may be arriving to another domain. (3) Storage of information in memory is distinct from the processes that underlie stimulus perception; rather, there is a two-stage process whereby

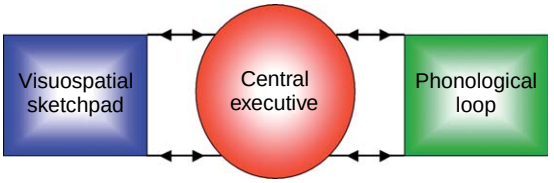


Figure 3 The Working Memory model comprises a control system, the central executive, and two storage systems, the visuospatial sketchpad and the phonological loop.

sensory information is first analyzed by perceptual modules and then transferred into specialized storage buffers that have no other role but to temporarily 'hold' preprocessed units of information. Moreover, the pieces of information that reside in such specialized buffers are subject to passive, time-based decay as well as interitem interference (e.g., similar sounding words such as 'man, mad, map, cap, mad' can lead to interference within a specialized phonological storage structure); finally, such storage buffers have no built-in or internal mechanism for maintaining or otherwise refreshing their contents – rather, this must occur from without, through the process of rehearsal, which might be a motor or top-down control mechanism that can sequentially access and refresh the contents that remain active within the store.

The initial Working Memory model proposed by Baddeley and Hitch (1974), but later refined somewhat (Salame and Baddeley, 1982; Baddeley, 1986), argued for the existence of three functional components of working memory (**Figure 3**). The 'central executive' was envisioned as a control system of limited attentional capacity responsible for coordinating and controlling two subsidiary slave systems, a phonological loop and a visuospatial sketchpad. The phonological loop was responsible for the storage and maintenance of information in a verbal form, and the visuospatial sketchpad was dedicated to the storage maintenance of visuospatial information.

3.13.2.3 The Central Executive

As has already been mentioned, working memory is viewed as a limited capacity system. There are a number of reasons for this capacity limitation, but an important one relates to what one might call the allocation of attention. Although many people are perfectly capable of walking and chewing gum at the same time, it is far more difficult to simultaneously perform more attention-demanding tasks, such as, to choose an unlikely example, monitoring the price of a stock for a quick trade while cogitating about one's next move in a highly competitive game

of chess. Thus, quite apart from the structural limitations inherent to memory storage systems (e.g., the natural inclination of memory traces to fade with time and interference), there also appear to be certain fundamental constraints on 'how much' attention can be allocated to the set of active tasks at any one time (Kahneman, 1973). The central executive component of working memory sits, as it were, at the helm of the cognitive apparatus and is responsible for the dispensation of attentional resources to the subsidiary components (e.g., the phonological loop) in working memory (Baddeley, 1986). Because total attentional capacity is finite, there must be a mechanism that intervenes to determine how the pool of attention is to be divided among the many possible actions, with their different levels of priority and reward contingencies, that are afforded by the environment. Thus, in dual-task paradigms, the central executive plays a crucial role in the scheduling and shifting of resources between tasks, and it can be used to explain the decline in performance that may be observed even when the two tasks in question involve different memory subsystems (Baddeley, 1992). Finally, it has often been pointed out that the central executive concept is too vague to act as anything other than a kind of placeholder for what is undoubtedly a much more complex system than is implied by the positing of a unitary and homunculus-like central cognitive operator (for a model of executive cognition, see Shallice, 1982). Provided, however, that the concept is not taken too literally, it can serve as a convenient way to refer to the complex and variegated set of processes that constitute the brain's executive system.

3.13.2.4 The Phonological Loop

Built into the architecture of the Working Memory model is a separation between domain-specific mechanisms of memory maintenance and domain-general mechanisms of executive control. Thus, the verbal component of working memory, or the phonological loop, is viewed as a 'slave' system that can be mobilized by the central executive when verbal material has to be retained in memory over some uncertain delay. Within the phonological loop it is the interplay of two components – the phonological store and the articulatory rehearsal process – that enables representations of verbal material to be kept in an active state. The phonological store is a passive buffer in which speech-based information can be stored for brief (approximately 2-s) periods. The articulatory control process serves to refresh and revivify the contents of the store, thus allowing the system to maintain short sequences of verbal items in memory for an extended interval. This division of labor between two interlocking components, one an active process and the other a passive store, is crucial to the model's explanatory power. For instance, when the articulatory control process is interfered with through the method of articulatory suppression (e.g., by requiring subjects to say 'hiya' over and over again), items in the store rapidly decay, and recall performance suffers greatly. The store, then, lacks a mechanism of reactivating its own contents but possesses memory capacity, whereas, conversely, the articulatory rehearsal process lacks an intrinsic memory capacity of its own, but can exert its effect indirectly by refreshing the contents of the store.

3.13.2.5 The Visuospatial Sketchpad

The other slave system in the Working Memory model is the visuospatial sketchpad, which is critical for the online retention of object and spatial information. Again, as is suggested by the term 'sketchpad,' the maintenance of visuospatial imagery in an active state requires top-down, or strategic, processing. As with the phonological loop, where articulatory suppression interferes with the maintenance of verbal information, a concurrent processing demand in the visuospatial domain, such as tracking a spot of light moving on a screen, random eye movements, or the presentation of irrelevant visual information during learning, likewise impairs memory performance. Although the symmetry between sensory and motor representations of visuospatial information is less obvious than it is in the case of speech, it has been demonstrated that saccadic rehearsal is important for the maintenance of spatial information (Postle et al., 2005). Baddeley (1986) initially proposed that in the context of spatial memory, covert eye movements can act as a way of revisiting locations in memory and thus operate very much like the articulatory rehearsal process known to be important for the maintenance of verbal information. Moreover, requiring subjects to perform a spatial interference task that disrupts or otherwise occupies this rehearsal component significantly impairs the performance of tests of spatial working memory, but has no effect on nonspatial visual memory tasks (Cocchini et al., 2002). In contrast, retention of visual shape or color information is interfered with by visual perceptual input, but not by a concurrent demand in the spatial domain (Klauer and Zhao, 2004). Thus, the principles that underlie the operation of the

phonological loop are qualitatively similar to those that underlie the operation of the visuospatial sketchpad; in both cases, maintenance processes consist of covert motor performance that serves to reactivate the memory traces residing in sensory stores. This mechanism might be most simply described as 'remembering by doing,' a strategy that is most effective when a motor code, which can be infinitely regenerated and which is under the subject's voluntary control, can be substituted for a fragile and less easily maintained perceptual memory code.

3.13.2.6 Summary

Working Memory is a system for the maintenance and manipulation of information that is stored in domain-specific memory buffers. Separate cognitive components are dedicated to the functions of storage, rehearsal, and executive control. Informational encapsulation and domain segregation dictate that auditory-verbal and visual information is kept in separate storage subsystems – the phonological loop and the visuospatial sketchpad, respectively. These storage subsystems themselves comprise specialized components for the passive storage of memory traces, which are subject to time and interference-based decay, and for the reactivation of these memory traces by way of simulation or rehearsal. Thus, storage components represent memory traces, but have no internal means of refreshing them, whereas rehearsal processes (e.g., articulatory, saccadic) have no mnemonic capacity of their own, but can reactivate the decaying traces held in temporary stores.

3.13.3 The Emergence of Working Memory as a Neuroscientific Concept

In the writings of the great neurologist Carl Wernicke, the idea that discrete pieces of cerebral cortex function as storehouses for 'memory images' is ubiquitous. For instance, the speech-perception deficit that accompanies lesions to the posterior superior temporal gyrus (STG), and that is one of the most characteristic symptoms of what is now referred to as Wernicke's aphasia, arises because this region of the auditory pathway, according to Wernicke, constitutes a location wherein 'auditory word images' are stored (Eggert and Wernicke, 1977). Indeed, Wernicke had a view of memory that shares much with some more modern formulations (Damasio, 1989; Wheeler et al., 2000; Cowan, 2001; Ruchkin et al., 2003) insofar as he viewed 'memories' as a reactivation of percepts originally formed during the sensory processing of an external stimulus:

> The sense impressions projected onto the cerebral cortex from the outside world last longer than the external stimulus affecting the sense organ; they can reappear in the form of memory images independently of the stimulus that produced them, although in less vivid form. (Eggert and Wernicke, 1977: 35)

Wernicke's ideas on memory, of course, predated the modern distinction between STM and LTM and were not intended to address the phenomenon that is today referred to as 'working memory.' Indeed, to Wernicke, perception and memory were part and parcel of the same functional–anatomical unit, whereby memory is perception evoked in the absence of direct external stimulation. The modern concept of working memory, however, distinguishes between stimulus recognition and discrimination, and the systems required to keep the products or residue of such sensory processes in the focus of attention during the temporal delays that often naturally intervene between stimulus perception and a contingent action. That is, unlike the memory images of Wernicke, which are punctate events, singular episodes, working memory entails sustained and persisting attention to an object or set of objects that exist in some upper register of the individual's consciousness. In this sense, then, the first insights into the neurobiological underpinnings of a memory whose purpose is to bridge cross-temporal contingencies (Fuster, 1997) comes from the work of Jacobsen, who studied nonhuman primate behavior after ablation to the prefrontal cortices. In comparing normal chimpanzees to those that had suffered extensive injury to the prefrontal cortex (PFC), Jacobsen (1936) noted:

> The normal chimpanzee has considerable facility in using sticks or other objects to manipulate its environment, e.g., to reach a piece of food beyond its unaided reach. It can solve such problems when it must utilize several sticks, some of which may not be immediately available in the visual field. After ablation of the prefrontal areas, the chimpanzee continues to use sticks as tools but it may have difficulty solving the problem if the necessary sticks and the food are not simultaneously present in the visual field. It exhibits also a characteristic 'memory' defect. Given an opportunity to observe a piece of food being concealed under one

> of two similar cups, it fails to recall after a few seconds under which cup the lure has been hidden. . . . (Jacobsen, 1936: 317)

In his pioneering experimental work, Jacobsen (1936) discovered that damage to the PFC of the monkey produces selective deficits in a task requiring a delayed response to the presentation of a sensory stimulus. The delayed-response tasks were initially devised by Hunter (1913) as a way of differentiating between animals on the basis of their ability to use information not currently available in the sensory environment to guide an imminent response. In the classic version of this test, a monkey is shown the location of a food morsel that is then hidden from view and placed in one of two wells. After a delay period of a few seconds, the monkey chooses one of the two locations and is rewarded if the choice corresponds to the location of the food. Variations on this test include the delayed alternation task, the delayed match-to-sample task, and the delayed nonmatch-to-sample task. The family of delayed-response tasks measures a complex cognitive ability that requires at least three clearly identifiable subprocesses: to recognize and properly encode the to-be-remembered item, to hold an internal representation of the item 'online' across an interval of time, and finally, to initiate the appropriate motor command when a response is prompted. Jacobsen showed that lesions to the PFC impair only the second of these three functions, suggesting a fundamental role for the region in immediate or short-term memory. Thus, monkeys with lesions to PFC perform in the normal range on a variety of tests requiring sensorimotor behavior, such as visual pattern discrimination and motor learning and control (i.e., tasks without a short-term mnemonic component). Although the impairments in the performance of delayed-response tasks in Jacobsen's studies were caused by large prefrontal lesions that often extended into the frontal pole and orbital surface, later studies showed that lesions confined to the region of the principal sulcus produced deficits equally as severe (Blum, 1952; Butters et al., 1972).

Fuster and Alexander (1971) reported the first direct physiological measures of PFC involvement in STM. With microelectrodes placed in the PFC, they measured the firing patterns of neurons during a spatial delayed-response task and showed that many cells showed increased firing, relative to an intertrial baseline period, during both cue presentation and the later retention period. Importantly, some cells fired exclusively during the delay period and therefore could be considered pure 'memory cells.' The results were interpreted as providing evidence for PFC involvement in the focusing of attention "on information that is being or that has been placed in temporary memory storage for prospective utilization" (p. 654). Many subsequent electrophysiological studies have demonstrated memory-related activity in the PFC of the monkey during delayed-response tasks of various kinds (e.g., Niki, 1974; Niki and Watanabe, 1976; Joseph and Barone, 1987; Quintana et al., 1988), although it was Patricia Goldman-Rakic who first drew a parallel (but see Passingham, 1985) and then firmly linked the phenomenon of persistent activity in PFC to the cognitive psychological concept of 'working memory.' In a monumental review of the existing literature on the role of the PFC in STM, Goldman-Rakic (1987), citing lesion and electrophysiological studies in the monkey, human neuropsychology, and the cytoarchitectonics and corticocortical connections of the PFC, argued that the dorsolateral PFC (the principal sulcus of the monkey) plays an essential role in holding visuospatial information in memory before the initiation of a response and in the absence of guiding sensory stimulation. In this and later work (especially that of Wilson et al., 1993), Goldman-Rakic developed a model of PFC in which visuospatial and (visual) object working memory were topographically segregated, with the former localized to the principal sulcus and the latter localized to a more ventral region along the inferior convexity of the lateral PFC (**Figure 4**).

This domain-specific view of the prefrontal organization, which was supported by observed dissociations in the responsivity of neurons in dorsal and ventral areas of the PFC during delayed-response tasks, could be viewed as an anterior expansion of the dorsal ('where') and ventral ('what') streams that had been discovered in the visual system in posterior neocortex (Ungerleider and Mishkin, 1982). In addition, the parallel and modular nature of the proposed functional and neuroanatomical architecture of PFC was in keeping with the tenet of domain independence in the Working Memory model of Baddeley and colleagues.

The connection between persistent activation in the PFC of the monkey and a model of memory developed in the field of cognitive psychology might seem tenuous, especially in light of the fact that the Working Memory model was originally formulated on the basis of evidence derived from behavioral

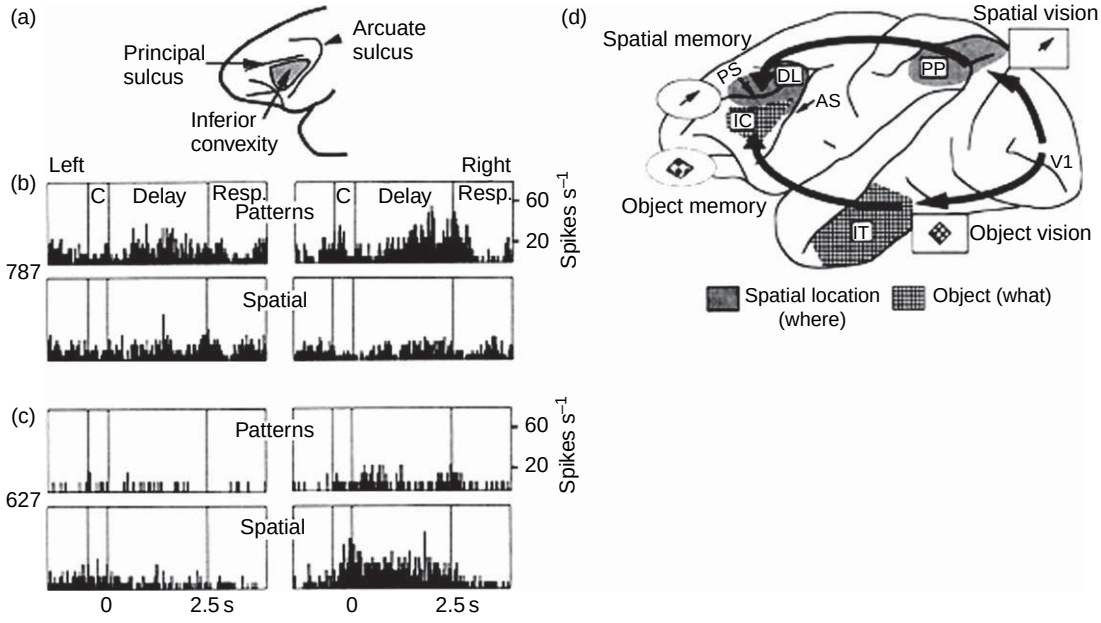


Figure 4 (a) Diagram of the frontal lobe and location of principal sulcus and inferior convexity. (b) Responses of inferior convexity neuron with visual object-specific activity in the delayed-response task. Upper panels show delay-period activity for object memory trials; lower panels show lack of response on spatial memory trials. (c) Responses of dorsolateral prefrontal neuron with spatial memory selectivity. Upper panels show lack of responsivity on object memory trials; lower panels show delay-period activity on spatial memory trials. (d) Schematic diagram illustrating the dorsal and ventral streams in the visual system and their connections with PFC. The posterior parietal (PP) is concerned with spatial perception, and the inferior temporal (IT) cortex with object recognition. These regions are connected with the dorsolateral (DL) and inferior convexity (IC) prefrontal cortices where, according to the Goldman-Rakic model, memory for spatial location and object identity are encoded in working memory. PS, principal sulcus; AS, arcuate sulcus. Adapted from Wilson FA, Scalaidhe SP, and Goldman-Rakic PS (1993) Dissociation of object and spatial processing domains in primate prefrontal cortex. *Science* 260: 1955–1958, with permission.

studies using linguistic material – an informational medium clearly unavailable to monkeys. For Goldman-Rakic, though, the use of the term 'working memory' in the context of nonhuman primate electrophysiology was not intended as an offhand or otherwise desultory nod to psychology (Goldman-Rakic 1990), but rather as a reasoned and deliberate effort to unify both our understanding of, and manner of referencing, a common neurobiological mechanism underlying an aspect of higher cognition that is well developed in primate species. Certainly, in retrospect, the decision to label the phenomenon of persistent activity in PFC with the term 'working memory' has had an immeasurable impact on memory research and indeed may be thought of as one of the two or three most important events contributing to the emergence of an integrated and unified approach to the study of neurobiology and psychology. Nowhere was this fusion between psychology and neurobiology more apparent, and nowhere were the ideas of Goldman-Rakic on visuospatial working memory more energetically tested and challenged, than in the realm of functional brain imaging.

3.13.3.1 Functional Neuroimaging Studies of Working Memory

At about the same time at which Fuster and Alexander (1971) recorded neural activity in the monkey PFC during a working memory task, Ingvar and colleagues (Ingvar, 1977; Ingvar and Risberg, 1965) examined variation in regional cerebral blood flow (rCBF) during tasks requiring complex mental activity. Indeed, Risberg and Ingvar (1973), in the first functional neuroimaging study of STM, showed that during a backward digit span task, the largest increases in rCBF, compared with a resting baseline, were observed in prerolandic and anterior frontal cortex. It was not, however, until the emergence of PET and the development of the O-15 tracer that the mapping of brain activity during the exercise of higher mental functions would become genuinely

amenable to the evaluation of complex hypotheses about the neural basis of cognition. In the middle and late 1980s, technological advances in the PET technique, with its relatively high spatial resolution (approximately 1 cm^3), were accompanied by a critical conceptual innovation known as 'cognitive subtraction' that provided the inferential machinery needed to link regional variation in brain activity to experimental manipulations at the task or psychological level (Posner et al., 1988). Thus, for any set of hypothesized mental processes (*a,b,c*), if a task can be devised in which one condition recruits all of the processing components (Task $1_{a,b,c}$) and another condition recruits only a subset of the components (Task $2_{a,b}$), subtraction of the observed regional activity during Task 2 from that observed during Task 1 should reveal the excess neural activity due to the performance of Task 1, and thus is associated with the cognitive component *c*. The Working Memory model of Baddeley, with its discrete cognitive components (e.g., central executive, phonological loop, and visuospatial scratchpad) was an ideal model with which to test the power of cognitive subtraction using modern neuroimaging tools. Indeed, in the span of only 2 years, the landmark studies of Paulesu et al. (1993), Jonides et al. (1993), and D'Esposito (1995), had mapped all of the cognitive components of the Working Memory model onto specific regions of the cerebral cortex. The challenge in successive years was to go beyond this sort of 'psychoneural transcription' – which is necessarily a unidirectional mapping between the cognitive box and the cerebral convolution – and begin to develop models that generate hypotheses that refer directly to the brain regions and mechanisms that underlie working memory. In the following sections, we review how neuroimaging studies of STM and executive control used the Working Memory model to gain an initial neural foothold on which later studies were buttressed and that would lead to insights and advances in our understanding of working memory as it is implemented in the brain.

3.13.3.2 Visuospatial Working Memory

The first study of visuospatial working memory in PET was carried out by Jonides and colleagues in 1993, using the logic of cognitive subtraction to isolate mnemonic processes associated with the maintenance of visuospatial information, in a task very similar to those used by Goldman-Rakic and her colleagues with monkeys (Goldman-Rakic 1987; Funahashi et al., 1989). During 'memory' scans, subjects were shown an array of three dots appearing for 200 ms on the circumference of a 14-mm imaginary circle and instructed to maintain the items in memory during a 3-s retention interval. This was followed by a probe for location-memory consisting of a circular outline that either did or did not (with equal probability) enclose one of the previously memorized dots, and to which subjects responded with a yes/no decision. In 'perception' scans, the three dots and the probe outline were presented simultaneously, so that subjects did not have to retain the location of the items in memory during a delay, but instead simply had to decide whether the outline encircled one of the three displayed dots (see **Figure 5**).

Subtraction of the 'perception' scans from the 'memory' scans revealed a right-lateralized network of cortical regions that would become a hallmark of neuroimaging studies of visuospatial working memory: the posterior parietal lobe, dorsal premotor cortex, occipital cortex (Brodmann area 19), and PFC. In their interpretation of the findings, the

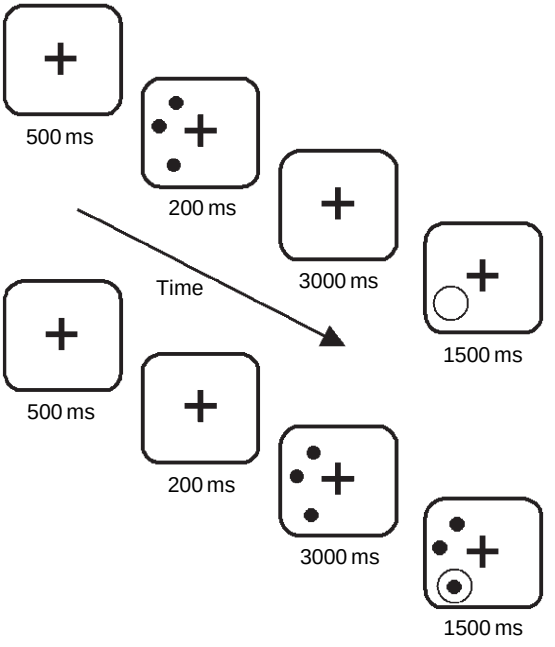


Figure 5 Schematic presentation of spatial memory task from Jonides J, Smith EE, Koeppe RA, Awh E, Minoshima S, and Mintun MA (1993) Spatial working memory in humans as revealed by PET. *Nature* 363: 623–625 and Smith EE, Jonides J, Koeppe RA, Awh E, Schumacher EH, and Minoshima S (1995) Spatial versus object working-memory – PET investigations. *J. Cogn. Neurosci.* 7: 337–356. Top panel shows example memory trial; bottom panel shows example perception trial.

authors suggested that the occipital activity reflected a role in the creation, but not necessarily the maintenance, of an internal visual image of the dot pattern, and that activity in the PFC might reflect one of two things: (1) the literal storage of a representation of the image in memory during the delay, or (2) the representation of a pointer or link to other brain circuitry, perhaps in the occipital or parietal lobe, that is actually responsible for maintaining the memory engram. These two explanations for the observation of prefrontal activity during working memory tasks, which in later years would often be pitted against each other, nicely frame the emerging debate on the division of labor among the cortical regions involved in the maintenance of information in working memory.

A major aim of many of the early neuroimaging studies of visuospatial working memory was to duplicate the canonical finding of Goldman-Rakic and colleagues of a dorsal-ventral dissociation in monkey PFC for spatial and object working memory. Studies by Petrides et al. (1993) and McCarthy et al. (1994) demonstrated with PET and functional MRI (fMRI), respectively, that middorsolateral PFC (Brodmann areas 9 and 46) shows increased activity during spatial working memory when compared with a control condition. An attempt to show a neuroanatomical double dissociation between spatial and object working memory was undertaken by Smith et al. (1995) in a PET study that used carefully controlled nonverbalizable object stimuli that were presented in both object and spatial task contexts. This study found distinct brain circuits for the storage of spatial and object information, with spatial working memory relying primarily on right-hemisphere regions in the prefrontal (BA 46) and parietal (BA 40) cortices, and object working memory involving only a left inferotemporal area. These results, however, only partially replicated the monkey study of Wilson et al. (1993), who had found distinct regions in PFC for spatial and object working memory. A similar pattern was found a year later in work by McCarthy et al. (1996), in which regional differences between object and spatial working memory were most pronounced across hemispheres rather than between dorsal and ventral divisions of the PFC. In a contemporaneous review and meta-analysis of all human neuroimaging studies of working memory, D'Esposito et al. (1998) showed that there was virtually no evidence for a neuroanatomical dissociation between spatial and object working memory. Indeed, establishing a correspondence between the functional neuroanatomy of visuospatial working memory in the monkey and human brains would prove remarkably difficult, leading to a protracted debate among and between monkey neurophysiologists and human neuroimaging researchers about the proper way to conceptualize the functional topography of working memory in the PFC (Goldman-Rakic, 2000; Miller, 2000). Increasingly, efforts were made to adapt human neuroimaging studies to resemble as closely as possible the kinds of tasks used in animal electrophysiology, such as the delayed match-to-sample procedure. The emergence of event-related fMRI, with its superior spatial and temporal resolution to O-15 PET, was critical to this new effort at cross-disciplinary synthesis and reconciliation and led to a number of fundamental insights on the brain basis of working memory, to the discussion of which we now turn.

Early PET studies of working memory relied exclusively on the logic of cognitive subtraction to isolate hypothesized components of a complex cognitive task. Thus, even for working memory tasks that consisted of a number of temporal phases within a given trial (e.g., stimulus presentation → memory maintenance → recognition decision), the low temporal resolution of PET prohibited separate statistical assessment of activity within a single task phase. Event-related fMRI, on the other hand, with its temporal resolution on the order of 2 to 4 s, could be used to examine functional activity in different portions of a multiphase trial, provided that each of the sequential task components was separated by approximately 4 s (Zarahn et al., 1997). This methodology permits the isolation of maintenance-related activity during the delay period of a match-to-sample procedure without relying on a complex cognitive subtraction (**Figure 6**).

Using event-related fMRI, Courtney et al. (1998) demonstrated a neuroanatomical dissociation between delay period activity during working memory maintenance for either the identity (object memory) or location (spatial memory) of a set of three face stimuli. Greater activity during the delay period on face identity trials was observed in the left inferior frontal gyrus, whereas greater activity during the delay period of the location task was observed in dorsal frontal cortex, a finding consistent with the spatial/object domain segregation thesis of Goldman-Rakic (1987). Unlike previous studies that had implicated human BA 46 – the presumed homologue to the monkey principal sulcus – in spatial working memory, Courtney observed enhanced delay-period activity for the location task, bilaterally, in the superior frontal sulcus, a region just anterior to the frontal eye fields.

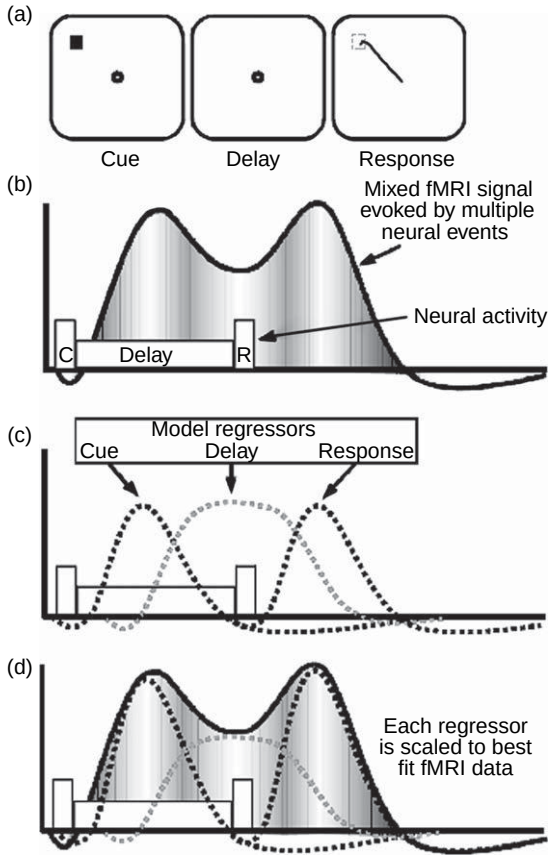
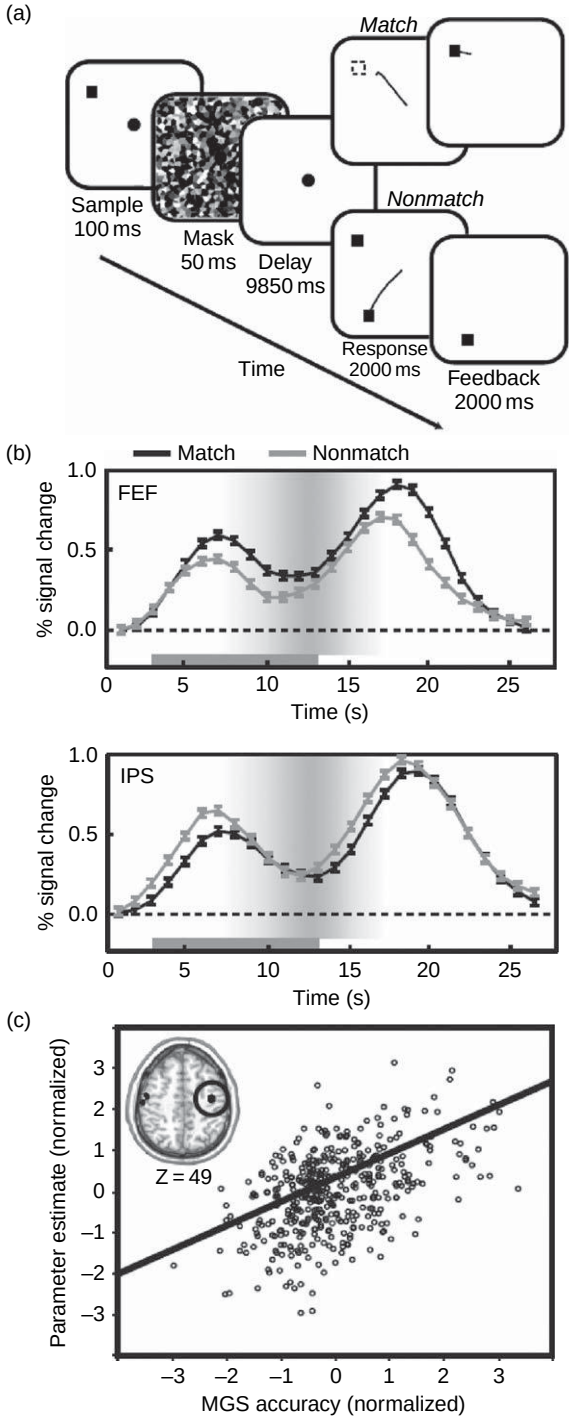


Figure 6 Delayed-response task and modeling with trial components of event-related fMRI data. (a) A prototypic spatial delayed-response task, like all delayed-response tasks, has three main epochs: a sample cue period where stimuli-to-be-remembered are presented, an unfilled delay period where stimuli are retained in memory, and finally a response period where a memory-guided response is required. (b) When multiple sequential neural events occur within a trial, the resulting fMRI response (R) is a mixture of signals arising from more than one time and more than one trial component. The gradient under the curve schematically represents the mixing or temporal overlap of the various signal components. For example, the white region at the peak of the first hump is evoked almost exclusively from neural processing during the cue phase of the task. However, just a few seconds later, in the darker portion just to the right, the signal is a mixture of processing at the cue phase and the beginning of the delay period. (c) In order to resolve the individual components of the mixed fMRI signal, separate regressors can be used to independently model the cue, delay, and response phases of the trial. (d) The magnitudes of the regressors scale with the degree to which they account for variance in the observed time series data. The magnitude of the delay regressor can be used as an index for maintenance-related activity.

A control task requiring sensory guided eye movements was used to functionally delineate the frontal eye fields and thus distinguish them from regions with a specifically mnemonic function. They concluded that the localization of spatial working memory in the superior frontal sulcus (posterior and superior to BA 46) indicated an evolutionary displacement in the functional anatomy of the PFC, possibly due to the emergence of new cognitive abilities such as abstract reasoning, complex problem solving, and planning for the future. In short, then, this study was the first functional neuroimaging study to fully replicate the object versus spatial working memory dissociation shown by Goldman-Rakic and colleagues, insofar as one accepts their proposal that the human homologue to the monkey principal sulcus is located not in the middle frontal gyrus or BA 46, but rather in the superior frontal sulcus.

The study by Courtney represented a high water mark in the level of agreement between human neuroimaging and monkey electrophysiological studies of visuospatial working memory and their mutual correspondence to the Goldman-Rakic conception of a domain-segregated topography of prefrontal cortical function. Although several subsequent studies of spatial working memory offered support (Munk et al., 2002; Sala et al., 2003; Walter et al., 2003; Leung et al., 2004) for a specifically mnemonic role of the superior frontal sulcus in tasks of spatial working memory, other studies failed to replicate the finding (Postle and D'Esposito, 1999; Postle et al., 2000; Postle, 2006). The primary disagreement concerns not whether delay-period activity is found in the superior frontal sulcus during spatial working memory – it is – but rather whether such activity subserves an exclusively mnemonic or storage function. For instance, although Postle et al. (2000) observed delay-period activity in this region during a spatial working memory task, they also found it to be equally active during the generation of two-dimensional saccades, a task that required visuospatial attention and motor control but placed no demands on memory storage. In addition, the neural circuitry underlying spatial selective attention largely overlaps with that of spatial working memory (Corbetta et al., 2002), including the superior frontal sulcus, the frontal eye fields, and the intraparietal sulcus (IPS). Curtis et al. (2004) scanned subjects while they performed an oculomotor delayed-response task that required maintenance of the spatial position of a single dot of light over a delay period after which a memory-guided saccade was generated. Both frontal eye fields (FEF) and IPS delay-period activity showed activity that spanned the entire delay period (**Figure 7**). In addition, the magnitude of FEF and IPS delay-period activity predicted the accuracy of the memory-guided saccade generated after the

delay. This relationship suggests that the fidelity of the stored location is reflected in the delay-period activity. One interpretation of this result is that the persistent activity observed in the PFC reflects the operations of control processes that do not store information *per se*, but rather act to focus attention via top-down signals to posterior parietal cortex, toward particular locations in space.



3.13.3.3 Visual Object Working Memory

A number of studies have investigated the maintenance of objects, mostly visually presented faces, houses, and line drawings that are not easily verbalizable (e.g., Smith et al., 1995; Courtney et al., 1996, 1997; McCarthy et al., 1996; Belger et al., 1998; Postle and D'Esposito, 1999; Druzgal and D'Esposito, 2001, 2003; Rama et al., 2001; Mecklinger et al., 2002; Linden et al., 2003; Postle et al., 2003; Sala et al., 2003). Consistently, posterior cortical areas within the inferior temporal lobe that normally respond to the visual presentation of select objects also tend to activate during object working memory tasks. Therefore, the temporal lobe appears to play an important role in short-term storage of object features. For example, the fusiform gyrus, the ventral convexity surface of the temporal lobe, shows greater activation when a subject is shown pictures of faces than when other types of complex visual stimuli such as pictures of houses or scenes or household objects are presented (Kanwisher et al., 1997). Indeed, given its selective response properties, the fusiform gyrus has been termed the fusiform face area or FFA.

Four important findings indicate that posterior extrastriate cortical regions like the FFA play an important role in the mnemonic storage of object features. First, the FFA shows persistent delay-period activity (Druzgal and D'Esposito, 2001, 2003; Rama et al., 2001; Postle et al., 2003) during working memory tasks. Second, the activity in the FFA is somewhat selective for faces; it is greater during delays in which subjects are maintaining faces compared to with other objects (Sala et al., 2003). Third, as the number of faces that are being maintained increases, the magnitude of the delay-period activity increases in the FFA (Jha and McCarthy, 2000; Druzgal and D'Esposito, 2001, 2003). Such load effects strongly suggest a role in STS because, as the number of items that must be represented increases, so should the storage demands. Fourth, using a delayed paired associates task, Ranganath et al. (2004) have shown that the FFA responds during an unfilled delay interval following the presentation of a house that the subject has learned is associated with a certain face. Therefore, the delay-period FFA activity likely reflects the reactivated image of the associated face that was retrieved from LTM despite the fact that no face was actually presented before the delay. Together, these studies suggest that posterior regions of visual association cortex, like the FFA, participate in the internal storage of specific classes of visual object features. Most likely, the mechanisms used to create internal representations of objects that are no longer in our environment are similar to the mechanisms used to represent objects that exist in our external environment.

There have been several reports of delay-period-specific activations in the PFC during object working memory tasks as well (e.g., Courtney et al., 1998; Postle et al., 1999; Jha and McCarthy, 2000; Nystrom et al., 2000; Stern et al., 2000; Rama et al., 2001; Munk et al., 2002; Pessoa et al., 2002; Druzgal and D'Esposito, 2003; Ranganath et al., 2003; Sala et al., 2003). However, the localization of the delay-period activity appears varied across the dorsal, ventral, and medial portions of the PFC. The most consistent

Figure 7 Event-related study of spatial working memory by Curtis et al. (2004). (a) Schematic depiction of the oculomotor delayed-response tasks where subjects used the cue's location to make a memory-guided saccade. Both the matching-to-sample (top) and nonmatching-to-sample (bottom) tasks began with the brief presentation of a small. During matching trials, the subject made a memory-guided saccade (depicted by the thin black line) after the disappearance of the fixation cue marking the end of the delay. Feedback was provided by the representation of the cue. At this point, the subject corrected any errors by shifting gaze to the cue. The difference between the endpoint fixation after the memory-guided saccade and the fixation to acquire the feedback cue was used as an index of memory accuracy. During nonmatching trials, the subject made a saccade to the square that did not match the location of the sample cue. (b) Average (± S.E. bars) bold time series data for matching (black) and nonmatching-to-sample (gray) oculomotor delayed-response tasks. The solid gray bar represents the delay interval. The gray gradient in the background depicts the probability that the bold signal is emanating from the delay period, where darker indicates more probable. The frontal eye fields (FEF) show greater delay period activity during the matching task where an oculomotor strategy is efficient. The right intraparietal sulcus (IPS) shows greater delay period activity during the nonmatching task when subjects are biased from using such a strategy. (c) Scatter plot showing the correlation between memory-guided saccade (MGS) accuracy and the magnitude of the delay period parameter estimates in the right FEF. More accurate MGS were associated with greater delay period activity. From Curtis CE, Rao VY, and D'Esposito M (2004) Maintenance of spatial and motor codes during oculomotor delayed response tasks. *J. Neurosci.* 24: 3944–3952; used with permission.

finding in that regard may be a greater bias toward right hemisphere activation for object working memory compared with verbal working memory.

3.13.3.4 Verbal Working Memory

Research on the neural basis of verbal working memory has, for a number of reasons, taken a rather different course from corresponding work in the visuospatial domain. First, whereas in visual working memory many of the most influential ideas and concepts have derived from work in the monkey, verbal working memory is a uniquely human phenomenon and has therefore benefited from animal research only indirectly or by analogy with the visual system. Even research on the primary modality relevant to verbal working memory, that of audition, is surprisingly scarce in the monkey literature, owing to the difficulty in training nonhuman primates to perform delayed-response tasks with auditory stimuli, which can take upwards of 15 000 learning trials (see Fritz et al., 2005). On the other hand, an entirely different state of affairs prevails in the field of human cognitive psychology, where verbal short-term and working memory has over the last 40 years been studied extensively, almost to the exclusion of other modalities, resulting in thousands of published articles, a host of highly reliable and replicated behavioral phenomena, and dozens of sophisticated computational models. Finally, the study of aphasic patients has provided a wealth of information about the neural circuitry underlying language, and systematic neurological and neuropsychological inquiries into the impairments that accompany damage to the language system have yielded detailed neuroanatomical models. The aphasia literature notwithstanding, the study of the neural basis of verbal working memory has depended, to a much greater extent than has been the case in the visuospatial domain, on pure cognitive models of memory, in particular the phonological loop of Baddeley and colleagues. Not surprisingly as it turns out, there are notable similarities between working memory for visual and linguistic material, despite the absence of an exactly analogous capacity in nonhuman primates.

Early neurological investigations of patients with language disturbances or aphasia revealed that lesions to specific parts of the cerebral cortex could cause extremely selective deficits in language abilities. Thus, lesions to the inferior frontal gyrus are associated with Broca's aphasia, a disorder that causes severe impairments in speech production. Broca's aphasia is not, however, a disorder of peripheral motor coordination, such as the ability to move and control the tongue and mouth, but rather is a disorder of the ability to plan, program, and access the motor codes required for the production of speech (Goodglass, 1993). The functions of speech perception and comprehension in Broca's aphasia are generally preserved, however. Lesions to the posterior superior temporal gyrus and surrounding cortex, on the other hand, are associated with Wernicke's aphasia, a complex syndrome that is characterized by fluent, but error-filled, production and poor comprehension and perception of speech. A third, less-studied syndrome called conduction aphasia, typically caused by lesions in the posterior sylvian region (generally less extensive and relatively superior to lesions causing Wernicke's aphasia), is associated with preserved speech perception and comprehension, occasional errors in otherwise fluent spontaneous speech (e.g., phoneme substitutions), and severe difficulties with verbatim repetition of words and sentences (Damasio and Damasio, 1980). From the standpoint of verbal STM, a number of important points can be drawn from these three classic aphasic syndromes. First, the neural structures that underlie the perception and production of speech are partly dissociable. Thus, it appears that the brain retains at least two codes for the representation of speech: a sensory, or acoustic, code and an articulatory, or motor, code; the former is necessary for the perception of speech, and the latter is required for the production of speech. It is tempting to postulate that posterior temporal lesions primarily affect receptive language functions, whereas anterior lesions affect productive language functions – but this is not quite true; both Wernicke's aphasia and conduction aphasia are caused by posterior lesions, yet only the former is associated with a receptive language disturbance (Hickok and Poeppel, 2000). Second, all the aforementioned disorders affect basic aspects of language processing, such as the comprehension, production, and perception of speech. Even conduction aphasia, for which a deficit in repetition of speech is often emphasized, is characterized by speech errors that occur in the course of natural language production. Finally, the classical Wernicke-Lichteim-Geschwind (Geschwind, 1965) model of language explains each of these three syndromes as disruptions to components of a neuroanatomical network of areas, in the inferior frontal and superior temporal cortices, that subserve language function.

In the 1960s a handful of patients were described that did not fit nicely into the classic aphasiological rubric. Both Luria et al. (1967) and Warrington and

Shallice (1969) described patients with damage to the temporoparietal cortex who were severely impaired at repeating sequences of words or digits spoken aloud by the experimenter. Luria referred to the deficit as an acoustic-amnestic aphasia, whereas Warrington and Shallice (1969), who were perhaps more attuned to extant information-processing models in cognitive psychology, referred to the deficit as a "selective impairment of auditory-verbal short-term memory" (p. 885) In both of these cases, however, the memory impairment was accompanied by a deficit in ordinary speech production (i.e., word-finding difficulties, errors of speech, and reading difficulty), which was, in fact, consistent with the rather routine diagnosis of conduction aphasia, and therefore complicated the argument in favor of a pure memory impairment. Several years later, however, a patient (J. B.) (Shallice and Butterworth 1977), also with a temporoparietal lesion, was described who had a severely reduced auditory–verbal immediate memory span (one or two items) and yet was otherwise unimpaired in ordinary language use, including speech production and even long-term learning of supraspan lists of words. Several other such patients have since been described (for a review, see Shallice and Vallar, 1990), thus strengthening the case for the existence of an auditory–verbal storage component located in temporoparietal cortex.

The puzzle, of course, with respect to the classic neurological model of language discussed earlier, is how a lesion in the middle of the perisylvian speech center could produce a deficit in auditory–verbal immediate memory without any collateral deficit in basic language functioning. One possibility is that the precise location of the brain injury is determinative, so that a particularly focal and well-placed lesion in temporoparietal cortex might spare cortex critical for speech perception and production, while damaging a region dedicated to the storage of auditory–verbal information. However, the number of patients that have been described with a selective impairment to auditory–verbal STM is small, and the lesion locations that have been reported are comparable to those that might, in another patient, have led to conduction or Wernicke's aphasia (Damasio, 1992; Goodglass, 1993; Dronkers et al., 2004). This would seem, then, to be a question particularly well suited to high-resolution functional neuroimaging.

Systematic investigations of STM patients on tests of verbal working memory were essential to the logical development of the phonological loop (Vallar and Baddeley, 1984). For instance, the phonological store component – a passive buffer capable of storing approximately 2 s worth of speech-based information – is a descendant of the auditory–verbal store of Warrington and Shallice. Although Baddeley and colleagues considered neuropsychological investigations to be an extremely useful source of evidence for the development of an information-processing model of verbal working memory, they did not explicitly link the hypothesized components of the loop to regions of the brain. Thus, when the first functional neuroimaging studies of the neural correlates of the phonological loop were carried out, they were done without strong *a priori* neuroanatomical predictions – unlike the early PET studies of visuospatial working memory, which were guided by a large body of monkey literature.

Before we discuss these neuroimaging efforts, it is important to review in slightly more detail certain key aspects of the phonological loop. As has already been discussed in this chapter, the concept of a buffer or a memory store implies an independence from perceptual or motor processing. Thus, the storage component in the phonological loop – the phonological store – plays no direct role in the sensory analysis and processing of acoustic input. How, then, does information arrive to the phonological store? It turns out that information can enter the store in various ways. Verbal information that is presented visually must first be subvocalized, before it enters the store. Subvocalization (or silent speech) is necessary to recode a visual-orthographic stimulus into a phonological form. In contrast, acoustic information has direct and obligatory access to the phonological store. Despite this asymmetry in the manner in which auditory- and visual-verbal information enters the store, the representational code of the store is not modality specific, in the sense that it is not strictly tied to an acoustic input source. Once verbal material has entered the phonological store, it begins to decay rapidly. Phonological traces within the store can be refreshed, however, through the operation of the articulatory rehearsal process, which can cycle or loop through the contents of the store, serially reviving each of the decaying memory traces.

Faced with this more or less abstract model of the cognitive architecture of verbal working memory, the functional neuroimager must formulate some simple heuristics that can help constrain the neuroanatomical space in which to search for the various components of the phonological loop. For instance, the articulatory rehearsal process is wholly dependent on the brain circuit that underlies speech production, and

therefore one would expect that the operation of this component would rely to a large extent on the contribution of the inferior frontal gyrus, or Broca's area. Clearly, because acoustic information has obligatory access to the phonological store, one should expect to find its neural correlate in a region that activates during passive auditory stimulation (Becker et al., 1999; Chein and Fiez, 2001). In addition, because visual–verbal information enters the phonological store only by way of the articulatory rehearsal process, one should expect both silent reading (accompanied by subvocalization) and subvocal rehearsal to activate the phonological store. On the other hand, one should not expect to find the neural correlate of the phonological store in a region that is known to be critical for speech perception proper because of the model's explicit separation of perceptual and storage modules. The last heuristic is probably the most important and probably the most philosophically problematic: It has to be assumed that the modular organization of the phonological loop is reflected by a similarly modular organization in the brain – which is to say that one must stipulate that the same brain region cannot simultaneously fulfill the role of more than one component of the model. Thus, one must assume that the articulatory rehearsal process and the phonological store are not both located in the same brain region or set of regions. In fact, though, there is ample evidence from neuropsychological investigations that the articulatory rehearsal and phonological store components do not share a common neuroanatomical substrate (e.g., Vallar et al., 1997).

The first study that attempted to localize the components of phonological loop in the brain was that of Paulesu and colleagues (1993). In one task, English letters were visually presented on a monitor, and subjects were asked to remember them. In a second task, letters were presented, and rhyming judgments were made about them (press a button if letter rhymes with 'B'). In a baseline condition, Korean letters were visually presented, and subjects were asked to remember them using a visual code. According to the authors' logic, the first task would require the contribution of all the components of the phonological loop – subvocal rehearsal, phonological storage, and executive processes – whereas the second (rhyming) task would only require subvocal rehearsal and executive processes. This reasoning was based on previous research showing that when letters are presented visually (Vallar and Baddeley, 1984), rhyming decisions engage the subvocal rehearsal system, but not the phonological store. Thus, a subtraction of the rhyming condition from the letter-rehearsal condition should isolate the neural locus of the phonological store. First, results were presented for the two tasks requiring phonological processing with the baseline tasks (viewing Korean letters) that did not. Several areas were shown to be significantly more active in the phonological tasks, including (in all cases, bilaterally) Broca's area (BA44/45), the supplementary motor cortex (SMA), the insula, the cerebellum, Brodmann area 22/42, and Brodmann area 40. Subtracting the rhyming condition from the phonological STM condition left a single brain area: Brodmann area 40 (BA 40) – the neural correlate of the phonological store.

Not surprisingly the articulatory rehearsal process recruited a distributed neural circuit that included the inferior frontal gyrus. The implication of multiple brain regions during articulatory rehearsal is not surprising, given the complexity of the process and the variety of lesion sites associated with a speech-production deficit. On the other hand, the localization of the phonological store in a single brain region, BA 40 (or the supramarginal gyrus), comports with the idea of a solitary receptacle where phonological information is temporarily stored. A number of follow-up PET studies, using various tasks and design logic, generally replicated the basic finding of the Paulesu study, namely, a fronto-insular-cerebellar network associated with rehearsal processes and a parietal locus for the phonological store (Awh et al., 1996; Salmon et al., 1996; Schumacher et al., 1996; Jonides et al., 1998; Smith and Jonides, 1999).

In a perspicacious review of these premillennial PET studies of verbal working memory, Becker et al. (1999) questioned whether the localization of the phonological store in BA 40 of the parietal cortex could be reconciled with the logical architecture of the phonological loop. They noted that because auditory material has obligatory access to the store, its neural correlate ought to show robust activation during simple auditory perception. Functional neuroimaging studies of passive auditory listening, however, do not show activity in the parietal lobe, but are typically circumscribed to the superior temporal lobe (e.g., Binder et al., 2000). In addition, efforts to show verbal mnemonic specificity to the parietal lobe activation were uniformly unsuccessful, showing instead that working memory for words, visual objects, and spatial locations all activated the area (Nystrom et al., 2000; Zurowski et al., 2002). Thus, it would appear that if there were a true neural correlate to the phonological store, it must reside within the confines of the auditory cortical zone of the superior temporal cortex.

As was the case in the visuospatial domain, the emergence of event-related fMRI, with its ability to isolate delay-period activity during working memory, was an inferential boon to the study of verbal working memory. Postle et al. (1999) showed, with visual–verbal presentation of letter stimuli, that delay-period activity in single subjects was often localized in the posterior superior temporal cortex rather than in the parietal lobe. Buchsbaum et al. (2001) also used an event-related fMRI paradigm, in which, on each trial, subjects were presented with acoustic speech information that they then rehearsed subvocally for 27 s, followed by a rest period.

Analysis focused on identifying regions that were responsive both during the perceptual phase and the rehearsal phase of the trial. Activation occurred in two regions in the posterior superior temporal cortex, one in the posterior superior temporal sulcus (STS) bilaterally and one along the dorsal surface of the left posterior planum temporale, that is, in the Sylvian fissure at the parietal-temporal boundary (area Spt). Notably, although the parietal lobe did show delay-period activity, it was unresponsive during auditory stimulus presentation. In a follow-up study, Hickok (2003) showed that the same superior temporal regions (posterior STS and Spt) were active both during the perception and delay-period maintenance of short (5 s) musical melodies, suggesting that these posterior temporal storage sites are not restricted to speech-based, or phonological, information (**Figure 8**).

In addition, Stevens (2004) and Rama et al. (2004) have shown that memory for voice identity, independent of phonological content (i.e., matching speaker identity as opposed to word identity), selectively activates the mid-STS and the anterior STG of the superior temporal region, but not the more posterior and dorsally situated Spt region. Buchsbaum et al. (2005) have further shown that the mid-STS is more active when subjects recall verbal information that is acoustically presented than when the information is visually presented, whereas area Spt shows equally strong delay-period activity for both auditory and

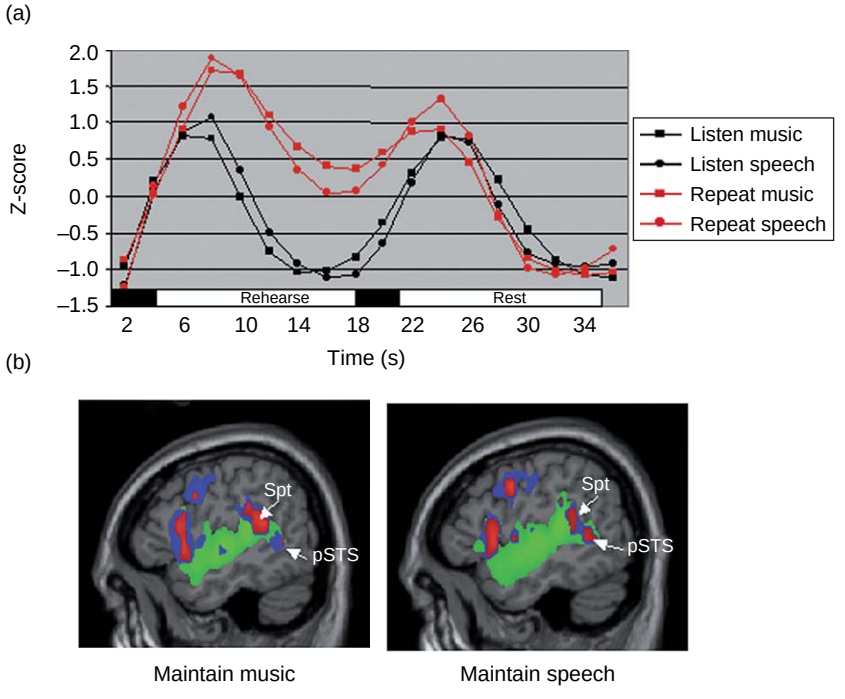


Figure 8 Main results from Hickok et al. (2003) study of verbal and musical working memory maintenance. (a) Averaged time course of activation over the course of a trial in area Spt for speech and music conditions. Timeline at bottom shows structure of each trial; black bars indicate auditory stimulus presentation. Red traces indicate activation during rehearsal trials, black traces indicate activity during listen-only trials in which subjects did not rehearse stimuli at all. (b) Activation maps of in the left hemisphere (sagittal slices) showing three response patterns for both music rehearsal (left) and speech rehearsal trials (right): auditory-only responses shown in green; delay-period responses shown in blue; and auditory + rehearsal responses shown in red. Arrows indicate the location of area Spt. pSTS, posterior superior temporal sulcus. From Hickok G, Buchsbaum B, Humphries C, and Muftuler T (2003) Auditory-motor interaction revealed by fMRI: Speech, music, and working memory in area Spt. *J. Cogn. Neurosci.* 15: 673–682; used with permission.

visual forms of input. Thus, it appears that different regions in the auditory association cortex of the superior temporal cortex are attuned to different qualities or features of a verbal stimulus, such as voice information, input modality, phonological content, and lexical status (e.g., Martin and Freedman, 2001) – and all these codes may play a role in the short-term maintenance of verbal information.

Additional support for a feature-based topography of auditory association cortex comes from neuroanatomical tract-tracing studies in the monkey that have revealed separate temporo-prefrontal pathways arising along the anterior–posterior axis of the superior temporal region (Romanski et al., 1999; Romanski, 2004). The posterior part of the STG projects to dorsolateral PFC (BA 46, 8), whereas neurons in the anterior STG are more strongly connected to the ventral PFC, including BA 12 and 47. Several authors have suggested, similar to the visual system, a dichotomy between ventral-going auditory-object and a dorsal-going auditory-spatial processing streams (Rauschecker and Tian, 2000; Tian et al., 2001). Thus, studies have shown that the neurons in the rostral STG show more selective responses to classes of complex sounds, such as vocalizations, whereas more caudally located regions show more spatial selectivity (Rauschecker and Tian, 2000; Tian et al., 2001). Hickok and Poeppel (2004, 2000) have proposed that human speech processing also proceeds along diverging auditory dorsal and ventral streams, although they emphasize the distinction between perception for action, or auditory-motor integration, in the dorsal stream and perception for comprehension in the ventral stream. Buchsbaum et al. (2005) have shown with fMRI time series data that, consistent with the monkey connectivity patterns, the most posterior and dorsal part of the superior temporal cortex, area Spt, shows the strongest functional connectivity with dorsolateral and posterior (premotor) parts of the PFC, whereas the midportion of the STS is most tightly coupled with BA 12 and 47 of the ventrolateral PFC (see **Figure 9**). Moreover, in a gross distinction between anterior (BA 47) and posterior (BA 44/6), parts of the PFC have been associated with conceptual-semantic and phonological-articulatory aspects of verbal processing (Poldrack et al., 1999; Wagner et al., 2001).

Earlier we posed the question of how a lesion in posterior sylvian cortex, an area of known importance for online language processing, could occasionally produce an impairment restricted to phonological STM. One solution to this puzzle is that subjects

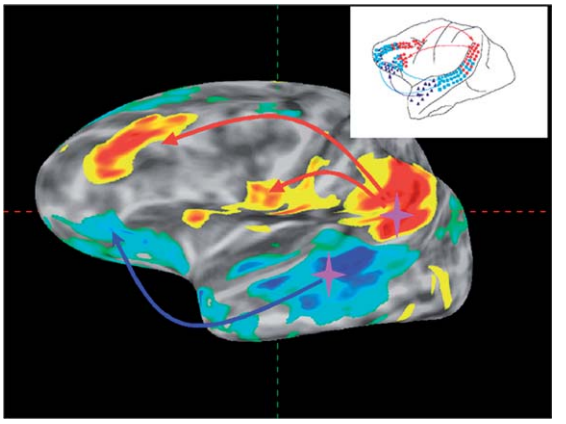


Figure 9 Map of functional connectivity delay-period maintenance of verbal stimuli from Buchsbaum et al. (2005). Seed regions for correlation analysis are denoted by stars located in area Spt and the middle part of the STS. Warm colors show areas more strongly correlated with Spt than with STS; cold colors show areas more strongly correlated with STS than Spt. Inset shows temporal-prefrontal connectivity in the monkey. From Buchsbaum BR, Olsen RK, Koch P, and Berman KF (2005) Human dorsal and ventral auditory streams subserve rehearsal-based and echoic processes during verbal working memory. *Neuron* 48: 687–697; used with permission. Inset from Scott SK and Johnsrude IS (2003) The neuroanatomical and functional organization of speech perception. *Trends Neurosci.* Feb; 26(2): 100–107; used with permission.

with selective verbal STM deficits from posterior temporal lesions retain their perceptual and comprehension abilities due to the sparing of the ventral stream pathways, whereas the preservation of speech production is due to an unusual capacity in these subjects for right-hemisphere control of speech. The STM deficit arises, then, from a selective deficit in auditory-motor integration – or the ability to translate between acoustic and articulatory speech codes – a function that is especially taxed during tests of repetition and STM (Hickok et al., 2003; Hickok and Poeppel, 2004).

The study of the neural basis of verbal working memory has proceeded from a large body of human neurological evidence pointing to the critical role of anterior regions (e.g., Broca's area) in speech production and posterior regions (e.g., temporoparietal cortex) in perceptual and mnemonic aspects of speech processing. This contrasts rather sharply with neurobiological investigations of spatial working memory, which was initially driven almost entirely by studies in the monkey and, in addition, posited a direct role for lateral PFC in mnemonic storage (Goldman-Rakic, 1987). Thus, for instance,

although there has been a great deal of debate as to whether the dorsolateral PFC stores information or whether it simply maintains pointers or links to posterior cortices where mnemonic representations are held (see Postle, 2006), there has, on the contrary, been little if any debate as to whether Broca's area is involved in verbal mnemonic storage *per se*, as it has generally been assumed that the inferior frontal region plays a specific role in motor-speech planning. Indeed, however, if we expand our conception of memory to include not just the percepts of the past, but the goals, plans, and intentions of the future – the "memory of the future" in David Ingvar's (1985) phrase – then the role of both dorsal and inferior frontal regions in spatial and verbal memory might relate to prospective operations that relate to objects held in working memory.

3.13.3.5 Models of Prefrontal Organization of Working Memory

Although there is strong support that the lateral PFC is critical for working memory maintenance processes, its precise role is still unclear. Goldman-Rakic and colleagues first proposed that different PFC regions are critical for active maintenance of different types of information. Based on monkey electrophysiological and lesion studies (Funahashi et al., 1989; Wilson et al., 1993), they theorized that persistent activity within the ventrolateral PFC would reflect the temporary maintenance of nonspatial codes (such as an object's color and shape), whereas dorsolateral PFC activity would reflect the maintenance of spatial codes (such as the location of an object in space). This hypothesis had the appeal of parsimony, as a similar organization exists in the visual system, which is segregated into what and where pathways (Ungerleider and Mishkin, 1982). Also, anatomical studies in monkeys have demonstrated that the parietal cortex (i.e., spatial vision regions) predominantly projects to a dorsal region of the lateral PFC (Cavada and Goldman-Rakic, 1989; Petrides and Pandya, 1984), whereas the temporal cortex (i.e., object vision regions) projects more ventrally within lateral PFC (Barbas, 1988).

Another possible axis along which the human lateral PFC may be organized is according to the type of operations performed on information being actively maintained, rather than the type of information being maintained. For example, Petrides proposed that there are two processing systems, one dorsal and the other ventral, within lateral PFC (Petrides and Pandya, 1994). It was proposed that ventral PFC (Brodmann's areas 45, 47) is the site where information is initially received from posterior association areas and where active comparisons of maintained information are made. In contrast, the dorsal PFC (areas 9, 46, 9/46) is recruited only when monitoring and manipulation of this information are required.

This model received initial support from an empirical PET study performed by Owen, Petrides, and colleagues (Owen et al., 1996) in which dorsal PFC activation was found during three spatial working memory tasks thought to require greater monitoring of remembered information than two other memory tasks that activated only the ventral PFC. We also tested this model of process-specific PFC organization using event-related fMRI (D'Esposito et al., 1999). In our study, subjects were presented two types of trials in random order in which they were required to either (1) maintain a sequence of letters across a delay period or (2) manipulate (alphabetize) this sequence during the delay to respond correctly to a probe. In every subject, delay-period activity was found in both dorsal and ventral PFC in both types of trials. However, dorsal PFC activity was greater in trials during which actively maintained information was manipulated. These findings suggest that the dorsal PFC may exhibit greater recruitment during conditions that require additional processing of actively maintained information, supporting a process-specific PFC organization.

On the surface, these two models of PFC organization seem incompatible, and to this day papers continue to be published pitting one against the other. However, a closer look at the empirical data from human functional imaging and monkey physiology studies over the past 10 years leads to the conclusion that both models accurately describe PFC organization. The persistence of the notion that these models are orthogonal to each other may result in part from a lack in precision of the anatomical definitions of the dorsal and ventral PFC that were being used. For example, as reviewed earlier, the principal evidence cited to support domain-specific PFC organization in humans (Leung et al., 2002) derives from studies by Courtney and colleagues, who found that the superior frontal sulcus (area 6/8) appears specific to spatial working memory, whereas regions within the inferior frontal gyrus (areas 45, 47) appear specific to nonspatial information (e.g., faces). Unquestionably, the superior frontal sulcus is anatomically dorsal to the inferior frontal gyrus. Thus, on the surface these data provide strong support for a dorsal-what versus a ventral-where,

domain-specific PFC organization. However, other data from monkey physiological and human functional imaging studies seem inconsistent with the domain-specific hypothesis because they provide evidence that certain dorsal and ventral PFC regions do not appear specific to one domain of information. For example, several single-unit recording studies during delayed-response tasks have found a mixed population of neurons throughout dorsal and ventral regions of lateral PFC that are not clearly segregated by the type of information (i.e., spatial vs. nonspatial) that is being stored (Rosenkilde et al., 1981; Fuster et al., 1982; Quintana et al., 1988; Rao et al., 1997). Also, cooling of PFC (Fuster and Bauer, 1974; Bauer and Fuster, 1976; Quintana and Fuster, 1993) and dorsal PFC lesions causes impairments on nonspatial working memory tasks (Mishkin et al., 1969; Petrides, 1995), and ventral PFC lesions cause spatial impairments (Iversen and Mishkin, 1970; Butters et al., 1973). Finally, another study found that ventral PFC lesions in monkeys did not lead to delay-dependent defects on a visual pattern-association task and color-matching task (Rushworth et al., 1997). Also, numerous human functional imaging studies have failed to find different patterns of PFC activation during spatial versus nonspatial working memory tasks (e.g., Owen et al., 1998; Postle and D'Esposito, 1999; Nystrom et al., 2000).

How can we reconcile all these findings? The answer emerges from a close examination of the particular PFC regions that do or do not exhibit persistent activity that is specific to a particular type of information. Thus, domain specificity may exist within the superior frontal sulcus (area 6/8) and portions of the inferior frontal gyrus (areas 44, 45, 47), but other lateral PFC regions such as middle frontal gyrus (areas 9, 46, 9/46) may not show domain specificity. A coarse subdivision of the PFC into dorsal and ventral regions fails to account for the possibility that both domain-specific and process-specific organization may exist. A hybrid model of PFC organization could accommodate the empirical findings (Postle et al., 2000). But a hybrid model may not be able to capture, in cognitive or neural terms, the specific type of processes that are being attributed to the middle frontal gyrus (areas 9, 46, 9/46). Are the processes attributed to this region (e.g., monitoring and manipulation) distinct from active maintenance processes? For example, one possibility is that monitoring and manipulation tasks recruit the middle frontal gyrus because they require active maintenance of more abstract relations (e.g., semantic, temporal) between items. In this view, the PFC is not organized by different types of processing modules, but by the abstractness of the representations being actively maintained. This organization could be hierarchic, ranging from features of an object (e.g., red), to more abstract dimensions (e.g., color), to superordinate representations such as goals or task context (e.g., color-naming task). Evidence from functional neuroimaging studies has begun to provide support for this idea.

A recent neuroimaging study has tested this model of hierarchical PFC organization, all within one set of experiments (Koechlin et al., 2003). In this fMRI study, the frequency of to-be-selected representations was manipulated in an effort to affect levels of PFC processing. Manipulation of the number of responses within a block primarily affected premotor cortex. Manipulation of the number of relevant stimulus dimensions within a block affected dorsolateral PFC. Finally, manipulation of the across-block frequency of cue-to-response or cue-to-dimension mappings affected PFC responses. Interestingly, structural equation modeling of the fMRI data revealed path coefficients from the PFC to the dorsal PFC to premotor cortex but not in the opposite direction, broadly consistent with a hierarchic organization. An important contribution from this study is that it considers the entire frontal cortex, from premotor regions to the most anterior portion of PFC (area 10), an area that has been relatively ignored in working memory research. This type of PFC organization is also consistent with data (O'Reilly et al., 2002), which demonstrated that a connectionist model possessing a concrete feature level and an abstract dimension level in its PFC could produce the double dissociations reported in the monkey data.

Miller and Cohen (2001) have presented a synthesis of empirical findings with a theoretical model regarding how basic maintenance processes subserved by the PFC can exert cognitive control. They propose that PFC delay activity is specific to those representations that are behaviorally relevant, enabling an animal or human to prospectively integrate across time when selecting an action. Automatic behaviors can be mediated by computations in posterior neocortices with little influence from internal goals maintained by the PFC. When bottom-up processes are insufficient for or in conflict with current goals, available cues may be insufficient to uniquely specify a response. Under such circumstances, the active maintenance of behaviorally relevant representations permits the appropriate selection for action.

The PFC has extensive reciprocal connections with most of the brain and is situated at the apex of mnemonic, affective, perceptual, and motor pathways arising from posterior and subcortical processors. Thus, it is in a privileged position to store behaviorally relevant representations and exert cognitive control. The frontal cortex appears hierarchically organized, not simply in a dorsal/ventral fashion, but in a posterior/anterior direction from premotor regions to frontopolar cortex. Future research must continue to determine the regional distinctions that define the functional topography of the frontal cortex and the principles by which these regions interact to produce controlled behavior.

In summary, goal-directed behavior, which is both intentional and flexible, requires the active maintenance of a broad range of perceptual, mnemonic, and motor representations. For example, imagine hitting a golf ball. If your ball is in the woods, you may need to maintain the location of the flag in the distance as you keep your eye on the ball. As you prepare to hit your ball, you also have to maintain the rules of the game because any movement of the ball as you address it may result in a penalty stroke. And finally, if you are playing poorly, it is important to maintain the original goal for taking up the game – to exercise and enjoy yourself.

3.13.4 Summary and Conclusions

Elucidation of the cognitive and neural architectures underlying STM has been an important focus of neuroscience research for much of the past two decades. The emergence of the concept of working memory, with its emphasis on the utilization of the objects stored in memory in the service of behavioral goals, has enlarged our understanding and broadened the scope of neuroscience research of STM. Data from numerous studies have been reviewed and have demonstrated that a network of brain regions, including the PFC, is critical for the active maintenance of internal representations. Moreover, it appears that the PFC has functional subdivisions that are organized according to the domain (verbal, spatial, object, etc.) of the topographical inputs arriving from posterior cortices. In addition, however, a level of representational abstractness is achieved through the integration of information converging in the PFC. Finally, working memory function is not localized to a single brain region but is rather an emergent property of the functional interactions between the PFC and other posterior neocortical regions. Numerous questions remain about the neural basis of this complex cognitive system, but studies such as those reviewed in this chapter should continue to provide converging evidence that may provide answers to the many residual questions.

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3.14 Prefrontal Cortex and Memory

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3.14.1 Introduction

Dating back at least to the work of Jacobsen (1935), researchers have been interested in characterizing the functional role of the lateral prefrontal cortex (PFC) in memory. In recent years, a wealth of evidence from neuropsychological, neurophysiological, and neuroimaging studies has accumulated, implicating the PFC in a wide variety of memory functions. Here, we will review this evidence and present a general framework for understanding the roles of different prefrontal regions in memory processing.

3.14.2 Anatomical Organization of the PFC

In order to understand how the PFC contributes to memory, it is useful to start by considering its anatomical characteristics. The PFC is situated in the frontal lobes, rostral to the premotor and motor cortices and, in humans, occupies approximately one third of the cortical mantle (Fuster, 1997). The lateral PFC consists of several highly interconnected subregions that can be distinguished based on cytoarchitectonic characteristics and anatomical connectivity. Many researchers (e.g., Goldman-Rakic, 1987; Petrides, 1994; Fuster, 1997) have proposed functional distinctions between mid-dorsolateral (DLPFC; Brodmann's areas [BA] 9 and 46) and ventrolateral (VLPFC; BA 44, 45, and 47) PFC. VLPFC is situated in the inferior convexity in the monkey brain and in the inferior frontal gyrus in humans. This region is highly connected to ventral posterior sensory areas, and especially to regions of the inferior and superior temporal lobe. DLPFC is situated in the cortex dorsal to the sulcus principalis in the monkey and in the middle frontal gyrus in the human brain. In contrast to VLPFC, DLPFC is more highly interconnected with dorsal stream regions (especially the posterior parietal cortex) and paralimbic cortical regions, including the retrosplenial and parahippocampal cortices.

Although little is known about more anterior regions of lateral PFC (APFC; BA 10), recent research suggests that these regions may be particularly critical for human cognition (Petrides, 1994; Fuster, 1997; Christoff and Gabrieli, 2000; D'Esposito et al., 2000; Koechlin et al., 2003; Ramnani and Owen, 2004). For example, recent comparative neuroanatomical work suggests a twofold increase in the size of APFC in humans relative to chimpanzees (Semendeferi et al., 2001), despite the fact that the relative size of the entire frontal lobe is similar between the two species (Semendeferi et al., 2002). As described in the following, VLPFC, DLPFC, and APFC have been implicated in human memory processes, and

there is some evidence that these regions may exhibit different functional characteristics (Wagner, 1999; Buckner and Wheeler, 2001; Fletcher and Henson, 2001; Rainer and Ranganath, 2002; Ranganath and Knight, 2003).

3.14.3 PFC and Working Memory

3.14.3.1 PFC Involvement in Working Memory: Short-Term Retention and Cognitive Control

Since the 1980s, a great deal of research has focused on the role of PFC in 'working memory' (WM) processes that support temporary retention and online processing of information. Much of this work has characterized the PFC as a global level, but more recent research has been devoted to specifying the different processes implemented by different subregions of PFC (Goldman-Rakic, 1987; Fuster, 1997; D'Esposito et al., 2000; Petrides, 2005). Psychological theories of WM generally distinguish between processes that support temporary retention of information across short delays (or what is now termed 'WM maintenance') and those that support attentional and cognitive control (Baddeley, 1986). The degree to which regions of PFC are necessary for maintenance versus control has been a topic of extensive debate in cognitive neuroscience.

The idea that PFC supports temporary retention of information has largely emerged from single-unit recording studies of monkeys performing the delayed response task, in which the monkey is shown a reward in one of two locations but must wait until after a delay before it can obtain the reward. The task is thought to require the monkey to maintain an internal representation of the remembered location or upcoming movement across the delay period. Critically, lateral prefrontal neurons exhibit persistent activity during the memory delay that is selective for the remembered location (Fuster and Alexander, 1971). This work was extended by Funahashi et al. (1989, 1990), who investigated prefrontal activity during an oculomotor delayed response task, in which a monkey is cued to make an eye movement to a spatial location, but it must wait until after a brief delay before making the saccade. Consistent with other studies of the delayed response task, lateral prefrontal neurons exhibited persistent activity during the memory delay that was specific to the spatial location that was to be remembered. Further research showed that PFC lesions in one hemisphere impaired memory performance specifically for locations in the contralateral hemifield (Funahashi et al., 1993). Based on these findings, it has often been assumed that lateral prefrontal neurons temporarily store information that is to be maintained across a delay (Goldman-Rakic, 1987).

Results from studies by Miller and Desimone seemed to further substantiate this idea by contrasting the activity of neurons in inferior temporal cortex (a region that is involved in visual object processing; cf. Miyashita and Hayashi, 2000) and PFC during a visual WM task. Their findings showed that both inferior temporal and prefrontal neurons showed persistent, stimulus-specific activity during memory delays (Miller et al., 1993, 1996). However, persistent delay period activity in inferior temporal neurons was abolished by the presentation of a distracting stimulus (Miller et al., 1993), whereas activity in PFC remained robust in the face of interference.

Many have interpreted Miller and Desimone's data to support the idea that short-term retention of information is supported by the PFC, rather than by posterior cortical areas that represent the information to be maintained. However, this interpretation rests on the assumption that persistent activity is the sole neural correlate of WM maintenance. This assumption was invalidated in a recent study, in which single-unit activity and local field potentials (LFPs) were recorded in occipital area V4 during a visual WM task (Lee et al., 2005). Unlike neurons in inferior temporal cortex, recordings from V4 did not reveal evidence of persistent, stimulus-specific activity during even an unfilled memory delay. However, results did reveal single-unit activity during the memory delay that was phase-locked to theta oscillations in the electroencephalogram. Critically, the phase-locking of single-unit activity and theta oscillations was selective to the remembered stimulus. These findings strongly suggest that posterior cortical regions can play important roles in WM maintenance even in the absence of overall changes in mean firing rate across the memory delay. Furthermore, converging lines of evidence now suggest that short-term retention of specific kinds of information is supported by activation of the posterior cortical areas that represent that information (*See* Chapter 3.13; Fuster, 1995; Miyashita and Hayashi, 2000; Lee et al., 2005; Ranganath and Blumenfeld, 2005; Ranganath and D'Esposito, 2005; Postle, 2006; Ranganath, 2006).

Results from lesion studies have additionally demonstrated that the PFC contributes to WM by

virtue of its role in cognitive control, rather than short-term retention (see D'Esposito and Postle, 1999; Curtis and D'Esposito, 2004; Postle, 2006, for detailed reviews of this topic). Jacobsen (1935) demonstrated that lateral prefrontal lesions caused impairments in the delayed response task, leading him to conclude that the PFC is critical for retaining information across short delays. However, this interpretation was later questioned by Malmo (1942), who demonstrated that prefrontal lesions only impaired monkeys' performance on the task if there was interference present between the instruction and the response. If the lights were turned off between the instruction and response (thus minimizing interference), performance was intact. Subsequent research in the 1960s substantiated the idea that monkeys with PFC lesions performed poorly because they were unable to suppress previously rewarded responses or because they could not maintain the appropriate task set (Mishkin, 1964; Pribram et al., 1964; Pribram and Tubbs, 1967; Nauta, 1971). For instance, Nauta (1971) noted that

> the initial impression that the 'frontal animal' suffers from a memory loss . . . has been effectively refuted, and it now seems certain that frontal-lobe ablation affects a response-guidance other than memory in the customary sense.

This impression has been supported by more recent lesion studies (Mishkin and Manning, 1978; Kowalska et al., 1991; Meunier et al., 1997; Rushworth et al., 1997; Petrides, 2000), as well as single-unit recording (Asaad et al., 2000; Lebedev et al., 2004) studies of monkeys and neuropsychological (Chao and Knight, 1995; D'Esposito and Postle, 1999; D'Esposito et al., 2006) and neuroimaging (Postle et al., 1999; Smith and Jonides, 1999) studies of humans. Indeed, consistent with the monkey literature, humans with prefrontal lesions do not exhibit significant impairments in short-term retention of information, but their performance is impaired on more complex tasks that involve inhibiting distraction and manipulating information (Chao and Knight, 1995, 1998; D'Esposito and Postle, 1999; Ranganath and Blumenfeld, 2005; D'Esposito et al., 2006).

How, then, does one explain the fact that prefrontal regions show persistent activity during WM maintenance? The most likely explanation is that prefrontal neurons do not temporarily store information that is to be maintained, but rather that they represent and maintain context-dependent rules or associations that dictate the kind of information that is currently goal relevant (Fuster, 1997; Miller, 2000). Indeed, this idea can explain not only why PFC neurons exhibit delay period activity, but also why they exhibit activity associated with virtually every relevant aspect of delayed-response tasks (Fuster, 1997). By representing context-dependent stimulus–response associations, PFC networks can use higher-order knowledge to guide behavior in novel situations and override prepotent responses. Because of its connections with posterior cortex, activation of prefrontal representations can increase or decrease the activation of posterior cortical representations, based on what is relevant for current or future goals. This view accords well with most theories of prefrontal function, which generally suggest a role for the PFC in the selection and maintenance of task-relevant information and the inhibition of irrelevant, distracting information (Pribram et al., 1964; Brutkowski, 1965; Luria, 1966a; Nauta, 1971; Stuss and Benson, 1986; Goldman-Rakic, 1987; Cohen and Servan-Schreiber, 1992; Cohen et al., 1996; Fuster, 1997; Knight et al., 1999; Miller, 2000; Shimamura, 2000; Miller and Cohen, 2001; Miller and D'Esposito, 2005).

3.14.3.2 Functional Imaging of Working Memory: Evidence for Functional Differentiation within PFC

Results from human neuroimaging studies of WM converge with the view described above, and these studies have also provided evidence for functional differentiations within PFC. Based on ideas outlined by Fuster (1997, 2004) and consideration of the available evidence, we suggest one such view, outlining a hierarchy of 'selection' processes implemented by different PFC subregions along the rostro–caudal and dorso–ventral axes (see **Figure 1**).

Anatomically, VLPFC subregions are well positioned to modulate activity in high-level auditory, visual, and multimodal association areas (Petrides and Pandya, 2002). Thus, the VLPFC may be a source of top-down signals that select (i.e., enhance or reduce the activation of) item representations in posterior cortical areas based on current task demands. Consistent with this idea, VLPFC activation is observed when a task requires inhibition of irrelevant or potentially distracting items (Konishi et al., 1999; Aron et al., 2004; Zhang et al., 2004), resolution of proactive interference (Jonides and Nee, 2006), resolution of competition among competing linguistic representations (Thompson-Schill

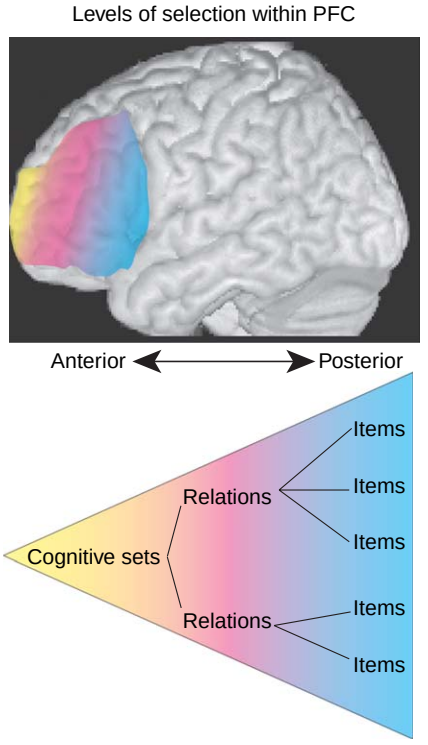

Figure 1 At top, a lateral view of the brain, with a gradient illustrating functional differentiation within the PFC along the rostro–caudal and dorso–ventral axes. At bottom, a hypothesized functional organization is depicted, such that regions in VLPFC (blue) may be involved in selecting relevant items, DLPFC (pink) may be involved in selecting relationships between items that are currently active, and APFC (yellow) may be involved in selecting cognitive sets that determine which items and relations are appropriate targets for selection.

et al., 1997; Wagner et al., 2001a), or activation of an item representation (i.e., WM maintenance; Curtis and D'Esposito, 2003). Furthermore, the topography of VLPFC activation in these studies is material dependent, such that tasks requiring selection, encoding, or maintenance of different kinds of items recruit different subregions of VLPFC (Schumacher et al., 1996; Kelley et al., 1998; Wagner et al., 1998; Poldrack et al., 1999; Wagner, 1999; Braver et al., 2001). In contrast, DLPFC and APFC activation is not material dependent, suggesting that these regions may act at a more abstract level (D'Esposito et al., 1998; Smith and Jonides, 1999; Wagner, 1999; Rainer and Ranganath, 2002; Ranganath and Knight, 2003; Ramnani and Owen, 2004).

Unlike VLPFC, DLPFC is not robustly recruited during tasks that solely require selection of task-relevant information. Instead, evidence from neuroimaging studies suggests that DLPFC is involved in using rules to activate, inhibit, or transform relationships among items that are active in WM. For example, DLPFC activation is reported in 'manipulation' tasks that involve sequencing of information that is being maintained in WM (D'Esposito et al., 1999; Postle et al., 1999; Wagner et al., 2001b; Barde and Thompson-Schill, 2002; Blumenfeld and Ranganath, 2006; Crone et al., 2006; Mohr et al., 2006) or monitoring of previous responses when selecting a future response (Owen, 1997; Owen et al., 1999). DLPFC activation has also been reported in 'chunking' studies which involve processing of relationships to build higher-level groupings among items that are active in memory (Bor et al., 2003, 2004; Bor and Owen, 2006). One parsimonious explanation for this diverse array of findings is that DLPFC may implement selection processes that accentuate or inhibit the activation of relationships among items that are active in memory.

Although APFC is not nearly as well characterized as DLPFC and VLPFC, available evidence is consistent with the idea that APFC is at the apex of the hierarchy of selection processes implemented by PFC. One way of conceptualizing this role is that APFC implements the selection of appropriate cognitive sets – that is, sets of goal-directed rules that determine which types of relations and items are appropriate for selection by DLPFC and VLPFC, respectively. Although virtually every type of task involves some kind of cognitive set, APFC recruitment specifically occurs during tasks that place demands on the *selection* of an appropriate cognitive set (Rogers and Monsell, 1995; Mayr and Kliegl, 2000; Rogers et al., 2000; Mayr, 2002). Consistent with this prediction, several functional magnetic resonance imaging (fMRI) studies have reported APFC activation using paradigms in which subjects must actively maintain a task set (Braver et al., 2003; Bunge et al., 2003; Sakai and Passingham, 2003) or hold a primary task set in mind while processing secondary subgoals (Koechlin et al., 1999; Braver and Bongiolatti, 2002; Koechlin et al., 2003; Kubler et al., 2003; Badre and Wagner, 2004). As we describe later, the role of APFC in selection and maintenance of cognitive sets might explain why APFC activation is routinely observed during long-term memory (LTM) retrieval tasks.

To summarize, it is clear that the PFC is involved in WM processes, but that role has been misinterpreted in recent years. Available evidence indicates that PFC is not necessary for short-term retention per se, and that it is more specifically critical for WM

control processes that guide behavior under a range of circumstances. These processes may emerge through prefrontal representations that use context information to select relevant information and inhibit irrelevant information. We suggest that this selection mechanism is common across prefrontal regions, and that different prefrontal regions act to select information at different levels (**Figure 1**). Specifically, VLPFC acts to activate or inhibit item representations, whereas DLPFC acts to activate or inhibit relationships between items that are actively selected or maintained through VLPFC operations. Finally, APFC may play a role in activating or inhibiting representations of cognitive sets that determine which types of items and relations are appropriate for selection (Bunge et al., 2003; Sakai and Passingham, 2003). As we will describe, this hypothesized division of labor corresponds well to the imaging literature on PFC and LTM processes (see also Fletcher and Henson, 2001, for a similar perspective).

3.14.4 Effects of Prefrontal Lesions on LTM Encoding and Retrieval

3.14.4.1 Neuropsychological Studies of Patients with Prefrontal Lesions

Clinicians have long noted that focal prefrontal lesions in humans produce subtle but noticeable memory deficits, and this impression accords well with results from neuropsychological studies (Stuss and Benson, 1986; Shimamura, 1995; Ranganath and Knight, 2003). In general, patients with PFC lesions show impairments on a wide range of memory tasks that tax executive control during encoding and/or retrieval (Stuss and Benson, 1986; Moscovitch, 1992; Shimamura, 1995; Ranganath and Knight, 2003). For instance, PFC patients exhibit impaired performance on unconstrained memory tests such as free-recall (Jetter et al., 1986; Janowsky et al., 1989a; Eslinger and Grattan, 1994; Gershberg and Shimamura, 1995; Wheeler et al., 1995; Dimitrov et al., 1999). In contrast to healthy control participants, PFC patients tend not to spontaneously cluster or group recall output according to semantic relationships within a categorized word list (Hirst and Volpe, 1988; Incisa della Rochetta and Milner, 1993; Stuss et al., 1994; Gershberg and Shimamura, 1995). Furthermore, when presented with several study-test trials with the same word list, healthy participants tend to recall items in the same order across recall trials, a phenomenon termed 'subjective organization.' Patients with prefrontal lesions, however, show less trial-to-trial consistency of recall output order compared to controls (Stuss et al., 1994; Gershberg and Shimamura, 1995; Alexander et al., 2003).

Patients with prefrontal lesions also exhibit impaired performance on tests of source memory (Janowsky et al., 1989c; Duarte et al., 2005), memory for temporal order (Shimamura et al., 1990; McAndrews and Milner, 1991; Butters et al., 1994; Kesner et al., 1994; Mangels, 1997), and judgments of frequency (Stanhope et al., 1998). Furthermore, PFC patients often fail to spontaneously use common memory strategies and lack insight into their own memory problems (Hirst and Volpe, 1988; Janowsky et al., 1989b; Moscovitch and Melo, 1997; Vilkki et al., 1998).

In contrast to these deficits, patients with prefrontal lesions can often perform at near-normal levels when given structured encoding tasks or tests that do not tax strategic retrieval processes. For instance, PFC patients perform better at cued recall compared to free recall and have only a mild impairment in item recognition (Wheeler et al., 1995). Moreover, patients can show marked improvements on a variety of recall measures if given sufficient practice or environmental support at encoding or retrieval (Hirst and Volpe, 1988; Incisa della Rochetta and Milner, 1993; Gershberg and Shimamura, 1994; Stuss et al., 1994).

3.14.4.2 Recollection and Familiarity in Patients with Prefrontal Lesions

As noted above, patients with prefrontal lesions tend to show only mild deficits in recognition memory performance. Behavioral research has supported the idea that item recognition can be supported either by the assessment of familiarity, or by the recollection of specific details associated with the item (Yonelinas, 2002). Some researchers have speculated that prefrontal damage may selectively affect the recollection process (Knowlton and Squire, 1995; Davidson and Glisky, 2002; Gold et al., 2006), based on reports of recollection deficits among the healthy elderly (Davidson and Glisky, 2002) and among amnesic patients with Korsakoff's syndrome (Knowlton and Squire, 1995) that may be correlated with performance on the Wisconsin card sorting task and other tasks thought to be dependent on PFC function. However, it is highly unlikely that performance on such

tests uniquely indexes the functioning of the PFC and no other brain region.

To directly test the role of PFC in recognition memory, it is necessary to directly assess recollection and familiarity in patients with focal prefrontal lesions. One methodological challenge in addressing this question is that prefrontal lesions are typically unilateral (due to stroke or tumor excision), and therefore patients might rely on the intact hemisphere to support performance. A recent study dealt with this issue by using a divided-field presentation method to specifically assess memory performance for information that was encoded in the visual field contralateral to the lesioned hemisphere ('contralesional') and the field ipsilateral to the lesioned hemisphere ('ipsilesional'). Thus, if PFC regions contribute to familiarity or recollection, one would expect deficits in these processes to be most substantial when objects were encoded in the contralesional visual field. Patients and controls were tested using the remember-know method, in which they decided whether each test object was shown during the study phase, and if so, whether they could recollect specific details about the study episode. These data were then used to create quantitative indices of familiarity and recollection for objects encoded in the contralesional and ipsilesional hemifields in each patient, and similar indices were created for each visual field in the corresponding age- and education-matched control participant. As shown in **Figure 2**, patients showed impaired familiarity for objects that were presented in the contralesional field at the time of encoding. Furthermore, although PFC patients did not exhibit deficits in subjective recollection, patients with left frontal lesions exhibited impairments in memory for the context in which each word was encountered (i.e., source memory).

Findings from this study demonstrate that, contrary to previous assertions, the PFC is necessary for normal familiarity-based recognition. Additionally, although patients with PFC lesions may have a subjective experience of recollection, they may still exhibit impairments in the ability to use recollected information to make source attributions (particularly following left frontal lesions – see the section titled 'Laterality of PFC activation during LTM encoding and retrieval' for more on this topic). This finding makes sense if one assumes that PFC damage affects control processes, rather than memory storage. That is, engagement of PFC-dependent control processes most likely impacts encoding of overall familiarity strength as well as encoding of distinctive contextual information that would support recollection (Ranganath et al., 2003b, 2005a; Blumenfeld and Ranganath, 2006). Furthermore, engagement of PFC-dependent control processes at retrieval most likely affect strategic search and decision processes that influence the retrieval and use of familiarity and recollective information (Ranganath et al., 2000, 2003a, 2007).

3.14.4.3 Theoretical Accounts of Memory Deficits Following Prefrontal Lesions

Theoretical accounts of memory deficits in patients with prefrontal lesions generally fall into two categories. Some theories emphasize the role of the PFC in selection processes that direct attention toward goal-relevant information and task-appropriate responses. Thus, memory deficits may arise in patients with prefrontal lesions because they are unable to select goal-relevant information or inhibit distracting or interfering items or responses during encoding or retrieval (Luria, 1966b; Perret, 1974; Shimamura, 1995). One finding consistent with this account comes from a study of paired associate learning in patients with focal PFC lesions and matched controls (Shimamura et al., 1995). In this study, participants learned a list of word pairs ('A–B') and then learned an overlapping list of word pairs ('A–C') across several trials. Recall success on the A–C list required subjects to inhibit the 'A–B' pairing and select the appropriate 'A–C' pairing. Critically, patients with PFC lesions showed a disproportionate decrease in recall performance between the first trial of A–B learning and the first trial of A–C learning (i.e., a measure of proactive interference), as compared to controls. Furthermore, during cued recall of the A–C list, PFC patients recalled significantly more intrusions from the A–B list. These finding suggest that patients with prefrontal lesions were unable to inhibit the influence of previously learned associations during encoding or retrieval of new associations.

The second category of theories to explain memory deficits following prefrontal lesions emphasizes the role of the PFC in guiding spontaneous organization of information (Milner et al., 1985; Hirst and Volpe, 1988; Incisa della Rochetta and Milner, 1993; Gershberg and Shimamura, 1995). In psychology, 'organization' refers to memory strategies that emphasize forming or utilizing relationships among items in a list during encoding and/or retrieval. Some common organizational strategies during encoding include

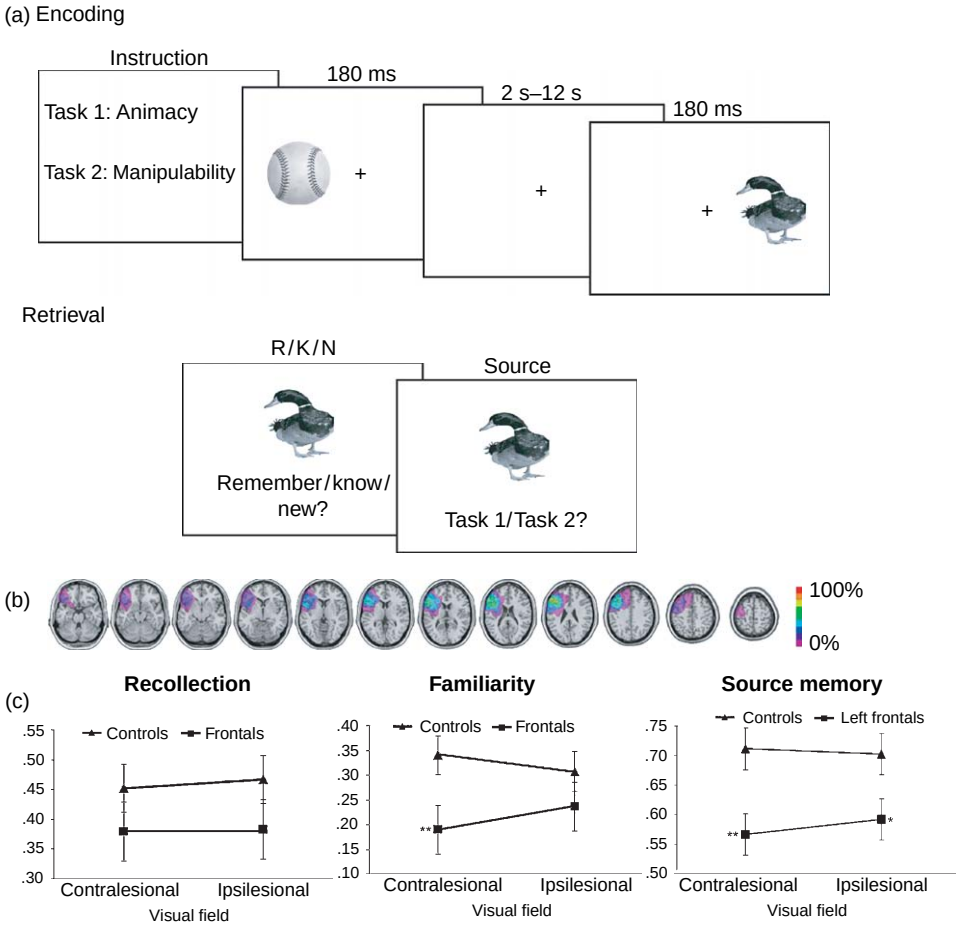

Figure 2 (a) Participants with lateral prefrontal lesions and controls encoded objects that were briefly flashed to the left or right hemifield, and then performed retrieval tests assessing memory for each item and the task that was performed during encoding. (b) Lesion overlap for patients. Right frontal lesions have been transcribed to the left hemisphere to determine the overlap across all patients. The color scale indicates the percentage of patients with lesions in a specific area. (c) Results showed that subjective measures of recollection (left) were relatively spared in the patients, whereas familiarity (middle) was impaired, particularly for objects encoded in the contralesional visual field. Source memory (right) performance was also impaired in patients with left frontal lesions. Experimental design and results from Duarte A, Ranganath C, and Knight RT (2005) Effects of unilateral prefrontal lesions on familiarity, recollection, and source memory. *J. Neurosci.* 25: 8333–8337.

categorizing words in a list according to semantic features, imagining two or more items interacting, or forming a sentence out of two or more words. Organizational processes do not facilitate LTM by enhancing features of specific items in memory, but rather by promoting memory for associations among items.

The organizational account described above can explain free recall deficits seen in patients with PFC lesions, because free recall is thought to rely heavily on organization of information in the study list. One study directly tested this hypothesis by comparing performance of patients with lateral prefrontal lesions and healthy controls on learning of lists of words that were either semantically related or unrelated (Gershberg and Shimamura, 1995). Patients with PFC lesions showed impaired recall of items from both related and unrelated lists. Furthermore, the patients failed to demonstrate normal levels of subjective organization and failed to show semantic clustering following study of semantically related lists, two indices of organizational processing during encoding. Interestingly, recall and clustering performance increased for semantically related word lists

when patients were explicitly asked to make a category judgment during encoding, when they were provided with the category names at test, or both. The same was not true for the healthy controls, who performed at similar levels regardless of whether they were given cues or instructions. This pattern of results suggests that patients were capable of using semantic information to guide encoding and retrieval, but that they lacked the ability to spontaneously use semantic organizational strategies. In contrast, controls were spontaneously using organizational strategies during encoding and/or retrieval. These findings, and others (Hirst and Volpe, 1988; Incisa della Rochetta and Milner, 1993; Stuss et al., 1994; Alexander et al., 2003) suggest that LTM deficits following prefrontal lesions may emerge partly from a failure to organize information during encoding and capitalize on organizational structure during retrieval.

Many researchers have suggested that both selection and organizational processes depend on the functioning of the PFC, and there are several studies that have found support for both the interference and organizational accounts in the same study (Hirst and Volpe, 1988; Stuss et al., 1994; Gershberg and Shimamura, 1995; Shimamura et al., 1995; Alexander et al., 2003). One question that cannot be addressed by the neuropsychological evidence is whether selection and organization depend upon the same regions of PFC, because these studies typically use subject groups that have significant heterogeneity in lesion size and location. However, in light of the evidence from imaging studies of WM control processes, it is possible that VLPFC is particularly critical for selection of relevant item information, whereas DLPFC is particularly critical for building of relationships between items in a manner that supports organization. As we will describe below, this hypothesis is consistent with results from neuroimaging studies of LTM encoding and retrieval.

3.14.5 Functional Neuroimaging of LTM Encoding and Retrieval

3.14.5.1 Subsequent Memory Effects and the PFC

Event-related fMRI studies have investigated LTM encoding by identifying 'subsequent memory' or 'Dm' (difference due to memory) effects (Paller and Wagner, 2002). In these paradigms, brain activity is monitored during the performance of an incidental encoding task (i.e., semantic processing of a single word). Following scanning, a surprise memory test is administered, and brain activation during encoding is analyzed as a function of later memory success or failure. For example, participants might be given a semantic encoding task in the scanner, and then once out of the scanner, they receive an item-recognition test on the items they studied. The results can then be used to contrast brain activity during successful versus unsuccessful encoding.

Results from studies using the subsequent memory paradigm have demonstrated significant pre-frontal involvement in LTM encoding. Inspection of the spatial distribution of activation peaks (or 'local maxima') from these studies, shown in **Figure 3**, reveals

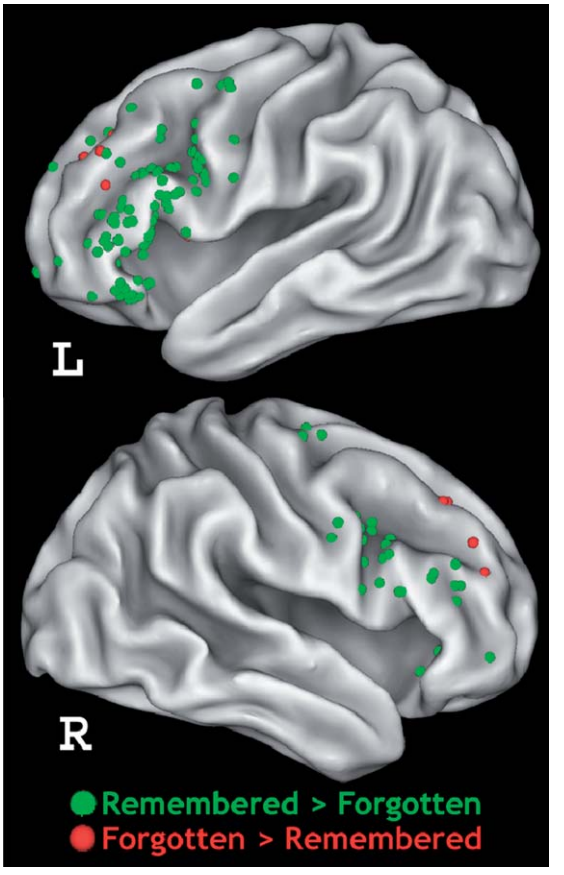

Figure 3 Activation peaks from fMRI studies investigating prefrontal activation during memory encoding. Each green dot represents an activation peak in an analysis that reported increased activation during encoding of items that were subsequently remembered, as compared with items that were subsequently forgotten (i.e., a 'subsequent memory effect'). Each red dot represents an activation peak in an analysis that reported increased activation during encoding of items that were subsequently forgotten, as compared with items that were subsequently remembered (i.e., 'a subsequent forgetting effect').

not only that PFC activation is routinely linked with successful LTM encoding, but also that the degree of involvement seems to differ between different PFC subregions. There is overwhelming support for the idea that VLPFC contributes to LTM formation. Out of 150 local maxima associated with subsequent memory, 132 fall within the VLPFC. Furthermore, 33 of the 35 studies that reported prefrontal subsequent memory effects find local maxima within the VLPFC. Given that the ability to select relevant item information is essential for many forms of goal-directed cognitive processing, including memory encoding, it makes sense that VLPFC should be strongly linked to memory encoding in a wide variety of behavioral contexts.

The imaging literature seems to tell a different story about the DLPFC. Out of 150 local maxima throughout the PFC, only 18 fall within the DLPFC. Furthermore, five studies have found increased DLPFC activation during processing of items that were subsequently forgotten, as compared with items that were subsequently remembered. Thus, based on the numbers alone, there does not appear to be much support for the idea that DLPFC contributes to LTM encoding. This pattern of findings could suggest either that DLPFC implements processes that do not contribute to successful LTM encoding, or that previous studies were insensitive to its role in LTM formation.

Relevant to the latter possibility, it is notable that imaging studies of WM have implicated DLPFC in processing of relationships between items, and that imaging studies of LTM encoding typically do not elicit relational processing. Most imaging studies have examined encoding of single items studied in isolation, using encoding tasks that orient attention toward specific attributes of a study item and away from relationships between items. In these studies, it is unlikely that participants would spontaneously process relationships among items in the study list, and engagement of relational processing might have even been deleterious to later memory performance (i.e., because allocating resources toward processing the relationships among items might take attentional resources away from processing the distinctive features of the items themselves). Accordingly, it is possible that the encoding conditions in many previous imaging studies were not conducive to revealing the contribution of DLPFC to successful LTM encoding. The retrieval tests used in subsequent memory paradigms might also be a relevant factor. Most imaging studies of encoding assess successful LTM formation with tests of item recognition memory. However, processing of relationships between items facilitates memory by enhancing interitem associations, and item recognition memory tests may not be sensitive to detecting these effects (Bower, 1970). Thus, sorting encoding activation by subsequent item recognition performance might mask the role of DLPFC in successful LTM encoding.

If DLPFC contributes to LTM encoding through its role in relational processing, the ability to detect this contribution may depend on the kinds of encoding and retrieval tasks that are used. Indeed, in imaging studies that used encoding tasks that encouraged relational processing or retrieval tests that are sensitive to memory for associations among items, DLPFC activity during encoding predicts subsequent memory (Addis and McAndrews, 2006; Blumenfeld and Ranganath, 2006; Staresina and Davachi, 2006; Summerfield et al., 2006). In one such study, we found evidence that DLPFC activation is related to successful LTM encoding specifically under conditions that emphasize processing of relationships between items (Blumenfeld and Ranganath, 2006). In this study, participants were scanned during the performance of two WM tasks (**Figure 4(a)**). On 'rehearse' trials, participants were presented with a set of three words and required to maintain the set across a 12-s delay period, in anticipation of a question probing memory for the identity and serial position of the items. On 'reorder' trials, participants were required to rearrange a set of three words based on the weight of the object that each word referred to. They maintained this information across a 12-s delay period in anticipation of a question probing memory for serial order of the items in the rearranged set. Although both rehearse and reorder trials required maintenance of the three-item set, reorder trials additionally required participants to compare the items in the set and transform the serial order of the items. Thus, reorder trials forced participants to actively process relationships between the items in the memory set, whereas rehearse trials simply required maintenance of the memory set across a delay. Analyses of subsequent recognition memory performance showed that there were significantly more reorder trials in which all three items were recollected than would be expected based on the overall item hit-rates alone (**Figure 4(b)**). The same was not true for memory for rehearse trials, for which the proportion of trials on which all three items were subsequently recollected was no different than would be expected by

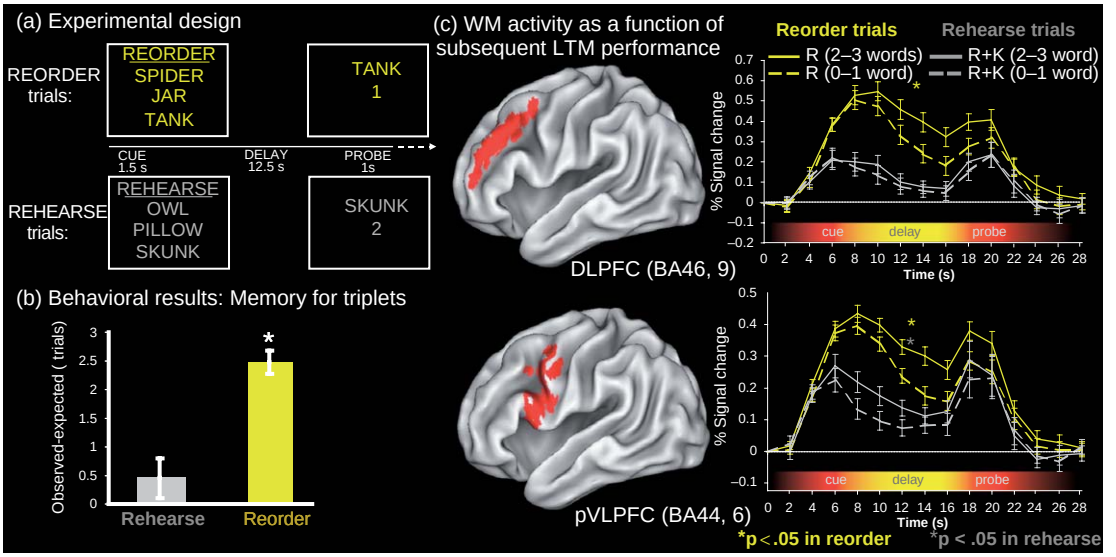

Figure 4 (a) Schematic depiction of the two tasks performed during fMRI scanning. (b) Behavioral results, showing that participants recalled significantly more triplets from each reorder trial (yellow) than would be expected based on the overall hit rate. This finding suggests that, on reorder trials, memory performance was supported by associations among the items in the memory set. (c) fMRI data showing that DLPFC (top) exhibited increased activation during the delay period of reorder trials for which 2–3 items were subsequently remembered (solid yellow), as compared with trials in which 0–1 items were remembered (dashed yellow). No such effect is seen on rehearse trials (gray lines). At bottom, activation in a region of posterior VLPFC (pVLPFC) is plotted, showing that delay period activation in this region during both rehearse and reorder trials was predictive of subsequent memory performance. Experimental design and results from Blumenfeld RS and Ranganath C (2006) Dorsolateral prefrontal cortex promotes long-term memory formation through its role in working memory organization. *J. Neurosci.* 26: 916–925.

the item hit-rates alone. These findings suggest that, on reorder trials, processing of the relationships among the items in each memory set resulted in successful encoding of the associations among these items.

Consistent with the idea that the DLPFC is involved in processing of relationships between items in WM, fMRI data revealed that DLPFC activation was increased during reorder trials, as compared with rehearse trials (**Figure 4(c)**). Furthermore, DLPFC activation during reorder but not rehearse trials was positively correlated with subsequent memory performance. Specifically, DLPFC activation was increased on reorder trials for which 2–3 items were later recollected, as compared with trials for which 1 or 0 items were later recollected. No such relationship was evident during rehearse trials. In contrast, activation in a posterior region of left VLPFC (BA 44/6) was correlated with subsequent memory performance on both rehearse and reorder trials. Thus, results from this study suggest that DLPFC and VLPFC may play dissociable roles in LTM encoding. DLPFC activation may specifically promote successful LTM formation through its role in processing of relationships among items, whereas VLPFC activation seems to promote LTM formation under a broader range of conditions.

Results from another recent study demonstrated the specific nature of DLPFC contributions to memory encoding by comparing the relationship between activation and subsequent performance on free recall and item recognition memory tests (Staresina and Davachi, 2006). As described earlier, item recognition tests are often insensitive to memory for inter-item associations in LTM, whereas recall performance is significantly influenced by encoding of interitem associations (Tulving, 1962). Consistent with a role for DLPFC in encoding inter-item associations, DLPFC activation was specifically enhanced during encoding of items that were recalled compared to those that were not. DLPFC activation was not correlated with subsequent item recognition performance. In contrast, encoding time activation in VLPFC was positively correlated with subsequent memory performance on both the recall and the recognition tests. These results are consistent with the idea that DLPFC activation will contribute to

subsequent LTM performance specifically under retrieval conditions that are sensitive to memory for associations among items.

The role of APFC in promoting LTM formation has not been well characterized in prior studies. We suspect that this is because activation in this region, like DLPFC, is not typically reported in studies of LTM encoding. Future work will be necessary to determine whether or how APFC contributes to successful LTM formation.

3.14.5.2 PFC Activation during LTM Retrieval

Numerous imaging studies have investigated the role of prefrontal regions in memory retrieval, showing that regions in DLPFC, VLPFC, and APFC are routinely activated during performance of such tasks (Fletcher and Henson, 2001; Ranganath et al., 2003a; Ranganath and Knight, 2003; Ranganath, 2004). Unlike studies of encoding, retrieval studies have not shown a consistent relationship between activation in any prefrontal region and successful retrieval. However, this is not surprising if PFC activation reflects control processes that are engaged during retrieval tasks even when retrieval fails (Ranganath et al., 2000, 2007; Dobbins et al., 2002; Simons et al., 2005a). If different prefrontal regions contribute to different control processes, then it may be more fruitful to compare and contrast activation between retrieval conditions that are more or less likely to engage these processes, rather than contrasting activation between successful and unsuccessful retrieval (Fletcher and Henson, 2001). Results from such studies have converged in many respects with results from studies of WM and LTM encoding in implicating VLPFC in item processing and DLPFC in relational processing.

One study conducted by Fletcher and colleagues is particularly relevant, in that they observed a double dissociation between the roles of VLPFC and DLPFC across two LTM retrieval tasks. In this study, positron emission tomography was used to measure prefrontal activation during two different retrieval tests. In one condition, the study lists were structured lists of 16 single words that were organized according to an overall theme and then broken down into four subcategories. For these lists, participants were given a free recall test, with the instruction to use the organizational structure of the list to guide retrieval. In the other condition, the study lists consisted of 16 category–exemplar word pairs (e.g., 'fruit–banana'). For these lists, participants performed a cued recall test, in which they had to recall the appropriate exemplar in response to each category name (e.g., 'fruit–?'). These two retrieval conditions differed in terms of the control processes that should be engaged during retrieval. In the free recall condition, participants were encouraged to use relational processing in order to generate appropriate retrieval cues, whereas in the cued recall condition, the specific cue was already provided. However, in the cued recall condition, presentation of a category name would presumably activate many semantic associations, and therefore, item-based selection processes would be required to resolve this conflict (Thompson-Schill et al., 1997; Wagner et al., 2001a). Critically, the authors found a double dissociation between activation within the PFC, such that DLPFC activation (BA 46) was increased during the free recall condition, whereas VLPFC activation (BA 44) was increased during the cued recall condition. This finding is consistent with findings from WM and LTM encoding studies suggesting that VLPFC regions implement processes that modulate activation of item representations, whereas DLPFC regions implement processes that activate representations of relationships among items.

In addition to more lateral regions of PFC, anterior prefrontal regions (BA 10) are also routinely activated in studies of LTM retrieval, and particularly during source memory tasks that require retrieval of detailed information (Nolde et al., 1998; Fletcher and Henson, 2001; Ranganath and Knight, 2003). An interesting finding to emerge from many of these studies is that APFC activation is often not contingent on successful retrieval, or even on the difficulty of the retrieval decision (Henson et al., 1999; Rugg et al., 1999; Ranganath et al., 2000, 2007; Dobbins et al., 2002, 2003; Simons et al., 2005a,b). As noted earlier, APFC activation during WM tasks tends to be associated with the demand to select or maintain a cognitive set that dictates what information is relevant for selection. Cognitive models of memory retrieval suggest that this may be particularly relevant for accurate performance on source memory tasks (Johnson et al., 1993, 1997a; Mather et al., 1997; Norman and Schacter, 1997; Marsh and Hicks, 1998). This is because episodic memories are complex and consist of multiple characteristics (e.g., records of perceptual information in multiple modalities, cognitive operations, actions, affective reactions; Johnson et al., 1993). In many instances, a potential retrieval cue can activate several potential memories, including information that is irrelevant to a particular source decision (Koriat and Goldsmith, 1996;

Johnson, 1997; Koriat et al., 2000). Source memory decisions therefore demand the selection of an appropriate cognitive set in order to constrain retrieval of information associated with a cue and to narrow down the criteria for subsequent decision processes (Johnson et al., 1993; Mather et al., 1997; Norman and Schacter, 1997; Marsh and Hicks, 1998). This set would be initiated in response to a retrieval cue in order to constrain retrieval of information associated with the cue and narrow down the criteria for subsequent decision processes (Johnson et al., 1993; Johnson and Raye, 1998; Rugg and Wilding, 2000). This process has been described as setting 'decision criteria,' 'feature weights,' or a 'retrieval orientation.' We hypothesize that APFC is critical for selecting cognitive sets, and that source memory decisions constitute an example of when this process must be engaged.

One way of testing this idea is to contrast APFC activation between retrieval tasks that vary in terms of the specificity of the memory decision that is to be made (Ranganath et al., 2000, 2007). In one such study (Ranganath et al., 2000), brain activity was contrasted between a retrieval task that required participants to make a general item recognition decision versus a retrieval task that required participants to make a recognition decision specifically based on the match between the visual features of test items relative to studied items (**Figure 5**). Not surprisingly, participants were slower and less accurate at making responses to previously studied items in the more specific test condition. However, for unstudied items, accuracy and reaction times were comparable across the two test conditions. Results showed that activation in left APFC was increased during the more specific test, as compared with the more general test. What is more remarkable, however, is that activation during specific test trials was also increased for new items that

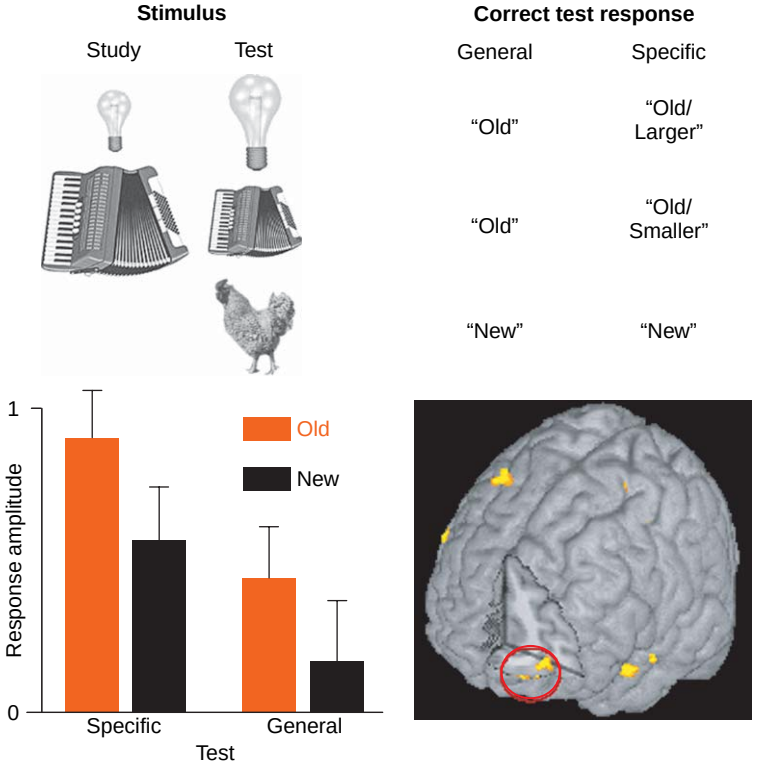

Figure 5 Participants studied objects that were shown in a large or small size, and then at test were shown objects that were either larger or smaller than the studied objects. In the 'General' test condition, participants were instructed to make a decision as to whether each object was studied, whereas on specific test trials, participants were additionally instructed to decide whether each test object was larger or smaller than the studied objects. In a region of left APFC, shown in the lower right panel, activation was increased for both old and new items in the specific test, as compared with the more general test condition (lower left panel). Experimental design and results from Ranganath C, Johnson MK, and D'Esposito M (2000) Left anterior prefrontal activation increases with demands to recall specific perceptual information. *J. Neurosci.* 20: RC108: 1–5.

were not seen during the study phase, despite the fact that behavioral performance was the same across the two test conditions. Thus, APFC activation during specific test trials reflected the need to constrain retrieval of information associated with each test item and to narrow down the criteria for subsequent decision processes. In this sense, APFC activation in source memory tasks might be analogous to activations in WM tasks in which one must select and maintain sets of rules that dictate the items or relationships that are currently relevant (Braver et al., 2003; Bunge et al., 2003; Sakai and Passingham, 2003).

Relevant to this idea, some researchers have suggested that APFC may be involved in maintaining an 'episodic retrieval mode' – a cognitive set which ensures that stimuli will be treated as cues for episodic retrieval (Lepage et al., 2000; Rugg and Wilding, 2000; Buckner, 2003). This view is supported by recent functional imaging studies showing sustained anterior prefrontal activity during episodic retrieval tasks, sometimes extending across multiple retrieval trials (Duzel et al., 1999; Lepage et al., 2000; Velanova et al., 2003). Additionally, retrieval mode-related activation in APFC appears to depend on the degree of control that is required in a given episodic memory test (Velanova et al., 2003). These findings are consistent with the idea that APFC is more generally involved in selection and maintenance of cognitive sets that support accurate episodic retrieval and source monitoring.

3.14.5.3 Laterality of PFC Activation during LTM Encoding and Retrieval

Another issue of interest that emerged from imaging studies of LTM retrieval concerns hemispheric asymmetries in the PFC. The question emerged from results of positron emission tomography studies of verbal memory that repeatedly observed left prefrontal activation during semantic decision tasks and right-lateralized prefrontal activation during tasks that engaged episodic retrieval processes (Tulving et al., 1994). Reviewing these findings, Tulving and his colleagues proposed the hemispheric encoding retrieval asymmetry (HERA) model, in which the left PFC was proposed to play a disproportionate role in episodic encoding (via its role in semantic processing) and the right PFC was proposed to play a disproportionate role in episodic retrieval (Tulving et al., 1994; Nyberg et al., 1996).

Shortly after its introduction, HERA was criticized, based on findings showing that left and right VLPFC are typically recruited during both encoding and retrieval tasks, and that the relative laterality of these effects is more dependent on the types of material that are being processed than on the type of memory operation (encoding or retrieval) being performed (Kelley et al., 1998; Wagner et al., 1998; McDermott et al., 1999; Golby et al., 2001). In response to this criticism, Habib et al. (2003) have argued that such findings

> . . . have no relevance to HERA. They provide good evidence in support of material-specific hemispheric asymmetry, but, because encoding and retrieval processes were not systematically varied and their interaction with hemispheres was not examined in these studies, the data are neutral with respect to HERA.

Another criticism of HERA is that, in focusing on left–right asymmetries, the model failed to account for perhaps more compelling functional differences along the rostro–caudal and dorsal–ventral axes (Buckner, 1996). Furthermore, laterality effects in DLPFC and APFC remain difficult to characterize. Laterality effects in these regions have typically not varied according to material, but also have not strictly followed predictions of the HERA model (Nolde et al., 1998; Ranganath and Knight, 2003; Ranganath, 2004).

Following up on the HERA model, Cabeza and colleagues (2003) recently proposed that the left PFC is engaged by tasks requiring semantically guided generation of information, whereas the right PFC is engaged by tasks requiring monitoring and checking of retrieved information. Another idea that has been proposed is that that left PFC is more engaged during the monitoring of specific memory characteristics, whereas right PFC is more engaged during the monitoring of undifferentiated information (Mitchell et al., 2004). A related idea is that the left PFC may disproportionately contribute to the use of specific contextual information to make a memory decision, whereas the right PFC may disproportionately contribute to the use of familiarity to make a memory decision (Dobbins et al., 2004). Like the HERA model, these accounts also do not specify whether the proposed hemispheric asymmetries would be expected to be constant across different subregions of PFC (e.g., DLPFC, VLPFC, APFC). At present, it is unclear if any of these models can fully account

for patterns of hemispheric asymmetry in PFC activation during LTM retrieval, but this is in part due to the fact that the models have not been directly contrasted in many studies (Ranganath, 2004).

3.14.6 Conclusions and Future Prospects

Converging evidence from neuropsychology and neuroimaging supports the idea that prefrontal regions play an important role in WM and LTM encoding and retrieval. Research on WM has shown that the PFC is not necessary for short-term retention of information per se, and instead that prefrontal regions contribute to WM performance by using goals and prior knowledge to guide activation of mnemonic representations. For example, regions in VLPFC may be more involved in modulating the activation of relevant items, whereas DLPFC may be more involved in modulating the activation of relationships between items that are currently being processed. Regions in APFC may be involved in selecting sets of rules that determine which items and relations are appropriate for selection.

Studies of LTM converge with the findings described earlier by demonstrating a role for the PFC in control processes that support encoding and retrieval. Neuropsychological research has shown that PFC regions may support LTM encoding by subserving controlled selection of attention toward goal-relevant items and by building or assessing relationships between relevant items. Imaging studies have shown that VLPFC may support LTM encoding by enhancing the strength and distinctiveness of memory for item information, whereas DLPFC may support encoding by building associations among items. During retrieval, VLPFC may support the ability to resolve competition in order to retrieve relevant items from memory, whereas DLPFC may support the ability to use relational information to guide successful retrieval and to inhibit previously learned associations. Additionally, some evidence suggests that APFC may support the selection of rules to determine the dimensions on which a retrieval cue and retrieved information should be processed.

Although our model can explain the extant evidence, future research will be needed to more extensively test it and to specify some important and currently unresolved issues. One important question is the nature of functional interactions within the PFC, and between different prefrontal and posterior cortical regions. Given the functional role for APFC suggested above, it would seem that APFC should modulate activation in corresponding regions of DLPFC and VLPFC, depending on the task set that is to be implemented (Sakai and Passingham, 2003). Furthermore, to the extent that VLPFC implements processes that select features of relevant items, one might expect that VLPFC should show increased connectivity with posterior areas that represent those features (Gazzaley et al., 2004). DLPFC regions, however, might process relational information in a number of ways. For instance, it is possible that DLPFC processes relational information by directly modulating activation in posterior cortical areas (Summerfield et al., 2006), perhaps by modulating the relative timing of neural firing within and across different areas (Shastri, 1996). Another possibility is that the posterior parietal cortex maintains dynamic relational bindings on line (Vogel et al., 2001), and that DLPFC can alter these bindings through its interconnections with parietal regions (Wendelken, 2001). A third possibility is that the DLPFC might modulate activation of relationships between items through its interactions with VLPFC (Blumenfeld and Ranganath, unpublished observations). Of course, none of the three accounts are mutually exclusive, and much more research needs to be done to address this fundamental question.

Another critical question for future research is to understand the role of orbitofrontal cortex (BA 11, on the ventromedial surface of the PFC) in memory processes. Research on orbitofrontal cortex and WM has generally focused on its role in integrating emotional and cognitive influences on behavior. However, given the extensive connectivity between the orbitofrontal cortex and medial temporal regions (perirhinal and entorhinal cortex) that are critical for LTM, it is reasonable think that orbitofrontal cortex should play an important role in LTM (Ranganath et al., 2005b). Evidence from patients with orbitofrontal lesions (due to anterior communicating artery aneurysms) is consistent with this idea (Rapcsak et al., 1996, 1999; Johnson et al., 1997b; Moscovitch and Melo, 1997; Schnider and Ptak, 1999; Schnider, 2000).

In conclusion, human neuropsychology and neuroimaging research has revealed significant insights into the roles of different regions of PFC in different kinds of memory processes. We have presented an

integrative framework to characterize these roles, but further research needs to be done to flesh out this framework and to address several important, and as yet unresolved, questions. Given the fact that disturbances in memory and prefrontal functioning are associated with normal aging (Tisserand and Jolles, 2003), cerebrovascular disease (Wu et al., 2002; Nordahl et al., 2005, 2006), and psychiatric (Cohen and Servan-Schreiber, 1992; Glahn et al., 2005) and neurological (Elliott, 2003; Levin and Hanten, 2005; Neary et al., 2005) conditions, addressing these questions will be of fundamental importance.

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3.15 Basal Forebrain and Memory

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3.15.1 Introduction

The basal forebrain is an aggregate of heterogeneous structures coursing along the ventral rostrocaudal extent of the brain. The anatomical connectivity of this collective allows it access to virtually the entire cortical mantle and to other subcortical structures thought to be essential to learning and memory. Often characterized by its large cholinergic projection neurons, the basal forebrain is home to a host of cell types and is regulated by a rich neurochemical background. The link between the basal forebrain and memory is frequently based on the decline of this structure in Alzheimer's disease. Yet, as in the medial temporal lobe system, there exists a characteristic amnestic syndrome associated with widespread damage to the basal forebrain. Animal models have been extensively used in an attempt to determine the role of this collective or of its subdivisions in memory and in other cognitive and behavioral functions. Further, the electrophysiological and rhythmic properties of the subdivisions of the basal forebrain are indicative of its extensive potential to function in a neuromodulatory capacity. Whereas analyses of the differential contribution of anatomical subdivisions of the basal forebrain to memory have been fruitful, further understanding of the organizational principles and functional properties of this region is likely to reveal additional clues to its role in memory and cognition.

3.15.2 Basal Forebrain Amnesia

A diversity of amnestic syndromes has been observed following insults to numerous loci in the neural system. As a result, a variety of neural circuits have been heavily implicated in underlying different aspects of memory, mostly indicated by the form of amnesia following disruption of the circuit. The circuits to which damage is traditionally observed to result in an amnestic syndrome are those including the prefrontal cortex, the cingulate cortices, the hippocampus, the rhinal cortices, multimodal cortical regions, and diencephalic regions (see Tranel et al., 2002; Milner, 2005; Frankland and Bontempi, 2006, for

review). Subregions of the basal forebrain share projections with each of these regions and are known for their neuromodulatory influence on these sites. Thus, not surprisingly, discrete lesions of the basal forebrain have also been observed to result in a profound amnestic syndrome. This basal forebrain amnestic syndrome shares many common features of the various forms of amnesia induced by damage to its projection sites.

Still, the manifestation of basal forebrain amnesia is dissociable from other amnestic syndromes based on a few hallmark features. In patients with basal forebrain lesions, severe anterograde amnesia for episodic memory is described as roughly equivalent to that of medial temporal and diencephalic patients. Yet, in basal forebrain amnesics, recognition of those episodic memories remains intact, demonstrating preserved function relative to medial temporal patients (Wais et al., 2006). This is illustrated by a case study, of patient JS, in which discrete damage to the basal forebrain resulted in a form of profound amnesia (Osimani et al., 2006). JS demonstrated severe anterograde amnesia, including diminished new learning and rapid forgetting, with relatively preserved recognition of episodic information. Also observed was temporally graded retrograde amnesia for episodic memory spanning several years in the face of preserved semantic and procedural memory. The general form of the amnestic syndrome described for patient JS thus overlaps with that of hippocampal and diencephalic amnesiacs who show impaired capacity for new declarative memory but preserved semantic and procedural memory. While each form of amnesia includes temporally graded retrograde amnesia, the temporal span of the retrograde amnesia in patient JS appears intermediate to that of medial temporal amnesiacs and diencephalic amnesiacs.

A further distinction between basal forebrain amnesiacs and medial temporal amnesiacs is a disproportionate deficit in their ability to determine the temporal distribution of episodic memories relative to their memories for those events. A study examining memory for episodes in contrast to memory for episodic timelines concluded that basal forebrain–damaged patients possess an equivalent deficit to medial temporal patients in knowing 'what' or retrieving information from autobiographical memories, but they have a disproportionate deficit in knowing 'when' or appropriate chronological placement of autobiographical memories (Tranel and Jones, 2006). Temporal knowledge is also disproportionately impaired in amnesiacs with retrosplenial or posterior cingulate damage who demonstrate profound impairments in the ability to remember the temporal order of recent events yet show spared order memory of remote events (Valenstein et al., 1987; Bowers et al., 1988). Thus, basal forebrain amnesiacs share the temporal disorientation of those enduring damage to cingulate cortices.

The most common source of basal forebrain damage is rupture and repair of aneurysms of the anterior communicating, the anterior cerebral, or the pericallosal artery (Myers et al., 2002). A comparison of subjects with basal forebrain damage resulting from anterior communicating artery aneurysms with those with hippocampal/medial temporal damage resulting from hypoxia demonstrated a double dissociation between medial temporal amnesics and basal forebrain amnesics in the ability to perform and reverse a conditional discrimination. The discrimination was a simple conditioning task in which subjects learned to associate a correct directional response (right or left) with a particular background color (light or dark). Hippocampal amnesiacs learned this conditional discrimination far better than did basal forebrain amnesiacs. When subjects were required to reverse the associations (direction to background color) in order to achieve a correct response, basal forebrain amnesiacs were able to reverse the contingencies, whereas hippocampal amnesiacs were rigid in their inability to perform such reversals (Myers et al., 2005). Thus, some of the most visible dissociations between medial temporal lobe amnesiacs and basal forebrain amnesiacs emerge in nondeclarative memory tasks (Myers et al., 2002).

The results put forth in this section reveal that there is a distinct amnestic syndrome that arises from damage to the basal forebrain. However, basal forebrain amnesia generally shares features of amnestic phenomena involved in damage to many other cortical and subcortical brain regions. This is most likely due to the vast anatomical connectivity in which the basal forebrain participates. The widespread influence of the basal forebrain on other regions involved in mnemonic processing suggests an integrated role of the basal forebrain and its associated structures in the determination of distinct memory dysfunction.

3.15.3 The Basal Forebrain and Alzheimer's Disease

The primary source of a role for the basal forebrain in memory is derived from years of research on

Alzheimer's disease (AD) (Perry et al., 1978; Bartus et al., 1982; Wenk, 1997; Francis, 2005). The cognitive signature of AD is progressive memory decline, whereas a striking neuroanatomical marker of AD is degeneration of the magnocellular cholinergic neurons in the basal forebrain (Wu et al., 2005; Wenk, 2006). In general, the observed deficits in AD parallel those of basal forebrain amnesia. There exists a deficit in explicit memory, a partial deficit for implicit memory, and preserved procedural memory (for review see Carlesimo and Oscar-Berman, 1992). An additional common feature is the recent finding of relatively preserved familiarity-based recognition memory in AD patients and intact familiarity-based recognition memory in patients with mild cognitive impairment (MCI) (Westerberg et al., 2006).

Pathology of the cholinergic basal forebrain is likely to occur in early stages of AD (Bowen et al., 1982), as is hypofunction of particular cortical target regions, including the posterior cingulate and entorhinal cortices (Huang et al., 2002; deToledo-Morrell et al., 2004; Pennanen et al., 2004; Borroni et al., 2006). Concomitant with posterior cingulate hypofunction in patients with MCI and in those with AD is a temporal disorientation that is manifested by temporal order or sequential processing problems (Hirono et al., 1998; deToledo-Morrell et al., 2001; Nestor et al., 2003). Entorhinal cortex hypofunction correlates with MCI, whereas the addition of further hippocampal dysfunction best correlates with a progression to AD (Pennanen et al., 2004). A postmortem study of individuals with AD and those with MCI demonstrates a reduction in (both groups) of P75 neurotrophin receptor (NTR) binding in the basal forebrain, indicating a reduced functional presence of the basal forebrain cholinergic neurons that bear this receptor in the brains of these individuals. This reduction is of particular interest, as the number of P75 (NTR) immunoreactive neurons in the basal forebrain is significantly correlated with tests of working memory and attention (Mufson et al., 2002). Thus, early indicators of memory decline include the basal forebrain and its projection sites. A recent clinical effort is aimed toward preserving the basal forebrain in an attempt to arrest the cognitive decline associated with AD. This effort involved the implantation of autologous fibroblasts genetically modified to express NGF directly into the basal forebrain of patients with early AD. The results are promising, as implanted patients show widespread increases in brain metabolism and markedly slower rates of cognitive decline (Tuszynski et al., 2005).

Despite the preponderance of research aimed at the cholinergic system, other transmitter systems demonstrate altered function in AD and may shed light on the role of the basal forebrain and its constituent circuits in memory (Gsell et al., 2004). For example, successful therapeutic efforts are currently being targeted at excitatory amino acid neurotransmission (for review see Wenk, 2006; also see Francis, 2005). Capitalizing on more recent anatomical findings that GABAergic neurons in the basal forebrain follow similar trajectories as cholinergic neurons may also provide an opportunity for delineating the status of GABAergic neurons in the basal forebrain of AD patients and their role in memory (McKinney and Jacksonville, 2005).

3.15.4 Basal Forebrain Anatomy

The basal forebrain is a group of heterogeneous clusters whose anatomical complexity and organization are constantly unraveling as new techniques are developed and applied to its study (for review see Zaborszky, 2002; also see Zahm et al., 2006). The basal forebrain is parsed into groups of functional anatomical subdivisions based on projection patterns, cell types, and transmitter content (for review see Alheid and Heimer, 1988). Historically, the striking loss of cholinergic function in AD led to extensive study of the basal forebrain cholinergic system and its role in memory. Thus, a large number of the anatomical subdivisions of this system are based on the distribution of cholinergic projection neurons. More recent investigations indicate that GABAergic and perhaps glutamatergic projection neurons also comprise this structure (Jones and Mühlethaler, 1999; Zaborszky et al., 1999).

The basal forebrain neurons are generally organized along a rostrocaudal axis, such that cholinergic nuclei are somewhat contiguous in nature (Semba et al., 1988; Zaborszky et al., 2005; see **Figure 1**). The majority of behavioral and anatomical studies subdivide the basal forebrain into primary subdivisions based on their differential projection systems. The differential projection patterns show a large degree of conservation from primate to rodent and have, thus, been heavily examined across multiple species.

The human basal forebrain has been subdivided and mapped according to the magnocellular projection neurons that reside as groups within the aggregate. The designation of subdivisions is based on cholinergic projection neurons, and the

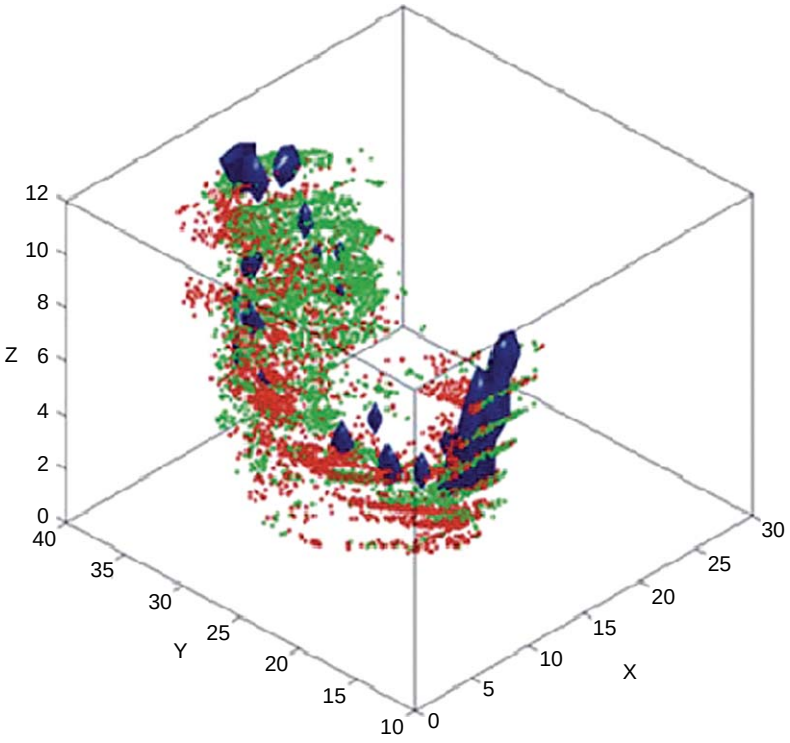

Figure 1 Scatter plots of both cholinergic (red) and parvalbumin (green) cells superimposed on the iso-relational surface (gradient of density changes) shows the relative cell density of the two neuronal types. From Zaborszky L (2002) The modular organization of brain systems. Basal forebrain: the last frontier. *Prog. Brain Res.* 136: 359–372.

subdivisions are referred to as Ch1-4 (for reviews see Mesulam et al., 2004; also see Zaborszky et al., 1999). These Ch subdivisions correspond to designated anatomical subdivisions in the rodent and primate. The Ch1 and Ch2 regions in the human correspond to the medial septum and ventral diagonal band complex (MS/VDB). The Ch3 region corresponds to the horizontal limb of the diagonal band of Broca (HDB), and the Ch4 region corresponds to the nucleus basalis of Meynert (nBM). Ch4 can be subdivided into six spatially distinct regions: Ch4am (anteromedial), Ch4ai (anterointermediate), Ch4id (intermediodorsal), Ch4al (anterolateral), Ch4iv (intermedioventral), and Ch4p (posterior). The Ch1 and Ch2 (MS/VDB) neurons provide the primary cholinergic input to the hippocampus. The Ch3 (HDB) neurons provide primary input to the olfactory bulb. The Ch4am provides the primary cholinergic input to medial cortical areas including the cingulate gyrus, whereas the Ch4al provides the primary input to frontoparietal, opercular areas, and the amygdala. Ch4i provides projections to lateral, frontal, posterior parietal, peristriate, inferotemporal, parahippocampal, and orbitoinsular regions, whereas Ch4p provides input to superior temporal and temporopolar regions (see Mesulam, 2004, for review; also see Zaborszky et al., 1999).

Much of the basal forebrain anatomy has been conserved in the rodent, and the bulk of recent anatomy has focused on the rodent system. Three primary subdivisions are most often referred to as the functional components of the basal forebrain: the medial septal nucleus in combination with the vertical limb of the diagonal band of Broca (MS/VDB), the horizontal limb of the diagonal band of Broca in combination with portions of the magnocellular preoptic area (HDB/MCPOA), and the corticopetal basal forebrain including the nucleus basalis magnocellularis (NBM), the substantia innominata (SI), and portions of the ventral pallidum (hereafter included in NMB/SI). Neurons within the MS/VDB complex provide the major cholinergic innervation of the hippocampus, cingulate cortex, and subiculum, with a projection to the entorhinal cortex. Cells within the HDB/MCPOA project primarily to the olfactory bulb and entorhinal cortex, with additional projections to the occipital cortex and the amygdala. The NBM/SI contains a continuum of

projection neurons that provides the major source of cholinergic input and some noncholinergic input (mostly GABAergic) to nearly all neocortical regions. Additionally, it provides a primary input to the basolateral amygdala and to the reticular nucleus of the thalamus (for a detailed review see Zaborszky, 2002).

The NBM/SI region serves to regulate cortex and, at the same time, receives input from nonisocortical paralimbic cortical areas (orbitofrontal-prefrontal, temporopolar, insular, parahippocampal, and cingulate), the amygdala, the hypothalamus, and various brain stem cell groups. As there is no evidence of direct thalamic projections to the basal forebrain, sensory information must reach the basal forebrain through either the prefrontal cortex or the central nucleus of the amygdala, although other unreported pathways cannot be discarded. Additionally, the basal forebrain makes reciprocal connections with the central nucleus of the amygdala, ventral tegmental area, locus ceruleus, and pedunculopontine tegmental nucleus (PPT) (a brainstem cholinergic nucleus that also projects to the thalamus).

By virtue of a convergence of afferent brainstem projections to the basal forebrain, diverse neurochemical systems can heavily modulate the basal forebrain and its constituent cortical targets. Dopaminergic influence can occur through projections arising from the ventral tegmental area (VTA) or by projections arising directly from the substantia nigra pars compacta, substantia nigra pars reticulata, or retrorubral field (Semba et al., 1988). Serotonergic influence gains access to the basal forebrain by way of dense projections arising from the dorsal raphe nucleus, lighter projections from the median raphe nucleus, or sparse projections directly from the raphe magnus (Semba et al., 1988; Gasbarri et al., 1999). The basal forebrain can receive noradrenergic influence either from the locus ceruleus or from the nucleus of the solitary tract (Semba et al., 1988; Espana and Berridge, 2006). Finally, the brain stem cholinergic system, which is also referred to as the pontomesencephalic cholinergic system (Shute and Lewis, 1967; Woolf, 1991), provides cholinergic input to the basal forebrain primarily by receipt of projections from the laterodorsal tegmental nucleus (LDT) (Satoh and Fibiger, 1986; Woolf and Butcher, 1986; Semba et al., 1988) and also from the PPT (Semba et al., 1988).

Thus, the NBM/SI corticopetal region is well positioned to exert influence on the cortex, providing a primary source of acetylcholine and other neurotransmitters in a regionally independent manner and in the service of other neural systems. Such a system is well suited to play a role in a variety of cognitive functions, including memory.

3.15.5 Basal Forebrain Cell Types

The ability to produce selective neurotoxic lesions of only the cholinergic neurons within the basal forebrain has led to a strong emphasis being placed on discovering the function of these cells, especially given their role in AD and in attentional processing. However, the cholinergic cells constitute only a portion of the cell population in the region. Several other cell types, including GABAergic, peptidergic, and most likely glutamatergic cells also reside within the basal forebrain (Jones and Mühlethaler, 1999; Zaborszky et al., 1999).The characterization of these noncholinergic cell populations is an important route of exploration. While the precise role of these other cell types is unknown, the anatomical localization of these cells within the cholinergic basal forebrain system and their projection patterns indicate that they provide a very important contribution to the functionality of the basal forebrain.

In an elegant series of studies, Zaborszky's group (Zaborszky, 2002; Gritti et al., 2003; Zaborszky et al., 2005) has found noncholinergic cells that contain the different calcium-binding proteins – calbindin, calretinin, and parvalbumin – and has determined the three-dimensional structure and clustering patterns of the four cell types within the entirety of the basal forebrain. These neuronal types form sheets or bands that are twisted and attached to each other and that are not randomly distributed but show location-dependent density profiles (see **Figure 1**). In almost all basal forebrain regions that contain large cholinergic neurons, the other cell types are interspersed in this manner.

There has been some discrepancy on the percentage of basal forebrain neurons that are cholinergic as opposed to containing other neurotransmitters. Zaborszky et al. (2005) found high ratios of GABAergic to cholinergic cells that varied according to structure, with higher ratios in the globus pallidus and SI than in the MS/VDB, HDB, ventral pallidum, and the internal capsule. While it is unclear what proportion of these GABAergic cells are cortically projecting, other studies (Gritti et al., 1997; Sarter and Bruno, 2002) found that there are roughly equal amounts of GABAergic and cholinergic cells that project to cortex. Additionally, Jones

and colleagues (Manns et al., 2001, 2003) found a large subset of cells that were neither GABAergic nor cholinergic but, rather, putatively glutamatergic. These cells also project to cortical regions. In a new study by this group using unbiased stereological estimates in rat, it was found that only a small minority of cells within the BF (5%) are capable of synthesizing acetylcholine, while 35% are capable of synthesizing GABA, and a vast majority (90%) are capable of synthesizing glutamate (Gritti et al., 2006).

The heterogeneity of cell types, and the interesting structural patterning they manifest, reveals a rich organization within the basal forebrain. The structure may consist of functionally distinct circuits constituted by basal forebrain subsections and their connected cortical regions. Further understanding of the organizational principles of this system and the role of GABAergic and glutamatergic, in addition to cholinergic, cells is likely to provide clues regarding the way in which the system may subserve aspects of cognition and memory.

3.15.6 Medial Septum/Vertical Limb of the Diagonal Band Electrophysiology and Memory

The MS/VDB is most well known for its role in the generation and maintenance of hippocampal oscillatory activity, especially the theta and gamma rhythms found therein (Petsche and Stumpf, 1962; Stewart and Fox, 1990a,b; Vinogradova, 1995). Initial evidence for this role came from studies that showed that lesions of the MS/VDB result in abolition of the hippocampal theta rhythm, whereas stimulation of the MS/VDB produces theta in the hippocampus (Lee et al., 1994; Jackson and Bland, 2006). Additionally, cells in the MS/VDB show oscillatory activity that correlates with the hippocampal theta rhythm. Ford et al. (1989) found that the majority of cells in the MS/VDB were theta-related and classified them as theta-on or theta-off cells. Dragoi et al. (1999) also found several types of theta-related neurons in the MS/VDB: rhythmic neurons phase-locked to theta, nonrhythmic but phase-locked cells, and a smaller group of cells that were neither rhythmic nor phase-locked to the hippocampal theta oscillation (see also Gaztelu and Buno, 1982; Alonso et al., 1987; King et al., 1998). The determination of which type of cells these were was not undertaken, and there is still some controversy over the role of different types of MS/VDB neurons on hippocampal theta. Given that there is a strong reciprocal connection from hippocampal interneurons to MS/VDB GABAergic neurons, and that hippocampal cells also show intrinsic theta rhythmic firing, it may be important to think of theta generation as a system-wide phenomenon involving several cell types and depending upon the reciprocal interrelationship between the MS/VDB and hippocampus.

Evidence for a role in hippocampal theta generation and maintenance by cholinergic MS/VDB cells is mainly indirect. Selective lesions of the cholinergic cells in the MS/VDB lead to a severe reduction of rhythmically bursting neurons within the MS/VDB (Apartis et al., 1998). Additionally, Lee et al. (1994) selectively lesioned the cholinergic MS/VDB cells and found a reduction in theta power in the hippocampus. However, a definite theta peak during active wake and rapid eye movement (REM) sleep remains in the face of an absence of cholinergic input, leading to the suggestion that GABAergic neurons could play a prominent role in theta rhythm generation. In a study in which only the noncholinergic cells in the MS/VDB were lesioned (Yoder and Pang, 2005), an attenuation of hippocampal theta was found, confirming this hypothesis. Additionally, juxtacellular labeling techniques have revealed parvalbumin (PV)-positive neurons exhibiting highly regular bursting activity correlated to either the peak or trough of hippocampal theta waves. Subsequent studies have shown that MS/VDB GABAergic cells have the intrinsic propensity to oscillate at theta frequency (Serafin et al., 1996). Furthermore, some of these neurons had an intraburst frequency in the gamma range (Manns et al., 2000a,b; Borhegyi et al., 2004), allowing for entraining of both theta and gamma rhythmicity in the hippocampus. These findings indicate that the septohippocampal GABAergic projection may be sufficient to maintain some hippocampal theta activity, while the cholinergic cells may have a role in determining the magnitude of hippocampal theta.

The MS/VDB's role in memory may be mainly through its role in theta generation, as there is considerable evidence that theta rhythm plays a vital role in information processing and memory formation (Winson, 1972; Givens and Olton, 1994; O'Keefe and Burgess, 1999; Seager et al., 2002). The role of theta in learning and memory has been a topic of interest since the finding that MS/VDB lesions that eliminated theta in the hippocampus produced severe memory deficits (Winson, 1978). A plausible mechanism by which theta rhythmicity could affect

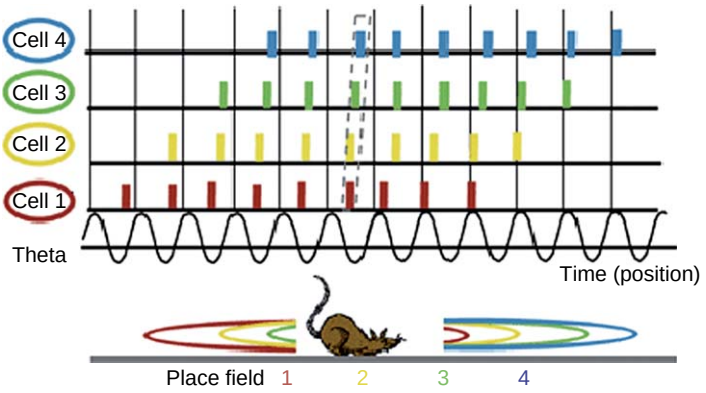

Figure 2 Theta phase precession observed in the place cells of the rat hippocampus. When the rat runs to the right, the phase shift in firing within each theta rhythm cycle occurs in place cells 1–4, which are activated sequentially. The phase is arranged in order of firing within one phase cycle (an example is represented by gray lines). The rat running environment is expressed in compressed form in each theta cycle. From Dr. Yoko Yamaguchi, Laboratory for Dynamics of Emergent Intelligence.

information-processing and memory is for it to act as a substrate for long-term potentiation (LTP) and long-term depotentiation (LTD). In fact, studies have indicated a faster decay of LTP with MS/VDB lesions and prolonged LTP maintenance with MS/VDB stimulation (Rashidy-Pour et al., 1996; Frey et al., 2003). While the role of LTP and LTD in learning and memory is still incompletely understood, there is mounting evidence for such a role (Moser et al., 1993; Rogan et al., 1997; Wilson and Tonegawa, 1997; but see Barnes, 1995, for a critique of this view). It is revealing that several studies have indicated that LTP occurs at the peak of the theta rhythm (Pavlides et al., 1988) and that stimulation at the peak of theta induces LTP, while stimulation at the negative phase of theta rhythm depotentiates previously potentiated synapses (Holscher et al., 1997). These findings have led to the construction of a model in which the two phases, the peak and trough of local theta, correspond to memory encoding and retrieval, respectively (Hasselmo et al., 2002).

Adding to the general idea that theta has a role in memory through its action on LTP and LTD is an intriguing finding concerning the nature of place-specific cells and their firing patterns with respect to the theta rhythm. Principal (pyramidal) cells in the hippocampus show location-specific discharge during exploration (place fields). O'Keefe and Recce (1993) found that the phase of the theta cycle at which such cells fire advances gradually as the animal passes through the cell's place field; that is, the initial spiking occurs at a particular phase of theta, but each successive spike moves to an earlier location on the theta wave (see **Figure 2**). This phenomenon has been called phase precession and has been postulated to result in the compression of temporal sequences of place cell firing, which could facilitate synaptic plasticity (Skaggs et al., 1996) and perhaps act as a neural substrate of path integration and episodic memory (Dragoi and Buzsaki, 2006).

The MS/VDB's effect on hippocampal rhythmicity is thus a well-studied and robust phenomenon. Additionally, through its effect on synaptic plasticity in the form of LTP and LTD, the MS/VDB can strongly affect the way in which hippocampal processes underlying learning and memory function.

3.15.7 The Nucleus Basalis Magnocellularis/Substantia Innominata Electrophysiology and Memory

The NBM/SI possesses interesting electrophysiological properties that render it highly important for cortical processing. It is involved in the general role of activation or desynchronization of cortex but also plays a more specific role in learning and memory, as single-unit, field potential, and stimulation studies have revealed. While some progress has been made in determining the electrophysiological properties of NBM/SI neurons and their role in learning and memory, there is still much to be done to fully explicate these properties.

There is an abundance of evidence indicating that the NBM/SI region of the basal forebrain is involved

in the activation or desynchronization of the cortical EEG, a state that corresponds to active, exploratory, and attentive behaviors. This capacity is dependent upon both cholinergic and GABAergic projections to cortex, although much of the initial evidence for this role came from studies on acetylcholine and its role in cortical activity. The first such study (Longo, 1966) blocked cortical acetylcholine through a muscarinic receptor antagonist, which resulted in an increase in slow-wave activity much like that found in non-REM sleep. Since this initial study, many others have corroborated a role for acetylcholine on the activation of cortex (Dringenberg and Vanderwolf, 1997; McCormick and Bal, 1997; Detari et al., 1999; Cape et al., 2000; Detari, 2000; Manns et al., 2000a; Linster and Hasselmo, 2001).

Further studies showed that lesions of the NBM/SI result in a marked reduction of cortical activation, leading to the conclusion the NBM/SI is necessary for such activation. While there is some evidence that selectively lesioning just the cholinergic cells in the NBM/SI results in an attenuation of cortical desynchrony (Berntson et al., 2002), more evidence points to a combinatorial action, with GABAergic and other transmitter types such as glutamate also being involved in this capacity. Given that an equal number of GABAergic and cholinergic neurons within the NBM/SI project to cortex (Walker et al., 1989; Gritti et al., 1997; Zaborszky et al., 1999), this is not surprising. Furthermore, as stimulation of NBM/SI GABAergic corticopetal projection neurons inhibits cortical GABAergic interneurons, this can in turn increase cortical disinhibition, resulting in increased cortical activation (Jones and Mühlethaler, 1999; Manns et al., 2001; Sarter and Bruno, 2002).

Single-unit studies have provided further evidence that the NBM/SI is involved in activating cortex. For example, enhancement of single-unit responses in the cortex occurs through stimulation of cholinergic cells (Metherate and Ashe, 1993; Herron and Schweitzer, 2000; Berntson et al., 2003). Additionally, many basal forebrain neurons show elevated discharge rates and a bursting pattern of firing during active wake and REM sleep (Cape et al., 2000; Manns et al., 2000a; Szymusiak et al., 2000). While many of these cells increase their firing rates during cortical desynchronization, some decrease their firing rates during this time. By utilizing simultaneous labeling and recording studies, Duque et al. (2000) discovered that both cholinergic and PV-labeled (GABAergic) neurons increased activity with cortical activation, while those that were labeled as neuropeptide Y (NPY), which are most likely local interneurons, decreased rates. These data lend support for a major GABAergic component to cortical activation. It is clear from the above that the basal forebrain has a complex effect on the cortex, with different neuronal types contributing differently to activation or desynchrony.

While the effect on cortical arousal is interesting in itself, there is also neurophysiological evidence supporting a more specific role for NBM/SI activity in learning and memory. This evidence originated with Kanai and Szerbs' work in 1965 on acetylcholine release in the cortex (Kanai and Szerb, 1965). They found that there were two distinct mechanisms of release in the cortex, one related to general arousal and another, more specific, function related to sensory stimuli that have obtained significance for the animal. Later work showed that stimulation of the NBM/SI increases the responsiveness of cortical neurons to sensory stimuli (Metherate and Ashe, 1991) and that Ach applied to cortical sensory neurons increases responses to sensory stimuli (Sillito and Kemp, 1983; Metherate et al., 1987), while lesions reduce the responsiveness of neurons in visual cortex to visual stimuli (Sato et al., 1987).

In addition to general increases in responsiveness, several studies have shown that NBM/SI neurons respond to particular associations formed during learning, as they respond to the learned significance of task events as well as to the novelty, familiarity, or recency of presentation of stimuli (Wilson and Rolls, 1990a,b; Whalen et al., 1994; Wilson, and Ma, 2004). Many studies have shown that stimulation of basal forebrain neurons also results in changes in the cortex that correspond to improvements in learning and increased plasticity (Metherate and Ashe, 1991; Edeline et al., 1994; Kilgard and Merzenich, 1998; Weinberger and Bakin, 1998; Rasmusson, 2000; Miasnikov et al., 2001; Kilgard, 2003). Conversely, lesions of the NBM/SI result in a reduction in such learning and plasticity (Conner et al., 2003, 2005). These changes may be subserved by the long-term enhancement of evoked potentials and the facilitation of multiunit activity that is also seen with stimulation of the region. One major remaining question is the determination of cell types that respond in these ways. To date, no study on the responses of NBM/SI neurons during behavioral tasks has determined the neurotransmitter type (cholinergic, GABAergic, or other) of the cells that have been recorded.

Cortical oscillations in the theta, beta, and gamma ranges are implicated in learning and memory.

Several studies have shown that cells within the NBM/SI are intrinsically oscillatory and that the NBM/SI can induce oscillatory activity in the cortex. Lee et al. (2004) showed that cholinergic neurons within the NBM/SI discharge in bursts at maximal rates during active waking and REM sleep, which is correlated with theta oscillations. They also found that subgroups of GABAergic neurons had very regular high-frequency tonic spiking within a gamma EEG frequency range and rhythmic bursting at theta-frequency range during cortical activation. In addition to these single-unit studies on oscillatory activity, Quinn et al. (2002) showed that local field potential recordings within the NBM/SI of freely behaving animals reveal rhythmic activity at the theta, beta, and gamma frequency ranges. Given its wide projection pattern to numerous cortical regions, oscillatory activity in the BF can have wide-ranging effects on the patterning of activity in cortex.

3.15.8 Animal Models of Memory

A characterization of the role of the basal forebrain and its subregions in memory has been provided through the utilization of primate and rodent models in which all or part of the basal forebrain has been removed and the resultant behavioral capabilities of the animal analyzed. A variety of techniques have been used for removal of this region, including electrolytic lesions, neurotoxic lesions, and more recently, immunotoxic lesions. Historically, observed deficits following removal of the basal forebrain were attributed to the cholinergic denervation of target structures of the basal forebrain, but with the advent of an immunolesion technique, reinterpretation of these results became necessary.

Direct investigation of the role of cholinergic neurons in memory became possible in the early 1990s, when a new immunotoxin, 192 IgG-saporin, was developed (Wiley et al., 1991). Delivery of 192 IgG-saporin directly into the basal forebrain results in selective destruction of cholinergic neurons, whereas neighboring noncholinergic neurons are left intact. This selective destruction is accomplished by taking advantage of the receptor profile of basal forebrain cholinergic neurons. Cholinergic neurons bear the P75 neurotrophin receptor that is absent from neighboring neurons. Preferentially binding to the P75 receptor, 192 IgG is an immunoglobulin that effectively delivers a cytotoxin (saporin) to the cell body, accomplishing selective destruction of the cholinergic neurons in the basal forebrain. Here it is important to note that a small subpopulation of neurons in the cholinergic basal forebrain do not bear the P75 neurotrophin receptor. This subpopulation of neurons projects primarily to amygdaloid nuclei, resulting in residual cholinergic innervation of various amygdaloid nuclei (including the basolateral nucleus) (Hecker and Mesulam, 1994).

A large body of work using this immunolesion technique has served to revise the role of the basal forebrain cholinergic system in various aspects of memory, as many of the deficits found with complete lesions have not been found with targeted removal of only cholinergic neurons. (see Baxter, 2001, for review). The basal forebrain contains several other neuromodulatory cell types, including GABAergic and glutamatergic cells, which together comprise a high percentage of the totality of neurons found in the region. Understanding the role of these and other neuromodulators is of great importance to fully explicate the role of the basal forebrain in memory.

3.15.9 The Medial Septum/Vertical Limb of the Diagonal Band and Memory

Total removal of MS/VDB neurons in rodents leads to profound deficits in spatial learning and memory tasks alongside an increase in reactive behavior (Gage and Olton, 1975; Mitchel et al., 1982; Dunnett et al., 1987; Hagan et al., 1988; Kelsey and Landry, 1988; Kesner et al., 1988). For example, on several spatial memory tasks that are classically disrupted by hippocampal lesions, including the Morris water maze and radial eight-arm maze, deficits in the ability to recall spatial locations following full lesions of the MS/VDB are robust. Additionally, temporary inactivation of the MS/VDB results in drastic memory deficits along with a reduction in hippocampal theta rhythm, which is important for memory (Mizumori et al., 1990; Givens and Olton, 1994; Hasselmo et al., 2002) Thus, historically, such deficits were thought to be a result of cholinergic denervation of the hippocampus and other structures to which the intact MS/VDB provides cholinergic input, building on a cholinergic hypothesis of memory. However, with the advent of selective lesioning techniques, the cholinergic basis of these deficits has been called into question.

3.15.10 Selective Cholinergic and GABAergic Lesions of the Medial Septum/Vertical Limb of the Diagonal Band: Implications for Attention, Learning, and Memory

A more recent body of work, in which discrete removal of the cholinergic neurons is accomplished by direct infusion of 192IgG-saporin into the MS/VDB complex, demonstrates that rodents maintain the ability to do standard spatial memory tasks, including various versions of the Morris water maze (Baxter et al., 1995, 1999a); McMahan et al., 1997). Rats with these lesions are also able to perform a standard radial maze task, even when long delays are inserted between training and testing (Chappell et al., 1998). Interestingly, selective lesions of just GABAergic cells within the MS/VDB also do not affect rats' ability to perform normally on the same tasks (Pang et al., 2001). Since neither type of lesion alone results in impairments on standard spatial memory tasks, it may be concluded that most spatial memory impairments found with lesions of the MS/VDB require at the least a depletion of both the cholinergic and GABAergic neurons within the region.

Whereas impairments in spatial memory are either mild or absent in rats who have sustained selective cholinergic lesions of the MS/VDB, a separate body of work indicates that these rats have memory deficits in tasks that emphasize other behavioral or sensory domains. Rats with selective cholinergic MS/VDB lesions demonstrate impairments on a social transmission of food preference task, a task that is dependent on olfactory associative memory (Berger-Sweeney et al., 2001; Vale-Martinez et al., 2002). Specifically, these rats demonstrate a deficit in delayed recall of a food preference learned prior to surgery, indicating a retrograde memory deficit. These data are taken to mean that the cholinergic projection to the hippocampus is involved in retrieval of social memories related to food preference.

Another arena in which rats with selective lesions of the MS/VDB demonstrate memory deficits is when they are presented with more than one potentially relevant feature. Rats were tested on a conditional associative learning task, termed environment-spatial conditional learning. In this task, the correct location of a spatial response depended on the array of local environmental cues. Rats with cholinergic lesions of the MS/VDB were impaired when the two parts of the conditional problem were presented concurrently, but not when one spatial environment was learned prior to the other. (Janisiewicz and Baxter, 2003).

These same lesions reliably produced impairments in tasks requiring that the rat learn to decrement attention to irrelevant stimuli, indicating that this system is critical for reducing attention to irrelevant stimuli and perhaps to reducing interference from irrelevant features in the environment. Although these functions do not directly address memory functions, they certainly come to bear on effective learning and memory. (Baxter et al., 1997, 1999b). Such changes in attentional processing are also evident in selective lesions of projection sites of the MS/VDB area. Discrete cholinergic denervation of the anterior cingulate, a projection region of this system, led to an increase in interference from distracting background flashes in a target detection task. This same manipulation also reduced a characteristic increase in the firing rate of medial prefrontal neurons to the distracting flashes (Gill et al., 2000), indicating that such firing works in the service of reducing processing of the flash and allowing salient information to be processed and remembered.

Discrete cholinergic lesions of the MS/VDB also impair various tasks that involve strategy selection or adaptively changing strategies or behavioral sets across both spatial (Cahill and Baxter, 2001; Bizon et al., 2003) and nonspatial (Baxter, 2001) memory tasks. This function of learning enables optimal performance in learning and memory tasks.

Selective cholinergic lesions of the MS/VDB produce domain-specific deficits in learning and memory that are insufficient to account for the sort of amnesia observed in humans but are sufficient to hamper the sort of optimal learning on which memory relies. Additionally, some very convincing studies have emphasized the importance of the GABAergic system not just as a modulator of the cholinergic system but as another primary projection system of the basal forebrain. Combined cholinergic and GABAergic lesions lead to global spatial memory impairments. This is a strong indicator that the conjoint activity of the system is essential to memory function.

3.15.11 The Nucleus Basalis Magnocellularis/Substantia Innominata and Memory

Complete lesions of the nucleus basalis/substantia innominata (NBM/SI) region of the basal forebrain

reveals similar learning and memory deficits to those of the MS/VDB region, despite the very different projection patterns of the two regions. Lesioning either region leads to difficulty on spatial discrimination tasks (Hepler et al., 1985) and spatial working memory tasks, including standard water maze and radial maze tasks (Bartus et al., 1985; Connor et al., 1991; Matsuoka et al., 1991). Thus, earlier studies viewed these two basal forebrain systems as having somewhat redundant memory functions while emphasizing a role for the total system in working rather than reference memory (Knowlton et al., 1985).

On nonspatial tasks, investigations have revealed differential disturbances of temporal memory between the MS/VDB and the NBM, indicating that rats with NBM lesions share deficits in temporal memory with those having frontal cortex lesions, rather than with those having MS/VDB lesions (Meck et al., 1987). The role of the NBM in aspects of temporal memory has also been explored through direct comparisons of the performance of rodents with NBM lesions to that of patients with AD on order memory tasks. Such comparisons revealed that complete lesions of the NBM result in order memory deficits that parallel those observed in patients with AD (Kesner et al., 1987).

With the development of a broader variety of neurotoxic lesion techniques, further studies indicated that, as with the MS/VDB, the substantive spatial working memory deficits observed in rodents with NBM lesions were most highly correlated with maximal destruction of basal forebrain neurons, but not with maximal depletion of acetylcholine (Wenk et al., 1989; Markowska et al., 1990). A direct comparison of different types of memory tasks across different neurotoxic lesions of the NBM indicated that maximal destruction of the NBM (in the absence of maximal depletion of cortical acetylcholine) led to spatial working memory deficits in the water maze and delayed match-to-position tasks. These lesions also impaired an attention-based serial reaction time and a passive avoidance task.

NBM lesions that maximally depleted cortical acetylcholine resulted in preserved performance on spatial working memory tasks. However, cortical acetylcholine depletion did result in impaired performance on the serial reaction task (Dunnett et al., 1990). These findings combined with contemporary findings from pharmacological studies, led to the proposal that the NBM may be preferentially involved in attentional processes rather than memory processes (Dunnett et al., 1990; Muir et al., 1993; Pang et al., 1993). One hypothesis set forth to explain the stark behavioral contrast between the substantive working memory deficits following total destruction of the NBM as opposed to the absent or mild working memory deficits following partial destruction of the NBM was the idea that the substantive memory deficits were a result of damage to the globus pallidus that included disruption of corticostriatal output pathways (for review see Dunnett et al., 1990; Muir et al., 1993). Alternatively, the mild deficits were taken to be a result of depleting cortical acetylcholine and thereby disrupting basic attentional processes (for review see Muir et al., 1993).

3.15.12 Selective Cholinergic Lesions of the Nucleus Basalis Magnocellularis/Substantia Innominata: Implications for Attention, Learning, and Memory

The advent of a selective immunolesion technique (192-IgG saporin) has allowed a thorough assessment of the necessity of the SI/NBM corticopetal cholinergic system in various aspects of attention as well as memory.

Behavioral and neurobiological data concur that cholinergic neurons in the basal forebrain help to fluidly and appropriately regulate attention to relevant stimuli (for review see Baxter and Chiba, 1999; McGaughy et al., 2000). The integrity of NBM/SI cholinergic neurons is also required for the typical facilitation in processing stimuli whose outcomes are uncertain (Chiba et al., 1995; Bucci et al., 1998), a strategy that has been proposed to underlie the learning of effective predictive associations between stimuli (Pearce and Hall, 1980; Holland and Gallagher, 1993). This facilitated learning of, or increasing of attention to, surprising events that are typical of intact rats but are absent in rats with NBM/SI cholinergic lesions. In addition, the integrity of NBM/SI cholinergic neurons has been found to contribute to the fluid allocation of visuospatial attention, perhaps by reducing the behavioral influence of misleading stimulus expectations from visuospatial cues. This was revealed in an experiment in which rats with NBM/SI cholinergic lesions demonstrated poorer performance than control rats on trials in which misleading cues were presented (Chiba et al., 1999). The integrity of the NBM/SI cholinergic system may also be essential for adequate detection, selection, vigilance, and processing of stimulus associations, in

addition to the proper allocation of processing to these attentional functions. This is indicated by a large literature in which NBM/SI cholinergic lesions selectively impair aspects of these functions. (for review see Sarter et al., 1999; McGaughy et al., 2000, 2002; Baxter, 2001).

Many of the described attentional deficits have been replicated by utilizing cortical infusions of 192IgG saporin to remove only those NBM/SI neurons that innervate particular cortical sites involved in these aspects of attention. Specifically, vigilance or sustained attention deficits have been replicated in animals with frontoparietal deafferentation (McGaughy et al., 1998), whereas failure to increase attention to surprising events and deficits in conditioned responding were replicated by posterior parietal deafferentation (Bucci et al., 1998; Bucci and Chess, 2005). Thus, there exists a large body of literature, based on this selective immunolesion technique, implying a role for the cholinergic NBM/SI and its cortical targets in various aspects of attention.

A large number of studies indicate that the classic working memory deficits observed in rats with total NBM/SI lesions are not observed in rats with selective cholinergic lesions of this system (for review see Baxter and Gallagher, 1996; Everitt and Robbins, 1997; Wenk, 1997; Baxter and Chiba, 1999; Baxter, 2001). For example, neither a deficit in water maze performance nor a deficit in radial maze performance (even with very long delays) was evident in these rats (Baxter et al., 1995; Chappell et al., 1998; Galani et al., 2002). Additionally, neither passive avoidance nor delayed alternation was impaired in rats with these lesions (Wenk et al., 1994). In order to eliminate the possibility that the MS/VDB neurons exert a compensatory function when the NBM/SI is lesioned, it was also important to examine the behavioral effects of combined lesions of the MS/VDB and NBM/SI cholinergic neurons on behavior. An investigation utilizing a cholinergic immunotoxin to create a combined lesion of MS/VDB, SI/NBM, and HDB indicated that, even in the face of removing this entire system, spatial working memory (as measured by performance in a standard water maze task) remained intact (Vuckovich et al., 2004). The aggregate of the NBM neurotoxic lesion work and the large body of work utilizing the selective cholinergic immunotoxin, 192 IgG saporin, refuted the long-standing hypothesis that basal forebrain cholinergic projections to the cortical mantle are essential to support classic working memory tasks.

Here it is important to note that the NBM/SI provides cholinergic innervation to the cortical mantle and also to the amygdala. The cholinergic immunotoxin, 192 IgG saporin, depletes the preponderance of cortical acetylcholine while sparing a large portion of the cholinergic innervation of the amygdala. Selected studies examining the importance of the NBM/SI projections to amygdala in memory highlight the potential importance of this basal forebrain axis in subserving aspects of memory. If the NMB/SI cholinergic neurons are lesioned simultaneously with either lesioning or cholinergic blockade of the amygdala, substantive working memory and/or emotional memory deficits are incurred, including aspects of memory consolidation and enhancement of emotional memory (see Beninger et al., 2001; Power et al., 2002). Further examination of the basal forebrain amygdalopetal pathway is likely to provide insight regarding an important role for basal forebrain cholinergic neurons in additional aspects of memory.

In light of the reliance of many of the classic working memory tasks on spatial information, a further set of studies set out to examine the role of the NBM/SI cholinergic projections in supporting forms of associative memory that rely on nonspatial sensory associations. The studies revealed that NBM/SI cholinergic lesions produce a deficit in social transmission of food preference on both immediate and 24-h retention intervals (Berger-Sweeney et al., 2000; Vale-Martinez et al., 2002), indicating a deficit in encoding new sensory associations. Another study assessing a form of configural associative memory, negative patterning, indicates that rats with NBM/SI cholinergic lesions demonstrated a deficit in this form of sensory associative learning (lights and tones) (Butt et al., 2002). These results were taken to indicate that the rats either were unable to attend to multiple sensory stimuli concurrently or were unable to cope with the different response strategies required by the task. In order to address the potential issue of cognitive flexibility, an additional study examined the ability of NBM/SI cholinergic lesioned rats to learn and reverse sensory associations. The results of this study indicate that NBM/SI lesioned rats were able to learn a simple discrimination (between two stimuli of different sensory modalities) but that they were impaired at learning multiple reversals (Cabrera et al., 2006). This is indicative of a general lack of cognitive flexibility, a function that is also impaired in primates with similar lesions. Thus, the NBM/SI appears to

play an important role in the learning, memory, and flexible use of sensory associations. Further clarity of this role will arise from additional investigation (*See* Chapter 3.11).

3.15.13 The Role of the Basal Forebrain in Regulating Cortical Targets

Behavioral learning and memory that depend on the formation and utilization of complex sensory associations have been shown to rely upon intact basal forebrain cholinergic projections to cortical regions. This is consistent with a large body of literature indicating that cortically projecting cholinergic neurons in the basal forebrain promote activity and plasticity in the cortex in response to sensory stimuli or actions that predict significant events in the environment (Wilson and Rolls, 1990a; Whalen et al., 1994; Bakin and Weinberger, 1996; Kilgard and Merzenich, 1998; Mercado et al., 2001; Conner et al., 2003, 2005; Oldford et al., 2003; Berg et al., 2005). The ability of the NBM/SI to promote plasticity in very restricted cortical regions (see Weinberger, 2003; Kilgard, 2003, for review) provides anatomical support for the idea that such associative learning deficits may rely on the basal forebrain cholinergic projections to the cortical targets that preferentially support a particular type of learning.

Further support for this hypothesis comes from studies showing that selective removal of only those cholinergic neurons that project to a particular target cortical region disrupts aspects of sensory learning or memory. For example, selective removal of the cholinergic innervation of the orbitofrontal cortex (OFC) in rats is sufficient to replicate the learning and memory deficits in social transmission of food preference observed in NBM/SI lesioned rats (Ross et al., 2005). This finding is in accordance with the strong anatomical olfactory inputs to OFC and the importance of the OFC in supporting various aspects of olfactory learning and memory (see Eichenbaum, 1998, for review). Removal of the HDB cholinergic neurons eliminated acetylcholine in the olfactory system (including cortical targets and the olfactory bulb), thereby decreasing a rat's ability to discriminate between perceptually similar odorants (Linster et al., 2001).

Whereas the aforementioned studies focus on the role of acetylcholine in permitting sensory associations and discriminations, other studies examining selective destruction of basal forebrain cholinergic neurons that project to multimodal cortical regions indicate that such lesions disrupt learning and memory of novel stimuli, but not familiar stimuli. For example, cholinergic deafferentation of the entorhinal cortex results in impaired working memory, as measured by a delayed nonmatch-to-sample task, for novel but not for familiar odorants (McGaughy et al., 2005). Cholinergic deafferentation of the perirhinal cortex disrupts object recognition memory for novel objects, a process that is also disrupted by complete lesions of the perirhinal cortex (Winters and Bussey, 2005). Interestingly, these cortical target structures receive cholinergic information from two or more of the basal forebrain regions, indicating redundancy of cholinergic input and an opportunity for convergence of basal forebrain input at these cortical sites. Taken together, investigations utilizing selective cortical deafferentation indicate that these selective lesions replicate impairments incurred by lesions to their cortical regions. This indicates that basal forebrain cholinergic modulation is basic to effective cortical function under conditions of learning, memory, and attention.

3.15.14 The Effects of Basal Forebrain Lesions in the Nonhuman Primate

The effect of basal forebrain lesions on learning and memory delineated above was gleaned primarily from rodent studies. Nonhuman primate studies utilizing neurotoxic lesion techniques that removed the majority of NBM cell bodies have revealed selective performance deficits that differ somewhat from those found in rodents. In general, unlike complete lesions in rodents, only mild or transient memory deficits ensued, whereas, as in the rodent studies, impairments in learning and attention persisted. A study of monkeys with restricted NBM lesions found a lack of memory impairment on a recognition memory test (Aigner et al., 1987). A study of visual discrimination learning in marmoset monkeys with neurotoxic lesions of the nucleus basalis indicated a transient impairment for retention (lasting only 1 week after lesion), with a persistent impairment in new learning (Roberts et al., 1990). An additional study, testing other aspects of learning and memory, demonstrated that marmoset monkeys were able to selectively attend to stimulus features in an intra- and extradimensional set shifting task. Still, they demonstrated a

transient impairment for new learning and were incapable of serial reversal learning, demonstrating a sort of cognitive inflexibility (Roberts et al., 1992). An additional study, examining the role of the basal forebrain in cynomolgus monkeys, employed neurotoxic lesion techniques targeted at both MS/VDB and NBM (with the end result being substantial damage to the NBM with lesser damage to the MS/VDB). These basal forebrain lesioned monkeys were tested on a variety of memory tasks and on aspects of attention such as orienting and target detection. The resultant data strongly indicate a role for this region in allocating visuospatial attention, in the absence of any distinct memory impairments (Voytko et al., 1994).

An immunotoxin (with similar action to 192-IgG saporin, described above) targeting cholinergic neurons in the primate was used to independently lesion the projections to hippocampus (MS/VDB) and to the majority of cortex (NBM) in the marmoset monkey. Monkeys with MS/VDB cholinergic lesions demonstrate intact performance on a simple visual discrimination task, with a persistent deficit in a visuospatial conditional discrimination task. Monkeys with NBM cholinergic lesions demonstrate intact visuospatial conditional discrimination learning, with a transient deficit in simple visuospatial learning (Ridley et al., 1999a).

Further studies indicate that combined cholinergic lesions of the MS/VDB and NBM led to persistent deficits in both of these tasks (Ridley et al., 1999b, 2005). Thus, cholinergic depletion of the primate basal forebrain appears to produce a persistent effect on new learning.

3.15.15 Behavior Summary

Certainly, the variety of work done on the basal forebrain indicates that it plays an important role in aspects of attention and in aspects of learning and memory. The role of the system in memory may rely, in part, on the fact that the integrity of the basal forebrain is essential in order for experience to exert change on fundamental stimulus representations (*See* Chapter 3.11). Although this sort of memory is distinct from the classic form of declarative memory to which people typically refer when discussing memory deficits, it remains a form of memory that can substantively affect many aspects of cognition, including working memory. Another way in which the system may play a role in memory is through facilitating acquisition and encoding of selected aspects of memories (see Hasselmo, 2006, for review). Thus, the basal forebrain is an essential structure in allowing new information to be fully sustained and flexibly used by the neural system.

3.15.16 A Comment on Theoretical Models of Basal Forebrain Function

A multitude of experiments across behavioral domains and species have been conducted in order to shed light on the essential role of the basal forebrain and memory. Anatomical work provides the rich neurochemical landscape and potential of this region to regulate target structures and serve as a pathway to cortical modulation. Electrophysiological recording provides clues to the timing functions of this structure, in addition to its potential to modulate brain dynamics under various behavioral states. Animal models present the potential for this structure to subserve various aspects of learning, memory, and attention. Disease states provide clues to the impoverished world in which subjects with damaged basal forebrains must exist. Despite the convergent evidence that multiple fields of study lend to the behavioral function of this region, reconstruction of this work into a unified framework has posed a difficult problem. Promising attempts to unify and concatenate these data into a formal structure have recently been attempted through theoretical modeling.

The important, but often contested, roles of the basal forebrain in supporting learning and memory have inspired formal theories of the computations performed by this region and the role of such computations in regulating aspects of neuromodulation and/or neural transmission in target structures. These theories hold promise in that they provide testable hypotheses and a means by which a multitude of data can be reconstructed into a larger theoretical framework. Of the many computational models that exist, those of Hasselmo and colleagues (see Hasselmo, 2006, for review; Linster and Hasselmo, 2001) and those of Dayan, Yu, and colleagues (Yu and Dayan, 2005) are particularly relevant to the behavioral work presented in this chapter. Among other things, models by Hasselmo and colleagues provide an account of how encoding might be enhanced in various target structures of basal forebrain neurons. Models by Dayan, Yu, and colleagues provide an account of how learning is

facilitated by basal forebrain influences on cortex, revealing the way in which stimulus uncertainty augments cortical processing according to prior expectations. Full explication of such models is beyond the scope of this chapter but bears high relevance to the role of the basal forebrain in learning and memory and provides a compelling direction for future investigation.

3.15.17 Conclusion

Given its widespread anatomical connectivity and provocative neurophysiological properties, the sphere of influence enjoyed by the basal forebrain is undoubtedly extensive. Accessing nearly all regions of cortex and subcortex through distinct subregions, each holding a rich and diverse compilation of cells and neurochemicals, the basal forebrain is in a position to affect neuronal function across the brain. The complexities and intricacies of this heterogeneous region have only begun to be discovered, but the knowledge already gleaned has provided us with insight into some of its functions. Many of the structures to which the basal forebrain is most highly connected are essential to learning and memory, and thus the basal forebrain is naturally positioned to have a large effect on mnemonic function. Evidence provided through behavioral, lesional, and electrophysiological methods has revealed just such an effect. Through further examination of the entirety of the basal forebrain, including the different cell types and their neuromodulatory action, and through continuing examination of the vast nexus of connectivity to cortical and subcortical areas involved in memory, subtleties concerning the exact role in mnemonic processing will most likely be revealed.

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3.16 Ascending Systems Controlling Attentional Functions

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3.16.1 Introduction: Attention in Learning and Memory

Neurobiological research has often attempted to differentiate between brain systems mediating learning and memory and attention. Indeed, the modulatory functions of brainstem and basal forebrain ascending systems, particularly the noradrenergic and cholinergic cortical projections, traditionally have been described in terms of contributing to 'arousal,' the 'gating' of cortical information processing, or to the general readiness of forebrain systems for input processing. In contrast, a role of these modulatory systems in learning and memory has remained a less salient subject (but *See* Chapter 3.26; McGaughy et al., 2005; Hasselmo and Stern, 2006). Some authors even rejected the hypothesis that these systems modulate learning and memory altogether (for a critical discussion of the evidence that supported such a dissociation see Sarter et al., 2003).

Perspectives concerning the relationships between attentional functions and capacities and learning and memory range from complete conceptual and experimental separation between the two cognitive domains, particularly in the context of theories that focus on storage mechanisms, to theories that consider the attentional processing of a stimulus as an integral variable for effective encoding (e.g., Craik and Lockhart, 1972). These latter theories question the degree to which brain systems subserving learning and memory and attention, respectively, can be dissociated (e.g., Gaffan, 2005). Contemporary connectionist views generally embrace this perspective and suggest that the attentional modulation of a cue represents an inherent function of a learning process during which outcome errors result in the redistribution of attentional resources to cues present (e.g., Kruschke, 2003).

The wide range of states and processes subsumed under the construct of 'attention' and the sometimes undefined use of this term have together complicated the theoretical development of relationships between attentional functions and learning and memory (*See* Chapters 1.13, 2.02). However, the grouping of attentional functions into basic, selective, and executive components readily gives rise to testable hypotheses about the role of attention in learning. First, at the most basic level, vigilance or alertness, that is, the ability to sustain attention to exteroceptive input sources, underlies all higher forms of attention. A general readiness for input processing, as opposed to a state of drowsiness or hyperarousal, presents a necessary condition for effective learning. Second, the ability to direct attentional resources to selected inputs is crucial for learning. This may be as a function of stimulus salience (often termed bottom-up

control of attention) or practice, knowledge, and expectation (top-down control of attention). Third, the management of attentional resources available for competing stimuli and tasks and in response to challenges to attentional performance (by, for example, distractors, extended time-on-task, sleepiness; see Sarter et al., 2006) concerns the most executive aspects of attentional functions.

The relationships between demands on attention and demands on memory are bidirectional. Attention to stimuli facilitates learning, and memories assist in allocating attentional resources to a particular location and/or modality, or away from distractors (Nissen and Bullemer, 1987; Summerfield et al., 2006; Turk-Browne et al., 2006). Attention-based rehearsal plays an active role in spatial working memory. If subjects' ability to direct their attention toward a memorized location is hindered, memory accuracy for location declines (Smyth and Scholey, 1994). Performance of a secondary task that requires shifts of attention also impairs memory performance on a primary task (Awh et al., 1998), supporting the idea that spatial attention has a functional role in the maintenance of location information. Furthermore, excessive demands on perceptual and attentional processes degrade learning rates and the performance of tasks involving previously learned rules. Conversely, high demands on working memory disrupt distractor filtering, reflecting that the attentional resources normally available for effective filtering were consumed by the demands on working memory task (e.g., Lavie, 2005; see **Figure 1**).

The role of attention in retrieval is less clear and has rarely been studied (e.g., Craik et al., 1996; Fernandes and Moscovitch, 2000). A common fronto-parietal-cingulate-thalamic network has been found for both visual attention and episodic retrieval, suggesting a role for attentional processes in episodic retrieval (Cabeza et al., 2003). It could be speculated that retrieval and reconsolidation mechanisms are influenced by attentional states and capacities, similar to the role of such states and capacities in the original encoding of stimuli (Craik and Lockhart, 1972).

Thus, it is not only appropriate but perhaps necessary to discuss brain systems controlling attentional

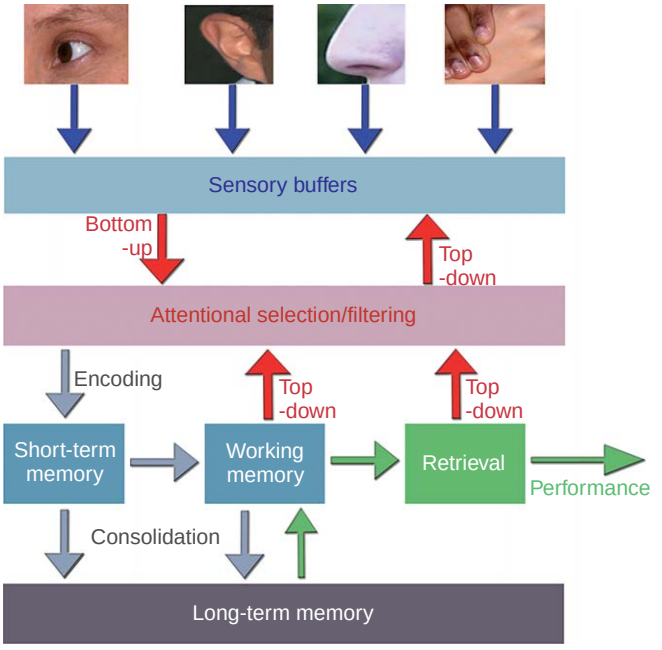

Figure 1 Schematic illustration of the role of attentional functions and capacities in learning and memory. Stimuli are selected for further processing, either as a result of their salience (bottom-up) or based on expectations, practice, and memory (top-down). Such top-down mechanisms include biasing of receptive fields toward stimulus shape, location, and modality, and perhaps also the suppression of the processing of irrelevant modalities and shapes (for examples see Table 1 in Sarter et al., 2006). Impairments in attentional processing weaken stimulus selection and detection (as defined by Posner et al., 1980) and therefore the effective formation of memories involving relevant stimuli. Conversely, excessive demands on working memory consume the attentional resources available for stimulus selection and detection, as indicated by the breakdown of 'noise'-filtering under such conditions (for more discussion see Sarter et al., 2003).

functions in a synopsis on learning and memory. As will be discussed in this chapter, recent evidence on the role of ascending noradrenergic and cholinergic projections in attention increasingly indicates that, in addition to mechanisms supporting the more traditional hypotheses about modulation of arousal and alertness, these systems modulate and indeed initiate defined cognitive operations. In other words, noradrenergic and cholinergic projections may not only modulate forebrain information processing, but 'decide' whether a stimulus will be integrated into the processing stream. The noradrenergic and cholinergic systems may therefore control the behavioral outcome, or whether an outcome is capable of redistributing the attentional resources toward stimuli and cognitive operations that optimize performance.

This evidence, discussed below, forces a departure from the conventional interpretation of effects of neuromodulators strictly in terms of 'alerting' and 'orienting' systems (Raz and Buhle, 2006). Rather, ascending modulator systems are integral components of distributed forebrain circuits mediating discrete cognitive functions. These cognitive functions range from the augmentation of sensory input processing to cue detection and outcome processing, thus constituting elementary stages of learning. Consequently, ascending neuromodulator systems play a necessary role in learning as well as the memory-based performance of tasks involving the detection, selection, and processing of stimuli that control motivated behavior.

In this chapter, we will focus on the regulation and functions of the noradrenergic projection system arising from the locus coeruleus (LC) and the basal forebrain (BF) cholinergic system. We will stress the organizational and functional similarities between these systems as well as the evidence indicating that cholinergic and noradrenergic projections mediate different aspects of attentional operations. As relatively little is known about the attentional functions of other brainstem ascending projections, including the serotonergic projections to the forebrain, these systems will not be discussed. Likewise, although the functions of dopaminergic systems in error detection and reward mechanisms are of relevance for attentional processes (Small et al., 2005; Sarter et al., 2006) and such mechanisms may be difficult to differentiate from attentional processes (Maunsell, 2004), the discussion of dopaminergic systems will be restricted to evidence on the dopaminergic modulation of the forebrain cholinergic system in the context of the mediation of attentional effort (defined in the section 'Acetylcholine').

3.16.2 Commonalities and Differences in the Organization of Noradrenergic and Cholinergic Systems

Noradrenergic and cholinergic systems exhibit several fundamental differences in their anatomical organization. Nonetheless, there are also important commonalities between the two systems, particularly with regard to the position of the locus coeruleus and basal forebrain within larger circuits including the prefrontal cortex.

Located in the dorsorostral pons, the LC represents the primary source of noradrenaline (NA) in forebrain, particularly in the cortex and basal forebrain. The LC contains a relatively small number of neurons (<50,000 in humans) but exerts immense influence over forebrain circuits, in part as a result of the extensive collateralization, or highly divergent efferents, of the noradrenergic projection system (Berridge and Waterhouse, 2003; Aston-Jones and Cohen, 2005b). In addition to collateralization, there is also substantial evidence that noradrenaline exerts widespread effects via extrasynaptic or 'volume transmission' (Seguela et al., 1990).

The cholinergic projections to hippocampus and cortex arise mainly from the septal nucleus, the vertical limb of the diagonal band of Broca, the nucleus basalis of Meynert (nbM), the substantia innominata (SI), the horizontal limb of the diagonal band (HDB), and the preoptic region (species-specific differences in the organization of these projection systems are discussed elsewhere, e.g., Mesulam et al., 1983; Woolf, 1991; Zahm, 2006. As little remains known about the regulation and functions of the septo-hippocampal cholinergic system, the cholinergic projections from the more lateral-dorsal basal forebrain regions to the cortex will be the focus of the present review on the role of ascending systems in the regulation of attention.

In contrast to the noradrenergic system, the degree to which the cholinergic projections from the nBM, SI, and HDB are collateralized remains disputed. However, several studies suggested that an individual cholinergic, cortically projecting neuron, if at all showing axonal collaterals, does not innervate multiple cortical areas (Price and Stern, 1983; Walker et al., 1985; Koliatsos et al., 1988). The view that individual cholinergic neurons have restricted terminal fields corresponds with the assumption that acetylcholine (ACh) is not transmitted extrasynaptically, although

this issue also remains controversial (Descarries and Mechawar, 2000; Mechawar et al., 2000; Turrini et al., 2001). The presence of a highly potent metabolizing enzyme, acetylcholinesterase (AChE) further challenges the degree to which ACh can reach distant, extrasynaptic receptors.

Finally, anatomical and functional considerations have formed the basis for a description of the cortical cholinergic input system in terms of consisting of modular, functionally distinct subcomponents (Zaborszky, 2002; Golmayo et al., 2003; see **Figure 2**). Evidence is not available to support such a conceptualization with respect to the noradrenergic projections to telencephalic regions (Saper, 1987). However, it should also be noted that earlier descriptions of a diffuse noradrenergic projection system were rejected on the basis of evidence indicating that noradrenergic innervation patterns differ between cortical target regions (Parnavelas and Papadopoulos, 1989; Papadopoulos and Parnavelas, 1991).

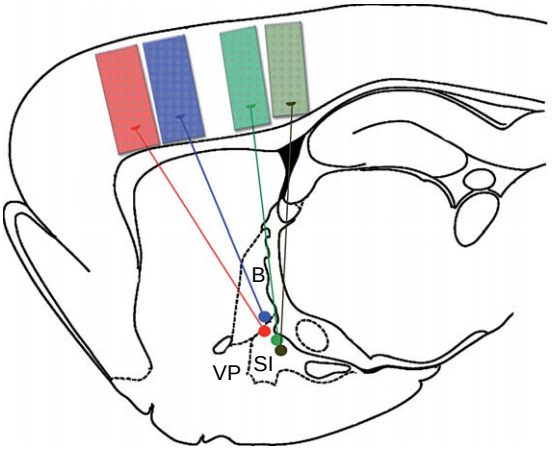

Figure 2 Schematic illustration of the organization of a modular basal forebrain cholinergic projection system to the cortex (Zaborszky et al., 1999; see also Zaborszky, 2002). Cholinergic inputs to cortical regions are thought to be activated in a modality-, cortical region-, and perhaps even column-selective manner. Although cholinergic projections follow a limited topographic organization and have been suggested to be arranged in longitudinally oriented, segregated projection bands, the selectivity of the recruitment pattern is also based on the hypothesis that prefrontal projections to the basal forebrain carry, for example, modality-specific information to cortical projection neurons (see also Golmayo et al., 2003). Although noradrenergic innervation patterns differ by cortical region, there is little evidence that would suggest the presence of similarly segregated projection bundle in the LC projection system. B, nucleus basalis; VP, ventral pallidum; SI, substantia innominata. Adapted and modified from Figure 13 in Zaborszky L, Pang K, Somogyi J, Nadasdy Z, and Kallo I (1999) The basal forebrain corticopetal system revisited. *Ann. N. Y. Acad. Sci.* 877: 339–367, with permission from Elsevier.

Thus, the anatomical organization of the two ascending modulator systems exhibits fundamental differences. The presence of a stricter topographic organization of cholinergic projections to the cortex (Luiten et al., 1987), combined with a very limited collateralization of axonal projections and the possibility that volume transmission does not apply to, or only to a very limited degree, ACh, collectively indicate a system that influences its target regions in a more specialized, differentiated manner when compared with noradrenergic projections. As we will see further, these contrasts between the two neuromodulator systems are not completely matched by analogous contrasts in their functions. However, we need to reiterate that the available evidence remains extremely incomplete and therefore limits direct, functional comparisons between the systems.

Furthermore, despite an anatomical description of these two systems that emphasizes organizational differences, several anatomical features suggest important commonalities between the noradrenergic and cholinergic systems. This is particularly true with respect to the position of the LC and the cholinergic BF within larger neuronal circuits involving the prefrontal cortex. Both the LC and the BF receive projections from prefrontal regions (Gaykema et al., 1991; Zaborszky et al., 1997; Jodo et al., 1998), and therefore, prefrontal operations can feed back and control the excitability of BF as well as LC neurons. Moreover, despite the morphological differences emphasized earlier, both systems can influence prefrontal as well as practically all other cortical regions. Assuming ACh, at least under certain conditions or in certain regions, is volume transmitted, both the noradrenergic and the cholinergic systems may support synaptic, rapidly changing (or phasic) as well as slower, extrasynaptic (or tonic) components of neurotransmission. In other words, both neuromodulator systems may support global mechanisms that can be described in terms of changes in arousal or readiness for input processing, as well as modulating highly defined, target area–specific cognitive operations. As will be discussed further, such dual modes of neurotransmission have emerged as a common theme in research on both the noradrenergic as well as the cholinergic system.

The possibility that commonalities in the basic operations of the two systems render any contrasts in their anatomical organization to be of secondary

importance is further supported by evidence demonstrating, anatomically and functionally, the significance of noradrenergic contacts with basal forebrain neurons (Chang, 1989; Zaborszky et al., 1993; Smiley and Mesulam, 1999; Smiley et al., 1999; Espana and Berridge, 2006). Noradrenergic neurons recruit the cholinergic cell bodies in this region primarily via α_1-receptors (Fort et al., 1995). We previously demonstrated that this LC–BF link is largely responsible for the modulation of cortical evoked potentials in response to activation of the noradrenergic system (Berntson et al., 2003; Knox et al., 2004). Thus, the available evidence, while remaining limited, strongly supports the general view that the effects of increases in activity in the ascending noradrenergic system on cortical information processing are mediated via stimulation of basal forebrain cholinergic neurons (see also Berridge, 2006).

3.16.3 Effects of Noradrenaline and Acetylcholine on Cortical Neuronal Excitability

NA and ACh are referred to as neuromodulators, meaning rather than directly producing direct excitatory or inhibitory effects on postsynaptic neurons, NA and ACh modulate the effects produced by other neurotransmitters like glutamate and gamma-aminobutyric acid (GABA). Numerous experiments determine the effects of local administration of NA or ACh on the excitability of cortical neurons. Based on these studies, the functions of both systems have often been described in terms of 'increasing signal-to-noise ratio,' with spontaneous activity being typically considered as 'noise' (Murphy and Sillito, 1991). Although some studies supported that, similar to ACh, NA enhances the responses of cortical neurons to sensory stimuli, there is also substantial evidence that, particularly as the activity of the LC or local cortical concentration of NA increases, NA suppresses such responses, (Foote and Aston-Jones, 1995; Edeline, 2003; Hurley et al., 2004).

3.16.3.1 Noradrenaline

The effects of cortically locally administered NA or stimulation of the LC on cortical neuronal activity can be summarized as follows:

1. Increases in noradrenergic activity enhance the response of cortical neurons to sensory stimuli (e.g., Waterhouse and Woodward, 1980; Waterhouse et al., 1990, 1998).
2. Further increases in the levels of LC stimulation suppress the sensory responsiveness of cortical target neurons in accordance with either a monotonic or bell-shaped function (Devilbiss and Waterhouse, 2004).
3. Paired noradrenergic and sensory stimulation preferentially attenuated the response to the sensory stimulus, indicating that brief increases in NA can modify the tuning curves of cortical neurons (Manunta and Edeline, 2004). These modifications in the tuning curves typically oppose those seen with ACh.

3.16.3.2 Acetylcholine

The evidence on the effects of local cortical administration of ACh or basal forebrain stimulation on cortical neuronal activity and cortical information processing is extensive and can be summarized as follows:

1. ACh suppresses intracortical or associational processing while amplifying thalamic input and enhancing intralaminar processing (e.g., Sato et al., 1987; Hasselmo and Bower, 1992; Xiang et al., 1998; Kimura et al., 1999; Oldford and Castro-Alamancos, 2003; Roberts et al., 2005).
2. ACh promotes synchronization of responses to sensory inputs (e.g., Rodriguez et al., 2004).
3. Paired cholinergic and sensory stimulation enhances the response to the sensory stimulus. Furthermore, the cortical area representing the stimulus is enlarged as a result of such pairings (e.g., Rasmusson and Dykes, 1988; Delacour et al., 1990; Metherate and Weinberger, 1990; Tremblay et al., 1990; Webster et al., 1991; Hars et al., 1993; Kilgard and Merzenich, 1998; Mercado et al., 2001; Kilgard et al., 2002; Penschuck et al., 2002; Weinberger, 2003; Ma and Suga, 2005). ACh appears to promote such neuroplasticity via cortical muscarinic M1 receptors (Zhang et al., 2006). However, cholinergic stimulation may not be necessary for such neuroplasticity to occur, as plasticity in the adult primary auditory cortex has been seen after removal of cholinergic inputs (Kamke et al., 2005). Thus, rather than acting as a gate on cortical plasticity, in this context the cholinergic system seems to act more as a gain control mechanism.

4. ACh preferentially suppresses previously potentiated fibers, therefore promoting the formation of new memories (Linster et al., 2003).

5. High levels of ACh suppress the 'signal-to-noise' ratio, suggesting a disruption of information flow under this condition (Zinke et al., 2006).

Although this evidence remains limited, particularly with respect to the effects of different levels of cholinergic stimulation, the two modulators produce overlapping as well as diverging effects on cortical target neurons. Furthermore, there is very little evidence on the effects of coactivation or coadministration of the two modulators, and the evidence described indicates that a prediction of such effects might be very difficult (Ahissar et al., 1996). Moreover, the physiological significance with respect to concentration and patterning of local administration of ACh and NA remains debated. Therefore, experiments that assess the effects of combined stimulation of noradrenergic and cholinergic projections on cortical target neurons (Florin-Lechner et al., 1996; Van Gaalen et al., 1997; Berridge and Abercrombie, 1999; Pudovkina et al., 2001) might generate particularly informative results. However, the interpretation of the results from such experiments is further complicated by evidence suggesting that local cortical mechanisms mutually influence the release of ACh and NA (e.g., Beani et al., 1986; Rao et al., 2003). Clearly, there is a pressing need for studies on the combined modulatory effects of ACh and NA on cortical neuronal responsivity and information processing.

3.16.4 Behavioral and Cognitive Functions Deduced from Neurophysiological Recordings in the Locus Coeruleus and Basal Forebrain

Contemporary theories about the cognitive functions modulated by LC projections to the forebrain are largely based on neurophsyiological recordings in the LC of task-performing animals. In contrast, there is a paucity of such recordings in the cholinergic basal forebrain, due in part to the challenges associated with the identification and recording from cholinergic neurons (Duque et al., 2000; Lee et al., 2004).

Recordings in the BF of primates indicated that neurons in this region encode the conditioned outcome of sensory stimuli (Richardson and DeLong, 1990; Wilson and Rolls, 1990). As will be discussed further in this chapter, such evidence corresponds with the more recent demonstration of transient increases in prefrontal ACh release in response to the detection of cues that predict reward. Therefore, the results from prior neurophysiological studies may be interpreted in terms of indicating that changes in BF neuronal activity reflect the enhanced attentional processing of behaviorally significant stimuli. Indeed, earlier suggestions that the phasic changes in activity in BF neurons reflect brief changes in arousal (Richardson and DeLong, 1990) correspond with the more specific hypothesis that such brief increases in cholinergic activity in prefrontal regions mediate the incorporation of attended signals into ongoing behavior and cognitive activity.

These early studies also suggested that telencephalic projections to the BF serve as a critical source for the convergence of sensory and reward information in the BF. Recently, we expanded this circuit to include prefrontal–nucleus accumbens–BF projections (Neigh et al., 2004; Sarter et al., 2006; see **Figure 3**). The nucleus accumbens is believed to serve as a link between reward information and generating behavior or behavioral change (see Robbins and Everitt, 1996, for a review). Based on the subject's motivation, the nucleus accumbens is capable of activating top-down effects to maintain the attentional performance needed to obtain the relevant reward (Sarter et al., 2006). The nucleus accumbens may thus activate the neuronal circuitry mediating the effects of increased attentional effort.

The general hypothesis that the BF encodes the learning of behaviorally significant stimuli likewise dominated the interpretation of earlier LC recording studies in monkeys and rats performing operant procedures (*See* Chapter 3.15), as the conditioned responses of LC neurons are alterable by changes in stimulus meaning (e.g., Sara et al., 1994; Aston-Jones et al., 1997). Extracellular recordings of LC noradrenergic neurons in primates performing a visual discrimination task revealed these neurons responded selectively to target stimuli (Aston-Jones et al., 1997). Upon reversal of task contingency, these neurons stopped responding to the initial stimulus and began responding to the new target stimulus. This reversal preceded any reversal in the behavioral responses. LC neurons' response to the new target increased in latency, and this increase was mirrored in an increased latency of behavioral responses as well, indicating these neuronal responses reflect stimulus meaning

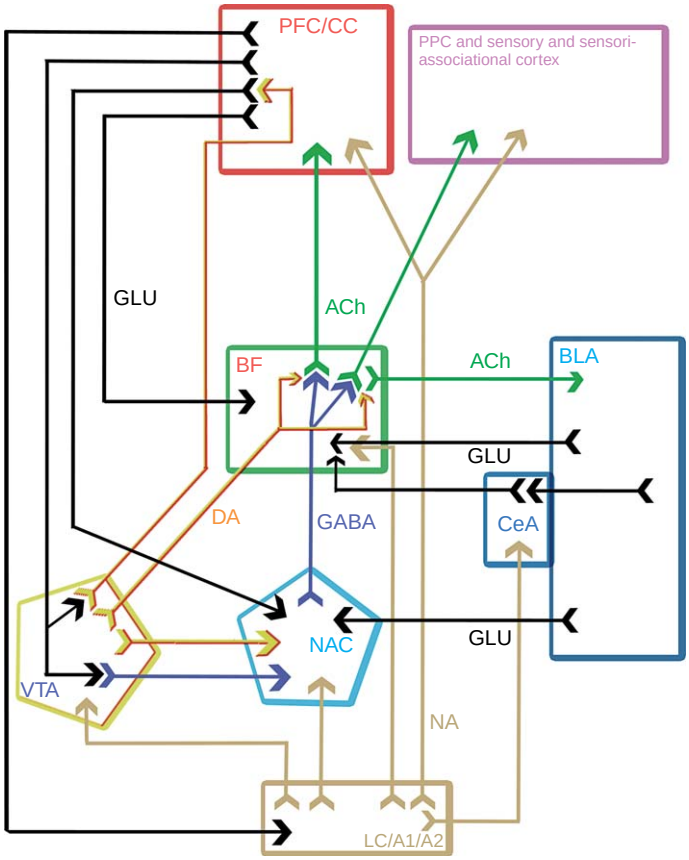

Figure 3 Schematic illustration of the neuronal network regulating the basal forebrain (BF) cholinergic system during attentional performance (Sarter et al., 2006). The figure describes connections and synaptic contacts selected on the basis of anatomical evidence and functional significance. During standard attentional performance, cholinergic neurons projecting to prefrontal and cingulated cortex (PFC/CC) contribute to the activation of the anterior attention system. Projections to sensory and posterior associational regions serve to amplify thalamic inputs while suppressing associational processing and synchronize the attentional operations maintained by prefrontal-posterior parietal cortex (PPC) circuits (e.g., Gill et al., 2000; Broussard et al., 2006). Information indicating performance decline (error detection, including prediction errors, reward loss) is generated on the basis of interactions between prefrontal (PFC) and anterior cingulate (ACC) circuits. Via glutamatergic (GLU) projections to midbrain dopamine (DA) cell groups, particularly the ventral tegmental area (VTA) and the nucleus accumbens (NAC), indicators of performance decline are integrated with incentive information. The VTA, directly and indirectly via projections to the NAC, activates cholinergic projections to the cortex. Activation of cholinergic projections to prefrontal regions under conditions that challenge attentional performance is hypothesized to further activate the anterior attention system and thereby initiate the top-down mechanisms designed to stabilize and perhaps recover attentional performance (Sarter et al., 2006). Based in part on prefrontal projections to the BF, cholinergic activation of the posterior cortex in response to performance challenges contributes to such top-down effects. The combined effects of increased cholinergic activity in the PFC/CC and posterior cortex are to enhance the detection and discrimination of target stimuli and to suppress the effects of distractors, thereby stemming further decline in attentional performance and fostering performance recovery. The attentional effects of changes in activity in ascending noradrenergic (NA) projections originating primarily from the locus coeruleus (LC) and the catecholaminergic cell groups in the medulla (A1/A2) are mediated in part via noradrenergic projections to BF cholinergic neurons (see text). Finally, glutamatergic (GLU) projections from the basolateral (BLA) amygdala, in part via the central amygdaloid nucleus (CeA), regulate the basal forebrain system. This circuit appears to be involved in the recruitment of attentional mechanisms during fear conditioning and, perhaps more generally, in situations involving the processing of salient affective stimuli (e.g., Power et al., 2002; Holland and Gallagher, 2006). Adapted and modified from Figure 3 in Sarter M, Gehring WJ, and Kozak R (2006) More attention must be paid: The neurobiology of attentional effort. *Brain Res. Rev.* 51: 145–160, with permission from Elsevier.

rather than being driven by physical attributes of the stimulus. Subsequent research by Foote and Aston-Jones and their coworkers identified phasic (bursts of LC activity) and tonic (elevation of LC baseline activity, absence of phasic bursts) changes in LC activity and proposed that intermediate levels of tonic LC activity promote selective responding to stimuli and thus practiced task performance. Intense LC activity, in contrast, may promote a shift in vigilance in situations characterized by new events or unexpected outcomes (Aston-Jones et al., 1991; Foote et al., 1991; Usher et al., 1999). Phasic activation of LC neurons was evoked by conditioned stimuli in monkeys performing an attention task, suggesting this phasic activation contributes to the enhanced attentional processing of such stimuli (Aston-Jones et al., 1994). More recent evidence further broadened the role of LC neurons in cognitive performance by demonstrating that they also encode the outcome of responses, thereby maintaining and facilitating performance (Clayton et al., 2004). Importantly, tonic and phasic LC activity do not represent distinct modes but are both part of a continuum: an inverted U-shaped relationship between LC activity and attentional task performance. Performance is poor with either low or high tonic LC activity, reflecting states of either too little or too much arousal, respectively. Attentional task performance is optimal at moderate levels of tonic LC activity, when there are also high levels of phasic LC activation following goal-relevant stimuli (phasic mode; Aston-Jones and Cohen, 2005a). We will come back to this conceptualization in the context of phasic ACh release in the prefrontal cortex below.

The studies by Aston-Jones and colleagues generated a detailed and perhaps unexpectedly sophisticated model of LC function. Within this model, phasic activity takes advantage of known reward sources by facilitating behavioral responses to task outcomes and filtering responses to distractors (Aston-Jones and Cohen, 2005b). Changes in tonic activity facilitate disengagement from task-related processes and exploration of the environment for other reward sources. These functions are implemented via NA's neuromodulatory effects on cortical targets, modifying the responsivity of processing in the cortical circuitry mediating task performance. This model may be interpreted as breaking the traditional boundaries of the role of a neuromodulator by attributing the processing of specific cognition operations to LC activity, or by considering the ability of the LC to modulate forebrain-based cognitive operations in a temporally highly specific, stimulus- and outcome-bound fashion. As little is known about the degree to which the functional specificity of LC activity translates into spatially and temporally constrained NA release in the forebrain, and as the high degree of collateralization predicts a fairly low spatial resolution of changes in noradrenaline release in the forebrain, it remains appropriate to continue discussing LC projections as a rather globally acting attentional modulator system (Aston-Jones and Cohen, 2005b; see also **Figure 3**). However, it is also clear that the stimulus- and outcome-based attentional modulation of forebrain cognitive operations by LC projections renders the more traditional and less defined description of noradrenergic 'arousal' functions no longer particularly useful.

It is unfortunate that corresponding analyses of performance-associated neuronal activity are not available with respect to BF (cholinergic) neuronal activity. However, given the available evidence on NA and the ACh release data to be discussed further, it is not difficult to predict that such research would generate a model describing BF neuronal activity in terms that correspond well with the contemporary conceptualization of LC activity.

3.16.5 Tonic Noradrenaline and Acetylcholine Release in Task-Performing Animals

In the past, the analysis of neuromodulator functions on the basis of the demonstration of changes in synaptic release was restricted largely to studies using microdialysis, a technique that allows monitoring of the neurochemistry of the extracellular space *in vivo*. The relatively low temporal resolution of evidence generated by microdialysis (typically at the order of minutes) indicates that this method mostly monitors tonic changes in release. However, measurements of neurotransmitter release in task-performing animals, including comparisons with release data from control tasks stripped of demands on cognitive, particularly attentional operations, yielded specific hypotheses about the functions of neuromodulators, especially the cortical cholinergic input system (Sarter et al., 2005, 2006).

3.16.5.1 Noradrenaline

The evidence relating telencephalic NA release with defined cognitive operations or, specifically, attentional performance has remained scarce. The majority of experiments demonstrated that cortical NA release increases in response to stressors or novelty (e.g., Dalley et al., 1996; McQuade et al., 1999). The study by Dalley et al. (2001) is of particular interest, as they demonstrated that NA release was not systematically elevated in association with attentional task performance, but robustly increased in response to changes in the stimulus-response contingencies. This supports the hypothesis that the attentional role of the noradrenergic system is a function of response outcome. A similar conclusion was drawn from the results of an experiment that measured noradrenaline release in animals performing a working memory task (Rossetti and Carboni, 2005). Although it will be important to exclude the possibility that these results are not merely reflecting unspecific arousal effects, it is noteworthy that these studies' interpretational focus on the mediation of the effects of response outcome on ongoing task performance corresponds with major aspects of the model by Aston-Jones discussed earlier.

3.16.5.2 Acetylcholine

Contrasting with the microdialysis evidence on NA, the functional significance of ACh release is better understood. While increases in cortical ACh release have been observed in numerous behavioral contexts and in relation to locomotor activity and sensory stimulation, the interpretation of evidence from several of these earlier experiments is confounded by uncontrolled variables, including stress brought by the experimental procedures and novelty (for review see Pepeu and Giovannini, 2004). However, a substantial number of experiments indicated that the performance of a task taxing attentional processes is selectively associated with increases in cortical ACh release. In contrast, during tasks that involved, for example, extremely high rates of operant responses, variable reward rates, and noncontingent stimuli and, generally, assessed basic components of operant performance, cortical ACh did not significantly increase over baseline, or the increases remained quite limited when compared with attentional task-performing animals (e.g., 30–50% increases in rats performing an operant schedule of reinforcement vs. 120–150% increases in attentional task-performing animals; Himmelheber et al., 1997, 2000; Passetti et al., 2000; Dalley et al., 2001; Arnold et al., 2002; McGaughy et al., 2002).

More recent experimental results further defined the variables responsible for performance-associated increases in ACh release, specifically in the prefrontal cortex. Behavioral or pharmacological challenges on attentional performance result in transient performance impairments that, in motivated subjects, subsequently stabilize or recover. However, despite impaired attentional performance relative to standard task levels, augmentation of task-associated increases in prefrontal ACh release was observed (Himmelheber et al., 2001; Kozak et al., 2006a,b). This augmented ACh release above levels seen during standard task performance is hypothesized to reflect increases in attentional effort (Sarter et al., 2006). Attentional effort is defined as cognitive incentive. In motivated individuals, increases in attentional effort are thought to activate top-down mechanisms to counteract performance declines following a challenge to attention. Examples of challenges to attention include distractors, increased time on task, sickness, and sleepiness.

These data support the broader view that the basal forebrain cholinergic projections to prefrontal regions, on account of their afferent connections with prefrontal, mesolimbic, and limbic regions (**Figure 3**), contribute to the activation of the anterior attention system believed to underlie effortful control capabilities in order to combat performance decline. Clearly, this hypothesis combines motivational and attentional mechanisms, as increases in attentional effort are a function of the subjects' motivation to maintain performance under challenging conditions. Therefore, such augmented increases in prefrontal ACh release observed in response to performance challenges are thought to be mediated primarily via prefrontal–mesolimbic circuits converging on BF neurons and recruiting these neurons in response to prediction errors and reward loss (Sarter et al., 2006). It is noteworthy in this context that glutamatergic manipulations in the nucleus accumbens exclusively affect ACh release in the prefrontal cortex (Zmarowski et al., 2005, 2007), confirming the view that reward-related information is selectively influencing cholinergic activity to this region.

Furthermore, evidence suggests that the cholinergic projections to the more posterior cortical regions

are a component of the prefrontal projection system that mediates top-down effects, such as enhanced input processing in order to combat challenge-provoked performance decline (Kozak et al., 2006a,b). These results support the conceptualization of a two-tiered cortical cholinergic input system, consisting of prefrontal inputs that contribute to the activation of the anterior attention system as a function of demands on attentional effort, and cholinergic projections to sensory and posterior associational regions that contribute to the mediation of top-down mechanisms in order to combat further performance decline following performance challenges (Sarter et al., 2006).

Collectively, the evidence from microdialysis studies on the role of NA and ACh release in attentional performance remains underdeveloped, particularly with respect to the demonstration of specific functional correlates of NA release. However, the available microdialysis evidence supports a potential dissociation between the functions of ACh versus NA release suggested by other experimental approaches. Specifically, increases in cholinergic activity are directly related to demands on attentional processing, particularly in response to challenging conditions. In contrast, increases in NA appear to be more directly related to the maintenance of ongoing task performance as a function of response outcome. Such a hypothesis, if corroborated by additional evidence, would yield a useful complementary scenario describing the combined, synergistic and antagonistic roles of ACh and NA in the mediation of attentional performance. Together, the two modulators span the full range of components of attentional performance, from general increases in arousal and readiness for cortical input processing (ACh and NA) to cue detection and regulation of attentional effort (ACh) and optimization of task performance as a function of response outcome (NA).

3.16.6 Phasic and Tonic Prefrontal Cortical Acetylcholine Release Mediates Cue Detection

Given the extensive evidence on the importance of phasic noradrenergic activity for optimal task performance, the development of a similar line of understanding in the cholinergic system is imperative. However, while techniques to monitor phasic activity of noradrenergic neurons have been available for years, analogous methods for the cholinergic system have been developed only recently. The recoding of brief, transient, or phasic changes in ACh release, at a subsecond level of temporal resolution, has become possible on the basis of an amperometric method. This method utilizes enzyme-selective microelectrodes to detect choline generated by hydrolysis of newly released ACh. Details about this technology as well as evidence in support of the fundamental assumption that choline signals recorded with this methods reflect ACh release have been previously reported (Parikh et al., 2004; Parikh and Sarter, 2006).

We recorded cholinergic activity using this method in the prefrontal cortex and, as a control region, in the motor cortex of rats performing a cued appetitive response task (Parikh et al., 2006). A trial consisted of the presentation of a light cue for 1 s that indicated the delivery of food reward 6 ± 2 s later at one out of two food ports (randomly selected). Importantly, the intertrial interval (ITI) was relatively long (90 ± 30 s), allowing the animals to disengage from performance and resume task-independent behavioral activities like grooming. Following the presentation of the cue, animals disengaged from ongoing behavior and oriented to and monitored the food ports. This effect of a cue is termed 'cue detection' (Posner et al., 1980). In 30–40% of trials, this behavior was not elicited by the cue, and therefore the cue was missed. Importantly, in trials involving missed cues, food reward delivery elicited port approach and reward retrieval. As would be expected, the latencies between reward presentation and pickup were longer in trials involving missed cues.

In the prefrontal cortex, transient increases in cholinergic activity were evoked by detected cues but not by missed cues (**Figure 4**). Port approach and reward retrieval did not elicit cholinergic activity. The increases in detected cue-evoked cholinergic activity coincided in time with the cue-evoked disengagement from ongoing behavior and orientation to the ports. Importantly, such disengagement and orientation to the ports was also elicited by food reward delivery in trials involving missed cues, but did not generate phasic increases in cholinergic activity in motor cortex.

Additional evidence supported the conclusions that these transient increases in cholinergic activity in the prefrontal cortex mediate the detection of cues, as opposed to reflecting the sensory properties of a perceived stimulus. First, the interval between the

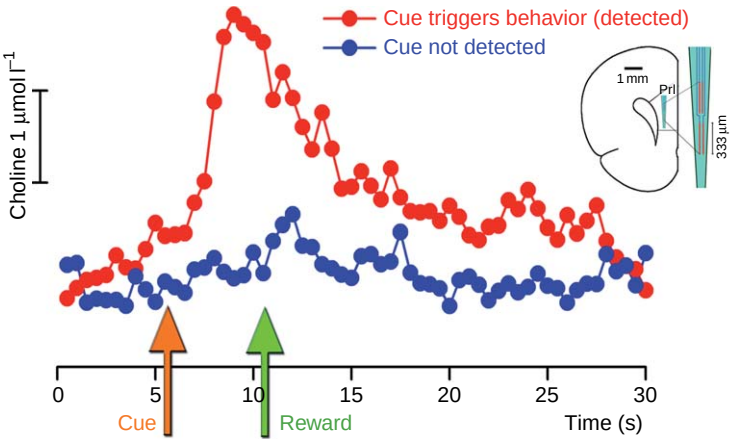

Figure 4 Representative traces depicting changes in cholinergic activity recorded by using enzyme-selective microelectrodes for the amperometric detection of changes in extracellular choline concentrations as a result of hydrolysis of newly released acetylcholine (ACh). The inset on the right depicts the four platinum recording sites on the ceramic-based microelectrode and the approximate placement of the electrode in the prelimbic cortex (Prl). Technical details and evidence in support of the reliability and validity of this method are described elsewhere (Parikh et al., 2004; Parikh and Sarter, 2006). In animals trained to perform a cued appetitive response task (see text for details), cues that triggered disengagement from ongoing behavior and orientation to, and monitoring of, the food ports ('detection') elicited a transient increase in cholinergic activity. Cues that failed to elicit such behavior did not produce such cholinergic spikes. Importantly, when animals missed the cue, food reward delivery still triggered food port approach, reward pickup, and consumption, but this was not associated with a cholinergic spike (see arrow depicting reward presentation). Moreover, a shortening of the interval between the cue and reward delivery resulted in a left shift of the timing of the peak amplitude of the cholinergic signal, indicating that this signal did not merely reflect sensory–perceptual mechanisms but the cognitive operation that were evoked by the cue. Finally, bilateral removal of cholinergic inputs to the prefrontal cortex decreased the cue detection rate, supporting the hypothesis that the phasic increase in cholinergic activity is necessary for cue detection (Parikh et al., 2006).

presentation of the cue and reward presentation was shortened to 2 ± 1 s, requiring the detection process to be moved closer to the actual presentation of the cue. This manipulation resulted in a significant left shift of the timing of the peak amplitude of the choline signal. The demonstration of this left shift indicates that the choline signal reflects a cognitive operation rather than the sensory processing of the cue. Second, the onset of the behavioral response to the cue correlated highly with the timing of a 25% increase in choline signal level. Third, bilateral removal of cholinergic projections to the medial prefrontal cortex resulted in a significant increase in the number of missed cues.

The detected cue-evoked spikes in cholinergic activity were superimposed over more slowly changing tonic cholinergic activity. We extracted data on tonic changes by expressing session-related changes relative to a presession baseline and boxcar-averaging data points in order to match the 8-min data point generated by microdialysis experiments conducted in parallel. This analysis roughly reproduced the release data measured by microdialysis and indicated increases in tonic levels beginning with the first trial and lasting throughout the session. Importantly, and in contrast to cue-evoked phasic cholinergic activity, session-related, tonic changes were also observed in motor cortex. This suggests tonic changes in ACh levels may be a cortex-wide phenomenon, reflecting a general increase in cortical input processing readiness and arousal. No such tonic changes in cholinergic activity were observed in trained animals placed into the test chamber but not allowed to perform, indicating that task performance is needed to elicit changes in tonic ACh release.

The origin and regulation of the two modes of cholinergic activity (phasic, tonic) in task-performing animals remain unknown. Nevertheless, one may speculate that the session-related tonic changes in cholinergic activity are influenced primarily by ascending noradrenergic projections (e.g., Berntson et al., 2003), while the cue-evoked phasic increases may be regulated by local prefrontal as well as direct and multisynaptic prefrontal projections to the BF (**Figure 4**).

3.16.7 Necessity of Noradrenergic and Cholinergic Neurotransmission for Attention: Implications for the Definition of a Neuromodulator

As already stressed, there are significant overlaps as well as differences in the characteristics of cognitive performance-associated alterations in noradrenergic and cholinergic activity. Adding to the available evidence, the results of experiments assessing the effects of cholinergic or noradrenergic lesions on performance indicate further important differences in terms of the necessity of these neuromodulator systems for attentional performance.

Neuromodulators are typically described as 'modulating' the cognitive operations conducted within the circuitry of the forebrain. Based on this definition one would not necessarily expect the removal of a modulator system to fundamentally disrupt cognitive operations. Rather, following lesions of a modulator system such operations would be expected to consume more time and be defective and insensitive to variations in task contingencies.

However, removal of the cortical cholinergic input system has been extensively demonstrated to result in persistent and elemental disruption of a wide range of attentional processes and capacities (Voytko et al., 1994; Chiba et al., 1995, 1999; McGaughy et al., 1996, 2000; Turchi and Sarter, 1997, 2000; McGaughy and Sarter, 1998; Burk and Sarter, 2001; Burk et al., 2002; Berntson et al., 2003; Dalley et al., 2004; Martinez and Sarter, 2004). For example, selective immunotoxic cholinergic lesions via 192 immunoglobulin G (IgG)-saporin showed that lesions disrupt the ability to modulate attention within an associative learning framework, leading to a disruption of increments in conditioned stimulus processing (Chiba et al., 1995). Saporin lesions also resulted in impairments in sustained attention performance (McGaughy et al., 1996) and an increase in response latencies as part of a speed–accuracy tradeoff in a divided-attention task (Turchi and Sarter, 1997).

Collectively, the results from these experiments strongly suggest that the modulatory effects of ACh mediate essential aspects of attentional performance, such as cue detection (see earlier discussion). Furthermore, these results suggest that the modulatory effects of ACh are necessary for certain cognitive operations to be initiated. Such a conclusion begins to blur the traditional dissociation between the effects of a neuromodulator system and a neuronal system processing discrete pieces of information and inserting this information into target circuits.

Additionally, the disruption of attentional performance resulting from cholinergic lesions was demonstrated to last for months. In our studies, as well as in the literature, there is very little evidence for a recovery of attentional performance following such lesions, even if animals' daily training is continued for up to about a year. Such a complete absence of functional compensation following cholinergic lesions contrasts with the general view that there is a degree of functional redundancy across the multiple neuromodulator systems. From this viewpoint, the overlapping effects of ACh and NA on cortical neuronal activity and responsivity support the idea that removal of one system would result in only mild, transient impairments, as the complementary functions of intact neuromodulator systems would limit the degree and duration of the functional consequences of such lesions. However, as the attentional impairments resulting from removal of the cholinergic input system to the cortex are profound and do not recover, one can conclude that, in the absence of the cholinergic system, noradrenergic and other modulatory projection systems are not capable of exerting compensatory effects.

The evidence on the necessity of noradrenergic projections for cognitive and specifically attentional performance is more scarce and ambiguous (Sara, 1985; Koger and Mair, 1992). Standard attentional performance generally is unaffected by extensive noradrenergic lesions (Carli et al., 1983; McGaughy et al., 1997). However, lesioned rats fail to respond normally to the alerting effects of stressors (Carli et al., 1983). Assuming such stressor effects normally activate the LC and therefore interfere with standard task performance, these effects correspond with a model that focuses on the ability of the LC to monitor sympathetic reactivity changes, via afferents from the paragigantocellular nucleus, and that suggests such sympathetic reactivity changes serve to 'import' information about stressors, novelty, and other salient events that are capable of modulating sympathetic activity into the noradrenergic ascending projection system (Aston-Jones et al., 1996). This scenario is of particular relevance for hypotheses linking psychopathological increases in emotional reactivity to attentional biasing and the subsequent development of affective disorders (Berntson et al., 1998).

Given the significance of noradrenergic–cholinergic interactions based on noradrenergic inputs to the

BF, the limited collateralization of the cholinergic system, and the open questions about the degree to which ACh acts extrasynaptically, the greater necessity of cholinergic compared to noradrenergic projections to the cortex for attentional performance may be conceptualized. Furthermore, considering the cholinergic system is the most evolutionarily recent and most dorsal addition to the modulator systems (Brockhaus, 1942; Gorry, 1963), the predominantly telencephalic origin of BF afferents, and the generally more discrete and topographic organization of cholinergic efferents (Zaborszky, 2002), it is possible to attribute more selective functions to this system and to describe the cholinergic system in part as a dorsal branch of the noradrenergic system. As such, the critical attentional functions mediated via the cholinergic system are subject to the gain-setting functions of noradrenergic projections (Aston-Jones and Cohen, 2005a,b). This scenario would explain the results reported by Carli et al. (1983) and corresponds with the collective evidence on the differential effects of lesions of the cholinergic and noradrenergic systems.

3.16.8 Future Research: Corecruitment and Comodulation

Noradrenergic projections modulate the cholinergic BF and, perhaps independently, the release of ACh in the cortex based on local regulation of synaptic mechanisms (Beani et al., 1986; Zaborszky et al., 1993; Fort et al., 1995; Berntson et al., 2003; Tzavara et al., 2006). Little is known about the converse, but it is highly likely local cortical NA release is influenced by cholinergic mechanisms (El-Etri et al., 1999). Therefore, the determination of the attentional functions of the cholinergic and noradrenergic ascending systems necessitates further research addressing and probing interactions between these systems in performing subjects. This chapter attempted to draw conclusions concerning the common properties of the effects of the two modulators as well as important dissociations between the regulation, activity, and function of cholinergic and noradrenergic projections to the forebrain. It cannot be excluded that, to a considerable degree, these conclusions represent an artifact that results from the isolated investigation of the individual neuromodulator systems. Measures and manipulations of corelease of ACh and NA as well as interactions between the two systems in the BF and cortex may well reveal the full range of the modulatory potency of these two systems. The current evidence suggests that the combined functions of ACh and NA are likely more complex than the relatively straightforward hypotheses available (e.g., as described in Figure 3 in Aston-Jones and Cohen, 2005b). Cholinergic and noradrenergic interactions may be characterized by antagonistic as well as synergistic relationships, depending on the levels of activity in these systems (Yu and Dayan, 2005). Obviously, this perspective readily extends to interactions with other ascending neuromodulator systems, particularly the mesolimbic dopamine system and serotonergic projections arising from the raphe nucleus (Doya, 2002).

Finally, neuronal systems modulating attentional functions are also, by conceptual necessity, involved in learning (see also Ridley et al., 1999; Power et al., 2002; McGaughy et al., 2005). Given the rather intricate and relatively limited evidence on the role of these systems in learning and memory, it will be important that future studies define and vary the attentional demands associated with the learning about stimuli. Such research would be expected to reveal that the learning of new contingencies necessitates noradrenergic–cholinergic comodulation as a function of the demand for attentional processing of relevant stimuli.

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3.17 Procedural Learning in Humans

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When one thinks of learning and memory in daily life, it is likely that one considers learning facts about the world and remembering events in the past as being important. However, our ability to function depends on knowing how to do things. From activities as mundane as walking and picking up a pencil, to more complex skills such as typing, playing the piano, or making basketball jump shots, perceptuomotor skills are procedures that we have learned over the course of experience. In addition to knowing how to perform motor skills, people also learn procedures for performing cognitive skills, such as the ability to discriminate your baby's cry from that of another, recognize the style of an artist, or become a chess master. In this chapter we focus on procedural learning and how it differs from learning about facts and events in terms of its behavioral characteristics and neural substrates. Because procedures are ubiquitous in daily life, disruptions in the ability to learn or retrieve procedures can have serious functional consequences.

3.17.1 Defining Procedural Knowledge

Procedural knowledge is generally defined as 'knowing how': It is one's knowledge of procedures that have been learned through experience (Newell and Simon, 1972; Anderson, 1976). Procedural memory is usually contrasted with declarative memory. Declarative memory is 'knowing that,' and is used to

describe memory for facts and events (Cohen and Squire, 1980; Squire, 1986). Declarative memory is distinguished by the ability to verbalize this knowledge. We are able to put our declarative memories into words, hence, we can declare them. We can recount to others happenings in the past or bits of information we have learned. In contrast, procedural memory is not easy to verbalize. It is difficult for someone to explain how to perform a motor skill, like riding a bicycle. Although we have learned the grammatical rules of our native language well enough to easily produce proper sentences, it is an effortful task to describe these grammatical rules. The difficulty in verbalizing procedural knowledge is one of its key features.

According to Anderson (1993, 1995), the difference in the verbalizability of declarative and procedural knowledge arises from differences in the accessibility of these types of knowledge to introspection and conscious awareness. These two types of knowledge are represented in fundamentally different ways. Declarative knowledge is represented as a network of interconnected bits of information, whereas procedural knowledge is represented as a set of condition–action links. This difference in mental representation between the two types of knowledge is derived from earlier concepts in artificial intelligence in which declarative computer languages specify relationships, whereas procedural languages specify steps to be carried out to achieve a goal (e.g., Minsky, 1975). The fact that the distinction between declarative and procedural learning can be made at the computational level is the basis for formal models of cognition such as ACT-R, which explicitly acknowledges the representational differences between the two types of learning (Anderson and Lebiere, 1998; Anderson et al., 2004).

3.17.2 What Are Procedures?

If we use the definition of procedural as 'knowing how,' it would seem to encompass an extremely wide range of knowledge. The knowledge underlying motor and perceptual skills would seem to be clearly procedural, especially after extensive practice (Newell and Rosenbloom, 1981; Anderson, 1983). Knowing how to use a manual transmission or produce your signature are skills that were learned through practice, and it is relatively difficult to verbally describe the knowledge that allows performance of the skill. However, when the right conditions are present (such as sitting in a car in the case of using a manual transmission), the skill can be executed.

Another kind of learning "'how'" is learning rules (Simon, 1975; Anderson, 1993). For example, to learn how to play checkers, one must learn a set of rules. In this example, the basic rules of checkers are simple and verbalizable. However, there are certainly more complex rules, or algorithms, that have been learned by expert checker players that may not be easy to verbalize, but that nevertheless have been learned through experience, are elicited during play, and lead to superior performance. Algorithms can also be learned to solve puzzles, like a Rubik's cube. Here, the algorithm is complex and difficult to state, even by people who are adept at solving the puzzle. Thus, learning rules, especially complex ones, can be an example of procedural learning. However, simple rules can certainly be learned declaratively (e.g., I know that white makes the first move in chess), underscoring the fact that many real-world activities combine procedural and declarative knowledge.

In addition to learning skills and rules, a third category of procedural learning is habit learning (Packard and Knowlton, 2002). Habit learning refers to the gradual acquisition of stimulus–response associations. This view of learning was promoted by Hull (1943), who suggested that all learning could be reduced to stimulus–response associations. Although subsequent work has shown that this is not the case, habit learning is nevertheless pervasive. An everyday example may be driving a well-traveled route to work. Each intersection along the route (or stimulus) comes to elicit a specific turn (or response). Habits are characterized by an independence from the goal state (Yin and Knowlton, 2006). This can be seen in the case where you find that you have made your usual left turn taking you to work when it is a Saturday and you were intending to go somewhere else. When confronted with the intersection stimulus, the response habit controlled behavior. In habit learning, the reinforcer, or goal, is not represented in the association with memory but, rather, plays a role in strengthening the association between the stimulus and response during learning.

Habit learning is less clearly a case of learning how than is skill or rule learning. However, habits are procedures in the sense of being actions elicited by conditions. Thus, the stimulus–response habit representation in memory is similar to the condition–action pair that is theorized to underlay procedural knowledge. Also, like other forms of procedural

knowledge, habits are not necessarily available to awareness (Bayley et al., 2005). You might get into the habit of flipping on a light switch when you first step into your apartment, and it may be the case that you are not aware of this habit until it is pointed out to you while you are performing it.

3.17.3 Motor versus Cognitive Procedures

Procedural learning includes both motor and nonmotor learning. By considering procedural learning to cover both motor and nonmotor domains, it suggests that there are fundamental similarities between them (see Rosenbaum et al., 2001). However, there are also important differences. For example, motor movements are first planned based on the geometry of the environment and then executed, with these processes depending on distinct brain regions (Willingham, 1998). Motor skills are also tied to specific effectors; for example, it is generally not possible to switch left and right hands when playing a well-learned piano piece. Control of motor movements also depends on feedback from the visual and proprioceptive systems. In nonmotor procedures, the planning and execution phases are not so clearly separable, and the mode of expression is more general – a master of go could presumably play equally well with either hand. Also, smooth performance of a cognitive procedure would not necessarily require the integration of feedback in real time. For motor procedures, learning may take place in the planning, execution, or integration of feedback in motor behavior. The brain structures responsible for procedural learning of motor behaviors may differ from those responsible for learning nonmotor procedures. Yet there may nonetheless be common ground in the nervous system. Motor behaviors begin as rather abstract action plans before they are translated into muscle movements. These initial action plans may have much in common with purely cognitive procedures like the learned algorithms invoked in solving puzzles or playing games, in that they may both be reduced to a series of condition–action links. Furthermore, both types of skills have the characteristics of procedural knowledge in that they are not readily transferred to new situations (Healy and Bourne, 1995). For example, learning to program a computer does not appear to facilitate logical reasoning in everyday life (Pea and Kurland, 1984).

3.17.4 What Are the Characteristics of Procedural Learning?

An important focus of behavioral research on procedural learning is to develop a list of the properties that distinguish it from other types of learning. In this chapter, we are using the 'knowing how' definition of procedural knowledge, which leads us to the question of how 'learning how' differs from 'learning that'. One characteristic described earlier is the lack of verbalizability of procedural knowledge. Learning must be measured through improved performance on the procedure; people can't really tell us what they have learned. Another characteristic discussed earlier is that procedural memories appear to be stored as condition–action pairs, or stimulus–response associations. This is clearly the case for habits, and even for complex skills it may be possible to break them down into a series of condition–action pairs.

An intriguing property of procedural learning is that learning is not necessarily accessible to awareness. This is linked to the lack of verbalizability of procedural knowledge, in that it is impossible to verbalize knowledge that you are unaware that you possess. The idea of inaccessibility to awareness is more general, though, in that it suggests that the difficulty is not simply in finding the right words to describe procedural knowledge but, rather, in the availability of this knowledge to other cognitive processes. According to this property, we are able to show improvement in performance on procedural tasks without being aware of getting better. As we discuss later, this dissociation between procedural learning and awareness is most striking in the case of amnesic patients, who can show normal procedural learning in the face of a lack of declarative memory for the training sessions that led to procedural learning. In the study of human learning, this distinction between aware and unaware, or explicit and implicit learning, has been useful in making predictions about new tasks in terms of the performance of amnesic patients and the brain systems that support learning. One conceptual drawback of this property, as well as the property of verbalizability, is that it is difficult to apply these criteria to tasks used in nonhuman animals. This has led to something of a separation between memory models derived from human studies and those derived from animal studies.

One property that may be applied to studies of procedural learning in both humans and animals is the lack of flexibility of the knowledge acquired. Unlike declarative knowledge, which can be used in

many ways, procedural knowledge often can only be used in the context of initial learning. For example, having learned a fact such as "The capital of France is Paris" will allow you to readily answer a question posed different ways, such as "What country is Paris the capital of?" or "Name some European capitals," or "Where would you be most likely to find the French president?" We can use declarative knowledge in different contexts – you may meet your friend's mother at her wedding and then have no trouble recognizing her in a store the following week. We can recombine bits of declarative knowledge to make inferences – you may infer that one of your coworkers is a vegetarian if you remember that each time you see him in the cafeteria he is eating a salad. In contrast, procedural knowledge is not flexible and is not readily applied to contexts outside the one in which learning took place. Even an expert driver often stalls initially when using a manual transmission in a different car. Often, it seems our procedural knowledge is encapsulated in the learning context. It is also often difficult to execute just part of a procedure; for example, it is often hard to start a golf swing or a tennis stroke in the middle. Thus, access to procedures can be limited to the manner in which they were learned.

The property of access flexibility is useful because it can be applied to studies using both human and nonhuman animal subjects. It may be the case that lack of flexible access is an overarching property of procedural knowledge. In humans, declarative knowledge can be accessed by cognitive processes that allow it to enter conscious awareness and allow it to be verbalized. In animals who do not possess the ability to verbalize or may not have conscious awareness, their declarative knowledge would still be accessible to the cognitive processes that are present, allowing the animal to use that knowledge in new situations.

Although the properties listed earlier describe the characteristics of procedural knowledge, the process of acquiring procedural knowledge, that is, procedural learning, appears to differ fundamentally from declarative learning. Although declarative learning can occur in an instant, procedural learning is gradual. When we think of a someone who possesses a high level of skill, whether it is in playing a musical instrument or solving chess puzzles, we marvel in the hours of practice that must have gone into achieving that level of skill. Habits are also learned gradually over time. When we talk about getting into a habit, it is understood that this occurred over a period of time. In contrast, we can have declarative memories for events that last a lifetime; by their very nature, these events occurred only once. Although memory for facts may benefit from repetition, it is certainly possible to remember a particularly interesting fact after hearing it once.

Although procedural learning may be slower than declarative learning, it does appear to require fewer attentional resources. Encoding declarative memories is clearly impaired by distraction. For example, you may not do well on an exam if you studied while chatting on the phone. However, learning skills and habits does not seem to depend on paying attention. So, it may be perfectly fine to practice your knitting while watching television. It appears that procedural learning can proceed without tying up working memory, unlike declarative learning, which does benefit from working memory. As working memory is of limited capacity, other tasks that require working memory will interfere with declarative, but not procedural, learning. Because declarative learning benefits from attention, it is sensitive to whether there is an intention to learn. When we intend to learn, we are able to divert attentional resources to the task. Procedural learning may not benefit as much from this intention to learn. This is most clearly seen in habit learning. We may not intend to develop a habit, but with repeated strengthening of a stimulus–response bond, the habit is acquired automatically.

The behavioral criteria for procedural learning are admittedly somewhat vague, and as we discuss later in the chapter, some of the criteria fit better with some tasks that supposedly measure procedural learning than with others. However, the behavioral differences between declarative and procedural learning suggest that the locus and nature of the neural change supporting learning differ as well.

3.17.5 Interactions between Procedural and Declarative Memory

The differences between procedural and declarative memory may lead one to think of them as independent, meaning that impaired or facilitated performance in one system would not influence the other. However, it is unlikely that different forms of memory are completely independent, given the level of interconnectedness of structures within the brain. According to one view, learning of procedures necessarily begins with learning some declarative knowledge. For example, when learning to drive a manual transmission, you must first learn which

pedal is the gas and where the clutch is located. This initial knowledge is then followed by the gradual acquisition of how to perform the skill. In this view, one would predict that a deficit in declarative memory would retard procedural learning, which generally does not seem to be the case. However, in many skills in the real world, declarative and procedural knowledge both make contributions, and in these cases, it may be that some declarative knowledge is needed to provide a foundation for procedural learning.

Although in some cases declarative knowledge can facilitate procedural learning, it is possible to imagine situations when they may compete with each other. For example, the persistence of an old habit may interfere with new declarative knowledge. One may end up following an old route by force of habit, even when you intend to go somewhere else. On the other hand, declarative knowledge may interfere with procedural knowledge. Athletes are familiar with the phenomenon of choking; performance of a skill fails when you think about what you are doing too much (Baumeister, 1984).

The fact that declarative and procedural knowledge may interfere with each other raises the question of where this interference occurs. According to one view, this competition occurs during learning, such that impairing the functioning of one system facilitates learning in the other system. In studies with human subjects, there is little direct support for this view. It seems more likely that competition is occurring at the level of response output. Thus, people may be able to simultaneously learn declarative and procedural knowledge that can be used to support task performance, with declarative knowledge supporting behavior under some circumstances and procedural knowledge supporting behavior under others. For example, driving may sometimes be based on procedural memory, as in when you are driving a familiar route. However, one could also be using declarative memory, as in when you are in an unfamiliar spot and you are recalling a set of directions to get to your destination. What factors influence which system is used to support behavior? It seems likely that the strength of knowledge is an important determinant. If you have not had the opportunity to develop relevant procedural knowledge, your performance is likely to be based on declarative knowledge, while after extensive practice, procedural knowledge may be stronger. Another factor may be the presence of distraction. As procedural memory appears to be less sensitive to distraction (Foerde et al., 2006), it may be more likely to support performance when someone is engaged in multiple tasks. Other factors, such as the presence of stress or emotional state, may also influence whether declarative or procedural knowledge is dominant. Understanding these factors is currently a key question in studies of optimizing human performance.

3.17.6 Procedural Learning and Brain Systems

The idea that there are different types of learning is the basis of the view that memory is composed of distinct systems that map onto different systems in the brain. In this view, procedural and declarative memory are accomplished using different brain regions. An important aspect of the memory systems view is that there are multiple memory systems, not just a dichotomy between procedural and declarative memory. This fits with the finding that many brain regions exhibit plasticity. Some forms of memory do not satisfy the criteria of declarative memory, in that people are not necessarily aware of what has been learned, but they also do not appear to satisfy the criteria of procedural learning, in that learning may occur rapidly.

A good example is the phenomenon of perceptual priming (Schacter et al., 1993). When people are preexposed to a stimulus, such as a word or picture, they are able to process it a little faster and more accurately than if it were novel. This form of learning occurs after a single exposure. Thus, priming may be a form of nondeclarative memory that is also not procedural. As such, it may depend on a different brain system than those that support declarative and procedural memory. A great deal of evidence has shown that declarative memory depends on structures in the medial temporal lobe. As detailed in other chapters, this includes the hippocampus and associated cortical regions in the parahippocampal gyrus. When these structures are damaged, declarative learning is impaired, but procedural learning is not. Thus, procedural learning appears to depend on other brain structures. As we discuss in the following sections of the chapter, converging evidence points to the basal ganglia as being an important system in at least some forms of procedural learning.

3.17.7 Neuropsychological Studies of Procedural Learning

A rich history of habit learning in animal research has suggested the importance of the basal ganglia in

procedural learning. Neuropsychological studies in patients with basal ganglia damage have provided evidence that procedural learning is impaired in these patients and have helped to establish which brain regions within the basal ganglia are crucial for procedural learning. Studies employing the brain imaging techniques of positron emission tomography (PET), functional magnetic resonance imaging (fMRI), and repetitive transcranial magnetic stimulation (rTMS) are identifying the neural substrates of procedural learning. Using a working definition that procedural learning is learning how to do something, one could identify scores of motor skills that could lend themselves to procedural learning studies; we will focus on only a few of the widely used paradigms.

3.17.7.1 The Pursuit Rotor Task

The pursuit rotor task has been extensively studied in motor learning. In the task, subjects attempt to maintain contact between a handheld stylus and a small (2 cm) metal disk that is mounted near the edge of a rotating turntable. The rotation speed can be adjusted to increase the difficulty of the task, and motor learning is assessed as an increase in time on target across training trials. Normally, subjects make jerky corrections early in training and then gradually increase their time on target and maintain smooth tracking of the metal disk with the tip of the target.

Neuropsychological studies employing the pursuit rotor task in patients groups provided evidence that basal ganglia, in addition to the motor cortex and supplementary motor areas, are important in performance. Patients with Huntington's disease and Parkinson's disease are impaired on the pursuit rotor task (Heindel et al., 1988; Heindel et al., 1989; Harrington et al., 1990).

The pursuit rotor task has been used to quantify motor impairments, but the fact that motor areas and basal ganglia were involved in the learning and performance of motor tasks was not surprising. Other investigators developed tasks to get at the nonmotor aspects of procedural learning – those behaviors that depend on learning new associations.

3.17.7.2 Sequence Learning in the Serial Reaction Time Task

Probably the most widely used experimental paradigm to study motor sequence learning is the serial reaction time (SRT) task (Knopman and Nissen, 1987; Nissen and Bullemer, 1987) (see **Figure 1**). In the SRT, subjects are presented with visual stimuli in one of four screen locations. Subjects are asked to respond as quickly as possible by pressing one of four buttons corresponding to the location of the stimulus. There are two types of testing blocks, sequenced and pseudorandom. During the sequenced blocks, the location of the stimulus follows a specific sequence (usually 8 to 12 items long); however, the subjects are

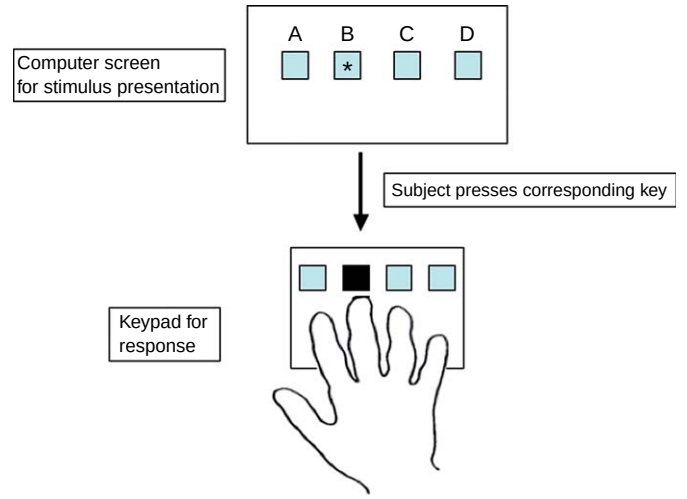

Figure 1 A schematic of the typical procedure of the Serial Reaction Time task, along with a sample sequence.

not informed that the stimulus locations follow a pattern. During the pseudorandom blocks, the stimuli appear in random locations with the stipulation that the stimulus is never shown in the same location on consecutive trials. Learning in the SRT is typically expressed as a reduction in reaction time during the sequenced blocks of trials as compared to the pseudorandom blocks of trials. Probe tests to measure the extent of declarative knowledge of the sequence are often performed, such as asking subjects to explicitly recall the sequence to contrast declarative and procedural learning in the task.

The question of what is learned in the SRT task is important for understanding the capabilities of procedural learning. It is possible that subjects could simply be learning that certain locations come up more frequently, and thus they elicit a quicker response. If so, this would be an example of nonassociative learning. However, most recent studies use sequences in which the occurrence of locations and transitions between locations is equivalent for the random and pseudorandom blocks.

The SRT task fits the criterion of procedural learning, in that at least a substantial subgroup of participants remain unaware of the underlying sequence, yet still show learning of it through their performance on the task (Willingham et al., 1989). A key issue has been what are the most appropriate measures of awareness of the sequence in this task (Shanks et al., 1994). It is possible that using difficult measures like the ability of subjects to generate the sequence or recognize it out of context may underestimate the amount of declarative knowledge that a subject has about the sequence. Data from amnesic patients helps answer the question of whether learning in the SRT could be accomplished without declarative knowledge. As discussed elsewhere in this volume, amnesic patients exhibit severe deficits in declarative memory because of damage to the medial temporal lobe and associated structures. Thus, the fact that amnesic patients show normal performance on the SRT task provides evidence that the task can be done independently of the declarative memory system. (Nissen and Bullemer, 1987; Nissen et al., 1989; Reber and Squire, 1994; Vandenberghe et al., 2006). The concept that sequence learning can be performed independently of the declarative memory system is also supported by other studies in patients with Alzheimer's disease. These patients have severe declarative memory deficits, but are nevertheless able to demonstrate learning in the SRT (Knopman, 1991).

Other behavioral studies using the SRT in patients with Parkinson's disease and Huntington's disease have implicated the basal ganglia as a substrate for sequence learning (Knopman and Nissen, 1991; Ferraro et al., 1993; Pascual-Leone et al. 1993; Willingham and Koroshetz, 1993; Jackson et al., 1995; Doyon et al., 1997, 1998). Even when task demands were modified to minimize the impact of patient motor deficits, by using a verbal version of the task, these patient groups show deficits (Westwater et al., 1998; Smith and McDowall, 2006a). Patients with lesions of the cerebellum show even more striking impairments on the SRT (Doyon et al., 1997, 1998; Molinari et al., 1997; Shin and Ivry, 2003). Thus, sequence learning appears to depend on contributions from two subcortical systems, the basal ganglia and the cerebellum.

3.17.7.3 Probabilistic Classification Learning in the Weather Prediction Task

Probabilistic classification tasks have been used extensively to study nonmotor procedural learning (Knowlton et al., 1994, 1996). A commonly used version of probabilistic classification is presented as a weather prediction game (see **Figure 2**). Subjects are asked to predict the weather, either sunshine or rain, by viewing sets of cue cards with geometric shapes on them. On each trial, after choosing one of the two outcomes, subjects receive feedback about whether their choice was correct or incorrect. The key to the task is that the cue-outcome relationships are probabilistic rather than deterministic. Due to the probabilistic structure of the cue-outcome relationships, memory for individual trials is not particularly useful, and subjects must acquire associations across many trials. Subjects are told to use a gut feeling to make their choices, and most participants report that they do not feel that they are learning early in training.

Studies using the weather task in patient groups have found interesting dissociations. Amnesic patients were found to perform as well as controls (Knowlton et al., 1994), suggesting that the task can be performed without an intact hippocampus. Evidence that the basal ganglia is involved in this type of learning came with other studies in patients with Parkinson's disease and Huntington's disease (Knowlton et al., 1996; Shohamy et al., 2004b; see Ashby and Maddox, 2005, for a review). Deficits in probabilistic classification have been found in patients with Tourette syndrome (Marsh et al., 2004), supporting the idea

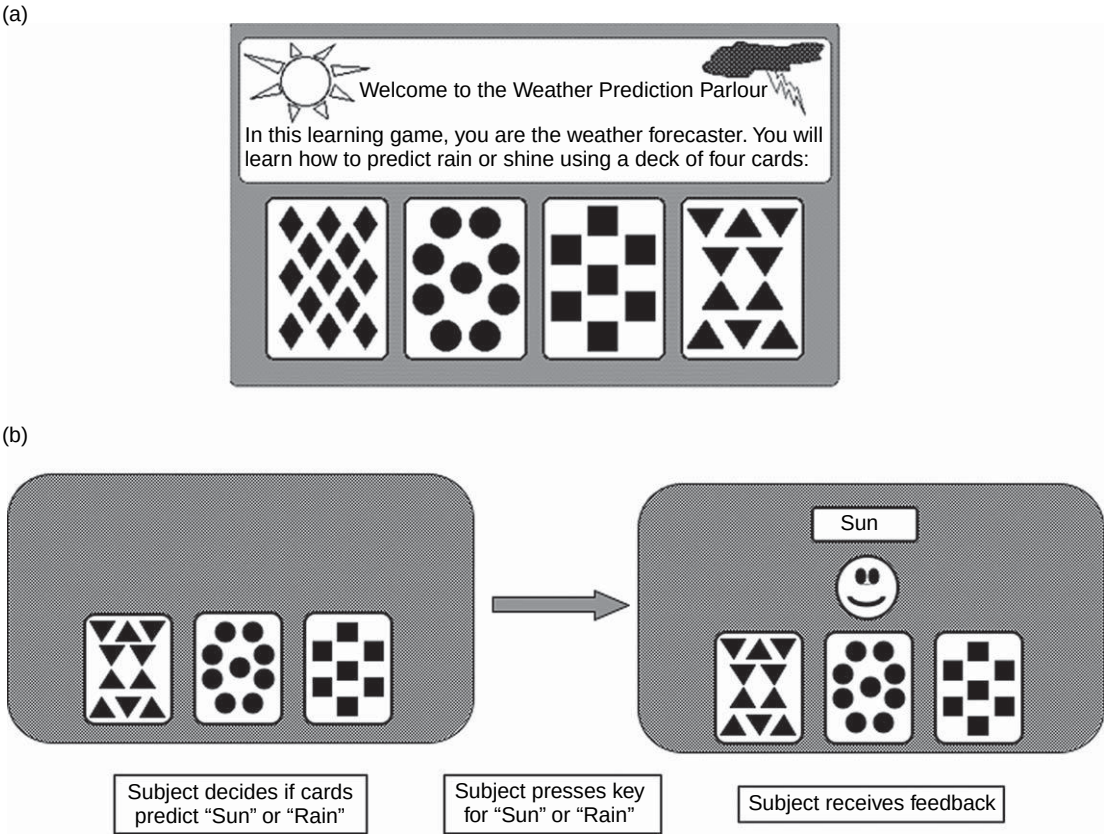

Figure 2 In (a), the instruction screen of the weather prediction task is shown. In (b), the events of a trial are shown.

that this disorder involves disruptions of basal ganglia function.

One important point to be made in interpreting the weather task data is that declarative knowledge of the cue-outcome associations is available and ultimately may be used by participants. Younger adult subjects, who presumably have better declarative memory abilities than older subjects, appear to rely on declarative memory in this task unless they learn while distracted by a secondary task (Foerde et al., 2006). Making the task slightly more deterministic also appears to result in more declarative learning in the task (Hopkins et al., 2004). With more training, even older subjects gain substantial declarative knowledge that can boost their performance above that of amnesic patients. The range of findings in this task underscore the idea that tasks are often not purely declarative or procedural, but, rather, one must examine the content of learning using criteria such as awareness and flexibility of knowledge (Reber et al., 1996).

To characterize the deficit in the weather prediction task in patients with Parkinson's disease, Shohamy et al. (2004b) discovered that a possible source of the lower performance for the patients is the use of a suboptimal strategy. Whereas control subjects based their responding on a single card early on, they shifted to a more optimal strategy as the task progressed in which they based their responding on multiple cards. In contrast, most patients continued using a single-cue strategy throughout the experiment. Since a multicue strategy involves integrating the responses from single-cue stimuli, one way to interpret this finding is that patients with Parkinson's disease might be impaired at response integration. Another way to interpret these findings is that single cards are likely to be learned declaratively because these tend to be easier to remember and are often highly associated with an outcome. Trials with multiple cards are generally less associated with an outcome and may be more difficult to memorize. Thus, learning to classify these trials may be more dependent on procedural memory. In line with the findings of Shohamy et al. (2004b), Sage et al. (2003) found that patients with Parkinson's disease who received a pallidotomy as treatment for motor

impairments exhibited deficits on the weather prediction task restricted to the multicue trials compared with their presurgical performance. By destroying part of the globus pallidus, the pallidotomy effectively eliminates a major source of output from the basal ganglia. Although the pallidotomy resulted in clinical improvement in these patients, removing this input appears to exacerbate the deficit in classifying multiple-cue stimuli.

In Parkinson's disease, the pattern of cell death in the substantia nigra over the course of the disease leads to greater loss of dopamine in the putamen than the caudate nucleus early in the disease (Morrish et al., 1998). As such, it suggests that the putamen plays a greater role in procedural learning in the weather prediction task than the caudate nucleus. However, performance in these patients is negatively correlated with severity of the disease. Patients with milder symptoms can perform within the normal range (Moody et al., 2004; Perretta et al., 2005). It may be that deficits in this task occur only when functioning of the caudate is compromised. Unfortunately, variability in the time course of the disease makes it difficult to use data from these patients to pinpoint which regions are important. Later in the chapter we discuss procedural learning and the anatomy of the basal ganglia in more detail.

3.17.7.4 Concurrent Discrimination

For each trial in a concurrent discrimination task, the participant is asked to choose between two objects of a pair. Subjects are trained across many trials, with one object of each pair being consistently rewarded. Concurrent discrimination tasks have been used extensively to study habit learning in animals. In monkeys, normal performance is seen after medial temporal lobe lesions using several different experimental procedures, suggesting that animals do not necessarily rely on declarative memory in concurrent discrimination, particularly under circumstances when it is difficult for the animal to memorize specific trials (Malamut et al., 1984; Phillips et al., 1988; Teng et al., 2000). Neuropsychological studies have yielded mixed results when the same tasks have been employed in humans to study the effects of medial temporal lobe damage. For example, amnesic patients were impaired in concurrent discrimination tasks in which monkeys with medial temporal lobe lesions are intact (Squire et al., 1988; Hood et al., 1999). In contrast, patients with hippocampal atrophy were shown to learn an 8-item concurrent discrimination as rapidly as controls (Myers et al., 2002). It seems quite possible that the concurrent discrimination task can be learned declaratively or as a set of procedural habits. This view is supported by a study showing that patients with severe amnesia were able to demonstrate learning in a concurrent discrimination task, although at a much slower rate than control subjects (Bayley et al., 2005). Importantly, the amnesic patients performed at chance on a subsequent task in which they were shown all objects on a table and had to sort them according to whether they were rewarded or not. In contrast, control subjects found this task to be easy, suggesting that they had gained declarative, flexible knowledge of which objects were rewarded. These results provide evidence that habit learning can occur for discrimination learning without conscious awareness and without the aid of an intact declarative memory system. However, it could be the case that this does not typically occur in subjects with intact declarative memory, in which discrimination learning may be more efficiently managed by medial temporal lobe structures.

Evidence for a role for the basal ganglia in concurrent discrimination learning comes from work in monkeys showing disruption by lesions of the striatum when 24-hour intervals are used between the presentation of stimuli to thwart declarative memory strategies (Teng et al., 2000; Fernandez-Ruiz et al., 2001). In humans, patients with Parkinson's disease show a deficit in acquiring stimulus-response associations necessary for discrimination learning. For example, Myers et al. (2003) found a deficit in acquisition of stimulus-reward associations in patients with Parkinson's disease.

3.17.7.5 Artificial Grammar Learning

In the typical artificial grammar learning task, subjects are presented with a series of letter strings and are asked to reproduce them. Only after studying the set of letter strings are subjects told that the letter strings were formed according to a set of complex rules, and their task will be to decide for a new set of items whether or not they are formed according to these rules. Although subjects feel that they are simply guessing, they are able to classify new letter strings at a level significantly above chance. Artificial grammar learning has many of the characteristics of procedural learning. It appears to proceed gradually without the awareness of what has been learned, and it does not depend on brain structures that support declarative memory (Reber, 1989; Knowlton et al.,

1992). However, unlike many procedural learning tasks, patients with basal ganglia dysfunction show normal learning (Reber and Squire, 1999; Witt et al., 2002), suggesting that performance of this task depends on other brain regions. One difference between this task and some of the others that do depend on basal ganglia structures is that artificial grammar learning does not require feedback. Thus, the need to integrate feedback during learning may be a key feature of striatal-dependent learning (Aron et al., 2004). In support of this idea, it has been found that patients with Parkinson's disease show deficits in artificial grammar learning when feedback was used (Smith and McDowall, 2006b).

3.17.7.6 Parkinson's Disease as a Model of Striatal Dysfunction

Parkinson's disease affects different brain circuits as the disease progresses and, as such, may reveal a succession of cognitive deficits. At the first sign of motor deficits, the disease has already insidiously destroyed between 70% and 80% of the dopaminergic cells projecting to the dorsal striatum (Agid et al., 1987, 1993; Pakkenberg et al., 1991). However, there is a gradient in dopamine depletion, with greater depletion in the putamen than in the caudate nucleus (Hirsch et al., 1999). Thus, both early- and late-stage Parkinson's disease patients suffer from severe dopamine depletion in the putamen. Later in the disease, the dopaminergic depletion progresses to the caudate nucleus (Agid et al., 1993, Damier et al., 1999). Patients with Parkinson's disease may not provide conclusive information about the role of specific regions in that the disease is somewhat variable in its progression.

The pattern of depletion and regional striatal dysfunction may be an explanation for the sometimes confusing pattern of learning deficits seen in Parkinson's disease populations. Although all Parkinson's disease patients should show impairment on tasks that depend on putamen function, other tasks may reveal dissociations between mildly and severely affected Parkinson's disease patients. Mildly affected Parkinson's disease patients may not show deficits on learning tasks that depend on an intact caudate, whereas deficits may be revealed in more severely affected Parkinson's disease patients who suffer from caudate damage. Few studies have reported data with results assessed separately for early- and late-stage Parkinson's disease patients. Another issue with these patients is the fact that most patients are receiving dopaminergic medications to treat their symptoms. These medications are likely to have wide effects throughout the brain if patients are being tested while on medication, and there are likely to be brain changes in those patients tested off their medication if they are accustomed to taking the medication. Although patients with Parkinson's disease are the most widely used group for assessing the contribution of the basal ganglia to cognition, there are numerous issues to consider in interpreting the data from this population.

3.17.8 Neuroimaging Studies

Neuroimaging techniques have complemented the study of procedural learning in neuropsychological patients. A major advantage of neuroimaging approaches is that they can provide insights into brain regions involved at different stages of learning, thus providing a dynamic picture of procedural learning. Initial studies using PET have shown that different brain regions participate in sequential phases of skill acquisition (sometimes referred to as early, automatic, and skilled). Here, automatic refers to performance that is not affected by the concurrent performance of an additional task – one can perform the automatic task while concentrating on something else. In studies involving simple sequential finger tapping, the cerebellum is activated in all stages of learning, whereas the basal ganglia are activated only in the later skilled stage – implicating a role for the basal ganglia in the performance of automatic skills, with the cerebellum being key for the execution of the movements. Similarly, Grafton et al. (1992) used PET to examine cerebral blood flow while participants were performing the rotor pursuit task. They found an increase in activity in motor areas and the cerebellum, providing evidence for the idea that the cortical–cerebellar loop that supports execution of motor tasks is also involved in learning the task. PET studies of performance on the SRT task with a complex embedded sequence showed sequence learning associated with increases in activity in a network of cortical and subcortical brain structures, with most studies showing increases in the striatum as well as in primary motor cortex, supplementary motor cortex, premotor cortex, thalamus, and cerebellum. In terms of the striatum, both PET and fMRI studies have provided evidence that the caudate head and the anterior putamen show activation during the task (Hazeltine et al., 1997; Peigneux et al., 2000).

As discussed earlier, the SRT is not always a purely implicit learning task, and explicit knowledge can often contribute to performance. A key experimental question is to what extent regions in the basal ganglia and cerebellum can support learning without awareness. Neuroimaging investigators have employed various manipulations to try to force learning without awareness and to compare patterns of performance and brain activity between subjects who did and did not acquire explicit knowledge of the sequence. Grafton et al. (1998) employed a version of the SRT task that added a secondary tone-counting task to make it more difficult for subjects to learn the underlying sequence explicitly. Under dual-task conditions, subjects were considered implicit learners, with only 1 of 20 subjects noticing the repeating sequence. This study revealed that activation in motor cortical areas was associated with sequence learning. However, when subjects transferred their sequence knowledge to a situation where they used different motor effectors, sequence-related activity was seen in the parietal lobe, suggesting that this is the site of abstract sequence knowledge. Using a number of similar tasks, many investigators have found a pattern of activation within corticostriatal networks that parallels procedural learning (Seitz et al., 1990; Doyon et al., 1996; Rauch et al., 1997).

More recent studies have focused on subregions of the basal ganglia to gain insight into how learning progresses. Lehéricy et al. (2005) found evidence for a shift in motor representations during learning in an SRT task. With practice, subjects exhibited a decrease in activity in the rostrodorsal putamen and an increase in the caudoventral putamen. Because the rostrodorsal regions have been implicated in associative learning and the caudoventral regions similarly implicated in sensorimotor tasks, these results suggest a shift of the representations of motor learning from associative to sensorimotor regions. It might be that the associative circuit plays the dominant role during acquisition of the motor skill, but that the sensorimotor circuit takes over to maintain a high level of automated performance. Other studies have provided evidence that these changes in the coritical-striatal circuits are accompanied by similar region-specific changes in the cerebellum (Doyon et al., 2003).

Other recent fMRI studies provide evidence that, in addition to the basal ganglia, the cerebellum and supplementary motor cortex play important roles in this type of procedural learning and that the location of activity may depend on the stage of learning. Some studies have shown that the pattern of sequence-specific activation during the SRT depended on the extent of explicit knowledge of the sequence (Hazeltine et al., 1997; Honda et al., 1998).

As with other recent neuroimaging studies of SRT learning, Poldrack et al. (2005) revealed a pattern of activation that differed early and late in training. There was a decrease in activity with extensive training that was regionally specific. They found that sequence-specific activity in the putamen was high early in training but decreased as training progressed, presumably as automaticity was developing. However, the caudate body showed only a trend for greater sequence-specific activity early in training (there was significant caudate activity under dual-task conditions). If the putamen is primarily involved in motor skill acquisition, this pattern of activation for the putamen is in agreement: The putamen comes online for acquisition of a motor skill, then gradually becomes less active with extensive training and the development of automaticity.

If the caudate were more important for the acquisition of motor sequence knowledge, one would expect early activity in the caudate nucleus during motor sequence learning. Jueptner et al. (1997) found a shifting pattern of striatal activation during sequence learning in a PET study, with early learning activating the caudate nucleus and anterior putamen, followed by a shift in activation to the posterior putamen and sensorimotor cortex when automated performance was achieved.

3.17.8.1 Neuroimaging of Probabilistic Classification

Recent fMRI studies using the weather prediction task shed some light on the issue of the neural substrates of procedural learning. Poldrack et al. (1999) found that normal subjects showed activity in the caudate nucleus (as well as in prefrontal and parietal regions) when performing the weather prediction task. They also found a deactivation in the medial temporal lobe compared with baseline during weather prediction task performance, suggesting that the striatum and medial temporal lobe have a mutually competitive relationship in the control of behavior. Interestingly, patients with Parkinson's disease show activation in the medial temporal lobe during performance of the weather prediction task, suggesting that striatal dysfunction in these patients allows for a greater role for the medial temporal lobe in learning in this task (Moody et al., 2004).

In the weather prediction task, feedback seems to be an important feature, which leads to striatal involvement in this task. Poldrack et al. (2001) showed that striatal activation was greater during performance of the weather prediction task when feedback was used compared with an observational learning condition in which subjects saw the cues presented alongside the outcomes on each trial, without making a choice. This condition was in fact associated with medial temporal lobe activation, suggesting that subjects learned the task declaratively under these circumstances. Consistent with this idea is the fact that patients with Parkinson's disease are able to learn the association between stimuli and responses in the observational condition (Shohamy et al., 2004a), while these patients show impairments in the weather prediction task when feedback is used (Knowlton et al., 1996; Shohamy et al., 2004a). Together, these studies point to the critical role of feedback in the recruitment of the striatum during learning.

Functional neuroimaging approaches have also been used to determine the role of attention in declarative and procedural learning. Using the weather prediction task, Foerde et al. (2006) examined the neural substrates of knowledge learned while subjects were focused on the task and when they were distracted by the need to perform a concurrent task. When subjects were able to focus on learning the weather prediction task, performance correlated with activation in the medial temporal lobe. It appears that these young, neurologically intact subjects were able to acquire declarative knowledge of these cue-outcome associations and were able to later use these associations flexibly in transfer tests. In contrast, for associations learned under distraction, performance appeared to rely on the striatum. For these associations, knowledge was more procedural in that it could not be used readily in transfer tasks in which subjects were asked questions about the associations in a form that was different from the training procedure. A key property of procedural habit learning may be the fact that learning can proceed normally when working memory resources are occupied by a concurrent task. This result has important implications for our multitasking society: We may be able to perform some of our daily activities in dual-task or multitask mode, but when declarative knowledge of new information is required, we need to focus on one task at a time, or the ability to flexibly use this knowledge will suffer.

Another example of the complex interaction of explicit and implicit learning can be found in a recent study examining the impact of basal ganglia damage on motor learning in a continuous tracking task with an embedded repeated sequence (Boyd and Winstein, 2006). The authors found that explicit information actually interfered with learning the motor task for the patients with basal ganglia damage. As expected, the control subjects performed better on the motor task when they were given explicit information of the repeated sequence. One interpretation of this result is that if the basal ganglia are important for switching between modes of processing, damage here may make it particularly difficult for procedural and declarative knowledge to interact.

3.17.8.2 Posttraining Disruptions of Procedural Learning Using TMS

The TMS technique allows researchers to effectively create a temporary lesion in a neurologically intact subject. This technique involves applying a repetitive magnetic field to regions on the surface of the brain using a coil held to the scalp. Although much of the focus on neural structures for procedural learning has been on the basal ganglia, TMS is particularly well suited to examine the involvement of cortical regions important for procedural learning that are part of critical corticostriatal loops. Although neuroimaging studies have shown activation of the dorsolateral prefrontal cortex (DLFPC) during procedural sequence learning, it was not clear whether this region is necessary for learning. Using TMS, Robertson et al. (2001) were able to disrupt the normal function of the DLFPC during a modified version of the SRT. Although this disruption did not cause a general impairment in sequence learning, it did appear to block learning of spatial sequence learning. Their results suggest that the DLPFC helps maintain spatial information in working memory until other cortical and striatal structures can learn the sequence. It appears that, unlike habit learning, spatial sequence learning does require working memory resources (Hsiao and Reber, 2001).

Other researchers have used TMS at different stimulation frequencies to enhance rather than disrupt neural function. Kincses et al. (2004) discovered that electrical stimulation of the prefrontal cortex could improve performance in a probabilistic classification task during direct transcranial stimulation of the left prefrontal cortex. The authors suggested that the weak

stimulation of the left prefrontal cortex increased neuronal excitability, and this facilitated the function of relevant corticostriatal loops. However, as seen recently by Foerde et al. (2006), probabilistic classification tasks can often be learned declaratively by normal subjects.

3.17.8.3 Shifts in Activation with Practice – an Emerging Theme in Basal Ganglia Neuroimaging

Another technique to examine the substrates of procedural learning employs intensive analysis of fMRI data to establish effective connectivity of distinct brain regions during task performance. Toni et al. (2002) have examined connectivity during procedural visuomotor learning and found specific learning-related increases of effective connectivity in temporostriatal and frontostriatal circuits. Connectivity among portions of the frontal cortex decreased with learning, whereas temporofrontal and parietofrontal connectivity did not change across learning trials. This result suggests that visuomotor associations can be established by strengthening specific corticostriatal circuits.

3.17.8.4 Who Is the Teacher?

Recent fMRI studies have allowed researchers to follow the patterns of activity in specific brain regions and to investigate a fascinating time-dependent interaction of different brain regions and possibly different memory systems. One can think of these interactions in terms of a symphony, with different brain regions playing their parts interactively throughout learning, with some regions playing a dominant role early in learning, and others later in learning – like a handoff of the melody between strings and wind instruments. However, an important question regarding the interplay of cortex and striatum in procedural learning is, "Who is teaching whom?" Some researchers have suggested that habit learning is acquired gradually, with the cortex teaching the caudate, and with a gradual buildup of habit representations in the striatum (Graybiel, 1998). In this model, cortical activation precedes striatal activation. However, recent studies provide evidence that this picture may not be so simple. Frank et al. (2001) described evidence that striatal activity can precede frontal activity in some cases. These researchers suggest that the striatum is providing contextual information to the cortex, so that the cortex can then choose an appropriate learning strategy.

3.17.8.5 Time Course of Basal Ganglia Activations

Some procedural learning studies have recently shown that within the basal ganglia there is also a temporally varying pattern of activity, with the head of the caudate playing the dominant role early in learning and the tail of the caudate taking the lead later in learning. Several recent studies have noted these variations that perhaps reflect learning-dependent changes in the coritical-striatal loops involved in the learning. Delgado et al. (2005), using the probabilistic classification task, found that the caudate was more highly activated in early learning than in later learning trials. Seger and Cincotta (2005) found dissociations in the pattern of activity in the head of the caudate versus the body and tail of the caudate. Using a version of the weather prediction task, the investigators also found a high level of activity in the head of the caudate that decreased as learning progressed. Activity in the body and tail of the caudate was found to correlate with successful learning and increased across the learning task. Thus, this pattern in the striatum may relate to the transformation of memories from declarative to procedural. In contrast to these findings, Poldrack et al. (2001) found increases in activation in the caudate nucleus as learning progressed in the probabilistic classification task. As discussed earlier, the probabilistic classification task may be learned declaratively under single task conditions. Thus, it is important to examine the nature of the learned representations in order to understand whether learning was procedural or declarative (Foerde et al., 2006).

3.17.8.6 Consolidation of Procedural Learning: Effects of Sleep

Many studies have pointed to an enhancement of procedural learning following sleep. For example, speed and accuracy in a finger-tapping sequencing task were improved following a night of sleep, but not after an equivalent period of wakefulness (Walker et al., 2002). There is also evidence that declarative learning shares the same benefit (Gais et al., 2006). Robertson (2004) found that whether sleep was of benefit in the performance of a motor sequencing skill depended on the level of declarative knowledge of the task. Those subjects with better declarative

memory for the task showed only overnight improvements in performance, whereas subjects with poorer declarative memory showed improvements in performance with or without sleep. Similarly, skill enhancement occurred following a nighttime retention interval only if there was declarative knowledge of the sequence (Walker, 2004). These results suggest that the benefits of sleep to skill learning are mediated by the contribution of declarative knowledge. However, there is also evidence that procedural and declarative learning can be enhanced by different stages of sleep (Rauchs et al., 2005). As the proportion of these phases may vary with many factors, including the duration and time of sleep, and age of the subjects, different effects on learning may be obtained.

3.17.8.7 Anatomical Basis of Procedural Learning

As discussed earlier, the basal ganglia have been closely associated with procedural learning based on both neuropsychological and neuroimaging studies. However, the basal ganglia are a major division of the brain with broad and heterogeneous functions. It is thus important to attempt to specify in more detail the circuits within the basal ganglia that subserve procedural learning. In addition, as there appear to be different types of procedural learning, these may depend on different circuits within the basal ganglia.

The basal ganglia receive input from all regions of the cortex. The major input structures are the neostriatum, including the caudate and putamen, which receive input from neocortex, and the ventral striatum, including the nucleus accumbens, which receives input from limbic cortices. The output of the basal ganglia is much more limited and is concentrated in projections to regions of the thalamus that project to the frontal cortex. The organization of the basal ganglia has been traditionally thought of in terms of corticostriatal loops (Alexander et al., 1986). These loops are considered to be somewhat segregated, with different regions of the cortex projecting to specific striatal regions, which in turn project to specific regions of the pallidum and substantia nigra. The output of the loops continues to be segregated from the nigra and pallidum to specific thalamic nuclei, which project to distinct cortical regions primarily in the frontal lobe. The segregation of these loops may be consistent with the relative inaccessibility of procedural knowledge from other cognitive processes.

There have been several different views of how these corticostriatal loops are organized. An influential model put forth by Alexander et al. (1986) divided the basal ganglia into five functional loops: skeletomotor, oculomotor, dorsolateral prefrontal, lateral orbitofrontal, and anterior cingulate circuits. These loops are thought to encompass the motor functions of the basal ganglia as well as cognitive, motivational, and emotional functions through the loops terminating in the dorsolateral prefrontal, lateral orbitofrontal, or anterior cingulate circuits. The parallel nature of these loops suggests that different types of procedural learning, such as motor skills, cognitive skills, and habits, may be instantiated in these different loops.

These loops have been grouped based on their targets in the frontal lobes, thus dividing them into motor, association, and limbic loops (Parent, 1990; see **Figure 3**). The motor loops include regions of frontal lobe involved in motor function, the association loop

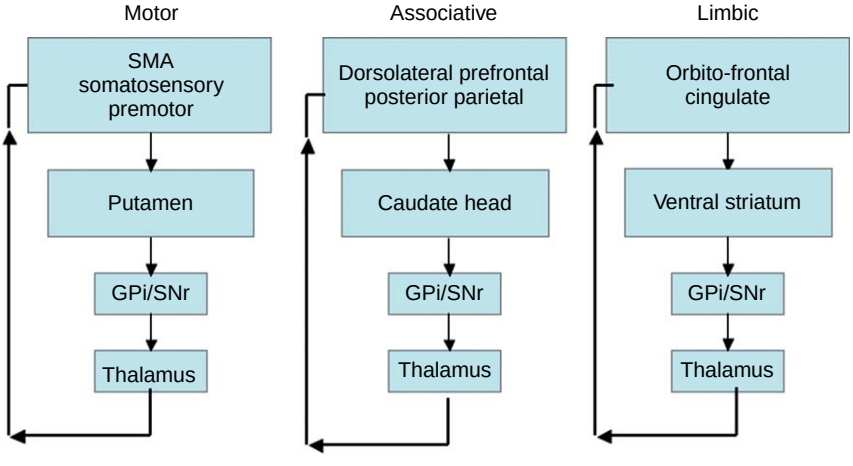

Figure 3 A schematic of the three major corticostriatal loops based on targets in the frontal lobes.

includes prefrontal regions that receive highly processed cortical input, and the limbic loops include frontal regions that have been associated with regulation of behavior and motivation. For the most part, these loops are closed, meaning that these cortical areas project to the striatal regions that eventually provide discrete basal ganglia input to the same cortical area. Such closed loops have recently been reported that include nonfrontal lobe regions including inferotemporal and posterior parietal cortices (Middleton and Strick, 2000; Reep et al., 2003). The closed-loop architecture of the corticostriatal system has been hypothesized to be an efficient means by which to select among and coordinate different actions or responses (Hikosaka, 1998; McHaffie, et al., 2005). By returning input to the original cortical region, responses originating in that cortical region can be inhibited or potentiated based on a central selection mechanism. In this view, the basal ganglia's role is not limited to procedural learning and memory but, rather, is key in selecting between responses based on procedural or declarative knowledge to smoothly produce ongoing behavior.

For the basal ganglia to be involved in response selection, there must also be integration across loops to coordinate their output. In addition to the prominent closed loops, there are additional open-loop projections by which functional subdivisions of the striatum can influence the output of other loops (Joel and Weiner, 1994). For example, the associative striatum can influence motor cortical regions via projections to pallidal regions that project to thalamic nuclei afferent to the premotor cortex.

In addition to the cortical input to the striatum, there is a prominent dopaminergic projection from the pars compacta region of the substantia nigra to the dorsal striatum and from the ventral tegmental area to the ventral striatum. Dopamine plays a key role in the function of the basal ganglia, and in turn, dopamine's greatest effect on cerebral processing is arguably in the basal ganglia. The limbic striatum in particular is able to influence other striatal loops via its projections to dopaminergic neurons in the substantia nigra (Haber et al., 2000; Joel and Weiner, 2000). This specialized role of the limbic striatum suggests that reward and motivation may be particularly important in response selection.

3.17.8.8 Caudate and Putamen

As discussed earlier, a comprehensive view of the basal ganglia holds that it has a general role in selecting between possible responses rather than a specific role in procedural learning and memory. It is thus important to identify those circuits within the basal ganglia that may be important for procedural learning. In the primate striatum, an initial distinction may be made between the caudate and putamen, which are the main input structures of the associative and motor loops. Differentiating the function of the caudate and putamen is somewhat difficult in human subjects, as diseases that affect the basal ganglia are not completely selective for one subset of the striatum, and neuroimaging techniques in the past have lacked the resolution necessary to differentiate striatal subregions. However, as spatial resolution improves, it is becoming possible to see activation within specific circuits using fMRI.

As a component of the motor circuit, the putamen would appear to be important for motor skill learning. However, it may be more accurate to think of the motor striatum as being involved in responses more abstractly, and not simply in effector-specific movements. Frontal cortical regions in the motor loop include premotor cortex, which has a role in motor planning. Thus, the putamen may be particularly involved in habits, in which particular responses become linked to environmental triggers (Yin and Knowlton, 2006). These responses may not be confined to specific movements, but they may also be more abstract actions (such as turning on a light switch or opening a cabinet) that have come to be automatically elicited by specific cues. Electrophysiological evidence from nonhuman primates indicates that neurons in the putamen respond during movements that are overlearned and habitual (Kimura et al., 1993). Also, fMRI studies of human habit learning tasks also demonstrate activation centered around the putamen (Foerde et al., 2006).

In contrast, the primate caudate nucleus appears to play an important role in executive functions. This is consistent with its connections with prefrontal cortex. Neurons in the caudate nucleus respond during goal-directed actions and working memory tasks (Levy et al., 1997). In patients with early Huntington's disease, in which degeneration is focused in the head of the caudate, poor performance is detected in learning cognitive skills (Watkins et al., 2000). It may be that acquiring rules and algorithms depends on this region, because of the importance of working memory in extracting structure from the environment.

3.17.8.9 Motor Cortex and Procedural Learning

In addition to the striatum, the cortical components of the corticostriatal loops are important for procedural learning. The motor cortex in particular shows plastic changes associated with learning motor skills (Monfils et al., 2005). For example, extensive practice of a motor sequence results in long-term increases in the extent of activation in primary motor cortex when the skill is performed. These data suggest that skill learning is accompanied by reorganization of primary motor cortex. These findings bring up the question of whether the motor cortex is the initial site of plasticity for motor skill learning, or if this representation is formed through signals originating from the striatum. According to this view, the basal ganglia show initial changes related to skill development, but with extended practice over the course of weeks or months, the learned skill is represented in the cortex (Ungerleider et al., 2002). These data suggest that the putamen may have a role in setting up the changes in motor cortex that support the performance of skills as well as setting up associations between stimuli and responses in habit learning. As motor skills may require extremely precise patterns of movement, changes in motor cortex may be necessary to support skilled behavior. The relationship between the striatum and cortex in skill learning underscores the idea that there may not be a single site of plasticity in procedural learning, but, rather, different structures become involved at different points during training.

3.17.8.10 The Role of Reinforcement

Dopamine has long been associated with reinforcement. Patients with Parkinson's disease who suffer from low levels of striatal dopamine exhibit deficits in learning trial-and-error tasks in which performance in shaped by feedback (Knowlton et al., 1996; Ashby et al., 1998; Frank et al., 2004; Shohamy et al., 2004a). In some forms of procedural learning, feedback plays a fundamental role. In habit learning, reinforcement is needed for the formation of the stimulus–response bond. Dopamine acts through D1 receptors in the direct pathway, which has the net effect of disinhibiting thalamic nuclei and facilitating behavior in the cortex, and through inhibitory effects on D2 receptors, which are part of an indirect pathway, which has the net effect of inhibiting thalamic nuclei and reducing behavior (Hikosaka, 1998). According to recent models of reinforcement learning, reward leads to dopamine bursts that increase synaptic plasticity in the D1 pathway and decrease plasticity in the D2 pathway, supporting learning to engage in a response (Frank et al., 2004; Frank, 2005). These models would predict that plastic changes within the striatum subserve habit learning. Other forms of procedural learning, such as motor and cognitive skill learning, may also be facilitated by reinforcement, but it would seem that additional mechanisms must come into play that are specific to the task. These would include programming the precise timing of movements and integrating sensory feedback in the case of motor skill learning, or gaining the ability to chunk different aspect of a problem so as not to exceed working memory limitations in cognitive skill learning.

3.17.8.11 Evolutionary Perspective on Procedural Memory

Considerable evidence indicates that multiple systems of learning depend on different brain systems. Procedural learning refers to one class of abilities that share features such as independence from awareness and lack of flexibility. The existence of these different systems raises the question of why we have evolved different forms of learning and what advantages this could have over a situation in which there was a single learning mechanism. Considering the brain from an evolutionary perspective, it would seem unlikely that there would be a general purpose learning and memory system. Rather, cognitive structures and their underlying neural circuitry would emerge through selection pressure (Sherry and Schacter, 1987). Procedural learning has been argued to have emerged earlier, based on the idea that knowing how to perform basic skills is more critical for survival than knowing that certain facts are true (Reber, 1992). Also, the striatum, which is likely to be important for many forms of procedural learning, is a phylogenetically older structure than the medial temporal lobe structures that are important for declarative memory. One of the most intriguing questions in cognitive neuroscience is what drove the evolution of declarative memory. What advantages does declarative memory convey over other forms of memory? One argument is that declarative memory evolved as a means to link spatial and nonspatial information – such as memory for items in locations, which would be useful in foraging behavior

(Manns and Eichenbaum, 2006). This required a learning system that received a confluence of information from different sources. This anatomical convergence may support the key feature of declarative memory – its accessibility to numerous cognitive processes. On the other hand, the evolution of declarative memory did not usurp the importance of procedural learning. Procedural learning is clearly well suited to acquiring skills and habits necessary for survival, and the fact that it does not rely on attentional resources makes it efficient.

Although procedural learning may be phylogenetically old, it is certainly the case that procedural learning has continued to evolve in humans. It is also the case that cognitive structures that evolved in the past may become exapted for different uses through time (Gould and Vrba, 1982). A learning system that evolved based on one set of environmental conditions may become adaptive in new situations as well. For example, it may be that complex skills like reading or playing a musical instrument exploit neural mechanisms that evolved for learning simple motor skills. As procedural learning mechanisms are likely to have emerged phylogenetically earlier than declarative learning mechanisms, and have been subject to a longer history of selection processes, they are likely to be more resilient to disruption. Thus, factors such as age, stress, and fatigue would be less likely to affect procedural learning than declarative learning, as has generally been found to be the case (Poolton et al., 2007).

3.17.8.12 Procedural Learning and Disorders of Cognition

Although procedural learning is robust to many of the changes that affect declarative learning, it is the case that disruptions of basal ganglia function result in procedural learning disorders. Are there functional consequences of disordered procedural learning? Patients with Huntington's disease or Parkinson's disease may have difficulty with learning complex skills, although motor limitations in these patients may make such difficulties less relevant for daily life activities than the primary difficulties in performing tasks. One possible consequence is that tasks that would normally become automatic may continue to draw on working memory resources in these patients. Thus, routines that could be accomplished with little mental effort after extensive practice (e.g., getting ready for work in the morning) would need each step to be accomplished deliberately in these patients and would make it more difficult to accomplish additional tasks concurrently. Thus, a procedural learning deficit may result in a lack of mental efficiency in everyday life.

In addition to neurological disorders, corticostriatal loops are involved in a number of psychiatric disorders as well. For example, schizophrenia appears to involve deficits in the cognitive loop and also to be accompanied by deficits in automating cognitive skills. It is likely that this impairment contributes to the difficulties these patients have in work and social settings (Granholm et al., 1991; Strauss, 1993). Although in general we implicitly learn how to behave around others, in patients with schizophrenia, these rules of social conduct must be consciously learned and applied. Other psychiatric disorders could be viewed as an overactive procedural system. Obsessive compulsive disorder is accompanied by overactivity in corticostriatal circuitry and involves the repetition of stimulus-bound behaviors such as washing, counting, and checking (Saxena et al., 1998). Addiction in those who would like to quit may also be seen as the triumph of habit over goal-directed action (Everitt and Robbins, 2005). Progress can be made in treating these disorders if we learn how the striatum affects the selection between habit and goal-directed responses.

From the time a baby is born, it is continuing to learn numerous procedures – both motor and cognitive skills. Reading is a skill that is learned during development and is crucial for success in our society. However, unlike evolutionarily older skills, including language production and understanding, reading difficulties are relatively common in otherwise normally developing children. It may be that in children with reading difficulties, the skill of reading may not become fully automatic. Understanding the process by which skills become automatized may yield insights into how this process may go awry and could lead to early identification of risk factors.

In all these cases, understanding the mechanisms of procedural learning is likely to lead to benefits to human health. Because of the central role procedures play in daily life, it is arguably as important, if not more so, to study the mechanisms of procedural learning as it is to study declarative learning. As our knowledge of basal ganglia function expands, we are gaining a grasp on knowing how we function in the world.

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3.18 Neurobiology of Procedural Learning in Animals

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3.18.1 Introduction

Research investigating learning and memory from a neurobiological perspective provides support for the hypothesis that multiple memory systems exist in the mammalian brain (for reviews, see Eichenbaum and Cohen, 2001; White and McDonald, 2002). Several theories outlining the psychological operating principles that define different types of memory have been proposed (e.g., Hirsh, 1974; O'Keefe and Nadel, 1978; Olton and Papas, 1979; Cohen and Squire, 1980; Mishkin and Petri, 1984; Graf and Schacter, 1985). In lower animals in particular, theoretical development of the sets of psychological mechanisms that distinguish different types of memory has been significantly influenced by the classic debate between cognitive (e.g., Tolman, 1932) and stimulus-response (e.g., Thorndike, 1933; Hull, 1943) learning theorists. One prominent dual-memory theory draws a distinction between declarative and procedural memory (Cohen and Squire, 1980; Squire and Zola-Morgan, 1991; Eichenbaum and Cohen, 2001). In lower animals, declarative memory involves the acquisition, consolidation, and retrieval of relational representations that allow for flexibility during behavioral expression (Eichenbaum and Cohen, 2001) in a manner analogous to the cognitive (Tolman, 1932) view of learning and memory. Declarative memory relies largely on a neuroanatomical system composed of the hippocampus and related structures in the medial temporal lobe (Squire et al., 1993; Eichenbaum and Cohen, 2001). In contrast, procedural memory involves the acquisition, consolidation, and retrieval of individual representations that are behaviorally expressed in an inflexible manner (Eichenbaum and Cohen, 2001), analogous, at least in part, to the stimulus-response or habit (Hull, 1943) view of learning and memory.

In rats, numerous findings from studies employing lesion, pharmacological, molecular, and electrophysiological approaches provide converging evidence supporting the hypothesis that the dorsal striatum (i.e., caudate-putamen) mediates the formation of stimulus–response habits. The goals of the present chapter are to provide a brief historical perspective to the development of this hypothesis and to summarize evidence of the role of the dorsal striatum in this form of procedural learning. Studies employing brain lesion techniques and pharmacological approaches in rats are emphasized, particularly in those instances in which dorsal striatal involvement in procedural and declarative memory tasks has been directly compared. However, it should be noted that neurobehavioral findings from electrophysiological (e.g., Graybiel et al., 1994; Rolls, 1994; Graybiel, 1998; Jog et al., 1999) and molecular (e.g., Colombo et al., 2003; Teather et al., 2005; Pittenger et al., 2006) experiments in rats and nonhuman primates also implicate the dorsal striatum in procedural learning.

In addition to dorsal striatal-dependent stimulus-response memory, learning that is selectively mediated

by other brain structures might also be considered procedural in nature, at least to the extent that they involve acquisition, consolidation, and retrieval of information that is behaviorally expressed in a relatively inflexible, stimulus-bound manner (Eichenbaum and Cohen, 2001). For example, under this definition, cerebellar-dependent classical conditioning of skeletal musculature (for review, see Thompson, 2005) and basolateral amygdala-dependent stimulus-affect learning (for review, see McDonald and White, 2002; *See* Chapter 3.24) might also be broadly construed as forms of procedural learning (Squire and Zola-Morgan, 1991). Findings of several studies have dissociated the roles of the dorsal striatum and basolateral amygdala in learning and memory. Therefore, following a consideration of dorsal striatal involvement in stimulus-response learning, data suggesting that learning based on stimulus-affect associations is mediated by the basolateral amygdala is briefly discussed.

3.18.2 The Neural Bases of Procedural Learning: Emergence of the Dorsal Striatal Hypothesis

In lower animals, the hypothesis that multiple memory systems exist was derived from an analysis of the effects of damage to the hippocampal system on behavior across a wide range of learning tasks (for reviews, see Hirsh, 1974; O'Keefe and Nadel, 1978; Olton, 1979). Similar to the selective behavioral pattern that is observed in rats, lesions of the hippocampal system in monkeys impair behavior in tasks requiring declarative/cognitive memory and spare acquisition of tasks that can be acquired by a putative procedural or habit learning mechanism (for review, see Mishkin and Appenzeller, 1987). In an attempt to identify the neural bases of this spared learning, the hypothesis that the primate dorsal striatum and associated putamen mediate procedural learning that involves the formation of stimulus–response habits was introduced (Mishkin et al., 1984; Mishkin and Petri, 1984; Mahut et al., 1984). At the time that it was originally proposed, this hypothesis was based largely on anatomical considerations. Specifically, the dorsal striatum receives sensory stimulus input from all regions of the cortex via topographically arranged corticostriatal projections and can readily influence motor output via downstream projections to brainstem structures and/or via corticothalamic-basal ganglia loops (e.g., Webster, 1961; Van Hoesen et al., 1981; Alexander et al., 1986). Thus, it was suggested that the dorsal striatum is anatomically well situated to mediate S-R habit learning across various sensory modalities (Mishkin and Petri, 1984; Mahut et al., 1984), although the behavioral evidence implicating the striatum in procedural learning in nonhuman primates was fairly meager (Divac et al., 1967; Buerger et al., 1974).

As noted by Iversen (1979), researchers interested in the neurobiology of learning and memory were not likely encouraged to examine a potential role for the dorsal striatum in view of Karl Lashley's premature conclusion that "the evidence seems conclusive that in mammals the basal ganglia are not an essential link in the patterning of learned activities" (Lashley, 1950, p. 454). Nonetheless, prior to the advent of multiple memory system theories, several experiments examining the effects of lesions of the dorsal striatum on learning were conducted in rats. When considered in retrospect, the findings of many of these studies are consistent with the hypothesized role of this structure in procedural learning and memory. For example, dorsal striatal lesions impair acquisition of various conditioned avoidance behaviors that can be readily acquired using a procedural learning mechanism (e.g., Kirkby and Kimble, 1968; Winocur and Mills, 1969; Neill and Grossman, 1971; Mitcham and Thomas, 1972; Allen and Davidson, 1973; Kirkby and Polgar, 1974). In addition, by the mid-1970s several studies in rats had demonstrated a modulatory effect of posttraining electrical stimulation of the dorsal striatum on memory consolidation using various tasks that could conceivably be acquired using a procedural learning mechanism (for review, see Kesner and Wilburn, 1974).

3.18.3 Dorsal Striatum and Procedural Learning: Dissociation Lesion Experiments

The impairments observed following dorsal striatal lesions in simultaneous discrimination learning in monkeys (Divac et al., 1967; Buerger et al., 1974) and in conditioned avoidance behaviors in rats (for review, see Oberg and Divac, 1979) are consistent with a putative role for this brain structure in procedural learning and memory. However, rather than impairing learning or memory processes per se, a lesion-induced deficit in acquisition of a single type of task may reflect an influence on nonmnemonic

factors that are involved in task performance (e.g., sensory, motor, or motivational processes).

Dissociation methodology provides an experimental design that more directly addresses the question of whether a particular brain structure plays a selective role in learned behavior. In a single dissociation design, the role of a single brain structure (X) in behavior is contrasted using a pair of behavioral tasks (A, B). For example, lesions of the dorsal striatum impair retention of an egocentric left-right discrimination in a radial maze but do not impair retention of an allocentric place learning task (Cook and Kesner, 1988). In addition, dorsal striatal lesions impair acquisition of a tactile discrimination procedural learning task in a T maze but do not impair declarative memory guiding goal-arm alternation (Colombo et al., 1989). These examples of single dissociations provide evidence consistent with a selective role of the dorsal striatum in procedural learning and are clearly more compelling than studies using only one behavioral task. Nonetheless, the presence of single dissociations may not reflect a strict functional independence of procedural and declarative memory systems in the brain (Eichenbaum and Cohen, 2001; Packard, 2002).

A more stringent test of the hypothesis that the dorsal striatum selectively mediates procedural learning employs double dissociation methodology. In this experimental design, the function of two brain structures in behavior is contrasted in two behavioral tasks. Ideally, this approach involves the use of a pair of tasks that possess similar nonmnemonic (e.g., sensory, motor, motivational) characteristics but that differ in terms of the type of learning and memory processes required. The first study to implement this design to investigate the role of the dorsal striatum in learning employed two radial maze tasks to compare the effects of dorsal striatum and hippocampal system lesions on procedural and declarative memory in rats (Packard et al., 1989). In a declarative or cognitive memory version of the task, rats obtained food rewards by visiting each arm of the radial maze once within a daily training session, and reentries into maze arms that were previously visited are scored as errors. This task requires rats to remember which maze arms have been previously visited within a trial and is essentially a test of declarative knowledge that may involve spatial working memory (Olton, 1979) and/or the use of a cognitive mapping strategy (O'Keefe and Nadel, 1978). In a procedural memory version of the task, rats obtained food rewards by visiting four randomly selected and illuminated maze arms twice within a daily training session, and visits to unlit maze arms are scored as errors. Every visit to an illuminated maze arm was reinforced, and thus there was no requirement to use declarative memory to remember specific arm entries. Rather, this task can be acquired by a procedural or habit learning mechanism by which a light cue (i.e., a stimulus) evokes approach behavior (i.e., a response). Pretraining electrolytic lesions of the dorsal striatum produce a dissociation in behavior in these two radial maze tasks, impairing acquisition of the procedural task and leaving acquisition of the declarative task unaffected (**Figure 1**). In contrast, lesions of the fimbria-fornix (a major input/output pathway of the hippocampus) selectively impaired acquisition of the declarative radial maze task, resulting in a double dissociation of the effects of dorsal striatal and hippocampal system lesions on learning (Packard et al., 1989). These findings were later replicated in a study examining the effects of neurotoxic lesions of the dorsal striatum and hippocampus (McDonald and White, 1993).

Although lesions of the dorsal striatum selectively impair acquisition of visual discrimination behavior in a procedural learning version of the radial maze task (Packard et al., 1989; McDonald and White, 1993), it is possible that this deficit reflects disruption of a stimulus–stimulus (light–food) association, rather than a stimulus–response (light–approach) association. The nature of the association guiding the expression of learned behavior can be assessed in a reinforcer devaluation paradigm (Adams and Dickinson, 1981). Rats exposed to reinforcer devaluation following acquisition of the dorsal striatal-dependent radial maze task continue to approach illuminated maze arms, indicating that performance of the task involves expression of a stimulus-response/habit form of procedural memory (Sage and Knowlton, 2000).

A second study used two water maze tasks to demonstrate that the selective role of the dorsal striatum in procedural learning generalizes to aversively motivated behavior (Packard and McGaugh, 1992). In these tasks, two rubber balls differing in visual appearance (vertical vs. horizontal black and white stripes) served as visual cues. One ball (correct) was located on top of a platform that could be used to escape the water, and the other ball (incorrect) was located on top of a thin rod that did not provide escape. In a declarative version of the task, the correct platform was located in the same spatial location on every trial. However, the visual pattern on the ball associated

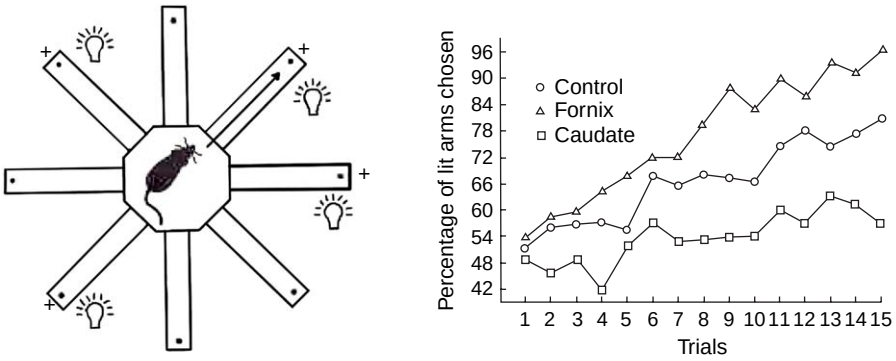

Figure 1 (a) In a radial maze task that can be acquired using a procedural learning mechanism, rats obtain eight food pellets by visiting each of four randomly selected and illuminated maze arms twice within a daily training session (Packard et al., 1989). This task can be acquired using a stimulus (light)–response (approach) form of procedural learning. Consistent with this suggestion, rats trained in this task and exposed to reinforcer devaluation continue to approach illuminated maze arms (Sage and Knowlton, 2000). Radial maze illustration used with permission from Eichenbaum H and Cohen NJ (2001) *From Conditioning to Conscious Recollection: Memory Systems of the Brain*. Oxford Psychology Series, no. 35. New York: Oxford University Press. (b) Lesions of the caudate (i.e., dorsal striatum) severely impair acquisition, consistent with the hypothesis that the dorsal striatum mediates procedural learning and memory. Note that lesions of the hippocampal system (i.e., fornix) actually enhance acquisition. This finding suggests that in some learning situations, hippocampus-dependent declarative memory interferes with dorsal striatal–dependent procedural memory (for a review of competitive interactions among multiple memory systems, see Poldrack and Packard, 2003). Data/graph used with permission from Packard MG, Hirsh R, and White NM (1989) Differential effects of fornix and caudate nucleus lesions on two radial maze tasks: Evidence for multiple memory systems. *J. Neurosci.* 9: 1465–1472.

with the correct platform varied across trials, and thus acquisition of this task required animals to use a spatial form of declarative memory. In a procedural version of the task, the visual pattern on the ball associated with the correct platform was consistent, but the platform was located in different spatial locations across trials. Therefore, this task can be acquired by a procedural learning mechanism that involved performing an approach response to a specific visual cue. Pretraining lesions of the dorsal striatum impair acquisition of the procedural task without affecting acquisition of the declarative task (**Figure 2**). An analogous dissociation is observed using a single-platform water maze task in which rats are trained to swim to a visible escape platform that is always located in the same spatial location. In this situation,

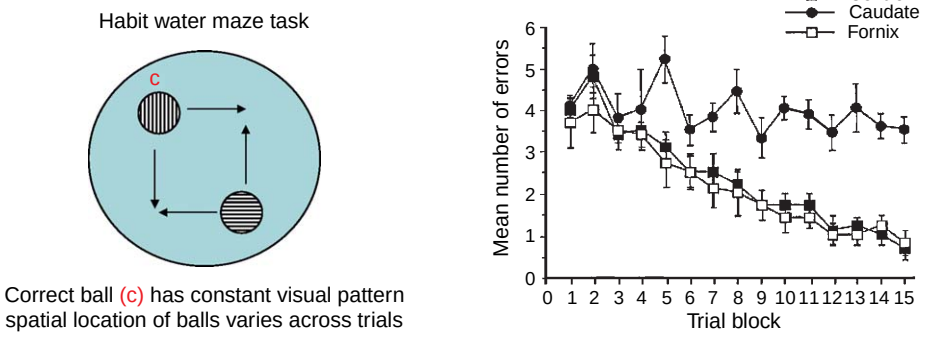

Figure 2 (a) A two-platform water maze task that can be acquired using a stimulus (visual pattern)–response (approach) form of procedural learning (Packard and McGaugh, 1992). Rats learn to swim to a correct (C) escape platform with a distinct visual pattern (ball with vertical stripes). Contact with an incorrect and inescapable platform (ball with horizontal stripes) is scored as an error. The spatial location of both balls varies across trials, and therefore a spatial form of declarative memory is not adequate for task acquisition. (b) Lesions of the caudate (i.e., dorsal striatum) severely impair acquisition, consistent with the hypothesis that the dorsal striatum mediates procedural learning and memory. Note that lesions of the hippocampal system (i.e., fornix) have no effect on acquisition. Data/graph used with permission from Packard MG and McGaugh JL (1992) Double dissociation of fornix and caudate nucleus lesions on acquisition of two water maze tasks: Further evidence for multiple memory systems. *Behav. Neurosci.* 106: 439–446.

when the visible platform is moved to a new spatial location following training, rats with pretraining lesions of the dorsal striatum exhibit a declarative/cognitive strategy and swim to the spatial location that the platform was previously located in, whereas control rats exhibit a procedural/habit strategy and swim to the visible platform in its new location (McDonald and White, 1994).

A third example of a lesion study providing evidence of a selective role for the dorsal striatum in procedural learning employed a plus-maze task that was originally introduced as a means of distinguishing between cognitive and stimulus-response habit theories of learning (Tolman et al., 1946, 1947). The plus-maze apparatus is arranged so that a goal box (e.g., east or west) can be approached from one of two start boxes (e.g., north or south). In a dual-solution version of the task, rats are trained to obtain food from a consistently baited goal box (e.g., east) from the same start box (e.g., north). According to a declarative or cognitive view of learning, rats trained in this task learn the spatial location of the reinforcer, and this relational information can be used to guide an approach response to the baited goal box. In contrast, according to a procedural or stimulus-response view of learning, rats can learn to approach the baited goal box by acquiring a response tendency (i.e., a specific body turn) at the choice point of the maze. Note that either a declarative or a procedural learning mechanism can be used to successfully acquire this dual-solution task. Following acquisition, a probe trial in which rats are given a trial starting from the opposite start box (e.g., north) can be used to assess the type of learning acquired. Thus, rats with declarative knowledge of the spatial location of the reinforcer should continue to approach the baited goal box on the probe trial (i.e., termed place learning), whereas rats that have learned a specific body turn should choose the opposite goal box on the probe trial (i.e., termed response learning). The hypothesis that the dorsal striatum may play a selective role in the expression of procedural/response learning in the plus-maze was examined using a reversible brain lesion technique (Packard and McGaugh, 1996). In this study, rats were trained in a daily session to obtain food from a consistently baited goal box and were allowed to approach this maze arm from the same start box on each trial. Following 7 days of training (i.e., on day 8), rats were given a probe trial to determine whether they had acquired the task using place information or had learned a specific body turn response. Prior to the probe trial, a localized and reversible brain lesion was produced via intradorsolateral striatal injections of the sodium channel blocker lidocaine. On the day 8 probe trial, rats receiving lidocaine or saline vehicle injections into the dorsal striatum were predominantly place learners. Thus, consistent with the findings from the radial and water maze studies described above, infusions of lidocaine into the dorsal striatum did not impair the expression of declarative/place learning. With extended training in the dual-solution plus-maze, intact rats eventually switch from the use of place learning to a response-learning tendency (Ritchie et al., 1950; Hicks, 1964). Therefore, the rats were trained for an additional 7 days, given a second probe trial on day 16, and again received intracerebral injections of lidocaine prior to the probe trial. On this second probe trial, rats receiving vehicle injections into the dorsal striatum were now predominantly response learners. However, rats receiving intradorsal striatal injections of lidocaine prior to the second probe trial exhibited place learning, indicating a blockade of the expression of response learning (**Figure 3**). The selective impairment in expression of response learning in the plus-maze following neural inactivation of the dorsolateral striatum is consistent with other evidence implicating this brain region in egocentric learning (e.g., Potegal. 1969; Cook and Kesner, 1988; Kesner et al., 1993). The results also suggest that the shift or transition from the use of place learning to response learning in a dual-solution plus-maze task involves the gradual recruitment of a dorsal striatal-based procedural learning system to guide behavior. The functional integrity of the dorsolateral striatum is also necessary for the acquisition of a single-solution plus-maze task that requires rats to use procedural response learning by varying the start point on each trial and reinforcing the same body turn at the choice point (Chang and Gold, 2004).

It is important to note that in the radial maze (Packard et al., 1989; McDonald and White, 1993), water maze (Packard and McGaugh, 1992; McDonald and White, 1994), and plus-maze, (Packard and McGaugh, 1996) tasks described above, lesions of the hippocampal system produce the opposite effect of dorsal striatal lesions. Specifically, hippocampal damage impairs acquisition of the declarative/cognitive versions of the tasks and spares (or in some cases enhances) acquisition of procedural learning. Moreover, the individual pairs of maze tasks used in these studies possess the same motivational, sensory, and motoric characteristics, suggesting that the double dissociations observed reflect differential roles of

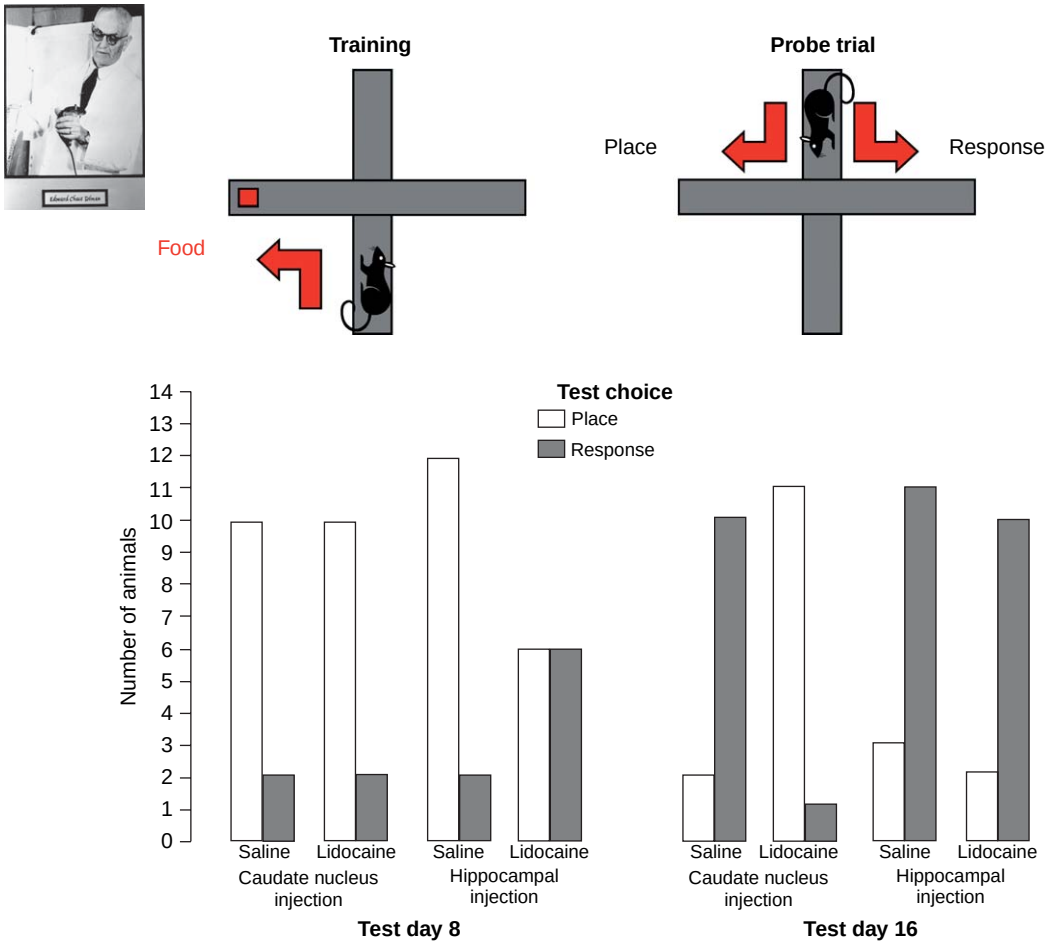

Figure 3 (a) In a dual-solution plus-maze task (Tolman et al., 1946), rats are trained to obtain food from a consistently baited goal box (e.g., west) from the same start box (e.g., south). Either a declarative (i.e., place) or a procedural (i.e., response) memory mechanism can be used to acquire this task. Following acquisition, a probe trial starting from the opposite start box (e.g., north) is used to assess the type of learning strategy employed. Rats approaching the maze arm that was baited during training are designated place learners, whereas rats that perform the specific body turn that was reinforced during training are designated response learners. Photograph of E.C. Tolman provided courtesy of James L. McGaugh. (b) On an initial probe trial (day 8), rats receiving intrastriatal saline or lidocaine are predominantly place learners, indicating that inactivation of the striatum does not impair expression of declarative memory. On a second probe trial given after additional training (day 16), rats receiving saline have switched to the use of response learning. In contrast, rats receiving intrastriatal lidocaine display place learning, indicating a blockade of the expression of procedural learning. Note that hippocampal injections of lidocaine produce the opposite effect, blocking expression of place (day 8), but not response learning (day 16). Thus, the transition to response learning involves an anatomical shift from the use of the hippocampus to the dorsal striatum. In rats receiving intrastriatal lidocaine, the use of place at the time point at which saline-treated rats have transitioned to response learning indicates a continued functional independence of declarative and procedural memory. Data/graph used with permission from Packard MG and McGaugh JL (1996) Inactivation of the hippocampus or caudate nucleus with lidocaine differentially affects expression of place and response learning. *Neurobiol. Learn. Mem.* 65: 65–72.

the hippocampus and dorsal striatum in declarative and procedural memory, rather than lesion-induced deficits in non-mnemonic factors. The observed double dissociations also allow for a more compelling argument that deficits in task acquisition following dorsal striatal lesions in studies employing a single task reflect a selective impairment of procedural learning. Examples include one-way and two-way active avoidance (e.g., Kirkby and Kimble, 1968; Neill and Grossman, 1971; Kirkby and Polgar, 1974; Mitcham and Thomas, 1972; Winocur, 1974), auditory discrimination learning (Adams et al., 2001), and straight-alley runway behavior (Kirkby et al., 1981; Salinas and White, 1998).

3.18.4 The Dorsal Striatum and Procedural Memory Revisited: Functional Heterogeneity

Although extensive evidence supports a role for the dorsal striatum in a stimulus–response form of procedural learning (for reviews, see White, 1997; Packard and Knowlton, 2002; Yin and Knowlton, 2006), increasing evidence from brain lesion studies in rats suggests that this function does not involve the entirety of this brain structure. Rather, lateral, but not medial, regions of the dorsal striatum may be particularly critical in procedural learning that results in the formation of stimulus–response habits. The hypothesis that functional heterogeneity exists in the learning and memory processes mediated by the dorsal striatum is based in part on recognition that this brain structure receives input from all areas of the neocortex (e.g., Webster, 1961; Carman et al., 1963; Webster, 1965; Kemp and Powell, 1970; Veening et al., 1980; McGeorge and Faull, 1989). This idea was explored behaviorally in several early lesion experiments investigating the functional relationship between the frontal cortex and the anteromedial regions of the dorsal striatum in delayed alternation behavior and reversal learning (Rosvold, 1968; Divac, 1968, 1972; Kolb, 1977; for reviews, see Iversen, 1979; Oberg and Divac, 1979).

In rats, damage to the medial dorsal striatum can in some cases produce cognitive learning deficits fairly similar to those observed following hippocampal system damage (e.g., Divac, 1968; Kolb, 1977; Whishaw et al., 1987; Devan et. al., 1999; Devan and White, 1999; but see also Packard and McGaugh, 1992; DeCoteau and Kesner, 2000; Adams et al., 2001; Sakamoto and Okaichi, 2001). Consistent with the idea that medial and lateral regions of the dorsal striatum are differentially involved in learning and memory, lesions of the posterior dorsomedial, but not the dorsolateral striatum, block the ability of reinforcer devaluation to reduce instrumental responding (Yin et. al., 2004, 2005, 2006). These findings suggest that the dorsomedial striatum selectively mediates expression of behavior based on action-outcome associations in instrumental conditioning, whereas the dorsolateral striatum selectively mediates stimulus–response habit formation. In addition, pretraining lesions of a posterior region of the dorsomedial striatum, but not the dorsolateral striatum, result in the predominant use of response learning in the dual-solution plus-maze (Yin and Knowlton, 2004). Further examples of dissociations between the role of medial and lateral regions of the dorsal striatum in learning include evidence that neurotoxic lesions of the dorsolateral, but not dorsomedial striatum, impair acquisition of an operant visual discrimination task (Reading et al., 1991) and of a conditional discrimination task that presumably involves stimulus-response procedural learning (Featherstone and McDonald, 2004a,b).

In addition to evidence suggesting that lateral, but not medial, regions of the rat dorsal striatum mediate procedural learning involving stimulus–response habit formation, other findings indicate that mnemonic function of the lateral dorsal striatum is organized based on the nature of the sensory information provided by cortical input. For example, lesions of the ventrolateral dorsal striatum, an area that receives olfactory cortical input, impairs procedural learning involving olfactory, but not visual sensory, information (Viaud and White, 1989). In contrast, lesions of the posteroventrolateral dorsal striatum, an area that receives visual cortical input, impairs procedural learning involving visual but not olfactory sensory information (Viaud and White, 1989). Similarly, the impairing effect of lesions of the dorsolateral striatum on response learning in the plus-maze may reflect a role for the vestibular/kinesthetic information that this striatal region receives from the somatosensory cortex.

In summary, modification of the hypothesis that stimulus-response procedural learning is mediated by the dorsal striatum is likely necessary to account for evidence indicating functional heterogeneity in the mnemonic functions of this brain structure. At least two levels of functional heterogeneity in the mnemonic functions of the dorsal striatum may exist. Specifically, a medial-to-lateral anatomical gradient may exist in the dorsal striatum that corresponds to a differential functional involvement in declarative and procedural memory, respectively. Second, within the lateral regions of the dorsal striatum, stimulus-response procedural learning appears to be organized based on the nature of the specific sensory input that is received from different cortical regions.

3.18.5 Dorsal Striatum and Procedural Learning: Pharmacological Experiments

The dorsal striatum contains several different neurotransmitters and neuropeptides (for review, see Graybiel, 1990). With regard to the function of this

brain structure in procedural learning and memory, several studies have focused on the role of dopaminergic, glutamatergic, and cholinergic neurotransmission. Dopaminergic projections to the dorsal striatum originate in the substantia nigra (Moore and Bloom, 1978; Gerfen and Wilson, 1996) and may provide a reinforcing signal that is critical in the initial formation of stimulus–response associations (White 1989a; White et al., 1994). Corticostriatal projections are primarily glutamatergic in nature (Fonnum et al., 1981) and are hypothesized to provide sensory or stimulus information embedded in stimulus–response associations (White 1989; White et al., 1994). Acetylcholine is present in the dorsal striatum within a substantial population of interneurons (Lynch et al., 1972) and can interact with dopaminergic transmission to modulate habit memory (White et al., 1994). In addition, the proportional amounts of acetylcholine release in the hippocampus and dorsal striatum may influence the use of habit memory relative to other available learning strategies (for review, see Gold, 2004).

Evidence implicating each of these three neurotransmitters in dorsal striatal-dependent procedural learning and memory is briefly described here. Particular emphasis is placed on studies investigating the effects of posttraining manipulations of neurotransmission in the dorsal striatum on the consolidation of procedural memory. This experimental approach is based on evidence that memory is in a labile state following early exposure(s) to new information (Muller and Pilzecker 1900) and can therefore be either strengthened or weakened by experimental manipulations (e.g., Duncan, 1949; Breen and McGaugh, 1961; McGaugh, 1966). Importantly, this approach avoids several potential nonmnemonic confounds that arise in interpretation of drug effects on memory when pretraining pharmacological treatments are administered (for review, see McGaugh, 1989).

3.18.5.1 Dopamine

Impairments in the acquisition of various conditioned avoidance behaviors following depletion of dopamine in the nigrostriatal pathway memory provided the initial evidence of a possible role for striatal dopamine in procedural learning and memory (Neill et al., 1974; Zis et al., 1974). Subsequent research demonstrated that posttraining electrical stimulation of the nigrostriatal bundle enhances memory (Major and White, 1978), and this effect is blocked by administration of the dopamine receptor antagonist pimozide (White and Major, 1978). An enhancement of procedural memory is also observed following posttraining injections of the indirect catecholamine agonist amphetamine directly into the dorsal striatum (Carr and White, 1984). Moreover, in a manner analogous to the double dissociation produced by irreversible lesions, posttraining intracerebral infusions of amphetamine (Viaud and White, 1989) or the dopamine D2 receptor agonist quinpirole (White and Viaud, 1991) into the ventrolateral dorsal striatum selectively enhance procedural memory involving olfactory sensory information, whereas infusion of these drugs into the posteroventrolateral dorsal striatum selectively enhances procedural memory involving visual sensory information. The latter findings suggest that dopamine release in the dorsal striatum enhances memory consolidation underlying procedural memory in a site-specific manner that is dependent on the nature of the sensory input provided by corticostriatal projections.

The first study to directly compare the role of dopaminergic function in the dorsal striatum in declarative and procedural learning and memory utilized versions of the two radial maze tasks described earlier (Packard and White, 1991). In a declarative memory version of the task, rats were first allowed to obtain food from four of eight randomly selected maze arms. They were then removed from the maze and received an intradorsal striatal injection of saline vehicle, amphetamine, the dopamine D1 receptor agonist SKF 38393, or the dopamine D2 receptor agonist quinpirole. Eighteen hours later, the rats were returned to the maze for a retention test with all eight maze arms open, and only the four arms that had not been visited prior to the delay contained food. In a procedural memory version of the task, rats obtained food rewards by visiting four randomly selected and illuminated maze arms. Rats were removed from the maze following training on day 5 and received an intradorsal striatal injection of either vehicle, amphetamine, SKF 38393, or quinpirole and were returned for a retention test 24 h later. Posttraining injection of all three dopamine agonists enhanced memory in the procedural memory radial maze task but had no effect on memory in the declarative memory radial maze task (Packard and White, 1991). Similarly, in a cued water maze task in which a visible escape platform is moved to a new spatial location on each trial, procedural memory consolidation is enhanced by posttraining peripheral injections of amphetamine and quinpirole

(Packard and McGaugh, 1994) and by intradorsal striatum infusions of amphetamine (Packard et al., 1994; Packard and Teather, 1998). In contrast, posttraining intradorsal striatal infusions of amphetamine have no effect on memory in a declarative/cognitive version of the water maze task in which rats are trained to swim to a hidden escape platform that is located in the same spatial location across trials.

In each of the above studies, posttraining intradorsal striatal infusions of amphetamine or direct dopamine agonists that were delayed until 2 h after training did not enhance memory. The time-dependent nature of the effects of the posttraining infusions suggest that the drug treatments enhanced the consolidation of procedural memory and argue against the possibility that the drugs affected behavior by influencing nonmnemonic factors (McGaugh, 1989). Posttraining intradorsal striatal injection of the dopamine receptor antagonist cis-flupenthixol impairs procedural memory in the radial maze (Legault et al., 2006), suggesting that intact dopamine function is a necessary component of habit formation.

Whereas extensive evidence implicates dorsal striatal dopamine in the initial consolidation of procedural memory, other findings suggest that dopaminergic function in this brain region may not be necessary for the expression of stimulus–response habits after they have been acquired (but see also Aosaki et al., 1994). For example, 6-hydroxydopamine lesions of the nigrostriatal pathway impair acquisition of active avoidance conditioning but do not affect task performance when the lesions are produced after acquisition has occurred (Zis et al., 1974). In addition, relative to early stages of training, significant decreases in neuronal responses of midbrain dopamine neurons are observed in monkeys following extended habit training (Ljungberg et al., 1992), and peripheral administration of dopamine receptor antagonists impair performance of a simple appetitive response only during early stages of training (Choi et al., 2005).

Taken together, evidence indicating a role for dorsal striatal dopamine in memory consolidation but not the expression of procedural memory is consistent with the hypothesis that dopamine release may act as a reinforcing signal in the initial formation of stimulus–response habits (White, 1989a). According to this view, dopamine release in the dorsolateral striatum may function as the proverbial stamp or glue (e.g., Thorndike, 1933) that binds stimulus–response associations rather than providing a representation of a specific stimulus or response. Note that the hypothesized reinforcing effect of dopamine on habit formation in the dorsal striatum is active in both appetitively and aversively motivated habit learning tasks (Packard and White, 1991; Packard and McGaugh, 1994; Packard and Teather, 1998). Therefore, this dopaminergic function is conceptually different (White, 1989b) than the putative reward signal often associated with dopamine release in the nucleus accumbens. In contrast to the stimulus–response learning mediated by the dorsal striatum, the nucleus accumbens has been implicated in stimulus-reward learning (for review, see Cador et al., 1989) and hippocampus-dependent declarative memory (for review, see Setlow, 1997).

3.18.5.2 Glutamate

Glutamatergic input to the dorsal striatum is supplied primarily via corticostriatal projections (Fonnum et al., 1981). Glutamate release in the dorsal striatum resulting from activation of corticostriatal pathways is hypothesized to provide sensory input critical to the formation of stimulus–response habits (White, 1989; White et al., 1994), and behavioral evidence is consistent with a role for this transmitter in procedural learning. For example, in a cued water maze task in which rats are trained to swim to a visible escape platform, procedural memory consolidation is impaired by posttraining intradorsal striatal injection of the glutamatergic *N*-methyl-D-aspartate (NMDA) receptor antagonist AP5 (Packard and Teather, 1997) and enhanced by injection of glutamate (Packard and Teather, 1999). In contrast, posttraining intradorsal striatal infusions of AP5 or glutamate have no effect on memory in a declarative version of the water maze task in which rats are trained to swim to a hidden escape platform that is located in the same spatial location across trials. The role of dorsal striatal glutamate in procedural memory is not limited to the fast excitatory neurotransmission mediated by NMDA receptor activation, as posttraining intrastriatal infusions of metabotropic glutamate receptor antagonists (i.e., MCPG, ACPD) also impair memory consolidation in procedural, but not declarative, memory water maze tasks (Packard et al., 2001).

Further evidence of a selective role for dorsal striatal glutamate in procedural learning and memory was obtained using the dual-solution plus-maze task (Packard, 1999). As described previously, rats that are given extended training in this task eventually transition from the use of hippocampus-dependent place learning to dorsal striatal-dependent response learning

(Packard and McGaugh, 1996). Interestingly, the transition to procedural learning can be facilitated by posttraining intradorsal striatal injections of glutamate. Specifically, intradorsal striatal injections of glutamate following initial training trials in the plus-maze result in the predominant use of response learning during an early probe trial on which control rats predominantly display place learning. Thus, in a task for which a declarative and a procedural learning strategy each provide an adequate solution, an increase in glutamatergic tone in the dorsolateral striatum during early learning appears to favor a more rapid adoption of procedural/response learning. In contrast, posttraining intrahippocampal infusions of glutamate following early training in the plus-maze prevents the transition to procedural learning that is normally produced by extended training. This finding suggests that a relative increase in glutamatergic tone in the hippocampus during early training is detrimental to the development of dorsal striatal-dependent procedural memory.

Consistent with an enhancing function of glutamate on memory consolidation underlying procedural learning (Packard, 1999), infusions of the NMDA receptor antagonist AP5 into the dorsolateral striatum impair acquisition of response learning in a plus-maze (Palencia and Raggozino, 2005). The region of the dorsal striatum targeted in these two latter studies receives glutamatergic input from somatosensory cortex (Fonnum et al., 1981; McGeorge and Faull, 1989). Therefore, similar to dopamine (Viaud and White, 1989, 1991), glutamatergic input to lateral regions of the dorsal striatum may enhance procedural memory in a site-specific manner that is organized based on the nature of the sensory information provided by the cortex. Moreover, infusions of AP5 into the dorsomedial striatum do not impair acquisition of response learning in the plus-maze (Palencia and Ragozzino, 2004), consistent with evidence from the lesion studies reviewed earlier indicating that this region of the dorsal striatum does not mediate procedural learning.

3.18.5.3 Acetylcholine

Prominent cholinergic systems (e.g., the basal forebrain cholinergic system) provide diffuse projections to widespread regions of the mammalian brain. In contrast, acetylcholine within the dorsal striatum is contained within a population of interneurons (Lynch et al., 1972). Numerous studies examining the effects of posttraining intracerebral injections of acetylcholine agonist and antagonist drugs on avoidance behavior implicate dorsal striatal cholinergic activity in consolidation of procedural memory (Haycock et al., 1973; Prado-Alcala and Cobos-Zapian, 1977, 1979; Prado-Alcala et al., 1981; Packard et al., 1996). Taken together, these several findings indicate that posttraining intrastriatal infusions of cholinergic agonists (e.g., oxotremorine, choline) enhance procedural memory consolidation in a time-dependent manner, whereas cholinergic antagonists (e.g., atropine, scopolamine) impair memory. In addition, immunotoxin-induced ablation of striatal cholinergic neurons impairs acquisition of a tone-cued T maze task but does not impair declarative memory in a hidden platform water maze task (Kitabatake et. al., 2003), suggesting that the facilitatory role of striatal acetylcholine may be selective for procedural memory.

A series of experiments using intracerebral microdialysis to monitor neurotransmitter release in the hippocampus and dorsolateral striatum during performance of the dual-solution plus-maze task suggests that the relative amount of acetylcholine release in these two brain regions influences the use of striatal-dependent procedural learning (for review, see Gold, 2004). For example, following training in the plus-maze, rats that exhibit response learning on a subsequent probe trial also display a higher ratio of acetylcholine release in the dorsolateral striatum relative to the hippocampus, whereas the opposite pattern of acetylcholine release is observed in rats displaying place learning (McIntyre et al., 2003). In addition, the transition from hippocampus-dependent place learning to dorsal-striatal response learning that occurs with extended training in the plus-maze (e.g., Packard and McGaugh, 1996; Packard, 1999) coincides temporally with an asymptotic value of acetylcholine release in the dorsolateral striatum (Chang and Gold, 2003). A similar relationship between increases in acetylcholine release and transition from a spatial strategy to a response learning strategy was observed in rats trained in a food-rewarded Y maze task (Pych et al., 2005).

Finally, the cholinergic interneurons in the striatum appear to correspond to the tonically active neurons (TANs) that have been identified in this brain structure using *in vivo* electrophysiological recordings (for review, see Zhou et al., 2002). During procedural sensorimotor learning, striatal TANs develop responsiveness to conditioned stimuli (reflected in part as a brief pause in tonic firing) and are hypothesized to integrate dopaminergic and

cholinergic transmission in the striatum and ultimately influence the activity of afferent projection neurons (Aosaki et al., 1994). The idea that dopamine and acetylcholine interact to influence striatal-dependent procedural learning is supported by several pharmacological studies in rats (for reviews, see Beninger, 1983; White et al., 1994).

In summary, several lines of evidence implicate dorsal striatal dopamine, glutamate, and acetylcholine in stimulus–response procedural learning. Similar to the effects of irreversible and reversible brain lesions, posttraining pharmacological manipulations also result in a double dissociation of dorsal striatal and hippocampal involvement in procedural and declarative memory, respectively. For example, in the radial maze, water maze, and plus-maze, intrahippocampal infusions of dopaminergic and glutamatergic drugs selectively affect consolidation underlying declarative memory. Thus, as multiple memory systems evolved in the vertebrate brain (Sherry and Schacter, 1987), a role for specific neurotransmitters in different types of memory appears to have been conserved. A challenge for future research is to understand how activity of essentially the same neurotransmitters can differentially influence the type of information acquired in multiple memory systems. The relative activation of transmitter systems produced by engaging in a particular learning task appears to shape the participation of different brain structures in guiding learned behavior (Packard, 1999; Gold, 2004; Korol, 2004). However, future research investigating potential differences in the physiological characteristics of the various forms of synaptic plasticity that have been observed in brain structures mediating declarative and procedural memory (e.g., long-term potentiation and long-term depression; Garcia-Munoz et al., 1992; Lovinger et al., 1993; Charpier and Deniau, 1997; Fino et al., 2005) may ultimately help define the neural mechanisms that allow independent brain systems to acquire different types of information in a given learning situation.

3.18.6 Procedural Learning Beyond the Dorsal Striatum: Amygdala and Stimulus-Affect Associations

In addition to findings implicating the dorsal striatum in procedural learning involving stimulus–response habits, other research suggests that the amygdala may mediate a form of procedural learning in which stimulus–affect associations are acquired. This hypothesis is consistent with extensive evidence indicating involvement of the amygdala in specific fear conditioning paradigms (for reviews, see Davis, 1992; LeDoux, 1995; *See* Chapter 3.24). However, the role of the amygdala in stimulus–affect procedural learning is clearly not limited to aversively motivated tasks (e.g., Cador et al., 1989). For example, the functional integrity of the basolateral amygdala is necessary for acquisition of conditioned place preference behavior for both natural rewards (e.g., food, McDonald and White, 1993; Schroeder and Packard, 2002) and addictive drugs (e.g., amphetamine; Hiroi and White, 1991; Hsu et al., 2002). In a conditioned place preference task, rats are confined on alternating days to one distinct environmental context paired with natural or drug rewards and a second context that is not paired with the rewarding treatment. On a reward-free test session given following training, the amount of time spent in the two environments is measured, and rats demonstrate a reliable preference for the environment previously paired with the rewarding stimulus. The conditioned place preference task has been used to demonstrate a double dissociation between the roles of the basolateral amygdala and dorsal striatum in stimulus–affect and stimulus–response procedural learning (McDonald and White, 1993). Specifically, lesions of the basolateral amygdala but not dorsal striatum impair acquisition of stimulus–affect learning underlying conditioned place preference behavior. In contrast, lesions of the dorsal striatum but not basolateral amygdala impair acquisition of stimulus–response learning underlying simultaneous visual discrimination behavior.

Finally, activation of efferent basolateral amygdala projections can act to modulate memory consolidation occurring in other brain structures, including declarative memory processes mediated by the hippocampus and procedural learning mediated by the dorsal striatum (e.g., Packard et al., 1994; for review, see Cahill and McGaugh, 1998; McGaugh, 2002). The modulatory role of the basolateral amygdala on memory consolidation is associated in part with hormonal influences on emotional arousal (for review, see McGaugh, 2004; *See* Chapter 3.26). Moreover, an organism's emotional state may interact with amygdala function to influence the relative use of multiple memory systems. For example, peripheral and intrabasolateral amygdala injections of anxiogenic drugs result in a predominant use of dorsal striatal-dependent procedural learning in the dual-solution plus-maze task (Packard and

Wingard, 2004). Finally, the memory storage and modulation views have at times been contrasted as mutually exclusive and competing theories of amygdala function (e.g., Cahill et al., 1999; Fanselow and LeDoux, 1999). However, a time-limited modulatory role for the basolateral amygdala in some types of learning and memory (e.g., hippocampus-dependent declarative or dorsal striatal-dependent habit memory) does not necessarily rule out a possible long-term role for this structure in stimulus–affect procedural learning and memory (or vice versa).

3.18.7 Conclusions

Significant progress has been made in identifying the neuroanatomical and neurochemical bases of stimulus–response habit learning in lower animals. Extensive evidence supports the hypothesis that this form of procedural learning is mediated by a neural system that contains the dorsal striatum as a primary component. Studies employing irreversible and reversible brain lesion techniques have dissociated the role of the dorsal striatum in procedural and declarative memory. In addition, pharmacological experiments indicate a selective role for dorsal striatal dopamine, acetylcholine, and glutamate in memory consolidation underlying stimulus–response procedural learning.

Finally, although the present chapter focused on studies involving rats, it is important to note that similar evidence for the role of the dorsal striatum in procedural learning also exists in nonhuman primates (e.g., Teng et al., 2000; Fernandez-Ruiz. et al., 2001) and humans (e.g., Knowlton et al., 1996; for reviews, see Packard and Knowlton, 2002; *See* Chapter 3.17). Understanding the influence of the dorsal striatal habit learning system on human behavior ranging from adaptive social interaction and self-regulation (e.g., Lieberman, 2000; Wood et al., 2002) to maladaptive psychopathology (e.g., White, 1996; McDonald et al., 2004; Marsh et al., 2004; Everitt and Robbins, 2005) is an exciting and challenging prospect for future research.

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3.19 Procedural Learning: Classical Conditioning

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3.19.1 Introduction

The two best understood and most extensively studied aspects of procedural learning are classical conditioning of discrete skeletal muscle responses and conditioning of fear. Indeed, more is known about the neural substrates of these two forms of learning than about any other aspects of learning and memory (*See* Chapter 4.11). A schema illustrating the different forms or aspects of memory is shown in **Figure 1**. The major distinction is between declarative and procedural memory (see Squire and Knowlton, 1994). Classical or Pavlovian conditioning is the *sine qua non* of procedural memory; it is defined by the procedure used. The "neutral" conditioned stimulus (CS) and the reflex-eliciting unconditioned stimulus (US) are presented paired together, with CS onset preceding US onset, and the outcome compared to various control procedures where the stimuli are not paired together. The stimuli are presented to the organism regardless of what the organism does, in contrast to instrumental or operant learning, where the organism's response can control the occurrence of the stimuli, e.g., responding so as to avoid presentation of the US.

Another way of distinguishing between declarative and procedural memory is in terms of awareness – declarative memory provides the capacity for conscious recollection of facts and events, whereas procedural memory is typically not accessible to conscious recollection – it can't be brought to mind and declared. Rather, procedural memory is expressed through performance (see Clark and Thompson, in press, for a more detailed discussion and history of these concepts).

Classical conditioning provides a temporal structure where the organism learns about the causal fabric of the environment. The CS provides the organism with information concerning the subsequent occurrence of another stimulus. However, mere temporal pairings of the CS and US, simple contiguity, are not sufficient for learning. Instead, it is the contingency, the probability that the CS will be followed by the US, that determines learning. As Rescorla showed in classic studies, if there are many presentations of the US alone, as well as paired presentations of CS and US, little or nothing may be learned (see Rescorla, 1988). Indeed the learning that occurs in classical conditioning is a good predictor of the probability one stimulus will be followed by

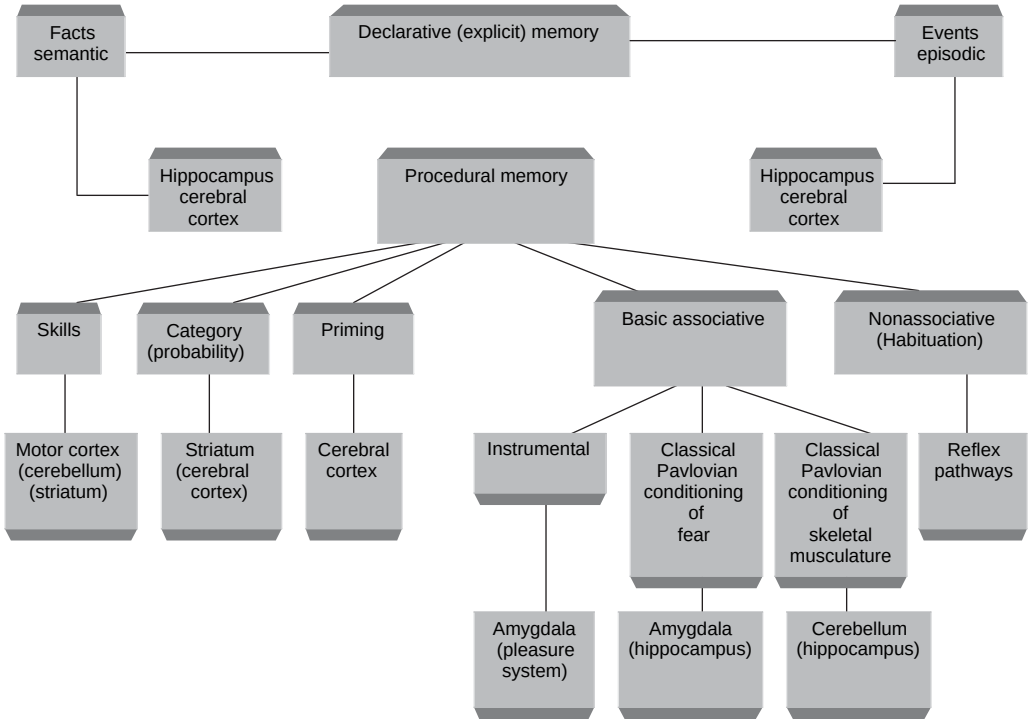

Figure 1 Taxonomy of long-term memory and the putative associated neural structures. Adapted from Squire LR (1998) Memory systems. *C. R. Acad. Sci. III* 321: 153–156, with permission.

another. This causal aspect of classical conditioning has led many to believe it is the most basic aspect of learning and memory.

Here, we focus on providing a comparative analysis of the behavioral and neurobiological mechanisms of two of the most well-understood forms of classical conditioning: eyeblink and fear conditioning.

3.19.2 Classical Conditioning of the Eyeblink Response

Eyeblink conditioning is perhaps the most widely studied form of procedural learning in mammals, including humans. In standard delay conditioning, a tone or a light precedes and coterminates with a reflex-eliciting stimulus such as a puff of air to the cornea. Following several presentations of these paired stimuli, an eyeblink conditioned response (CR) will develop that reflects the learned contingency between the CS and US. Extensive investigation into the neural substrates of this associative memory has resulted in perhaps the most complete description of mammalian memory formation to date (see Thompson and Krupa, 1994; Thompson and Kim, 1996; Kim and Thompson, 1997; Yeo and Hesslow, 1998; Nores et al., 2000; Steinmetz, 2000a,b; Woodruff-Pak and Steinmetz, 2000; Christian and Thompson, 2003, for reviews).

3.19.2.1 The Nature of the Eyeblink Conditioned Response

Gormezano et al. (1962) showed some years ago in separate studies in the rabbit that conditioned eyeball retraction, nictitating membrane (NM) extension, and external eyelid closure all had essentially identical acquisition functions. Simultaneous recording of NM extension and external eyelid closure (electromyographic (EMG) recordings from *orbicularis oculi*) during acquisition and extinction showed that they were, in essence, perfectly correlated, both within trials and over training (McCormick et al., 1982c; Lavond et al., 1990). Furthermore, some degree of conditioned contraction of facial and neck musculature also developed and was also strongly correlated with NM extension. These observations led to characterization of the CR as a "synchronous facial 'flinch' centered about closure of the eyelids and extension of the NM" (McCormick et al., 1982c: 773; see Thompson and Krupa, 1994, for overview). Substantial learning-induced increases in neuronal unit activity that correlate very closely with the conditioned NM extension response have been reported in several motor nuclei: oculomotor, trochlear, motor trigeminal, abducens, accessory abducens, and facial. These are all components of the same global CR involving, to the extent studied, essentially perfectly coordinated activity in a number of muscles and associated motor nuclei. The NM extension response is but one component of the CR. The suggestion that different motor nuclei might somehow exhibit different CR in the eyeblink conditioning paradigm (Delgado-Garcia et al., 1990) is not supported by evidence.

The CR and the unconditioned response (UR) are similar in eyeblink conditioning in the sense that, to a large extent, the same muscles and motor nuclei are engaged. However, the CR and the UR differ fundamentally in a number of respects. The minimum onset latency of the CR to a tone CS in well-trained rabbits, measured as NM extension, is about 90–100 ms; the minimum onset latency of the NM extension UR to a 3-psi corneal airpuff US in the rabbit is about 25–40 ms. Perhaps most important, the variables that determine the topographies of the UR and CR are quite different. The topography of the UR is under the control of the properties of the US: for example, stimulus intensity, rise-time, and duration. In marked contrast, the topography of the CR is substantially independent of the properties of the US and is determined primarily by the interstimulus interval (the CS-US onset interval) – the CR peaking at about the onset of the US over a wide range of effective CS-US onset intervals (Coleman and Gormezano, 1971; Steinmetz, 1990a). This key property of the CR cannot be derived from the properties of the US or the UR. The CR and the UR also differ in that certain components of the UR can be elicited separately by appropriate peripheral stimuli, but the CR always occurs as a global coordinated response (McCormick et al., 1982c). Another important difference is that the CR exhibits much greater plasticity in recovery from lesions of the motor nuclei that impair performance of the UR than does the UR itself (Disterhoft et al., 1985; Steinmetz et al., 1992a).

In sum, the conditioned eyeblink response involves highly coordinated activity in a number of motor nuclei and muscles; it is one global defensive response that is conditioned to a neutral stimulus as a result of associative training. Electrical stimulation of the small critical region of the cerebellar interpositus nucleus (see following) elicits this full complement of coordinated behaviors; it is a 'higher motor program.' The very small lesion of interpositus nucleus, which is effective in completely and permanently abolishing

the conditioned NM extension response, also completely and permanently abolishes all other components of the CR that have been studied—eyeball retraction, external eyelid closure, orbicularis oculi EMG—without producing any impairment in performance of the reflex response (Steinmetz et al., 1992a).

3.19.2.2 Brain Systems Engaged in Eyeblink Conditioning

Electrophysiological recordings of neural activity in eyeblink conditioning indicate that a number of brain areas and systems become engaged, most prominently motor nuclei, the hippocampus, and the cerebellum (see Thompson and Kim, 1996; Steinmetz et al., 2001; Christian and Thompson, 2003, for overviews). In all these systems neuronal activity in the CS period increases over the course of learning and precedes the onset of the behavioral CR within trials. Indeed, this increased pattern of action potential discharges in the hippocampus and cerebellum predicts the occurrence and the actual temporal form of the CR (but not the reflex response). In standard delay conditioning when the CS and the US overlap, the cerebellar system is critical, but the hippocampal system is not (see below and Berger et al., 1986; Thompson and Krupa, 1994, for overviews.) Within the cerebellar system, the following regions developed the predictive response: relevant motor nuclei, a region of the fifth nucleus, various reticular brainstem regions, the cerebellar cortex (ansiform and anterior lobes), the cerebellar interpositus nucleus, the pontine nuclei, and the red nucleus (McCormick et al., 1983).

3.19.2.3 The Cerebellar System

3.19.2.3.1 Lesions

The initial discovery of the key role of the cerebellum in eyeblink conditioning involved both large aspiration lesions including cerebellar cortex and nuclei and electrolytic lesions of the dentate-interpositus nuclear region (McCormick et al., 1981). Neuronal recordings in both cortex and nuclei showed learning-induced increases of neuronal unit activity that preceded and predicted the occurrence of the behavioral CR, as noted earlier. Stimulation of the critical nuclear region elicited the eyeblink before training; the circuit is hard-wired from nuclei to behavior, as noted. In a subsequent series of studies it was found that the key nuclear region is the anterior lateral interpositus nucleus ipsilateral to the trained eye. Very large lesions of the cerebellar cortex that did not damage the interpositus nucleus did not abolish the behavioral CR, although CR latency was altered such that the eye closed and opened before the onset of the US – the timing of the behavioral response was no longer adaptive (McCormick and Thompson, 1984a,b; Logan, 1991).

In these initial studies it was shown that the interpositus lesion effect was ipsilateral – it abolished the eyeblink CR on the side of the lesion but did not impair eyeblink conditioning of the contralateral eye, providing a control for possible nonspecific or state variables (McCormick et al., 1982a). Strikingly, no recovery of the CR is observed even with extensive postlesion training (Steinmetz et al., 1992b). Furthermore, if the lesion is made before training, learning is completely prevented. These lesions had no effects at all on performance of the UR. There have now been more than 30 studies on several species of mammals showing that appropriate lesions of the anterior interpositus nucleus before training completely prevent learning of the eyeblink and other discrete responses and completely and permanently abolish already learned responses (Christian and Thompson, 2003).

Kainic acid lesions of the interpositus as small as about $1\,mm^3$ in the anterior lateral region of the nucleus abolished the CR, indicating extreme localization of the critical region and ruling out the possibility that fibers of passage were involved (Lavond et al., 1984b). Finally, lesions of the output of the cerebellar nuclei, the superior cerebellar peduncle (scp), abolished the behavioral CR with no effect on the UR (McCormick et al., 1982b; Rosenfeld et al., 1985; Voneida, 2000 (limb flexion CR in cat)). It is important to emphasize that the cerebellar interpositus nucleus is essential for the learning of all discrete movements trained with an aversive US: eyeblink, limb flexion, head turn, etc. To the extent tested, the memory traces for all those learned movements are stored at separate loci in the interpositus nucleus. Eyeblink conditioning has simply been the most widely studied, although there is also considerable evidence for head turn and for limb flexion conditioning (see Voneida, 2000; Mintz and Wang-Ninio, 2001; Christian and Thompson, 2003).

Results of clinical studies of eyeblink conditioning in humans with brain damage are strikingly parallel to the infrahuman animal literature. Appropriate

cerebellar lesions markedly impair or completely prevent learning of the standard delay eyeblink conditioning task; if the cerebellar damage is unilateral, only the ipsilateral eye is affected (Daum et al., 1993; Woodruff-Pak, 1997). In general, effective lesions are large, including damage to cerebellar cortical regions and nuclei, although exact extent of damage is difficult to determine from the brain scans. Schugens et al. (2000) conclude that damage to cerebellar nuclei appears likely in most of these studies.

3.19.2.3.2 Recordings

As noted briefly, recordings of neuronal unit activity from the interpositus nucleus during eyeblink conditioning revealed populations of cells in the critical region of the nucleus that, as a result of training, discharged prior to the execution of the learned eyeblink response and fired in a pattern of increased frequency of response that predicted the temporal form of the behavioral CR (the 'neuronal model' of the CR in at least 20 studies to date in several species of mammals (Christian and Thompson, 2003). Single-unit activity sampled from the interpositus and immediately adjacent regions can be categorized into several distinct response patterns (Tracy, 1995). Some cells show stimulus-evoked activity to the CS and/or the US over the course of training, a pattern that demonstrates appropriate convergence of sensory information in the interpositus but does not directly support a role for the interpositus in CR generation. Likewise, some cells show behavior-related changes in firing patterns coincident with but not prior to the onset of the CR. However, many cells, particularly in the critical region of the anterior dorsolateral interpositus, significantly increase firing in a precise temporal pattern that is delayed from the onset of the CS, occurs before the onset of the behavior, and is temporally correlated with the onset of the behavior. It is clear from recordings such as these that neurons in the interpositus are capable of contributing to the generation of the CR. While there are certainly several distinct response profiles for single cells that are likely to reflect functional subdivisions within the population of neurons in the deep nuclei, it is compelling that multiple- and single-unit activity recorded from the critical interpositus region reflects an increase in activity appropriately timed to effect the downstream motor pathway and culminate in the well-timed CR.

3.19.2.4 The Pathways

A large literature is in general agreement in identifying the essential circuitry for classical conditioning of eyeblink and other discrete responses. This circuitry follows closely the well-established anatomy of the cerebellar system (Brodal, 1981) and is in general accord with classical theories of cerebellar learning (Marr, 1969; Albus, 1971; Ito, 1972; Gilbert, 1975; Eccles, 1977). We summarize this work only briefly here. See Christian and Thompson (2003) for detailed citation of evidence.

3.19.2.4.1 The UR pathways

The eyeblink reflex in the rabbit is a coordinated response involving simultaneous and perfectly correlated external eyelid closure, eyeball retraction, and resulting passive extension of the NM, as noted.

In terms of the reflex pathways, there are direct projections from neurons in regions of the trigeminal nucleus to the accessory abducens (and abducens) nuclei and to the facial nucleus, as well as indirect projections relaying via the brainstem reticular formation, at least to the facial nucleus. Although the CR and UR share many common features, there are critical qualitative differences between the two that include both variations in the intrinsic properties and the neural substrates responsible for each response (see earlier discussion). Standard eyeblink training procedures typically result in a progressive increase in the amplitude of the UR due to both associative and nonassociative factors (Steinmetz et al., 1992a). Interpositus lesions can impair the associative component of the UR increase (Wikgren et al., 2002).

Lesions of the interpositus that are successful in abolishing the CR have only a transient depressive effect on UR amplitude in initial postlesion assays at the same US intensity level used in prelesion training (Steinmetz et al., 1992a). Subsequent training fully restored UR amplitudes to prelesion levels, demonstrating a lack of interpositus involvement in the sustained eyeblink reflex modification following conditioning. Steinmetz et al. (1992a) investigated numerous parameters of the UR including amplitude, rise time, frequency, and latency in the same animals at several US intensities and found no significant lasting effects of interpositus lesions in any of these properties. Ivkovich et al. (1993) lowered US intensity levels to threshold to equate amplitudes of prelesion CRs and URs, and it was again

shown that interpositus lesions that completely abolished the CR had no significant effect on postlesion UR amplitude.

3.19.2.4.2 The CR pathway

Neurons in a localized region of the interpositus nucleus ipsilateral to the trained eye develop a neuronal model of the learned behavioral CR; lesions of this region selectively abolish the CR with no effect in the UR; electrical stimulation of this region evokes the eyeblink response before training (as noted). This region of the interpositus projects via the superior cerebellar peduncle to a region of the contralateral magnocellular red nucleus. Lesions of the peduncle abolish the CR, as do lesions of the key region of red nucleus, where neurons also show a model of the learned response. Stimulation of this rubral region also elicits the eyeblink response. The descending rubral pathways project contralaterally to premotor and motor nuclei, seventh for external eyelid closure, and accessory sixth and sixth for NM extension. Overwhelming evidence identifies this circuit as the efferent CR pathway for the conditioned eyeblink response (see detailed review in Thompson and Krupa, 1994). A similar pathway projecting to the spinal cord subserves classical conditioning of the limb flexion response in the cat (Voneida, 1999, 2000).

3.19.2.4.3 The CS pathway

The pontine nuclei send axons as mossy fibers directly to the cerebellar cortex and interpositus nucleus, mostly contralaterally. The pontine nuclei in turn receive projections from auditory, visual, somatosensory, and association systems, both cortical and subcortical. Appropriate lesions of the pontine nuclei can abolish the CR established to a tone CS but not a light CS, i.e., can be selective for CS modality (interpositus lesions abolish the CR to all modalities of CS) (Steinmetz et al., 1987). Lesions of the region of the pons receiving projections from the auditory cortex abolish the CR established with electrical stimulation of auditory cortex as a CS (Knowlton and Thompson, 1992; Knowlton et al., 1993). Extensive lesions of the middle cerebellar peduncle (mcp), which conveys mossy fibers from the pontine nuclei and other sources to the cerebellum, abolish the CR to all modalities of CS (Lewis et al., 1987).

Electrical stimulation of the pontine nuclei serves as a 'supernormal' CS, yielding more rapid learning than does a tone or light CS (Steinmetz et al., 1986; Tracy et al., 1998; Freeman and Rabinak, 2004). Stimulation of the mcp itself is an effective CS (Steinmetz, 1990a; Svensson and Ivarsson, 1999), and lesion of the interpositus nucleus abolishes the CR established with a pontine or middle peduncle stimulation CS (Steinmetz et al., 1986). When animals are trained using electrical stimulation of the pontine nuclei as a CS (corneal airpuff US), some animals show immediate and complete transfer of the behavioral CR and of the learning-induced neural responses in the interpositus nucleus to a tone CS (Steinmetz, 1990b) and complete transfer from peripheral CSs to mossy fiber stimulation in the mcp (Hesslow et al., 1999). These results indicate that the pontine–mcp stimulus and tone must activate a large number of memory circuit elements (neurons) in common. In sum, the mossy fiber system, coming mostly from the pontine nuclei, is the CS-activated pathway to the cerebellum (Thompson et al., 1997).

3.19.2.4.4 The US pathway

Neurons in the inferior olive (IO) send climbing fiber projections contralaterally directly to cerebellar cortex and interpositus nucleus. The critical region of the IO for eyeblink conditioning is the dorsal accessory olive (DAO), which receives predominantly somatosensory input relayed from the spinal cord and appropriate cranial nuclei, including nociceptive input (Brodal, 1981). Lesions of the critical region of the IO, the face representation in the DAO, completely prevent learning if made before training and result in extinction of the CR if made after training (McCormick et al., 1985; Mintz et al., 1994). Neurons in this critical DAO region do not respond to auditory stimuli (CS), respond only to US onset, and show no learning-related activity, and the US-evoked response decreases as animals learn (Sears and Steinmetz, 1991). Electrical microstimulation of this region serves as a very effective US (Mauk et al., 1986). All these data argue that the DAO-climbing fibers system is the essential US-reinforcing pathway for the learning of discrete responses (Thompson et al., 1998).

In a classic but largely forgotten study, Brogden and Gantt (1942) reported that stimulation of cerebellar white matter elicited discrete behavioral movements, e.g., limb flexion, head turn, eyeblink, and these movements so elicited could easily be conditioned to any neutral stimulus, e.g., light or sound. These observations have been replicated and extended in recent years (Thompson et al., 2000).

Swain et al. (1992) used a tone CS and showed that stimulation of cerebellar white matter in lobule HVI (rabbit) did indeed elicit movements: eyeblink, NM extension, and movements of the head and upper lip. These movements all conditioned, extinguished, and reconditioned to a tone CS in a manner identical to CRs established with aversive peripheral USs. Further, kainic acid lesions of the interpositus that spared fibers abolished both the CR and the white matter stimulation–elicited UR, thus ruling out antidromic activation via pontine nuclei or IO of the UR (Swain et al., 1999).

Shinkman et al. (1996) stimulated cerebellar cortical parallel fibers as a CS (white matter US) with similar results. In this study the parallel fiber stimulus (CS) intensity was well below movement threshold. With sufficiently intense stimulation, movements could be evoked by this parallel fiber stimulus. Interestingly, these were often quite different from the behavioral response (UR) evoked by the white matter US. However, as a result of training, the earlier subthreshold parallel fiber CS now evoked a CR that was identical to the white matter UR and often quite different from the suprathreshold response evoked by the parallel fiber stimulus prior to training. There is extraordinary plasticity in the organization of the parallel fiber actions on the cerebellar circuitry (see also Poulos and Thompson, 2004).

Evidence is consistent with stimulation of climbing fibers in cerebellar white matter as the US effective for learning. To our knowledge the IO-climbing fiber system is the only system in the brain, other than reflex afferents, where the exact response elicited by electrical stimulation can be conditioned to any neutral stimulus. Thus, such movements elicited by stimulation of the motor neocortex cannot be so conditioned (Loucks, 1935; Wagner et al., 1967; Thompson et al., 2000). We therefore argue that this system is the essential reinforcing pathway for the learning of discrete responses.

The interpositus nucleus sends direct GABAergic (GABA: gamma-aminobutyric acid) projections to the DAO (Nelson and Mugnaini, 1989). Hence, as learning-induced increases in interpositus neuron activity develop, inhibition of the DAO neurons will increase (Hesslow and Ivarsson, 1996). This accounts for the fact that US-evoked activity in the DAO decreases as learning develops (Sears and Steinmetz, 1991), consistent with the Rescorla and Wagner (1972) formulation. This also appears to serve as a part of the neural circuit essential for the behavioral learning phenomenon of 'blocking,' where prior training to one CS, e.g., tone, prevents subsequent learning to a light CS when it is then presented together with the tone in paired compound stimulus training (Kamin, 1969). Infusion of picrotoxin in the DAO to block the GABA inhibition from the interpositus during compound stimulus training completely blocks the development of behavioral blocking (Kim et al., 1998).

3.19.2.4.5 Conjoint activation of CS and US pathways

If the aforementioned hypotheses concerning the identities of the CS and US pathways are correct, it should be possible to train behavioral conditioned responses by conjoint stimulation of these pathways. Steinmetz et al. (1989) stimulated the pontine nuclei – mossy fibers as a CS (below movement threshold) and DAO-climbing fibers as a US. Stimulus (US)-elicited movements included eyeblink, head turn, and limb flexion. The stimulus-elicited movements were learned to the mossy fiber stimulation CS just as was the case for peripheral CSs. In an even more reduced preparation, the CS was stimulation of parallel fibers by an electrode (concentric pair of ovoids) resting on the surface of the cortex of lobule HVI, and the US was a stimulation delivered through an electrode pair in the white matter directly beneath the surface electrode, as noted (Shinkman et al., 1996). Again, the movements elicited by white matter stimulation were learned in a normal fashion to the parallel fiber stimulation CS. It is tempting but premature to conclude that this procedure established a memory trace in the localized region of cortical tissue activated.

3.19.2.4.6 Reversible inactivation

Although the evidence cited is consistent with the interpositus nucleus being the site of the conditioned eyeblink memory trace, it does not prove this hypothesis. Predictive neuronal models of the CR develop in the interpositus nucleus, the red nucleus, and the motor nuclei, as noted. Further, lesions of the interpositus, of the scp (that conveys all the efferent projections from the interpositus), and of the red nucleus all abolish the CR with no effect on the UR. Lesions of the motor nuclei of course abolish both the CR and the UR. The technique of reversible inactivation was used to determine where within the circuit the memory trace was formed and stored (see Clark and Lavond, 1993; Thompson

et al., 1993; Thompson and Krupa, 1994; Christian and Thompson, 2003, for details).

Several parts of the cerebellar circuit, illustrated in **Figure 2**, have been reversibly inactivated for the duration of training (eyeblink conditioning) in naive animals. Motor nuclei essential for generating UR and CR (primarily seventh and accessory sixth and adjacent reticular regions) were inactivated during standard tone–airpuff training. The animals showed no CRs and no URs during this inactivation training; indeed, performance was completely abolished. However, the animals exhibited asymptotic CR performance and normal UR performance from the very beginning of postinactivation training. Thus, performance of the CR and UR is completely unnecessary and makes no contribution at all to formation of the memory trace – the CR and the UR are completely efferent from the trace. Inactivation of the magnocellular red nucleus during training had no effect on the UR, but completely prevented expression of the CR. Animals showed asymptotic learned performance of the CR from the beginning of postinactivation training. Consequently, the red nucleus must be efferent from the memory trace.

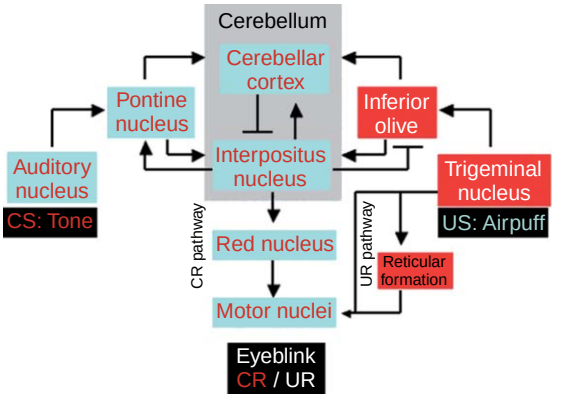

Figure 2 Simplified schemata of the neural circuits underlying Pavlovian eyeblink conditioning. In auditory eyeblink conditioning, information pertaining to a tone conditional stimulus (CS) is relayed to the cerebellar cortex and interpositus nucleus via auditory projections from the pontine nucleus, while nociceptive information about the airpuff unconditional stimulus (US) is conveyed by the trigeminal nucleus to the inferior olive, which in turn projects to both the cerebellar cortex and interpositus nucleus. Conditional eyeblink responses established and maintained within the cerebellar cortex and interpositus nucleus are relayed to the red nucleus. The final common out for both the conditional and unconditional eyeblink response is expressed via facial motor nuclei. Modified from Thompson DF and Krupa DJ (1994) Organization of memory traces in the mammalian brain. *Annu. Rev. Neurosci*. 17: 519–549, with permission.

Inactivation of the dorsal anterior interpositus and overlying cortex resulted in no expression of CRs during inactivation training and in no evidence of any learning during inactivation training. In subsequent postinactivation training, animals learned normally as though completely naive; they showed no savings at all relative to noninactivated control animals. If any part of the memory trace were established prior to the interpositus nucleus in the essential circuit, then the animals would have shown savings, but they did not. None of the methods of inactivation had any effect at all on the performance of the UR on US-alone trials. Infusions of very low doses of muscimol (1.0 nmole in 0.1 µl of vehicle) limited to the anterior lateral interpositus nucleus, (with no significant ^{3}H label in cerebellar cortex) completely prevented learning of the eyeblink CR.

Finally, the output of the cerebellum was inactivated by infusion of tetrodotoxin (TTX) in the scp during training. This inactivates both descending and ascending efferent projections of the cerebellar hemisphere. TTX infusion in the scp completely prevented expression of the CR (with no effect on the UR) during training. In subsequent postinactivation training the animals immediately showed asymptotic learned performance of the CR. Collectively, these data strongly support the hypothesis that the memory trace is formed and stored in the interpositus nucleus.

3.19.2.5 Mechanisms of Memory Storage in the Interpositus Nucleus

Infusion of GABA antagonists, picrotoxin or bicuculline methiodide, into the interpositus in trained animals blocks performance of the behavioral eyeblink CR and the neuronal unit model of the CR in the interpositus in a dose-dependent manner with no effects on the UR (Mamounas et al., 1987). Picrotoxin infusion in the interpositus during training completely prevents learning (Bao et al., 2002). Infusion of the $GABA_B$ antagonist baclofen in the interpositus during training similarly prevents learning and performance of the CR (Ramirez et al., 1997). These results suggest that GABA and its actions on GABA receptors in the interpositus are important for both learning and performance of the CR. However, equivalent doses of strychnine had no effect on the CR or the UR, suggesting that glycine receptors are not involved (Mamounas et al., 1987). Infusion of AP5 into the interpositus markedly impaired acquisition of the eyeblink CR (Chen

and Steinmetz, 2000). However, after learning, AP5 infusion had little effect on CRs. This argues for a role of glutamate *N*-methyl-D-aspartate (NMDA) receptors in the interpositus in acquisition but not performance of the CR.

Bracha et al. (1998) reported that infusion of anisomycin into the region of the interpositus nucleus impaired acquisition of the eyeblink CR but had no effect on expression of the CR. Gomi et al. (1999) reported that infusion of actinomycin D into the interpositus nucleus completely prevented acquisition of the eyeblink CR but had no effect on performance of the learned response. By the same token Chen and Steinmetz (2000) reported that infusion of the protein kinase inhibitor H7 into the interpositus markedly impaired acquisition but had no effect on performance of the learned response. Results of all these studies argue strongly that protein synthesis (both transcription and translation) in the interpositus nucleus is necessary for learning of the eyeblink CR, but not for its expression once learned. Indeed, Gomi et al. (1999) identified a kinase whose expression was increased in interpositus neurons following eyeblink conditioning. The cDNA was isolated, and the deduced amino acid sequence of the kinase contains the KKIAMRE motif, conserved among cell division cycle 2–related kinases. All these results argue for the formation of neuronal/synaptic plasticity, a memory trace, in the interpositus itself. Even with 1 month of overtraining or rest, the memory trace remains in the interpositus (Christian and Thompson, 2005).

Extremely important direct evidence for a strengthening of the mossy fiber–interpositus neuron synapses has been presented by Kleim et al. (2002), using eyeblink conditioning in the rat. They demonstrated a highly significant increase in the number of excitatory synapses in the interpositus nucleus but no change in inhibitory synapses following eyeblink conditioning, compared to unpaired stimulation control animals. We note that some years earlier Racine et al. (1986) reported the development of LTP in interpositus following mossy fiber tetanus in the rat *in vivo,* a result recently replicated *in vitro* by Linden (Aizenman and Linden, 2000).

In sum, the evidence is now very strong from behavioral, physiological, pharmacological, anatomical, and inactivation studies that the basic associative memory trace in eyeblink conditioning is established in the interpositus nucleus. The next step is to elucidate the causal chain from behavioral training to increased synaptic efficacy and synapse formation.

3.19.2.6 Cerebellar Cortex

After more than two decades of research, the role of the cerebellar cortex in eyeblink conditioning is still unclear and embedded in controversy (see Yeo and Hardiman, 1992; Christian and Thompson, 2003, for reviews). However, it is clear that it plays a very important role in normal learning – animals with large cortical lesions learn more slowly than normal and not as well. In the first study to explicitly address cerebellar cortical function in eyeblink conditioning, large aspiration lesions of ansiform or ansiform and paramedian lobules in well-trained rabbits resulted in the expression of short-latency, small-amplitude CRs (McCormick and Thompson, 1984b). Yeo and associates reported that lesions of lobule HVI of cerebellar cortex abolished the conditioned eyeblink response (Yeo et al., 1984, 1985). However, subsequent studies in several laboratories, including Yeo's, have been unable to replicate this result (e.g., Lavond et al., 1987; Clark et al., 1990; Harvey et al., 1993; Perrett and Mauk, 1995; Yeo and Hardiman, 1992).

In an experiment designed to assess the role of the cortex in acquisition, extensive unilateral lesions including complete removal of HVI and HVIIa lobules and significant portions of the anterior lobe were shown to significantly impair the rate of learning (Lavond and Steinmetz, 1989). Lesioned animals took seven times longer to reach criterion, and asymptotic levels of CR frequency were much reduced. Despite these specific learning deficits, animals were able to achieve significant levels of conditioned responding in the absence of HVI. This result was confirmed by another study in which extensive cortical lesions were shown to significantly impair the rate of acquisition but not prevent learning (Logan, 1991). Animals with complete or near-complete removal of HVI and crura I and II of the ansiform lobe took six times longer to reach criterion. Rabbits with less extensive damage to HVI and the ansiform lobe showed moderate decreases in learning rates. Both moderate and extensive lesions decreased asymptotic CR frequency, whereas extensive lesions also impaired CR magnitude and timing.

Using a different methodological approach to investigate cortical function, the HVI lobule has been temporarily inactivated via infusions of the $GABA_A$ receptor agonist muscimol, during both acquisition and retention. Muscimol acts to hyperpolarize neurons, prohibitively raising the firing threshold and thus disrupting the cortical output mediated by Purkinje cells. Muscimol inactivation

restricted to the ventral HVI region has no effect on the rate of acquisition or quality of the CRs (Krupa, 1993). Extensive cortical inactivation encompassing both HVI and portions of the anterior lobe significantly impairs but does not prevent acquisition and has no effect on the CR performance once the animal reaches asymptotic levels of conditioned responding (Krupa, 1993; Thompson et al., 2001). Similarly, reversible inactivation of the HVI region achieved with a cortical cooling probe significantly decreased the learning rate but did not disrupt CR expression in well-trained animals (Clark et al., 1997).

The great difficulty in all these studies is the limitation of the permanent lesion approach. The depths of cortical tissue in lobule HVI are only a mm or so above the critical region of the interpositus nucleus. It is impossible to remove all relevant cerebellar cortex without damaging the interpositus nucleus. To circumvent these anatomical complications, several attempts have been made to investigate the effects of functionally inactivating the entire cerebellar cortex. The most definitive of these studies tested acquisition of eyeblink conditioning in mutant mice (pcd mice) in which Purkinje cells degenerate approximately 2 weeks postnatally (Chen et al., 1996). Elimination of these sole-output neurons in the cortex effectively eliminates the cerebellar cortex itself. pcd mice show significant deficits in both the rate of acquisition and the asymptotic level of conditioning, but nevertheless express significant levels of learning and extinction in the absence of cortical input to the interpositus. Peak latencies of the CRs in the mutants were significantly decreased compared to wild-type mice, although the effect was not great. A later study confirmed that the interpositus was responsible for the residual learning, by demonstrating that bilateral lesions of the interpositus in pcd mice completely block acquisition (Chen et al., 1999). In a recent study, OX7-saporin, an immunotoxin selective for Purkinje cells, was infused intraventricularly prior to conditioning, and deficits in both acquisition and extinction were observed which correlated with cell loss in HVI and the anterior lobe (Nolan and Freeman, 2006).

Functional and reversible lesions of the entire cerebellar cortex can also be achieved through a targeted disruption of the Purkinje cell afferents at the level of the interpositus. In one study, application of the GABA antagonist picrotoxin into the anterior interpositus nucleus resulted in short-latency, reduced-amplitude, prolonged CRs but at a frequency similar to that observed in the absence of infusion (Garcia and Mauk, 1998). These results are contradicted by other studies in which picrotoxin infusions into the interpositus blocked the expression of CRs completely (Mamounas et al., 1987; Bao et al., 2002). A much more precise elimination of cortical input can be achieved through sequential infusions of muscimol and picrotoxin. Muscimol first blocks the synaptic transmission from the cortex, and the baseline level of excitability in the interpositus neurons is restored through application of picrotoxin (Bao et al., 2002). With this procedure, onset and peak latencies were decreased in well-trained animals, CR amplitudes were increased, and no effects (i.e., no CRs) were observed in naive animals. Although studies of cerebellar patients cannot address these questions with anatomical precision, it was recently reported that damage primarily to the cerebellar cortex impaired the acquisition and timing of conditioned eyeblink responses (Gerwig et al., 2005).

In sum, it is clear that the cerebellar cortex plays a critical role in normal learning and adaptive timing of the eyeblink CR. The cortex exerts inhibitory control over the interpositus neurons; this inhibition could control precisely the timing of interpositus neurons' activation by the CS to generate the CR. Further, a decrease in this inhibition could permit the necessary activation of the interpositus neurons to express the CR. The most prominent mechanism for decreasing Purkinje neuron activity is long-term depression (LTD) at parallel fiber synapses on Purkinje dendrites, first discovered by Masao Ito (see Ito, 1984).

The majority of evidence suggesting a role for cortical LTD in learning and memory comes from behavioral assays of mutant mice with deficits in LTD expression. There is a consistent relationship between deficits in eyeblink conditioning and deficits in cerebellar cortical LTD (Kim and Thompson, 1997). It has been shown that activation of metabotropic and ionotropic glutamate receptors (mGluR and AMPAR) on the Purkinje cell dendrites is necessary for the induction of LTD (Linden and Connor, 1993; Jeromin et al., 1996). Although the downstream signaling pathway is not entirely known, several key molecules have been identified. Factors required for LTD include Ca^{2+} influx via voltage-activated channels and transient protein kinase C (PKC) activation (Hansel et al., 2001). Mutant mice in which the mGluR1 subunit is not expressed, rendering the receptor nonfunctional, have shown deficient LTD and impaired eyeblink conditioning (Aiba et al., 1994). Phospholipase Cbetas (PLCbetas) are

downstream signaling molecules of mGluR activation, and the PLCbeta4 isoform is expressed selectively in Purkinje cells in the rostral cerebellum, including portions of HVI. Mutant mice deficient in PLCbeta4 also show impaired LTD and an impairment in acquisition of CRs (Kishimoto et al., 2001a; Miyata et al., 2001). Mutants lacking the $\delta 2$ subunit of the glutamate receptor exclusively in the cerebellar cortex are likewise impaired in LTD and delay eyeblink conditioning (Kishimoto et al., 2001b,c).

3.19.2.7 Eyeblink Conditioning and the Hippocampus

In eyeblink conditioning, neuronal unit cluster recordings in hippocampal fields CA1 and CA3 increase in discharge frequency in paired (tone CS–corneal airpuff US) training trials very rapidly, shift forward in time as learning develops, and form a predictive 'temporal model' of the learned behavioral response, both within trials and over the trials of training (Berger et al., 1976). To summarize a large body of research, the growth of the hippocampal unit response is, under normal conditions, an invariable and strongly predictive concomitant of subsequent behavioral learning (see reviews in Berger et al., 1986). This increase in neuronal activity in the hippocampus becomes significant by the second or third trial of training, long before behavioral signs of learning develop, as would be expected of a declarative memory system. This initial hippocampal unit increase is in the US period; increases in the CS period appear at about the time point in training when behavioral CRs appear. With continued training the hippocampal neuronal model eventually declines (Katz and Steinmetz, 1994).

Many neurons that could be identified as pyramidal neurons in CA1 and CA3 (antidromic stimulation and collision) showed learning-related increases in discharge frequency in the trial period. Typically, a given neuron modeled only some limited time period of the trial. Cumulating many such single pyramidal neuron responses produced the typical unit cluster model of the behavioral learned response. So the pyramidal neuron representation of the behavioral learned response is distributed over both space and time in the hippocampus. The high percentage of learning-influenced pyramidal neurons and their spatially distributed loci have been strikingly verified in studies by Disterhoft and associates (Disterhoft et al., 1986; de Jonge et al., 1990) using *in vitro* studies of hippocampal slices from trained versus control animals. The work described was all done using the basic delay paradigm, where hippocampal lesions do not impair simple acquisition (Schmaltz and Theios, 1972; Solomon and Moore, 1975). Similarly, humans with hippocampal-temporal lobe anterograde amnesia are able to learn simple acquisition of the eyeblink CR, but cannot describe it (Weiskrantz and Warrington, 1979).

3.19.2.7.1 Trace conditioning

Trace conditioning was first described by Pavlov; the CS terminates and there is a period of no stimulation between CS offset and US onset (as Pavlov stressed, the organism must maintain a 'trace' of the CS in the brain in order for the CS and the US to become associated). In eyeblink conditioning in animals, a typical trace interval is 500 ms. The trace CR is more difficult to learn than the standard 'delay' procedure where the CS and US overlap in time.

McEchron and Disterhoft (1997) reported marked increases in hippocampal neuronal activity in trace conditioning, as was reported earlier for delay conditioning. Further, with extensive training, the neuronal model declines. Very large bilateral removal of the dorsal plus some ventral hippocampus in rabbits markedly impaired subsequent acquisition of the 500-ms trace CR, an example of anterograde amnesia (Solomon et al., 1986; Moyer et al., 1990). Consistent with this finding, scopolamine at doses sufficiently low to have little effect on delay learning completely prevents acquisition of the trace CR (Kaneko and Thompson, 1997). If rabbits are first trained in the trace procedure, large bilateral hippocampal lesions made immediately after training completely abolish the trace CR (such immediate lesions have little effect on the delay CR). However, if these same hippocampal lesions are made a month after training, they do not impair performance of the trace CR at all (Kim et al., 1995).

To summarize, large bilateral lesions of the hippocampus made before training markedly impair learning of the trace CR. If the animals are first trained, lesions immediately after training abolish the trace CR, but lesions made 1 month after training have no effect on memory of the trace CR. These results are strikingly consistent with the literature concerned with the declarative memory deficit following damage to the hippocampal-medial temporal lobe system in humans and monkeys. These deficits have two key temporal characteristics: (1) profound and permanent anterograde amnesia, and (2)

profound but clearly time-limited retrograde amnesia. Subjects have great difficulty learning new declarative tasks/information and have substantial memory loss for events for some period just preceding brain damage (1 or more years in humans, 2–3 months for monkeys) but relatively intact memory for earlier events (Zola-Morgan and Squire, 1990). Very similar results were found for classical conditioning of fear to context in rats (Kim and Fanselow, 1992).

These results from studies on animals suggest that trace eyeblink conditioning provides a simple model of hippocampal-dependent declarative memory, a possibility strongly supported by studies of humans with hippocampal-medial temporal lobe amnesia. In brief, such patients are markedly impaired on acquisition of trace eyeblink conditioning if the trace interval is sufficiently long (see reviews by Clark and Squire, 1998; McGlinchey-Berroth, 2000). It is important to emphasize that lesions of the cerebellar interpositus nucleus completely and permanently abolish the trace conditioned eyeblink response (rabbits) (Woodruff-Pak et al., 1985).

Clark and Squire (1998, 1999) made the striking observation that awareness of the training contingencies in normal human subjects correlated highly with the degree of trace conditioning. They showed that awareness played no role in delay conditioning, that awareness does play a role in both single-cue and differential trace conditioning and that expectancy of US occurrence influenced trace but not delay conditioning (Clark and Squire, 2000; Manns et al., 2000a,b; Clark et al., 2001). They conclude that delay and trace conditioning are fundamentally different phenomena, delay inducing nondeclarative or procedural memory and trace inducing declarative memory. It would seem that this very simple procedural classical conditioning task, where the CS and US are separated by a very brief period of time, has converted the memory from a procedural memory to a declarative memory! Interestingly, new neurons persist in the dentate gyrus in trace but not delay eyeblink conditioning, and blocking formation of new neurons impairs trace but not delay conditioning (Gould et al., 1999a,b; Shors et al., 2001).

3.19.3 Classical Fear Conditioning

Within the past few decades Pavlovian fear conditioning has become one of the most intensely studied forms of mammalian procedural learning (*See* Chapter 4.11). In fear conditioning a discrete stimulus such as a tone or static features of the training context (i.e., shape, feel, lighting, and smell) is paired with an aversive stimulus. Upon return to the original training environment or presentation of the previously paired tone, fear is expressed as an array of autonomic, hormonal, and behavioral responses. The development of neurobiological, behavioral, molecular, and genetic methods and their application to rodent models of fear conditioning has yielded tremendous insight into our understanding of the neural substrates of Pavlovian fear conditioning (see Maren and Quirk, 2004; Walker and Davis, 2004; Fanselow and Poulos, 2005; Phelps and LeDoux, 2005, for reviews).

3.19.3.1 Nature of Conditional Fear

As described some years ago by Bolles (1970) the ability to rapidly predict impending danger is crucial for the survival of any organism; therefore mammals have evolved a series of preprogrammed species-specific defensive reactions. For example in response to threat, the chameleon changes color, the opossum plays dead, the skunk releases an odor, the turtle retreats in its shell, and the rat or mouse freezes (Bolles, 1967).

Fear has been described as the perception and recognition of danger, the learning and remembering about dangerous experiences, and the coordination of defensive behaviors to environmental threat (Fanselow and Gale, 2003). In addition to more overt behavioral responses, conditional fear has been measured by collective changes in heart rate (Antoniadis and McDonald, 2000), body temperature (Godsil et al., 2000), defecation (Antoniadis and McDonald, 2000), cortisol release (Goldstein et al., 1996), and opiate-related analgesia (Fanselow, 1986). In rodent models of fear, arguably the most reliable measure of learned fear is freezing, a defensive posture exhibited by some mammals (e.g., rats, mice, rabbits, and deer) that is best described as the absence of movement with exception of those related to respiration. Other prominent indices of conditional fear include enhancement in startle to loud acoustic stimuli (Brown et al., 1951; Rosen and Davis, 1988) as well as suppression of previously reinforced lever press in rats (Estes and Skinner, 1941; Angladа-Figueroa and Quirk, 2005). In addition, work by McGaugh and colleagues has made tremendous strides in our understanding of the role of the

amygdala in memory consolidation using an inhibitory avoidance procedure (McGaugh, 2004). Here, to limit the scope of this chapter, much of the work described will primarily focus on freezing as a measure of fear, as described in rodents, unless otherwise noted.

In contrast to eyeblink classical conditioning, the UR to footshock and the CR as measured by freezing are notably different in that footshocks evoke a reflexive burst of locomotor activity (Fanselow, 1980). Following even a single pairing of the CS and US, postfootshock freezing begins to emerge soon after the offset of the activity burst and has been used as a reliable online measure of fear learning (Fanselow, 1980).

3.19.3.2 Brain Systems Engaged in Fear Conditioning

The primary brain area engaged during fear conditioning is the basolateral amygdala complex (BLAc), a region composed of several heterogeneous subnuclei, including the lateral (LA), basomedial (BM), and basolateral nuclei (BL). Other brain regions that play a prominent role include the hippocampus, medial geniculate nucleus, anterior cingulate cortex, and ventral periaqueductal gray. In auditory fear conditioning, the BLAc is essential; however, under conditions in which fear to the original training context is assessed, not only is the BLAc important, but so is the hippocampus.

3.19.3.3 The Amygdalar System

3.19.3.3.1 Lesions

The primary foundation for the role of the amygdala in fear conditioning was laid by Brown and Shafer (1888) in humans and Kluver and Bucy (1937) in monkeys: damage to the medial temporal lobes that included the amygdala resulted in profound changes in emotional responsiveness and most notably a loss of fear. Later, work by Weiskrantz et al. (1956) showed that lesions specific to the amygdala could produce similar changes in emotional reactivity. Subsequent work by Kellicutt and Schwartzbaum (1963) showed that lesions of the amygdala attenuated fear-motivated bar-press suppression. The crucial finding for the key role of the amygdala in fear conditioning was provided by Blanchard and Blanchard (1972), who demonstrated that electrolytic lesions of the amygdala in rats prevented contextual fear conditioning as measured by freezing. In addition, McGaugh and colleagues revealed posttraining electrical stimulation of the amygdala resulted in deficits in inhibitory avoidance memory retention (Gold et al., 1973). Since then, further work in a number of laboratories has demonstrated that discrete lesions of the BLAc prior to training prevent the development of both contextual and cued fear responses (LeDoux et al., 1990; Campeau and Davis, 1995; Cousens and Otto, 1998). Conversely, similar lesions in previously trained animals completely abolish all measures of fear responding to all CS modalities tested (Phillips and LeDoux, 1992; Sananes and Davis, 1992; Campeau and Davis, 1995; Lee et al., 1996; Cousens and Otto, 1998; Koo et al., 2004). In an interesting experiment by Gale et al. (2004), lesions of the BLA 17 months following fear training, nearly equivalent to the entire life span of the rat, completely abolished the expression of conditional fear responses, suggesting that, once Pavlovian fear memories are established, they are permanently maintained by the BLAc. It should be noted that the lack of fear responding such as freezing is not due to an inability to perform the response, given that extensive overtraining (>75 footshocks) can yield significant freezing (Maren, 1999; Gale et al., 2004).

Neuropsychological studies in humans with Urbach-Weithe disease, a condition resulting in degeneration of the amygdala that leaves intact surrounding temporal lobe structures, reveal deficits in delay fear conditioning (Bechara et al., 1995). Consistent with patients with amygdala damage, magnetic resonance imaging reveals activation of the amygdala during both fear conditioning and expression (Cheng et al., 2003; Knight et al., 2005).

3.19.3.3.2 Measures of neuronal activity

Electrophysiological recordings and quantification of immediate early gene expression in neurons of the BLAc are consistent with lesion studies and reveal that these neurons are actively engaged during and as a result of fear conditioning. Indeed, populations of neurons in the LA nuclei respond to both the CS and US. LeDoux and colleagues have shown that responding of these individual neurons to a tone CS is enhanced as a result of fear conditioning (Quirk et al., 1995, 1997; Li et al., 1996). Similar learning-related changes have been also observed in neurons of BL nucleus (Maren and Quirk, 2004). Consistent with these findings, measurements of immediate early gene *c-fos* are elevated in regions of the amygdala (Beck and Fibiger, 1995) and interestingly show a significant

lateralization within the right BLA and central nucleus of the amygdala (CeA) (Scicli et al., 2004).

3.19.3.3.3 The pathways

There is a general agreement within the literature that the neural circuitry underlying Pavlovian fear conditioning is centered around the BLAc (Fanselow and LeDoux, 1999; but see McGaugh, 2004) and that distinct neural efferents support the expression of different components of the conditional fear response (**Figure 3**). In contrast, examinations of pathways by which CS- and US-related information converges upon the amygdala suggest that multiple pathways are sufficient, none of which seem solely necessary for fear conditioning (Fanselow and Poulos, 2005).

3.19.3.3.3.1 The CS pathway In auditory fear conditioning, both the auditory cortex and medial geniculate nucleus (mGN) convey CS-related information to the lateral nucleus of the amygdala (LeDoux et al., 1990). Lesions of afferents to the LA or the LA itself disrupt the acquisition of tone-cued fear conditioning (LeDoux, et al., 1990; Romanski and LeDoux, 1992). Moreover, tone-evoked responses in the mGN occur within 12 ms, whereas learning-related increases in tone-evoked spike firing in the lateral nucleus occur around 15 ms (Li et al., 1996), suggesting that mGN activity precedes the development of learning-related LA activity.

Under conditions in which the fear response is signaled by the training context, both hippocampus and perirhinal cortex play an important role in conveying environmental cues to the BLA neurons. Either posttraining lesions of the ventral angular bundle, a primary source of hippocampal and perirhinal input to the BL, or single lesions of the hippocampus and perirhinal cortex produce deficits in the expression of contextual fear responses, but fail to disrupt tone-cued fear responses (Maren and Fanselow, 1995; Bucci et al., 2000; Burwell et al., 2004). Finally, blockade of hippocampal NMDA receptors prior to training severely attenuates contextual fear conditioning (Young et al., 1994; Quinn et al., 2005).

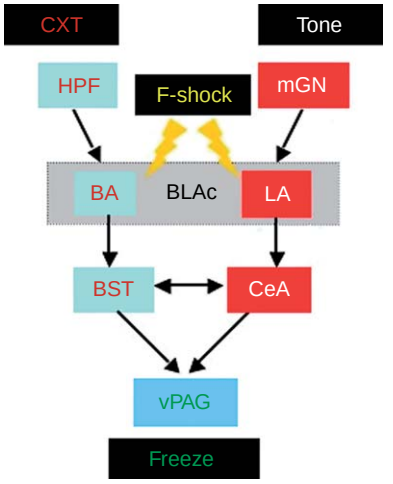

Figure 3 Simplified schemata of the neural circuits underlying Pavlovian fear conditioning. In contextual fear conditioning, information pertaining to the training context (CXT) is relayed to the basal amygdala (BA) via the hippocampal formation (HPF), where it converges with footshock-related (F-shock) information. In auditory fear conditioning, information pertaining to tone is relayed to the lateral amygdala (LA) via the medial geniculate nucleus (mGN) and converges with F-shock. Contextual and auditory fear established and maintained within the basolateral amygdala complex (BLAc) are relayed to the bed nuclei of the stria terminalis (BST) and central nucleus of the amygdala (CeA), respectively. The final common output for the generation of freezing is expressed via the ventral periaqueductal gray (vPAG).

3.19.3.3.3.2 The US pathway In order for the BLAc to support fear conditioning, pathways conveying information pertaining to the US must reach this region. Unlike the CS pathway, relatively less is known about how information related to the footshock is conveyed. Regions of the thalamus that respond to somatosensory information receive input from the spinothalamic tract and send projections to the lateral amygdala. In addition, insular cortex seems to play a role in relaying pain-related information as well (Shi and Davis, 1999). Interestingly, combined but not single lesions of the posterior thalamus and insular cortex attenuate tone-cued fear conditioning, but leave contextual fear conditioning intact (Brunzell and Kim, 2001). The central nucleus receives nociceptive input from the parabrachial nucleus and nucleus of the solitary tract and directly from the dorsal horn of the spinal cord (Bernard and Benson, 1990; Burnstein and Potrebic, 1993; Gauriau and Bernard, 2002). The function of such inputs at the central nucleus needs further investigation, given that this region does not project back to any regions of the basolateral amygdala complex in rats (Fanselow and Poulos, 2005).

3.19.3.3.3.3 The CR pathway Neurons within the lateral amygdala which undergo auditory fear learning–related plasticity project to intercalated

inhibitory neurons, which project to the CeA. Lesions limited to lateral amygdala or central nucleus disrupt tone-cued fear conditioning (Nader et al., 2001). In parallel, environmental cues encoded by the hippocampus in correspondence with activation by US input drive the development of learning-related plasticity among neurons of the BLA and send axonal projections which course through the central amygdala nucleus and terminate at the bed nuclei of the stria terminalis. Electrolytic lesions of the BLA, CeA, or bed nuclei of the stria terminalis disrupt the expression of contextual fear responses (Phillips and LeDoux, 1992; Sullivan et al., 2004). Excitotoxic lesions of the CeA that spare fibers of passage, as confirmed by fiber staining methods, selectively attenuate cued but not contextual fear responses (Koo et al., 2004). Whereas, if these lesions (electrolytic) include damage to fibers of passage, both context and cued-fear responses are disrupted. This suggests that projections from the lateral amygdala → central nucleus and basolateral amygdala → bed nuclei of stria terminalis may represent separate response pathways for freezing to tone and contextual fear conditioning, respectively. Conversely, both the central nucleus and bed nuclei of the stria terminalis heavily innervate the periaqueductal gray, a region critical for the expression of defensive responses such as a freezing. Lesions of the periaqueductal gray, which abolish the expression of freezing, do not disrupt the expression of other conditional fear responses, such as alterations in blood pressure, while damage to the lateral hypothalamus affects blood pressure but not freezing (LeDoux et al., 1988). Therefore, the important point here is that distinct neural pathways initialized within the BLAc mediate the expression of different components of the conditional fear responses and to the some extent the signals that predict danger.

3.19.3.3.4 Reversible inactivation

Although the aforementioned studies strongly implicate the BLAc in fear conditioning, they do not indicate whether effects of lesions result from learning or performance deficits or whether learning-related changes in neuronal responding are due to plasticity efferent to the BLAc. Therefore, as previously described, reversible inactivation has become a valuable tool in localizing memory traces in the brain. Reversible inactivations targeting the BLAc via direct microinfusion of the $GABA_A$ receptor agonist muscimol prior to fear conditioning prevent the development of context and cued-fear memories. Reversible inactivations of the BLAc after training and prior to tests of memory retention completely abolish the expression of cued and contextual fear responses. These results indicate that functions of BLAc neurons are vital for the development and expression of Pavlovian fear memory. In an attempt to distinguish the relative contribution of the LA and BL in cued versus contextual fear conditioning, Jaffard and colleagues demonstrated that reversible inactivation (via the sodium channel blocker lidocaine that inactivates both soma and axons) targeting the LA attenuated the acquisition of cued, but not context fear, while inactivations of the BL attenuated the acquisition of context, but not cued fear (Calandreau et al., 2005). Finally a study by Maren and colleagues showed that both development of learning-related activity in mGN and cued-fear conditioning are disrupted by reversible inactivation of the BLAc (Maren et al., 2001).

3.19.3.3.5 Mechanisms of storage in the basolateral amygdala complex

Since the initial demonstration of long-term potentiation (LTP) by Bliss and Lomo (1973) showing that normally weak synapses in the hippocampus could be strengthened by high-frequency stimulation, LTP has become an extremely attractive cellular model of Pavlovian learning. For example, in fear conditioning, CS-generated input could be strengthened by US activation of the BLA, and thus subsequent presentations of the CS could more readily activate the amygdala and promote expression of conditional fear responses. If it were the case that amygdalar LTP represents a substrate of conditional fear memories, then manipulations that attenuate LTP should correlate with deficits in fear conditioning. A number of *in vitro* experiments have demonstrated the establishment of LTP within amygdala circuits (Racine et al., 1983; Chapman et al., 1990; Clugnet and LeDoux, 1990; Maren and Fanselow, 1995). Stimulation of previously described CS pathways, the mGN (Clugnet and LeDoux, 1990) or the hippocampus (Maren and Fanselow, 1995), are able to support LTP at BLAc synapses. Consistent with the LTP hypothesis, McKernan and Shinnick-Galagher (1997) demonstrated that brain slices taken from fear-conditioned rats with the LA and auditory nucleus intact showed larger excitatory postsynaptic potentials (EPSPs) in the LA following stimulation of the auditory nucleus than untrained control rats.

Further studies have demonstrated that, as in the hippocampus, the induction of amygdala LTP

requires the activation of NMDA receptors (Bliss and Collingridge, 1993; Maren and Baudry, 1995; Fanselow and Maren, 1995; Huang and Kandel, 1998). Indeed, the induction of LTP is blocked when amygdala slices are treated with NMDA receptor antagonist aminophosphonovaleric acid (APV), whereas once LTP is established APV fails to affect its expression.

Consistent with this line of thought, Davis and colleagues demonstrated that infusion of NMDA receptor antagonist APV into the BLAc blocked the acquisition but not the expression of fear-potentiated startle (Miserendino et al., 1990). Further studies measuring freezing have yielded similar effects, with NMDA blockade disrupting the acquisition but not the expression of conditional fear (Fanselow et al., 1994; Rodrigues et al., 2001). Other forms of the glutamate receptor also implicated in LTP and fear conditioning are mGluRs, in particular mGluR5. Antagonism of this receptor not only impairs the induction of amygdala LTP, but also selectively blocks the acquisition, while leaving expression of conditional fear intact (Rodrigues et al., 2002). Both of these receptors are thought to trigger an influx of extracellular and intracellular Ca^{2+}, resulting in the activation of calcium/calmodulin-dependent protein kinase II (CaMKII). In turn, auditory fear conditioning and the induction of amygdalar LTP by stimulation of mGN result in an increased activation of αCaMKII in the lateral amygdala (Rodrigues et al., 2004). Conversely, BLAc infusion of a CaMKII blocker disrupts fear conditioning (Rodrigues et al., 2004). Along with αCaMKII, protein kinases A and C (PKA and PKC) and Akt are known signals that converge upon the microtubule-affinity regulating kinase (MARK) signaling pathway. BLA infusion of St-Ht32, which blocks PKA anchoring to scaffolding proteins, disrupts the establishment of conditional fear memory (Moita et al., 2002). In addition, blockade of tyrosine kinase B (TrkB) receptor activation of PKC signaling pathways via pharmacological blockade of brain-derived neurotrophic factor (BDNF) binding or in heterozygous BDNF knockout mice results in severe fear conditioning deficits (Liu et al., 2004; Rattiner et al., 2004). Moreover, if mitogen-activated protein kinase (MAPK) expression, which is elevated following fear conditioning, is blocked, fear memory at 24 h following training is markedly attenuated (Schafe et al., 2000). Consistent with these data, amygdala slices bathed in MAPK inhibitor U0126 have impaired LTP (Schafe et al., 2000). Both MAPK and PKA are thought to activate transcription factors such as cyclic adenosine monophosphate (cAMP) response element binding protein (CREB), which are critical for establishment of memory (Rodrigues et al., 2004). Indeed, levels of phosphorylated CREB are increased in the BLAc following fear conditioning (Hall et al., 2001; Stanciu et al., 2001), while overexpression of CREB in the amygdala enhances fear conditioning (Hall et al., 2001) and fear potentiated startle (Josselyn et al., 2001). Moreover, direct BLAc infusion of transcription inhibitor actinomycin-D blocks the acquisition of fear conditioning (Bailey et al., 1999). Further studies have demonstrated that lateral amygdala LTP results in the phosphorylation of CREB and that inhibition of protein synthesis blocks the late phase of LTP as well as the long-term fear memories (Huang et al., 2000; Schafe and LeDoux, 2000; Maren et al., 2003). Finally, *de novo* protein synthesis has been thought to promote the maintenance of both LTP and fear memory by the insertion of postsynaptic alpha-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA) receptors. Indeed, Malinow and colleagues, in a striking set of experiments, have demonstrated that both LTP and fear conditioning drive the insertion of AMPA receptors, and that if insertion of receptors is inhibited, fear memory is reduced (Hayashi et al., 2000; Rumpel et al., 2005).

3.19.3.4 Fear Conditioning and the Hippocampus

3.19.3.4.1 Contextual fear conditioning

As described earlier, the hippocampus composes part of the CS pathway, which converges with US input at the BLAc in contextual fear conditioning. A number of studies suggest that in contextual fear conditioning the hippocampus is not just a passive relay of multisensory information pertaining to the organism's immediate environment, but rather encodes a unified time-limited mnemonic representation of the context CS (Kim and Fanselow, 1992; LeDoux and Phillips, 1992; Fanselow, 2000; Anagnostaras et al., 2001; Rudy et al., 2004). Indeed, this account is in accord with other views that the hippocampus plays an important role in memory (Milner and Ettlinger, 1970; O'Keefe and Nadel, 1978; Zola-Morgan et al., 1986).

The initial study implicating the hippocampus in fear conditioning was by Blanchard and Fial (1968), demonstrating that electrolytic lesions of the hippocampus disrupted fear conditioning. Years later, Phillips and LeDoux (1992) showed that amygdala lesions disrupted both cued and context fear, while lesions of the hippocampus selectively produced

anterograde amnesia of contextual fear conditioning, suggesting that the amygdala had a more general role in fear conditioning, whereas the hippocampus appeared to relay information about the training context in fear conditioning. Kim and Fanselow (1992) further showed that electrolytic lesions of the hippocampus 1 day after training abolished contextual fear, whereas lesions made at 7, 14, and 28 days showed significant contextual fear. Moreover, in the same animals, lesions failed to affect fear responses to tone at any time. Together these results were consistent with the hippocampus playing a time-dependent role in the consolidation of memory and at the same time encoding spatial cues in the animals' immediate environment. However, further studies employing excitotoxic hippocampal lesions, which continued to demonstrate retrograde memory deficits, failed to show anterograde amnesia of contextual fear (Philips and LeDoux, 1994; Maren et al., 1997; Frankland et al., 1998). A number of explanations have been posited based on these discrepancies (Anagnostaras et al., 2001). (1) Excitotoxic infusion could result in damage to neighboring areas such as the amygdala (Mintz and Knowlton, 1993). (2) Ventral hippocampal lesions could result in damage to projections that may affect normal amygdalar function (Maren and Fanselow, 1995). (3) Excitotoxic lesions may result in a sustained seizure-like activation of efferents such as the amygdala, resulting in partial damage (McCelland et al., 1995). Given this, the resulting amygdala dysfunction could result in memory deficits most apparent for recently encoded information (Anagnostaras et al., 2001). A more recent study by Fanselow and colleagues demonstrated that complete excitotoxic lesions of the hippocampus in naive rats failed to disrupt the acquisition of contextual fear, whereas in previously fear-conditioned rats, similar lesions abolish the expression of contextual fear responses (Wiltgen et al., 2006). However, these posttraining deficits could be overcome with retraining.

An alternate possibility is that under normal conditions a slowly established configural mnemonic representation of the training context is encoded by the hippocampus; however, in the absence of the hippocampus, single elements of the context, like cued conditioning, may be sufficient to produce associative plasticity within the BLAc. However, if lesions of the hippocampus are made after BLA plasticity has already been established using a configural representation, then the remaining elemental system may not be sufficient to support the retrieval of contextual fear memories (Fanselow, 2000). Indeed, a number of studies are consistent with this view. (1) Immediate delivery of footshock upon placement into the training context does not yield contextual fear conditioning (Landeira-Fernandez et al., 2006). (2) Posttraining lesions of the hippocampus are more detrimental than pretraining lesions. (3) Preexposure to the training context followed by fear conditioning 35 days later mitigates retrograde amnesia effects of hippocampal lesions (Anagnostaras et al., 1999). Moreover, an elegant study by Rudy and colleagues showed that mRNA of *Arc* and immediate early gene *c-fos* mRNA are induced in the hippocampus following context exposure or context plus shock experience, but not after immediate shock (Huff et al., 2006). However, if the BLA is inactivated, *Arc* and *c-fos* mRNA were attenuated following contextual fear training, but *Arc* mRNA levels remained unaffected by context preexposure alone. Perhaps under normal conditions hippocampal LTP may be critical for assembling elemental components of the training context into a cognitive and/or configural representation of the context (Anagnostaras et al., 2001), which is further consolidated via amygdala-dependent fear conditioning.

3.19.3.4.2 Trace fear conditioning

In fear conditioning, as in eyeblink conditioning, the addition of a stimulus-free period or 'trace' (>10 s) between the auditory CS and footshock requires the hippocampus (Chowdhury et al., 2005; Misane et al., 2005). Pretraining lesions of the hippocampus disrupt the acquisition of trace fear conditioning (McEchron et al., 1998; Burman et al., 2006), while posttraining lesions attenuate the expression of trace fear conditioning (Chowdhury et al., 2005; Burman et al., 2006). Subsequently Fanselow and associates revealed that blockade of dorsal hippocampal NMDA receptors disrupted the acquisition but not the expression trace fear memories (Quinn et al., 2005). Interestingly, the presentation of a visual distracter disrupts trace, but does not delay fear conditioning, suggesting that attention is required for the acquisition of trace fear memory. Finally, trace fear conditioning, which is associated with an increase in *c-fos* activation in the anterior cingulate cortex, is attenuated by lesions of this same region (Han et al., 2003).

3.19.3.4.3 Recent versus remote fear memories

An important question posed by the results of Kim and Fanselow (1992) is that, if the hippocampus is

transiently involved in the consolidation of contextual fear memory, what brain region(s) are involved in retrieving more remotely established contextual fear memories? A series of elegant studies by Silva and colleagues suggests that a number of regions of the cortex including the anterior cingulate cortex may be required (Frankland et al., 2001, 2004, 2006). In genetically engineered mice with reduced levels of αCaMKII, recent contextual fear memory tested 24 h later is normal, whereas remote fear memory tested at 10 to 50 days is completely absent (Frankland et al., 2001). Interestingly these mice, which failed to show remote fear memory, revealed no changes in cortical immediate early gene expression following recent or remote memory tests, whereas wild-type mice showed correlated increases of cortical gene expression and remote context fear memory (Frankland et al., 2004). Conversely, reversible inactivation of the anterior cingulate cortex at remote, but not recent, retention intervals disrupts the retrieval of contextual fear memories. Moreover, αCaMKII mutant mice, which showed normal levels of hippocampal LTP, have impaired cortical LTP. These experiments suggest that αCaMKII expression within a number of regions, notably the anterior cingulate cortex, plays an important role in the retrieval of remote contextual fear memory.

3.19.4 Interactions Between Conditioned Fear and Eyeblink Conditioning: The Two-Stage Hypothesis

A number of authors have distinguished between two classes of CRs: diffuse or nonspecific preparatory CRs and precise, specific, adaptive CRs (e.g., Konorski, 1967; Rescorla and Solomon, 1967; Thompson et al., 1984). According to the 'two-stage theory,' the association between the CS and the aversive US is formed within the first few conditioning trials and results in the acquisition of emotional CRs taking the form of nonspecific, autonomic arousal. Nonspecific responses are usually autonomic but also include generalized body movements, are learned rapidly, and prepare the organism to do something. Such responses are viewed as manifestations of a 'conditioned emotional state,' e.g., conditioned fear. Conditioning of specific responses, for example, eyelid closure or leg flexion, involves learning precise, adaptive CRs that deal specifically with the US and requires more extensive training. The two learning processes proceed not only at different rates but also at different brain sites.

The two-stage hypothesis suggests that the initial conditioned fear may be necessary or at least play a role in subsequent learning of discrete movements and that the learning of discrete movements may impact subsequent retention of conditioned fear. Powell and associates (1974) showed that, when heart rate and eyeblink conditioning were given simultaneously, conditioned heart rate developed very rapidly in a very few trials, but then as eyeblink conditioning developed, the conditioned heart rate diminished. As noted earlier the essential brain substrates for these two aspects of learning are quite different: amygdala for fear and cerebellum for eyeblink. Neufeld and Mintz (2001) showed that prior fear conditioning facilitated subsequent eyeblink conditioning and amygdala lesions abolished this facilitation. Weisz et al. (1992) showed that amygdala lesions could actually impair rate of learning of the eyeblink response. Lavond et al. (1984a) showed that appropriate cerebellar interpositus lesions that abolished the conditioned eyeblink response had no effect on initial acquisition of the conditioned heart rate response, as expected. However, Mintz and Wang-Ninio (2001) showed that interpositus lesions, which prevented discrete response conditioning, also prevented the subsequent decline in fear conditioning with extensive eyeblink training that normally occurs in intact animals. If the animal cannot learn to deal with the aversive US, then fear does not extinguish. Patterns of gene expression over the course of eyeblink conditioning in the interpositus nucleus (mouse) support the two-stage hypothesis (Park et al., 2006). Indeed, further work examining the relative activation of gene expression in both regions of the amygdala and cerebellum over the course of simultaneous eyeblink and heart rate conditioning could directly test the two-stage hypothesis of Pavlovian conditioning.

3.19.5 Conclusions

The findings described here indicate that two forms of procedural learning, eyeblink conditioning, a slowly acquired fine motor behavior, and fear conditioning, a rapidly acquired global defensive response, are established, maintained, and expressed by two different sets of neural circuits centered around the cerebellum and amygdala, respectively. Moreover, the mechanisms of plasticity within each of these brain regions at cellular and genetic levels suggest that different forms of Hebbian plasticity (LTP vs. LTD) and gene expression

may be crucial for each form of procedural learning. However, at the systems level, there are similarities in eyeblink and fear conditioning in that increasing task difficulty or the interval of time between training and testing seems to differentially engage the hippocampus and regions of the neocortex. In addition, accumulating data seem to suggest that eyeblink conditioning may develop normally in a two-stage process, where initial training establishes a conditioned emotional component requiring the amygdala, which with further training facilitates the acquisition of cerebellar-dependent eyeblink responses. Collectively, these studies strongly indicate that each of these forms of procedural learning, which depend on different neural loci and mechanisms of memory, may indeed work in conjunction to promote the establishment of appropriately timed and coordinated adaptive behaviors.

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3.20 Procedural Learning: VOR

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3.20.1 Introduction

Humans can learn skilled motor tasks, such as riding a bike, playing the piano or even hitting an accurate topspin serve in tennis. This long-term 'motor memory' is referred to as procedural memory. Although practice can improve performance, memories of these learned movements remain remarkably intact, even after years of disuse. Procedural or 'motor' learning also serves an important role in calibrating simpler movements such as eye movements and reflexes. One class of eye movements, the vestibulo-ocular reflex (VOR), is a particularly useful model system. The VOR produces compensatory eye movements that are required to stabilize gaze and ensure clear vision during the head movements that are generated during everyday activities such as walking and running. The VOR shows impressive adaptation in response to environmental requirements. Furthermore, the relative simplicity of the neural circuit that mediates this reflex has proven to be well suited to linking systems and cellular levels of analyses of motor learning.

Procedural memory is generally contrasted with declarative memory, which is the memory of facts or experiences. Over the past decade, work on learned

reaching movements has provided evidence to support the idea that procedural memory, like declarative memory, passes through multiple stages before becoming stable, including encoding, consolidation, and retrieval (see a recent review by Robertson, 2004). Similarly, the findings of recent behavioral, single-unit recording and lesion studies have established that VOR motor learning passes through distinct stages. Moreover, neural substrates underlying the progression of learning in this model system have been localized, and significant progress has been made in understanding the cellular mechanisms that control their formation. In addition, the commonly held idea that procedural learning is characterized by the transformation of a memory from a fragile state to a new permanent memory has been challenged. Recent studies of the VOR, in particular, have emphasized the importance of contextual cues in guiding the retrieval of learned responses.

This chapter will review the findings of behavioral, single-unit recording and lesion studies of VOR motor learning. First, the current understanding of the neural mechanisms that underlie motor learning in the VOR will be reviewed. Then recent evidence supporting the idea that VOR motor learning is characterized by a progression of changes in multiple brain areas will be discussed. Finally, these findings will be integrated in relation to recent experiments that have characterized context-dependent learning in the VOR.

3.20.2 Why Do We Need to Adapt the Gain of the VOR?

3.20.2.1 A Brief Introduction to the VOR

The simplicity of the three-neuron arc that produces the VOR is reflected in its fast response time (**Figure 1(a)**); compensatory eye movements lag head movements by only 5–6 ms in the primate (reviewed in Cullen and Roy, 2004). This reflex produces eye movements in response to both the angular and linear components of head movement and functions to move the eyes in the opposite direction of the concurrent head motion. For example, if a subject undergoes passive head rotation in darkness, the gain of the VOR, which is defined as compensatory eye velocity divided by head velocity, is ~1.0. The VOR initiates eye movements at much shorter latencies than those of visually mediated eye movements. As a result, the VOR is fast enough to stabilize gaze during common activities such as locomotion. Bilateral loss of vestibular function is extremely debilitating because without a functional VOR, simple activities such as walking or driving are accompanied by oscillopsia – the illusion that the environment is moving when we move our heads.

3.20.2.2 The Adaptive Capabilities of the VOR

The VOR is capable of impressive adaptation in response to environmental requirements. To date, perhaps the most dramatic illustration of the adaptive capabilities of this reflex was provided by a study in which participants wore prisms, which reverse the world such that left was right and vice versa (Gonshor and Jones 1976; **Figures 1(b)–1(c)**). Within minutes of wearing the prisms, the gain of the VOR substantially declined. Moreover, when reversing prisms were worn for extended periods (3–4 weeks), the response of the VOR actually reversed; head movements induced an eye movement in the same rather than opposite direction. This change was appropriate to account for the new demands that were imposed on the reflex by the spectacles and accordingly produced improved stabilization of the world on the retina during head movements.

In more commonly encountered situations, the demands on the VOR are less challenging. Nevertheless, the adaptive capabilities of the VOR are essential to guarantee gaze stability throughout life. In humans the VOR must be continuously adjusted in the first years of life to compensate for significant changes in head circumference (~30% in the first year). Later in life, adaptive changes in VOR performance are required to compensate for the magnification of corrective lenses that are worn for common visual conditions. For example, the VOR gain in myopic individuals who wear convex lenses will be lower than for individuals with normal vision. The adaptability of the VOR is also critical to compensate for the effects of aging, disease, and trauma on the nervous system. Notably, the VOR shows remarkable recovery following the loss of unilateral labyrinthine input as a result of injury. Similarly, the reflex shows robust adaptation to the incremental loss of the receptor cells that occur naturally during aging.

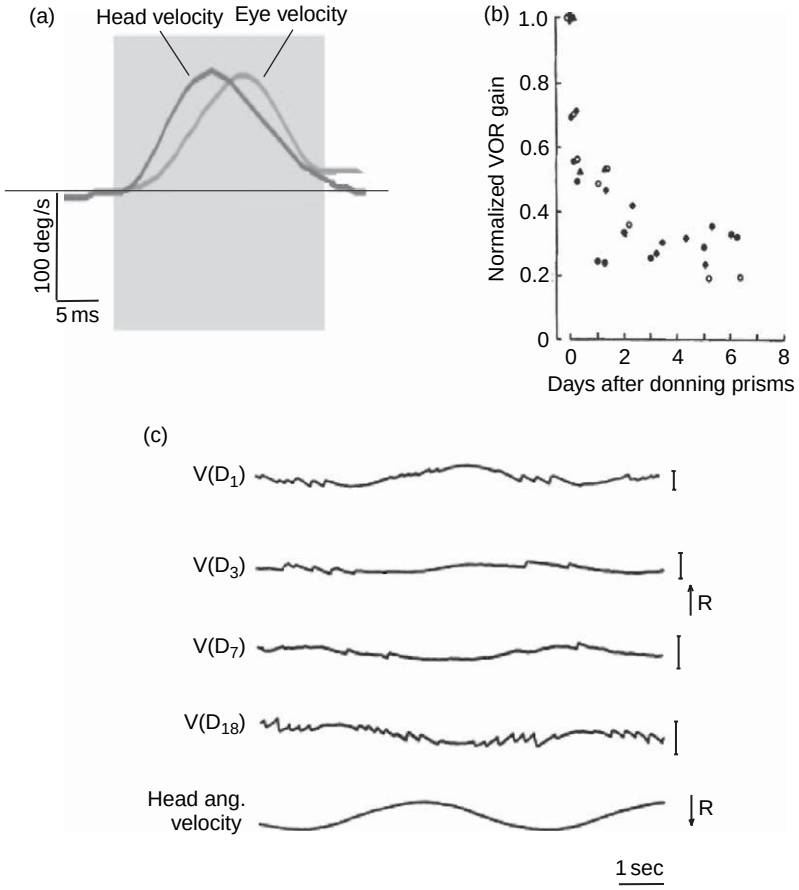

Figure 1 (a) Eye response following a transient head perturbation. Following the onset of head rotation, the eye begins to counterrotate after a latency of 5–6 ms. The gain of the reflex is eye velocity divided by head velocity. Note that eye velocity has been inverted to facilitate comparison. (b) Changes of VOR gain after wearing reversing prisms for 8 days. (c) Example of the VOR in a subject who continuously wore reversing prisms for 49 days. By day 18, the mean response phase was nearly inverted (i.e., −∼140 deg phase shift) relative to the correct nonreversed compensatory response. From (a) Marko Huterer M and Kathleen E. Cullen KE (2002) Vestibuloocular reflex dynamics during high-frequency and high- acceleration rotations of the head on body in rhesus monkey. *J Neurophysiol.* 88: 13–28; 10.1152/jn.01034.2001. (b, c) Gonshor A and Jones G (1976) Extreme vestibulo-ocular adaptation induced by prolonged optical reversal of vision. *J. Physiol* 256: 381–414.

3.20.3 Historical and Current Models of VOR Adaptation

3.20.3.1 The Cerebellum and Motor Learning

The cerebellum is required for VOR motor learning; new gains cannot be learned after the vestibular cerebellum is removed. The basic circuitry of the cerebellum is highly organized and consists of repeating motifs of five primary cell types: Purkinje cells, granule cells, basket cells, stellate cells, and Golgi cells which are organized into the same basic circuit all across the cerebellum (Eccles et al. 1976). The Purkinje cells are the only neurons whose axons leave the cerebellum, and it is noteworthy that these cells send inhibitory projections to their targets within the vestibular and deep cerebellar nuclei. The other cell types are local circuit neurons whose axons make synaptic connections within the cerebellar cortex.

There are two main input pathways to the cerebellum – mossy fibers and climbing fibers. Mossy fiber inputs arise from many regions of the brainstem and spinal cord, including those from the vestibular system via direct projections from the vestibular nerve and vestibular nuclei. Mossy fibers affect the discharges of Purkinje cells through cerebellar interneurons called granule cells. These latter neurons

send projections to Purkinje cells via parallel fibers. Climbing fiber inputs arise from the inferior olive and make powerful excitatory synaptic connections with Purkinje cells. Typically each Purkinje cell receives only one climbing fiber input, but each climbing fiber spike evokes a powerful EPSP, which causes repetitive discharge in Purkinje cells. The Purkinje cell discharge is then followed by a prolonged pause in its firing rate due to additional climbing fiber synapses on inhibitory interneurons (**Figure 2(a)**).

Mossy fiber and climbing fiber inputs to the cerebellum each shape the discharge patterns of Purkinje cells in a very specific way. Inputs from the climbing fibers result in generation of complex spikes (or alternatively climbing fiber responses) in the Purkinje cells. In contrast, mossy fiber inputs are responsible for the simple spike activity of Purkinje cells. Simple spikes (SS) occur much more frequently (discharge rates reaching up to 300 sp/s) as compared to complex spike activity (~1 sp/s). In addition SSs are shorter in duration than complex spikes, which can last as long as 2–5 ms.

The conceptual framework put forth by Marr (1969) and Albus (1971) more than three decades ago has been extremely influential in shaping our current view of the role of the cerebellum in motor learning. In this framework, the role of the climbing fiber input to the cerebellum is to modify the response of Purkinje cells to mossy fiber inputs. The specific hypothesis is that climbing fibers signal errors in motor performance and alter parallel fiber (i.e., mossy fiber-related) Purkinje cell synaptic efficacy. There is considerable evidence to support this idea: Lesions of the cerebellum abolish several types of motor learning, climbing fibers can carry signals that are related to motor performance error, and lesions of the inferior olive abolish some types of motor learning (reviewed in Boyden et al., 2004).

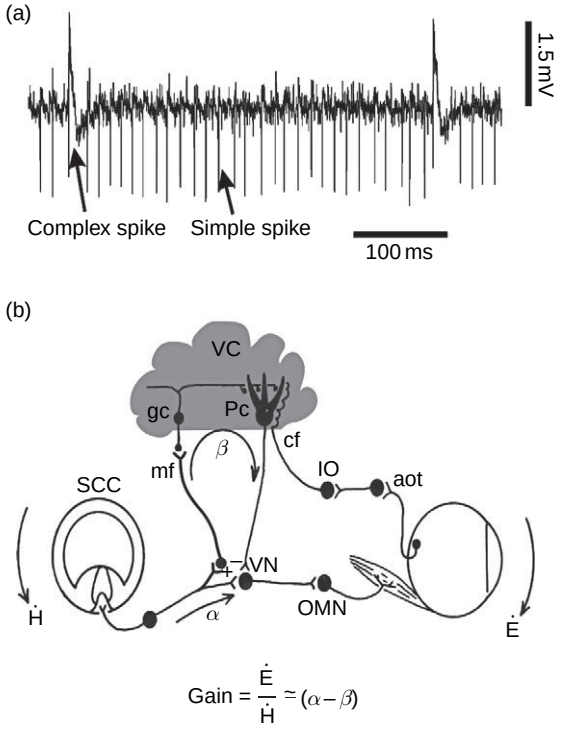

Figure 2 (a) Extracellular recording from an example floccular Purkinje cell showing its high-frequency simple spike (SS) activity as well as complex spike activity (CS) which is due to the climbing fiber input. (b) Two pathways control the gain of the VOR. The main reflex path of gain is paralleled by an inhibitory side branch through the vestibulocerebellum with a gain (beta) so that total gain is proportional to (alpha-beta). Abbreviations: CF, climbing fibers; GC, granule cells; IO, inferior olive; MF, mossy fibers; OMN, oculomotor nuclei; PC, Purkinje cells; VN, vestibular nuclei; aot, accessory optic tract; SCC, semicircular canal; $\dot{E}$, eye velocity; $\dot{H}$, head velocity. From (a) Courtesy of Simpson J and Maruta J. (b) Modified from Robinson DA (1976) Adaptive gain control of vestibuloocular reflex by the cerebellum. *J. Neurophysiol.* 39:954–969.

3.20.3.2 Two Historically Influential Models of Motor Learning in the VOR

The results of single recording and more confined lesion studies have more precisely established that the flocculus and ventral paraflocculus (herein called the floccular complex) of the vestibulocerebellum mediate motor learning in the VOR (e.g., Ito et al., 1974, 1982; Robinson, 1976; Nagao, 1983; Lisberger et al., 1984). Mossy fiber projections from the vestibular nuclei provide a source of head movement-related information to the floccular complex. In turn, the Purkinje cells of the neurons of the floccular complex send monosynaptic inhibitory projections back to neurons in the vestibular nucleus that mediate the VOR (Fukuda et al., 1972; Highstein, 1973; Ito et al., 1977; Sato et al., 1988). Effectively, this vestibular–cerebellar–vestibular pathway provides a parallel inhibitory side loop that can modulate the gain of the direct VOR pathway (**Figure 2(b)**).

To change the gain of the VOR response, there must be adaptive adjustment(s) of the synaptic efficacy between the neuronal elements that mediate the reflex. The question of where and how this adaptation occurs has been debated, and in

particular two hypotheses of motor learning in the VOR have dominated the literature. The principal difference between these two models is the location of the modified synapses that underlie motor learning.

First, in 1972, Ito introduced an influential theory of VOR adaptation based on the Marr–Albus model of cerebellar motor learning. In Ito's adaptation of the Marr–Albus model to VOR learning, the climbing fiber input from the inferior olive provides an error signal that alters the parallel fiber (i.e., mossy fiber-related) Purkinje cell synaptic efficacy. This error signal modifies the head velocity-related modulation of Purkinje cells to change the gain of this cerebellar loop of the VOR circuitry so that the VOR is compensatory (**Figure 3(a)**; red star). Consistent with this theory, climbing fiber inputs to the floccular complex chiefly encode visual slip information, which provides an indication of the reflex performance (Maekawa and Simpson, 1973; Simpson and Alley,

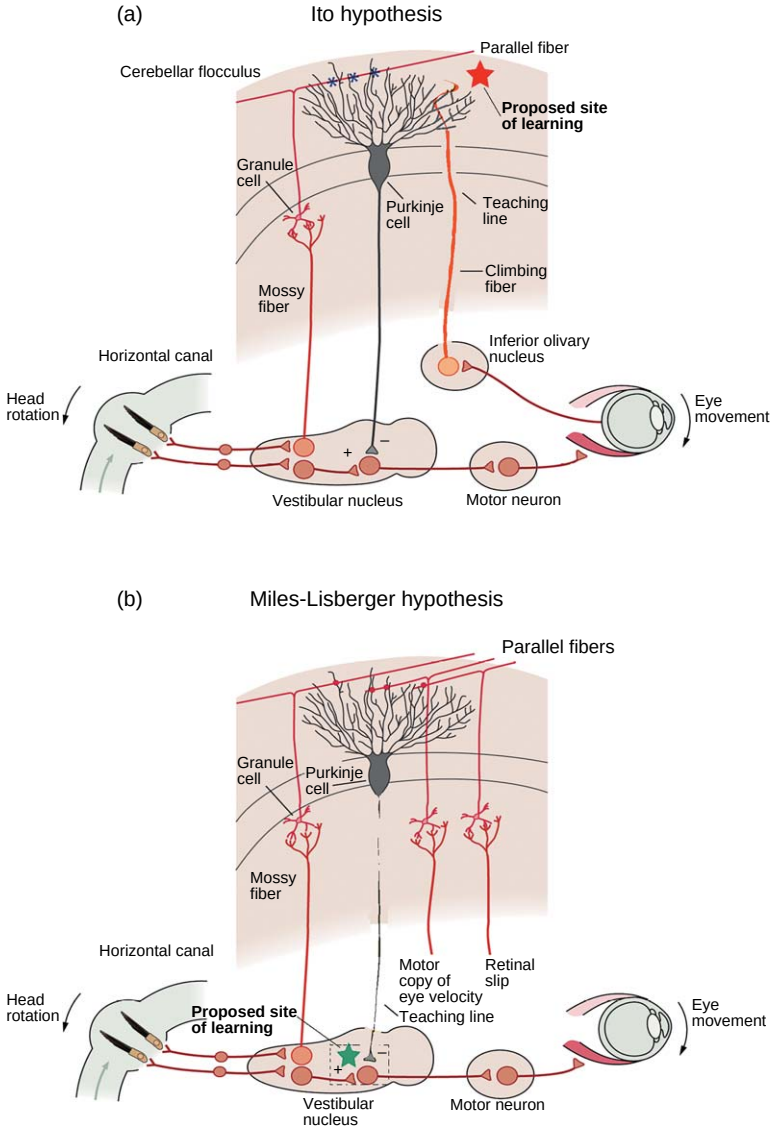

Figure 3 Two models of VOR motor leaning. (a) In Ito's model, climbing fibers encode a visual error signal, which induces learning at the synapses of vestibular parallel fibers onto the Purkinje cells. (b) In the Miles–Lisberger model, floccular Purkinje cells encode an error signal, which supports the modification of synapses in the target neurons of the vestibular nuclei. See text for additional details. From Kandel ER, Schwartz JH, and Jessell TM. *Principles of Neural Science,* 4th edn. Copyright © 2000 by The McGraw-Hill Companies; Chapter 41.

1974; Ghelarducci et al., 1975; Graf et al., 1988). According to Ito's theoretical framework, learning continues until the visual slip signal (which is a measure of performance error) encoded by the climbing fibers becomes zero. At this point, the VOR would be fully compensatory and so no further adaptation would be required.

Alternatively, Miles and Lisberger (1981) proposed that the floccular complex provided an instructive signal to the vestibular nuclei, to guide plastic changes that took place exclusively in the brainstem (**Figure 3(b)**; green star). This hypothesis incorporated the finding that the Purkinje cells of the floccular complex receive significant eye as well as head movement-related information from their mossy fiber inputs via projections from the vestibular, reticular, raphe, and perihypoglossal nuclei. These investigators hypothesized that when the VOR is in the normal state (i.e., when there is no retinal slip during head movements), eye and head movement signals would be equal and opposite and thus would cancel each other out. As a result, no net drive would be encoded by Purkinje cells, and thus no instructive signal would be sent to the vestibular nuclei. However, when retinal slip is present, as would be the case after donning magnifying or minimizing lenses, visual tracking responses (i.e., pursuit) would be recruited to adjust the eye movement response. As a result a significant eye movement signal would be sent back to the floccular complex, and so the output of the Purkinje cells would code an error signal that represents the difference between eye and head movement. In this schema, the error signal guides plastic changes at the level of neurons in the vestibular nuclei, so that the modified VOR pathways will produce stable gaze.

3.20.3.3 More Recent Evidence and the Emergence of the Multisite Hypothesis of Motor Learning

More recently, substantial evidence has accumulated to support an alternative view, namely that the site of motor learning is not exclusively restricted to either the cerebellar cortex of the floccular complex nor to the vestibular nuclei. Evidence for this proposal has been provided by the results of single-unit recording experiments as well as lesion and transgenic studies. These findings are discussed in the sections titled 'Neuronal networks and single-unit recording studies' and 'Current models of long-term VOR modulation' in relation to the Ito and Miles–Lisberger models of motor learning in the VOR.

3.20.4 Neuronal Networks and Single-Unit Recording Studies

3.20.4.1 Review of Direct VOR Pathways

The three-neuron arc responsible for mediating the direct VOR pathway consists of projections from vestibular afferents to interneurons in the vestibular nuclei, which in turn project to extraocular motor neurons. Because it is experimentally less challenging to rotate an alert subject in the yaw axis than in the pitch or roll axes, the circuit that mediates the horizontal VOR has been best characterized. Hair cells within the horizontal semicircular canals are activated by ipsilaterally directed head rotations. In turn these receptor cells excite the vestibular afferents, which project via the VIII nerve to neurons within the vestibular nuclei. In particular, neurons in the rostral medial and the ventrolateral subdivisions of the vestibular nuclei receive direct inputs from horizontal semicircular afferents (Fuchs and Kimm, 1975; Keller and Daniels, 1975; Chubb et al., 1984; Scudder and Fuchs, 1992; Cullen et al., 1993). These neurons in the vestibular nuclei in turn project to the extraocular motor neurons that project to the lateral and medial recti. To date, single-unit studies have focused on the responses of vestibular afferents and neurons in the vestibular nuclei, but not the motor neurons.

3.20.4.2 Changes in Neural Responses in the Adapted State: Vestibular Afferents

In addition to their centrally projecting connections, vestibular receptors receive innervation from centrifugally projecting efferent neurons located near the abducens nucleus (Rasmussen and Gacek, 1958; Gacek and Lyon, 1974; Goldberg and Fernandez, 1980). Electrical activation of the vestibular efferent pathway results in an increase in resting discharge and decrease in sensitivity of vestibular afferents in toad fish and squirrel monkeys (Goldberg and Fernandez, 1980; Highstein and Baker, 1985). Thus, in theory, the vestibular efferent system could be used to alter the dynamic range of the input that is available at the level of the nerve to guide changes in VOR gain.

Miles et al. (1980a) investigated this possibility in monkeys following VOR learning. No change in

afferent responses was observed after motor learning was induced by having the animals wear magnifying or minimizing prisms for more than a month. Neuronal responses were tested over a limited range of frequencies 0.1–1 Hz (peak velocity of 20 deg/s). A more recent study (Sadeghi et al., 2006) has extended these findings to show that the responses of vestibular afferents are similarly not altered following contralateral vestibular damage. In this latter study rotational frequencies up to 15 Hz were used, thus extending the range of rotational frequencies to match those produced during natural head movements. Taken together, these investigations show that changes in responses of vestibular afferents do not appear to play a significant role in supporting long-term adjustments of the VOR gain.

3.20.4.3 Changes in Neural Responses in the Adapted State: Vestibular Nuclei

The second-order neurons in the rostral medial and ventral lateral subdivisions of the vestibular nuclei can be grouped into distinct classes based on idiosyncratic constellations of discharge properties in response to voluntary eye movements and passive whole-body rotations. Of these, two neuron classes contribute to the direct three-neuron arc that mediates the VOR, namely: (1) position-vestibular-pause (PVP) neurons, and (2) eye-head (EH), which are also called floccular target neurons (FTNs).

3.20.4.3.1 PVP neurons

PVP neurons are thought to constitute most of the intermediate leg of the direct VOR pathway; they receive a strong monosynaptic connection from the ipsilateral semicircular canal afferents and project directly to the extraocular motor neurons (McCrea et al., 1987; Cullen et al., 1991; Scudder and Fuchs, 1992; Cullen and McCrea, 1993). These neurons derive their name from the signals they carry during head-restrained head and eye movement paradigms; their firing rate increases with contralaterally directed eye position; they are modulated in response to ipsilaterally directed head velocity during passive whole-body rotations; and they stop firing or pause during ipsilaterally directed saccades and vestibular quick phases.

To address whether changes in the direct VOR pathway mediate motor learning, single-unit recordings were made from PVP neurons in monkeys before and after they had worn magnifying or minimizing spectacles (Lisberger and Miles, 1980, Lisberger et al., 1994a, b). After wearing these spectacles, head movements will evoke larger/smaller eye movements (i.e., an adapted VOR) relative to control conditions to facilitate stabile vision. Thus, it is important to differentiate changes in neuronal modulation that result from the altered movement from those that genuinely reflect changes in a neuron's sensitivity to head movement. To explicitly address this concern, neuronal responses were characterized using a paradigm in which the monkey 'cancels' its VOR by tracking a target that moves with the head. The resulting vestibular stimulation does not lead to eye motion in the opposite direction to the head motion because trained subjects can accurately follow the target at frequencies < 1.5 Hz. Because the PVP responses to VOR cancellation were comparable before and after motor learning, it was concluded that the vestibular nerve synapse on the PVP neurons is not a primary site of plasticity during visually induced motor learning.

Taken together these results have been taken as evidence that PVP neurons receive feedback signals that are related to the adapted eye movements. It is important to note, however, that cancellation of the VOR is largely accomplished via visual pathways that drive a pursuit signal (reviewed in Cullen and Roy, 2004). These pathways are of cerebellar origin and function to cancel the input from the vestibular nerve at the level of the vestibular nuclei. Because PVP neurons are sensitive to smooth pursuit eye movements as well as head velocity, it is important to note that the strength of PVP responses during VOR cancellation will not provide a completely unbiased estimate of their actual sensitivity to vestibular nerve inputs.

3.20.4.3.2 Floccular target neurons

Integration of the results of more recent studies support the idea that changes occur in pursuit as well as VOR pathways after VOR learning (Miles et al., 1980a, b; Lisberger et al., 1994b; Blazquez et al., 2003, 2006). A subset of neurons in the rostral medial and ventral lateral subdivisions of the vestibular nuclei receive direct inhibitory projections from the floccular complex (Lisberger and Pavelko, 1988; Broussard and Lisberger, 1992; Lisberger et al., 1994a, b). These neurons are distinct from PVP neurons and are called floccular target neurons. Their responses largely correspond with those of a distinct physiological subclass of cells, termed

eye-head (EH) neurons, which have been well characterized during eye and head movements in the head-restrained monkey (Tomlinson and Robinson, 1984; McFarland and Fuchs, 1992; Scudder and Fuchs, 1992; Cullen et al., 1993; McCrea et al., 1996; Chen-Huang and McCrea, 1999; Gdowski and McCrea, 1999, 2000; Gdowski et al., 2001; Roy and Cullen, 2003). EH neurons are the most significant premotor input to the extraocular motor neurons of the abducens nucleus during smooth pursuit eye movements (McFarland and Fuchs, 1992; Scudder and Fuchs, 1992; Cullen et al., 1993; Lisberger et al., 1994a, b).

The primary characteristic of EH neurons is that they respond to eye and head movements in the same direction during horizontal smooth pursuit and cancellation of the VOR, respectively. Many EH neurons receive monosynaptic projections from the ipsilateral vestibular nerve (Broussard and Lisberger, 1992; Scudder and Fuchs, 1992), as well as from the floccular complex. EH neurons that show increased firing for eye and head movements away from the side of recording (cEH neurons) can also send direct projections to extraocular motor neurons (Scudder and Fuchs, 1992). Thus, cEH neurons work together with PVP neurons to support the intermediate leg of the direct VOR pathways. Single-unit recordings, made from EH neurons in the brainstems of monkeys before and after wearing magnifying or minimizing spectacles, show that changes in the VOR gain are accompanied by corresponding changes in the head-velocity related modulation of FTNs during cancellation of the VOR (**Figure 4**, Lisberger et al., 1994b). These changes in response gain are more

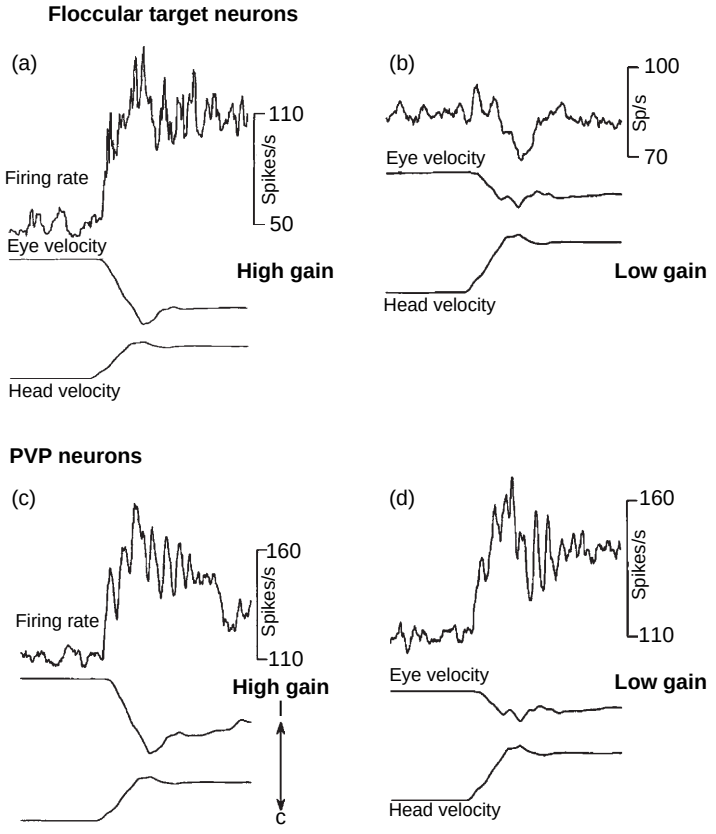

Figure 4 Responses of FTN (a,b) and PVP (c,d) neuron to rapid changes in head velocity after motor learning. Typical response are shown for ipsiversive head motion when the gain was high (a and c) and low (b and d). Upward deflections of the eye and head velocity traces indicate ipsiversive motion. On average the modulation of FTN neuron was significantly different for the two gain states (2.0 versus −0.8 sp/s per deg/s, respectively). In contrast, the modulation of PVP neurons was unchanged. From Lisberger SG, Pavelko TA, and Broussard DM (1994) Neural basis for motor learning in the vestibuloocular reflex of primates. I. Changes in the responses of brain stem neurons. *J. Neurophysiol.* 72: 928–953.

marked for neurons that are activated by the contraversive eye during head-restrained pursuit (i.e., the cEH cells, which contribute to the direct VOR pathway).

EH neurons are sensitive to orbital eye position, as well as smooth-pursuit eye movements and head movement. Thus, as discussed earlier in relation to PVP neurons, changes in an EH neuron's modulation during the VOR or even during cancellation of the VOR do not provide an unbiased estimate of neuronal sensitivity to vestibular nerve inputs. Nevertheless, comparison of the results of the analyses of different groups of brainstem neurons during these conditions does indicate that VOR-related modulation of the cEH neurons is the most altered following motor learning. Furthermore, the responses of these neurons cannot be fully explained by the changes that are seen in the flocculus following learning. In particular their response latencies, unlike those of the floccular Purkinje cells, are appropriate to cause the earliest component of motor learning in the VOR (Lisberger et al., 1994a, b). More recently, the idea that changes at the level of the EH neurons underlie VOR motor learning in the horizontal VOR has been furthered by the finding that the responses of FTNs in the subdivisions of the vestibular nuclei that mediate the vertical VOR (i.e., the y group) are similarly modified during learning in the vertical VOR (Blazquez et al., 2005).

3.20.4.4 Changes in Neural Responses in the Adapted State: Flocculus and Ventral Paraflocculus

Floccular Purkinje cells show both short-term and long-term changes in their responses following VOR learning (Miles et al., 1980a; Watanabe, 1984, 1985; Nagao, 1989; Lisberger, 1994; Raymond and Lisberger, 1996, Blazquez et al., 2003). Work by several laboratories, using different time courses of gain adaptation, demonstrated that changes in Purkinje cell responses are appropriate to induce VOR adaptation either over the course of hours (Watanabe, 1984, 1985; Raymond and Lisberger, 1997; Hirata and Highstein, 2001) or weeks (Miles et al., 1980a; Lisberger et al., 1994a; Blazquez et al., 2003). Because Purkinje cells, like neurons in the vestibular nuclei, are sensitive to eye as well as head movements, these results are difficult to interpret. VOR adaptation will alter the eye movements that are produced in response to head movement, and so it follows that changes in Purkinje cell responses can be a consequence of the altered eye movement-related inputs that are sent to the flocculus.

A number of different approaches have been used in an effort to isolate the vestibular input to the Purkinje cells so that their head velocity sensitivity could be measured following VOR adaptation. First, as described earlier for the vestibular nuclei, neuronal responses were compared during cancellation of the VOR before and after VOR motor learning. Using this approach, changes in Purkinje cell modulation appeared to be in the wrong direction to support the current VOR gain state (Miles et al., 1980a; Lisberger et al., 1994a; Hirata and Highstein, 2001). Accordingly, this finding was originally used in support of the proposal that the floccular complex was not the site of plasticity in VOR motor learning, but instead that changes in the vestibular nuclei were responsible for VOR adaptation (i.e., see Miles et al., 1980a). More recently, it has been established that models of VOR learning that incorporate gain changes at the level of both the brainstem and cerebellum can account for the results of these single-unit recording experiments. In particular, the results of systems identification-based analyses, which incorporate the feedforward and feedback connectivity of the visual and vestibular pathways in the brainstem and floccular complex, indicate that Purkinje cells not only change their head velocity sensitivities, but also their eye position and eye velocity sensitivities after chronic learning (Blazquez et al., 2003). The resultant responses of these combined effects at the level of floccular Purkinje cells provide a modulation of the brainstem pathways (see earlier section titled 'Changes in neuronal responses in the adapted state: vestibular afferents') that is appropriate to support the new VOR gains.

3.20.5 Current Models of Long-Term VOR Modulation

3.20.5.1 Support for a Multiple-Site Hypothesis

As reviewed earlier, evidence from single-unit recording experiments indicates that the site of VOR motor learning is not exclusively restricted to the floccular complex or the vestibular nuclei. Immediately following learning, changes in the simple and complex spike activity of Purkinje cells are appropriate to drive this learning in both the horizontal (Raymond and Lisberger, 1997) and vertical

(Hirata and Highstein, 2001) systems. Moreover, the responses of floccular neurons (Miles et al., 1980a; Watanabe, 1984; Lisberger et al., 1994b; Partsalis et al., 1995a, b; Hirata and Highstein, 2001) as well as FTNs in the vestibular nuclei (Lisberger et al., 1994a; Blazquez et al., 2005) remain modified once a new VOR gain state has been firmly established. Analytical solutions of simple models of the brainstem and cerebellar pathways further indicate that gain changes at the levels of both the cerebellum and vestibular nuclei are required to ensure that the eye movements produced by both the visual tracking (pursuit and optokinetic) and VOR pathways remain accurate after VOR motor learning (Lisberger and Sejnowski, 1992; Lisberger et al., 1994a,b; Blazquez et al., 2003, 2006).

The results of lesion studies have furthered the proposal that the site of VOR motor learning is not restricted to either the floccular complex or the vestibular nuclei. These studies provide strong evidence that the gain changes required for VOR adaptation are initially stored in the floccular complex and in turn drive the formation of long-term synaptic changes at the level of the vestibular nuclei such that long-term memory is chiefly consolidated in the brainstem rather than the cerebellum (reviewed in Broussard and Kassardjian, 2004). This process has been termed the transfer or consolidation hypothesis (Galiana, 1986; Peterson et al., 1991; Raymond and Lisberger, 1996; Broussard and Kassardjian, 2004).

Consistent with this proposal is the finding that learned changes in VOR gain are completely abolished by inactivation of the floccular complex immediately following learning (McElligott et al., 1998; Nagao and Kitazawa, 2003), while inactivation of the floccular complex does not completely abolish long-term changes in VOR gain (Luebke and Robinson, 1994; Partsalis et al., 1995a; Broussard and Kassardjian, 2004; Shutoh et al., 2006). Behavioral and single-unit recording studies have provided further evidence for the proposal that memory storage is transferred from the cerebellum to the vestibular nuclei, but only in the long term. This is consistent with the recent observation that chronically acquired VOR gain changes are better retained than acutely acquired VOR gain changes (Kuki et al., 2004). In addition, the relationship between the head velocity sensitivity of floccular Purkinje neurons and VOR gain after learning differs following short-term versus long-term training (**Figure 5**; Hirata and Highstein, 2001; Blazquez et al., 2003). The cellular mechanisms that are thought to mediate short-term versus long-term changes in VOR gain are considered in more detail in the section titled 'Cellular mechanisms of VOR motor learning: evidence for LTD versus LTP.'

3.20.5.2 The Role of the Flocculus versus Ventral Paraflocculus in VOR Learning

In primates, the flocculus proper is defined as the four most medial lobules caudal to the posterolateral fissure. Adjacent to the fissure is a lobule that constitutes a transition zone termed the medial extension, which is then followed by the five to six more rostral lobules, which constitute the ventral paraflocculus. The ventral paraflocculus is particularly well developed in primates, and most single-unit recording experiments have focused on it rather than the flocculus proper. Selective lesions of these different portions of the floccular complex suggest differences in their relative contributions to the generation of smooth-pursuit eye movements and VOR adaptation (Rambold et al., 2002; Nagao and Kitazawa, 2003). Surgical or irreversible chemical lesions of the ventral paraflocculus results in deficits in both behaviors (Rambold et al., 2002). On the other hand, permanent bilateral lesions, which remove only the flocculus proper, do not produce major deficits in smooth pursuit or changes in gain after long-term VOR motor learning (Rambold et al., 2002). In contrast, acute bilateral inactivation of the flocculus proper immediately following VOR short-term adaptation impairs learned VOR gains (Nagao and Kitazawa, 2003; Shutoh et al., 2006). Taken together, these findings have led to the suggestion that the flocculus makes a greater contribution to the acquisition of VOR motor learning than to its long-term consolidation or retention.

3.20.6 Cellular Mechanisms of VOR Motor Learning: Evidence for LTD versus LTP

3.20.6.1 Cellular Mechanisms for VOR Motor Learning in the Flocculus

As described earlier in the section titled 'Current models of long-term VOR modulation,' there is now general agreement that the gain changes required for VOR adaptation are initially stored in the floccular complex, and the modulation of the Purkinje cell response in turn drives the formation

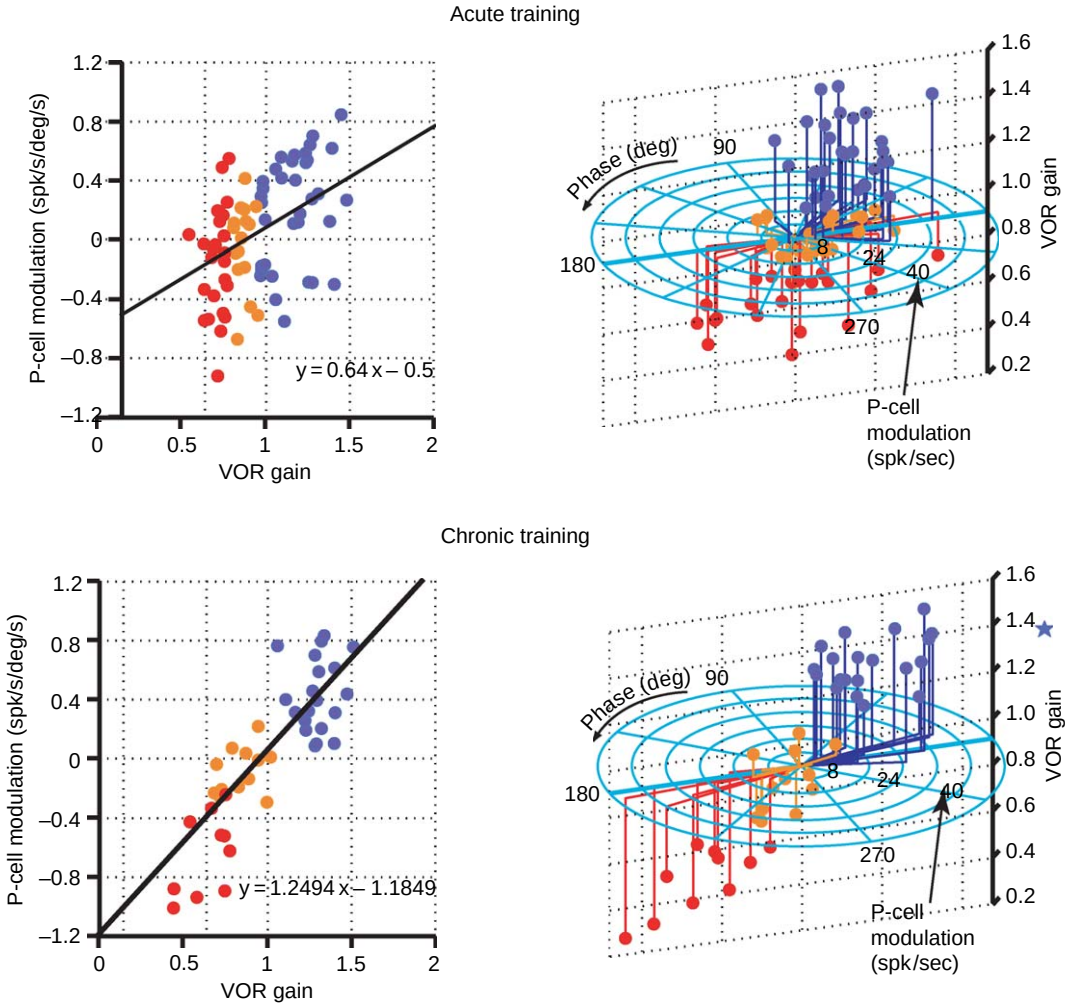

Figure 5 The effects of acute (top row) and chronic (bottom row) training on Purkinje cell responses during VOR for normal (yellow circles), versus low gain (red circles) and high gain (blue circles) adapted animals. Plots on the left show Purkinje cell response sensitivity during head rotation (i.e., (sp/s) per (deg/s)) as a function of VOR gain. Figures on the right are three-dimensional representations where the modulation amplitude for each cell is the length of the line running flat along the circle. The phase of modulation is the degree of the circle, where zero degrees is in phase with head velocity. VOR gain is plotted along the vertical axis. Courtesy of Steve Highstein.

of long-term synaptic changes at the level of the vestibular nuclei. Much recent work has focused on the cellular mechanisms that underlie VOR motor leaning at the level of the flocculus and vestibular nuclei.

The existence of long-term depression (LTD) at the Purkinje cell-parallel fiber synapse was first established by Ito et al. (1982) using electrophysiological techniques. Stimulation of the climbing fiber input to the flocculus reduced the strength of the Purkinje cell-parallel synapse when parallel fibers were simultaneously activated. Subsequent to Ito's original experiments, substantial additional evidence has accumulated in support of the proposal that cerebellar LTD plays a critical role in VOR motor learning. Inhibition of the signaling pathways that are required for induction of cerebellar LTD in normal animals prevents short-term motor learning in the VOR (Nagao and Ito, 1991; Li et al., 1995). Moreover, transgenic mice that express an inhibitor of protein kinase C, an enzyme that is involved in LTD, cannot adapt their VOR gain during short-term motor learning (de Zeeuw et al., 1998; van Alphen et al., 2002). Similarly, transgenic mice

lacking the $\delta 2$ subunit of the glutamate receptor (GluR$\delta 2$), which is required for LTD, do not show VOR motor learning (Katoh et al., 2005).

3.20.6.2 Cellular Mechanisms for VOR Motor Learning in the Vestibular Nuclei

The results of recent *in vitro* experiments have provided insights into the cellular mechanisms that underlie VOR motor learning at the level of the vestibular nuclei. High-frequency stimulation of vestibular nerve can evoke both LTD and long-term potentiation (LTP) in the vestibular nuclei (Caria et al., 1996, 2001; Grassi et al., 2001). The induction of either form of synaptic plasticity requires the activation of *N*-methyl-D-aspartate (NMDA) (Capocchi et al., 1992; Grassi et al., 1995) as well as metabotropic glutamate (Grassi et al., 1998, 2002, 2005) receptors. Interestingly, an animal's past visual or vestibular experience can alter the mechanisms that underlie cellular synaptic plasticity in the vestibular nucleus. For example, neurons in the ventral vestibular nuclei normally exhibit a shift from LTD to LTP during development (Grassi et al., 2004). This shift does not occur in animals that have experienced early visual deprivation or limited vestibular stimulation. Moreover, lack of visual input early in maturation alters the balance of mGluR1 versus mGluR5 receptors (Puyal et al., 2003). Accordingly, it is possible that the presence of visual signals such as retinal slip error (**Figure 3**) might induce changes in the strength of vestibular afferent–vestibular nuclei synapses to facilitate VOR motor learning.

3.20.6.3 Cellular Mechanisms Underlying Increases versus Decreases in VOR Gain

The results of behavioral analysis of VOR learning provide support for the idea that learned increases versus decreases in VOR gain are achieved by means of different plasticity mechanisms. Following learning, increases in VOR gain decay far more rapidly (Miles and Eighmy, 1980) and can be reversed more easily (Boyden and Raymond, 2003) than decreases in VOR gain. This has been furthered by recent studies that have addressed the underlying mechanisms of these differences using genetic manipulations and pharmacological approaches (reviewed in Boyden et al., 2004). Increases and decreases in VOR gain appear to be mediated by plastic changes in common locations because cerebellar lesions have comparable effects in both directions (Michnovicz and Bennett, 1987; Luebke and Robinson, 1994; Pastor et al., 1994; Partsalis et al., 1995b; McElligott et al., 1998). Ito's original model of VOR motor learning (1972) assumed that increases and decreases in VOR gain are governed by a single synaptic plasticity mechanism in the cerebellum, namely LTD. Most recently, however, the results of electrophysiological experiments that have shown that LTP as well as LTD can be induced at parallel fiber–Purkinje cell synapses (Coesmans et al., 2004; Lev-Ram et al., 2003). Thus, it has been suggested that cerebellar LTD at the parallel fiber–Purkinje cell synapse mediates increases in VOR gain, whereas LTP of the same synapse mediates decreases in VOR gain (**Figure 6**; Boyden and Raymond, 2003).

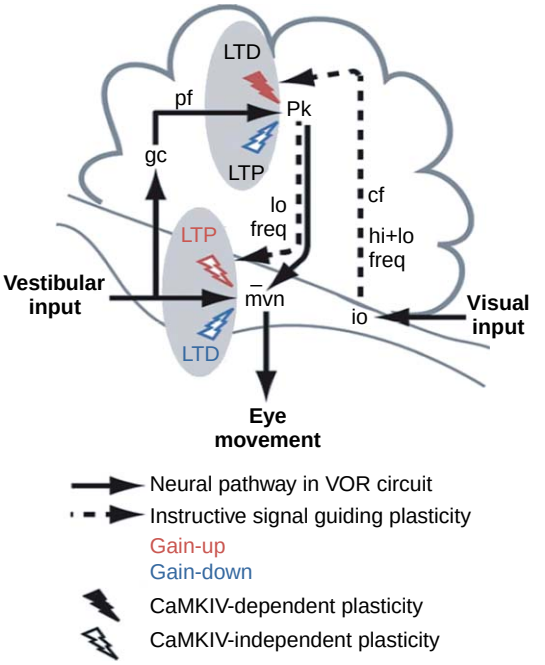

Figure 6 A model that explains differences in gain-up versus gain-down learning in the VOR. Purkinje cells carry only information about the required direction of learning for low-frequency training, whereas climbing fibers carry instructive signals during both high- and low-frequency training. As a result, during low-frequency training Purkinje cells induce plasticity (open lightning bolts) in the vestibular nuclei. In contrast, climbing fiber activity in the cerebellum (filled lightning bolts) also contributes to plasticity during high-frequency training. Learning in the cerebellum that requires LTD could increase VOR gain (red), whereas that which requires LTP could decrease VOR gain (blue). Abbreviations: cf, climbing fibers; gc, granule cells; io, inferior olive; pf, parallel fibers; Pk, Purkinje cells; mvn, medial vestibular nuclei. From Boyden ES, Katoh A, Pyle JL, Chatila TA, Tsien RW, and Raymond JL (2006) Selective engagement of plasticity mechanisms for motor memory storage. *Neuron* 51: 823–824.

3.20.6.4 Cellular Mechanisms: Open Questions

Although both LTD and LTP can also be induced in the vestibular nuclei, it is unclear whether the firing of a Purkinje cell induces depression and/or potentiation at these synapses (Babalian and Vidal, 2000). When LTD is induced in floccular Purkinje cells in an isolated whole-brain preparation, traces of flocculus-dependent plasticity cannot be detected in the FTNs within vestibular nuclei (Babalian and Vidal, 2000). Moreover, recent studies in transgenic mice have further shown that LTD is not required for all forms of VOR plasticity. As discussed earlier in the section titled 'Cellular mechanisms for VOR motor learning in the flocculus,' when LTD is blocked via inhibition of protein kinase C, neither short- nor long-term learning can be induced by visual-vestibular mismatch training. In contrast, vestibular compensation following unilateral damage to the vestibular sensory organs is not altered by the absence of LTD (Faulstich et al., 2006). Thus it appears that non-LTD-dependant pathways can mediate compensation. One possibility is that the synaptic plasticity of excitatory mossy fiber inputs to cerebellar nuclear neurons (Pugh and Raman, 2006; Zhang and Linden, 2006) contributes to non-LTD-dependent motor learning. Moreover, noncerebellar pathways can make a significant contribution to long-term changes in VOR gain following vestibular compensation (Cullen et al., 2006). Further studies will be required to fully understand the mechanisms that underlie VOR plasticity, particularly for learning that occurs following vestibular damage.

3.20.7 Context-Dependent VOR Motor Learning

3.20.7.1 Stimulus-Dependent VOR Motor Learning: Behavior

VOR adaptation can be specific for the stimulus parameters that are used during the induction of motor learning. Numerous studies have established that the amount of adaptive gain change exhibited by the angular VOR is related to the frequency of head rotation that was used to induce motor learning during training (Lisberger et al., 1983; Raymond and Lisberger, 1996; Kramer et al., 1998). The VOR shows the greatest changes at the adapting frequency, and gain changes induced by adaptation at higher frequencies are less frequency specific (i.e., more generalized) than those induced using lower frequencies. This idea has been furthered by the demonstration that the dominant linear component of the VOR response and a frequency-dependent nonlinearity (a rise in gain with increasing velocity of rotation at frequencies more than 2 Hz), which is observed only at higher frequencies (Minor et al., 1999) can be differentially adapted (Clendaniel et al., 2002). The nonlinear component of the VOR was only modified by the training protocols that evoked this component of the response.

The mechanism underlying these frequency-dependent differences in generalization of VOR adaptation are not yet well understood but several possibilities (see discussion by Boyden et al., 2004) can be reconciled with the results of behavioral investigations. On the one hand it is possible that a single plasticity mechanism underlies this frequency-specific adaptation. This could be the case if most neurons in a given structure (i.e., floccular Purkinje cells and/or vestibular nuclei neurons) respond to high-frequency stimulation, but only a subset of these are also sensitive to lower frequencies of stimulation. In this condition, more generalized plasticity would be induced in the circuit by higher-frequency stimulation because it would recruit most neurons. On the other hand, it is tempting to consider the alternative possibility that the dependence of generalization on training frequency results from the existence of two or more distinct plasticity mechanisms.

Evidence that blocking LTD alters the ability to adapt to high- versus low-frequency training protocols is consistent with the latter idea. Knockout mice that lack a protein kinase (CaMKIV) required for LTD (Boyden et al., 2006) show that VOR learning induced by higher-frequency (1 Hz) training is impaired, whereas adaptation induced by lower-frequency stimulation is not altered (0.5 Hz). The results further suggest that increases in gain induced with high-frequency training are mediated by different cellular/molecular plasticity mechanisms than those recruited by decreases in gain or increases in gain induced with low-frequency training. Findings from previous single-unit recording from floccular Purkinje cells provide additional support that different mechanisms guide learning for high- versus low-frequency training (Raymond and Lisberger, 1998). Climbing-fiber responses contained the information required to guide learning only at high-stimulus frequencies, whereas at lower frequencies both

climbing-fiber and simple-spike signals contained the information required to appropriately guide learning. This has led to the proposal that changes in the cerebellum (i.e., changes in the efficacy of the parallel fiber Purkinje cell synapse) specifically induces VOR gain changes at higher frequencies, whereas changes at the level of the vestibular nuclei (driven by the simple spike output of Purkinje cells) can induce gain changes during low-frequency training.

3.20.7.2 Stimulus-Dependent VOR Motor Learning: Neuronal Pathways

The VOR is capable of remarkable adjustments in response to environmental challenges that include lesions of the vestibular system as well as the use of magnifying or minimizing optical lenses. Immediately following unilateral labyrinthectomy, there is a marked asymmetry in gain characterized by diminished responses to rotations toward the lesioned side. Although the VOR shows nearly complete functional recovery for head rotations at lower frequencies (<4 Hz) and velocities (<50 deg/s) the VOR never fully compensates in response to rotations with higher frequencies and/or velocities (reviewed in Sadeghi et al., 2006).

Previous work in squirrel monkeys has demonstrated the presence of linear and nonlinear components to the horizontal vestibulo–ocular reflex (VOR). The nonlinear component is a velocity-dependent gain enhancement (see earlier discussion in the section titled 'Stimulus-dependent VOR motor learning: behavior'). In normal squirrel monkeys, the nonlinear pathway makes only a small (10–15%) contribution to the overall VOR response. The influence of this pathway, however, increases significantly following adaptation to unilateral vestibular damage or motor learning magnifying spectacles, suggesting it plays an important role in VOR adaptation and compensation (Lasker et al., 1999, 2000). Recent work provides support for this idea in old world monkeys and humans (Della Santina et al., 2001; Sadeghi et al., 2006).

Combined *in vivo*/*in vitro* studies have shown that neurons in the vestibular nuclei can be roughly separated into two groups (Type A and B) based on the shape of their action potentials and the subsequent after hyperpolarizations (Serafin et al., 1991a, b). Analysis of the intrinsic membrane dynamics of each cell class suggests that Type B neurons function as active filters, which promote high-frequency responses, whereas Type A neurons behave more like low-pass filters (Ris et al., 2001; Sekirnjak and du Lac, 2002; Beraneck et al., 2003). Thus, it has been proposed that Type A and Type B neurons can be considered channels for encoding low- and high-frequency signals, respectively (Av-Ron and Vidal, 1999; Ris et al., 2001).

Interestingly, the responses of Type A and B neurons resemble the dynamics of the linear (tonic) and nonlinear (phasic) VOR pathways, respectively, described by Minor and colleagues (1999). In addition, some Type B neurons preferentially receive inputs from the flocculus (Babalian and Vidal, 2000; Sekirnjak et al., 2002). Thus, the Type B cells that have been described in *in vitro* studies correspond to the FTN/EH neurons that have been shown to play an important role in lens-induced adaptation in *in vivo* studies (see discussion earlier in the section titled 'Changes in neural responses in the adapted state: vestibular afferents'). Taken together, these findings provide support for the idea that neurons with more phasic membrane properties play the principal role in mediating vestibular compensation and adaptation at the level of the vestibular nuclei.

3.20.7.3 Context-Specific Changes in VOR Gain

A number of recent studies provide firm evidence that subjects can store more than one VOR gain state, where each state is associated with a particular context. Eye position, vergence angle and otolith signals (i.e., head orientation) have all been shown to provide cues that can be used for inducing contextually dependent changes in VOR gain. Shelhamer and colleagues (1992) showed that when the angular VOR was adapted using magnifying and minimizing prisms with subjects' eyes looking up and down, respectively, the gain of the VOR following the adaptation was consistently reduced or increased when subjects looked downward or upward. Comparable results were shown for the translational VOR by the same group (Patel et al., 1998). Similarly, the angular VOR can be trained to store two different gain states by training that requires different gains for two vergence angles (i.e., diverged versus converged conditions; Lewis et al., 2003). The adapted VOR is characterized by different gain states, which are immediately accessed in a vergence specific manner that corresponds to the training context.

Similarly, several studies have shown that static otolith signals can provide strong contextual cues for gating the expression of different VOR gain states.

When the VOR is adapted with the head in a specific orientation relative to gravity, gain changes following adaptation of the angular VOR (Baker et al., 1987; Tiliket et al., 1993; Yakushin et al., 2000) and translational VOR (Shelhamer et al., 2002) are maximal when the head position is in the same position in which the gain had been adapted. These VOR gain changes are stored in a manner that is linked to the context of the head orientation in which changes were induced and fall off to zero when the head is in the opposite position (**Figure 7**; Yakushin et al., 2005). Gravity-specific adaptation of the VOR, like vergence-specific adaptation, can also be adapted to so that the VOR is adapted to two or even three specific gain states, each of which will be optimal for a different specific head position (e.g., Shelhamer et al., 2002, Xiang et al., 2006).

The context-specific VOR adaptation described appears to be strongly associated with the specifics of the stimulus parameters that are used during VOR adaptation (e.g., frequency of stimulation or eye movements that are induced during the training, see the section titled 'Stimulus-dependent VOR motor learning: behavior'). For example, the context-specific adaptation of translational VOR is greatest for head movement stimuli that are similar to those used during training (Shelhamer and Zee, 2003), and this frequency dependency is even more striking than for animals trained to a single gain state (Shelhamer et al., 2000). Other recent studies have

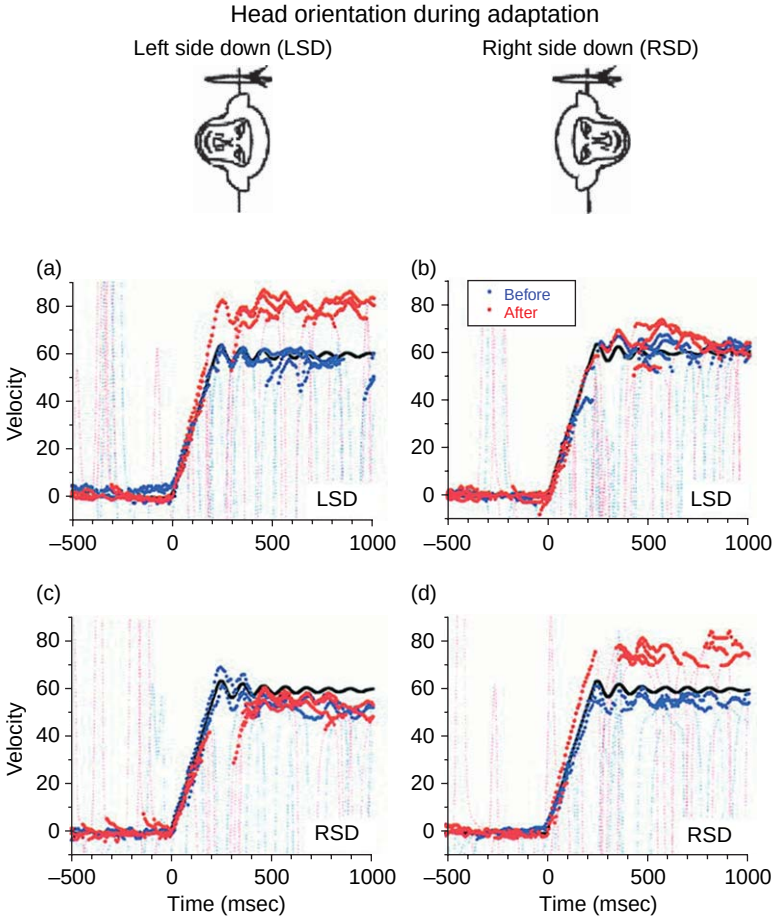

Figure 7 Step responses of the VOR when the animal was adapted left side down (a and c), and right side down (b and d). Eye velocities evoked by rotation before and after adaptation are shown in blue and red, respectively. The head velocity stimulus is shown in black. The direction of the stimulus was reversed to facilitate comparison. When the animal was adapted on one side, increases in eye velocity were significant when the animal was tested with the same side down (a and d), but there were no increases in eye velocity when the contralateral side was down (b and c). From Yakushin SB, Raphan T, and Cohen B (2000) Context-specific adaptation of the vertical vestibuloocular reflex with regard to gravity. *J. Neurophysiol.* 84: 3067–3071.

shown that motor learning can be specific to the particular combination of head motion and evoked eye movement that occurred during training. For example, when one eye is aligned with direction of head movement during adaptation of the translational VOR, it will remain motionless during training (Zhou et al., 2003). In this condition, VOR adaptation is only expressed in the eye that moved during the training.

3.20.8 Conclusions

The results of recent behavioral, single-unit recording and lesion studies have established that VOR motor learning induced by visual-vestibular discrepancy passes through distinct stages. Synaptic changes in the floccular complex underlie the initial induction of VOR adaptation. These changes, in turn, drive the formation of long-term synaptic changes at the level of the vestibular nuclei such that long-term memory is, for the most part, consolidated in the brainstem rather than in the cerebellum. Significant progress has also been made toward understanding the cellular mechanisms that underlie VOR adaptation in both the floccular complex and vestibular nuclei. Experiments in *in vitro* and isolated brain preparations as well as transgenic animals have shown that LTD and LTP can be induced at the parallel fiber–Purkinje cell as well as vestibular afferent–vestibular nuclei synapses. This has been furthered by recent studies that provide support of the idea that different cellular mechanisms induce changes at the parallel fiber–Purkinje cell synapse to induce increases versus decreases in VOR gain. Further investigation will be needed to address many important open questions. For example, we do not yet understand the mechanisms that ultimately drive the formation of long-term synaptic changes at the level of the vestibular nuclei. In addition, the mechanisms that underlie another form of VOR motor learning, namely compensation of the VOR following vestibular damage, are not fully understood.

Although we have gained considerable insights into the neural mechanisms that induce and encode gain changes in the VOR circuitry to support its adaptation and compensation, recent findings by several laboratories have highlighted another key feature of VOR adaptation. Current vergence angle or eye position, otolith-derived signals (i.e., head position), as well as attributes of the head velocity stimulus itself provide cues that can be used for inducing contextually dependent changes in VOR gain. This implies that, at a given instant in time, more than one VOR gain state is stored, and that the particular context will determine which state is retrieved.

Context-specific adaptation has similarly been demonstrated in other motor systems, for example, during ocular pursuit (Takagi et al., 2000), as well as reaching (Lewis and Tamargo, 2001), pointing (Welch et al., 1993), and throwing (Martin et al., 1996) movements. The ability to retrieve motor memories in a context-dependent manner is important for everyday activities. For example, during head rotation, the eyes translate as well as rotate relative to space because they cannot both be perfectly aligned with the axis of rotation. Consequently, a larger VOR gain is necessary to stabilize a near than a far earth-fixed target as a result of the differences in the translation of the target relative to the eyes. Thus, vergence-specific adaptation of the angular VOR is required to ensure gaze stability over a wide range of viewing conditions. Similarly, people who wear bifocal spectacles require a different VOR gain state for each lens magnification, each of which could be retrieved on the basis of current vertical eye position. It remains a significant challenge to understand the mechanisms that underlie context-specific adaptation; nevertheless, the relative simplicity of the vestibular system and the pathways that mediate the VOR make it an excellent model system for bridging the gap between brain and behavior.

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3.21 Neurophysiology of Motor Skill Learning

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3.21.1 Introduction

Motor performance improves with practice. Movements are initially slow, variable, uncoordinated, and fractionated. After numerous repetitions, movements become fast, stereotyped, coordinated, and smooth, so that individual joint contractions blend into a single, transitionless action. Several aspects of cognitive and motor processing must be refined in order for such high levels of motor performance to be attained. Over the past several years, neurophysiological studies in experimental animals, especially in nonhuman primates, as well as neuroimaging studies in humans, have provided a wealth of data, which has helped to clarify the neural processes and structures underlying the development of skilled motor behavior. It now appears that a broad network of structures in the cerebral cortex, striatum, and cerebellum coordinate their activity to enable skilled motor behavior. Each of these structures probably plays a different role in either motor planning or execution, and some of these structures are critical to the learning of new motor tasks. This is evident since activity patterns are correlated with specific phases of learning, and this activity is altered as tasks are practiced and become highly skilled. In this chapter, we review the evidence from

neurophysiological, neuroanatomical, and neuroimaging studies that shed light on brain mechanisms underlying motor skill learning.

3.21.2 Definition of Motor Skill Learning

As used in this chapter, motor skill refers primarily to fine motor skill, such as might be required in picking up small objects, writing, typing, and so on, and not gross motor skill, such as might be required to sit upright, crawl, or walk. While the learning of gross motor skills is important during development, during activities of daily living, and during recovery from certain severe neurological disorders, the current chapter focuses on fine motor skill and its acquisition. Skill learning is sometimes referred to as habit learning, defined as the process of successive adaptation of behavioral responses to contingencies in environmental stimuli (Mishkin et al., 1984). Habit or skill learning is one of several forms of implicit learning thought to be mediated by neural networks in the cerebellum, frontal cortex, and basal ganglia. Implicit learning is contrasted with declarative learning, which involves the temporal lobe (Squire, 2004).

Although a general sense of what is meant by motor skill exists among researchers, there is no general agreement regarding what constitutes the acquisition of motor skill. In our view, motor skill learning is operationally defined as a change in motor behavior, specifically referring to the increased use of novel, task-specific joint sequences and combinations, resulting from practice and/or repetition. Others have argued that changes in the speed of movement, even if not accompanied by reciprocal changes in the accuracy of movement, indicate skill learning (Fitts, 1954; Hallett et al., 1996). However, it should be cautioned that speed as the sole criterion for evidence of skill learning may, at least in some situations, be confounded, as they may occur as a function of motivational state, also called dispositional learning (Amsel, 1992).

3.21.3 Central Nervous System Structures Involved in Motor Skill Learning

It is now clear that the motor cortex does not execute motor tasks in isolation. A broad network minimally involving the primary and secondary motor areas, the dorsolateral prefrontal cortex, the posterior parietal cortex, the striatum, and the cerebellum is involved in both skill acquisition and in motor execution. One goal of recent research has been to differentiate the role of various structures in the actual movement execution versus the role of the structure in learning the task (usually a movement sequence) or storage of the learned motor program. While both cortical and subcortical structures are clearly involved in motor learning, this chapter focuses on cortical motor areas and their role in skill learning.

Classically, motor skill was viewed as a serial process. Premotor areas were thought to be critical for the learning and storage of motor sequences. Premotor areas were thought to send information to the primary motor cortex for execution of motor behavior via descending pathways to the spinal cord. While certain neuroanatomical and neurophysiological features of premotor and primary motor areas support this concept, the more modern view recognizes that each of the cortical motor areas are, to some extent, involved in motor learning and the storage of motor commands, and that motor skill does not reside in any one place. However, these areas may differ in their degree of involvement in skilled motor behaviors, depending upon various task demands (e.g., role of visual or somatosensory guidance, cognitive demands, bimanual involvement, etc.). Here we review both the basic anatomy and physiology of these various motor areas and evidence for their differential roles in motor skill learning.

3.21.3.1 Cortical Motor Areas in Nonhuman Primates

It is generally accepted that no single feature is sufficient for characterizing an area as a distinct region. Features used to define cortical motor fields include cytoarchitectonics, patterns of afferent and efferent connections, features of intrinsic connectivity, chemoarchitectonics, behavioral effects of ablation, and, particularly for motor cortical areas, the ability to elicit movements upon electrical stimulation.

A differentiated motor field has a unique cytoarchitecture, traditionally defined by stains for Nissl bodies and myelin. Additionally, areas have been examined and characterized based on cytochrome oxidase staining, acetylcholinesterase staining, neurofilament antibody staining, and receptor binding. Unique characteristics of cell types, laminar organization, cell density, fiber density, and various staining densities

are all used for characterization. Extensive tract tracing studies have been used to identify subareas of motor cortex based on differential afferent and efferent connections with the thalamus, basal ganglia, and other cortical areas, as well as their projections to the spinal cord. Physiological criteria have also been used for differentiation. Intracortical microstimulation mapping procedures have helped to define somatotopic organization within each motor area, with attention paid to minimal threshold requirements for initiation of movements, as well as the characterization of the movements themselves. The relationship of single-unit activity to behavioral aspects of motor tasks in awake, freely moving animals has also been an important approach to distinguishing motor fields. Finally, motor areas have been characterized based on functional differences in ablation-behavior studies in nonhuman primates and functional imaging studies in humans.

The nomenclature used for subdivisions of primate motor areas has varied across laboratories. The generalized current scheme includes M1 (or Brodmann's area 4); four subdivisions of the lateral premotor cortex (PMd-c, PMd-r, PMv-c, and PMv-r, or F2, F7, F4, and F5, respectively); two premotor subdivisions on the mesial surface of the hemisphere (SMA and pre-SMA, or F3 and F6), and three subdivisions of the cingulate motor area within regions lining the cingulate sulcus (CMAr, CMAd, and CMAv, or area 24c, area 6c, and area 23c). M1 is the most easily recognizable area in histological stains, as it contains very large pyramidal neurons in layer V, the so-called Betz cells. Also, it contains a greatly reduced layer IV, compared with sensory cortex that contains a thick layer IV with substantial numbers of granule cells. For this reason, M1 is sometimes referred to as agranular cortex. **Figure 1** illustrates the location of the main cortical motor areas.

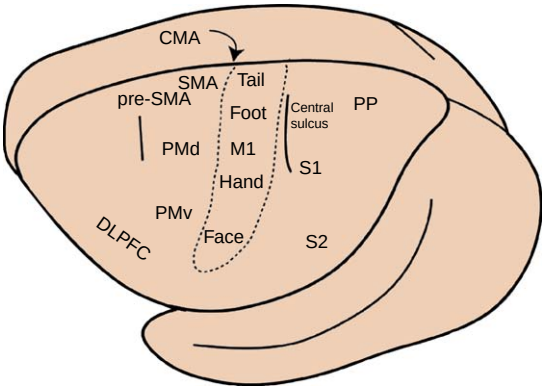

Figure 1 Location of cortical areas involved in motor skill learning and execution. Cortical areas are depicted on the brain of a squirrel monkey, a primate with few convolutions in its cortex. The areas depicted can be subdivided further on the basis of anatomical and physiological criteria. For example, SMA can be divided into a caudal component (SMA proper) and a rostral component (pre-SMA). CMA, cingulate motor areas; SMA, supplementary motor area; PMd, dorsal premotor cortex; PMv, ventral premotor cortex; DLPFC, dorsolateral prefrontal cortex; M1, primary motor cortex; PP, posterior parietal cortex; S1, primary somatosensory cortex; S2, second somatosensory area.

3.21.3.2 Cortical Motor Areas in Rodents

Because rodents are often used to study the role of motor cortex in motor skill learning, a brief comparative account of cortical motor areas in rodents is instructive. (While motor cortex in cats is also frequently a focus of motor learning experiments, this species will not be discussed in this review.) Intracortical microstimulation studies of sensorimotor cortex in the rat have shown a complete motor representation that is cytoarchitectonically defined as agranular cortex (Hall and Lindholm, 1974; Donoghue and Wise, 1982) and commonly called M1 based on its similarities to primate M1. In rodents, the portion of the agranular cortex in caudal portions of frontal cortex that is devoted to forelimb movements is referred to as the caudal forelimb area (CFA). In addition, a second motor representation of the forelimb has been identified in more rostral portions of the frontal cortex. This second forelimb representation, referred to as the rostral forelimb area (RFA), is smaller than the CFA (Neafsey et al., 1986). The RFA is separated from the CFA by a zone where intracortical microstimulation elicits vibrissa or neck movements.

Because the presence of a secondary motor area in rats would appear to parallel the differentiation of motor areas in primates, suggestions have been made that the RFA is a homolog of one of the primate secondary motor areas. Tract tracing studies of motor cortical connections in rat have shown differences in the thalamic, striatal, and cortical connections of CFA and RFA (Rouiller et al., 1993). Comparison of these connections to the pattern of connections of primate motor areas suggests that RFA has some similarities to primate premotor areas. Overall, based on its connections, CFA is more similar to the M1 forelimb area in primates, and RFA in some ways appears to be more similar to nonprimary motor cortex in primates. It is not currently possible to

decide whether RFA is a homolog of primate premotor cortex, supplementary motor areas, or a combination of secondary motor areas in primates (Rouiller et al., 1993). A more comprehensive review of primate and rodent motor areas can be found in Nudo and Frost (2006).

3.21.3.3 Role of Somatosensory Cortex in Motor Skill Learning

While the present chapter focuses on motor output structures, it should be recognized that sensory information plays a critical role in the learning of new motor skills. For example, the somatosensory system is critical for skilled motor behavior. Various somatosensory regions in the parietal lobe of the cerebral cortex are intimately interconnected with motor cortical regions in the frontal lobe. Somatosensory information can be recorded from neurons in M1, and this information is segregated by submodality. Cutaneous inputs arrive in the more caudal aspects of M1, while proprioceptive inputs arrive in the more rostral aspects. Focal experimental lesions in rostral or caudal M1 result in distinct sensory-related deficits in skilled use of the hands. Lesions in either primary somatosensory cortex or M1 in nonhuman primates result in similar deficits. Thus, while there are clear structural and functional distinctions between M1 and S1, they are functionally codependent with regard to skilled motor behaviors.

3.21.4 Organization of Primary Motor Cortex and Its Role in Motor Skill Learning

The vast majority of studies examining the neurophysiological bases for motor skill learning have been performed in the primary motor cortex, or M1. The reasons for this bias are numerous: M1 is very accessible because of its location in the precentral gyrus. The portion of M1 that is most often examined, the M1 hand representation, is located on the dorsolateral surface. In most primates, however, a large portion of the M1 hand representation is buried in the anterior banks of the central sulcus, rendering accurate reconstruction of two-dimensional topographic maps more challenging. Nevertheless, neuronal population studies, especially from cells within the more rostral portions of M1, and to a lesser extent, the caudal portions of M1 within the depths of the central sulcus, are numerous. Thus, from the first functional localization studies in the 1800s to cellular responsivity studies of the present day, M1 is the primary focus of most cortical studies of motor control.

In primates at least, M1 is equivalent to Brodmann's area 4, based on cytoarchitectonic criteria. M1 contains a complete representation of skeletal and orofacial musculature organized in a topographic fashion, but with an exaggerated representation of the hands and face. This organization has been known since the late 1800s, primarily because of muscular contractions evoked from electrical stimulation of the cortical surface and, more recently, by more direct stimulation of the output neurons located in layer V, demonstrated by intracortical microstimulation. The most direct access to motor neurons in the spinal cord is via the corticospinal tract, originating in layer V pyramidal cells and terminating in intermediate and deep laminae of the spinal cord. In many primate species (and possibly other mammalian species), a subset of corticospinal neurons, so-called corticomotoneuronal cells, terminate monosynaptically onto spinal motor neurons. It is now known that corticomotoneuronal cells diverge to innervate multiple (on average, about four or five) motor neuron pools (**Figure 2**). Furthermore, corticomotoneuronal cells with different projection patterns intermingle within each local zone within M1 (Rathelot and Strick, 2006). Thus, the highly organized topographic arrangement inferred from electrical stimulation studies is valid only on a macroscopic scale. Substantial divergence and convergence exist in the connections between M1 and spinal cord motor neurons at a more focal level. It is possible that this anatomical arrangement confers on M1 a certain capacity for functional plasticity, since, depending upon the subset of neurons in a given zone that is activated, a different motor output might result.

M1 can access motor neurons in the spinal cord via more indirect routes also. For example, corticofugal neurons project monosynaptically to the red nucleus, which in turn projects monosynaptically and disynaptically to spinal cord motor neurons. These pathways are topographically organized on a macroscopic scale, much like the direct corticomotoneuronal pathway. Somewhat more diffuse access can be accomplished via M1 projections to the pontine and medullary reticular formation, which in turn projects to the spinal cord.

M1 also contains a rich array of intracortical connections that are thought to modulate the output of corticofugal cells. The role of local corticocortical fibers in shaping responses in the execution of skilled motor tasks is not well known. However, this

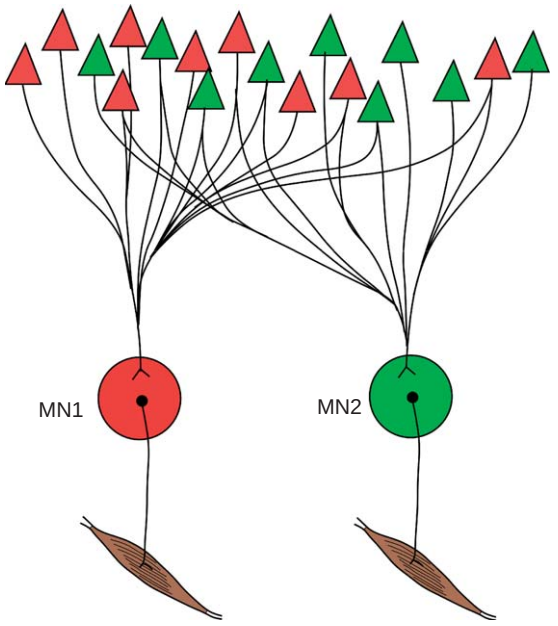

Figure 2 Divergence and convergence of corticospinal neurons in M1. Corticospinal neurons diverge to form monosynaptic connections with up to four or five motor neuron pools. Two such pools, MN1 and MN2, are shown in the figure. Corticospinal neurons projecting to a single motor neuron pool are represented over a large territory within M1. Thus, different corticospinal neuron populations (red and green triangles) are intermixed across the spatial domain of M1.

modulatory influence may provide another important substrate for plasticity in the output properties of motor cortex.

Virtually all mammalian species contain a cortical structure analogous (if not homologous) to primary motor cortex. Corticospinal neurons originating in layer V exist in every mammalian species studied to date, including primates, carnivores, rodents, insectivores, and marsupials. However, the number of corticospinal neurons, their penetration into the cord, their trajectory in the cord, and the proximity of their terminals to motor neuron cell bodies vary widely across species. A motor cortex, as defined by an area of the cerebral cortex whose stimulation results in movements of muscles of the body, seems to be universal among mammals.

3.21.4.1 Neurophysiological Changes in M1 Associated with Motor Skill Learning

Historically, it has been thought that since M1 is so intimately connected to motor neurons in the spinal cord, its role in motor behavior was concerned primarily with low-level execution of muscular contractions. However, several lines of evidence now suggest that it plays a very important role in skill acquisition and motor learning.

Neurophysiological studies in animal models, especially nonhuman primates, provide unique insight into the role of various cortical and subcortical networks in motor skill learning, since the activity of neuronal populations can be tracked over time in the same animals, as they perform a specific task. However, until recently, the most common paradigm was to examine neuronal activity in relation to movement only after an animal had been highly trained for several weeks to months. While this paradigm allows examination of the relationship of neuronal activity to control of movements, it does not provide much insight into the actual learning of the task. This is important since the acquisition of various aspects of motor skill might be mediated by different neuronal structures and processes.

Several human studies using transcranial magnetic stimulation have demonstrated that increased use of muscles expands their motor representations (Pascual-Leone and Torres, 1993; Tyc et al., 2005). Even simple movements repeated over a short period of time are effective in inducing cortical representational changes (Classen et al., 1998). Recent experiments in nonhuman primates have demonstrated that motor representations of hand movements expand in concert with the acquisition of new motor skills (Nudo et al., 1996). In these studies, monkeys were trained on an operant task requiring retrieval of food pellets from small wells. Behavioral performance was initially poor based on timing of retrievals and numbers of digit flexions required per retrieval. Behavioral performance increased asymptotically and reached plateau in about 10 days. At the end of the training period, both individual joint movements and specific joint movement combinations and sequences used by individual animals became represented over larger cortical territories (**Figure 3**). As digit skills were acquired, very little expansion of the total hand representation occurred. Instead, digit and wrist representations became redistributed. These effects were reversible, to some extent, though not completely. This suggests that once a novel motor task is learned, certain aspects of cortical topography are altered for an extremely long period of time. Changes in face motor cortex have also been

Figure 3 Alterations in motor representations in M1 as a result of motor skill training. After several days of practice at a novel motor task (pellet retrievals from small wells), monkeys display larger representations of the fingers and wrist. In addition, representations of multijoint movements (fingers + wrist) emerge. Current thresholds required for activating movements at multijoint sites are particularly low compared with at single-joint sites. From Nudo RJ, Milliken GW, Jenkins WM, and Merzenich MM (1996) Use-dependent alterations of movement representations in primary motor cortex of adult squirrel monkeys. *J. Neurosci.* 16: 785–807.

demonstrated after learning a novel tongue protrusion task (Sessle et al., 2007).

One of the more interesting aspects of these results is that movement combinations that were used in the task became represented over a larger cortical territory. This suggests that temporal correlation of movements (and presumably muscles) drives changes in cortical motor organization. It is possible that local intracortical connections couple various separate modules together to form muscle or movement synergies. After training, at sites where multiple joints are represented (as defined by similar thresholds for activation), the current levels required for evoking the multijoint movements are significantly lower than those required to evoke single-joint movements. Recently, analogous results have been demonstrated using functional magnetic resonance imaging after motor training in humans (McNamara et al., 2007). Thus, training may in some way prime specific intracortical connections to enhance the excitability of subsets of corticomotoneuronal cells that must fire concurrently for skilled tasks to be completed.

Subsequent microelectrode stimulation studies in monkeys and rats confirmed these initial findings and further suggested that skill acquisition was a necessary feature of the behavioral experience for neurophysiological map plasticity to occur, rather than simple movement execution alone. Monkeys that retrieve food pellets from larger wells than those used in the study described do not exhibit the slowly developing asymptotic learning curve compared with monkeys trained on the small-well task. Even though they execute the same number of digit movements, these animals demonstrate no systematic change in motor map topography in M1 (Plautz et al., 2000). Similar findings have been found in rodents. Rats trained in a novel task to retrieve food pellets from a rotating platform demonstrate expansions of distal forelimb representations in M1. However, rats trained to press a bar repeatedly with their distal forelimb show no map changes (Kleim et al., 1998). Presumably, the large-well pellet retrieval task in the monkey and the bar pressing task in the rat are skills that had already been acquired in the normal behavior of the animal. Only when novel tasks are taught that require the acquisition of new joint movement combinations is the motor cortex topography altered significantly.

Another paradigm for motor training, strength training, also does not appear to alter motor maps in M1. In comparison to nontrained control animals, rats that are trained to repetitively grasp and break a strand of dried pasta show expanded representations of the distal forelimb, presumably due to the novel skill required by the task. However, if rats are trained to break progressively larger bundles of pasta strands, a similar task that simply requires more force, the same expansions occur. That is, no further reorganization in distal forelimb representations occurs with strength training (Remple et al., 2001).

At the cellular level, both long-term potentiation (LTP) and long-term depression (LTD) can be generated in somatosensory and motor cortex of rats under specific conditions (Castro-Alamancos et al., 1995; Rioult-Pedotti et al., 2000). It has been suggested that extensive intracortical connections in superficial layers of motor cortex might undergo changes in synaptic efficacy that underlie neurophysiological changes in motor cortex during motor learning. In rats, when one limb is trained on a motor task, the amplitude of field potentials contralateral to the trained limb is significantly increased relative to the cortex opposite the untrained limb. Further, the trained cortex is less amenable to subsequent LTP induction (Rioult-Pedotti et al., 1998; Hodgson et al., 2005). However, others have argued that learning motor skill acquisition produces bidirectional changes in synaptic strength distributed throughout the intracortical networks of motor cortex. Unidirectional changes in the population of neural elements are suggested to occur during certain behavioral states not directly related to the learning process (i.e., stress; Cohen and Castro-Alamancos, 2005).

3.21.4.2 Neuroanatomical Correlates of Motor Skill Training in M1

Skill training is also associated with changes in the microanatomy within motor cortex, including dendritic reorganization, synaptogenesis, and changes in

synapse morphology. Training rats on an acrobatic task that requires them to traverse a series of obstacles (horizontal ladder, grid platform, rope, barriers) results in increases in synapse to neuron ratios and dendritic processes in motor cortex (Jones et al., 1999; Bury and Jones, 2002). Similar synaptic and dendritic changes occur with reach training in rats (Withers and Greenough, 1989; Kleim et al., 2002). Training-induced structural reorganization is limited to the motor cortex region that undergoes neurophysiological reorganization (Kleim et al., 2002). More recent immunohistochemical studies have attempted to determine the proteins that are involved in these morphological changes. In rats, synaptophysin and GAP-43 expression appear to be correlated with the first 5 days of motor skill learning. However, microtubule-associated protein 2 (MAP-2) was not influenced by learning (Derksen et al., 2006). Brain-derived neurotrophic factor (BDNF) appears to be involved in learning-related plasticity in motor cortex. If BDNF is inhibited by injection of antisense oligodeoxynucleotides, receptor antagonists, or BDNF receptor antibodies, motor skill is impaired and neurophysiological organization is disrupted (Kleim et al., 2003; Adkins et al., 2006).

While studies at the genetic and molecular level of analysis are still few in number, recent studies suggest a time-dependent change in corticostriatal expression patterns of immediate early genes during motor training (Hernandez et al., 2006).

3.21.5 Secondary Motor Areas and Their Role in Motor Skill Learning

In addition to M1, there are several secondary motor areas (SMAs) recognized in the primate cortex (**Figure 1**). These areas have been defined as having direct connections to both M1 and the spinal cord. The premotor cortex, the SMA, and the cingulate motor cortex have been identified in all primate species examined, including prosimian primates. Each of these secondary areas has been divided into subareas based on differences in cortical architecture that are related to hodological and functional differences. The lateral premotor area is divided into ventral and dorsal areas (PMv and PMd, respectively), the SMA into SMA proper and pre-SMA, and the cingulate motor cortex into rostral (CMAr) and caudal (CMAc) divisions. As many as 10 motor fields emerged early in primate evolution (Wu et al., 2000).

The premotor and supplementary motor areas appear to be involved in different aspects of movement compared to M1. This has been suggested simply from the timing of activity in the various cortical motor regions. Using time-resolved fMRI during a delayed cued finger movement task, activity in M1 was substantially weaker during movement preparation compared with during movement execution. However, activity in PM and SMA was equally high during both phases of the task (Richter et al., 1997).

3.21.5.1 Role of the SMA in Motor Skill Learning

A significant role in the planning, preparation, and initiation of voluntary movement has been ascribed to the secondary motor areas on the medial wall of the hemispheres, especially the supplementary motor area. Early stimulation studies demonstrating complex movements evoked at higher currents from stimulation of SMA first suggested that the medial motor areas are involved in more complex aspects of motor behavior (Thickbroom et al., 2000). Later, neuroimaging studies implicated the role of SMA in higher-order processes. In one very influential study examining cerebral blood flow, for example, the SMA was the only area activated when subjects mentally rehearsed a finger tapping sequence without actually executing it (Roland et al., 1980).

Based on these early results, the focus for SMA studies has been on its role in complex aspects of motor behavior, such as movement planning and sequencing. In daily activities, discrete movements must be coordinated in the proper sequence, and transitions from one movement to the next must be accomplished smoothly and rapidly. As movement sequences are practiced, the individual movements become more stereotyped (i.e., with decreased variability in kinematics and kinetics), and the time between different movements decreases. Eventually, one movement component blends into the next, and a smooth, coordinated action results. The SMA is thought to participate in this sequencing function. But it should be recognized that sequence learning requires several neural systems that are involved in cognitive, perceptual, motoric, and temporal aspects of learning (Grafton et al., 1998). Motor behaviors are controlled by a distributed network, and it may be too simplistic to think in terms of compartmentalized units that have mutually exclusive functions. For example, a recent 2-deoxyglucose study in nonhuman primates

showed substantial metabolic activity in both SMA and pre-SMA associated with visually guided reaching movements. The intensity of activation was comparable to that of M1 in the same animals (Picard and Strick, 2003). Most neurons in SMA are active in relation to effector-related variables. However, a modest number appear to represent target direction independent of reach direction. Further, the largest number of neurons in SMA appears to be context-dependent, that is, activity is directional in one visuomotor mapping condition, but not others (Crutcher et al., 2004). While it is now clear that these areas are involved in diverse aspects of movement control, planning and timing are the major focus of investigations.

Many studies in nonhuman primates have demonstrated that the supplementary motor area is important in the control of sequential movements. Early studies demonstrated that SMA neurons are active during movements of either extremity (Brinkman and Porter, 1983), and SMA lesions result in deficits in bimanual coordination (Brinkman, 1981). More recent studies demonstrated that removal of SMA in monkeys does not cause paralysis or akinesia. However, monkeys were impaired at performance of a simple learned task in which they were required to raise their arm to obtain a food reward below (Thaler et al., 1995). They were less impaired if the task was paced by an external cue (tone). These deficits are similar to those observed in humans with damage to SMA. Patients with lesions involving the SMA, but sparing the lateral hemispheric surface, demonstrated difficulties in producing rhythmic motion unless the task was guided by auditory pacing (Halsband et al., 1993). These data have been cited as evidence for the role of SMA in self-initiated actions.

SMA is thought to play a role in modulating motor output based on kinesthetic inputs. A large percentage of neurons in SMA respond differentially to different instructions during a preparatory state (Tanji et al., 1980; Passingham, 1987). Also, neuronal discharge within SMA typically precedes activity in M1 (Deecke et al., 1985), but SMA response properties differ from those in M1 in that SMA activity is related to factors other than the execution of movement to a greater extent than neurons in M1 (Kurata and Tanji, 1985).

Neuronal activity in SMA is also thought to reflect internal models of movement dynamics, or the forces exerted by contracting muscles. Dynamic related signals are nearly comparable in SMA and M1 (Crutcher and Alexander, 1990). Movement dynamics are significantly represented in SMA during both motor planning and execution. This property is likely to play a particularly important role in motor learning, as SMA neurons shift their preferred direction in the direction of an external force when monkeys adapt to a perturbing force field, and back in the other direction when the monkeys readapt to the nonperturbed direction. This shift occurs during the instructed delay and during the movement-related time window (Padoa-Schioppa et al., 2004).

3.21.5.2 Two SMAs: Different Roles for SMA and Pre-SMA

Two SMA representations can be differentiated in primate species based on distinct cytoarchitecture, intracortical microstimulation, neuronal response properties, and connection patterns. These two areas, located on the mesial aspect of the frontal cortex, are referred to as SMA proper (or simply SMA; also called F3) and the pre-SMA (also called F6), situated more rostrally (Wu et al., 2000). Typically, in human neuroimaging studies, the SMA is differentiated from pre-SMA based their location relative to the anterior commissure, with the SMA proper lying caudal to the level of the commissure, and the pre-SMA lying rostral to the commissure. However, diffusion tensor imaging studies have revealed different patterns of connections in SMA and pre-SMA, and thus, more refined identification methods in humans are now available (Lehericy et al., 2004).

3.21.5.2.1 Basic differences in physiology and anatomy of SMA/pre-SMA

SMA contains a complete motor representation with the face-arm-leg regions arranged rostrocaudally (Luppino et al., 1991). Movements are elicited at low current thresholds. Arm movements can be elicited from pre-SMA, though larger currents are required (Matsuzaka et al., 1992). Compared to SMA, pre-SMA has only sparse spinal projections but connects more heavily with prefrontal cortex. For this reason, some investigators consider pre-SMA (as well as pre-PMd) to be a prefrontal cortical region rather than a premotor area. In general, SMA proper is thought to be involved primarily in simple tasks, while pre-SMA is activated during relatively complex tasks (Picard and Strick, 2001).

3.21.5.2.2 Role of SMA/pre-SMA in learning of motor sequences

Many cells in SMA and pre-SMA fire preferentially during the performance of new sequences (Nakamura et al., 1998). Studies of the role of SMA and pre-SMA in sequence learning have focused primarily on either movement sequences with the upper extremity or sequences of saccades (Muri et al., 1994). Neuronal responses to visual stimuli predominate in pre-SMA, while responses to somatosensory stimuli predominate in SMA proper (Matsuzaka et al., 1992). However, the current review focuses exclusively on movements of the upper extremity, and not on saccades.

Neurons in SMA/pre-SMA that are related to sequence learning start discharging after the illumination of the stimuli for each set; the discharge rate increases until the monkey presses the first button. As learning proceeds, these neurons discharge progressively less, so that in highly learned sequences, almost no activity is evident. Such neurons that preferentially are activated during new sequences are more common in pre-SMA. This role has often been referred to as reprogramming. In fact, neurophysiological studies in monkeys demonstrate that many pre-SMA cells are preferentially active only during the single trials where the animals are required to update to a new movement sequence (Shima et al., 1996). The activity is not related to a new association, since the sensory stimuli and the associations with different movements are already well learned. This suggests that pre-SMA is involved in updating motor plans or reprogramming.

Many SMA neurons display a gradual change in activity related to experience with a particular movement sequence. This suggests that activity in SMA is dynamically reorganized by experience (Lee and Quessy, 2003). It is possible for learning about motor tasks to occur in the absence of motor performance changes, for example, as measured by reaction time. This might occur during the observation of a motor task. The subject later demonstrates more rapid improvements in performance since some perceptual/cognitive aspect of the task was learned during the observation. It has been argued that SMA may play a role in sequence learning independent of the relationship with performance changes (Lee and Quessy, 2003).

One behavioral paradigm that is popular for studying motor sequence learning in nonhuman primates is the sequential button task, in which monkeys are required to press pairs of buttons in the proper sequence. This task has been used extensively by Hikosaka and colleagues, who generally train monkeys in sets of five consecutive pairs of button presses. Using a similar task in a PET study of cerebral blood flow in humans, SMA was more active (more blood flow) during the performance of a prelearned sequence compared with a new sequence (Jenkins et al., 1994). In contrast, pre-SMA is active during the process of learning the sequence (Hikosaka et al., 1996).

These results may explain the earlier lesion results. If SMA is more active after a motor sequence is learned (and thus, after external cues are no longer necessary to pace the task), damage to SMA would then result in deficits in performing the learned sequence. However, if external cues are then provided, the lateral premotor areas are engaged and can compensate for the deficit.

But many brain regions are active during new learning, such as dorsolateral prefrontal cortex, parietal cortex, lateral premotor area, cerebellum, and basal ganglia (Nakamura et al., 1999). Are either SMA or pre-SMA necessary for learning new sequences? In monkey studies, these areas can be differentially and temporarily inactivated using muscimol, a GABA agonist. Inactivation of either area increases reaction time of button presses for both novel and learned sequences. However, inactivation of pre-SMA, but not SMA, increases errors for novel sequences, but not learned sequences (Nakamura et al., 1999). These data provide further support that pre-SMA is more involved in the learning of new motor sequences. What specific factors related to new learning are disrupted by the inactivation (novelty detection, selective attention, decision making, error correction, switching motor plan, memory coding, retrieval, etc.) are not completely known.

Cells have also been found in SMA that appear to be responsive to a particular order of forthcoming movements (Tanji and Shima, 1994), supporting the view that SMA is particularly important in the relational order of sequence components (i.e., neurons fire during movement A only if movement A is preceded or followed by movement B). In addition, both SMA and pre-SMA neurons show selectivity for the numerical order of sequence components, although the incidence of such neurons is higher in pre-SMA. That is, these neurons may fire during movement A only if movement A is the second movement in the sequence (Clower and Alexander, 1998).

Before leaving this discussion of motor sequence learning, it should be emphasized that SMA and pre-SMA are probably not the only cortical motor

areas involved in coding movement sequences. Recent data from monkeys have demonstrated that after long-term training on a sequence task, a large proportion of neurons in M1 were differentially active during a repeating sequence versus a random sequence (Matsuzaka et al., 2007). A large number of M1 neurons are active during the instruction period, similar to findings in premotor areas. When cells were sought that showed an interaction between movement direction and serial order (sequence-related cells), such cells were the most common type (40%). Only 17% of these cells were related to movement direction alone (Lu and Ashe, 2005). This suggests that M1 may represent sequential movements, as has been suggested for SMA and pre-SMA. It would appear that M1 participates in motor skill learning beyond the simple kinematic level, and thus, the neural network participating in more cognitive aspects of motor tasks may be broadly distributed.

A model for a more global network view of sequence learning has been proposed by Ashe and colleagues (2006). The basic principle of this model is that motor sequences involve both implicit and explicit learning depending upon the stage of the learning process (**Figure 4**). These two forms of learning are thought to employ somewhat different neural structures. Much of motor sequence learning is implicit, as elements of the movements are made in sequential order without specific awareness about the sequence. Ashe's model suggests that in these conditions, implicit processes originate in M1 and propagate to premotor areas. With repeated practice, learning may become explicit, as the subject is aware of the instructional set. In this case, explicit processes originate in prefrontal cortex and then propagate to premotor areas. In reality, both implicit and explicit processes probably interact, and thus neural correlates can be found across broad neuronal networks.

3.21.5.2.3 Role of SMA/pre-SMA in self-initiated versus externally guided movements

One of the central issues concerning the role of SMA/pre-SMA in movement is its role in self-initiated movements as opposed to movements triggered by external stimuli, or externally guided movements. While a large percentage of neurons in SMA are active during IG movements, approximately 90% of task-related neurons are active before or during EG movements (Mushiake et al., 1991).

Both self-initiated and externally guided movements activate a common network of cortical areas including SMA, pre-SMA, cingulate cortex, M1, superior parietal cortex, and insular cortex. Neurophysiological data in animals seem to indicate that the mesial motor areas are more involved in self-initiated movements, while lateral premotor areas are involved in externally guided movements (Romo and Schultz, 1987; Thaler et al., 1988; Mushiake et al., 1991; Cunnington et al., 2002). Motor preparatory activity appears to arise primarily from mesial motor areas, supporting this view. Neuroimaging studies have demonstrated that mesial motor areas show greater activation with self-initiated compared with externally triggered movement (Rao et al., 1993; Wessel et al., 1997; Deiber et al., 1999; Jenkins et al., 2000; Hoshi and Tanji, 2006), and greater activation of lateral premotor areas for externally triggered (EG) movements.

Activity increases in both mesial motor areas and lateral premotor areas during a movement preparation period (Richter et al., 1997). M1 shows weak activation

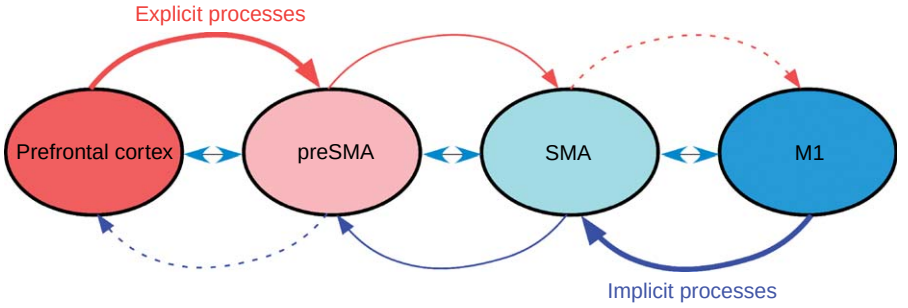

Figure 4 Cortical structures involved in the control of motor sequences. In this model, both explicit and implicit processes operate in the learning and execution of movement sequences. The degree of involvement of the two processes, and the underlying neural structures participating in the behavior, are a function of the stage of the learning process. Adapted from Ashe J, Lungu OV, Basford AT, and Lu X (2006) Cortical control of motor sequences. *Curr. Opin. Neurobiol.* 16: 213–221.

during this preparation period, and follows that of premotor areas in time (Wildgruber et al., 1997). Using rapid event-related functional magnetic resonance imaging that allows the timing of different motor cortical areas to be examined, activity has been examined using a finger sequence movement task. Using this approach, activity in SMA does not differ between self-initiated and externally guided sequences during movement, or between self-initiated premovement and movement. Both self-initiated movements and externally cued movements show strong activation in mesial motor areas. However, the timing of the activity in pre-SMA was significantly earlier for self-initiated compared with externally triggered movements. (Cunnington et al., 2002). Sequence complexity had a greater effect on SMA before rather than during movement, suggesting that SMA is more involved in preparatory processes (Elsinger et al., 2006).

Pre-SMA appears to be preferentially involved in self-initiated movements. Pre-SMA also appears to be activated when a mental representation of a movement is engaged, in the absence of actual movement (Koski et al., 2003). However, imagined movements are also correlated with activation in SMA, but the SMA was more active during movement execution (Cunnington et al., 2006).

Whether the SMA is involved in self-initiated movements is subject to debate (see discussion in Deiber et al., 1999), but other variables may modulate activity of SMA, in addition to the mode of movement initiation. Pre-SMA (as well as PMd) and not SMA was activated preferentially during an auditory conditional motor task, in which two different tones triggered different movements (Kurata et al., 2000). Pre-SMA activity appears to be rate dependent, with more extensive activation with faster movements. SMA is preferential for sequential rather than fixed movements, that is, the type of movement (Deiber et al., 1999).

One interesting aspect of the timing of movements focuses on whether motor areas are concerned with ordinal or interval properties of the timed movements. Timing control appears to be independent of sequence representation. That is, timing can be changed without changing the sequential order. During a sequential movement task, subjects had to attend to either the interval or the ordinal information of a sequence of visually presented stimuli. While the same motor areas were activated in both conditions, pre-SMA, lateral PMC, frontal opercular areas, basal ganglia, and left lateral cerebellar cortex were activated more strongly by interval information, while SMA, frontal eye field, M1 and S1, cuneus, and medial cerebellar cortex were more active for ordinal information. (Schubotz and von Cramon, 2001). Thus, different aspects of impending movement sequences may be processed by different areas.

3.21.5.2.4 Shift-related cells in pre-SMA

In monkeys, activity in response to visual cue signals during a preparatory phase that indicates the direction of an upcoming arm movement is abundant in pre-SMA. Also, pre-SMA contains an abundance of shift-related cells that respond when monkeys are instructed to shift the target of a future reach from a previous target to a new target (Matsuzaka and Tanji, 1996). Relatively few shift-related cells are found in SMA. The majority of SMA neurons are time-locked to movements.

3.21.5.2.5 Role of SMA in kinetics and dynamics of movement

Two aspects of reaching movements have been described based on kinematics versus dynamics of the action (Padoa-Schioppa et al., 2002). Kinematics refers to the evolution in time of the joint angles and hand position. Dynamics refers to the set of forces exerted by the muscles. The accomplishment of skilled reaching can be defined from a neural perspective as the generation of appropriate forces (dynamics) for a desired hand trajectory (kinematics). Thus the role that the central motor structures plays in the transformation of desired kinematics into dynamics is critical to our understanding of motor skill learning. Dynamics-related neural activity has been found in several motor structures including dentate and interpositus nuclei in the cerebellum, M1, SMA, PMd, PMv, and putamen. Neurophysiological studies suggest that dynamics-related activity in SMA occur during the motor planning phase. Thus, SMA is likely to participate in this kinetics-to-dynamics transformation (Padoa-Schioppa et al., 2002).

3.21.5.3 Lateral Premotor Cortical Areas and Their Role in Motor Skill Learning

3.21.5.3.1 Comparative aspects of lateral premotor areas

Recent studies indicate that premotor areas play an important role in sensory guidance of movement and some aspects of motor preparation. Premotor areas probably first appeared with the divergence of

prosimian primates, as evidenced by lateral premotor representations coincident with distinct cytoarchitectonics rostral to the M1 representation. Two major subdivisions, PMd and PMv, have been identified in the prosimian Galago (Wu et al., 2000). PMd has been further subdivided in Galago into rostral and caudal components (PMd-r and PMd-c, respectively). These two PMd areas appear to be functionally distinct, as saccades are evoked from stimulation of PMd-r, and forelimb and body movements are evoked from PMd-c (Fujii et al., 2000). To the extent that Galagos represent a primordial state of primate motor cortex, it is likely that PMd-r, PMd-c, and PMv are homologs in all extant primates. Intracortical microstimulation studies have also identified PMv and PMd in New World and Old World monkeys (Gould et al., 1986; Stepniewska et al., 1993; Preuss et al., 1996; Frost et al., 2003; Hoshi and Tanji, 2004). The PMd has been shown to consist of representations of both hindlimb and forelimb (He et al., 1993; Ghosh and Gattera, 1995; Preuss et al., 1996; Raos et al., 2003), while PMv contains representations of the forelimb and orofacial muscles (Stepniewska et al., 1993; Preuss et al., 1996).

In addition to the areas noted that have been identified in prosimian primates and New World monkeys, the PMv is further differentiated in Old World monkeys. A total of four subareas of lateral premotor cortex are identifiable in macaques: PMd-c, PMd-r, PMv-c, and PMv-r (or F2, F7, F4, and F5, respectively) based on cytoarchitectonics and intracortical microstimulation results and connections (Rizzolatti and Fadiga, 1998; Rouiller et al., 1998; Morel et al., 2005).

Unlike macaque monkeys, there have been no delineations of subareas F4 and F5 (Pmv-c and Pmv-r) in PMv of prosimian primates or New World monkeys. In macaques, PMv is subdivided into caudal and rostral divisions based on cytoarchitectonic, connectional, histochemical, and physiological distinctions (Matelli et al., 1985, 1991; Luppino et al., 1999; Rizzolatti and Luppino, 2001; Rizzolatti et al., 2002; Morel et al., 2005).

Since direct corticospinal projections originate from dorsal and ventral premotor areas (as from M1, SMA, and CMA), it is possible that all frontal cortical areas may participate in movement dynamics.

3.21.5.3.2 Role of the ventral premotor cortex in motor control

The ventral premotor cortex was first characterized functionally by its involvement in visually guided motor behavior as its neurons discharge during preparation and execution of movements under visual guidance (Godschalk et al., 1981). PMv neurons also respond to tactile and proprioceptive stimulation (Graziano et al., 1997; Graziano, 1999). Thus, PMv has been implicated in the initiation and control of limb movements based on visual and somatosensory information (Kurata and Tanji, 1986; Gentilucci et al., 1988; Rizzolatti et al., 1988; Mushiake et al., 1991, 1997). PMv is important for the integration of visual information derived from extrapersonal three-dimensional space and involved in the spatial guidance of limb movements (Kakei et al., 2001). PMv neurons respond to somatosensory stimuli applied to either face or arm and to visual stimuli corresponding to peripersonal space (Gallese et al., 1996; Graziano et al., 1997). PMv neurons are selective for the three-dimensional shape of objects to be grasped (Murata et al., 1997), the direction or movement trajectory in visual/extrinsic space (Kakei et al., 2001; Schwartz et al., 2004), the attention to visuospatial stimuli (Boussaoud et al., 1993), and decisions making based on somatosensory signals (Romo et al., 2004).

Specific lesions of PMv in the area responding to visual object presentation (F5) result in deficits in visually guided grasping movements. Hand preshaping preceding grasping is markedly impaired. Monkeys were able to grasp objects, but only after compensation based on tactile correction (Fogassi et al., 2001). Animals show a reluctance to use the affected hand (Rizzolatti et al., 1983; Schieber, 2000), though the ability to make a precision grip appears unaffected (Schieber, 2000). During conditional visuomotor association tasks, movements have smaller amplitudes and slower velocities, but few direction errors are made (Kurata and Hoffman, 1994). Monkeys appear to lose the ability to perform a prism adaptation task after muscimol injection in PMv (Kurata and Hoshi, 1999). Lesions of the periarcuate region of frontal cortex (premotor cortex, probably primarily PMv) result in deficits in selecting the proper response to a conditional cue (Petrides, 1986).

In macaques, F4 appears to code goal-directed actions mediated by spatial locations (Rizzolatti et al., 2002), while F5 has been shown to be involved in motor-action recognition (Umilta et al., 2001). Human area 44 has been shown to be involved in sensorimotor transformations for grasping and manipulation (Binkofski et al., 1999) and is thought

to be homologous to F5 in macaques (Rizzolatti et al., 2002).

Neurons in PMv reflect processes in extrinsic coordinates more often than neurons in M1 (Kakei et al., 2001). Effector-independent activity is more frequent in PMv than in PMd (Hoshi and Tanji, 2002). Premovement activity is less frequent in PMv than PMd (Boudreau et al., 2001). Activity of neurons in PMv co-varies with external force in a precision grip task (Hepp-Reymond et al., 1994, 1999). Dynamics of movement appear to be coded in both PMd and PMv (Xiao et al., 2006).

3.21.5.3.3 Role of dorsal premotor cortex in motor control

PMd is involved in movement parameters (Fu et al., 1993; Kurata, 1993; Crammond and Kalaska, 2000) and in the integration of internal body representation and target information for the preparation of motor actions (Kurata, 1994; Hoshi and Tanji, 2004). PMd neurons are active during a preparatory motor-set period (Weinrich and Wise, 1982) and in relation to visuomotor-association tasks (Kurata and Wise, 1988; Mitz et al., 1991). Neurons in PMd are active before expected visual signals (Mauritz and Wise, 1986) and during motor preparation (Wise and Mauritz, 1985; Kurata and Wise, 1988). Target-related activity prior to and during movement is more abundant in PMd than in M1 (Shen and Alexander, 1997). However, there is considerable overlap in function (Riehle and Requin, 1989; Kalaska et al., 1997).

Like SMA, neurons in PMd appear to reflect dynamics during motor planning, or the dynamics of upcoming movement. PMd may also be involved in the kinetics-to-dynamics transformation, though not to the degree that SMA is. During the premovement period, neurons in PMv are mostly not directionally tuned, which is more like M1.

Monkeys with PMd lesions exhibit increased direction errors during conditional visuomotor association tasks. However, movement amplitude and velocity are normal (Kurata and Hoffman, 1994). This contrasts with effects of PMv lesions.

3.21.5.3.4 Direct comparison of ventral and dorsal premotor cortex response properties

In a few studies, direct comparisons have been made between these two premotor areas. The results show that set-related activity is mainly found in PMd, while movement-related activity is found in both PMd and PMv (Kurata, 1993). PMv neurons mainly respond to visual target location, while PMd neurons respond to target location and arm direction (Hoshi and Tanji, 2004). PMv neurons predominantly reflect locations of visuospatial signals (Hoshi and Tanji, 2002, 2006). About half of PMd neurons reflect motor instructions about the cue (arm or target to be selected) (Hoshi and Tanji, 2006).

3.21.5.3.5 Role of dorsal and ventral premotor cortex in motor skill learning

Based on the response properties of premotor neurons and the effects of lesions to PMd and PMv, it would appear that premotor cortex plays a role in retrieval of movements from memory based on environmental context (Mitz et al., 1991). This would suggest an important role for premotor cortex in learning conditional associations between sensory stimuli and appropriate motor actions. When monkeys were trained to learn new conditional motor associations, over half the neurons tested in PMd changed their activity during the learning of the association (Mitz et al., 1991). As learning progressed, the activity of novel associations began to resemble familiar ones (Buch et al., 2006). Interestingly, after the response choice had been made, but prior to any feedback, activity increased in the putamen, but not in PMd (Buch et al., 2006). In an auditory conditional association task in humans, PMd and pre-SMA exclusively showed preferential activation, suggesting that these are sites where general sensorimotor integration for learning new associations takes place (Kurata et al., 2000).

In another popular paradigm for motor learning, neuronal activity is recorded in motor areas as animals learn to adapt to a novel force field. In this task, premotor neurons display plasticity related to learning of kinematic, dynamic, or memory properties (Xiao, 2005). A progressive change in movement-related representations have been reported in PMd in an instructed-delay paradigm (Messier and Kalaska, 2000).

3.21.5.3.6 Learning through observation: role of premotor cortex

There is now considerable interest in the role of motor cortical areas in learning new motor skills through observation. While motor skills are typically learned through physical practice, it is well known that motor performance can be improved simply by observing a skilled motor task (Vogt, 1995; Mattar and Gribble, 2005). It has been suggested that observing the movements of another

individual is not simply the observation of visual patterns but the generation of an image of oneself for performing the action (Petrosini et al., 2003). Thus, it is likely that the same structures that are involved in motor execution are active during the observation.

The neural basis of imitation is becoming increasingly clear. In macaque monkeys, so-called mirror neurons located in the ventral premotor cortex (area F5) and posterior parietal cortex (area PF) fire not only when the monkey performs a particular motor action but also when the monkey observes someone else performing the same action (Rizzolatti et al., 1996). This phenomenon has been implicated in the human ability to imitate. Functional magnetic resonance imaging studies in humans confirm that similar regions may be active during both observed and executed actions (Koski et al., 2003). Further, these regions appear to be more active during specular imitation (i.e., the actor moves the left hand and the imitator moves the right hand, as if looking in a mirror), more so than during anatomic imitation (i.e., the actor and imitator move the right hand) (Koski et al., 2003).

3.21.5.4 CMAs and Their Role in Motor Behavior

The primate cingulate cortex, buried in the cingulate sulcus, has traditionally been divided into rostral and caudal architectonic subdivisions (areas 24 and 23). More recently, in the macaque, the caudal CMA has been further differentiated into three distinct areas – rostral (CMAr), dorsal (CMAd), and ventral (CMAv) – based on cytoarchitectonics and neuronal response properties (Walsh and Ebner, 1970; Vogt et al., 1987; Takada et al., 2001; Hatanaka et al., 2003). Because the CMAs are in close proximity to SMA and pre-SMA, it is likely that in many older studies in both nonhuman primates and humans, neuronal activation anywhere on the medial wall was attributed to SMA. Thus, caution should be taken when interpretating findings in which this distinction is not made clear. In general, the rostral cingulate zone is activated by complex tasks, while the caudal cingulated zone is activated during simple tasks (Picard and Strick, 1996).

CMAv receives proprioceptive input from the arm and hand (Cadoret and Smith, 1995). As with other secondary motor areas, the CMA areas send somatotopic projections directly to M1 and the spinal cord (Muakkassa and Strick, 1979; Dum and Strick, 1991, 1996; Luppino et al., 1993; He et al., 1995; Wang et al., 2001). The somatotopy of CMA has been examined using intracortical microstimulation, demonstrating at least a forelimb representation in each of the three subareas (Mitz and Wise, 1987; Luppino et al., 1991, 1994; Takada et al., 2001; Wang et al., 2001; Hatanaka et al., 2003). CMA also receives prominent afferent input from limbic structures and the prefrontal cortex, conveying information regarding motivation. Functional studies examining CMAs suggest that CMAr plays a unique role in the cognitive control of voluntary movements, while the caudal CMA (CMAd and CMAv) is directly involved in the execution of voluntary movement (Devinsky et al., 1995; Picard and Strick, 1996, 2001; Carter et al., 1999; Tanji et al., 2002).

Both the rostral and caudal cingulate areas are more active for self-initiated as opposed to externally triggered movements. In the caudal cingulate zone, activation is greater for sequential rather than for fixed movements, much like SMA proper (Deiber et al., 1999).

On the basis of deoxyglucose labeling techniques in monkeys, it has been suggested that CMAd is highly active during the preparation for and/or execution of a highly rehearsed, remembered movement sequence but is less active if the movement sequence is performed under visual guidance (Picard and Strick, 1997). In the same remembered movement sequence task, the CMAr and CMAv were not active. Instead, these areas are active during simple motor tasks (Shima et al., 1991).

CMAs are likely to play a role in motor learning. A learning paradigm that is popular in rabbits is trace eyeblink conditioning. Lesions of the caudal anterior cingulate cortex in rabbits prevent trace eyeblink conditioning (Weible et al., 2000). However, based on inputs from limbic and prefrontal structures, the role of cingulate areas in motor learning is likely to be related to transmitting information regarding motivation and memory of previous events, rather than simple sensorimotor transformations.

3.21.6 Phases of Motor Learning and Differential Activation of Motor Structures

Significant improvements in reaching accuracy occur in rats after less than 1 week of training. However, significant expansion of movement representations does not occur for at least 10 days after training is initiated (Kleim et al., 2004). This

mismatch between behavioral and neurophysiological endpoints suggests that plasticity in cortical maps reflects only certain aspects of the motor learning experience. Therefore, it is important to differentiate the various phases of motor learning.

Memory has often been described in terms of a short-term memory that is labile and susceptible to interference and a long-term memory that is more stable. The process of transferring information from short-term to long-term memory is referred to as consolidation. It has been argued that motor learning proceeds through similar stages and that different neural mechanisms may account for the two distinct functional stages (Shadmehr and Brashers-Krug, 1997). Stages of motor skill learning have also been described as consisting of a fast, within-session phase that occurs on a time scale of minutes and a second, slowly evolving phase that takes hours to occur. Whether these stages of motor learning that have been proposed based on human experiments have any relationship to neurophysiological and neuroanatomical findings from experimental animal models is still unclear. While the human studies are typically conducted over the course of several hours or a few days, the cortical plasticity demonstrated in animals takes place over 1 week or more. It is possible that the stages of motor learning described in human studies are the initial events in a much more protracted process.

Early and late phases of motor learning have been associated with different neuronal networks. In motor sequence learning, the early phase is thought to involve the corticostriatal and corticocerebellar networks, while the late phase is attributed to the corticostriatal network (see Doyon and Benali, 2005). In a pursuit rotor task requiring human subjects to track a moving target with a stylus, positron emission tomography studies have shown increased blood flow involving a large distributed network in cortex, striatum, and cerebellum during the execution of the task (Grafton et al., 1992). These included motor areas (M1, SMA, putamen, substantia nigra, anterior lobe, and inferior vermis of the cerebellum) and visual association areas (fusiform gyrus and extrastriate area 18). However, sequential positron emission tomography scans over the course of learning the pursuit task demonstrated increased cerebral blood flow in a smaller subset of motor structures including contralateral SMA, M1, and pulvinar thalamus. Thus, procedural motor learning is accomplished by a subregion of the larger network that is critical to the movement execution.

3.21.7 Summary

Significant advances have been made in the past several years in understanding the role of the various cortical motor areas in motor learning. It is clear that motor skill acquisition is associated with alterations in topographic maps of motor representations, structural changes in neuronal elements (synapses, dendrites, etc.), and differential expression of growth-related proteins. We now recognize that motor learning is a distributed process. While differentiation in the response properties of various cortical areas demonstrates their unique functions, there is considerable overlap. Primary motor cortex is most associated with movement-related aspects of learning motor tasks. The mesial areas are more related to learning motor sequences, especially when they are self-initiated. The lateral motor areas are more related to externally guided movements and the integration of sensory cues with motor commands, especially in forming conditional motor associations. Finally, the CMAs are involved in more cognitive and motivational aspects of motor learning.

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3.22 Cerebral Cortex: Motor Learning

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3.22.1 Introduction

The proposition that mechanisms in the cerebral cortex contribute to motor skill learning seems relatively straightforward. A learning-related role for motor related structures of the frontal lobe, including the primary motor cortex (M1), the premotor area (PMA), and the supplementary motor areas (SMA) might once have generated significant controversy. However, inspection of the historical and recent record provides considerable evidence that many neocortical regions, even those with clear roles in supporting motor performance, exhibit plasticity (Brown and Sherrington, 1912; Lashley, 1923; Sanes and Donoghue, 2000). The observations of plastic phenomena in these regions and their highly organized intracortical network suggest that adaptive modifications are a feature of motor structures, and that they operate in parallel with mechanisms that control movement output parameters. Significant recent advances have occurred in detailing how the neocortex contributes to motor skill learning, and these are reviewed here with a focus on human physiology and related work for experimental animals. Subcortical structures have key roles in various aspects of motor learning, such as the vestibular-ocular reflex, but these are not discussed in extensive detail.

Despite long-standing indications that motor structures of the brain exhibit plastic changes, traditional views hold that neocortical regions such as M1, PMA, and SMA function only to mediate the kinematics and dynamics of voluntary skeletal movements. To be sure, the interconnected afferent and efferent neocortical and subcortical targets of these regions form a highly integrated neural network that emits precise neural commands to mediate limb positions, velocities, and forces needed to implement the fluid movements that humans (and other mammals) readily perform. The range of interconnections among neocortical and subcortical motor-related areas that include inputs from so-called association areas (Wise et al., 1997) suggests that motor cortical regions should express functions beyond motor control. The diffuseness of intrinsic connectivity and the functional representation pattern in M1 could also provide a ready substrate for roles in motor learning for M1 and other nearby related neocortical motor areas (Sanes and Donoghue, 2000; Sanes, 2003). This chapter reviews the static and dynamic organization of motor-related neocortical areas with a view that this organization supports their participation in acquisition, retention, and expression of motor skills. The chapter in particular reviews evidence that processing in motor-related neocortical and selected subcortical regions contributes to motor learning and reviews recent studies on the possible cellular mechanisms of motor learning.

Any discussion of motor learning necessarily commences with its definition. Motor learning entails acquiring novel movement patterns, which can occur simply by repeating a simple action when the muscles that generate the movement(s) develop a stereotyped pattern of agonist and antagonist activity, either gradually or emergently (Corcos et al., 1993). Developing expertise for more complex skills such as for numerous sport or artistic activities that involve multiple joint actions and across-limb coordination likely entails

developing novel simultaneous and sequential action patterns. Motor learning also includes the acquisition of pairings between external events and voluntary actions – sometimes the events and actions are common and perhaps part of an individual's normal experience, but they have not been previously combined. Examples are learning to manipulate a newly found tool or responding for the first time to a sensory stimulus that directs movement. The constituent actions, such as grasping, applying forces, and the sensory cues that stimulated the actions have already been acquired or experienced but not necessarily connected in a new context. Independently of whether the motor learning requires adaptation or forming new sensory-motor links, new patterns of neural activity or activation must accompany these changes. Understanding the behavioral and neural concomitants of sequential actions has been an active area of recent motor learning research and is considered here.

3.22.2 Motor Cortical Representations

Following upon pioneering observations from the late nineteenth and early twentieth centuries (e.g., Fritsch and Hitzig, 1870; Ferrier, 1876; Jackson, 1884; Sherrington, 1911), researchers working in the middle of the twentieth century used focal application of electrical stimulation to map output functions of M1. These studies collectively revealed a somatotopically ordered movement map that represented the body; the representational form of this map resembled a distorted cartoon of the body (Penfield and Rasmussen, 1950; Woolsey et al., 1952), with body-part joints or movements laid out upon the surface of M1. The principal organizing feature of this representation incorporated a topographic ordering of the leg (hindlimb in quadrupedal mammals), arm (forelimb, including digits), and head and face from the medial convexity to more lateral and inferior sections of the cerebral hemisphere.

While the general rubric of a medial to lateral, leg, arm, and face representation has appeared consistently in all early and current studies, significant commentary exists that casts doubt upon the notion that the homunculus cartoon ever represented the actual findings (Schott, 1993; Sanes and Schieber, 2001). Indeed, Penfield and Woolsey merely used the cartoon and figurine representations to depict in summary form some aspects of orderly patterning of the body superimposed upon the neocortical surface. Referring to the homunculus cartoon for M1, Penfield and Rasmussen noted that,

> A figurine of this sort cannot give an accurate indication of the specific joints in which movement takes place, for in most cases movement appears at more than one joint simultaneously. (Penfield and Rasmussen, 1950: 56)

Instead, they suggested that the summary illustration should become used as a heuristic for further investigation. With a similar sentiment, Woolsey et al. expressed caution similar to that of Penfield and Rasmussen (1950) by stating that an illustration depicting the results of their electrical stimulation mapping

> *is an inadequate representation of the localization pattern, since in a line drawing one cannot indicate the successive overlap which is so characteristic a feature of cortical representation, not only in the motor but also in the sensory areas.* (Woolsey et al., 1952: 251–252; italics appearing in the original)

Despite these admonitions, the widely adopted corporeal schema has profoundly influenced concepts of motor cortex organization. Specifically, the homunculus plan suggests that representations for each body part have a high degree of order, with a point-to-point plan and without overlap in cortical space. An orderly somatotopic arrangement would suggest dedication of specific brain elements, such as a cortical column, for the control of one body part (Asanuma and Ward, 1971), such as an individual finger, perhaps precluding a flexible arrangement required for plasticity or learning.

While controversy still remains, a critical review of experimental data derived from a variety of sources, including intracranial, surface, and transcranial stimulation methods, lesions, and neuroimaging, upholds the segregation of functional subregions within M1, that is, the separation of representations for head, arm (forelimb in quadrupeds), and leg (hindlimb in quadrupeds). However, the same data resolutely discards precise topography within M1 as an organizing principle. The alternate view holds that each M1 subregion has an organization resembling a network with distributed functions (Sanes and Donoghue, 1997; Sanes and Schieber, 2001); neural elements – actual spiking neurons, local field potentials recorded from neurons, functional magnetic resonance imaging (MRI) voxels, or electroencephalogram sources – within these

subregions may have highly specific functional features, though according to contemporary notions of network properties, the elements can have flexible and shared functions. The most complete data sets to assess whether ordered topography represents voluntary movements have been obtained for the M1 arm region, with the most extensive descriptive maps derived from evoked movements via electrical stimulation techniques. Intracranial electrical stimulation as applied to M1 can reveal the movement or muscle coupling of the stimulated site by current deposited by a microelectrode inserted amongst cortical neurons (Stoney et al., 1968). New movements evoked when the electrode traverses the cortex in small steps suggested the existence of an underlying pattern of organization. However, when assembled into a comprehensive map, sites for any particular body part appear widely distributed, multiple, and overlapping (Kwan et al., 1978; Sessle and Wiesendanger, 1982; Gould et al., 1986; Donoghue et al., 1992; Nudo et al., 1992; Park et al., 2004). It should be noted that even the cruder surface stimulation data derived by Penfield and his colleagues, using much lower resolution than feasible with intracortical stimulation also has supported this form of distributed organization (Penfield and Boldrey, 1937). Additionally, the pioneering studies of Jankowska and coworkers predicted the recognition that M1 has a distributed pattern representation of motor control (Jankowska et al., 1975). The available data for M1 representations other than that for the arm – M1 organization for the face (Huang et al., 1989) and leg areas (Gould et al., 1986) – each show a similar distributed internal organization while maintaining separation from the adjacent arm region.

Reservations about the nonbiological nature of electrical stimulation are clearly warranted when defining the organizing features of the motor map. However, evidence from neural recordings, pharmacological inactivation, and connectional studies all reveal the distributed nature of M1 subregion organization consistent with that identified by intracortical electrical stimulation mapping. Spike-triggered averaging has demonstrated that single neurons in the M1 arm area influence multiple arm muscles (Buys et al., 1986; Fetz et al., 1989), including combinations of proximal with distal muscles (McKiernan et al., 1998; Park et al., 2004). In addition, recordings of single M1 neurons show that they rarely modulate with the action of a single joint or part; each neuron appears to participate in multiple hand motor actions, and neurons influencing different digits or the wrist appeared to have a seemingly random distribution (Schieber and Hibbard, 1993). This pattern does not easily find reconciliation with a discrete, topographically segregated organization for M1. As predicted by distributed functional relationship of M1 to movement and muscle control, focal inactivation of monkey M1 produces more global effects on arm actions but does not block the action of individual parts (Schieber and Poliakov, 1998).

Contemporary neuroimaging methods that detect local changes in blood flow have also allowed exploration of the organization of human motor cortex. Low-resolution studies with positron emission tomography (PET) found overlapping activation patterns for distal and proximal arm movements within the M1 arm area (Colebatch et al., 1991; Grafton et al., 1991). The higher-resolution fMRI method has also revealed overlapping, distributed activation in M1 for distinctive movements of the fingers, wrist, and elbow (Rao et al., 1995; Sanes et al., 1995; Hlustik et al., 2001; Indovina and Sanes, 2001), thus showing consistency with a distributed organization within the M1 arm subregion. Finally, all the functional data appear to have an anatomical correlate insofar as the M1 arm subregion has vast horizontal interconnectivity without an obvious topographic plan for fingers, wrist, or other parts of the arm (Huntley and Jones, 1991).

Taken together, this body of experimental data reveals a pattern of M1 organization having discrete gross subdivisions. However, each subdivision has an internal distributed network in which control emerges from broad activity patterns (Sanes and Donoghue, 1997, 2000). Not only can this organization provide the immense storage capability and richness of function characteristics of distributed networks, but it also provides a basis for flexibility if the organization of this network has modifiability. In the next sections, we provide evidence that M1 representations have plasticity and discuss its implications for a M1 role in motor learning.

3.22.3 Motor Cortical Plasticity

One of the most interesting aspects of behavior of mammals, particularly primates, is their remarkable, seemingly limitless flexibility in motor behavior. The range of skills, precision, and adaptability of motor control evident in animals with a large forebrain seems unmatched. Behavioral flexibility often becomes attributed to the neural mechanisms embedded in the

cerebral cortex, though to be sure, subcortical structures have key roles in motor learning. This connection suggests that some sort of modifiable architecture exists within motor cortical circuitry. The idea of a dynamic organization in motor cortex has a lengthy history and seemingly coexisted with the development of notions that a portion of the neocortex has a movement specialization (Brown and Sherrington, 1912). Furthermore, the concept that M1 circuits have flexibility has appeared throughout the experimental literature of the twentieth century (Lashley, 1923; Gellhorn and Hyde, 1953). In the past 20 years, application of intracortical electrical stimulation mapping, and then related mapping methods using transcranial stimulation in humans, has shown that functional maps within M1 can exhibit rapid and sometimes even long-lasting reorganization. In a series of these electrical stimulation mapping experiments, we demonstrated that transection of the facial motor nerve, which supplies the rat's facial whisker musculature, led to a functional loss of the M1 whisker area. This region was supplanted by representations of the adjacent forelimb or eye/eyelid regions (Donoghue et al., 1990; Sanes et al., 1992). This reorganization emerged abruptly within hours of the nerve lesion, and the basic form of the reorganization that was revealed soon after the lesion persisted for months (Sanes et al., 1990). The ability to evoke movements at similar thresholds in both the reorganized and normal areas of M1 suggested that these areas acquired functions of the normal cortex as part of an expanded representation. In experimental animals, this pattern generalizes to peripheral nerve (Sanes et al., 1990; Franchi and Veronesi, 2006) or central (Nudo and Milliken, 1996) lesions, repetitive M1 stimulation (Nudo et al., 1990), or changes in limb configuration (Sanes et al., 1992). The availability of transcranial stimulation methods has extended M1 mapping to humans. A variety of manipulations including amputations (Hall et al., 1990; Cohen et al., 1991), spinal cord injury (Topka et al., 1991), transient modifications in sensory inputs (Brasil-Neto et al., 1992), immobilization (Liepert et al., 1995), and repetitive M1 stimulation (Berardelli et al., 1998) all have been shown to modify M1 movement representations. This diverse set of results has clearly established the fact that motor maps in humans and nonhuman animals have dynamic and adaptive features.

The demonstrations of motor plasticity with electrical stimulation do not come without controversy. A complex issue of electrical stimulation mapping is defining the site of effect. It is essential to understand the site(s) and underlying basis for reorganized motor representations prior to concluding that changes observed in an M1 output map related to changes in an M1 neural circuit. A key issue concerns understanding that intracortical stimulation applied to M1 activates intrinsic and especially output neurons, such as the pyramidal tract neurons of layer 5 that descend to the spinal cord and via excitation of alpha motor neurons produce the muscular activity with appropriate temporal and spatial summation. However, electrical stimulation also activates intracortical dendritic and axonal processes in and around the vicinity of the stimulating electrode(s), and perhaps most significantly, stimulation activates the recurrent axon collateral system for each neuron brought to threshold. Additionally, the precise location of the stimulating electrode governs, in part, the neural circuits activated; an electrode in layer 5 would predominantly activate pyramidal tract neurons, whereas an electrode in layers 2/3 of M1 would preferentially activate intrinsic and corticocortical circuits. Moreover, a striking feature of neocortical pyramidal neurons is that these projection neurons have extensive local collateral branches in addition to the efferent axon (Ghosh and Porter, 1988). The intracortical M1 branches extend locally and appear to synapse in the immediate vicinity of the neuron and typically have substantial horizontal, or lateral, connection systems that can extend upward of 1 cm within M1 (DeFelipe et al., 1986; Huntley and Jones, 1991; Keller 1993a; Hess and Donoghue, 1994). Thus, one must take into account the fact that projection neurons likely also participate in internal processing within M1 in addition to delivering its output message to subcortical systems. Finally, electrical stimulation of pyramidal tract neurons sends signals not only to the spinal cord but also to a vast set of supraspinal, subcortical targets within the brainstem, striatum, and thalamus (Shinoda et al., 1979; Canedo, 1997).

In humans, transcranial stimulation with electrical and magnetic devices has also been used profitably to study M1 map plasticity (Hall et al., 1990; Cohen et al., 1991; Topka et al., 1991; Boroojerdi et al., 2001). These more global transcranial stimulation methods, in comparison to intracortical electrical stimulation, while essential to study the potential for modification in the human cortex, suffer even more severely from the lack of precise knowledge of which neurons, and their related connections, are activated by stimulation. Transcranial magnetic stimulation (TMS) is assumed to activate neurons superficial to output layer V, while transcranial

electrical stimulation (TES) likely activates neurons mainly in layers V and VI and axons in the underlying white matter (Day et al., 1987). Although the observation that TMS but not TES yields certain types of map reorganization has consistency with an intracortical site for plasticity (Chen et al., 1998; Ziemann et al., 1998; Boroojerdi et al., 2001), both TMS and TES would activate immensely complex sets of cortical and subcortical circuits. Thus, it remains unproven that reorganization observed in the cerebral cortex using any of these forms of stimulation necessarily means that the cerebral cortex is the site of modification.

3.22.4 Substrates for Motor Cortical Plasticity

In the past 20 years, evidence has accrued that directly demonstrates substrates and mechanisms within M1 to implement plasticity. Thus, these findings have placed M1 intrinsic circuitry in a strategic position to mediate and to account for representational organization that stimulation mapping has revealed. The candidate substrate for M1 plasticity is the system of horizontal connections that spans M1. Evidence from several sources demonstrates that horizontal connections in M1 can serve to functionally associate intrinsic neurons into dynamically structured assemblies that can form a flexible set of motor maps. The existence of latent motor output maps in M1 has been revealed using focal and local blockade of GABAergic inhibition that then apparently unmasks existing horizontal connections likely normally blocked by feedforward inhibition (Jacobs and Donoghue 1991). Local GABAergic release yielded map changes parallel to those occurring following facial nerve lesions (Sanes et al., 1990). The results of this experiment demonstrated that M1 contains the intrinsic circuitry necessary to support reorganization. Furthermore, they suggested that the moment-to-moment details of M1 organization likely depended on the precise balance of excitatory and inhibitory influences within the network of M1 connections. Plasticity similar to that observed in experimental animals also occurs in humans using the indirect methods of transcranial stimulation and pharmacological manipulation through systemic application (Ziemann et al., 1998; Di Lazzaro et al., 2006).

Another relevant finding that links M1 map reorganization to modification of the horizontal M1 circuitry relates to the distribution of intracortical connections within M1 and the sites of map changes following nerve damage (Huntley, 1997). In this work, facial nerve lesion in rats resulted in M1 reorganization only at sites that had strong horizontal connections that spanned the reorganized facial whisker area and the forelimb areas. No significant reorganization occurred in M1 zones without any or with only sparse connections between the forelimb and whisker regions. This anatomical-physiological finding indicated that M1 reorganization revealed by electrical stimulation mapping reflected the architectural pattern of the horizontal connection network in M1 and that reorganization could reflect synaptic plasticity of these fibers.

A third important set of results obtained from motor cortex slice preparations has provided additional support for an intracortical substrate that mediates restructuring of M1 maps. Slice preparations can directly evaluate local connections under controlled circumstances using both intracellular and, on a grosser scale, field potential recordings. Field potentials can reveal summed synaptic effects within a small patch of cortex (Aroniadou and Keller, 1993; Donoghue et al., 1996). The studies using slices to investigate the functional architecture of M1 have demonstrated the existence of fairly extensive and functionally strong horizontal pathways in this region that not only spanned superficial layers 2/3 but also traversed the deeper layer 5. Pharmacological manipulations demonstrated that glutamate receptors mediate these horizontal excitatory connections (Keller, 1993b; Hess et al., 1994), consistent with pathways in other systems that might exhibit plasticity. Further, feedforward GABA inhibition likely regulates the strength of excitation, as was predicted by earlier work (Hess et al., 1994, 1996; Donoghue et al., 1996).

While these intracortical connections could provide a basis for dynamic and even instantaneous modulation of the functional architecture of M1, persistent changes in the efficacy of horizontal connections over longer periods likely require a stable form of synaptic modification. Activity-dependent modification through processes leading to long-term potentiation (LTP) and depression (LTD), which has been documented extensively in the hippocampus and other central nervous system (CNS) structures, could also provide a mechanism for long-lasting synaptic modification in the neocortex. The possibility for activity-dependent synaptic plasticity has been long established and well documented in M1

(Baranyi and Feher, 1978; Baranyi et al., 1991). The horizontal pathways in M1 that may mediate formation of new associations among populations of M1 neurons do indeed exhibit the capacity for long-lasting synaptic modification (Hess and Donoghue, 1994; Aroniadou and Keller, 1995). Similar to other systems, the pattern of stimulation regulates the connection strength of the horizontal M1 pathway, either up for LTP with high-frequency stimulation or down for LTD with low-frequency stimulation (Hess and Donoghue, 1996b). As in other systems that exhibit plasticity (Malenka and Bear, 2004), *N*-methyl-D-aspartate (NMDA) receptors appear to mediate the plasticity occurring in M1, thereby suggesting that common mechanisms regulate synaptic efficacy across the CNS.

LTP induction in the mature M1 appears somewhat more constrained than similar mechanisms elsewhere in the adult CNS and processes transpiring during development. LTP in M1 horizontal pathways cannot be induced unless inhibition is transiently reduced during the LTP-inducing high-frequency stimulation (Hess et al., 1996). LTP in the horizontal pathway can occur when tetanization of vertical pathways accompanies horizontal activation (Hess et al., 1996). This vertical activation presumably recruits thalamocortical fibers and a mixture of other inputs and output fibers with recurrent collaterals. These results suggest that modifications in adult M1 representations related to increases in synaptic efficacy require a specific context: Alterations in the level of inhibition or concomitant activity of extrinsic pathways appear to allow a permissive situation for functional restructuring of intracortical connections that can lead to changes in the M1 output map. These permissive relationships raise the possibility that the cerebello-thalamocortical pathway, which forms one important vertical input to motor cortex, provides a gating signal for synaptic reorganization. By contrast to LTP, LTD in the horizontal M1 pathway is readily induced by repetitive low-frequency stimulation independent of contexts (Hess and Donoghue, 1996a). The differences in induction of LTP and LTD in horizontal pathways of M1 suggest a set of complex network relationships that could operate during formation and rehearsal of rules that govern acquisition of motor skills. These results have demonstrated the M1 has a propensity to exhibit plastic phenomena, but they do not provide a direct link between LTP or LTD and behavior. While these linkages are discussed in more detail in the section titled "Learning Mechanisms," it is worth noting here that induction of LTP in a subzone of rat M1 enlarges the output representation for this region of cortex (Monfils et al., 2004).

Through activity-sensitive mechanisms, the synaptic efficacy of the interconnections among M1 neurons can be continuously adjusted up and down, presumably throughout life, as a consequence of their exposure to appropriate activity patterns. The extent of these adjustments is likely strongly regulated by context. These findings seem to place M1 at the center of an arena where functional reorganization of motor representations occurs, since M1 contains both the substrate, that is, the horizontal connection system, and the mechanisms, that is, LTP/D, for reorganization. Nevertheless, one cannot rule out subcortical sites as participants in lesion-induced reorganization of M1 because the potential for synaptic modification is widespread in the nervous system (Randic et al., 1993).

3.22.5 Movement Practice

A simple form of motor learning entails practice-related performance changes. Considerable evidence exists suggesting a neocortical role in both short-term learning, on the order of minutes to hours (Karni et al., 1995), and longer-term learning, from hours to days or months and even beyond (Karni et al., 1995; Pascual-Leone et al., 1995; Pearce et al., 2000; Hlustik et al., 2004). Many of these studies have focused upon learning-related changes in M1, perhaps due to its propensity to exhibit shifts in representation patterns and evidence of plasticity (Sanes and Donoghue, 2000).

A common finding is that practice changes the size of cortical representation of a particular movement (Pascual-Leone et al., 1995) or the size of the motor-evoked potential (Pearce et al., 2000) following transcranial stimulation. In its simplest form, repetitive practice of a known motor sequence appears to increase the area of cortical territory related to individual finger movements (Pascual-Leone et al., 1995). In these studies, participants repetitively performed a task akin to simple piano keying sequence across several days, using only one hand. As expected, performance improved both in speed and accuracy. In parallel to the improved performance, the scalp region over M1 from which TMS elicited motor-evoked potentials (MEPs) from finger flexor and finger extensor muscles increased. These effects do not generalize to the untrained hand, but

interestingly, mere mental practice yielded comparable expansion of the motor cortical representation of digit muscles when compared to actual movement. Numerous groups have replicated these findings (e.g., Classen et al., 1998; Hlustik et al., 2004; Jensen et al., 2005). Hlustik et al. (2004) extended them to functional studies and observed expanded activation with functional MRI in M1 and S1 following extensive practice of a sequential movement. These types of practice effects have been interpreted as indicating plastic processes occurring at the neocortical level, some of which might become consolidated.

The cortical basis of the practice-related modifications in motor cortical output find support from differences in the effects of practice on MEPs evoked by TMS and TES (Classen et al., 1998). In this study, participants repeatedly performed a thumb movement opposite in direction to the movement evoked by TMS over presumed M1. After 30 min of such training, or practice, the movement evoked by TMS had shifted toward the direction of the previously opposite-trained movement; this effect was transitory, enduring, on average, for 20–30 min. The cortical basis of the effect lies in the observations that movements evoked by TES posttraining resembled those evoked prior to training and the differences between the effects of TES and TMS. TES is generally thought to activate neurons and fibers in deep cortical layers and the white matter, whereby neurons and fibers activated by TMS are located more superficially (Day et al., 1987); therefore, while TES yields its effects on muscles directly through activating M1, TMS exerts its spinal effects indirectly by first activating layer II/III neurons and then transsynaptically activating M1 output layers.

In general, M1 appears to figure prominently in development of practice-related motor skills, as might be predicted from its propensity to exhibit rapid shifts in output representation patterns (Sanes and Donoghue, 2000). As noted, simple repetitions of finger movements can yield modifications of M1 output for tens of minutes (Classen et al., 1998), though in this study the specificity of the modified M1 output pattern was not established. More recent data have extended these findings to indicate that M1 practice-related plasticity occurs for rapid but not slow-ramp movements, suggesting a functional specialization for M1 (Muellbacher et al., 2001). Furthermore, it appears that M1 has a role in early consolidation of practice-related changes in motor skills (Muellbacher et al., 2002). Disruption of M1 processing by repetitive transcranial magnetic stimulation (rTMS) substantially reduced performance practice-related improvements. These effects did not seem to have relation to M1 output, since it appeared normal upon testing with standard TMS evaluation and was restricted to disruption of M1, since rTMS delivered to prefrontal cortex (PFC) or occipital cortex had no effect. Other studies, however, have noted, not surprisingly, that the role of M1 networks in consolidation may not extend to all motor learning tasks, such as learning novel limb dynamics (Baraduc et al., 2004). In this study, rTMS delivered to M1 did not disrupt a participant's ability to retain previously learned adaptations to modifications in limb dynamics.

Practice-related effects occur in situations other than repetition of simple movements and in structures other then M1. Humans readily perform rhythmic movements paced by a metronome in a syncopated or synchronized mode, though syncopation degrades as the beat frequency increases beyond a critical threshold and transforms into a synchronized sensory-motor rhythm. Humans improve their syncopation ability with practice, as demonstrated by an elevation of the threshold, allowing it to occur at higher rhythmic frequencies; therefore, the transition from syncopation to synchronized pacing can become a useful tool to evaluate brain mechanisms of practice. Using magnetoencephalographic methods, Jantzen et al. (2001) found that syncopation training reduced differences in magnetic field power in the alpha and beta bands recorded over M1 when comparing syncopation and synchronized rhythm production. The same group assessed neocortical network activation patterns with functional MRI, using the same behavioral assay (Jantzen et al., 2002). Prior to practice-induced improvements in syncopation performance, syncopation activated a wider set of structures, including SMA, the inferior frontal gyrus, superior temporal gyrus, and subcortical structures, than occurred during the synchronization task. The greater network of activation might have related to the increased complexity of the syncopation task, which would seem to require greater need to attend to sensory-motor timing. Analogous to findings in other domains, speech in this case (Raichle et al., 1994), and practice on the syncopation task, reduced activation in SMA and the inferior frontal gyrus, yielding a more similar activation pattern between the two tasks. Practice-related changes in neocortical activation also occurred with improvements in accuracy on a targeted movement

task. In an event-related potential (ERP) study, the amplitude of frontal ERPs decreased while parietal ERPs increased with improvements in accuracy (Staines et al., 2002), a finding reminiscent of the frontal-to-parietal shift in activation pattern found with acquisition of motor sequences by trial-and-error learning (Sakai et al., 1998; Eliassen et al. 2003) or motor adaptation (Shadmehr and Holcomb, 1997). In summary, it appears that practice yields convergent neocortical processing resembling that occurring for the simplest possible action, a process that might be described as finding the lowest common denominator coupled with a shift in activation from frontal to parietal regions.

Much of the prior discussion focused mostly on short-term changes in M1 output and neocortical networks related to discrete bouts of practicing movement-tasks. Plasticity in motor-related areas of neocortex has also been observed in groups of individuals who practice specific motor tasks for years; these groups include professional and semiprofessional musicians, athletes, and blind individuals using Braille for reading. Both stimulation and imaging methods have been used to document effects of long-term practice on cortical physiology. Long-term musical training clearly changes a variety of the brain's anatomical features, some subtly. Morphometric methods based on anatomic MRI have revealed increased volume of M1 parietal motor areas and the cerebellum of both professional and amateur musicians compared to controls (Gaser and Schlaug, 2003; Hutchinson et al., 2003). Prolonged musical training also appears to increase the volume of the pyramidal tract, as assessed with the noninvasive MRI method of diffusion tensor imaging (Bengtsson et al., 2005). Musical training and extensive athletic performance also influence the size of MEPs evoked by TMS applied to M1 (Sparing et al., 2007), extent of cortex activated (Tyc et al., 2005), and intracortical facilitation and inhibition using paired-pulse TMS (Nordstrom and Butler, 2002), again supporting a role for localizing plasticity to M1. Functional MRI studies with musicians have also revealed substantive changes in activation related to motor tasks; a common result is that musicians exhibit less movement-related activation than nonmusicians in neocortex and cerebellum (Jancke et al., 2000; Koeneke et al., 2004; Meister et al., 2005). These results supported the general concept that prolonged practice improves the efficiency of brain processing.

3.22.6 Movement Sequence Learning

Investigating the brain correlates and mechanisms related to learning and performing movement sequences has generated considerable interest, undoubtedly related to the recognition that motor behavior commonly involves performing and coordinating a highly organized set of independent movements that become precisely sequenced in space and time. Learning movement sequences can occur explicitly or implicitly and may or may not observe rules established for declarative learning (Rosenbaum et al., 2001). Researchers have most commonly used a specialized task – the serial reaction time task (SRTT) – that has implicit and explicit components to investigate the neurobiology of motor sequence learning (Nissen and Bullemer, 1987); other sequential movement situations have also been used, but less frequently than the SRTT, to assess brain mechanisms of sequential movements (Hikosaka et al., 2002). The SRTT typically requires discrete responses to cues presented visually and sequentially. Commonly, reaction time (RT) decreases as an ordered sequence repeats, with evidence of learning occurring in about 100–200 trials. While repetition alone would also yield performance improvements, the decreases in RT can be shown to reflect learning, and this sequence learning is not purely perceptual (Willingham, 1999), as might be conjectured from a task that presents a set of ordered visual cures. Additionally, since the improvements in RT initially occur without explicit knowledge of the ordered sequence, these early RT improvements ostensibly index implicit knowledge. As exposure to the order sequence continues, participants develop explicit knowledge of the sequence that can become probed by specially designed generation tasks or verbal query. Thus, the SRTT can assess brain changes occurring during both the implicit and explicit phases of motor acquisition.

Prior work using the SRTT has found widespread neocortical activation, as assessed with PET and functional MRI, during both the implicit and explicit phases of the task. Subcortical structures, including those in the basal ganglia and cerebellum, also become activated during the learning that occurs during the SRTT. Despite the wealth of information about neural substrates of the SRTT, numerous questions remain unanswered. These uncertainties include whether the same areas mediate learning

and expression of implicit and explicit knowledge and whether brain areas implicated in mediating SRTT have a general or specific role in variants of the task, that is, sequence learning for spatial, temporal, or object attributes.

Different brain networks become activated during development of implicit and explicit knowledge during performance of the SRTT (Grafton et al., 1995; Hazeltine et al., 1997; Honda et al., 1998). Accrual of implicit knowledge activated neocortical motor-related areas, including M1 and SMA; these data had consistency with observations of expansion of finger movement representations in M1, as assessed with TMS, during development of implicit knowledge (Pascual-Leone et al., 1994). By contrast, development of explicit knowledge yielded activation in PFC, PMA, and posterior parietal cortex, among other areas. Activation related to acquisition of implicit knowledge during motor sequence performance may extend beyond M1 and SMA, since recent data indicated activation in ventral PMA, midcingulate, PFC, and posterior parietal cortex when implicit sequence knowledge developed (Seidler et al., 2002). Consider though the findings of Nitsche et al. (2003), who extended earlier results of Pascual-Leone et al. (1994) of an M1 role in mediating development of implicit knowledge. In this work, Nitsche et al. (2003), also using TMS, confirmed a role for M1 in acquisition of implicit knowledge during the SRTT but failed to find any effect of TMS to PMA or PFC in modifying decreases in response time, the marker for development of implicit knowledge. Subsequent work by Seidler et al. (2005) provided support for a differential role of neocortical and basal ganglia during the encoding of procedural knowledge of the ordered sequence. In this work, M1 activation, as indexed with functional MRI methods, showed increases in the earliest phases of learning; by contrast, the putamen, the motor component of the striatum, exhibited activation during the later phase of implicit learning. The involvement of parietal and frontal networks during rapid development of explicit knowledge has been confirmed (Eliassen et al., 2001; Muller et al., 2002). The Eliassen et al. (2001) work has particular importance, since it provided indirect evidence of interactions between the implicit and explicit phases of the SRTT task. The data from that study provided clear evidence for prefrontal and parietal involvement in explicit learning. The experiment used a variant of the SRTT to address brain activation patterns during acquisition of explicit knowledge without the possibility of interactions with development of implicit knowledge. Participants were informed that a sequence existed, but not its pattern, and learned this sequence while functional MR images were obtained. Prefrontal and posterior parietal cortical areas became active during acquisition of explicit knowledge, without involvement of the implicit system. Evidence from neuropsychology also suggests segregation of neural mechanisms for implicit and explicit motor sequence learning. Patients with damage to the left parietal lobe fail to exhibit implicit knowledge in standard versions of the SRTT even when provided with extensive practice and failure to gain explicit knowledge (Boyd and Winstein, 2001).

Despite indications of independence of implicit and explicit knowledge systems for motor sequence learning, evidence from behavioral and neuropsychological sources provides clues that these systems have interactions. For example, providing explicit knowledge to patients with a variety of neocortical damage in the frontal and parietal neocortical regions, basal ganglia regions, or the cerebellum prior to performing the SRTT yields a decreasing RT time profile as the sequence progresses that resembles development of implicit knowledge (Molinari et al., 1997; Boyd and Winstein, 2001, 2004, 2006). These data suggest that neocortical–cerebellar loops, with likely mediation of the basal ganglia, have key roles in how the implicit and explicit knowledge systems interact during learning of sequential actions. Behaviorally, RT advantages occur for elements of explicitly trained sequences covertly imbedded in a skein of random response elements, even though participants evince no awareness of the explicitly trained sequence elements (Willingham and Goedert-Eschmann, 2002). The results of this study appear to provide evidence that dorsal PFC has a specific role for developing knowledge about spatial sequences. Temporary inactivation of the PFC with rTMS prevented response time decrements that accompany repeated exposure to a covert motor sequence. PFC inactivation did not prevent improvements in motor performance to sequences cued by colored visual stimuli. The only caveat about this work is that the baseline response time for the spatial sequence may have been too low to reveal further response time decrements. Further work should be done to clarify this point. A functional MRI study using analogous methods provided clear evidence of interactions between mechanisms related to implicit and explicit sequence knowledge (Willingham et al., 2002). Activation related to implicit knowledge and covert-explicit knowledge occurred in portions of the left inferior

parietal lobule, left inferior frontal gyrus, and right putamen. Performance of an overt-explicit task activated these same areas and many more in neocortex and subcortical regions, including thalamic areas and the cerebellum. The activation occurring for the covert-explicit task and accompanying reduced response time, even when participants had no awareness of the sequence elements, indicates that explicit knowledge can improve performance (and activate the same brain areas), even without awareness. Brown and Robertson (2007) provided additional evidence of interactions between the explicit and implicit systems for accrual of sequence knowledge, though they did not localize the site of these interactions. In this study, Brown and Robertson (2007) followed up on prior work showing that the procedural skill of improved response times becomes enhanced with sleep, but not during wakefulness, if and only if the declarative (or explicit) aspects of the sequence also were learned (Robertson et al., 2004; Spencer et al., 2006). They hypothesized that disruption of explicit sequence knowledge would enhance response time, a marker for implicit knowledge; the results supported the hypothesis, supplying support to claims that mechanisms for developing implicit and explicit knowledge interact.

A neocortical involvement in motor sequence learning extends beyond development of implicit and explicit knowledge for discrete movements cued by visual-spatial stimuli. In addition to disrupting SRTT performance, PFC damage impairs learning of continuously performed movement sequences (Gomez Beldarrain et al., 2002), as does low-level rTMS (Pascual-Leone et al., 1996). This impairment appears to occur independently of processes underlying learning of discrete movements sequences – as in the SRTT – and has a relationship to overall planning abilities (Gomez Beldarrain et al., 2002). Additionally, the more superior portions of the PFC, perhaps homologous to the dorsal-lateral PFC of monkeys, seems to have a specific role in development of motor sequence learning cued by spatial stimuli. Reversible inactivation of this PFC area by rTMS disrupted development of implicit knowledge when responding to spatial cues, as assessed by response times; comparable stimulation when participants responded to color cues or rTMS delivered over the posterior parietal cortex when participants responded to spatial or color cues had no effect on response times (Robertson et al., 2001). Explicit knowledge development was not assessed with this protocol, but rTMS of PFC might be expected to disrupt explicit sequence knowledge. These data have consistency with a working model of PFC functional domains (Levy and Goldman-Rakic, 2000).

Two additional findings have clarified the neocortical role in motor sequence learning beyond the 'standard' discrete visual-spatial responding required for the SRTT. Sequences performed by continuous tracking yield neocortical activation related to improved performance in a region spanning the precentral and postcentral gyri, the precuneus, and a region spanning the parietal–occipital fissure (Grafton et al., 2001). Since the improvements in tracking performance can be considered development of implicit knowledge, the pre- and postcentral activation has consistency with implicit knowledge-related activation occurring during the standard SRTT (Grafton et al., 1995; Hazeltine et al., 1997; Honda et al., 1998). Discrete sequence tasks have typically evaluated visuospatial cuing and responding, yet accurate performance of sequential movements also requires precise timing. Ramnani and Passingham (2001) found that progressive acquisition of temporal sequences yielded increasing activation in lateral and medial sections of the posterior parietal cortex, preSMA, and PMA in neocortex and also in the posterior lateral cerebellum. Decreasing activation in connection with temporal sequence acquisition occurred in inferior temporal cortex (IT) and prestriate cortex. Direct comparison of spatial with temporal sequence learning yielded overlapping activation in the parietal lobe, but only the cerebellum and inferior temporal gyrus became active during learning of timed sequences (Sakai et al., 2002). Combined learning of spatial and temporal sequences yielded novel activation in PMA and PFC, suggesting that these frontal areas participated in coordinating choice of effector (the spatial component) and when to start a sequence element (the temporal component).

3.22.7 Arbitrary Sensory-Motor Associative Learning

Neocortical structures also appear to participate in learning arbitrary relationships between sensory events and voluntary movements. Lesion, neural recording, and neuroimaging data have all provided evidence of involvement of PMA and PFC and, more recently, IT, basal ganglia, and the hippocampus in forming and maintaining arbitrary sensory-motor

associations (Wise and Murray, 2000). Neural recording studies in monkeys have found linkages to the dorsal portions of PMA and PFC to forming visual-motor associate rules (Mitz et al., 1991; Asaad et al., 1998; White and Wise, 1999), though evidence exists that other cortical areas, such as frontal and supplementary eye fields (Chen and Wise, 1995) and hippocampus (Cahusac et al., 1993; Wirth et al., 2003) also participate in this type of learning. Since visual-motor rule formation and expression typically requires object identification, it has plausibility that ventral visual pathways involved in visually based object identification should also participate in forming learned relationships between visual stimuli and voluntary movements. Bussey et al. (2001) tested this hypothesis more extensively by assessing whether bilateral lesions in the ventral and orbital PFC in monkeys affected formation and retention of arbitrary visual-motor associations. Bilateral damage to this region, an efferent target of IT, abolished rapid acquisition of new association, and impaired association retention. However, the ventral and orbital PFC lesion did not prevent new learning, indicating that PFC alone does not have a critical role in establishing or retaining visual-motor associations. A subsequent study, using the cross-disconnection procedure (staged unilateral damage in different hemispheres to two neocortical structures), revealed that unilateral ventral and orbital PFC damage had little or no effect on rule formation. The addition of an IT lesion in the opposite hemisphere severely impaired learning of visual-motor association (Bussey et al., 2002). This lesion study in monkeys provides clear evidence of the important roles of IT and ventral PFC in forming visual-motor associative rules. Unilateral lesion of PFC did not alter retention of acquired or learning of new arbitrary visual-motor associations. Addition of a contraversive lesion of IT, an input structure to PFC, impaired retention and learning. The results suggest that information processed in IT and passed to PFC is critical in learning new visual-motor rules. These data indicate the necessity of a functional pathway between IT and PFC to form and retain visual-motor associations.

Neuroimaging work has reinforced these views by showing that the human homologue of monkey IT and ventral PFC exhibit dynamic activation changes during sustained performance of initially (and then learnt) visual-motor couplings (Toni et al., 2001). Further support for interactions between temporal and frontal structures during rule formation derive from studies showing learning-related changes in effective connectivity in a processing stream originating in occipital regions then to the middle temporal gyrus through the anterior striatum ending in the superior PMA (Toni et al., 2002). Effective connectivity related to visual-motor associative learning also changes in other pathways, with increases occurring between regions of the inferior frontal sulcus to the anterior striatum. By contrast, decreasing effective connectivity occurred in a frontal pathway from the inferior frontal gyrus to the opercular region of the precentral gyrus, as mediated by a presumed connection through the anterior striatum. Despite the emphasis on a temporal-striatal-ventral frontal pathway on forming and maintaining sensory-motor associations, other neocortical areas of humans also participate in these processes, particularly interconnected regions in the parietal and frontal cortex (Eliassen et al., 2003; Grol et al., 2006). Different structures in these regions participate in early, later, and over-learned aspects of visual-motor mappings.

The basal ganglia also participate in development and retention of arbitrary sensory-motor associations. Neural recording from various nuclei in the basal ganglia have shown that spiking changes during the acquisition of visual-motor associations (Hadj-Bouziane et al., 2003; Brasted and Wise, 2004). Buch et al. (2006) have provided evidence of a key role for putamen neurons in the process of forming such associations. They examined population activity of neurons in putamen and dorsal PMA, comparing the firing rates of these two areas between the stimulus and feedback cues during trial-and-error learning of stimulus-response mappings. Early in learning, the population discharge of putamen neurons became differentiated from that of dorsal PMA neurons after behavioral selection occurred but before the feedback signal appeared. Putamen neurons exhibited sustained spiking in this interval, whereas dorsal PMA discharge rates fell to baseline. Additionally, putamen neurons showed selectivity for particular mappings. The maintenance of selective ephemeral information about stimulus response mappings by the putamen until reinforcement occurs suggests that this brain region operates to first form and likely also to maintain arbitrary sensory-motor associations. As noted previously, functional connectivity between the temporal and frontal cortex, as mediated via the anterior striatum, exhibits learning-related changes during formation and rehearsal of visual-motor associations. These results provide support for a key role of the striatum in development of sensory-motor mappings.

3.22.8 Learning Mechanisms

A remaining question concerns how use-dependent and sensory-driven motor learning occurs. Similar to the mechanisms discussed for M1 plasticity, candidate mechanisms include fundamental modification in neural spiking properties, formation of new intrinsic or extrinsic synaptic contacts, LTP (LTD) of network synapses, and changes in intracortical processing (Sanes and Donoghue, 2000). Support for each of these mechanisms having a role in supporting motor learning exists, and therefore they should not be considered as a feature only for motor plasticity, or even learning, but complementary processes. Understanding mechanisms for motor learning have commonly focused upon M1 and relatively simple motor tasks, though similar mechanisms likely mediate a wide range of motor learning phenomena across a variety of neural structures.

A useful tool to examine how M1 might participate in learning is to characterize potential changes in the firing profiles of M1 neurons. Cells in M1 commonly exhibit a preferential increase in spiking related to movement in a particular direction (Georgopoulos et al., 1982). The preferred direction can index changes in M1 coding in response to a monkey adapting movements when exposed to an artificial force field (Gandolfo et al., 2000). A more systematic treatment of these data revealed that a substantial fraction of M1 neurons exhibited memory-like changes in a neuron's preferred direction (Li et al., 2001); a subsequent study by the same group (Padoa-Schioppa et al., 2004) found similar results for neurons in SMA. About half of these memory-like cells retained a profile similar to that developed during exposure to the new force field after it was removed, while the remaining half changed the preferred direction only after the new force field was removed but in a direction consistent with the novel force field. The difference between these two memory-like cells is that one expressed (and then retained) modification in the preferred direction upon exposure to the change in force field, whereby the second type expressed the modified preferred direction only after the novel force field was removed. The results provide important insights into a M1 role in fundamental processes of memory and consolidation. However, a weakness of these studies relates to an inability to determine the durability of these shifts in preferred direction; recalling the findings of Classen et al. (1998) on only short-term changes in M1 output due to practice, one might expect that the changes observed by this group of researchers (Gandolfo et al., 2000; Li et al., 2001; Padoa-Schioppa et al., 2004) might not endure. Therefore, it remains an important issue to resolve in determining how M1 and SMA code motor learning.

Another feature of M1 physiology is synchronous firing between nearby or even relatively distant neurons (Murthy and Fetz, 1996; Donoghue et al., 1998), a property that can be revealed in the characteristics of post-spike averages recorded in skeletal muscles (Smith and Fetz, 1989). Recent data indicate that the probability of synchronous firing of M1 neurons, as determined from examining spike-triggered averages in hand muscles, increases with longevity and complexity of training (Schieber, 2002), suggesting that within-area communication, as well as between-area connectivity, likely has importance as an important mechanism underlying development of motor skill. Future work should likely address whether synchrony within the cortex is an antecedent or a consequence of motor skill acquisition. Recent work provides evidence that enhanced functional connectivity between M1 and spinal targets parallels development of motor skills (Kargo and Nitz, 2003, 2004). First, Kargo and Nitz (2003) demonstrated a change in the correlation between M1 neural spiking and muscle synergy patterns as reach-to-grasp learning improved in rats. Second, they showed that the signal-to-noise ratio of M1 neurons improved as motor skill learning progressed and that M1 firing more reliably recruited muscles related to task performance (Kargo and Nitz, 2004). The improved recruitment of muscles by M1 indicated that learning improved, albeit by a currently unknown mechanism, the excitatory transmission from M1 to spinal cord.

LTP and LTD have been proposed as mechanisms for learning and memory functions throughout the brain, though definitive proof of the relationship between synaptic plasticity and learning often is lacking (Martin and Morris, 2002). As noted, modification of internal circuitry and synaptic plasticity has been amply demonstrated in M1 (Jacobs and Donoghue, 1991; Hess and Donoghue, 1994). Motor skill learning and the amount of LTP and LTD induced in M1 appear associated (Rioult-Pedotti et al., 1998, 2000; though see Cohen and Castro-Alamancos, 2005) to suggest that synaptic plasticity mediates motor skill acquisition. Induction of LTP in M1 can increases the size of M1 output representations (Monfils et al. 2004). In this study, LTP was induced in the caudal forelimb zone of the rat M1 by high-frequency stimulation of the

corpus callosum across multiple days. Intracortical stimulation mapping revealed an expansion of the caudal forelimb representation and local changes in dendritic morphology. Other work from this group has demonstrated modifications in synaptogenesis (Kleim et al. 2004) following prolonged motor training; however, the increased synaptogenesis preceded map reorganization, suggestion that synaptic rearrangement stimulated the functional consequences of motor skill learning.

In addition, work in humans paralleling that in experimental animals has suggested the possibility of LTP-like mechanisms in human M1 (Stefan et al., 2000; Ziemann et al., 2004). In this work, peripheral nerve stimulation can induce lasting enhancement – for tens of minutes – of motor-evoked potentials from M1 TMS. Systemic pretreatment of human participants with pharmacologic agents that block NMDA receptors reduces this LTP-like phenomena (Stefan et al., 2002). Practice-related changes in TMS-induced M1 output can also be modified by GABA transmission blockade (Ziemann et al., 2001; McDonnell et al., 2007), as can agents that generally reduce cortical excitability (Sohn et al., 2002). Modification of GABA and NMDA function also impairs adaptation to novel sensory-motor environments but not recall of already-learned sensory-motor perturbations (Donchin et al., 2002). In this work, the authors provide additional information that modifications in GABAergic and glutaminergic neurotransmission affected motor learning, in particular sensory-motor adaptation. Coupled with earlier studies showing that manipulation of GABAergic and glutaminergic neurotransmission affects learning of simple movements, the results of this study suggest that these neurotransmitter systems have a general function across a variety of motor skill situations. Thus, it appears that manipulation of neurotransmitter systems, albeit via systemic routes, that foster LTP or LTD affect the underlying physiology of human motor learning.

3.22.9 Concluding Remarks

The understanding of how neocortex and its associated subcortical structures contribute to generating motor learning and its expression has advanced significantly in the past 20 years. Neuroimaging, novel approaches using neural recording in experimental animals, and interventional studies that use transcranial stimulation have provided new insights into how a variety of cerebral cortical and subcortical targets contribute to performance-related improvements of voluntary movements following practice, development of implicit and declarative knowledge of movement sequences, and how sensory-motor rules become formed and retained. The learning-related changes in activation history or even neuronal spiking cannot directly address underlying synaptic mechanisms that drive motor learning. Not unlike other learning systems, LTP and LTD have been proposed as mechanisms of motor learning, as have changes in neural synchrony within and across structures. In M1, horizontal connections show plastic phenomena that correlate with improvement in skilled performance, an example of procedural motor learning. Since neural plasticity occurs in many brain sites, balancing LTP and LTD could represent a mechanism for the synaptic modifications that may underlie many forms of motor learning. Thus, while procedural motor learning might mostly involve M1, associative motor learning might involve cortical regions other than M1, even though LTP and LTD or perhaps neuronal synchrony might mediate both forms of motor learning, with the inputs to the involved cortical areas determining the form of motor learning.

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3.23 Neurophysiology of Birdsong Learning

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3.23.1 Synopsis

Songbirds are one of the few nonhuman animals that learn to vocalize. Juvenile songbirds first memorize a tutor song and then match this memorized model using auditory feedback. The close parallels between song learning in birds and speech learning in humans have piqued interest in the mechanisms of song learning. The neurobiological analysis of birdsong was revolutionized by the discovery of a specialized constellation of brain nuclei necessary to singing. Part of this 'song system' includes a basal ganglia pathway necessary to song plasticity, providing an important insight into neural mechanisms of song learning. Other important issues, such as where tutor song memories are stored and how and where auditory feedback registers in the brain of the singing bird, are now beginning to be addressed, with many of the most exciting results about to unfold. This chapter discusses song's function as a communication signal, the role experience plays in song development, peripheral and central song mechanisms, and neural mechanisms of song learning. *See* Chapter 1.17 for additional discussion of bird song learning.

3.23.2 The Song Behavior

3.23.2.1 Taxonomy of Songbirds

Oscine songbirds (Aves, Passeriformes, Oscini) comprise over half of the approximately 8000 extant avian species. Oscine songbirds learn to sing, which distinguishes them from most other birds – and indeed almost all nonhuman animals. Most neurobiological studies of singing and song learning have focused on a few domesticated or partially domesticated songbirds that breed readily in captivity. These include zebra finches (*Taeniopygia guttata*), a colonial and nomadic species native to the Australian Outback, Society finches (*Lonchura domestica*), which have been fully domesticated for centuries, and canaries (*Serinus canaria*), a seasonally breeding cardueline finch native to the Canary Islands (**Figure 1**). Although these species will continue to be useful, the great diversity of singing-related behaviors in other wild songbirds provides a vast and currently underexploited resource for comparative analysis of song learning mechanisms.

3.23.2.2 Calls versus Songs

Birds utter both calls and songs (Marler, 2004b; Williams, 2004). Calls are brief sounds (~100 ms) with relatively simple acoustic structure. Songs are longer in duration (typically 1–3 s) and typically more complex in structure, often consisting of extensive and rapid frequency and amplitude modulations. Song complexity does not always reflect learning: The songs of some suboscine birds (Aves, Passeriformes, Tyranni) are complex but innate. Moreover, although calls are simpler than songs, some fine acoustical features of calls also may be learned.

3.23.2.3 Song Nomenclature

Songs can be visualized with a sonogram, which plots frequency as a function of time (**Figure 1**). The smallest song unit is the note, a short burst of sound (~5–100 ms) that is separated by brief (5–10 ms) silence and that appears as a continuous trace on a

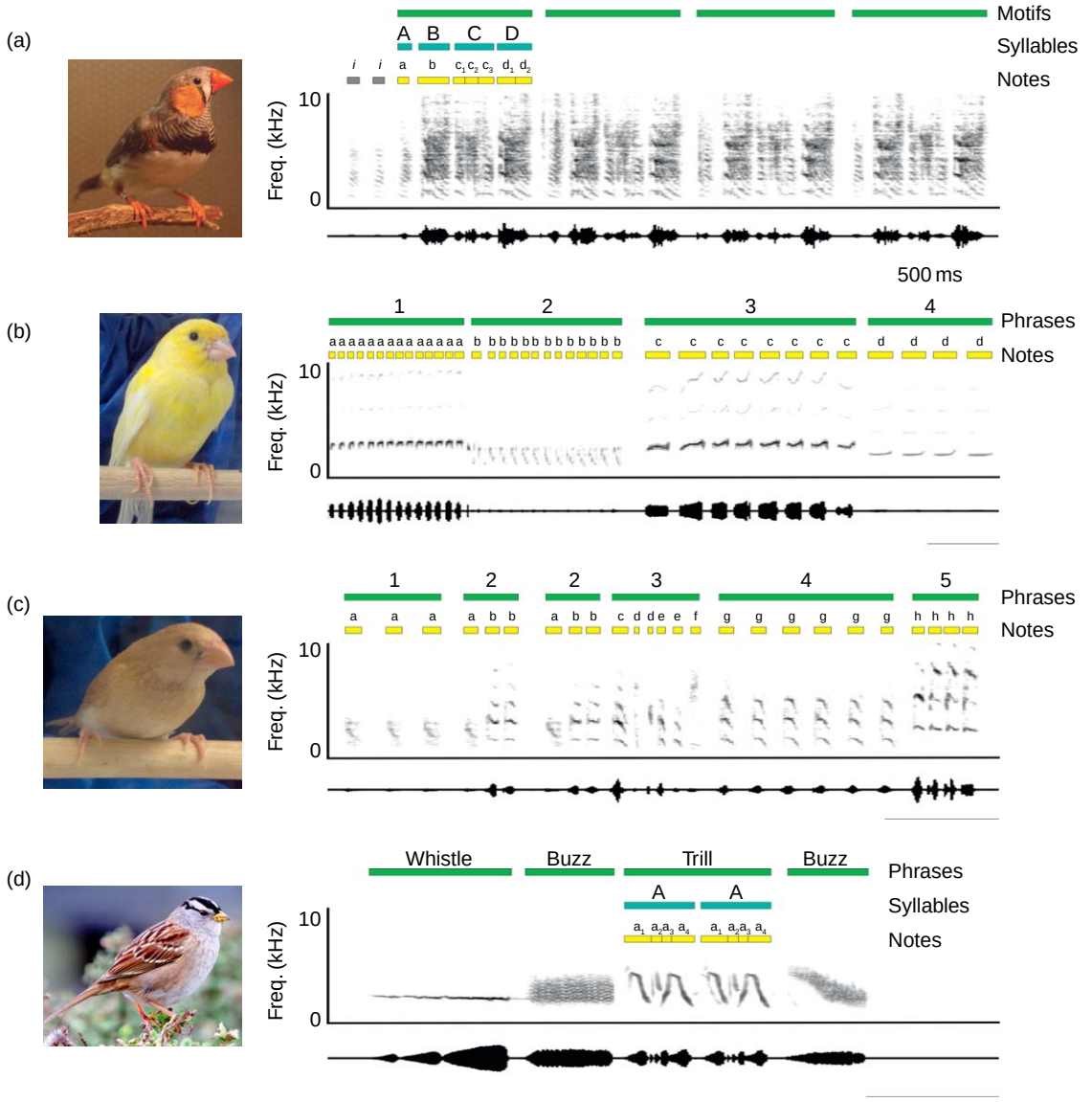


Figure 1 Four male songbirds and their crystallized songs, depicted by an oscillogram (bottom) and a sonogram (top). Colored boxes above each sonogram delineate song components for each exemplar. (a) Zebra finch (*Taeniopygia guttata*) song bouts begin with a series of brief introductory notes (denoted by '*i*' and gray boxes) followed by one or more identical motifs (green boxes). Each motif consists of a stereotyped sequence of syllables (blue boxes), with each syllable comprising one or a few notes (yellow boxes). (b) Canary (*Serinus canaria*) song consists of a series of phrases (green boxes), each of which consists of a trilled single-note (yellow boxes) or multinote syllable (not shown). Male canaries can sing a large number of different phrases, which can be combined in different sequences to produce a large repertoire of songs. (c) Society finch (*Lonchura domestica*) song is characterized by phrases (green boxes) consisting of a sequence of notes or syllables (yellow boxes). Although the note sequence that defines each phrase is typically stereotyped, the phrase sequence can vary across song bouts. (d) White-crowned sparrow (*Zonotrichia leucophrys*) song begins with a whistle phrase (green boxes) a combination of other phrases referred to as buzzes and syllables (a repeated sequence of one or more notes). Zebra finch image courtesy Daniel D. Baleckaitis, canary and bengalese finch images courtesy of David Kloetzer and Jon Prather, white-crowned sparrow image courtesy Vladimir Pravosudov (University of Nevada Reno).

sonogram. One or more notes are grouped to form syllables, which are arranged in specific sequences known as motifs, phrases, or songs, depending on the species (**Figure 1**) (Marler, 2004a). In those birds that produce several distinct songs, each song is referred to as a song type. Song typically lasts ~2 s, but it can be as long as 30 s in canaries and European starlings. Songs are uttered one to several times in quick

succession to form a bout, which can be separated from the next bout by many seconds or even minutes.

3.23.2.4 Function of Song as Communication Signal

3.23.2.4.1 Territorial defense

In most songbirds, song is produced by males, primarily during breeding season. Song serves two primary functions: territorial defense and mate attraction (Catchpole and Slater, 1995). Song's role in territorial defense can be shown by poking a small hole in one of the air sacs in the bird's specialized respiratory system. Such air sac rupture temporarily mutes the bird, and he quickly loses his territory to neighboring males (McDonald, 1989). In a similar vein, neighboring males are reluctant to invade territory vacated by a fellow conspecific bird if the absent male's songs are played through speakers positioned in the vacant territory (Krebs, 1977). Thus, song is necessary to enable a bird to defend his territory against other males and can even serve this function in the absence of visual displays or physical combat.

Distinguishing neighbors from strangers also is important to territorial defense. During the breeding season, a male will aggressively approach and even attack speakers broadcasting unfamiliar songs (Catchpole and Slater, 1995). These territorial displays habituate when the male is repeatedly exposed to several 'virtual neighbors' simulated by songs played through separate speakers. However, when the male hears a 'new' bird's song through one of the speakers, he again attacks, indicating he detected a stranger's song on a background of familiar songs (Nelson and Marler, 1989).

3.23.2.4.2 Mate attraction and female song preferences

The other major function of male song is to attract and arouse conspecific females. Song is a powerful acoustic aphrodisiac that can draw in females literally from out of sight. Song's arousing qualities can be revealed by simply playing it through a loudspeaker. For conspecific female birds in breeding condition, this acoustical stimulation is sufficient to evoke a lordotic response, known as a copulation solicitation display (CSD).

The CSD has helped reveal song features females find attractive. Females tend to favor longer songs containing more complex syllables (Clayton and Prove, 1989), features that distinguish learned songs from those produced by untutored birds (Searcy et al., 1985). Moreover, females prefer the highly stereotyped (crystallized) songs of adult males in breeding condition. In swamp sparrows, females favor songs most challenging for males to produce (Ballentine et al., 2004). Finally, although females often do not sing, their capacity for song discrimination can surpass that of males. For example, female redwing blackbirds make CSDs to the songs of a male conspecific but not to a mockingbird's imitations of this song, while male redwing blackbirds attack speakers broadcasting either song (Searcy and Brenowitz, 1988).

From an evolutionary perspective, female songbirds select males partly based on learned features of song (Searcy et al., 1985). Consequently, sexual selection in songbirds has exerted extraordinary selective pressure on brain structures specialized for singing and song learning. As discussed in greater detail later in this chapter, the elaboration of specialized neural circuits for song has proven to be a great boon to neurobiologists interested in vocal learning mechanisms.

3.23.2.5 Acoustic Features of Song Vary with Function and Context

3.23.2.5.1 Broadcast versus local songs

A basic distinction can be made between broadcast songs, which are highly tonal and typically produced by territorial songbirds to propagate over large distances, and local songs intended for a more intimate audience (**Figure 1**). The acoustic energy in broadcast songs is focused in a narrow-frequency band centered at ~3–4 kHz, avoiding interference with lower-frequency environmental sounds. When broadcast songs propagate over long distances, their tones become 'blurred.' Males disregard spectrally blurred songs, even when played at sound pressure levels approximating songs of nearby birds, indicating that the degree of spectral blurring, rather than absolute loudness, is used to estimate the singer's distance (Naguib and Wiley, 2001). In contrast to broadcast songs, the songs of birds that sing only for a local audience, such as male zebra finches, often are broadband signals that propagate only several to a few tens of meters (**Figure 1**).

3.23.2.5.2 The importance of social context

Song can vary with social context. The songs of adult male zebra finches directed to another individual are slightly more stereotyped than undirected songs

(Kao and Brainard, 2006). The functional significance of these context-dependent changes is unclear, but activity in the male's brain changes depending on whether he is singing directed or undirected songs (Jarvis et al., 1998; Hessler and Doupe, 1999b). Dueting songbirds also afford another example of social contributions to song structure. In some tropical wrens (*Thryothorus spp.*), both males and females sing a small repertoire of song types, and breeding partners sing precisely coordinated duets thought to facilitate breeding synchrony in their equatorial environment (Langmore, 1998).

3.23.3 Song Learning

3.23.3.1 General Themes

Songbirds learn to sing during a juvenile sensitive period comprising two distinct phases, both dependent on auditory experience (**Figures 2** and **3**). During the first phase, known as sensory acquisition, the young bird listens to and memorizes one or more tutor songs. During the ensuing phase of sensorimotor learning, the pupil relies on auditory feedback to match its song to the memorized model. Song crystallization, wherein the song becomes highly stereotyped and usually much less dependent on auditory feedback, signals the end of sensorimotor learning.

In seasonally breeding songbirds that populate temperate regions, 6–10 months separate sensory acquisition and sensorimotor learning (**Figure 2**). In contrast, these periods overlap in species such as the zebra finch, which crystallize their songs only 3 months after hatching (**Figure 2**). Regardless of the pace of song learning, young birds evince sensory plasticity, by memorizing one or more tutor songs, and motor plasticity, by vocally imitating one or more song model.

The great diversity of songbird species is paralleled by minor variations on these major song

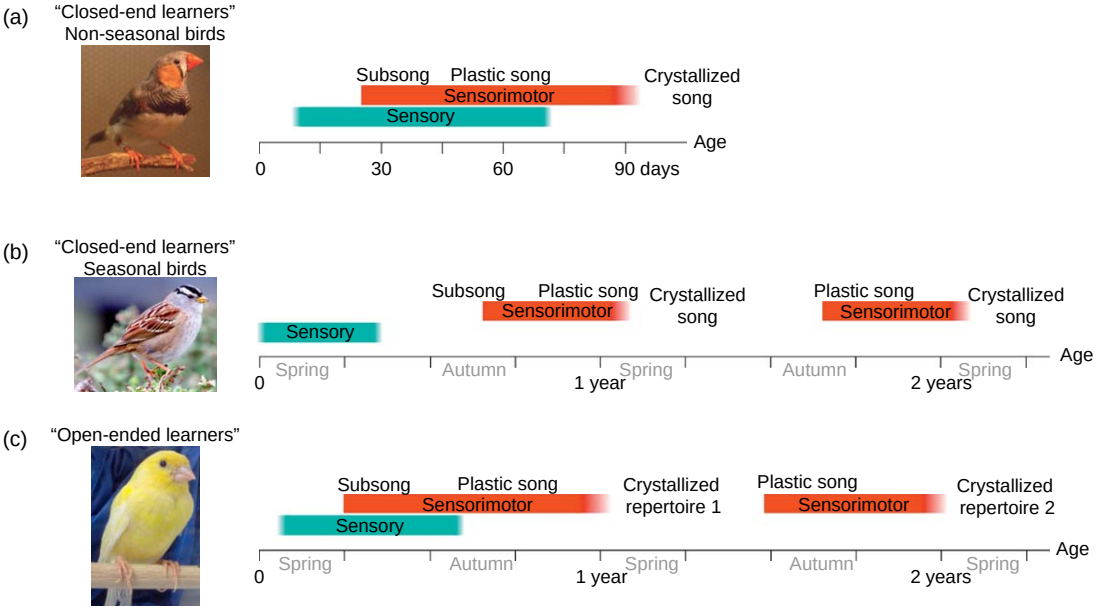


Figure 2 Developmental timelines of song learning in different songbird species illustrate similarities and variations in the song learning process. During sensory acquisition (blue box), the juvenile bird listens to and memorizes one or more tutor songs. During sensorimotor learning (red box), the juvenile matches its own song to the memorized model using auditory feedback. Song crystallization marks the end of sensorimotor learning. 'Closed-end learners,' such as the zebra finch and white-crowned sparrow (a and b), retain the same crystallized song repertoire throughout adulthood, while in 'open-ended learners,' such as the canary, the crystallized repertoire can change from one year to the next. (a) In the zebra finch, the sensory (blue box) and sensorimotor periods (red box) overlap extensively, and song crystallization is complete between 90 and 120 days after hatching. (b) In the white-crowned sparrow, sensory acquisition and sensorimotor learning are separated by many months, indicating that the tutor songs are stored in memory without rehearsal. Song crystallization occurs in the spring, at the end of the first year. Early each ensuing spring, adult male white-crowned sparrows again sing plastic songs, but they recrystallize the same song type as that crystallized in their first year. (c) Canaries are seasonally breeding birds, like white-crowned sparrows, but can exhibit changes in their repertoire of crystallized songs from one year to the next. Zebra finch image courtesy of Daniel D. Baleckaitis, white-crowned sparrow image courtesy of Vladimir Pravosudov (University of Nevada Reno), canary image courtesy of David Kloetzer and Jon Prather.

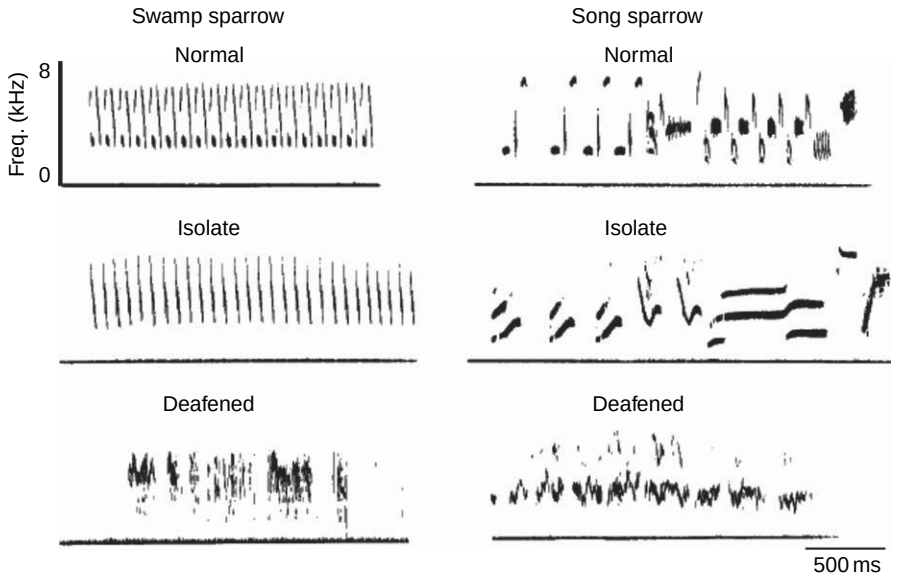


Figure 3 Development of species-typical song structure requires auditory experience of an appropriate song model and experience of singing-related auditory feedback. These sonograms depict songs of adult swamp (left) or song (right) sparrows raised with normal experience of a tutor and with their hearing intact (top row), raised in isolation from others birds' songs with hearing intact (middle) and raised with experience of a tutor song but deafened before sensorimotor learning (bottom). Isolate songs display rudimentary species-typical song features but lack the acoustical complexity of normal wild-type song, underscoring the important role of auditory experience of the tutor song. Songs of deafened birds lack even the rudimentary features of the isolate song, revealing the important role auditory feedback plays in sensorimotor learning Reprinted from Marler P and Doupe AJ (2000) Singing in the brain. *Proc. Natl. Acad. Sci. USA* 97: 2965–2967. Copyright (2000) National Academy of Sciences, U.S.A, with permission.

learning themes (**Figure 2**). 'Closed-ended' learners, which include the white-crowned sparrow and the zebra finch, retain one crystallized song throughout adult life. 'Open-ended' learners, such as the canary, continue to modify their songs as adults, although whether such adult plasticity involves copying of new tutor songs remains uncertain.

3.23.3.2 Sensory Acquisition

3.23.3.2.1 Cross-fostering and isolates

Several lines of evidence show that songbirds learn to sing. First, young songbirds transplanted into the nest of another species develop songs resembling those of their foster parents (Immelmann, 1969; Baptista and Petrinovich, 1984, 1986). Second, local song dialects distinguish different breeding populations of the same songbird species (Marler and Tamura, 1964). Third, birds raised without a tutor subsequently fail to sing species-typical songs and, instead, produce rudimentary 'isolate' songs (**Figure 3**) (Thorpe, 1954, 1958; Immelmann, 1969; Marler, 1970; Price, 1979). This dependence on auditory instruction distinguishes birdsong from most other animal vocalizations that, regardless of their acoustical complexity, develop through innate processes (Konishi and Nottebohm, 1969; Kroodsma and Konishi, 1991).

3.23.3.2.2 Sensory acquisition: born to learn

Young songbirds are prodigious song mnemonists. The number of models that may be memorized during sensory acquisition can range into the hundreds in some species, and the total exposure required for accurate recall can be remarkably limited. In perhaps the most impressive example of this learning capacity, young nightingales accurately learn 10–20 song types (comprising a total of 75–100 syllables) after hearing them fewer than only 20 times (Hultsch and Todt, 1989a,b). Moreover, these memories are stored for many months before the bird actually begins to sing. This astounding feat of auditory memory is reminiscent of other forms of sensory imprinting and suggests that the juvenile songbird is predisposed to memorize certain sounds almost effortlessly.

Cross-fostering experiments show that juvenile songbirds are flexible in the range of songs they will memorize and subsequently copy (Immelmann, 1969; Baptista and Petrinovich, 1984, 1986). Nonetheless, they prefer to learn conspecific songs when given the opportunity. After being tutored on a recorded medley of conspecific and heterospecific songs, juvenile swamp and song sparrows preferentially copy their conspecific songs (Marler and Peters, 1987, 1989; Marler, 1990). Interestingly, these experiments used recorded tutor songs, indicating that juveniles must innately recognize acoustic cues present in the conspecific songs. In the wild, such innate recognition of conspecific song may help naïve juveniles avoid spurious imprinting on heterospecific songs.

3.23.3.2.3 A sensitive period for sensory acquisition

Sensory acquisition closes between the end of the second and third month after hatching in many species (**Figure 2**) (Thorpe, 1958; Immelmann, 1969; Marler and Peters, 1987, 1988). Young birds typically fledge at the end of the first month, so in natural settings juveniles socialize with other potential tutors in addition to their fathers. The timing of sensory acquisition has been mapped most precisely in zebra finches, where juveniles were initially raised with tutors, then removed to an isolated environment at various ages (Bohner, 1990). These controlled tutoring experiments suggest that much of what will be copied can be memorized by the end of the fifth week. Complementary experiments in which birds were sequentially exposed to a series of tutors show that juvenile birds become refractory to further copying from new tutors by about the end of the second month (Eales, 1985). The closure of sensory acquisition is not strictly age limited, because raising birds in isolation extends sensory acquisition 1 to 2 months (Eales, 1987; Morrison and Nottebohm, 1993). Nevertheless, birds subjected to late tutoring copy less extensively than do normally tutored birds, and eventually individuals become totally resistant to learning from a tutor.

3.23.3.2.4 Sensory acquisition results in long-lasting memories of the tutor song

A remarkable feature of sensory acquisition is that the memory of the tutor song can be stored for long periods prior to the first attempts at vocal imitation. In seasonally breeding species, such as swamp and song sparrows, imprinting on the tutor song occurs in the late spring immediately after hatching, but the earliest attempts at vocal imitation do not begin until early in the following spring, a full 8 to 10 months later (**Figure 2**) (Marler and Peters, 1981, 1982b). This capacity to store the tutor song memory for a long period prior to imitation is especially impressive and is one way in which songbirds may differ from humans, where auditory experience of the vocal model and attempts at vocal imitation overlap.

Long-term storage of the tutor song memory may be a general feature of song learning. Despite their normally compressed song learning schedules, juvenile zebra finches briefly exposed to a tutor, then prevented from hearing their own songs for several months by exposing them to a loud masking noise, imitate the tutor song when the noise is turned off (Funabiki and Konishi, 2003). The long delays between tutor song imprinting and subsequent vocal recall afford an opportunity to search for neural correlates of long-lasting auditory memories.

3.23.3.3 Sensorimotor Learning

3.23.3.3.1 General themes including the role of auditory feedback

During sensorimotor learning, the juvenile matches its own song to the memorized tutor model (**Figures 3 and 4**). Although sensorimotor learning typically occurs without ongoing exposure to the tutor, pioneering studies by Mark Konishi showed that juvenile birds deafened after sensory acquisition but before or during sensorimotor learning subsequently developed highly abnormal songs (**Figure 3**) (Konishi, 1965). This observation supports the idea that the juvenile uses auditory feedback to evaluate differences between its own song and a 'template' initially created upon hearing the tutor. Interestingly, Konishi also discovered that birds raised without tutors also develop highly abnormal songs following juvenile deafening (Konishi, 1965). Apparently, even isolate birds use auditory feedback to match their rudimentary songs to an innate 'template.'

Sensorimotor learning comprises several stages (**Figure 4**) (Immelmann, 1969; Marler and Peters, 1982a). Subsong, which constitutes the young bird's earliest song efforts, is a soft and rambling vocalization with little resemblance to the species-typical song. Subsequently, birds produce plastic songs, which contain recognizable notes and syllables that vary in their acoustical structure and sequence from one bout to the next. This bout-to-bout variability is thought to facilitate exploration of the vocal-acoustic space, enabling the pupil to better match the tutor song. In distinction from sensory acquisition, sensorimotor

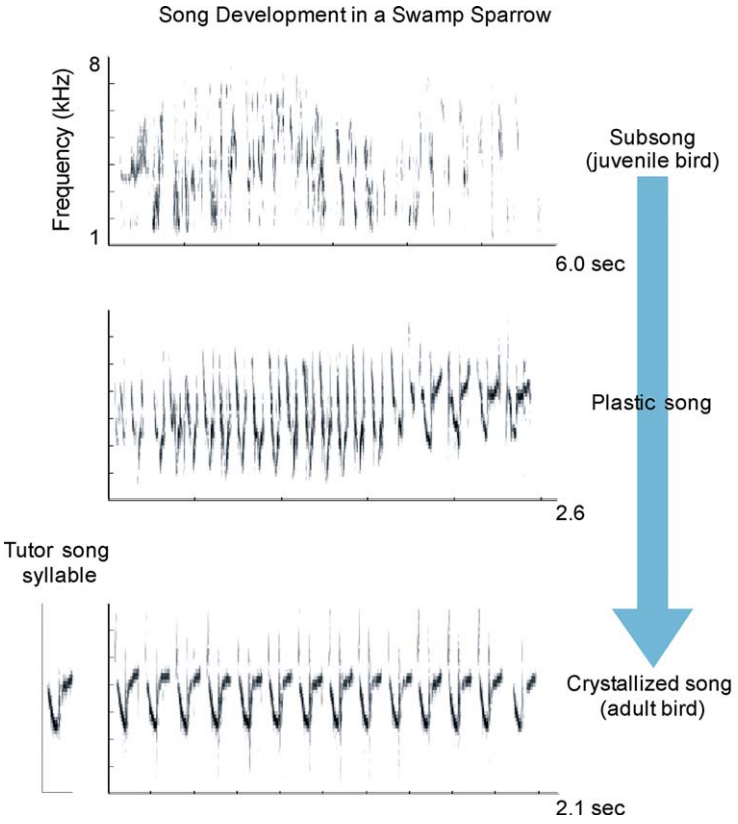


Figure 4 During sensorimotor learning, song progresses from a string of sounds with little recognizable structure ('subsong') to the stereotyped species-typical pattern characteristic of crystallized song. These sonograms depict sensorimotor learning in a male swamp sparrow, a species that in adulthood produces several distinct song types, each consisting of a monosyllabic trill. Subsong contains a variable sequence of notes that bear no obvious resemblance to the tutor song. 'Plastic song' is defined by acoustic features typical of adult song, in this case a trilled syllable similar to one of the tutor songs, and variability in the structure and sequence of notes and syllables. Upon crystallization, the structure and sequence of syllables becomes highly stereotyped. Image created from swamp sparrow data provided by Susan Peters.

learning is a slow process. Over many weeks and tens or hundreds of thousands of vocal renditions, the juvenile's song undergoes plastic changes that render it similar to the tutor model. Thus, juvenile songs display acute variability and adaptive plasticity. Juvenile birds also generate improvised notes and make mistakes in memorization and/or imitation of the tutor song, with the result that their imitations, though highly accurate, are not perfect copies of the tutor. Innate mechanisms also can play a role: juvenile white-crowned sparrows tutored only on overlapping syllable pairs eventually 'stitch' these syllables together to form a complete song phrase (Rose et al., 2004). This interplay between imitation, improvisation, innate constraints, and error yields songs that are unique yet still species typical.

With the onset of sexual maturity, the structure and sequence of notes and syllables becomes highly stereotyped, or 'crystallized.' In contrast to the slow pace of sensorimotor learning, crystallization can occur very rapidly, often in less than 1 week. In many seasonally breeding songbirds, the male sex hormone testosterone is thought to be the catalyst for crystallization (Marler et al., 1988). Because testosterone levels can fluctuate with changes in day length, adult males of seasonally breeding species sing plastic songs early each spring and then recrystallize their songs as spring days lengthen.

3.23.3.3.2 Syllable overproduction and attrition during sensorimotor learning

Much of what we know about sensorimotor learning stems from studying sparrows, songbirds that breed seasonally and that display an 8-month gap between sensory acquisition and the first stages of plastic song. Syllable overproduction and subsequent attrition are major features of sensorimotor learning in these birds

(Marler and Peters, 1982c). In contrast to crystallized songs, plastic songs in sparrows are not only more variable but also contain a wider range of material, much of it learned from various tutors during sensory acquisition. Thus, the juvenile's plastic songs effectively report the numerous tutor songs stored in memory. Upon crystallization, much of this learned material is deleted, with the consequence that the adult crystallized repertoire represents only a subset of what was actually learned.

3.23.3.3.3 Selection-based models of sensorimotor learning

The sequence of syllable overproduction followed by attrition supports a selection-based model of sensorimotor learning (Marler and Peters, 1982c). In this model, overproduction provides a palette of songs from which the young adult chooses its crystallized repertoire. But is this 'choice' random or instead guided by an instructive process? One idea is that slight variations in late plastic songs help the juvenile gain breeding territory by providing it with the necessary behavioral flexibility to match the dialect of the older, more established breeding males in the neighborhood. Indeed, juvenile white-crowned sparrows crystallize a plastic song most like a white-crowned sparrow song broadcast to them repeatedly through a speaker and delete their other plastic songs (Nelson and Marler, 1994).

3.23.3.3.4 A fine time-scale analysis of sensorimotor learning

The pioneering studies of sensorimotor learning relied on tape recordings, which were scanned by human listeners in real time, limiting the numbers of songs that could be analyzed. The advent of cheap mass storage devices coupled with the development of automated song analysis methods, especially those developed by Ofer Tchernichovski and his coworkers, have provided a blow-by-blow account of sensorimotor learning in zebra finches (Tchernichovski et al., 2000, 2001; Deregnaucourt et al., 2005). Such comprehensive analyses have yielded several important insights into vocal learning strategies (Deregnaucourt et al., 2005). First, the match between the pupil's song and the tutor model varies systematically over the course of the day, being poorest in the morning but quickly rising to a plateau by early afternoon. In contrast, daily variations are much more modest following crystallization. Second, the quality of the match partially declines overnight, so that the pupil begins each day slightly worse than it left off the evening before. Third, and perhaps most intriguingly, the greater the night-to-morning song deterioration during sensorimotor learning, the better the final match between the pupil's song and the tutor song. These observations suggest that during juvenile life, sleep triggers song deconsolidation, generating increased variability that enables the pupil to more fully 'search' vocal space in the quest to match the memorized tutor song.

3.23.3.4 Song Crystallization

3.23.3.4.1 A changing role for sensory feedback

Crystallized songs are not only more stereotyped than plastic songs but they also depend less acutely on auditory feedback (Konishi, 1965; Price, 1979). Deafening in juveniles leads to rapid song deterioration, whereas adult deafening in some species can exert little or no effect on the crystallized song (Konishi, 1965; Price, 1979). This implies that crystallization either transforms the song motor program into a feedback-independent 'read-only' system or that crystallized song maintenance is mediated by nonauditory (i.e., proprioceptive) forms of sensory feedback. Notably, when brief puffs of air are injected into the respiratory system of singing birds, respiratory and vocal muscles rapidly (~10 ms) compensate to maintain stable song output; these effects are seen even in deafened birds, indicative of proprioceptive feedback (Suthers et al., 2002). However, whether proprioceptive feedback is used for crystallized song maintenance remains untested.

The degree to which crystallized songs depend on auditory feedback varies across species. Pioneering studies by Mark Konishi indicated that crystallized songs of adult white-crowned sparrows were impervious to deafening (Konishi, 1965). In contrast, crystallized songs of adult zebra finches are maintained actively via auditory feedback: Adult deafening (Nordeen and Nordeen, 1992) or chronic exposure to distorted auditory feedback (Williams and McKibben, 1992; Leonardo and Konishi, 1999) triggers a slow process of song deterioration and plastic changes to the vocal pattern, known as decrystallization. Nonetheless, as with humans, deafening-induced vocal deterioration in zebra finches is slower in adults than in juveniles (Price, 1979; Cowie and Douglas-Cowie, 1992). Notably, adult Society finches, close relatives of the zebra finch, remain acutely dependent on auditory feedback, as their crystallized songs start to deteriorate several days to a week after deafening (Okanoya and Yamaguchi, 1997; Woolley and Rubel,

1997). In both zebra finches and Society finches, one idea is that the neural mechanisms enabling feedback-dependent learning in the juvenile also are employed to maintain stable song patterns in the adult.

3.23.3.4.2 Crystallization can impose innate constraints on song structure

A major insight is that crystallization can impose innate, species-typical constraints on song structure. Juvenile canaries tutored on synthetic songs provide an especially elegant demonstration of this effect (Gardner et al., 2005). In normal adult canaries, short stereotyped syllables are repeated to form phrases, which are linked together in the crystallized song. Synthetic 'phraseless' songs, in which syllables continuously vary in duration and amount of frequency modulation, can be used to tutor young canaries. Remarkably, juveniles first produce faithful imitations of such phraseless tutor songs but then impose phrasing with crystallization. Such innate constraints may explain why the songs of birds deafened early in life still exhibit crude but species-typical song features and why isolate songs are more similar within rather than across species. These innate mechanisms also could account for the conservation of song phenotype across geographically isolated breeding populations of the same species. If the brain of the naïve juvenile was truly a tabula rasa, then songs would randomly diverge across isolated populations, making such song conservation improbable.

3.23.3.4.3 Crystallization and critical periods for motor learning

Most evidence indicates that crystallization is not simply the result of successful learning but is the result of other factors – specifically testosterone. Male swamp and song sparrows castrated as juveniles develop plastic songs containing imitations of tutor songs, but they fail to undergo song crystallization unless implanted with testosterone (Marler et al., 1988). More generally, many seasonal songbirds crystallize their songs as vernal days lengthen and their testosterone levels rise but 'decrystallize' their songs as autumnal days shorten and their testosterone levels drop (**Figure 2**) (Nottebohm et al., 1987; Smith et al., 1997a). Thus, crystallization can be regulated by photoperiod-sensitive endocrine factors rather than the quality of the match to the tutor song.

Some evidence of an age-dependent component of crystallization comes from experiments in which botox injections were used to reversibly paralyze syringeal muscles of juvenile zebra finches (Pytte and Suthers, 2000). When vocal paralysis spanned the period bracketing crystallization, the birds crystallized abnormal songs. In contrast, permanent disruptions in song quality were not observed when reversible paralysis was induced either earlier in sensorimotor learning or after crystallization.

Although this age-dependent effect may point to a critical period for sensorimotor learning, different aspects of this learning process may be regulated independently. As mentioned earlier, juvenile zebra finches can be prevented from copying previously memorized Society finch tutor songs by chronically exposing them to masking noise (Funabiki and Konishi, 2003). Such 'reversibly deafened' birds successfully imitated syllables from the memorized tutor songs when the noise was turned off between 100 to 200 days after hatching, one to several months after crystallization normally occurs in this species. However, the phrase structure of the Society finch tutor song was only imitated if the masking noise was turned off prior to day 80; otherwise, imitations consisted of Society finch notes organized into motifs typical of normal zebra finch songs. These results indicate that the closure of sensorimotor learning is not strictly age limited, at least when auditory feedback is blocked, and also hint that different aspects of sensorimotor learning, particularly note versus phrase imitation, are regulated independently.

3.23.3.4.4 Vocal plasticity following song crystallization

In seasonal birds such as swamp sparrows, syllables 'lost' during the initial round of crystallization may reappear in plastic songs in subsequent years, suggesting that they persist as auditory or motor memories (Marler and Peters, 1982a). Despite transient reexpression of 'lost' syllables, swamp sparrows nevertheless recrystallize the same subset of song types each summer. Those species that never alter their crystallized songs, which include zebra finches as well as North American sparrows, are referred to as 'closed-ended' or 'age-limited' learners (**Figure 2**) (Marler and Peters, 1987). In contrast, 'open-ended' or 'age-independent' learners, such as canaries, change their songs with each round of recrystallization (**Figure 2**) (Nottebohm and Nottebohm, 1978; Nottebohm, 1984). From a neurobiological perspective, this ongoing pattern of vocal 'exuberance' followed by attrition suggests that brain mechanisms engaged during sensorimotor learning in the juvenile can be reengaged in the adult.

3.23.4 Peripheral Mechanisms of Song Production

3.23.4.1 General Themes

Birds and mammals vocalize by inducing pressure waves in the expiratory air column. In general, this is achieved by passing air over vibrating membranes within a vocal organ. In humans, the vibratory elements are the vocal folds in the larynx. Although birds have a larynx, their vibratory element is in the syrinx, a specialized vocal organ unique to birds (**Figure 5**) (King, 1979).

The structure of the syrinx varies greatly across different avian taxa (King, 1979). General features of the syrinx are that it is located in the airway below the larynx, and it can be moved by sets of intrinsic and extrinsic muscles. The syrinx of oscine songbirds is a bipartite structure located at the confluence of the trachea and the two bronchi and contains a larger number of intrinsic muscles than in nonoscines (**Figure 5**). This sophisticated intrinsic musculature, the bipartite structure of the syrinx, and the highly specialized avian respiratory system enable virtuosic song displays.

A current view is that vocal output is determined by expiratory air pressure, syringeal muscle tension as air passes through the bronchial lumen, and filtering by the upper vocal tract (Suthers and Margoliash, 2002; Goller and Cooper, 2004; Suthers and Zollinger, 2004). Indeed, mathematical models indicate that small variations in the timing and magnitude of expiratory pressure and syringeal tension are sufficient to generate many of the acoustic features of birdsong (Mindlin, 2005).

3.23.4.2 The Syrinx: A Vibrating Vocal Organ

3.23.4.2.1 Anatomy and function of the syrinx

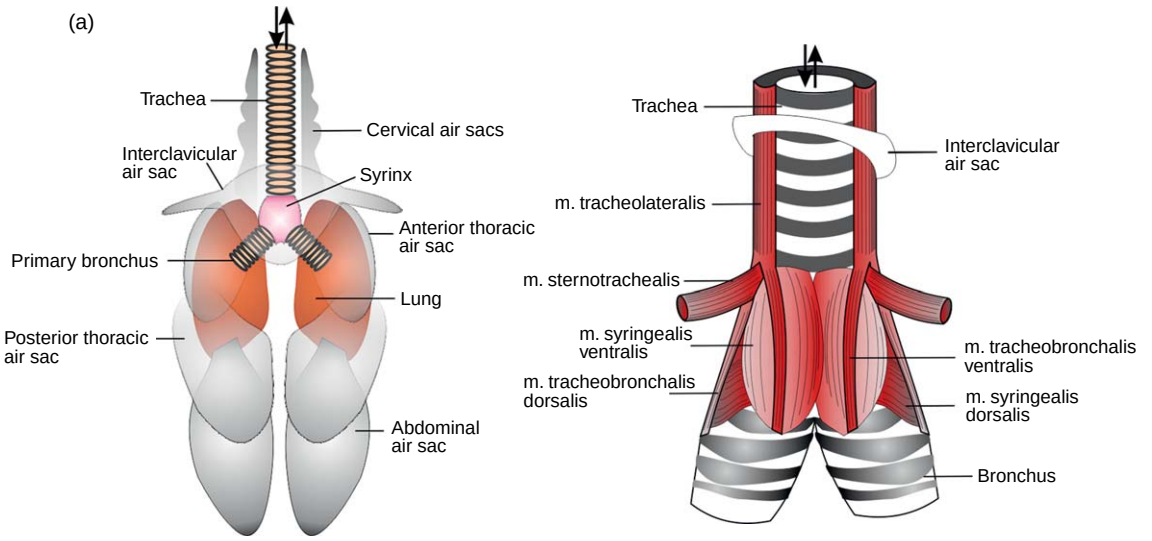
The oscine syrinx consists of a group of intrinsic muscles attached to specialized cartilaginous rings in caudal portions of the trachea and/or the primary bronchi (**Figure 5**) (King, 1979). In the upper bronchi, the medial parts of the rings are absent and instead consist of a sheer membrane, known as the medial tympaniform membrane (MTM). The cranial end of each bronchus is characterized by thickenings known as the medial and lateral labia. Over a century ago, Setherwall (1901) suggested that the syringeal labia were functionally analogous to the laryngeal vocal folds. Miskimen (1951) later suggested that the MTM was the primary vibratory source of sound in the syrinx, an idea that dominated for several decades. The highly tonal quality of many birdsongs also led to an alternate, 'aerodynamic' hypothesis (Nottebohm, 1976; Gaunt et al., 1982; Gaunt, 1983; Casey and Gaunt, 1985), namely, that birdsong is not produced by a vibratory source but, instead, by air passing through a constricted bronchial lumen, which would act like a hole-tone whistle. Over the last several decades, Franz Goller, Roderick Suthers, and their colleagues used bronchial airflow measurements (Suthers, 1990; Goller and Suthers, 1996a,b) and endoscopic examination of the syrinx (Goller and Larsen, 1997; Larsen and Goller, 2002) in singing birds to show that song results from rapid vibrations in the syringeal labia, rather than by vibrations in the MTM or aerodynamic effects (Goller and Cooper, 2004; Suthers and Zollinger, 2004).

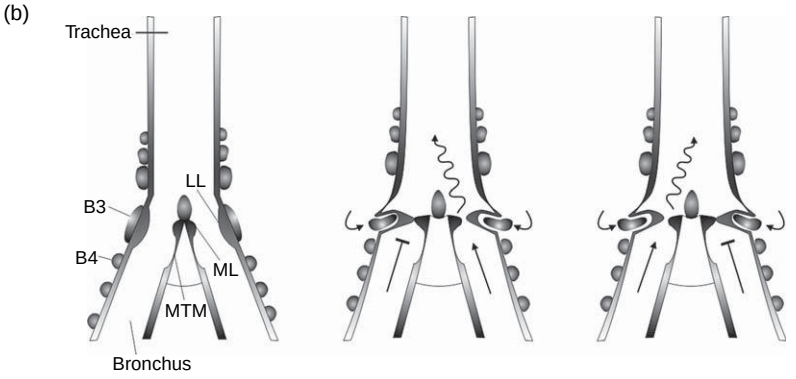
3.23.4.2.2 Ventral versus dorsal syringeal muscles

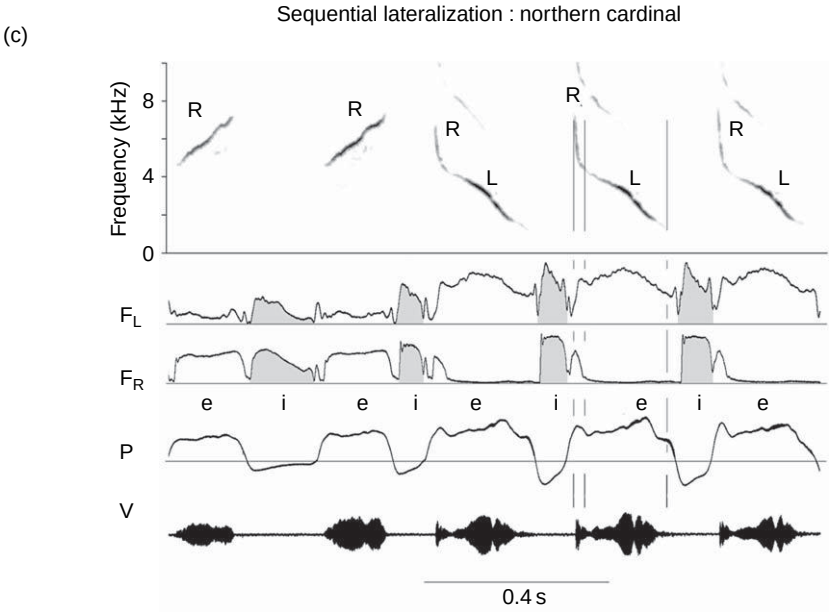
Studies by Suthers and his colleagues also lend considerable insight into the role of different syringeal muscles in birdsong. By using fine wires to record syringeal muscle activity while simultaneously measuring bronchial air flow and subbronchial air pressure in singing birds, Goller and Suthers determined that ventral syringeal muscle activity correlates with the song's fundamental frequency, while dorsal syringeal muscle activity controls air flow through the syrinx and thus sound output (Goller and Suthers, 1996a,b). This suggests that the different syringeal muscles can independently regulate the pitch and fine temporal features of song.

3.23.4.2.3 Independent control of the two sides of the syrinx

Songbirds exploit the bipartite structure of the syrinx to maximum effect. In several species, the two sides of the syrinx have been shown to move independently (Suthers, 1990; Goller and Suthers, 1995), greatly increasing fluency and vocal range (Zollinger and Suthers, 2004). Several species, including the brown thrasher, independently control the two sides of the syrinx to simultaneously sing two harmonically distinct sounds, a vocal effect termed the 'two-voice phenomenon' (Greenwalt, 1968; Suthers et al., 1994).The two sides of the syrinx also differ slightly in diameter, and thus in resonant frequency. The different resonant frequencies of the two sides allow


(a)
Trachea
Cervical air sacs
Interclavicular air sac
Syrinx
Anterior thoracic air sac
Primary bronchus
Lung
Posterior thoracic air sac
Abdominal air sac
Trachea
Interclavicular air sac
m. tracheolateralis
m. sternotrachealis
m. syringealis ventralis
m. tracheobronchalis dorsalis
m. tracheobronchalis ventralis
m. syringealis dorsalis
Bronchus


(b)
Trachea
LL
B3
B4
ML
MTM
Bronchus


(c)
Sequential lateralization : northern cardinal
Frequency (kHz)
8
4
0
R
L
F_L
F_R
e i e i e i e i e
P
V
0.4 s

rapid alternation between notes of greatly contrasting pitch, an effect difficult to achieve with a unipartite vocal organ. The cardinal exploits these different resonances, skillfully and seamlessly switching from one side to the other to sing extremely broad frequency-modulated sweeps, or glissando notes (**Figure 5(c)**) (Hartley and Suthers, 1990; Suthers et al., 2004). Intriguingly, mockingbirds use a similar strategy when imitating the cardinal's glissando notes, suggesting constraints on peripheral song mechanisms (Zollinger and Suthers, 2004).

3.23.4.2.4 Nonlinear oscillatory properties intrinsic to the syrinx

Notably, some of birdsong's acoustical complexity results from nonlinear oscillatory properties intrinsic to the syrinx (Fee et al., 1998), rather than highly elaborate patterns of neuromuscular control. Experiments involving isolated syringes have shown that the syrinx displays nonlinear oscillatory dynamics in response to continuous variations in respiratory drive or syringeal activity (Fee et al., 1998). In terms of neural codes for song, modeling studies show that even simple and continuous changes in neuromuscular activity can trigger nonlinear modes of syringeal vibration, resulting in spectrally and temporally complex sounds (Mindlin et al., 2003; Mindlin, 2005).

3.23.4.3 The Avian Respiratory System and Temporal Control of Song

When songbirds sing, they exploit their specialized respiratory systems, which display impressive adaptations to the metabolic demands of flight (Scheid and Piiper, 1979). Unlike the tidal action of mammalian respiration, in which the lungs fill and empty with each respiratory cycle, avian respiration is unidirectional, with oxygenated air always flowing from the caudal to the rostral margin of the lung. This unidirectional flow is achieved by a series of highly inflatable air sacs that act like bellows to perfuse the relatively inflexible lungs (**Figure 5(a)**). The air sac system and very rapid inspiratory activity (i.e., minibreaths) enable songbirds such as the canary to generate rapidly (5–30 Hz) trilled songs lasting tens of seconds (Suthers et al., 2004).

Measurements of air sac pressure can be used to estimate changes in the pressure head that drives air through the syrinx during singing (Suthers, 1990; Goller and Suthers, 1996a,b; Suthers and Zollinger, 2004). These measurements show that respiratory patterns determine the temporal structure of birdsong. In almost all species studied to date, sounds are produced during expiration, while silent intervals between notes and syllables correspond to inspiration (**Figure 5(c)**). Expiratory pulses may constitute

Figure 5 Anatomy of the songbird respiratory system and the syrinx, and their integrated activity during singing. (a) (left) The avian respiratory system is distinguished by a series of air sacs (gray) that function as bellows to move air through the relatively inflexible lungs. The songbird vocal organ, the syrinx, is a bipartite structure located at the junction between the primary bronchi and the trachea. (a) (right) Ventral view of the songbird syrinx and associated muscles. The syrinx is affected by six bilaterally paired muscles, each innervated by the tracheosyringeal branch of the ipsilateral hypoglossal nerve (XIIts). These muscles control the movement and tension of the medial and lateral labia (ML and LL, respectively), thickenings at the cranial end of each bronchus within the syrinx (see b). (b) A cross section through the syrinx schematizing quiet respiration (left) and sequential lateralized airflow during phonation (middle and right). The trachea and bronchi consist of a series of cartilaginous rings; in the syrinx, the medial parts of the rings are absent and are replaced by the medial tympaniform membrane (MTM). During quiet respiration (left), the syringeal lumen is open on both sides. During singing (middle and right), contraction of the syringeal muscles (m. syringealis dorsalis and m. tracheobronchalis dorsalis) rotates the third bronchial cartilage (B3) into the syringeal lumen, forcing the LL and ML into the airstream and causing them to vibrate, resulting in sound (wavy lines). Phonation can be bilateral (not shown) or unilateral, when contraction of the syringeal muscles on one side of the syrinx is sufficient to completely block airflow. (a, b) Adapted from Suthers RA and Zollinger SA (2004) Producing song: The vocal apparatus. *Ann. N.Y. Acad. Sci.* 1016: 109–129. (c) The song of a northern cardinal illustrates sequential unilateral phonation during singing. The sonogram (top) shows a series of five syllables consisting of long frequency sweeps spanning as much 5 kHz. The lower panels are: airflow through the left (FL) and right (FR) sides of the syrinx (horizontal lines = zero air flow; e, expiration; i, inspiration, shaded grey); P, cranial air sac pressure (horizontal line = ambient pressure); V, oscillogram. The first two syllables, consisting of upward frequency sweeps, were generated largely by the right side of the syrinx. The following three syllables consist of long downward FM sweeps, with the initial high-frequency portion (between first and second vertical lines) produced through the right side of the syrinx and the final lower-frequency portion of the syllable produced by the left side. Figure courtesy of Rod Suthers. Images (a) and (b) courtesy of Todd Roberts. Image (c) courtesy of Rod Suthers.

fundamental motor units of song: Singing birds startled by brief stroboscopic flashes complete the ongoing syllable, only becoming silent at the end of the current expiratory cycle (Franz and Goller, 2002).

Ultimately, the song's temporal pattern is a product of precise control of expiration. Therefore, neural circuitry for song must control and coordinate both syringeal and respiratory motor activity. Indeed, separate neural pathways for respiratory and syringeal control exist in the songbird's brain (Wild, 1997a; Suthers and Margoliash, 2002), a point discussed in greater detail in following sections.

3.23.4.4 Syringeal Dynamic and Upper Vocal Tract Filtering

Songbirds and humans use their upper vocal tracts to filter vocal output. Indeed, simultaneous free field and syringeal recordings show that highly tonal sounds emitted at the beak correspond to harmonic series of tones emanating from the syrinx (Beckers et al., 2003). This discrepancy suggests that the trachea and beak selectively filter out certain harmonics emanating from the syrinx. In essence, the bird's upper vocal tract acts as a filter matched to certain wavelengths of sound produced by the syrinx. One way this filtering effect can be revealed is by placing birds in a helium–oxygen atmosphere (heliox); when notes are sung in heliox (Nowicki, 1987), they sound higher in pitch because the energy of the fundamental decreases while the energy of certain harmonics increases. This effect arises because the speed of sound increases in heliox while the fundamental frequency of syringeal vibration remains unchanged. As a consequence, the sound wavelength associated with syringeal vibration lengthens, no longer matching the filter. Instead, upper harmonics of the syringeal vibration better match the resonant properties of the upper vocal tract, imparting a higher pitch to the note.

The upper vocal tract of birds is a highly dynamic structure that can rapidly match the changing vibratory modes of the syrinx. The beak is part of this variable filter (Goller et al., 2004; Nelson et al., 2005; Fletcher et al., 2006); when the beak is opened wide, the effective length of the upper vocal tract shortens, raising the resonant frequency (Westneat et al., 1993). High-speed X-ray films of singing birds (Riede et al., 2006) show that the oropharyngeal cavity also is actively manipulated to dynamically alter the upper vocal tract resonance. These various observations imply that the bird's brain must actively coordinate respiratory patterning, syringeal tension, and upper vocal tract dynamics to produce song.

3.23.5 Neural Circuits for Singing and Song Learning

3.23.5.1 General Themes

The foundation of songbird neurobiology rests on several major discoveries made over the past three decades. Arguably the most important of these was that the songbird's telencephalon exerts executive control of brainstem vocal-respiratory networks during singing, much as human language cortices command brainstem vocal-respiratory networks during speech (Doupe and Kuhl, 1999). This veritable epiphany stemmed from pioneering neuroanatomical and behavioral studies conducted by Fernando Nottebohm and his colleagues (Nottebohm et al., 1976, 1982; Nottebohm, 2005), who found that the songbird's brain contains a 'song system' – a constellation of interconnected nuclei necessary to singing (**Figure 6**).

Two telencephalic song nuclei, HVC and RA, are essential to singing and form a descending pathway that links the telencephalon to the brainstem vocal-respiratory network (**Figure 6**) (Nottebohm et al., 1976; Wild, 1997b). Intensive studies have yielded much information about the organization of this song motor pathway (SMP) and the nature of song motor 'codes' (Yu and Margoliash, 1996; Fee et al., 2004). Although the SMP has traditionally been viewed as a feedforward circuit, recent findings suggest that recurrent pathways from the brainstem to HVC contribute to song patterning (Ashmore et al., 2005).

A second key discovery was that the song system contains an anterior forebrain pathway (AFP) unnecessary to crystallized song but essential to song plasticity in both juvenile and adult birds (**Figure 6**) (Bottjer et al., 1984; Williams and Mehta, 1999; Brainard and Doupe, 2000; Olveczky et al., 2005). The AFP indirectly links HVC to RA and resembles mammalian cortical–basal ganglia (BG) pathways (Doupe et al., 2005). Experiments have revealed that the AFP helps generate acute song variability (Kao et al., 2005; Olveczky et al., 2005) and also acts over longer timescales to regulate the strength of synaptic connections between HVC and RA (Kittelberger and Mooney, 1999).

A final key discovery was that song nuclei display sensory as well as motor activity (**Figure 6**) (McCasland and Konishi, 1981). Auditory responses

highly selective for the bird's own song can be detected in the song system (Margoliash, 1983; Margoliash and Konish, 1985; Doupe and Konishi, 1991; Margoliash and Fortune, 1992; Mooney, 2000), and behavioral studies show that birds exhibit perceptual as well as vocal motor deficits when HVC or AFP nuclei are damaged (Brenowitz, 1991; Del Negro et al., 1998; Scharff et al., 1998a; Burt et al., 2000). Notably, the sensorimotor nature of HVC and the AFP resembles the expressive and receptive functions performed by language cortices in humans (Doupe and Kuhl, 1999). A major goal of current research is to understand whether auditory activity in the song system relays information about the memorized tutor song and/or auditory feedback.

3.23.5.2 Brainstem Vocal Respiratory Networks

3.23.5.2.1 General themes

Two key determinants of song structure – syringeal tension and respiratory patterning – are mediated by vocal respiratory networks in the brainstem and spinal cord (**Figure 6**). Substantial progress has been made in characterizing the anatomy of this vocal respiratory network. However, we still know relatively little about how these networks function during singing and the neural mechanisms that mediate upper vocal tract filtering (Wild, 2004).

3.23.5.2.2 The tracheosyringeal motor nucleus

The syringeal muscles are innervated by motor neurons in the tracheosyringeal part of the hypoglossal motor nucleus (XIIts) (Nottebohm et al., 1976), a midline medullary nucleus situated caudal to the obex (**Figure 6**). XIIts is myotopically organized: caudal XIIts motor neurons innervate dorsal syringeal muscles, whereas rostral XIIts motor neurons innervate ventral syringeal muscles (Vicario and Nottebohm, 1988). Syringeal motor neurons project ipsilaterally onto the muscles of the syrinx, providing one substrate for lateralized syringeal control. In certain songbird species, unilateral section of the XIIts nerve exerts different effects on song depending on which side is cut (Nottebohm, 1971, 1977; Williams et al., 1992). This observation initially suggested that the central structures controlling song also may be lateralized, as cortical control of speech is lateralized in humans.

Syringeal motor neurons drive highly dynamic syringeal muscle activity during singing. When the XIIts nerve is cut, the spectral and fine temporal features of song are severely disrupted, while the global temporal features of song, which are determined by expiratory musculature, remain largely intact (Williams et al., 1989, 1992; Simpson and Vicario, 1990). The sparing of song temporal structure following XIIts nerve section indicates that central pathways controlling syringeal and respiratory activity are independent. Indeed, these two control systems are partly segregated at the brainstem and at higher levels of the song system (Wild, 1993a,b; Reinke and Wild, 1998b; Suthers and Margoliash, 2002).

3.23.5.2.3 Brainstem and spinal cord respiratory networks

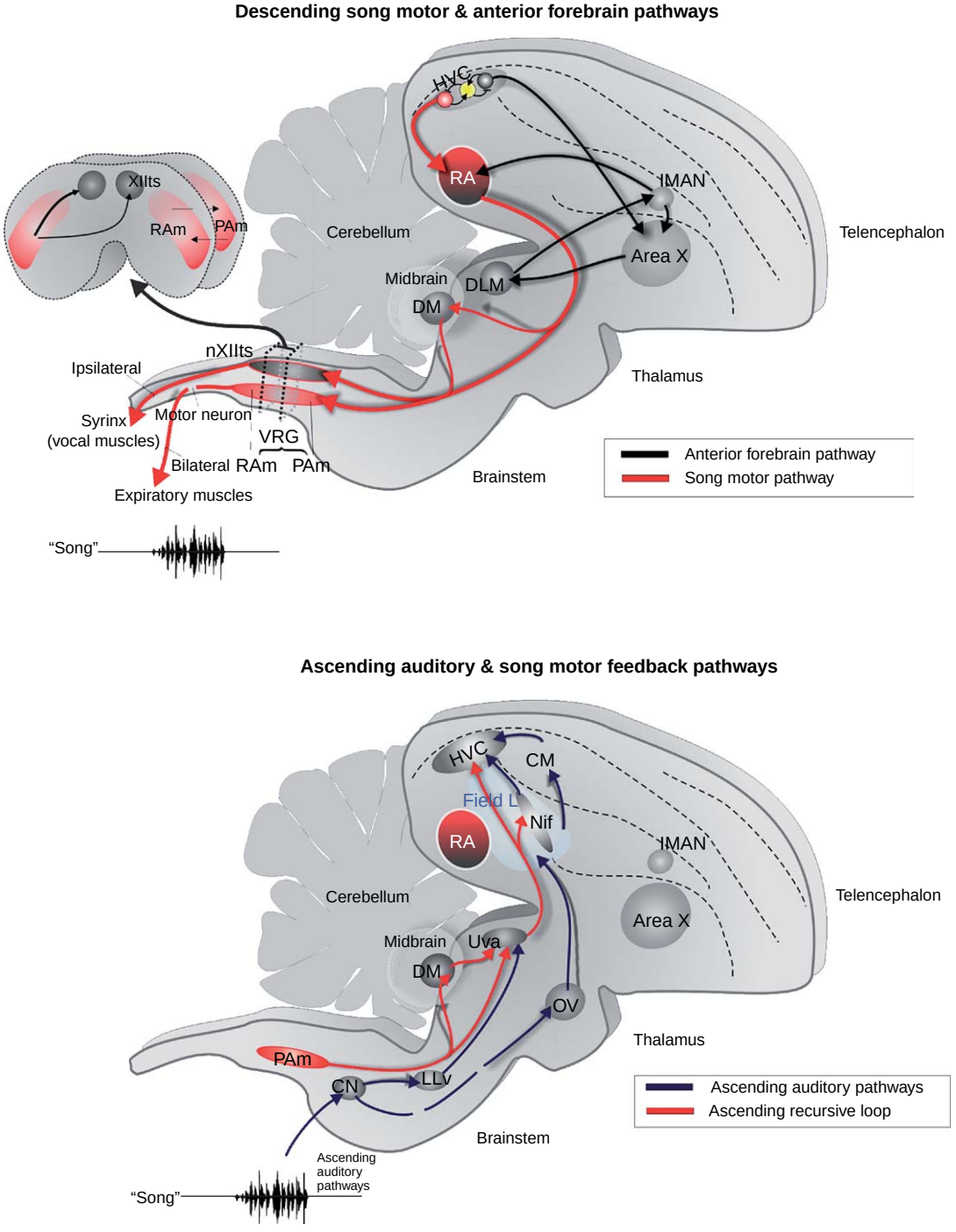
Expiratory and inspiratory motor neurons reside in the thoracolumbar and upper thoracic spinal cord, respectively (Wild, 2004). A cell column in the ventrolateral medulla, known as the ventral respiratory group (VRG), contains premotor neurons that innervate these expiratory and inspiratory motor neurons, thus controlling respiration (**Figure 6**) (Wild, 1994). The caudal VRG (nucleus retroambigualis [RAm]) controls expiration (Wild, 1993a,b), while the rostral VRG (nucleus parambigualis [PAm]) controls inspiration (Reinke and Wild, 1998b). The VRG is bilaterally organized and reciprocally interconnected, with RAm neurons projecting throughout the ipsilateral and contralateral VRG (**Figure 6**). These bilateral projections, as well as the bilateral spinal projections of VRG axons, provide an anatomical substrate for bilateral coordination of respiration during song.

Vocalization requires precise coordination of respiratory and syringeal activity. The bilateral synaptic connections that VRG neurons make onto XIIts motor neurons provide a substrate for this coordination and also are the likely source of the respiratory rhythm that can be recorded from XIIts in nonvocalizing birds (Manogue and Paton, 1982; Williams and Nottebohm, 1985; Vicario, 1991a). Recordings made in brain slices show that XIIts motor neurons receive inhibitory and excitatory inputs from the VRG (Sturdy et al., 2003); the inhibitory input may help establish the observed phase delays between onset of expiratory and syringeal muscle activity (Vicario, 1991a). Although XIIts motor neurons are highly linear in their intrinsic firing properties (Sturdy et al., 2003), the extensive interconnectivity of the brainstem vocal-respiratory network may contribute to pattern generation beyond that provided by inputs from the telencephalic song premotor nucleus RA (Sturdy et al., 2003; Kubke et al., 2005).

3.23.5.3 The Song System: Song Motor and Anterior Forebrain Pathways

3.23.5.3.1 The dawn of songbird neurobiology

Songbird neurobiology took flight in the mid-1970s, when Fernando Nottebohm, Tegner Stokes, and Christiana Leonard discovered that specialized nuclei in the songbird's telencephalon are essential to singing. In a landmark study (Nottebohm et al., 1976), Nottebohm and his coworkers showed that bilateral lesions to one of these nuclei, now referred to simply as 'HVC,' rendered an adult male canary



mute for song, even though it readily assumed a singing posture when presented with a female canary and produced unlearned calls normally. Anterograde tracing revealed two efferents of HVC: the robust nucleus of the arcopallium (RA), in the caudal telencephalon, and area X, in the avian basal ganglia. As its rather mysterious-sounding name might imply, lesions to area X exerted no obvious effect on crystallized song. However, RA lesions profoundly disrupted song, and tracing studies revealed that RA axons terminated in XIIts, suggesting that RA was a song premotor nucleus. This study showed that birdsong, unlike most other animal vocalizations save perhaps human speech, involves direct telencephalic control of vocal motor neurons.

3.23.5.3.2 Current overview of song system anatomy

In the past 30 years, a fully fledged song system has taken wing (Nottebohm, 2005). This system can be divided into two major components: a song motor pathway (SMP) and an anterior forebrain pathway (AFP) (Nottebohm et al., 1976), which both emanate from HVC and converge in RA (**Figure 6**). The SMP and the AFP arise from distinct pools of projection neurons (PNs) located in HVC (Katz and Gurney, 1981; Kirn et al., 1991; Fortune and Margoliash, 1995; Mooney, 2000). One HVC PN type (HVC_{RA}) provides excitatory input onto RA PNs (Mooney, 1992), which innervate syringeal motor neurons and respiratory premotor neurons (Nottebohm et al., 1976; Wild, 1993b). The other PN type (HVC_X) innervates area X, which is part of a serially connected pathway that indirectly links HVC to RA and includes the thalamic nucleus DLM and the anterior telencephalic nucleus LMAN (Nottebohm et al., 1982; Okuhata and Saito, 1987; Foster and Bottjer, 1998). The axons of LMAN PNs bifurcate, with one branch innervating area X (Nixdorf-Bergweiler et al., 1995) and the other forming excitatory synapses on the same RA PNs that receive input from HVC (Mooney, 1992). Targeted photoablation of HVC_{RA} but not HVC_X neurons grossly disrupts song (Scharff et al., 2000), mirroring the differential effects of RA versus area X lesions (Nottebohm et al., 1976) and reinforcing the idea that HVC_{RA} neurons drive song premotor activity in RA. Ultimately, the SMP and AFP arise from different pools of HVC PNs and converge on song premotor neurons in RA that constitute the sole forebrain output of the song system.

The nucleus RA displays anatomical features likely to facilitate selective control of syringeal and respiratory activity. First, neurons located in ventral and medial RA project onto rostral and caudal XIIts (Vicario, 1991b), respectively. Because these different parts of XIIts ultimately innervate different syringeal muscle groups (Vicario and Nottebohm, 1988), activity in different parts of RA could recruit different

Figure 6 Specialized neural circuits in the songbird's brain, collectively referred to as the 'song system,' enable singing and song learning. (a) The song motor pathway (SMP; red) and the anterior forebrain pathway (AFP; black) are schematically illustrated in a parasagittal section through the songbird brain. The SMP arises from neurons in HVC (HVC_{RA} neurons) that project directly to the robust nucleus of the arcopallium (RA). RA in turn provides song motor output from the telencephalon through its projections onto syringeal motor neurons in the tracheosyringeal portion of the hypoglossal motor nucleus (XIIts) and onto respiratory premotor neurons in a column of cells in the ventrolateral medulla known as the ventral respiratory group (VRG). The VRG comprises the nucleus retroambigualis (RAm), which controls expiration, and the nucleus parambigualis (PAm), which controls inspiration. RA also projects onto the dorsomedial intercollicular nucleus (DM) in the midbrain, which also innervates XIIts and the VRG; DM plays a role in call generation in birds. The anterior forebrain pathway (black arrows) arises from a distinct population of HVC neurons (HVC_X neurons) that innervate area X (part of the songbird basal ganglia). Large inhibitory neurons in area X project axons onto the medial nucleus of the dorsolateral thalamus (DLM), which in turn provides excitatory input to the lateral portion of the magnocellular nucleus of the anterior nidopallium (LMAN). Axons from LMAN innervate area X and also innervate the same song premotor neurons in RA that receive input from HVC_{RA} neurons. Thus, the SMP and AFP arise from distinct pools of HVC projections neurons and innervate the same RA song premotor neurons. (b) Pathways that are believed to convey auditory and recurrent song motor information to HVC. Auditory information (blue arrows) originates in the inner ear and passes via the eighth cranial nerve to the cochlear nucleus (CN) in the medulla, where it is relayed indirectly to HVC through two pathways. The first pathway includes the ventral portion of the lateral lemniscus (LLv) and the thalamic nucleus uvaformis (Uva). The second pathway includes an indirect pathway (broken line) through the auditory hindbrain and midbrain (not shown) to the thalamic nucleus ovoidalis (Ov); axons from Ov terminate in the massively interconnected telencephalic area Field L, which is analogous to mammalian primary auditory cortex. From Field L, activity is relayed through an interconnected network comprising the caudal medial nidopallium (NCM) and the caudal mesopallium (CM), which in turn projects directly to HVC and indirectly to HVC through the nucleus interfacialis (NIf). Song motor-related feedback and possibly respiratory-related activity from the brainstem are thought to reach HVC through a recurrent circuit (red lines) that includes PAm, DM, Uva, and NIf. Images courtesy of Todd Roberts.

syringeal muscles. Second, neurons in dorsal RA terminate on regions of the lateral medulla containing respiratory premotor neurons (Wild, 1993b). This segregated organization may enable dorsal and ventral RA neurons to independently modulate respiratory and syringeal activity. Third, RA axons terminate on several structures in the thalamus and midbrain, including the dorsomedial intercollicular nucleus, an area implicated in the generation of innate calls (Wild, 1993b; Wild et al., 1997). Although the function of this latter connection is unknown, one possibility is that RA interacts with DM to suppress call generation during singing. Finally, RA neurons do not directly innervate the glossopharyngeal, facial, and trigeminal motor nuclei that control the upper vocal tract (Wild, 1993b), which suggests that RA influences the upper vocal tract indirectly via the VRG.

The gross structure of HVC and RA correlates with song function. First, these nuclei are absent in birds that do not learn their songs, including flycatchers (Kroodsma and Konishi, 1991), close cousins to the oscines. Second, in those species where only the male sings, HVC and RA are greatly reduced in the female (Nottebohm and Arnold, 1976); such dimorphisms are lacking in duetting species where both sexes sing (Brenowitz et al., 1985). Finally, in seasonal breeders, HVC and RA expand in volume in the spring, when the bird sings a crystallized song, and shrink in the fall, when the bird sings less frequent and more acoustically variable songs (Nottebohm, 1981; Smith et al., 1997b; Brenowitz, 2004).

Despite these gross variations in song system structure, anatomical correlates of song lateralization have remained elusive (DeVoogd and Nottebohm, 1981; Nottebohm et al., 1981). Therefore, peripheral asymmetries in the vocal apparatus and more subtle central specializations underlie the lateralized effects of XIIts nerve section. More generally, the descending projections of the forebrain song nuclei are entirely ipsilateral, and in some species the projections from RA to the brainstem also are ipsilateral (Wild et al., 2000). This arrangement presumably enables distinct motor programs to be sent to the two sides of the syrinx.

3.23.5.3.3 Singing-related neural activity in the SMP

Chronic electrophysiological recordings made in singing birds have illuminated the neural dynamics underlying song. A pioneering effort by McCasland and Konishi showed that bursts of activity in HVC and RA occurred before and during the utterance of individual syllables; premotor activity also could be detected in the nucleus interface (NIf), a telencephalic structure presynaptic to HVC (McCasland and Konishi, 1981). A latency analysis supported the notion of a song motor hierarchy, with a feedforward flow of song motor activity propagating from NIf through HVC, RA, and the brainstem.

Although far ahead of its time, McCasland's study relied on multiunit recording methods, making it difficult to determine how song is encoded at different levels of the SMP. A series of elegant single-unit recording studies overcame this limitation, providing us with a detailed picture of how song motor codes change between HVC and RA (**Figure 7**). A particularly elegant study by Richard Hahnloser, Alex Kozhevnikov, and Michale Fee used a miniature motorized microdrive in the zebra finch to show that single HVC_{RA} neurons fire one brief (~10 ms) burst of action potentials at precisely the same time in each motif, with different HVC_{RA} neurons firing at different times during the motif (**Figures 7(a) and 7(c)**) (Hahnloser et al., 2002). Although only a relatively small number of neurons were sampled from any one bird, this finding implies that sparse activity propagates in a rapid (~100 Hz) clocklike fashion rapidly through the entire HVC_{RA} population, spanning the whole motif. Other groundbreaking studies by Albert Yu and Dan Margoliash, and by Anthony Leonardo and Michale Fee, found that single RA PNs burst at many (~10) precise times during a motif, in contrast to the temporally sparse firing patterns of individual HVC_{RA} neurons (**Figures 7(b) and 7(d)**) (Yu and Margoliash, 1996; Leonardo and Fee, 2005). Intriguingly, recordings made in sleeping birds show that HVC_{RA} and RA neurons generate spontaneous activity patterns similar to those they exhibit during singing (Dave and Margoliash, 2000), suggesting that the SMP 'replays' song motor programs during sleep.

3.23.5.3.4 Models of song patterning networks

The acoustic features of song span many timescales: milliseconds for internote intervals, tens to hundreds of milliseconds for notes and syllables, and one to tens of seconds for an entire song. Several circuit models have been put forth to account for patterning of notes, syllables, and songs. Aspects of all of these models find at least partial support in experimental observations.

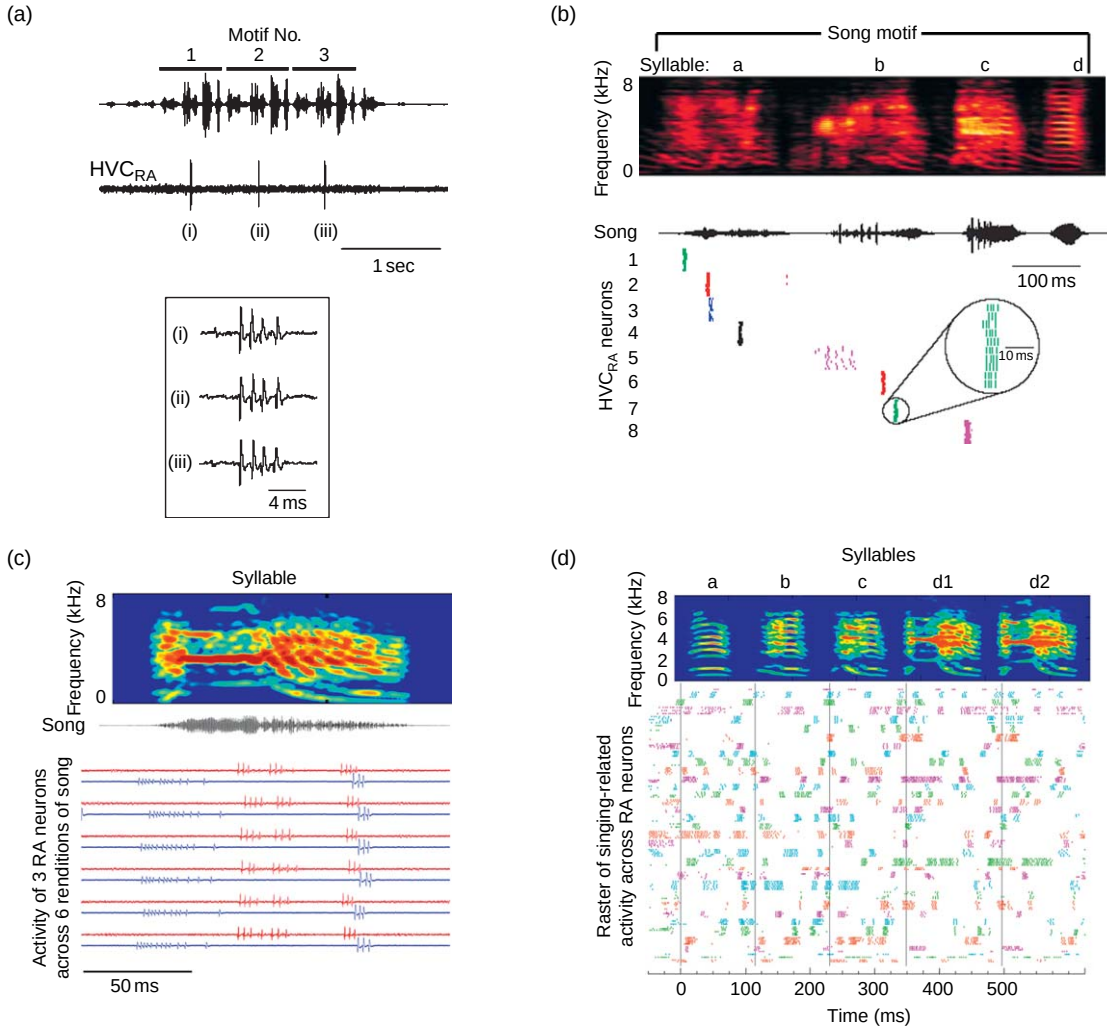


Figure 7 Chronic recordings of single-unit activity in singing zebra finch suggest that neuronal codes for song are transformed from an ultrasparse temporal representation in HVC to a more continuous representation in RA. (a) Individual HVC$_{RA}$ neurons fire a single brief burst of action potentials at one precise time in each motif (top, song oscillogram; bottom, simultaneously recorded neural activity; inset, expanded time base showing structure of the burst). (b) Different HVC$_{RA}$ neurons are active at different points in the motif, suggesting that the entire ensemble of HVC$_{RA}$ neurons provides a fine timescale representation of song (top, sonogram; middle, oscillogram; bottom, raster display of action potential activity for eight different color-coded HVC$_{RA}$ neurons over ten renditions of the motif). (a, b) Reprinted by permission from Macmillan Publishers Ltd: Hahnloser RH, Kozhevnikov AA, and Fee MS (2002) An ultra-sparse code underlies the generation of neural sequences in a songbird. *Nature* 419: 65–70, copyright 2002. (c) Singing-related activity in RA neurons is temporally precise over multiple renditions of the same syllable but is temporally more continuous than in HVC$_{RA}$ neurons (top, sonogram of the syllable; bottom, blue and red records show data aligned to the syllable acquired from different electrodes in RA). (d) Motif-aligned activity of 34 RA neurons recorded from the same bird (top, sonogram of the motif) reveals that each RA neuron exhibited multiple (~10) bursts of activity, the timing of which was similar across different renditions of the motif but generally different from the timing of burst activity in other RA neurons. Different neurons are represented by different colors, with different motif renditions represented by different rows of the raster display. (c, d) Reprinted from Leonardo A and Fee MS (2005) Ensemble coding of vocal control in birdsong. *J. Neurosci.* 25: 652–661.

In the music-box model, the entire HVC$_{RA}$ population can be likened to the programming cylinder in a music box, with the subset of HVC$_{RA}$ neurons active at any instant constituting an individual pin on this cylinder (**Figure 8**) (Fee et al., 2004). A pattern of divergent and convergent feedforward connections transforms temporally sparse activity from these HVC$_{RA}$ 'pins' into more continuous activity in RA,

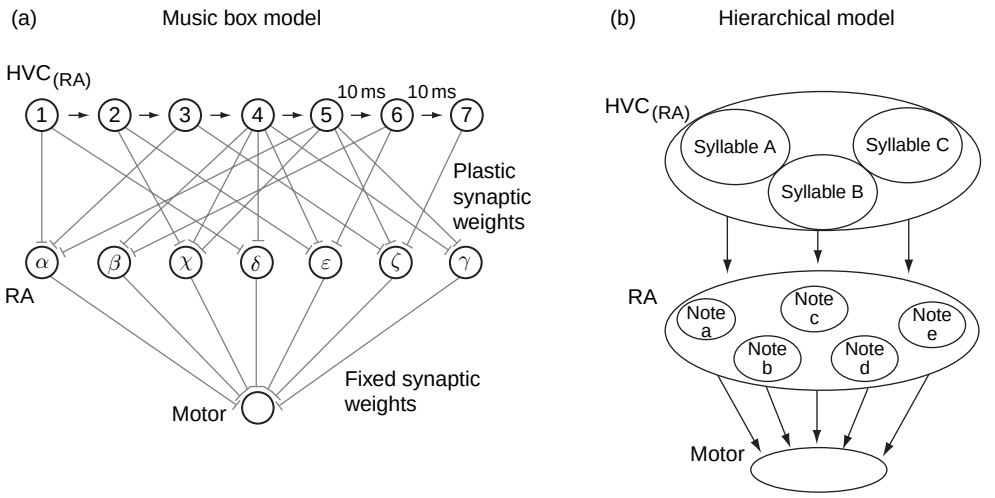


Figure 8 Schematic diagrams illustrating the music box (A) and hierarchical (B) models for song generation. (a) In the music-box model, each HVC_{RA} neuron is active at only one time in the song motif and activity propagates in 10-ms steps through the entire HVC_{RA} ensemble to span the entire motif. A pattern of divergent and convergent HVC / RA synapses translates this temporally sparse activity into a more continuous activity pattern in individual RA neurons, the output from which is transformed by the brainstem motor system into song. A central component of this model is that HVC encodes timing information about song but does not explicitly represent any acoustical features of the song, such as syllables or notes. Reprinted from Leonardo A and Fee MS (2005) Ensemble coding of vocal control in birdsong. *J. Neurosci.* 25: 652–661. (b) In the hierarchical model, HVC encodes large-scale song features, such as syllables. Neurons within RA code for finer-grain song features, such as individual notes.

which then modulates the vocal respiratory system to generate song. In its simplest form, the HVC_{RA} ensemble constitutes a relatively fast (~100 Hz) metronome that, via its synaptic connections with RA, dictates the timing of syllables, notes, and even intersyllable gaps. Interestingly, some HVC_{RA} and RA neurons do burst during silent periods between notes and syllables, suggesting that these song premotor nuclei could encode the timing of silent gaps as well as audible components in the song. Because song output in the music-box model is determined by the specific pattern of synapses HVC_{RA} neurons make in RA, error signals presumably act within RA to alter these connections during sensorimotor learning. In fact, modeling studies show that temporally sparse activity in HVC optimizes learning rates, because error signals that 'correct' the connections of an HVC_{RA} neuron only introduce changes at a single time in the motif (Fiete et al., 2004).

Another feedforward model involves a hierarchical patterning network, with syllables or motifs encoded in 'higher' areas, such as HVC, and lower-level features, such as notes, encoded in RA and the brainstem (**Figure 8**) (Yu and Margoliash, 1996). One early observation supportive of a hierarchy was that HVC microstimulation in the singing zebra finch could reset the entire motif, while RA stimulation only interrupted the ongoing note (Vu et al., 1994). Although consistent with a hierarchical model, electrical stimulation in HVC also activates areas presynaptic to HVC, such as NIf and the thalamic nucleus Uva, and thus any higher-level patterning mechanisms may not be localized to HVC. A recent comprehensive temporal analysis of zebra finch songs shows that, as the song tempo varies, syllables scale much less elastically than do intersyllable gaps (Glaze and Troyer, 2006), implying these features are regulated independently. These behavioral observations are consistent with a hierarchical model and contrast with the music-box model, where syllables and gaps should scale proportionally with variations to the overall song tempo.

In contrast with these two feedforward models, a third idea is that recurrent pathways from the brainstem modulate forebrain song patterning networks, establishing a distributed and possibly circular hierarchy. Indeed, a recent study showed that motif resetting could be triggered by microstimulation in HVC, RA, and even the RVG (Ashmore et al., 2005), a set of observations difficult to reconcile with the strictly feedforward architectures of the music box or hierarchical models. Instead, these results indicate that recurrent pathways from the brainstem to the forebrain likely contribute to song temporal

structure. An anatomical substrate for this recurrent pathway is provided by a subset of RVG axons that project bilaterally to the thalamic nucleus Uva, which in turn innervates HVC and NIf (Reinke and Wild, 1998a; Striedter and Vu, 1998). Because the RVG contains respiratory premotor neurons and receives descending input from RA, it may provide forebrain song nuclei with respiratory information and recurrent song-related motor activity important to setting the song temporal pattern, including the timing of syllable transitions. In addition, because songbirds lack a cerebral commissure, the bilateral projections from RVG to Uva are the most likely substrate for the precise bilateral coordination of song premotor activity seen in HVC. Notably, this recurrent model does not rule out a role for HVC in generating a fine timescale code for song patterning, but such a code would be under the influence of the recurrent loop.

Regardless of which model is most accurate, HVC displays network features that could serve an important role in song patterning. In an isolated HVC preparation, trains of electrical pulses evoke sustained trains of quasi-rhythmic synaptic potentials in HVC neurons (Solis and Perkel, 2005). Additionally, HVC PNs and inhibitory interneurons are reciprocally connected (Mooney and Prather, 2005), a synaptic motif known to sustain oscillatory activity in other pattern-generating networks (Selverston and Moulins, 1985). Whether endogenous pattern generation is unique to HVC is unclear, however, because complex local synaptic networks exist within RA (Spiro et al., 1999) and in the respiratory-vocal brainstem (Sturdy et al., 2003; Kubke et al., 2005) and may be capable of generating rhythmical activity independent of input from HVC.

3.23.5.4 The Role of the Anterior Forebrain Pathway in Song Plasticity

3.23.5.4.1 The AFP is a basal ganglia pathway necessary to song plasticity

The AFP forms intimate links with the SMP: area X is innervated by HVC, and the AFP output, nucleus LMAN, innervates RA (Nottebohm et al., 1976, 1982). Despite direct connections between the AFP and song premotor structures, initial behavioral studies showed that adult crystallized songs were unaffected by lesions to either area X or LMAN (Nottebohm et al., 1976; Bottjer et al., 1984; Sohrabji et al., 1990). A breakthrough came in 1984, when Sarah Bottjer and her coworkers discovered that bilateral LMAN lesions in juvenile zebra finches caused their plastic songs to degrade rapidly and become much less variable, assuming a highly repetitive and simplified form (Bottjer et al., 1984). A subsequent analysis of area X lesions made in juvenile birds showed that songs in these birds remained highly variable into adulthood, never achieving the stereotypy typical of crystallized song (Sohrabji et al., 1990; Scharff and Nottebohm, 1991).

These age-dependent effects of AFP lesions lent support to the idea that the AFP plays a developmentally restricted role in song learning. However, more recent findings show that the AFP also is necessary to adult forms of song plasticity. Rather remarkably, LMAN lesions block the song deterioration (i.e., decrystallization) normally triggered in adult zebra finches by deafening (**Figure 9**) (Brainard and Doupe, 2000) or by exposure to chronically distorted auditory feedback (Williams and Mehta, 1999). An important implication is that decrystallization is an active process requiring the AFP and not simply a degenerative process. The AFP also plays an ongoing role in adult song recrystallization; adult white-crowned sparrows subjected to LMAN lesions during the winter, prior to the annual reexpression of plastic song, failed to successfully recrystallize their songs (Benton et al., 1998). Finally, song learning in adult isolates also has been shown to be blocked by LMAN lesions (Morrison and Nottebohm, 1993), further underscoring that the AFP's role in song plasticity is not age limited.

The specific means by which the AFP contributes to song plasticity is of great interest. One way the AFP could enable song plasticity is by generating acute song variations that serve as 'stepping stones' for larger and more gradual changes to song. Over longer timescales, the AFP also might enable song plasticity by exerting trophic effects on synapses in the SMP. In either case, the AFP could play either a permissive or instructive role to enable song learning.

3.23.5.4.2 LMAN plays an acute role in generating song variability

Understanding the synaptic connections that LMAN makes with RA can inform how the AFP could influence song plasticity. Morphological and electrophysiological studies reveal that RA song premotor neurons receive convergent excitation from both HVC and LMAN axon terminals (Canady et al., 1988; Mooney and Konishi, 1991; Mooney, 1992). Both inputs excite ionotropic glutamate receptors on RA PN dendrites, but near the resting membrane potential, those from LMAN predominantly activate

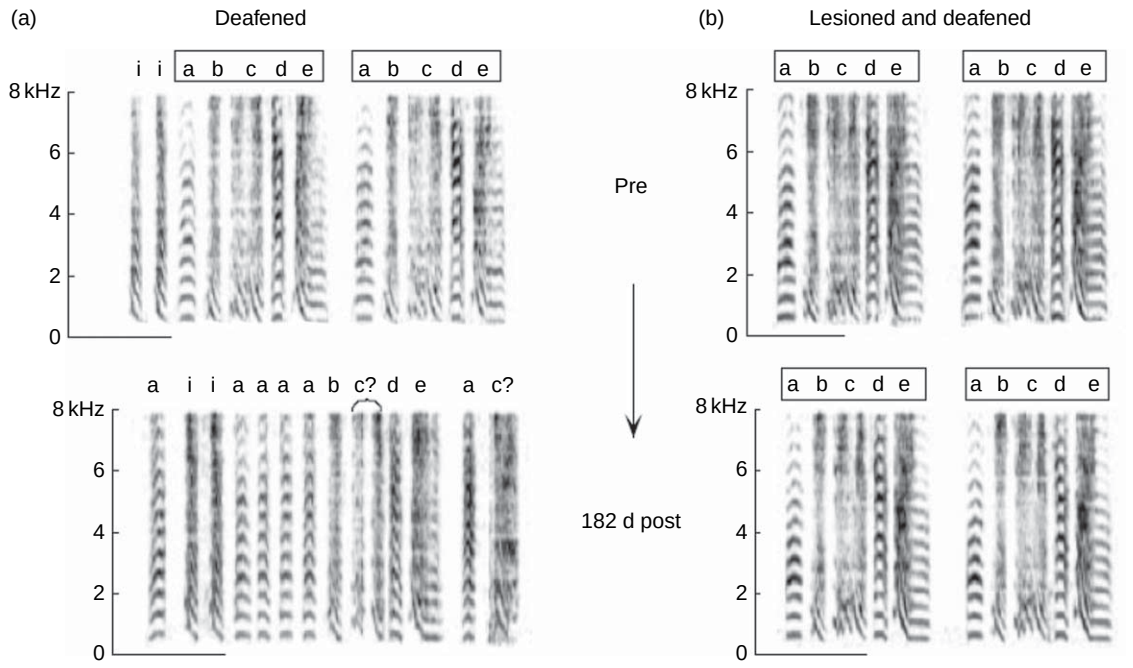


Figure 9 An intact AFP is necessary for deafening-induced song decrystallization in adult zebra finches. (a) Sonograms of an adult zebra finch before and 182 days after deafening reveal changes in the structure of the bird's song, including stuttered syllables ('a'), altered syllable morphology ('?'), and changes to the syllable sequences ('a–c –?'). (b) Bilateral lesions in LMAN made prior to adult deafening prevent changes to song structure. Reprinted by permission from Macmillan Publishers Ltd: Brainard M and Doupe A (2000) Interruption of a forebrain-basal ganglia circuit prevents plasticity of learned vocalizations. *Nature* 404: 762–766, copyright 2000.

postsynaptic *N*-methyl-D-aspartate (NMDA) receptors, while those from HVC predominantly activate alpha-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA) receptors (Kubota and Saito, 1991; Mooney and Konishi, 1991; Mooney, 1992). Differential activation of NMDA and AMPA receptors in other pattern-generating networks leads to different output patterns (Dale and Roberts, 1984; Dye et al., 1989), suggesting that differential activation of LMAN and HVC inputs onto RA neurons could lead to song variability.

Indeed, several observations show that LMAN drives acute song variability. First, pharmacologically inactivating LMAN in juvenile zebra finches rapidly and reversibly reduces note and sequence variability in their plastic songs (Olveczky et al., 2005). Blocking NMDA receptors in RA exerts similar effects, underscoring that LMAN drives song variability via its synaptic connections with song premotor neurons (Olveczky et al., 2005). Second, microstimulation in LMAN can drive slight variations in song of adult zebra finches (Kao et al., 2005). Third, LMAN neurons are active during both directed and undirected singing in adult zebra finches, but during the more variable undirected singing, they burst at higher frequencies and in more variable patterns (Kao and Brainard, 2006). Finally, the acoustic variability of undirected songs is abolished by LMAN lesions (Kao and Brainard, 2006). Ultimately, these various findings indicate that in both the juvenile and the adult, LMAN operates over a short timescale (tens to hundreds of milliseconds) to drive song variability.

The details of AFP connectivity provide a useful context in which to interpret the effects of AFP lesions on song variability. The AFP bears strong similarities to mammalian cortical–basal ganglia pathways (Doupe et al., 2005), with HVC and LMAN providing the 'cortical' input and output and area X and the medial part of the dorsolateral thalamus (DLM) the interposed basal ganglia and thalamic 'relays.' The basal ganglia homologue area X comprises many different cell types, including a smaller GABAergic cell type (SN) that resembles mammalian striatal medium spiny neurons and a larger pallidal-like GABAergic cell type (AF) that makes massive inhibitory synapses onto DLM neurons (Luo and Perkel, 1999; Ding and Perkel, 2002; Farries and Perkel, 2002). These thalamic neurons make excitatory synapses onto LMAN neurons

(Livingston and Mooney, 1997; Boettiger and Doupe, 1998), which in turn form excitatory synapses on RA neurons (Mooney and Konishi, 1991). Because AF neurons fire spontaneously at high rates (Farries and Perkel, 2002), a reasonable assumption is that, at 'resting' levels, DLM neurons are tonically inhibited and LMAN activity remains low. Conversely, factors that suppress AF neuron firing would release DLM neurons from inhibition, ultimately rendering LMAN neurons more active (Person and Perkel, 2005). Indeed, this connectivity provides a useful context in which to understand the contrasting behavioral effects of LMAN and area X lesions: LMAN lesions abolish activity necessary to driving acute song variability, whereas lesions to area X remove tonic inhibition on DLM, increasing LMAN activity and generating higher levels of song variability. If this model is correct, then a key to understanding endogenous regulation of song variability will rest on determining how afferents to area X, which include HVC and midbrain dopamine neurons, influence AF neuron activity.

3.23.5.4.3 Trophic regulation of HVC–RA connectivity by LMAN

In addition to driving bout-to-bout variability, LMAN also could affect song plasticity by exerting trophic effects on RA. As seen more widely in the developing vertebrate central nervous system (CNS), synaptic density in RA describes an inverted 'U' over development, with numbers of HVC axon terminals and RA dendritic spines reaching a peak during the height of sensorimotor learning (Herrmann and Arnold, 1991; Kittelberger and Mooney, 1999). Both of these parameters decline markedly by crystallization, indicative of synapse elimination, while the remaining HVC > RA synapses increase in strength, suggestive of synapse consolidation (Herrmann and Arnold, 1991; Kittelberger and Mooney, 1999).

One idea is that, in the juvenile, LMAN actively maintains RA microcircuitry in a state permissive for song plasticity. Consistent with this idea, LMAN lesions trigger a rapid consolidation of HVC > RA synapses like the consolidation that occurs gradually over normal development (Kittelberger and Mooney, 1999). These experiments show that HVC > RA synapses in juvenile songbirds are highly plastic, and that LMAN plays a role in regulating this synaptic plasticity. These studies also suggest that a normal developmental decline in the number or efficacy of LMAN synapses in RA could consolidate HVC > RA synapses, leading to persistent changes in song. Candidates for mediating this trophic effect include the brain-derived neuronotrophic factor (BDNF), which is expressed in both LMAN and HVC_{RA} neurons (Johnson et al., 1997; Akutagawa and Konishi, 1998; Li et al., 2000), and which at earlier stages of development has been shown to rescue RA neurons from cell death triggered by LMAN lesions (Johnson et al., 1997). Consistent with the idea that BDNF acts as a permissive signal for song plasticity, BDNF injections into the RA of the adult zebra finch simultaneously elevate song variability and augment the density of HVC terminals in RA (Kittelberger and Mooney, 2005).

3.23.5.4.4 The AFP and critical periods for song plasticity

The means by which LMAN enables song plasticity also suggest mechanisms of song crystallization. One possibility is that crystallization arises as a result of decreased variability in LMAN firing patterns during singing. In support of this idea, the acoustic variability of undirected song and the variability of LMAN activity decline in parallel as adult zebra finches grow older (Kao and Brainard, 2006). Crystallization also could arise because the 'gain' of LMAN synaptic currents decreases in adults. In fact, LMAN > RA synaptic currents shorten in duration between juvenile and adult life (Stark and Perkel, 1999; White et al., 1999; Livingston et al., 2000), likely because of an elevation in the NR2A:NR2B subunit ratio of postsynaptic NMDA receptors (Scott et al., 2004). A shortening of LMAN > RA synaptic timecourse also can be precipitated rapidly in juvenile zebra finches by testosterone implants (White et al., 1999), a treatment that also impairs sensorimotor learning (Korsia and Bottjer, 1991). Moreover, the NR2A:NR2B subunit ratio in the RA of the adult canary waxes and wanes in parallel with changes in day length, testosterone titers, and song stereotypy (Singh et al., 2003). Thus, changes to the firing patterns of LMAN neurons and the efficacy of their synapses in RA could underlie the decline in song variability that occurs with song crystallization.

3.23.5.5 Auditory Roles of the Song System: Templates, Feedback, and Error Signals

3.23.5.5.1 General themes

The neural components underlying a template model of song learning include a motor pathway for song production, an auditory memory of the tutor song (i.e., a template), and a mechanism for comparing singing-related auditory feedback to the template (Konishi, 1965, 2004). When this 'comparator' detects

mismatches between the feedback signal and the template, it generates an error signal that modifies motor activity in the song production pathway, with the eventual result that the bird's song comes to resemble the tutor song. In addition to a song motor role, several lines of evidence suggest that the song system also may serve one or more auditory roles, including song recognition, template storage, conveying auditory feedback, or processing the resulting error signal. One piece of evidence is that auditory responses can be detected throughout the song system (Katz and Gurney, 1981; Margoliash, 1983; Williams and Nottebohm, 1985; Williams, 1989; Doupe and Konishi, 1991). Second, some song system neurons respond selectively to the bird's own song (BOS) and to the tutor song, indicating that they encode aspects of the bird's auditory experience (Margoliash, 1986; Solis and Doupe, 1999). Third, lesions to HVC or the AFP impair song recognition, suggesting auditory activity in the song system serves a perceptual role (Brenowitz, 1991; Scharff et al., 1998b; Burt et al., 2000). Finally, the presence of auditory activity in the AFP, a pathway necessary to song learning, raises the possibility that it conveys either feedback or the resulting error signal to the SMP (Doupe and Konishi, 1991; Brainard and Doupe, 2000).

3.23.5.5.2 *Auditory responses in the song system*

Intriguingly, the earliest chronic recordings of singing-related neural activity conducted in HVC also detected robust responses to auditory presentation (i.e., playback) of the BOS (McCasland and Konishi, 1981). Subsequent playback studies, mostly in anesthetized zebra finches, detected auditory responses throughout the AFP and the SMP, even in the hypoglossal nerve (Margoliash, 1983, 1986; Williams and Nottebohm, 1985; Doupe and Konishi, 1991). The source of this widespread auditory activity is HVC, which transmits auditory activity to the SMP and the AFP via its two populations of projection neurons (Doupe and Konishi, 1991; Vicario and Yohay, 1993; Mooney, 2000).

Song system neurons exhibit some of the most selective sensory responses yet described. Many HVC neurons are 'BOS selective,' firing vigorously to BOS playback but not to playback of conspecific songs or time-reversed BOS (**Figure 10**) (Margoliash,

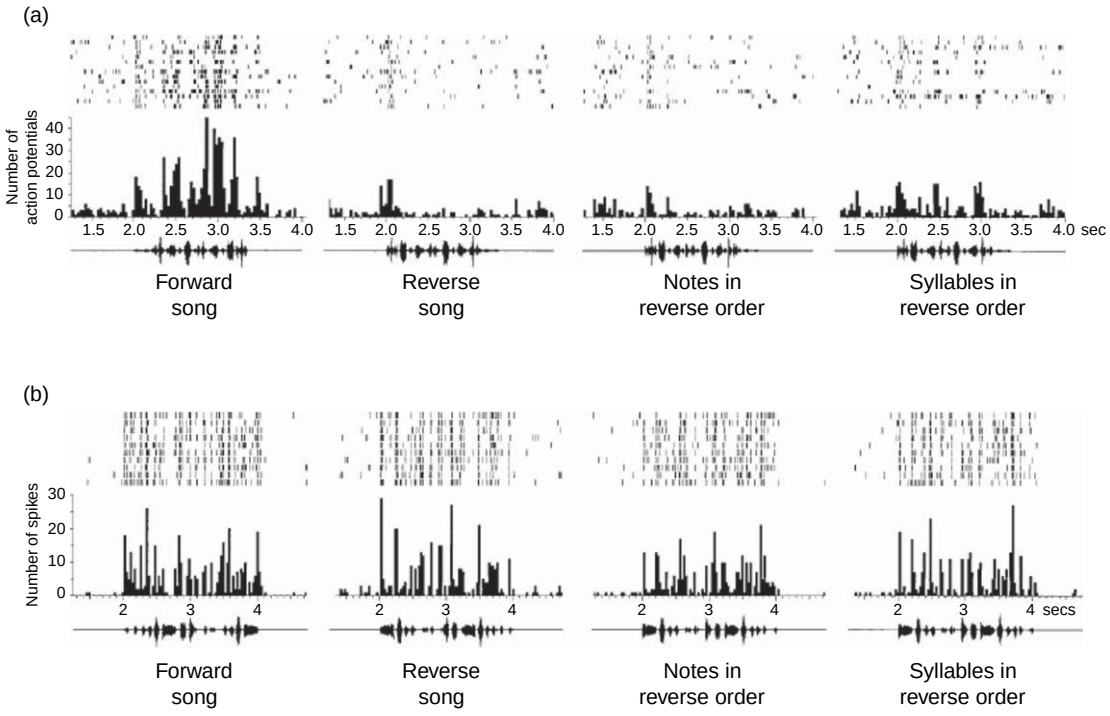


Figure 10 Auditory selectivity for the bird's own song (BOS) emerges between Field L and HVC. Each panel contains the raster of a single neuron's action potential response to the given auditory stimulus (top), the cumulative histogram of those responses (middle), and an oscillogram of the stimulus (bottom). (a) An HVC neuron responds strongly to playback of the BOS but weakly or not at all to temporally manipulated versions of the BOS. (b) In contrast, a Field L neuron responds strongly to playback of the BOS and to temporally manipulated versions of the BOS. Reprinted from Lewicki MS and Arthur BJ (1996) Hierarchical organization of auditory temporal context sensitivity. *J. Neurosci.* 16: 6987–6998.

1983, 1986; Doupe and Konishi, 1991; Volman, 1996). BOS-selective neurons are sensitive to temporal cues, as revealed by their selectivity for forward over reverse BOS, two stimuli with equivalent spectral content but contrasting temporal features. Some BOS-selective neurons also are sensitive to harmonic features of song (Margoliash and Fortune, 1992). Studies using synthetic songs have found that HVC neurons typically are more sensitive to degradation of temporal rather than spectral features, and that their temporal acuity is remarkable given HVC's distance from the auditory periphery (Theunissen and Doupe, 1998). Furthermore, a subset of HVC neurons responds exclusively to note-combinations in the BOS; studies where internote intervals were artificially manipulated show that these combination-sensitive neurons can integrate auditory information over hundreds of milliseconds (Margoliash, 1983; Lewicki and Konishi, 1995). Finally, in the swamp sparrow, a bird with multiple song types, different HVC neurons respond in an all-or-none fashion to different song types in the bird's repertoire (Mooney et al., 2001). From a functional standpoint, BOS-selective neurons are well suited for a feedback role, because they can respond differentially to slight variations in local (i.e., within-note) and global (i.e., note or syllable sequence) temporal structure of the BOS. BOS-selective neurons also could play an important communicative role by facilitating discrimination of the fine syntax variations that distinguish songs produced by conspecific birds from different breeding populations.

One clue that auditory activity in the song system actually plays a perceptual role is that lesions made either in HVC or in the AFP impair song recognition (Brenowitz, 1991; Scharff et al., 1998b; Burt et al., 2000). This perceptual deficit is perhaps most striking in female songbirds, which typically do not respond to playback of other species' (i.e., heterospecific) songs. However, following bilateral HVC lesions, females generate 'promiscuous' CSDs in response to heterospecific song playback (Brenowitz, 1991). In male songbirds, deficits in discrimination following lesions to the AFP are most pronounced for songs resembling the BOS, implicating BOS-selective neurons in perceptual processes (Scharff et al., 1998b). More generally, the mixed sensorimotor roles of HVC are strongly reminiscent of the mixed expressive and receptive roles in human speech served by language cortices in humans.

Another clue that auditory activity in the song system serves an important function is that it can be gated in a state-dependent fashion (Dave et al., 1998; Schmidt and Konishi, 1998). In the adult zebra finch, playback-evoked auditory responses in NIf, HVC, and RA are most robust when the bird is asleep or anesthetized and diminished and more variable (but not altogether absent) during wakefulness (Dave et al., 1998; Schmidt and Konishi, 1998; Cardin and Schmidt, 2003, 2004). One idea is that sleep–wake changes in auditory activity in the song system reflect the presence of an auditory 'gate' that operates more dynamically in the waking bird as a function of arousal or changes in attention or saliency. Although the function of auditory gating in the song system is unknown, one idea is that it prevents auditory signals from altering vocal activity in sensorimotor neurons (Williams, 1989; Konishi, 2004). Additionally, auditory gating in HVC also may vary in a species-dependent manner; in notable contrast to the zebra finch, robust auditory activity is present in the HVC of both canaries and swamp sparrows during periods of wakefulness (McCasland and Konishi, 1981; Prather et al., in revision). In contrast to male zebra finches, which are colonial animals that sing unidirectionally to females, male canaries and sparrows are solitary animals that rely on song to identify neighboring males and defend territory, behaviors that may necessitate a more active role for the song system in auditory perception.

3.23.5.5.3 Sources of auditory input to the song system

The mechanisms that generate BOS electivity must to some extent be influenced by auditory experience, because the BOS is a learned behavior. Thus, locating where BOS selectivity arises in the brain can point to sites that encode aspects of auditory experience, particularly singing-related auditory feedback. Notably, BOS selectivity is largely absent from Field L, the avian equivalent of the mammalian primary auditory cortex, and the indirect source of auditory input to HVC (**Figure 10**) (Lewicki and Arthur, 1996; Amin et al., 2004; Theunissen et al., 2004). Current evidence indicates that BOS selectivity arises in areas interposed between Field L and HVC (Theunissen et al., 2004; Theunissen and Shaevitz, 2006) and that HVC integrates both selective and nonselective inputs from a variety of sources in the forebrain (Cardin and Schmidt, 2004; Coleman and Mooney, 2004; Rosen and Mooney, 2006; R. Mooney, unpublished observations).

Anatomical and functional studies indicate that HVC receives auditory inputs from three other song nuclei – NIf, Uva, and mMAN – and from the secondary auditory telencephalic region CM (Vates et al., 1997; Cardin and Schmidt, 2004; Coleman and

Mooney, 2004; R. Mooney, unpublished observations). At the population level, NIf and mMAN neurons are BOS selective, whereas Uva neurons are nonselective (Vates et al., 1997; Coleman and Mooney, 2004; M. Coleman personal communication). Similar to HVC, these three song nuclei are sensorimotor structures, and thus selectivity in these areas may reflect aspects of motor as well as auditory experience. In contrast, CM is embedded in the auditory telencephalon and thus may constitute a 'pure' source of auditory information to the song system. CM is densely interconnected with primary and secondary regions of the auditory telencephalon, including Field L and NCM (Vates et al., 1996). At the population level, CM neurons are not BOS selective, although they respond more to conspecific songs than to synthetic sounds, and their sensitivity to conspecific songs is enhanced relative to Field L neurons (Grace et al., 2003; Theunissen et al., 2004; Theunissen and Shaevitz, 2006). An especially fascinating study in starlings showed that the response properties of CM neurons can be altered during auditory learning tasks (Gentner and Margoliash, 2003). Furthermore, some CM neurons are BOS selective, and robust auditory activity can be detected in CM during quiet wakefulness and singing (R. Mooney, unpublished observations). Thus, CM may convey auditory feedback to the song system and could weight this information as a function of the bird's auditory experience of its own song and the songs of other birds.

Although a response bias to the BOS is established at least as early as NIf and in some CM neurons, cells in both areas show elevated firing rate responses to a wide range of non-BOS stimuli (Coleman and Mooney, 2004; R. Mooney, unpublished observations). In contrast, note combination–sensitive HVC neurons appear to fire only to the BOS and to the songs of conspecific birds with similar note sequences (Margoliash, 1983; Lewicki and Konishi, 1995). This all-or-none selectivity arises in HVC through synaptic interactions between HVC's BOS-selective excitatory afferents (i.e., NIf and possibly CM) and BOS-selective inhibitory interneurons in HVC (Mooney, 2000; Rosen and Mooney, 2003, 2006). Specifically, excitatory and inhibitory inputs onto HVC_X neurons generate highly nonlinear responses to the BOS through both thresholding effects and priming mechanisms (Rosen and Mooney, 2003). Thus, auditory representations of the BOS are enhanced locally in HVC, and both HVC and areas immediately presynaptic to HVC are likely to encode aspects of auditory experience.

3.23.5.5.4 Does auditory activity in the song system encode the template?

In the context of a template model, one idea is that auditory activity in the song system encodes experience of the tutor song. In fact, studies in juvenile zebra finches found that blocking NMDA receptors in LMAN during tutoring sessions subsequently impaired copying (Basham et al., 1996). One potential confound is the close overlap between sensory acquisition and sensorimotor learning in zebra finches, which makes it difficult to rule out an effect of drug treatment on motor aspects of song learning. Another potential confound is a nonspecific impairment of attention or arousal, due perhaps to diffusion of the drug into brain regions surrounding LMAN.

A related idea is that auditory selectivity in the song system reflects experience of the tutor song. Nonetheless, several findings suggest that BOS selectivity reflects the bird's experience of its own song, rather than of its tutor. First, most HVC and AFP neurons in adult birds respond best to the BOS (Margoliash, 1986; Doupe and Konishi, 1991; Volman, 1996), whereas template neurons presumably would respond best to the tutor song. Second, juvenile zebra finches sequentially tutored by two different birds develop transient responses in LMAN to the first tutor's song and the bird's own imitation of this model, but these responses are lost or overwritten as the bird copies the second tutor (Yazaki-Sugiyama and Mooney, 2004). Thus, even when young birds demonstrate they have learned from a tutor, LMAN neurons do not permanently encode memories of these songs. Third, recordings made in HVC and the AFP of anesthetized juvenile songbirds reveal that song selectivity emerges only after the bird begins to sing; before this time, auditory responses in HVC and LMAN are typically weak and nonselective, despite experience of the tutor song sufficient to enable subsequent copying (Volman, 1993; Doupe, 1997). Moreover, in juvenile birds singing plastic song, most HVC and AFP neurons are BOS selective (Volman, 1993; Solis and Doupe, 1997), although many BOS-selective neurons also respond more strongly to the tutor song than to the songs of other conspecific birds (Solis and Doupe, 1997).

Neurons with 'dual selectivity' for the BOS and the tutor song could potentially encode tutor song experience, with the qualification that, when learning is successful, these two songs share acoustic features.

Thus, a potential caveat is that neurons in which selectivity was specified solely by the BOS might respond to these shared features, rather than encoding features unique to the tutor song. To investigate this possibility, Solis and Doupe unilaterally cut the syringeal nerve in juvenile zebra finches, spectrally distorting their songs and rendering them dissimilar from their tutors' songs based on several criteria (Solis and Doupe, 1999). Notably, most LMAN neurons in such 'dysphonic' juvenile birds developed strong selectivity for the distorted BOS, reinforcing the idea that the bird's experience of its own song is the primary factor influencing selectivity. Nonetheless, some neurons responded equally well to the distorted BOS and to the tutor song, raising the possibility that they encoded different BOS and tutor song features. However, the features in the distorted BOS and the tutor song that evoked responses were not characterized and were not necessarily those judged to be dissimilar in the two songs. Thus, it remains plausible that selectivity in LMAN is shaped by the bird's experience of its own song, and dual-selective neurons in 'dysphonic' birds respond to features common to the BOS and tutor song.

An important concern is that most studies of auditory selectivity in the song system have been conducted in anesthetized animals. Indeed, a recent study using chronic multiunit recordings in the awake juvenile zebra finch found evidence of mild selectivity for the tutor song in HVC (Nick and Konishi, 2005). Further studies are needed in freely behaving birds to confirm that auditory selectivity, as well as auditory responsiveness, may change in a state-dependent fashion. However, the present weight of evidence points away from the song system and the AFP in particular as sites where tutor song memories are stored.

These largely negative findings advance regions outside the song system as candidates for storing the song template (Bolhuis and Gahr, 2006). Foremost amongst these is NCM, which is reciprocally connected to CM and thus provides an indirect source of auditory input to the song system (Vates et al., 1996). Both IEG and electrophysiological studies show that auditory responses of NCM neurons habituate to repeated playback of the same song (Chew et al., 1995; Mello et al., 1995), consistent with NCM being a site of experience-dependent plasticity. A specific role for NCM in template storage is hinted at by the finding that tutor song playback can induce IEG expression in the adult zebra finch NCM, with expression levels correlating with how well the bird copied the tutor song (Bolhuis et al., 2000, 2001; Terpstra et al., 2004). Similarly, NCM neuronal firing rates in adult finches habituate more slowly to playback of tutor song than to novel songs, with the slowest habituation rates for those tutor songs copied most accurately (Phan et al., 2006). As with studies of auditory selectivity in the song system, one necessary caveat is that these closely copied tutor songs are acoustically similar to the BOS. Thus, more definitive experiments, perhaps using vocal nerve section to increase the acoustical 'distance' between the BOS and the tutor song, are needed to determine to what extent NCM neurons encode tutor song memories as opposed to self-experience.

3.23.5.5.5 Does the song system process auditory feedback and/or error signals?

Singing-related auditory feedback is essential to sensorimotor learning and, in certain songbirds, to adult song maintenance. One idea is that feedback is evaluated by a neural comparator, which generates an error signal when it detects mismatches between the feedback signal and the template. The error signal could either provide 'simple' reinforcement, acting in an all-or-none fashion to 'punish' vocal errors (or to 'reward' correct performances), or provide information about the direction and magnitude of the vocal error. In either case, the error signal would adaptively modify song during sensorimotor learning, but it also could drive maladaptive changes, as when deafening or delayed feedback triggers song decrystallization. Although feedback must be integrated by the brain in real time as the bird sings, questions remain as to the timescale over which the resultant error signal operates. In an online model, auditory feedback acts over a short timescale (perhaps within a single song or song bout) to generate the error signal. In an offline model, the error signal arises more slowly, driving changes in song only after a substantial delay of hours, days, or even weeks.

The AFP is an attractive site to look for both auditory feedback and error signals because it conveys BOS-selective auditory information and because it is necessary to song plasticity. Anthony Leonardo directly tested whether the AFP in adult zebra finches encoded feedback or error signals by measuring the singing-related activity of individual LMAN neurons in the presence and absence of delayed auditory feedback (DAF), a treatment that decrystallizes song gradually over several weeks (Leonardo and Konishi, 1999; Leonardo, 2004). Notably, the singing-related activity patterns of

individual LMAN neurons were unchanged by DAF, at least over the relatively short timescales at which single-unit isolation could be maintained (tens of minutes) (Leonardo, 2004). This finding reinforced an earlier study, also performed in adult zebra finches, which found that singing-related multiunit activity patterns in LMAN were unaltered immediately after deafening (Hessler and Doupe, 1999a). These results are difficult to reconcile with a feedback function for the AFP, and instead show that most or all of the singing-related activity in the AFP is song motor corollary discharge. Indeed, the local HVC circuit contains direct and indirect synaptic pathways from HVC_{RA} to HVC_X neurons, providing a robust substrate for relaying song premotor activity to the AFP (Mooney and Prather, 2005). These findings also suggest that LMAN does not transmit an acute error signal but, instead, either conveys an offline error signal or plays a permissive rather than instructive role in song learning.

One technical limitation to these studies is that neural activity was monitored over only short timescales, whereas DAF and deafening induce song plasticity in adults only after many weeks (Nordeen and Nordeen, 1992; Leonardo and Konishi, 1999). The slow onset of adult plasticity raises the possibility that the 'gain' of feedback and/or error signals decreases markedly in the adult. One hint that feedback may act slowly in the adult AFP comes from the finding that, following syringeal nerve section in adult zebra finches, selectivity in LMAN can shift to the spectrally distorted song over a 1- to 2-week period, prior to the onset of decrystallization (Roy and Mooney, in press). Another hint that the AFP may convey an error signal over longer times is that auditory responses of LMAN neurons were absent in adult zebra finches that sustained syringeal nerve section as juveniles (Solis and Doupe, 2000); such depression of sensory-evoked activity could be caused by the actions of an error signal arising from the chronic mismatch between auditory feedback and the memorized model.

Another potential concern is that most attempts to detect feedback or error signals have been undertaken in the adult, because the crystallized song provides a stable motor 'background' on which to detect these signals. Nevertheless, feedback and error signals are likely to be most robust in juveniles singing plastic songs. Especially relevant in this regard are chronic recording studies in juvenile zebra finches, which report that short-term exposure to DAF failed to perturb singing-related activity of HVC_X neurons (Kozhevnikov et al., 2006). Here an important consideration is that even in auditory-vocal specialists such as humans and bats, vocal modulation by auditory feedback is in some sense offline, arising only after a delay of 150 ms to several seconds following feedback perturbations (Schuller et al., 1975; Donath et al., 2002; Konishi, 2004). Thus examining activity patterns just during the motif may set too narrow a time window to detect feedback- or error signal–related signals.

Behavioral studies may highlight the best times during sensorimotor learning to search for error signals. In juvenile zebra finches, the most dynamic changes in song structure occur during the first few hours of the morning and overnight (Tchernichovski et al., 2001; Deregnaucourt et al., 2005). The rapid improvement in syllable matching seen each morning hints that online or fast offline processes adaptively modify song. Conversely, the nightly deterioration of the match between the BOS and the tutor song suggests that an offline error signal drives song deconsolidation during sleep. One plausible idea is that song deconsolidation is actively driven by a comparator that interprets bursting activity in HVC and RA during sleep as song motor activity lacking any sensory feedback (Dave and Margoliash, 2000; Deregnaucourt et al., 2005). The contrasting processes of daily improvement followed by nightly deconsolidation also underscore that song learning could involve multiple error signals, acting online as well as offline, rather than a single reinforcement or directional error signal acting entirely on- or offline.

3.23.6 Future Directions and Conclusions

Important insights into birdsong have been gleaned at many different levels, ranging from behavioral aspects of song learning to biomechanical and neural mechanisms of singing and song learning. Despite these important advances, some of the most basic and exciting questions remain to be answered, and comparative approaches remain vastly underexploited.

To date, the search for neural correlates of song has largely been a top-down affair. Consequently, we know much more about singing-related activity of neurons in HVC, RA, and LMAN than in the brainstem. It is unlikely that song motor codes can be fully deciphered without analyzing brainstem and

neuromuscular components of the song system in singing birds. Moreover, we still know remarkably little about coding strategies in the HVC and RA of species with larger and syntactically more variable repertoires than displayed by the zebra finch. To what extent is the sparse singing-related activity seen in the zebra finch HVC_{RA} neurons a general song-coding strategy, especially in species with large song repertoires? What are the mechanisms that generate variations in syntax that characterize songs of species other than the zebra finch? What are the mechanisms contributing to the remarkably sparse singing-related activity seen in HVC_{RA} neurons?

An especially important line of future research pertains to the synaptic basis of song learning. Notably, acute forms of synaptic plasticity have been detected in the AFP, and consolidation has been observed at HVC > RA synapses over development (Kittelberger and Mooney, 1999; Boettiger and Doupe, 2001; Ding and Perkel, 2004). Moreover, the architecture of the AFP suggests that RA is a major site of synaptic modification underlying song learning. However, whether classical forms of synaptic plasticity, such as LTP, contribute to song learning remains unknown, and the field is still in its infancy as far as relating synaptic and cellular mechanisms to the song behavior.

Arguably the most exciting questions in songbird research – the nature of the template, auditory feedback, and error signals – are still largely unanswered. The current body of largely negative evidence indicates that feedback or error signals arise in the song system of the adult only over a slow timescale or are mediated largely outside the song system. Nevertheless, concerns remain that we have not focused on the most appropriate species, employed the best methods for triggering an error signal, or concentrated on the right time during development. First, it may be useful to focus on those species, such as the Bengalese finch, that in adulthood depend acutely on auditory feedback to maintain their songs. Second, though DAF ultimately triggers song plasticity, initial exposure may not be detected by the bird as vocal error. Thus it may be useful to induce actual vocal errors, perhaps by stimulating the syringeal nerve during singing. Although technically challenging, more chronic recording studies are needed in juvenile birds learning to sing. The search for singing-related auditory feedback signals should be expanded to include those regions of auditory forebrain that ultimately provide auditory drive to the song system. Such a bottom-up approach may reveal the degree to which vocalization gates auditory activity in the auditory system, as has been seen in other vocalizing animals, and help establish the degree to which these auditory areas can register changes in feedback in an online fashion. Finally, the nightly process of song deconsolidation seen during sensorimotor learning and the evidence of song motor replay seen in sleeping birds have lent support to the idea that these sleep-related patterns of activity are necessary to song learning. Therefore, a future goal should be to directly test whether spontaneous bursting activity in the sleeping bird plays a role in song learning.

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3.24 Emotional Learning: Animals

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3.24.1 Introduction

The capacity to learn and retain information, whether it is the sequence and rhythm of muscle movements required for a motor skill or the details of a traumatic emotional experience, imbues organisms with an enormous advantage for coping with an ever-changing world. How the brain forges memory from experience has been a question of considerable interest to psychologists and neuroscientists for decades. What has become clear in the last several decades is that memory representations are not monolithic and are therefore not wrought by a singular core of specialized brain tissue. Rather, multiple brain systems and regions participate in the encoding and storage of many different types of memories for skills, emotions, facts, and episodes, and so on.

The parsing of memory into different domains is probably best illustrated by the dissociation in memory systems apparent after brain lesions in people. In the 1960s, Milner described the most instructive and well-studied case of dissociable brain systems for memory in a man known by his initials as H.M. (Milner, 1962). Patient H.M. came to Milner's attention when he presented with severe amnesia, both for past events and newly experienced events, after he received a bilateral medial temporal lobe resection for intractable epilepsy. Despite his profound amnesia for episodes in his daily life, he exhibited apparently normal learning and memory for a variety of motor tasks, including mirror-tracing and rotary pursuit. The critical observation made by Milner was that H.M.'s memory deficit was selective for certain types of information. She necessarily concluded that there must be multiple memory systems in the brain. In particular, the medial temporal lobe the hippocampus, which was the major site of brain damage in H.M., was clearly critical for episodic memory, but not motor memory. Years of research have now confirmed this view and revealed dissociable neural systems underlying memory for emotional events, pleasurable rewards, skeletal motor responses, sensory percepts, and facts and episodes, to name but a few.

This implies that any single experience is always a hybrid of memorable events traced in different neural circuits in the brain. Consider the memory of a traumatic experience, such as an armed robbery by a masked man in a convenience store. The victim of

such a crime is likely to vividly recollect the time and place of the crime, as well as the events leading up to and following the event. In addition, there may be visceral emotional responses, such as a racing heart and sweating palms, that are evoked by aspects of the robbery – such as the sight of a man in a ski mask on a snowy day years after the crime. The victim might also form the opinion that convenience stores are dangerous places after the experience. Hence, the armed robbery filters through several memory systems, leaving its trace in memories about where and when the episode occurred, the emotional consequences of the event, and factual knowledge about the event, among others.

The memory system that is the focus of this chapter is that concerned with emotional information. Emotions, of course, come in several varieties – fear, love, anger, joy, and so on. However, the most productive neurobiological analysis of emotional learning has been in the context of aversive emotions such as fear. There has been considerable work in both human and animals on the anatomy and physiology of the memory system for fear. This focus on fear memory has been driven by many factors, including the isomorphism of fear responses in humans and animals, the rapid acquisition and long-term stability of fear memory, and the primacy of fear memory in the context of behavioral systems adapted to defense and self-preservation. Moreover, from a clinical perspective, dysfunction in emotional memory systems is at the heart of the most prevalent psychiatric disorders in humans. Specifically, anxiety disorders including posttraumatic stress disorder, panic disorder, and specific phobias are rooted in pathological fear memories. The aim of this chapter is to explore the emotional memory system, focusing on the contribution of experimental studies in animals to fostering our understanding of the nature and properties of emotional learning and memory, particularly for aversive events, in humans. The approach that is followed in this chapter is to use studies of brain function and anatomy to inform the properties of emotional memory and, similarly, to leverage psychological theory and animal behavior to inform the nature of the brain systems underlying emotional learning and memory. This behavioral neuroscience perspective on the emotional learning and memory systems in animals has had great influence on modern thinking about the neurobiology of memory, in a general sense. This chapter will not consider the extensive literature on the cellular, genetic, and molecular mechanisms underlying learning, which are the focus of other chapters in this volume.

3.24.2 Behavioral Models for Exploring the Neural Substrates of Emotional Learning

Before considering in depth the neural systems underlying emotional learning and memory, it is important to understand the paradigms and methods used to investigate aversively motivated learning and memory in the laboratory. The experimental investigation of emotional learning has its roots in Watson and Rayner's classic demonstration of fear learning in the human infant, Little Albert (Watson and Rayner, 1920). In this case study of human fear learning, Watson presented Albert with a live, white rat and subsequently hammered an iron rod to produce a loud, startling sound that sent Albert into tears. After several conditioning trials, Albert came to fear the rat and burst into tears and attempted to avoid the rat when presented with the animal, even in the absence of the loud noise. Watson and Rayner (1920) argued that emotional responses could be conditioned in much the same way as discrete motor or salivary responses, as described by Pavlov. This provided an important demonstration of the use of Pavlovian procedures to study emotional learning and laid the groundwork for subsequent studies in both humans and animals of the nature and properties of aversively motivated learning.

Although Watson and Rayner's examination of Little Albert was a noteworthy proof of concept that fear could be established through conditioning procedures, the successful analysis of emotional learning and memory systems required the development of behavioral tasks that could be adapted to laboratory animal subjects and brought under rigorous experimental and parametric control. In most cases, these tasks were developed in efforts to understand the psychological properties of learning and memory systems (rather than brain substrates *per se*), whether in the context of motor skill acquisition, appetitive learning, or aversively motivated learning, for example. Indeed, the work that emerged from psychological laboratories in the early to mid-twentieth century proved to be crucial for understanding the process and content of learning. These advances in psychological learning theories were essential to adapting behavioral tasks to the analysis of learning and memory systems in the brain.

3.24.2.1 Instrumental Conditioning Paradigms

The types of learning tasks used in analyzing the psychological and neural substrates underlying emotional learning can be classified in general terms according to their associative contingencies. Broadly speaking, one class of task requires that the animal learn that the delivery of an aversive stimulus is a consequence of its behavior. These tasks therefore involve an instrumental contingency and are variously referred to as instrumental conditioning or operant conditioning tasks. In the context of aversive learning, animals are often instrumentally trained to avoid a noxious stimulus, such as the delivery of footshock on an electrified grid floor. Hence, one might arrange that a conditioned stimulus (CS) will signal the occurrence of a footshock unconditioned stimulus (US). In some tasks, such as a shuttle-box avoidance task in rats, the animal can avoid the aversive US if it learns to move from one side of the box to the other when the CS is initiated. In this case the animal learns an active response to avoid shock. Active avoidance tasks may take several forms, from one-way active avoidance (in which the animal always shuttles in one direction from a consistently dangerous compartment to a consistently safe compartment) to two-way or shuttle avoidance (in which either compartment might be associated with shock and the animal learns to shuttle, in either direction, on alternate trials). Other active avoidance tasks might take advantage of entirely different response requirements, such as wheel-running avoidance, in which rabbits step in a running wheel to avoid shock, or conditioned taste avoidance, in which animals learn to avoid a novel taste that is followed by gastrointestinal malaise induced with an emetic such as lithium chloride.

In other instrumental avoidance tasks, such as inhibitory avoidance conditioning, the animal learns that making a response, such as walking from an illuminated compartment to a darkened compartment of a chamber, will result in shock delivery. In this case, the animal learns to avoid footshock by inhibiting its tendency to move to the dark side of the apparatus when it is returned to the illuminated chamber. Thus, the animal learns a passive avoidance response in this situation. There are many variants of passive avoidance tasks, including step-through passive avoidance of the sort just described, or step-down avoidance, in which movement from a small, elevated platform to a larger arena is punished with footshock. Animals that have learned to press a bar for food will suppress that response if it comes to yield footshock. Similarly, thirsty animals will avoid licking a water spout if contacts with the spout lead to a shock. In another variant of passive avoidance training, rats are placed in a chamber that houses an exposed, electrified shock probe. Upon contacting the probe, the animal receives a shock and subsequently avoids the probe. In addition to avoiding contact with the probe, the animal will spray bedding or any other substrate at the probe in an effort to bury it. Defensive burying is an ethologically relevant avoidance behavior observed by animals in response to noxious stimuli in their environments.

In every instrumental task described, the animal learns that its behavior has consequences for the likelihood of experiencing an aversive event. In one case, the animal learns that its behavior causes the aversive event and then learns to withhold that response in the future (i.e., passive avoidance tasks), and in another case the animal learns that its behavior terminates an aversive stimulus, and it can avoid the aversive event by making a behavioral response (i.e., active avoidance tasks). Thus, in passive avoidance tasks, the probability of various approach behaviors is decreased by punishment, yielding passive avoidance. In active avoidance tasks, the probability of an active avoidance response is increased through negative reinforcement (removing an aversive stimulus). In both cases, it is important to appreciate that although the task is orchestrated to reinforce one behavioral response or another, the instrumental association between a stimulus and a response (the so-called stimulus–response or S–R association) is certainly not the only thing learned in the situation. Of course, for successful performance in such tasks, animals must learn associations between stimuli (stimulus–stimulus or S–S associations, such as the CS–US association), associations between behavioral responses and their outcomes (response–outcome or R–O associations), and any other information incidental to task performance that defines the episode in the stream of the animal's experience. So, simple conditioning tasks are often much more complicated than they would appear to be at first glance.

3.24.2.2 Pavlovian Conditioning Paradigms

The other major class of aversive conditioning tasks that has been adapted to study emotional memory systems does not require the instrumental contingency that is at the heart of avoidance conditioning

tasks. In classical or Pavlovian conditioning, just as in instrumental conditioning, animals learn an association between stimuli and their consequences, such as an auditory CS and a footshock US that it predicts. However, in Pavlovian conditioning, the animal's behavior is inconsequential to the delivery of either stimulus; CSs and USs are delivered irrespective of the animal's behavior, and the animal cannot engage in behavior to avert or avoid the US. In this sense, Pavlovian conditioning simplifies the analysis of the underlying brain systems because it eliminates some of the associative processes that operate in the context of instrumental conditioning tasks (namely, instrumental S–R and R–O associations).

Pavlovian conditioning tasks designed to characterize emotional learning and memory, such as learning fear, typically utilize noxious USs such as electric shock applied to the feet or, in some cases, electric current delivered through fine wires implanted in the neck or around the eye. Other aversive USs that are effective for conditioning fear include loud sounds, such as bursts of white noise (Watson actually used a loud sound to condition fear of rats in Little Albert), air puffs, illness induced by emetics such as lithium chloride, and inhalation of carbon dioxide. These aversive USs can be signaled by a variety of CSs including lights, sounds (most typically pure tones or white noises), olfactory stimuli, or even the places or contexts in which the animal encounters the US. What tends to vary across different Pavlovian fear conditioning tasks is the behavioral (or autonomic) response used to index fear. The choice of response system depends on many factors, not the least of which is the animal species used in the experiments. In rats and mice, a variety of response measures have been used. In a naturalistic setting, rodents show a hierarchy of defensive behavior in response to predatory threat. When a predator is near, but not yet in contact with, the prey, a rat or mouse will exhibit freezing behavior, which is characterized by nearly complete immobility, as a means to avoid detection by the predator. In the laboratory, freezing behavior is readily conditioned to places in which aversive USs are delivered (Blanchard and Blanchard, 1969). Interestingly, unlike other notable forms of Pavlovian conditioning, the learned fear response (the conditioned response or CR) does not take the form of the activity burst that is generated by the shock US. Running, jumping, and vocalization characterize the activity burst of a rat to footshock; this collection of responses resembles a rat's circa-strike response to a predatory attack. It is widely believed that the activity burst reflects the sensory properties of the shock US, whereas the freezing response is a function of the affective (fear-engendering) properties of the aversive event. Hence, many studies of fear conditioning in rodents rely on freezing behavior as an index of fear. In addition to freezing, rats exhibit a number of other behavioral and autonomic responses that can be used to index fear. For instance, they show less recuperative behavior to a painful formalin injection into their paw because of fear-induced hypoalgesia. Fear is also associated with increased heart rate and blood pressure and the release of stress hormones such as glucocorticoids.

Another popular measure for indexing fear is the change in the acoustic startle reflex that is observed in the presence of a fear-provoking stimulus (Brown et al., 1951). In the fear-potentiated startle paradigm, rats or mice are exposed to a series of loud noise bursts to index their acoustic startle amplitudes. After this phase they are exposed to Pavlovian fear conditioning procedures in which a visual CS is paired with a footshock US. To assess the fear engendered by the CS, the rats are once again exposed to the acoustic startle stimuli, but now in the presence of the visual CS on some trials. Under these conditions, the acoustic startle response is greatly elevated on trials in which the fearful CS is present relevant to trials without the CS. Fear, then, is indexed indirectly by potentiation of the startle response. Another indirect measure of fear described by Estes and Skinner is the suppression of appetitive responding in the presence of a fearful CS (Estes and Skinner, 1941). In this case, animals were first instrumentally trained to press a bar for a food reward. When the animals were reliably pressing the bar for food, they underwent (typically in another chamber) fear-conditioning procedures. The animals were then once again allowed to respond instrumentally for food. When presented with the fearful CS in this situation, instrumental responding during the CS dropped considerably relative to intervals before or after delivery of the CS. This bar-press suppression by a fearful CS has historically been used to index the conditioned emotional response (CER), which is synonymous with the conditioned fear response.

3.24.2.3 Naturalistic Conditioning Paradigms

In addition to these traditional models for studying emotional learning, there are some less widely used,

yet more naturalistic, paradigms that have been used for studying aversively motivated learning. For example, social defeat by a dominant conspecific appears to yield a conditioned fear state that shares many of the properties of fear motivated by artificial aversive stimuli, such as footshock (Potegal et al., 1993). In this paradigm, typically conducted in Syrian hamsters, a younger, subordinate animal (the intruder) is introduced into the cage of an older, dominant animal (the resident), which arranges a social interaction whereby the resident behaves aggressively toward the intruder, chasing and biting the subordinate animal. Subsequent to this antagonistic interaction, the intruder exhibits submissive behavior around the dominant animal as well as other, nonthreatening conspecifics. Although the pattern of behavior that emerges from social defeat does not include freezing behavior, there are other commonalties with conditioned fear responses such as a reduction in pain sensitivity (hypoalgesia). Clearly, negative social interactions can yield an aversive state that conditions fear to other conspecifics and even the places where defeat occurs (Razzoli et al., 2006).

Of course, one function of fear is not only to motivate defensive behavior but also, at least in the case of overt fear responses, to communicate an individual's emotional state to nearby conspecifics. This might serve to protect genetically related individuals from external threats bearing on one member of the group. Learned fear through social transmission has been demonstrated in both mice and rats. For instance, mice that witness a conspecific being attacked by biting stable flies will subsequently exhibit hypoalgesia and attempt to bury themselves (to avoid being bitten) when exposed to flies whose mouthparts have been removed (to prevent biting) (Kavaliers et al., 2003). Animals that were never exposed to flies did not exhibit these responses. Social transmission of fear may also influence subsequent aversively motivated learning. In a recent study, rats were housed in pairs, and then one member of the pair was removed and subjected to fear conditioning (Knapska et al., 2006a). After the fear-conditioning episode, the conditioned animal was allowed to interact with its naïve cage-mate. During the interaction, the nonshocked cage-mate exhibited behaviors similar to that of the shocked rat and subsequently acquired a conditioned avoidance response faster than rats whose cage-mate left home but did not undergo fear conditioning. Collectively, these studies suggest that fear can be socially transmitted, can be learned, and can influence subsequent behavior to novel aversive events. Fear learning would appear to promote defensive behavior in not only the individual experiencing an aversive stimulus but also others with whom that animal interacts.

3.24.3 Historical Perspective on Brain Mechanisms of Emotional Learning

To appreciate modern work on the neural mechanisms of aversively motivated learning, it is worthwhile to trace the development of work linking emotion to the brain. There is no doubt that our modern appreciation of the brain circuits involved in aversive learning and memory emerged from early observations on the effects of brain damage on emotional behavior in humans and animals. Emotional learning in humans will be covered in depth elsewhere, but it is worthwhile mentioning one human case as a prelude to the discussion of work in animals. Prior to the nineteenth century, there had been an explosion of detailed descriptions of human brain anatomy, but only small gains in appreciating the function of various brain areas in psychological function. Perhaps the most influential one was the discovery of profound emotional changes in a man named Phineas Gage, whose brain was penetrated by an iron spike in a railway construction accident in 1848. Amazingly, Gage survived the injury to his head, but he was not the same person after recovering from the wound. The surgeon that oversaw Gage's case noted that Gage's personality showed marked changes after the injury. Relative to his gentle demeanor prior to the accident, he angered easily, used profanity, and could not maintain interpersonal relationships after his accident. Emotions, it would appear, had their roots in the brain. Gage's case would not inform the question of brain systems involved in emotional learning and memory *per se*, but it did set the groundwork for understanding the brain mechanisms of emotion, which was then actively pursued in experimental studies in animals.

The experimental investigation of emotional changes after brain injury in animals was heralded by Goltz's work in dogs (Goltz, 1892). Goltz surgically removed large portions of the dog cerebral cortex (a decortication) and noted profound changes in emotional reactivity after the animals recovered from the procedure. He found that any disturbance of the animal, however small, provoked an acute rage

that included barking, biting, deflection of the pinnae, and piloerection among other responses. The animals appeared to assume an emotional state that was only provoked in normal animals by highly aversive stimuli. Cannon and Britton later termed this behavioral state 'sham rage,' to distinguish it from the emotional reaction displayed by normal animals. The importance of Goltz's work was to begin a series of more systematic functional neuroanatomical studies that sought to localize emotional centers in the brain.

In parallel with Goltz's work in Germany, Brown and Schäfer, working in London, described profound alterations in emotional reactivity following temporal lobe lesions in monkeys (Brown and Schafer, 1888). Klüver and Bucy subsequently extended this work by making more discrete lesions and recording detailed behavioral observations of their operated animals (Klüver and Bucy, 1937). Both groups found that temporal lobe resections, which damaged both cortical and subcortical tissue, produced marked behavioral changes, including hyperorality, hypersexuality, visual agnosia, and notably, a loss of fear. For example, monkeys with temporal lobe lesions readily consumed novel and normally avoided foods, such as meat, and they would mouth inedible objects including metallic screws. Moreover, monkeys that once cowered in the presence of humans readily approached and contacted their caretakers after surgery. This work heralded the study of the neural substrates of emotion and focused intense interest on the role of the temporal lobes in the mediation of fear.

Papez, and later MacLean, built on the functional studies in animals and proposed what were to become highly influential neuroanatomical models of emotion systems in the brain (Papez, 1937; MacLean, 1949). Papez used anatomical methods to trace axonal connections between brain structures. He found that brain structures involved in generating emotional responses were highly interconnected and circuitous. His anatomical circuit for emotion included a prominent role for the hippocampus (a medial temporal lobe structure), the anterior thalamus and mammillary bodies in the diencephalon, cingulate lobe of the neocortex, and the major fiber tracts connecting these structures (fornix and mammilothalamic tract). MacLean expanded on the Papez circuit to include other brain structures in the emotional circuit, including the septum and amygdala, in what he termed a larger limbic system that he implicated in emotion. Central to MacLean's theory was the triune brain, which he contended described a systematic pattern of brain evolution along three neural axes: primitive, regulatory centers in the brain stem (the reptilian R-complex); emotional centers in the subcortical limbic system; and higher cognitive centers in the neocortex.

Although the Papez circuit and related limbic system had a potent influence on thought concerning the neural basis of emotion, it is now generally understood that there is no singular emotional center in the brain. Even specific emotions, such as fear, appear to involve brain circuitry that is not constrained by the anatomical circuits described by Papez and MacLean. In the context of aversive learning and memory, for example, it has become clear that a critical brain structure is the amygdala, a temporal lobe structure that was omitted from the Papez circuit. The central role of the amygdala in fear emerged from early work focusing on the consequence of temporal lobe damage on emotional reactivity. Specifically, Weiskrantz found that the loss of fear that Klüver and Bucy described in monkeys with temporal lobe lesions was the consequence of damage to the amygdala (Weiskrantz, 1956). Indeed, recent work has shown that a selective loss of amygdala neurons results in a fear reduction similar to that observed by Klüver and Bucy (Zola-Morgan et al., 1991; Meunier et al., 1999; Kalin et al., 2004). Numerous other studies have demonstrated reduced fear (taming) after amygdala damage in several mammalian species, including rats, cats, rabbits, dogs, and humans (Goddard, 1964). Moreover, electrical stimulation of the amygdala in animals and amygdaloid seizures in humans are associated with autonomic and behavioral changes characteristic of fear (Gloor, 1960; Gloor et al., 1982; Iwata et al., 1987; Davis, 1992). Hence, consensus emerged from these studies that the amygdaloid complex has an indispensable role in the regulation of fear.

3.24.4 Neural Mechanisms of Instrumental Avoidance Conditioning

The discovery of the central role for the amygdala in fear heralded an era of systematic examination of the neural substrates of aversively motivated learning and memory tasks in animals (Sarter and Markowitsch, 1985). Moreover, the observation by Watson and Rayner that fearful emotions could be conditioned in humans led to considerable interest in

how acquired states of fear and anxiety might influence normal and pathological behavior (Watson and Rayner, 1920). Hence, the mid-twentieth century witnessed a convergence of neuroanatomical work on emotion centers in the brain with psychological work on the properties of emotional learning that provided the foundation for systematic studies of the neural circuitry and mechanisms involved in learning about fearful experiences. Several investigators set out to further quantify this function by employing learning and memory tasks. The earliest work in this domain used instrumental avoidance conditioning tasks to investigate the neural mechanisms of learned emotions. And although there has been considerable debate concerning the nature of avoidance learning (Bolles, 1972; Fanselow, 1997), active and passive avoidance conditioning tasks have remained an important method for characterizing an animal's propensity to alter its behavior to avoid aversive stimuli.

3.24.4.1 Active Avoidance Conditioning

The earliest studies to examine the neural substrates of fear-motivated learning used instrumental avoidance tasks, in which animals could avoid an aversive stimulus by making the appropriate behavioral response. Not surprisingly, the amygdala was the focus of this early work, given the important role it had been shown to play in emotional behavior. In the first study of its kind, Brady and colleagues trained cats in a footshock-motivated shuttle avoidance task and found that large amygdala aspirations impaired the acquisition, but not the retention, of the avoidance response (Brady et al., 1954). Subsequent studies extended the active avoidance conditioning deficits after amygdala damage to rats. These deficits were reported in two-way active avoidance (McNew and Thompson, 1966; Campenot, 1969; Bush et al., 1973; Yeudall and Walley, 1977; Schutz and Izquierdo, 1979) and wheel-turning avoidance (Robinson, 1963).

Of course, the amygdala is a heterogeneous structure composed of several anatomically distinct nuclei (Krettek and Price, 1974; Krettek and Price, 1978; Pitkanen et al., 1997; McDonald, 1998; Swanson and Petrovich, 1998; Sah et al., 2003). The basolateral complex of the amygdala is the primary cortical interface of the amygdala and consists of the lateral, basolateral, and basomedial nuclei. These nuclei in turn project heavily to the central nucleus of the amygdala, which is the primary interface of the amygdala to hypothalamic and brainstem structures involved in generating various fear responses. Many of the earlier studies, with some exceptions, damaged the amygdala in its entirety without attention to the different contributions individual amygdaloid nuclei make to avoidance learning and memory. However, some of the early studies attempted to examine the contribution of individual nuclei to avoidance learning. For example, discrete electrolytic lesions to the basolateral complex of the amygdala impaired the acquisition and retention of a shuttle-box avoidance in cats (Horvath, 1963), one-way active avoidance in rats (Werka et al., 1978), and two-way avoidance in rats (Coover et al., 1973). The central nucleus of the amygdala was also shown to be critical for the acquisition, but not retention, of one-way (Werka et al., 1978) and two-way (Roozendaal et al., 1993) active avoidance. Importantly, selective neurotoxic lesions of the central nucleus of the amygdala that spare axonal fibers of passage result in active avoidance conditioning deficits (Sanchez Riolobos, 1986). Interestingly, however, cellular markers of neuronal activity in the amygdala indicate that avoidance training engages primarily the lateral and basolateral nuclei of the amygdala, with little evidence for central nucleus activation (Savonenko et al., 1999; Radwanska et al., 2002; Knapska et al., 2006b). This pattern of activity is consistent with recent work that indicates that the basolateral, but not central nucleus, of the amygdala is particularly involved with instrumental avoidance under some conditions (Killcross et al., 1997; Amorapanth et al., 2000).

In contrast to the amygdala, damage to the hippocampus, the fornix (the major subcortical projection tract of the hippocampus), or the entorhinal cortex (the major cortical interface of the hippocampus) typically produces a facilitation of active avoidance learning (Myhrer, 1975; Weiner et al., 1998; Pouzet et al., 1999; Guillazo-Blanch et al., 2002). As will be discussed next, the enhancement in active avoidance conditioning might be the result of two factors. First, hippocampal lesions increase locomotor activity in a variety of test situations (Douglas, 1967; Blanchard et al., 1977; Maren and Fanselow, 1997). Increases in locomotor activity are likely to foster the emission of escape responses in response to shock during avoidance training and are thus permissive to the acquisition of avoidance responses. Second, hippocampal lesions produce impairments in the acquisition of contextual fear, while having minimal effects on fear to discrete CSs (e.g., Phillips and LeDoux, 1994). Contextual fear tends to retard acquisition of two-way active avoidance insofar as it generates freezing behavior (which

competes with escape and avoidance responding), and it encourages passive avoidance of the compartment of the apparatus that was associated with shock on each subsequent trial. Hence, hippocampal rats unencumbered by contextual fear, accruing CS fear normally, and primed to emit active avoidance responses, outperform normal rats in active avoidance tasks. The hippocampal system does not appear to play a direct role in supporting avoidance learning but, rather, modulates performance in a way that is constrained by the contextual demands of the task.

Gabriel and colleagues have conducted one of the most systematic neural analyses of instrumental avoidance learning in rabbits performing wheel-running responses in a discriminative avoidance task (Gabriel, 1993). In this task, one auditory conditional stimulus (CS+) signals the onset of footshock, whereas a different auditory conditional stimulus (CS−) does not. Rabbits come to respond discriminatively to the two cues, only making stepping responses in a running wheel to avoid footshock in response to the CS+. Consistent with work in rats and cats in other avoidance conditioning tasks, lesions or pharmacological inactivation of the amygdala impair the acquisition of discriminative avoidance conditioning in rabbits (Poremba and Gabriel, 1997a, 1999, 2001). Damage to the lateral and central nuclei of the amygdala appears to be particularly predictive of avoidance deficits in rats with amygdala lesions (Poremba and Gabriel, 1997a). Indeed, discrete lesions of the central nucleus severely impair acquisition of discriminative avoidance learning without affecting instrumental responding for an appetitive reward (Smith et al., 2001). And as noted in earlier reports, the amygdala appears to be particularly important during the acquisition of instrumental avoidance behavior, insofar as reversible inactivation of amygdala neurons with muscimol, a $GABA_A$ receptor agonist, in well-trained rabbits does not impair avoidance responding (Poremba and Gabriel, 1999).

Electrophysiological recordings in a number of interconnected structures reveal that there is a systematic engagement of neuronal populations in encoding the conditioning contingencies and mediating the performance of well-learned behavior (Gabriel, 1993). Earliest to engage during avoidance conditioning are the basolateral amygdala, mediodorsal nucleus of the thalamus, and anterior cingulate cortex; later avoidance performance in well-trained animals is correlated with discriminative neuronal activity in the anterior thalamic nuclei and the posterior cingulate cortex (Gabriel et al., 1991; Maren et al., 1991). Both thalamic structures are critically involved in acquisition and maintenance of avoidance behavior (Gabriel et al., 1989; Steinmetz et al., 1991). Auditory information that drives neuronal firing to the CSs during avoidance training reaches the amygdala, thalamus, and cingulate cortex by way of the auditory thalamus (Poremba and Gabriel, 1997b). Amygdala lesions or inactivation, in addition to impairing acquisition of avoidance responses, impair electrophysiological correlates of avoidance conditioning that develop in the auditory thalamus, particularly the medial division of the medial geniculate nucleus, the anterior and mediodorsal thalamic nuclei, and the anterior and posterior cingulate cortices (Poremba and Gabriel, 1997a; Poremba and Gabriel, 1999; Poremba and Gabriel, 2001; Smith et al., 2001). Electrophysiological correlates of avoidance conditioning have also been observed in the central and basolateral nuclei of rats during both active (Rorick-Kehn and Steinmetz, 2005) and passive (Chang et al., 2005) avoidance.

As in other active avoidance conditioning tasks, damage to the hippocampal formation improves the acquisition of instrumental avoidance conditioning in rabbits (Gabriel and Sparenborg, 1986; Gabriel et al., 1987). Interestingly, hippocampal areas, particularly the subiculum, appear to have an inhibitory influence on anterior thalamic neuronal activity; accordingly, subicular lesions facilitate both CS-elicited spike firing in the thalamus and behavioral performance (Gabriel and Sparenborg, 1986; Gabriel et al., 1987). As suggested earlier, the hippocampus appears to be particularly germane to encoding contextual representations during conditioning. Indeed, hippocampal neurons exhibit highly context-dependent spike firing when auditory CSs acquire multiple meanings (Freeman et al., 1996). Lesions placed in the entorhinal cortex, which is the primary cortical interface of the hippocampus, impairs context-dependent behavioral performance and CS-elicited neuronal activity (Freeman et al., 1997).

Collectively, the results from instrumental avoidance conditioning tasks suggest that the central and basolateral nucleus of the amygdala are involved in acquiring active avoidance responses but are not required for their retention. It has been argued that two processes contribute to the acquisition of avoidance responses (Mowrer, 1947). First, animals acquire Pavlovian fear of the CS and subsequently learn to terminate the CS by making an instrumental avoidance response (see Bolles, 1972, for an alternative interpretation). According to this view, avoidance responses are reinforced by fear reduction when the CS is terminated. Once learned, performance

is maintained by the instrumental contingency, and conditional fear to the CS plays a relatively minor role in supporting performance (Mineka and Gino, 1980). Hence, one explanation for the differential role of the amygdaloid nuclei in the acquisition and retention of instrumental avoidance is that lesions made before training disrupt acquisition because they interfere with Pavlovian fear conditioning, whereas lesions made after training have little effect because Pavlovian fear is not required to sustain performance at that point in training (Maren, 1998).

3.24.4.2 Passive Avoidance Conditioning

Unlike active avoidance tasks, the instrumental contingency in a passive avoidance task is arranged to punish active behavioral response and encourage shock avoidance through inhibition of responding. Similar to active avoidance learning, several studies have indicated a critical role for the amygdala in the acquisition and retention of this form of learning. Large, bilateral amygdala lesions impair the acquisition of a variety of passive avoidance responses in rats (McNew and Thompson, 1966; McIntyre and Stein, 1973; Bresnahan et al., 1976; Nagel and Kemble, 1976; Russo et al., 1976; Liang et al., 1982; Jellestad and Bakke, 1985), mice (Slotnick, 1973), and cats (Horvath, 1963; Ursin, 1965). Smaller electrolytic lesions centered on the basolateral nucleus (Coover et al., 1973; Grossman et al., 1975; Werka et al., 1978) or central nucleus (Grossman et al., 1975; Werka et al., 1978) of the amygdala reproduce these passive avoidance deficits. In contrast, only excitotoxic lesions of the central or lateral nuclei, but not the basolateral nucleus, have been reported to impair step-through inhibitory avoidance (Tomaz et al., 1992); this is an interesting contrast to other studies that find that basolateral, but not central, lesions influence active avoidance responses (Killcross et al., 1997; Amorapanth et al., 2000). In addition to acquisition deficits, impairments in the retention of a passive avoidance response after amygdala lesions have also been reported (Nagel and Kemble, 1976; Liang et al., 1982; Parent et al., 1994, 1995), and reversible inactivation of the amygdala with lidocaine, a voltage-gated sodium channel blocker (Parent and McGaugh, 1994), or muscimol (Holahan and White, 2004a,b) impairs the retention of inhibitory avoidance.

Given the importance of the amygdala, particularly the lateral and central nuclei, in passive avoidance, it is reasonable to consider the possibility that the memory for some aspect of passive avoidance training resides within the amygdala. Passive avoidance conditioning depends on both Pavlovian associations between context and shock and instrumental associations between approach behavior and its aversive outcome (Randall and Riccio, 1969). Unlike active avoidance conditioning, it is likely that Pavlovian associations play a key role in maintaining passive avoidance behavior (most passive avoidance tasks involve only a single training trial) (Randall and Riccio, 1969). As discussed earlier, considerable evidence shows that the central and lateral nuclei of the amygdala are involved in encoding and storing Pavlovian fear memories (e.g., Maren and Quirk, 2004). Therefore, disruption of these structures would be expected to impair the acquisition and expression of passive avoidance behavior. Spared passive avoidance performance that is observed after damage to the basolateral nucleus may be maintained by conditioned fear, which can survive these lesions under some conditions (Nader et al., 2001; Anglada-Figueroa and Quirk, 2005). Nonetheless, basolateral nucleus lesions do impair the acquisition of fear conditioning in other studies (Goosens and Maren, 2001), and these lesions disrupt the expression of conditioned fear when made after conditioning (Anglada-Figueroa and Quirk, 2005). Thus, the contribution of individual amygdaloid nuclei to both the acquisition and retention of passive avoidance warrants further attention, particularly in relation to the effects of these manipulations on Pavlovian fear conditioning.

In addition to the amygdala, the hippocampus plays an important role in the acquisition and retention of passive avoidance conditioning. Hippocampal lesions or electrical stimulation disrupt the acquisition and consolidation of passive avoidance memory (Blanchard and Fial, 1968; Winocur and Bindra, 1976; Munoz and Grossman, 1981; Kesner and Hardy, 1983). Moreover, reversible inactivation of the hippocampus with tetrototoxin, a voltage-gated sodium channel blocker, impairs the acquisition, consolidation, and retrieval of passive avoidance memories (Lorenzini et al., 1996; Ambrogi Lorenzini et al., 1997). The important role of the hippocampus in passive avoidance conditioning is likely due to the prominent role contextual conditioning plays in standard passive avoidance paradigms. That is, step-through and step-down versions of the task, which

are the most commonly used passive avoidance paradigms, essentially consist of an animal-initiated context-shock conditioning trial. The Pavlovian association between context and shock is an important component to passive avoidance performance, insofar as forced extinction of the shock context after avoidance conditioning degrades conditional responding (Randall and Riccio, 1969). Of course, the instrumental approach-shock contingency is also important in shaping passive avoidance, and delay of shock after animals enter the dangerous context (which degrades the response-outcome contingency) reduces passive avoidance performance (Randall and Riccio, 1969). Indeed, the hippocampal contribution to encoding contexts (which is discussed later), as opposed to processing the aversive shock event, appears to play a critical role in the influences of hippocampal manipulations on passive avoidance memory (Malin and McGaugh, 2006). Recent work shows that the representation of contexts by the hippocampus depends, in part, on activity in the amygdala (Roozendaal and McGaugh, 1997; Huff and Rudy, 2004; Huff et al., 2006; Malin and McGaugh, 2006).

Because passive avoidance conditioning can be acquired in a single trial, it has been become one of the most extensively used tasks to explore the pharmacology of emotional learning and memory. Indeed, a variety of pharmacological manipulations within the amygdala can bidirectionally influence (either enhancing or impairing) the retention of passive avoidance memory (Gallagher et al., 1977; McGaugh, 2000, 2004). In this regard, there is substantial evidence that the amygdala is also involved in consolidating memories for aversive experiences outside the amygdaloid circuitry (Cahill and McGaugh, 1998), and the majority of work indicates that the basolateral nucleus of the amygdala in particular is instrumental in this function (Roozendaal and McGaugh, 1997; Roozendaal et al., 1998). It has been argued that the amygdala may not be involved in the local storage of memories for aversive events but, rather, is preferentially concerned with modulating aversive memory in other brain structures (Cahill et al., 2001). However, the roles for the amygdala in Pavlovian association formation and memory consolidation are dissociable. For example, posttraining inactivation of the amygdala with muscimol produces deficits in the retention of inhibitory avoidance conditioning, but not Pavlovian fear conditioning (Wilensky et al., 2000). Therefore, the nature of the amygdala's involvement in aversive learning, whether it is local memory storage or remote memory consolidation, depends importantly on the associative structure of the conditioning situation and the behavioral measures used to index memory (Kapp et al., 1978; Maren, 2003b).

3.24.4.3 Defensive Burying and Shock-Probe Avoidance

Similar to traditional passive avoidance tasks, large lesions of the hippocampus or amygdala lesions impair avoidance of an electrified probe once the probe has been contacted (Treit and Menard, 1997). Neither lesion, however, affects how much time animals spend burying the probe after they have received shock. Small lesions of the amygdala centered on the central nucleus do not affect probe avoidance but do affect immobility that occurs after contact with the electrified probe (Roozendaal et al., 1991). Interestingly, lesions or inactivation of the amygdala, despite reducing probe avoidance during the conditioning session, do not affect subsequent avoidance of the probe during retention testing (Lehmann et al., 2000, 2003). Although this has been interpreted to indicate that the amygdala is not necessary to form probe-shock associations, another possibility is that instrumental contingencies related to the training procedure maintain avoidance in the absence of associative fear of the probe (Maren, 2003b).

3.24.5 Neural Mechanisms of Pavlovian Fear Conditioning

In addition to instrumental avoidance conditioning tasks, Pavlovian fear conditioning has been used extensively to examine the brain substrates of emotional learning and memory (LeDoux, 2000; Maren, 2001; Fanselow and Poulos, 2005; Davis, 2006). Studies of the neural mechanisms of Pavlovian fear conditioning have focused on the contribution and interaction of several interconnected structures, including the amygdala, hippocampus, and prefrontal cortex (McIntosh and Gonzalez-Lima, 1994, 1998). Sensory information reaches each of these structures via both thalamic and cortical routes. To understand the neural basis of fear conditioning, we will consider the contribution of each of these brain areas to various fear conditioning paradigms. This review will focus on the anatomy and physiology of these forms of learning, insofar as the synaptic and cellular basis

of fear conditioning is considered in detail elsewhere (*See* Chapter 4.11). The majority of the work described was conducted in rats, and work in other species will be identified where appropriate.

3.24.5.1 Conditioned Freezing

It has long been appreciated that aversive stimuli evoke freezing behavior in several animal species, particularly rodents. Robert and Caroline Blanchard pioneered the use of freezing behavior as an index of conditioned fear (Blanchard and Blanchard, 1969). Not surprisingly, they were also the first to systematically examine the neural systems involved in the acquisition and retention of conditioned freezing. In early work, they demonstrated a role for the amygdala in the acquisition of conditioned freezing (Blanchard and Blanchard, 1972a). In this case, they used a contextual fear conditioning procedure in which footshocks were delivered in a specific environmental context, and freezing behavior in that context served as the measure of conditional fear. They found that large, bilateral amygdala lesions completely eliminated shock-elicited freezing behavior, as well as unconditional freezing to a cat (Blanchard and Blanchard, 1972a). Interestingly, they also found in related studies that damage to the hippocampal formation produced a similar loss in contextual fear conditioning (Blanchard et al., 1970, 1977; Blanchard and Blanchard, 1972b), but such lesions also elevated motor activity in a number of test situations. Nonetheless, as will be discussed shortly, the hippocampus proves to have a special role in learning about contextual stimuli, and this role comes to influence the acquisition of contextual fear. The use of freezing behavior as an index of learned fear has become the most widely used paradigm for studying the neural mechanisms of emotional learning and memory. This is due in large part to the ease of measuring freezing behavior and the simplicity of the training regimen (appetitive training to establish an operant baseline is not required). For this reason, the most published literature is on this paradigm, and this will be reflected in the extensive coverage of conditioned freezing in this section.

Building on the Blanchards' original work, several laboratories in nearly countless studies have confirmed the critical role for the amygdala in both the acquisition and expression of conditioned freezing behavior using selective lesions of individual amygdaloid nuclei. For example, selective lesions of the basolateral complex, particularly the lateral nucleus, produce severe deficits in both the acquisition and expression of conditioned freezing to discrete CSs (whether auditory, visual, or olfactory) and contexts (LeDoux et al., 1990; Ambrogi Lorenzini et al., 1991; Maren et al., 1996a; Cousens and Otto, 1998; Maren, 1998, 1999b; Amorapanth et al., 2000; Antoniadis and McDonald, 2000; Cahill et al., 2000; Goosens and Maren, 2001; Nader et al., 2001; Blair et al., 2005). It is noteworthy that lesions of the basolateral amygdala made long after conditioning (from a month to over a year) produce a complete retrograde amnesia for conditioned fear manifested as a loss of conditioned freezing (Maren et al., 1996a; Gale et al., 2004). Reversible pharmacological inactivation of the basolateral amygdala with agents such as muscimol (a $GABA_A$ receptor agonist) or lidocaine (a voltage-gated sodium channel blocker) also eliminates the acquisition and expression of conditioned freezing (Helmstetter, 1992a; Helmstetter and Bellgowan, 1994; Muller et al., 1997; Wilensky et al., 1999; Wilensky et al., 2000; Maren et al., 2001; Goosens and Maren, 2003; Blair et al., 2005). Furthermore, the amygdala plays a prominent role in the ontogeny of fear conditioning in rats (Moriceau and Sullivan, 2005, 2006; Moriceau et al., 2006).

An important observation is that deficits in conditioned freezing after basolateral complex lesions can be overcome with extensive training so long as overtraining occurs in a brain-damaged animal; basolateral lesions made after extensive overtraining still yield complete deficits in conditioned freezing (Maren, 1998, 1999b). This argues that although the basolateral complex of the amygdala, including the lateral nucleus of the amygdala, is critical for the acquisition of conditioned freezing, another brain region can compensate for loss of the basolateral complex under some conditions. One possibility is that the central nucleus of the amygdala, which receives a major projection from the basolateral complex (Krettek and Price, 1978; Paré et al., 1995; Savander et al., 1995), has a critical role in the acquisition and expression of conditioned freezing (Paré et al., 2004). This possibility has been supported in preliminary work (Zimmerman et al., 2005).

The possibility that the central nucleus has a critical role in fear conditioning is not novel. Indeed, it has long been appreciated that the central nucleus of the amygdala has a critical role in fear behavior. For example, electrical stimulation of the

central nucleus produces behavioral responses similar to those evoked by stimuli paired with shock (Applegate et al., 1983; Iwata et al., 1987; Kapp et al., 1992). Lesions of the central nucleus of the amygdala, like those of the basolateral complex, prevent the acquisition and expression of conditioned freezing in rats (Amorapanth et al., 2000; Goosens and Maren, 2001; Nader et al., 2001), although there is some evidence of spared fear conditioning with pharmacological manipulations of the central nucleus during conditioning (Goosens et al., 2003; Goosens and Maren, 2003). As mentioned earlier, lesions placed in central amygdala efferents produce selective deficits in certain fear responses such as conditioned freezing in the case of the periaqueductal gray (LeDoux et al., 1988; Kim et al., 1993a; De Oca et al., 1998; Amorapanth et al., 1999) or arterial pressure in the case of the lateral hypothalamus (Iwata et al., 1986b; LeDoux et al., 1988). Because lesions in the central nucleus of the amygdala impair all these fear responses (LeDoux et al., 1988), the evidence suggests that the central nucleus is the final common pathway for the generation of learned fear responses.

There is extensive sensory convergence in both the basolateral complex and central nucleus in the amygdala (Krettek and Price, 1974, 1978; Pitkanen et al., 1997; McDonald, 1998; Swanson and Petrovich, 1998; Sah et al., 2003). In the past, the possibility that associations between sensory inputs occurred exclusively in the basolateral complex, particularly the lateral nucleus, rather than the central nucleus had been emphasized (LeDoux, 1993a,b, 1994, 1995, 2000; Maren, 1996, 1999a,b, 2001, 2003a; Fanselow and LeDoux, 1999; Fendt and Fanselow, 1999). However, it has recently been appreciated, given both anatomical considerations and spared learning in rats with basolateral complex lesions, that the central nucleus has a more important role in fear conditioning than previously thought (Paré et al., 2004). A recent series of experiments indicates that many of the molecular processes believed to operate in the service of long-term memory storage are required in the central nucleus to acquire conditional fear (Wilensky et al., 2006). The important role for the central nucleus in the acquisition of conditioned affective states has also been emphasized in appetitive conditioning paradigms and in conditioned suppression (Cardinal et al., 2002; Balleine and Killcross, 2006). As a result, there is mounting evidence that the central nucleus and basolateral complex might perform different functions in aversive conditioning, at least under some conditions.

Sensory inputs to the amygdala arise from a number of areas, including the medial thalamus (LeDoux et al., 1984; Doron and LeDoux, 2000a,b), hippocampal formation (Ottersen, 1982; Aggleton, 1986; Canteras and Swanson, 1992; Maren and Fanselow, 1995), rhinal cortices (Aggleton, 1986; McDonald and Mascagni, 1997; Shi and Cassell, 1999), and spinal cord (Ma and Peschanski, 1988; Cliffer et al., 1991; Burstein and Potrebic, 1993; Newman et al., 1996). Consistent with this anatomy, single neurons in the basolateral complex and central nucleus of the amygdala respond to auditory, visual, and somatic (shock) stimuli (Applegate et al., 1982; Pascoe and Kapp, 1985a,b; Kapp et al., 1992; Romanski et al., 1993), which indicates that the amygdala is a locus of convergence for information about CSs and USs. Thus, the amygdala is anatomically situated to integrate information from a variety of sensory domains. Extensive pharmacological evidence indicates that synaptic plasticity in the amygdala contributes to both the acquisition and extinction of conditioned freezing (Maren et al., 1996b, 2003; Lee and Kim, 1998; Rosen et al., 1998; Bailey et al., 1999; Weisskopf et al., 1999; Goosens et al., 2000; Nader et al., 2000; Schafe et al., 2000, 2005; Fendt, 2001; Lee et al., 2001, 2006; Malkani and Rosen, 2001; Bauer et al., 2002; Lamprecht et al., 2002; Moita et al., 2002; Rodrigues et al., 2002, 2004; Goosens and Maren, 2003, 2004; Apergis-Schoute et al., 2005; Maren, 2005b; Rumpel et al., 2005; Merino and Maren, 2006; Wilensky et al., 2006).

As mentioned earlier, it is well documented that amygdala damage disrupts not only learned fear but also innate fear under some conditions. For example, rats with amygdala lesions do not exhibit freezing or analgesia in the presence of a cat (Blanchard and Blanchard, 1972a; Fox and Sorenson, 1994); they do show attenuated unconditional analgesia and heart rate responses to loud noises (Bellgowan and Helmstetter, 1996; Young and Leaton, 1996), and they exhibit reduced taste neophobia (Nachman and Ashe, 1974). Amygdala damage does not disrupt all unconditional fear responses, however. Amygdala lesions do not affect open arm avoidance in an elevated plus maze (Treit et al., 1993; Treit and Menard, 1997), unconditional analgesia (Watkins et al., 1993), or unconditioned freezing to a predator odor (Wallace and Rosen, 2001), or after ejaculation (Choi and Brown, 2003). Thus, although amygdala damage may reduce unlearned fear responses under some conditions, it does not appear that a general loss of fear accounts for the memory impairments

observed after lesions or inactivation of the amygdala, as has been suggested by some (Cahill et al., 2001, 1999).

Electrophysiological recordings of amygdaloid neuronal activity support a role for the amygdala in representing conditional fear memories (Maren and Quirk, 2004). Auditory fear conditioning induces short-latency plasticity in amygdala neurons (Quirk et al., 1995, 1997; Armony et al., 1998; Collins and Paré, 2000; Maren, 2000a; Pelletier et al., 2005). This plasticity takes the form of enhanced spike firing elicited by acoustic CSs. Fear conditioning also increases the amplitude of synaptic potentials in lateral amygdala neurons recorded either intracellularly (Rosenkranz and Grace, 2002) or extracellularly (McKernan and Shinnick-Gallagher, 1997; Rogan et al., 1997). The short latency of learning-related changes in spike firing is consistent with plasticity in thalamo-amygdala projections, specifically, projections from the medial division of the medial geniculate nucleus. Amygdala neurons exhibit plasticity earlier in training than auditory cortical neurons, further suggesting that direct thalamo-amygdala projections, rather than cortico-amygdala projections, mediate neuronal plasticity in the lateral amygdala (Quirk et al., 1997). Although spike-firing changes with conditioning are only correlative, it is difficult to determine if they represent fear memories or are consequent to changes in fear and arousal engendered by auditory CSs. A recent study, however, indicates that learning-related changes in the amygdala are independent of conditioned freezing behavior, suggesting that they reflect the associative properties of CSs paired with shock (Goosens et al., 2003). Electrophysiological plasticity also develops in both the auditory thalamus (Weinberger et al., 1972; Supple and Kapp, 1989; Edeline and Weinberger, 1991a,b; McEchron et al., 1996), and the auditory cortex (Edeline et al., 1993; Edeline and Weinberger, 1993; Weinberger, 1995) after auditory fear conditioning. The latency of CS-elicited plasticity in the lateral amygdala is not consistent with transmission of plasticity from the cortex (Quirk et al., 1997; Maren, 2000a); however, transmission of plasticity from the auditory thalamus cannot be ruled out (Weinberger and Bakin, 1998; Cahill et al., 1999). Although thalamic plasticity might modulate the memory formation in the amygdala during fear conditioning (Apergis-Schoute et al., 2005; Parsons et al., 2006), evidence suggests that cellular activity in the amygdala is necessary for both fear conditioning and auditory thalamus plasticity (Maren et al., 2001).

As mentioned earlier, the hippocampus is also involved in conditioned freezing under some conditions. In particular, there is extensive evidence that the hippocampus is involved with encoding the contexts in which aversive stimuli occur (Maren et al., 1998; Anagnostaras et al., 2001; O'Reilly and Rudy, 2001; Sanders et al., 2003). Several investigators have found that lesions of the hippocampus produce rather selective deficits for the acquisition of fear to contextual stimuli, as opposed to discrete CS (Sutherland and McDonald, 1990; Selden et al., 1991; Phillips and LeDoux, 1992, 1994, 1995; Kim et al., 1993a). However, others have not found deficits in the acquisition of contextual fear conditioning after hippocampal damage (Maren et al., 1997; Cho et al., 1999). This has led to the suggestion that contextual fear can be mediated by discrete stimuli within the conditioning chamber (such as the smell of the chamber) (Maren et al., 1997; Rudy and O'Reilly, 1999; Rudy et al., 2002). Interestingly, the amount of training appears to be critical for obtaining deficits in the acquisition of contextual fear insofar as deficits are obtained with limited, but not more extensive, training (Wiltgen et al., 2006). Hippocampal lesions also impair the consolidation of contextual fear memory when made within a month of fear conditioning (Kim and Fanselow, 1992; Maren et al., 1997; Anagnostaras et al., 1999). Although dorsal hippocampal lesions tend to spare auditory fear conditioning, neurotoxic lesions that include the subiculum or ventral hippocampus produce deficits in auditory fear conditioning in many cases (Maren, 1999c; Maren et al., 1997; Richmond et al., 1999; Maren and Holt, 2004). Nonetheless, considerable evidence indicates that contextual and auditory fear conditioning is mediated, at least in part, by dissociable neural systems (Rudy et al., 1999; Venton et al., 2006).

In addition to its role in encoding contextual representations, the hippocampus is involved in contextual memory retrieval (Holt and Maren, 1999; Maren and Holt, 2000; Maren, 2005a). Although the hippocampus is not necessary to retrieve context memories *per se* (e.g., Kim and Fanselow, 1992), it is necessary for using contextual information to retrieve the meaning of an ambiguous CS. For instance, in two related paradigms, latent inhibition and extinction, animals are exposed to a phase of CS-alone presentations either before (in the case of latent inhibition) or after (in the case of extinction) fear conditioning. With these procedures, the CS acquires two different meanings: it predicts a fearful US in the

conditioning phase but does not predict the US during the latent inhibition or extinction phase of training. Importantly, considerable work indicates that both latent inhibition and extinction are context dependent, and contexts are used to inform the animal what a CS means in a particular context (Bouton, 1993). Lesion or reversible inactivation of the dorsal or ventral hippocampus disrupt the context dependence of latent inhibition and extinction and also disrupt the context dependence of lateral amygdala spike firing after extinction (Holt and Maren, 1999; Corcoran and Maren, 2001, 2004; Corcoran et al., 2005; Ji and Maren, 2005; Bouton et al., 2006; Hobin et al., 2006; Maren and Chang, 2006). Based on this work, it appears that the amygdala is involved in associating CSs and USs, and the hippocampus encodes contextual representations and uses those representations to tag CS–US associations to enable their retrieval under conditions in which the CS memory has become ambiguous.

In addition to the hippocampus, considerable attention has been directed at the role of the prefrontal cortex in the extinction of fear conditioning (Quirk et al., 2006). It has been reported that lesions or pharmacological manipulations in the prefrontal cortex impede the recall of extinction without effecting extinction *per se* (Morgan et al., 1993; Quirk et al., 2000; Hugues et al., 2006; Sierra-Mercado et al., 2006). Moreover, prefrontal neurons increase their activity to CSs that have undergone extinction (Milad and Quirk, 2002), an effect that may be regulated by the amygdala (Garcia et al., 1999). Despite the explosion of interest in prefrontal cortical contributions to fear extinction, not all investigators report that prefrontal cortical lesions influence the extinction of conditional fear (Gewirtz et al., 1998; Garcia et al., 2006). Additional work is required to fully understand the contribution of the hippocampus, prefrontal cortex, and amygdala to fear extinction.

3.24.5.2 Conditioned Suppression of Appetitive Responding

As noted earlier, the amygdala has a critical role in the acquisition and expression of Pavlovian fear conditioning as indexed by conditioned freezing. Insofar as it has been argued that conditioned fear contributes to conditioned suppression of appetitive responding (Estes and Skinner, 1941), one would expect a similarly important role for the amygdala in this form of responding. Consistent with this possibility, Kellicutt and Schwartzbaum demonstrated a critical role for the amygdala in the acquisition of a conditioned emotional response, which they assessed by measuring bar-press suppression to an auditory CS previously paired with shock (Kellicutt and Schwartzbaum, 1963). This study used large lesions of the amygdala that were not specific to particular nuclei, but nonetheless it was the first to establish that fear learning requires the amygdala. More recent studies have also found that large lesions of the amygdala or electrical stimulation of the amygdala disrupts conditioned bar-press suppression (Lidsky et al., 1970; Spevack et al., 1975; Kopchia et al., 1992; Mintz and Wang-Ninio, 2001). Importantly, fiber-sparing excitotoxic lesions of amygdaloid nuclei also disrupt the acquisition of conditioned suppression to auditory CSs, such as lick suppression (Selden et al., 1991).

Subtotal lesions of the amygdala, focused on either the basolateral complex or the central nucleus of the amygdala, have also been reported to produce deficits in the acquisition of bar-press suppression (Killcross et al., 1997). In an unconventional paradigm, rats initiated delivery of two different CSs on each of two bars (one bar yielded a CS+ that signaled footshock, and the other bar yielded a CS− that did not signal shock). The paradigm was designed to yield measures of both instrumental avoidance (preferential pressing on CS− bar) and conditioned suppression (reduction in appetitive responding on trials in which the animal delivered a CS+). In this paradigm, both types of lesion impaired conditioned suppression early in training, but ultimately animals with basolateral complex lesions acquired the response. In contrast, animals with basolateral lesions were unable to acquire instrumental avoidance, whereas rats with central nucleus lesions were unimpaired on this measure of aversive conditioning. Based on these results, it has been argued that there is a functional dissociation between the central nucleus and basolateral complex of the amygdala, with the central nucleus mediating Pavlovian conditioned aversive states required for appetitive suppression and the basolateral complex mediating specific sensory representations of biologically relevant outcomes required for instrumental choice (Blundell et al., 2001; Cardinal et al., 2002; Balleine et al., 2003; Balleine and Killcross, 2006).

However, as we have seen, there are considerable data supporting a role for both the central and basolateral nuclei in the acquisition and expression of

Pavlovian conditioned freezing. These data argue that a serial circuit in the amygdala underlies fear memory. A critical factor in revealing dissociations between the central nucleus and the basolateral complex in aversive conditioning may be the extent of training. For instance, deficits in conditioned suppression are equally robust early in training in rats with central or basolateral complex lesions but recover with additional training in some cases (Killcross et al., 1997; Lee et al., 2005). Moreover, as mentioned earlier, deficits in conditioned freezing in rats with basolateral complex lesions, but not central nucleus lesions, recover with additional training (Zimmerman et al., 2005). Hence, these amygdaloid nuclei may operate cooperatively and in a serial manner early in fear conditioning, but functionally dissociate after more extensive training. It remains to be determined how central or basolateral complex lesions affect the expression of conditioned suppression when made after extensive training.

The potent influence of amygdala lesions on the acquisition of conditioned suppression is not a general effect of damage to limbic system structures. For example, electrolytic or excitotoxic lesions of the hippocampus do not impair the acquisition of conditioned bar-press suppression (Wilson et al., 1995; Frohardt et al., 2000; Talk et al., 2002) or lick suppression (Selden et al., 1991) to auditory CSs. Lesions of the septo-hippocampal cholinergic system do not themselves affect the acquisition of conditioned suppression but may alter the competition between discrete CSs and contextual cues in gaining control of behavior (McAlonan et al., 1995; Calandreau et al., 2006). Interestingly, damage to the dorsal noradrenergic bundle, which is one of the major forebrain sources of the catecholaminergic neurotransmitter, norepinephrine, impairs the acquisition of conditioned suppression (Cole and Robbins, 1987). An important issue is whether deficits in bar-press suppression with any neural intervention are secondary to disruptions in conditioned freezing behavior, for example. That is, rats may suppress appetitive responding in the presence of a fear CS because that CS elicits freezing behavior, which competes with licking for water or pressing for food. However, there is evidence that bar-press suppression can occur in rats that otherwise do not show conditioned freezing behavior. Rats with lesions of the midbrain periaqueductal gray, which completely eliminate the expression of conditioned freezing responses (De Oca et al., 1998), while sparing other indices of conditional fear, such as increases in arterial pressure (LeDoux et al., 1988), do not effect bar-press suppression (Amorapanth et al., 1999). This suggests that fear-induced suppression of appetitive responding is not dependent on conditioned freezing in all circumstances.

Relatively little work has been done on the neurophysiological correlates of conditioned suppression. However, a recent study in rats has revealed that auditory CSs paired with footshock produced changes in CS-elicited firing in the dorsal portion of the lateral amygdala (Repa et al., 2001). Interestingly, in some lateral nucleus neurons, these changes occurred before the appearance of conditioned suppression, suggesting that neuronal changes in the amygdala precede appearance of the behavioral CR. In ventral regions of the lateral nucleus, another population of cells was slower to exhibit learning-related changes but showed persistent learning-related activity, even after the behavioral fear response had been extinguished. The development of learning-related changes in amygdala spike firing during the acquisition of conditioned lick suppression has also been shown in monkeys (Rolls, 2000; Paton et al., 2006).

3.24.5.3 Conditioned Hypoalgesia

In addition to conditioned freezing, considerable work has examined the consequences of emotional learning and memory for pain sensitivity. As first described by Bolles and Fanselow (Bolles and Fanselow, 1982), fear has an important role in modulating endogenous opiate levels, thereby influencing pain sensitivity. Hypoalgesia after fear conditioning has been demonstrated in numerous studies (Fanselow and Bolles, 1979), and several investigators have explored the neural mechanisms underlying this effect. As with conditioned freezing, amygdala lesions or pharmacological manipulations prevent the acquisition and expression of conditioned hypoalgesia to contexts that have been paired with footshock (Helmstetter, 1992b; Helmstetter, 1993; Helmstetter and Bellgowan, 1993; Watkins et al., 1993; Good and Westbrook, 1995; Harris and Westbrook, 1995). Also like freezing, the periaqueductal gray is the primary descending target of the amygdala that is required for the expression of conditioned hypoalgesia (Helmstetter and Landeira Fernandez, 1990; Helmstetter and Tershner, 1994; Harris and Westbrook, 1995; Bellgowan and

Helmstetter, 1998; Helmstetter et al., 1998; Tershner and Helmstetter, 2000).

3.24.5.4 Fear-Potentiated Acoustic Startle

Another important model system for analyzing the neural mechanisms of Pavlovian fear conditioning is the fear-potentiated acoustic startle paradigm (Davis, 1992, 2006; Davis and Whalen, 2001). Similar to conditioned freezing, lesions placed in the amygdala, including excitotoxic lesions in the basolateral complex or central nucleus, produce severe impairments in the acquisition and expression of fear-potentiated startle to a visual CS in rats (Hitchcock and Davis, 1986; Sananes and Davis, 1992; Kim and Davis, 1993; Campeau and Davis, 1995b; Lee et al., 1996). Similarly, pharmacological inactivation of either the central nucleus or basolateral complex of the amygdala prevents the acquisition and expression of fear-potentiated startle (Kim et al., 1993b; Walker and Davis, 1997a; Walker et al., 2005). Both thalamic and cortical afferents of the amygdala transmit sensory information to the amygdala for the acquisition and expression of potentiated startle (Rosen et al., 1992; Campeau and Davis, 1995a; Shi and Davis, 1999, 2001). Extensive pharmacological evidence indicates that synaptic plasticity in the amygdala contributes to both the acquisition and extinction of fear-potentiated startle (Miserendino et al., 1990; Falls et al., 1992; Gewirtz and Davis, 1997; Walker and Davis, 2000; Lu et al., 2001; Josselyn et al., 2001; Lin et al., 2001, 2003a,b; Walker et al., 2002; Chhatwal et al., 2006).

In addition to learning-induced potentiation of acoustic startle, ambient illumination (bright light) can lead to nonassociative increases in acoustic startle (Davis, 1998). Unconditioned increases in potentiated startle also involve the amygdala, but interestingly there is a double dissociation in the circuitry for conditioned and unconditioned potentiated startle. Inactivation of the central nucleus of the amygdala affects conditioned increases in startle without influencing unconditioned increases in startle, whereas inactivation of the bed nucleus of the stria terminalis (which receives input from the amygdala) produces the converse pattern of results (Walker and Davis, 1997a). Inactivation of the basolateral complex of the amygdala influences both conditioned and unconditioned potentiated startle.

Although fear-potentiated startle is a widely used measure of fear conditioning, it displays some properties that differentiate it from other indices of conditioned fear. Unlike many of the other measures of conditioned fear, the magnitude of fear-potentiated startle decreases with increases in US magnitude. This decrease in the amplitude of the acoustic startle response is not predicted by formal models of learning and appears to be due to competition with freezing behavior. That is, lesions of the periaqueductal gray that eliminate freezing behavior permit the expression of potentiated acoustic startle by CSs trained with high US intensities (Walker et al., 1997; Walker and Davis, 1997b). Another factor that differentiates the acoustic startle response from other measures of fear is the timing of the conditioned response relative to the CS. Whereas freezing or hypoalgesic responses are tonic and expressed for minutes after the delivery of a brief CS, acoustic startle is only potentiated within a narrow time window that envelopes the expected time of US delivery (Davis et al., 1989; Burman and Gewirtz, 2004). These factors prove valuable for the analysis of temporal relationships that modulate fear expression but also suggest that the neural network involved in the expression of fear-potentiated startle is quite different from that involved in the expression of other fear responses.

3.24.5.5 Cardiovascular Conditioned Responses

In addition to somatic responses, learned fear is associated with the expression of many autonomic and hormonal responses. The most extensively studied autonomic correlates of fear conditioning are changes in heart rate and blood pressure to fearful CSs. Kapp was one of the first to systematically examine the neural basis of heart rate CRs in rabbits during Pavlovian fear conditioning. He found that the central nucleus was critical for the acquisition and expression of heart rate CRs (bradycardia in this case), but not unconditioned responses to either the auditory CS or periorbital shock US (Kapp et al., 1979). Considerable electrophysiological work has revealed that single neurons in the central nucleus exhibit plasticity during the acquisition of heart rate conditioning (Applegate et al., 1982; Pascoe and Kapp, 1985a,b). Subsequent work has shown that cell-specific lesion of the central nucleus, and the auditory afferent areas in the thalamus, also disrupt the acquisition and expression of heart rate conditioning in rabbits (Gentile et al., 1986; Jarrell et al., 1986a,b; McCabe et al., 1992, 1993). Foreshadowing recent work implicating the prefrontal cortex in fear

CRs, there is considerable evidence suggesting a role for prefrontal and cingulate cortices in the acquisition of conditioned bradycardia in rabbits (Buchanan and Powell, 1982; Powell, 1992; Powell et al., 1994).

Another brain structure that has been implicated in cardiovascular conditioning in rabbits is the cerebellar vermis, including the medial cerebellar cortex (Supple and Leaton, 1990a,b; Supple and Kapp, 1994). This finding is somewhat surprising because although the cerebellum has an established role in Pavlovian conditioning of discrete motor responses (Christian and Thompson, 2003), it has typically not been implicated in emotional CRs (Lavond et al., 1984; Lee and Kim, 2004). Most of these studies, however, have focused on the lateral cerebellar cortex and its projections to the interpositus nucleus. Moreover, previous studies in rats had indicated a role for the cerebellar vermis in unconditioned fear reactions but found little evidence of the vermis in conditioned fear (Supple et al., 1987, 1988). However, recent work suggests the vermis may have a role in conditioned freezing in rats (Sacchetti et al., 2002, 2005). This raises the possibility that the vermis has a role not only in the cardiovascular components of learned fear responses but also in other somatic fear CRs.

An important role for the auditory thalamus and amygdala has also been observed for heart rate and arterial pressure CRs in rats (LeDoux et al., 1984, 1986; Iwata et al., 1986a; Sananes and Campbell, 1989). In this case, both the lateral and central nuclei of the amygdala are critical for hear rate conditioning (LeDoux et al., 1990; Romanski and LeDoux, 1992). The projections from the amygdala that are involved in the expression of heart rate CRs are distinct from those involved in the other fear CRs that have been discussed. The expression of cardiovascular CRs involves both the lateral and peri-fornical regions of the hypothalamus (Iwata et al., 1986b; LeDoux et al., 1988; Furlong and Carrive, 2007).

3.24.5.6 Social Defeat and Social Transmission of Fear

Laboratory studies of fear conditioning using artificial stimuli under rigid parametric control are highly useful for analyzing the neural substrates of emotional learning and memory. Fear conditioning, of course, is a form of learning with relevance to an animal's niche and is the product of interactions with members of other species (e.g., predators) and even members of the same species (e.g., aggressive conspecifics). For example, in Syrian hamsters social defeat by a resident, dominant conspecific has been shown to yield later submissive behavior in the defeated individual that has some similarities with conditioned fear (Potegal et al., 1993). Interestingly, pharmacological inactivation of the amygdala in the subordinate animal during the aggressive encounter impairs the development of fear-related submissive behavior (Jasnow and Huhman, 2001; Jasnow et al., 2004a,b). In addition, augmenting molecular pathways that foster amygdala plasticity facilitate the development of submissive behavior that follows social defeat (Jasnow et al., 2005). Although the neurobiological analysis of this form of social fear conditioning is relatively young, it is interesting that it too requires the amygdala.

Conditioned fear is not only the product of certain social interactions but is also the source itself for generating fear in conspecific animals that have not themselves experienced aversive stimuli. There are multiple routes by which fear in one individual might be communicated to another. In rodents, olfactory stimuli and ultrasonic vocalizations are potent in this regard (Blanchard and Blanchard, 1989). Lesions of the amygdala, but not hippocampus, prevent the acquisition of fear-conditioned ultrasonic vocalizations (Koo et al., 2004; Lee and Kim, 2004). Moreover, both conditioned and unconditioned vocalization after discharges are sensitive to central amygdala damage (Borszcz and Leaton, 2003). The fact that conditional fear-induced vocalizations depend on the amygdala is not surprising insofar as all the Pavlovian fear CRs we have discussed depend on the amygdala.

However, an interesting new discovery is that the amygdala of a naïve observer appears to be engaged by the fearful behavior of a conspecific that has undergone an aversive fear-conditioning procedure (Knapska et al., 2006a). In this case, molecular markers of cellular activity (c-fos expression) were upregulated in several amygdaloid nuclei of rats merely exposed to a cage-mate that had undergone fear conditioning, even though the observers themselves never experienced the aversive conditioning procedure. This is similar to the activation of the human amygdala that has been observed by verbal warnings of potential fear experiences without actual presentations of an aversive stimulus (Phelps et al., 2001). Interestingly, subsequent emotional learning and memory in the observer rats, which was assessed in a shock-motivated shuttle avoidance task, was facilitated. This suggests that a brief social interaction

with a cage-mate that undergoes an aversive learning experience promotes aversive learning in an otherwise naïve animal. Apparently, fear conditioning has an important role in promoting adaptive defensive behavior in both the individual experiencing an aversive event, as well as others that are in proximity to the affected individual. In both cases, amygdala activity appears essential.

3.24.6 Conclusions

Animal models have proved incredibly informative for understanding the neural basis of emotional learning and memory. In fact, animal work has provided the groundwork for understanding the neural systems underlying emotional memory in humans. Consistent with the results that have emerged from animal studies, several investigators have now revealed an important role for the human amygdala in fear conditioning (Davidson and Irwin, 1999). Patients with amygdala pathology do not exhibit Pavlovian fear conditioning to either visual or auditory cues paired with loud noise (Bechara et al., 1995; LaBar et al., 1995), and patients with amygdala damage fail to recognize fear in facial expressions (Adolphs et al., 1995, 1999; Young et al., 1995). Functional neuroimaging has extended these lesion studies by revealing amygdala activation to visual or vocal expressions of fear (Morris et al., 1996; Phillips et al., 1997; Whalen et al., 1998; Whalen et al., 2004) and during Pavlovian fear conditioning (Buchel et al., 1998; LaBar et al., 1998; Morris et al., 1998; Morris and Dolan, 2004). Thus, the neural mechanisms of fear conditioning appear to exhibit homology across several mammalian species.

Of course, it is also important to stress that the amygdala does not encode every aspect of an aversive learning experience. For example, humans with amygdala damage exhibit intact declarative memory for a fear-conditioning experience, despite failing to exhibit conditional fear responses to stimuli paired with loud noise (Bechara et al., 1995). Similarly, rats with amygdala lesions avoid a compartment in which they have received footshock, despite failing to exhibit conditional freezing to the contextual cues associated with shock (Vazdarjanova and McGaugh, 1998). These results indicate that multiple memory systems are engaged during relatively simple learning and memory tasks. Thus, the amygdala operates to encode certain aspects of an emotional event, whereas other brain structures including the hippocampus and prefrontal cortex encode other aspects of the event that together integrate a robust representation of the emotional experience.

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3.25 Hormones and Memory

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3.25.1 Introduction and Scope

Hormones are ubiquitous and important. Even though the topic of hormonal effects is considered somewhat marginal in the study of memory, every experiment described in these volumes (at least in mammals) has been subject to hormonal modulation. Occasionally, these influences were noted and manipulated (*See* Chapters 3.26, 3.27); most often they were not. Hormones usually were not the most important determining factor in memory, which may account for much of the field's ability to overlook them. It is safe to assert that no hormone is secreted primarily for its effects on learning and memory. However, a complete understanding of the learning of any task, as well as the memory that an individual forms, does require an understanding of hormones.

Hormones are chemicals secreted into the bloodstream, which carries them to target tissue. All hormones have multiple effects both on the body and directly on the brain, and they generally act as integrators of bodily functions. Hormones are only effective if there is an appropriate receptor with which they chemically interact. Once this hormone–receptor interaction occurs, it facilitates other chemical reactions that change the physiology of the cell. These alterations in physiology can include which genes are active. To add to the complications, hormones often interact with more than one type of receptor. These receptors are dynamic in that their numbers, and even their presence, can change depending on the amount of the hormone that has been available recently, the presence of other hormones, and the age of the organism. This means that hormones usually act in interaction with other factors, which complicates their study. It also complicates any simple generalization about the effect or role of any given hormone.

This chapter is an overview of this recently burgeoning field. It is not, and indeed it cannot be,

exhaustive because of the breadth and depth of this field. Our primary focus is on ovarian and adrenal steroids, as the bulk of the research has centered on these. There is an emphasis on recent data. We concentrate on mammals not only because of our own expertise but also to be concordant with most of the other chapters in this volume series. Neurohormones, substances that are secreted in the brain and that act on the brain, will not be covered. The review is not aimed at neuroendocrinologists. Rather, the audience is assumed to be investigators and students interested in memory in humans and other mammals – behavioral neuroscientists and psychologists.

3.25.2 Gonadal Steroid Hormones

Clearly, the hormones secreted by the gonads – the testes in males and the ovaries in females – have a primary role in the behavioral aspects and physiology of reproduction. Their effects on learning and memory may be indirect by-products of this role. Although this may be the origin of the effects, the gonadal steroids influence many situations that appear to have little relationship to reproduction.

Although the gonadal steroids are characterized as 'male' (testosterone, androgens) or 'female' (estrogen, progesterone), they are closely related chemicals, as are all of the steroid hormones (**Figure 1**). The steroids are produced from one another, and as a result, steroid-producing glands secrete small amounts of many steroids, in addition to the principal secretions. This further complicates interpretation of steroid effects.

3.25.2.1 Ovarian Steroids

3.25.2.1.1 Effects on cognitive behaviors – animal models

Estrogen and progesterone are the two primary steroid hormones secreted by the ovaries. Of the two, estrogen is by far the most heavily investigated in learning and memory. There appear to be several reasons for this. One is that the secretion of estrogen starts the estrous/menstrual cycles (**Figure 2**), and progesterone is not secreted without estrogen first being present, which gives it a secondary role. Another is that estrogen has been found to increase spine and synaptic density in a portion of hippocampus (see section 3.25.2.1.3.) with or without progesterone. The series of studies by Woolley, McEwen, and colleagues (e.g., Woolley et al., 1990) legitimized the investigation of estrogenic effects on cognitive tasks. Prior to these studies, the role of the estrous cycle and estrogen had only been investigated in active and passive avoidance tasks (reviewed in Dohanich, 2002). Finally, the medical implications of the drop in estrogen, and its replacement, at menopause have stimulated research (see section 3.25.2.1.5).

During the estrous cycle, both estrogen and progesterone rise and peak during the proestrus phase and then drop to low levels during the estrous phase (**Figure 2**). This drop is precipitous, occurring in less than 24 h in rodents, the major animal model for learning and memory. **Table 1** shows some of the mnemonic effects of the phase of the cycle. The effects tend to be subtle, which means that no differences will be found if the task is made easier, for

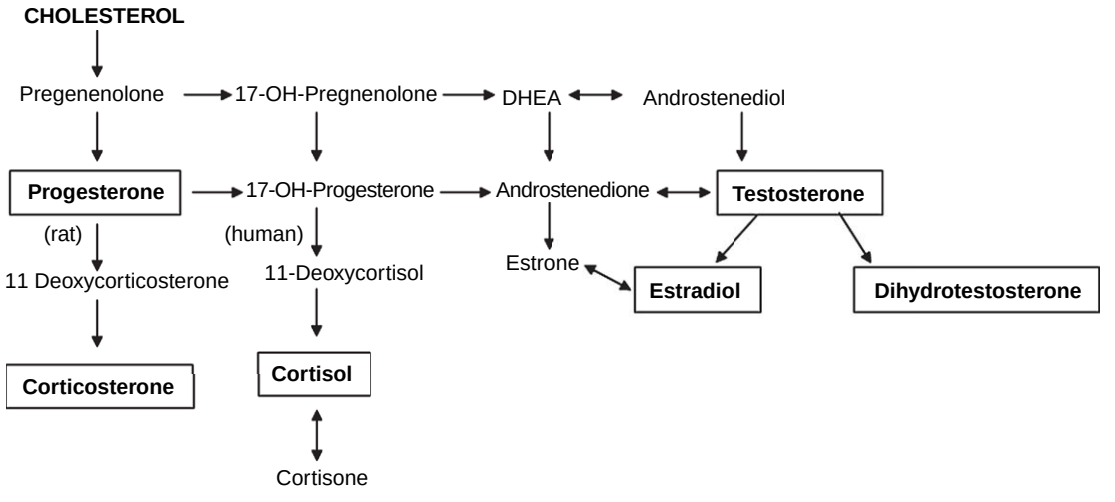

Figure 1 The synthesis pathways of the steroid hormones, illustrating their close interrelationships.

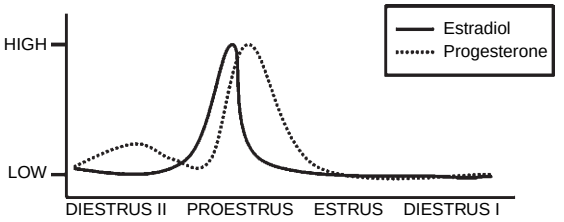

Figure 2 A schematic of the rise and fall of estrogen and progesterone during the 4- to 5-day estrous cycle. The menstrual cycle has the same pattern of rise and fall of hormones, with portions of the cycle temporally extended over 28 days.

example, by using a curtain with cues surrounding the water maze (Berry et al., 1997). Effects also tend to be lost if learning of the task is spread over several cycles (Stackman et al., 1997). Thus the water maze, which can be learned in a single day, has often been employed. The tasks that are facilitated by high hormones (proestrus) or having hormones (intact) defy simple classification. This is a point to which we will return. The studies in **Table 1** illustrate that the cycle of intact female lab rodents would affect the results of learning and memory experiments if this half of the population had been included. The effects of the cycle, which have been investigated by removing the ovaries and then replacing hormones, are due mostly to estrogen itself.

Table 2 shows the results of replacing estrogen (sometimes with progesterone) in females following removal of the ovaries. This can either be done acutely, usually with injections that mimic the cycle, or chronically, with pellets containing estrogen implanted under the skin, which allows the use of tasks that take longer to learn, such as the radial arm maze. In general, the effects are somewhat stronger than in the cycle, perhaps because of the supraphysiological doses that are often employed. Estrogen facilitates the performance of many cognitive tasks, but there are exceptions, and a generalization about what types of tasks are facilitated by estrogen has not been obvious. Some variability is caused by the dose, the pharmacokinetics of the method of replacement, and the length of time between the removal of the ovaries and replacement. The stressfulness of the task is also a factor (see section 3.25.3.5). There are some discrepancies between rats and mice. Still, even for data gathered on rats, there is no simple story.

There have been several attempts at a unifying explanation or construct for the role of estrogen in cognitive behavior. The first explanation, derived from estrogen's effects on hippocampal spines during proestrus and with replacement (Gould et al., 1990; Woolley et al., 1990), was an enhancement of spatial ability (Desmond and Levy, 1997), but this was negated by performance on the water maze, where high levels of estrogen resulted in poorer performance (Warren and Juraska, 1997; Chesler and Juraska, 2000). In a thorough review, Dohanich (2002) articulated the distinction in terms of working and reference memory, with estrogen aiding working memory while mildly hindering reference memory. This accounts for the negative effects on the water maze and on reference memory (unbaited arms) in the radial arm maze and the positive effects on working memory (baited arms) in the radial arm maze (Fader et al., 1999). The working/reference memory distinction did fail to explain the place/response

Table 1 Estrous cycle/intact effects in adult females

Species	*Task*	*Performance*	*Citation*
Rat	Water maze	Diestrus > estrus	Frye, 1995
Rat	Water maze: spatial	Estrus > proestrus	Warren and Juraska, 1997
Rat	Water maze: cued	Proestrus > estrus	Warren and Juraska, 1997
Rat	Water maze	OVX[a] > intact	Daniel et al., 1999
Rat	Radial arm maze	Intact > OVX	Daniel et al., 1999
Rat	Contextual fear conditioning	Estrus > proestrus	Markus and Zecevic, 1997
Rat	Eye blink conditioning	Proestrus > estrus	Shors et al., 1998
Rat	Spontaneous alternation	Proestrus > estrus	Korol et al., 2004
Rat	T-maze: place	Proestrus > estrus	Korol et al., 2004
Rat	Broad cue utilization	Proestrus > estrus	Sava and Markus, 2005
Mouse	Radial arm maze	Intact > OVX	Wilson et al., 1999
Mouse	Water maze	Proestrus > estrus	Frick and Bergner-Sweeney, 2001
Meadow vole	Water maze	High E[b] > low E	Galea et al., 1995

[a]OVX = ovariectomized.
[b]E = estrogen.

Table 2 Estrogen replacement to ovariectomized adult females

Acute replacement (8 days or less)			
Species	*Task*	*Estrogen beneficial?*	*Citation*
Rat	Radial arm maze: delay	Yes	Luine et al., 1998
Rat	Water radial arm maze	Yes	Bimonte and Denenberg, 1999
Rat	Radial arm maze	No	Galea et al., 2001
Rat	Radial arm maze	Yes	Daniel and Dohanich, 2001
Rat	Water maze	No	Chesler and Juraska, 2000
Rat	Contextual fear conditioning	No	Gupta et al., 2001
Rat	Water delayed match to sample	Yes	Sandstrom and Williams, 2001
Rat	Cued win-stay	No	Galea et al., 2001
Rat	Conditioned place preference	No	Galea et al., 2001
Rat	T-maze: place	Yes	Korol and Kolo, 2002
Rat	Eye blink conditioning	Yes	Leuner et al., 2004
mice	Object recognition	Yes	Vaucher et al., 2002
Chronic replacement (more than 8 days)			
Species	*Task*	*Estrogen beneficial?*	*Citation*
Rat	Active avoidance	Yes	Singh et al., 1994
Rat	Radial arm maze	Yes	Daniel et al., 1997
Rat	Radial arm maze	Yes	Luine et al., 1998
Rat	Radial arm maze: baited arms	Yes	Luine et al., 1998
Rat	Radial arm maze: baited arms	Yes	Fader et al., 1999
Rat	Radial arm maze: unbaited arms	No	Fader et al., 1999
Rat	Radial arm maze	LowE > highE	Holmes and Galea, 2002
Rat	Delayed match to position	Yes	Gibbs, 1999
Rat	Cued operant discrimination	Yes	Daniel et al., 2002
Rat	Water maze	No	Daniel and Lee, 2004
Mice	Radial arm maze: baited arms	Yes	Heikkinen et al., 2004

t-maze findings of Korol and Kolo (2002), in which estrogen facilitated place learning, but not response learning, even though both are reference memory. Korol and Kolo (2002) proposed that estrogen predisposed hippocampal (place) strategies. However, the water maze data do not fit into this scheme, especially when the standard reference version of the water maze is impeded by high estrogen (Warren and Juraska, 1997; Chesler and Juraska, 2000), while a working memory version of the maze is facilitated (Sandstrom and Williams, 2001). Further, estrous cycle effects on the reference memory version of the water maze are influenced by task stressfulness (Rubinow et al., 2004; see also section 3.25.3.5). Daniel (2006) has further elaborated the hippocampal view by proposing that estrogen enhances performance on tasks that involve relational representations and a flexible use of memory. This enlarged construct is supported by the author's own work with a cued operant discrimination that is not a spatial task (Daniel et al., 2002), and this view can handle the above paradox in water maze

paradigms. Another interesting proposal is by Sava and Markus (2005), who present evidence that estrogen increases the breadth of cue selection. This formulation can also account for much of the existing data, including the impairing effects of high estrogen on contextual fear conditioning (Markus and Zecevic, 1997). Thus, it is not just the type of task but the amount and placement of cues within the task that influence the effect of estrogen. This formulation also may extend the effects of estrogen beyond the hippocampus into neural areas involved in attention.

3.25.2.1.2 Effects on cognition during aging – animal models

Table 3 lists the studies that examined hormonal effects in females that were middle aged or older. Aging females, until recently rarely tested in the study of aging, have become a focus of attention as a model for the hormonal effects of human menopause. This research area has become important enough that there is a considerable literature on primates. Some primate species have menstrual cycles, but researchers usually do not wait for menopause to occur, rather, removing the ovaries in middle age. The ovaries are also often removed in aging rodents because, although the estrous cycle stops in female rodents in middle age, unlike human menopause, the ovaries continue to secrete estrogen and progesterone in a noncyclic fashion. This has been most thoroughly documented in rats (reviewed in Warren and Juraska, 2000), and two estropausal states have been described. One, termed constant estrus, is characterized by moderate amounts of estrogen and progesterone secretion. The other is constant diestrus (sometimes called pseudopregnancy), in which progesterone secretion is high, but estrogen is moderate to low. We have verified the hormonal bases of these stages in our own work on aged females (Markham and Juraska, 2002).

As can be seen in Table 3, replacement estrogen is often beneficial for the performance of cognitive tasks in aging females across the species that have been examined. The question in this part of the literature is less on how to characterize what type of tasks estrogen benefits and more on the parameters of the replacement that are most beneficial: chronic or cyclic administration, the length of time after the loss of estrogen during which replacement is still beneficial, the type of estrogen that is optimal, and the role of progesterone. All of these issues are under

Table 3 Effects of estrogen in females from middle through old age

Species	*Task*	*Better performance*	*Citation*
Rat	Water maze	Constant estrus > constant diestrus	Warren and Juraska, 2000
Rat	Water maze	Chronic E, acute E, acute E + P > OVX[a]	Markham et al., 2002
Rat	Working memory water maze	Cyclic E or intact > chronic E	Markowska and Savonenko, 2002
Rat	Water maze	Low chronic or cyclic E > OVX or high chronic E	Bimonte-Nelson et al., 2006
Rat	Delayed match to position	Cyclic E + P > chronic E or OVX	Gibbs, 2000
Rat	Delayed alternation	Cyclic E or intact > chronic E	Markowska and Savonenko, 2002
Rat	Inhibitory avoidance	OVX > high E	Foster et al., 2003
Rat	Water radial arm maze	OVX > chronic E	Bimonte-Nelson et al., 2003a
Rat	Radial arm maze: acquisition and delay	Immediate chronic E > delayed E	Daniel et al., 2006
Mouse	Object recognition	Chronic E > OVX	Fernandez and Frick, 2004
Mouse	Object recognition	Chronic E > OVX	Vaucher et al., 2002
Mouse	Radial arm maze: unbaited arms	Chronic E > OVX	Heikkinen et al., 2004
Mouse	Radial arm maze: baited arms	No effect	Heikkinen et al., 2004
Mouse	Water radial arm maze	Chronic E or OVX > cyclic E	Gresack and Frick, 2006
Mouse	Spatial recognition	Chronic E or OVX > cyclic E	Gresack and Frick, 2006
Primate	Delayed spatial recognition	Chronic E > OVX	Lacreuse et al, 2002
Primate	Delayed response	Cyclic E > OVX	Rapp et al., 2003
Primate	Delayed response	Chronic E > OVX	Tinkler and Voytko, 2005
Primate	Visuospatial attention	Chronic E > OVX	Tinkler and Voytko, 2005

[a]OVX = ovariectomized.

investigation, and none are considered settled. There is some evidence that progesterone tends to impede the beneficial effects of estrogen in aged rats, both in intact females in different estropausal states (Warren and Juraska, 2000) and when given with estrogen following ovariectomy (Bimonte-Nelson et al., 2006), but more work is warranted.

It is important to note that the effects of estrogen in adult females of reproductive age are often not the same as the effects during aging. Comparisons between ages are especially powerful if the studies were run by the same investigator with parameters held as constant as possible. We have found that adult females showed a small deficit in the acquisition of the water maze when estrogen and progesterone were high both at proestrus and when replaced following ovariectomy (Warren and Juraska, 1997; Chesler and Juraska, 2000), but estrogen replacement in aged rats, with or without progesterone, facilitated performance on the same task (Markham et al., 2002). Likewise, Bimonte and Denenberg (1999) found that estrogen facilitated performance on the water radial arm maze in adult females, whereas Bimonte-Nelson et al. (2003a) found that both endogenous and exogenous estrogen impeded performance on the task in aged females. There also is evidence for changes in the subcellular localization of estrogen receptors in the hippocampus of the aged female rat (Adams et al., 2004). These examples indicate that young adult female rats cannot serve as a model for aging.

3.25.2.1.3 Neural mechanisms

The inquiry into the role of estrogen in cognition became a major field after Woolley et al. (1990) found that dendritic spines increased on a portion of the dendritic tree of CA1 pyramidal neurons during proestrus (high estrogen and progesterone), and Gould et al. (1990) found the same spine changes after estrogen replacement. The increases were large (20–30%), happened over the course of 3–4 days, and were subsequently seen in the density of synapses in the region (Woolley and McEwen, 1992).

How does high estrogen translate into new spines that may affect the processing of the hippocampus, and thus behavior? Estrogen has several paths to influencing neuronal functions (**Figure 3**). Most of its actions are through two known receptor subtypes, estrogen receptor (ER) α and β, that are widely distributed in the nervous system – including areas that are involved in cognitive behavior. Most receptors are in the cell and act by altering gene expression. In addition, there are ERs in the neuronal membrane that are influenced quickly by estrogen (**Figure 3**) (reviewed by Boulware and Mermelstein, 2005). ERα has been found in dendritic spines in hippocampal CA1 (Milner et al., 2001). Estrogen is also known to increase glutamate *N*-methyl-D-aspartate (NMDA) receptors in CA1 (Weiland, 1992). Interestingly, there are several ties to cholinergic input, including a study finding that a muscarinic receptor antagonist placed in the medial septum blocked the estrogen-mediated rise in NMDA receptors in the hippocampus and the improvement on the radial arm maze (Daniel et al., 2006). In addition, in cell culture estrogen decreases the inhibitory transmitter GABA, and treatment with a blocker of GABA synthesis mimics estrogenic increases in dendritic spines on hippocampal CA1 neurons (Murphy et al., 1998). Further details of intracellular signaling are reviewed by Woolley (2007). Less directly, estrogen levels modulate growth factors in the hippocampus such as BDNF (Gibbs, 1998).

All of this makes a compelling story for the interrelationship between estrogen, hippocampal dendritic spines, and behavior. It is impossible to deny that the

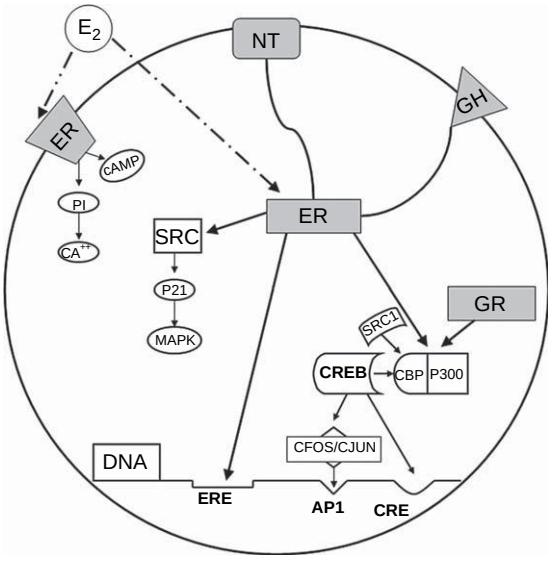

Figure 3 The intracellular mechanisms for estrogen (E_2) interactions with the estrogen receptor (ER). These interactions include several proteins that are second messengers such as cAMP, PI, SRC, and CREB (which also is influenced by the glucocorticoid receptor [GR]). Ultimately, response elements on the DNA are activated. Neurotransmitter receptors (NT) and growth hormone receptors (GH) interact with the estrogen receptor. Adapted from David Rubinow, 2006, with permission.

hippocampus is playing an important role in the effects of estrogen on behavior. However, there has been far less work on estrogenic effects on other important neural areas, such as the cerebral cortex, that may complicate easy correlations. Also, there are some dissociations between estrogen-induced spine increases and behavioral effects. One comes from our own laboratory, in which we have consistently failed to find increases in spine density at proestrus in the hippocampus (Warren et al., 1996; Markham et al., 2005). We should add that we handle all of the rats in our experiments extensively, which interferes with estrogen-induced spine increases (Garza-Meilandt et al., 2002, 2006), as does behavioral training (Frick, et al., 2004). In spite of this, we find increased long-term potentiation (LTP) (presumably NMDA dependent) during proestrus (Warren et al., 1995) and estrogenic influences on behavior (e.g., Warren and Juraska, 1997). There also is a dissociation between increases in dendritic spines and NMDA receptors in aging. Adams et al. (2001) found that, unlike young female rats, aged females do not have increased hippocampal spines when given estrogen, but they do have an increase in NMDA receptors. It is possible that estrogenic influences on NMDA receptors, as often reflected in spines, are more important for behavior than the spines themselves. It is also reasonable to assume that other neural areas, in addition to the hippocampus, are involved in the behavioral response to estrogen. Recent evidence that estrogen increases spines in the prefrontal cortex of aged female rhesus macaques (Hao et al., 2006) underscores this possibility.

3.25.2.1.4 Nonmnemonic effects

Given that estrogen plays a critical role in reproduction and a modulatory role in cognition, it is not surprising that it influences other aspects of behavior as well. It has long been known that estrogen increases activity in rodents, and much of its influence on simple tasks such as active and passive avoidance appears partially caused by this (reviewed by Beatty, 1979). The confound with activity would seem to be controlled to some degree in tasks in which a good deal of activity is called for, such as the radial arm maze and the water maze. Indeed, swimming speed in the water maze does not appear to be influenced by estrogen in any of the studies that have measured it.

Estrogen may interact with several motivational factors. Estrogen does influence pain perception, but the direction of influence varies considerably between inbred and outbred strains of both rats and mice, so no simple generalization is possible (Mogil et al., 2000). Thus the use of several strains in a task serves as converging evidence. Food motivation can also be influenced by estrogen, as estrogen decreases food intake and weight (Wade, 1975). It is not understood how this may interact with various learning tasks. Again, the convergence of tasks using food and nonfood motivation is critical. Another potential confound is the stressfulness of a task because of interactions between stress hormones (especially the corticosteroids) and the gonadal steroids. This is covered in section 3.25.3.

3.25.2.1.5 Humans

The naturally occurring menstrual cycle affects cognitive behavior, as does exogenous estrogen, which is often given to postmenopausal women. The generalization has been made that estrogen facilitates tasks on which females have an advantage over males (e.g., verbal tasks) and impedes tasks on which males have the advantage (e.g., spatial tasks) (Hampson, 1990a). As more tasks have been tested, this generalization has given way to a more complex view (Janowsky et al., 1998; Maki et al., 2002; Hampson et al., 2005). The differences in the effect of estrogen between young, cycling, and postmenopausal women are not as readily evident as they are between young and old rats, perhaps because women in early postmenopause are not as aged as females in the rat studies. In both cycling and menopausal human populations, estrogen tends to enhance verbal tasks (Hampson, 1990b; Kimura, 1995; Sherwin, 1998; Wolf and Kirschbaum, 2002). Even in nonreplaced postmenopausal women, endogenous estrogen levels have a positive correlation with verbal memory (Hogervorst et al., 2004). There also is work indicating that estrogen facilitates motor skills (Hampson, 1990b; Szekely et al., 1998; Maki et al., 2002) in young women. On the other hand, tests of spatial ability tend to be more variable, with negative correlations between estrogen and spatial ability in cycling (Hampson and Kimura, 1988) and elderly women (Drake et al. 2000), as well as positive effects in cycling (Janowsky et al., 1998) and postmenopausal women (Kimura, 1995). It may be that 'spatial' tasks are not really a unitary category. Adding to the range of effects are studies showing that estrogen inhibits perceptual object priming and a fragmented-object discrimination (Maki et al., 2002; Hampson et al., 2005). It is evident that, like the

nonhuman animal literature, it is difficult to make generalizations about the role of estrogen, and there are examples of failure to find differences (e.g., Gordon and Lee, 1993), presumably because of methodological differences between studies and the relatively modest size of the effects.

It is not known whether estrogen increases the number of dendritic spines in the human hippocampus, as it can in the rat. Certainly the range of tasks influenced by estrogen indicates that more than one neural area is involved. fMRI studies indicate that estrogen alters the pattern of cortical activation (e.g., Shaywitz et al., 1999), implicating a cortical contribution.

Contrary to the many positive effects of estrogen that have been found in naturally cycling and in nonrandomized, postmenopausal estrogen users, the Women's Health Initiative Memory Study found an increase in dementia/mild cognitive impairment when women aged 65 or older were given either estrogen plus progesterone or estrogen alone (Shumaker et al., 2004). However, in a study from Denmark, randomized replacement of both types in women younger than 65 years resulted in less cognitive impairment than controls after 65 years of age (Bagger et al., 2005). This distinction is supported by studies showing that initiation of replacement around the time of menopause aids verbal memory, whereas replacement after 65 years of age does not (reviewed by Maki, 2006). It has been suggested that changes in both the blood–brain barrier and immune system in the aging brain could change the neural response to estrogen (Sohrabji and Bake, 2006).

3.25.2.2 Androgens

The testes are the principal source of androgens, and testosterone is the most abundant androgen secreted. Testosterone binds to the androgen receptor, as does one of its principal metabolites, dihydrotestosterone. Estrogen is another metabolite of testosterone (**Figure 1**), and it acts through the estrogen receptors (see section 3.25.2.1.3). Perhaps because male production of testosterone does not change radically after puberty (relative to the female cycle and menopause), there is far less work on its effects on cognitive behavior or even on its cellular mechanisms. There is a large literature on its role in forming the male brain and behavior during early development of rodents. However, this literature is confounded with a significant problem for any behavior that depends on the cerebral cortex or hippocampus. Neonatal anesthetics (necessary for neonatal castration) result in neuronal death in the rat cortex and hippocampus, and the loss is larger in males than in females (Nunez et al., 1998, 2000; Nunez and Juraska, 2000). Performance in the water maze is also impeded (Nunez et al., 2000). Therefore, only manipulations of testosterone in males that are past puberty are reviewed here.

In adult rats, castration often impairs performance, and testosterone replacement brings performance back to the level of intact controls in several tasks: delayed alternation (Kritzer et al., 2001), object recognition (Ceccarelli et al., 2001), radial arm maze (Daniel et al., 2003), inhibitory avoidance (Edinger and Frye, 2004), and delayed matching to position (Gibbs, 2005). Because testosterone is readily converted to estrogen, it is possible that some of these effects are the result of this conversion, but there is no consensus on this. For example, on similar tasks, Kritzer et al. (2001) found that both testosterone and estrogen reversed the effects of castration on a delayed alternation task, whereas Gibbs (2005) examined a delayed matching-to-sample task and found that testosterone, but not estrogen, brought delayed performance back to controls. Thus, even small differences between tasks can alter whether androgenic or estrogenic effects are responsible.

There is evidence that testosterone can affect several neural areas relevant to cognition. Castration decreases spine density on CA1 hippocampal pyramidal neurons, as does removal of the ovaries in females. Testosterone and one of its androgenic metabolites, dihydrotestosterone (**Figure 1**), reverse the decrease to control male levels, whereas estrogen is without effect in males (Leranth et al., 2003). There is also evidence that both the loss and replacement of testosterone changes markers of acetylcholine levels in the cortex and hippocampus (Nakamura et al., 2002) and dopamine levels in the cortex (Kritzer, 2003).

Little work has been done in aged male rats, but there are indications that testosterone can improve performance on a water radial arm maze to levels better than aged controls and similar to young adult males (Bimonte-Nelson et al., 2003b). Dihydrotestosterone did not have this effect, implying that testosterone was acting through conversion to estrogen. Testosterone did not reverse aging effects on the standard water maze, however (Goudsmit et al., 1990), so broad generalizations about the antiaging effects of testosterone cannot be made.

In human males, the lack of a cycle or natural cessation of secretion makes studies more difficult. Correlational studies across both young men and women indicate that highest spatial and mathematical ability correlated with the low range of testosterone in men but the high range in women (Gouchie and Kimura, 1991; Moffat and Hampson, 1996). The degree to which testosterone is converted to estrogen appears to benefit verbal memory in men (Kampen and Sherwin, 1996). Confirmation of these correlational results occurs in studies where extra testosterone has been administered and has improved performance of visual-spatial tasks in older, but not younger, men (reviewed in Janowsky, 2006). Supplemented older men also show improvements in spatial memory that are not caused by conversion to estrogen, and verbal memory improvements that are the result of increases in estrogen (Janowsky, 2006).

3.25.3 Adrenal Steroids

Glucocorticoids are the steroid hormones produced by the adrenals. The primary adrenal steroid is cortisol in humans and other primates, and its close relative corticosterone in rats (both abbreviated CORT; **Figure 1**). CORT release is regulated by the hypothalamic-pituitary-adrenal axis, which exhibits a circadian rhythm, with plasma CORT peaking around the onset of the active period (morning for humans; prior to dark cycle onset in rats). CORT release is also increased under stress. In addition to multiple actions in the periphery coordinating the body's energetic priorities under stress, CORT readily passes the blood–brain barrier and acts on specific receptors throughout the brain. The well-characterized brain glucocorticoid receptors are classified as mineralocorticoid (MR) or glucocorticoid (GR). As with the estrogen receptor, newer studies have provided evidence for membrane glucocorticoid receptors (Makara and Haller, 2001; Johnson et al., 2005), which likely account for some of the fast actions of CORT. GRs, though most concentrated in hypothalamic and pituitary areas, which regulate the hypothalamic-pituitary-adrenal axis, are ubiquitous in the brain. By contrast MRs – initially characterized by their affinity for aldosterone – are most concentrated in the hippocampus, where they lack aldosterone specificity and exhibit very high affinity for CORT, approximately 10× greater than that of colocolized GRs. Hippocampal MRs are largely occupied at basal levels, whereas GRs are activated only at the circadian peak and under stress (De Kloet et al., 1998). As discussed below (in section 3.25.3.1.1), the dynamic between MR and GR occupation may be one of the critical factors determining the way CORT affects cognitive processes. Several excellent reviews provide more detailed discussion of CORT actions in the brain (e.g., De Kloet et al., 1998).

Literally thousands of studies on humans and experimental animals attest to the potent influence of stress hormones on learning and memory, but the direction of these cognitive effects – enhancing or impairing – depends on several factors, including the subject's age and sex, the timing of stress exposure, brain memory systems involved in a given task, and whether the stressor is acute or chronic. Each of these mediating factors is discussed here. Perhaps a more basic – and a long-acknowledged – variable involves the amount of stress hormones released: How much stress is optimal for enhancing cognition?

3.25.3.1 Acute Exposure: Effect of Amount

This question was addressed as early as 1908, when Yerkes and Dodson published their landmark paper, 'The relation of strength of stimulus to rapidity of habit formation,' in which they reported the effects of utilizing different shock intensities in a discrimination task on the rate of learning in mice (Yerkes and Dodson, 1908). Their results showed that, for moderate and difficult discriminations, shock intensity affected performance according to an inverted-U function, with lowest and highest shock intensities impairing the rate of learning, and intermediate intensities enhancing learning. The suggestion that a moderate level of arousal represents the optimal peak for performance along an inverted-U function has come to be known as the 'Yerkes–Dodson principle.' Glucocorticoids are likely to at least partially underlie this phenomenon, as CORT levels – which certainly represent one measure of arousal – also exhibit an inverted-U relationship to performance, at least for some types of tasks (see below). The same appears true of the other major adrenal stress hormone, epinephrine (Gold and Van Buskirk, 1975). Although our discussion here is focused on glucocorticoids, those studies that examined effects of behavioral stressors, rather than manipulations specific to glucocorticoids, may be presumed to involve adrenal catecholamine as well as glucocorticoid effects (*See* Chapters 3.26, 3.27, for discussion of epinephrine effects on memory).

3.25.3.1.1 Animal models

Studies of experimental animals (Pugh et al., 1997; Sandi and Rose, 1997; Sandi et al., 1997; Conrad et al., 1999) demonstrate nonlinear relationships between CORT levels and memory formation, which typically take the form of an inverted-U function: optimal mnemonic effects are seen with moderate CORT levels, and either no effect or memory impairment is seen at very low and very high CORT levels. In rats, this phenomenon is often seen in studies with hippocampal-dependent tasks (Pugh et al., 1997; Sandi et al., 1997; Conrad et al., 1999). For example, male rats trained in the spatial water maze task with water temperature of 19° C had higher levels of posttraining plasma CORT and exhibited better memory the following day than rats trained in 25° C water. When rats trained at these temperatures were administered posttraining CORT, performance benefits were only seen in the rats trained in warmer water, suggesting that when the rats trained in cold water were given CORT on top of already elevated levels, they were pushed past the peak of CORT's mnemonic effects for this task (Sandi et al., 1997).

Electrophysiological studies likewise demonstrate an inverted-U relationship of CORT levels to hippocampal function, as seen in studies of LTP, long-term depression (LTD), and primed burst potentiation (Diamond et al., 1992; Kerr et al., 1994; Pavlides et al., 1995). LTP studies that manipulated MR and GR binding with selective agonists and antagonists provided strong evidence for the theory that MR occupation accounted for the rising part of the inverted-U curve, and GR occupation for the falling part (Pavlides et al., 1996; Pavlides and McEwen, 1999). However, several behavioral studies that manipulated MR and GR effects suggest a dissociation between the mechanism underlying the inverted-U seen for behavior versus for electrophysiology (e.g., Conrad et al., 1999) and generally implicate GRs rather than MRs in beneficial effects on memory consolidation (Sandi, 1998; Roozendaal, 2000).

3.25.3.1.2 Human studies

Perhaps the most direct evidence for an inverted-U relationship between CORT and human memory comes from a recent study that applied cold-pressor stress (immersion of one hand in ice water) immediately following the reading of a relatively neutral story, and tested memory for the story 1 week later. Among male subjects, individual salivary CORT response to the cold-pressor stress showed a significant quadratic relationship to subsequent recall performance (different results for the female subjects in this study are discussed below in section 3.25.3.5; Andreano and Cahill, 2006).

Less direct evidence from human studies demonstrates the way that seemingly minor endogenous factors can alter CORT effects on cognition. A recent meta-analysis found mixed effects of hydrocortisone administration prior to learning, until the studies were broken down by time of day: The studies that administered hydrocortisone in the afternoon, when basal CORT is relatively low tended to find enhancing effects on subsequent memory, whereas studies that gave hydrocortisone in the morning, near the circadian peak, were generally impairing to memory formation (Het et al., 2005). The same finding was reported in a direct study of time-of-day effects in glucocorticoid administration (Maheu et al., 2005).

The social significance of high stress levels negatively affecting memory is quite broad, including the suggestion that glucocorticoid therapy may be helpful in impairing memory formation following traumatic experience (De Quervain, 2006; Schelling et al., 2006) and the finding that experimentally induced stress increases susceptibility to false recognition memory (Payne et al., 2002).

3.25.3.2 Timing

Studies with experimental animals show that the timing of stress hormone administration in relation to different stages of learning, memory, and performance of a task is another key to whether the effects are beneficial or harmful. Task-associated stress (e.g., lower water maze temperature or higher shock intensity) and CORT manipulations administered prior to learning suggest generally enhancing effects of CORT on learning (Sandi et al., 1997; Cordero et al., 1998; Conrad et al., 1999), at least for male rats performing hippocampal-dependent tasks. Memory consolidation is also enhanced by CORT: Immediate posttraining administration of stress hormones, which eliminate performance effects and are thought to mimic endogenous stress effects on memory consolidation, enhances subsequent performance in male rats (Roozendaal, 2000). However, stress hormones administered prior to retrieval testing or during the delay interval between training and testing impair memory retrieval (De Quervain et al., 1998; Roozendaal, 2002). Although most of these studies also used hippocampal-dependent tasks, numerous studies by Roozendaal, McGaugh, and colleagues have provided elegant

demonstration that both enhancing and impairing glucocorticoid effects on these tasks depend on the modulatory influence of the basolateral amygdala (Roozendaal, 2003; McGaugh, 2004).

Timing of stress exposure is also important in human studies. For example, the meta-analysis cited above that found that pretraining CORT exposure interacted with time of day also looked at several studies that administered CORT prior to retention testing, and in all cases, memory impairment resulted (Het et al., 2005). This makes some intuitive sense, as preretention testing (unlike post- or peritraining) stress would not be expected to influence central processes associated with the task itself.

3.25.3.3 Chronic Effects

Until now, our discussion has examined the effects of acute glucocorticoid exposure. In many ways, chronic exposure is quite different. In rats, monkeys, and other experimental animals, deleterious effects are most often reported in studies of spatial and working memory, mediated by the hippocampus and prefrontal cortex, respectively (see excellent review in Wolf, 2003). Although it is not feasible to administer glucocorticoids long-term in human studies, chronic stress effects on cognition have special relevance for people who have undergone long-term glucocorticoid exposure, either because of a medical condition requiring long-term corticosteroid medication or in people with conditions such as Cushing's syndrome or major depression, both of which are associated with chronic elevations in plasma CORT. Studies of these populations have described cognitive deficits in hippocampal and neocortical function (Forget et al., 2000; Starkman et al., 2001; Bourdeau et al., 2005).

In both humans and rats, chronic stress-related cognitive impairments are paralleled by anatomical changes. In rats, chronic stress effects are best documented in the hippocampus and include dendritic atrophy in area CA3 (Watanabe et al., 1992), decreased neurogenesis in the dentate gyrus (Cameron and Gould, 1994), and sometimes CA3 neurotoxicity (Sapolsky, 2000). Chronic stress also induces dendritic atrophy in the prefrontal cortex (Cook and Wellman, 2004). In humans, volume reductions associated with chronic glucocorticoid exposure (e.g., in major depression and Cushing's disease) are most often seen in the hippocampal formation, but also in the prefrontal cortex (Starkman et al., 1992; Duman et al., 1999; Wolf, 2003). At the other end of the spectrum, long-term glucocorticoid deprivation, as in Addison's disease (Maehlen and Torvik, 1990) or experimental adrenalectomy (Conrad and Roy, 1993), can induce hippocampal dentate granule cell loss and memory deficits.

Chronic stress effects on amygdala structure and function are markedly different from those seen in the hippocampus and prefrontal cortex. Also, cognitive effects of both chronic and acute stress interact potently with sex and ovarian hormones and change with lifespan age. Each of these interacting variables is reviewed in the following sections.

3.25.3.4 Memory System

When deleterious effects of acute stress are seen, they are, as with chronic stress, most often seen in relation to hippocampal or prefrontal function. For example, acute stress can impair or block LTP in both the hippocampus and prefrontal cortex, at levels that may enhance amygdalar LTP (reviewed in Diamond et al., 2004). Furthermore, a study that examined dendritic morphology in both the hippocampus and amygdala following chronic stress found that losses in the hippocampus were accompanied by dendritic growth in the basolateral amygdala (Vyas et al., 2002). Behavioral studies with rats suggest that high levels of stress hormones may also bias the relative use of different memory systems, from hippocampal-dependent strategies toward caudate nucleus–dependent strategies, and this shift appears to be mediated by the basolateral amygdala (Packard and Wingard, 2004).

The dose-dependent effects of acute CORT exposure discussed above (see section 3.25.3.1) also appear to be memory system dependent. The inverted-U function describing acute CORT effects on memory and plasticity is best supported for hippocampal function. By contrast, acute and even chronic CORT can enhance amygdala-dependent memory in a linear fashion (reviewed in Conrad, 2005). In the case of prefrontal function, evidence from human and nonhuman primates suggests a general deleterious effect of acute stress on working memory (reviewed in Wolf, 2003). Thus, glucocorticoid effects on cognition are sensitive to several factors (e.g., amount, timing) in locally specific manners.

3.25.3.5 Sex and Ovarian Steroids

Oddly enough, a growing literature describes quite different and frequently opposite effects of stress in male versus female rats. Luine and colleagues have done extensive research on chronic stressor effects in males and females, and on the modulating effects of ovarian hormones in females (reviewed in Bowman et al., 2006). Generally, they observe that the same chronic stress regimen that is deleterious to spatial memory in male rats is enhancing to female rats. This enhancement is abolished by ovariectomy, suggesting that estrogen has an important role in this striking interaction of sex and stress. Further, chronic stress effects on CA3 dendrites are both attenuated and different (occurring in the basilar rather than the apical tree) in female rats (Galea et al., 1997).

Striking sexual dimorphisms have also been found in studies of acute stress. For example, acute stressors that enhance eyeblink conditioning in male rats are impairing to female rats (Shors, 2004). Endogenous ovarian hormone fluctuations across the estrous cycle interact with acute stress effects in a parallel fashion (Wood et al., 2001; Rubinow et al., 2004). For instance, in a study comparing water maze performance of estrous versus proestrous rats using warm (30° C) or cold (19° C) water temperatures, we found a significant interaction such that overall performance was impaired in proestrous rats in the cold water maze and enhanced in the warm maze, while estrous rats (similar to male rats) showed the opposite pattern (Rubinow et al., 2004).

Human studies of acute stress that have examined the factor of sex also report significant interactions. For example, salivary CORT in response to a psychological stressor showed a strong negative association with word list recall among young men but showed no such relationship among young women (Wolf et al., 2001b). Another recent study found that acute psychological stress enhanced fear conditioning in men but produced no effect or impairment in women. The same study showed different salivary CORT responses in men versus women (Jackson et al., 2006). Finally, in the study discussed above (see section 3.25.3.1.2), which found an inverted-U relationship between cold-pressor salivary CORT response and subsequent recall in men, women showed no clear relationship between salivary CORT and subsequent memory. However, unlike the results of Jackson et al. (2006), this study found similar CORT responses to stress in men and women (Andreano and Cahill, 2006). Further complicating this story are studies showing that sexually dimorphic effects of stress on cognition change across the lifespan.

3.25.3.6 Interactions with Age

In a series of studies on acute stress effects on eyeblink conditioning across the lifespan of male and female rats, Hodes and Shors (2005, 2006) found that sexually dimorphic effects of stress on this task (favoring males) were limited to adulthood. Prior to puberty and in old age, stress exposure produced no effects on conditioning in either sex, whereas during puberty, stress enhanced performance for both males and females. Other laboratories using different stress and task paradigms showed different kinds of alterations in stress effects in old age. For example, chronic stress remains impairing to aged male rats, but the enhancing effects in adult females are lessened in old age (Bowman, 2005).

In humans, Wolf and collaborators found sexually dimorphic effects of CORT on working memory that were reversed in old age: Young men were impaired under CORT, whereas young women were unaffected; among the elderly, aged men were unaffected, but aged women were impaired (Wolf et al., 1998, 2001a).

Taken as a whole, these findings defy easy explanation. Leaving aside the vast and important literature on the lasting effects of developmental stress on cognition, findings that discrete episodes of stress exposure have markedly different effects on cognition across the male and female lifespan further point to the complexity of glucocorticoid effects on cognitive processes.

3.25.4 Other Hormones

The peptide hormones, which are composed of a string of amino acids, are the other major class of hormones besides the steroids. In fact, most mammalian hormones are peptides, and many of them have been shown to influence learning and memory. We highlight those hormones that have been most investigated.

3.25.4.1 Oxytocin and Vasopressin

Oxytocin and vasopressin are structurally similar and secreted from the same subregion, the intermediate zone, of the pituitary gland. They also are secreted as

neurotransmitters in the central nervous system. As demonstrated by intracerebral injections and in gene knockout mice, these hormones can influence many types of learning, including social recognition (Argiolas and Gessa, 1991; Alescio-Lautier et al., 2000; Winslow and Insel, 2004). However, these effects may be part of the role of the peripheral hormones, because neither peptide appears to directly cross the blood–brain barrier, although they can influence the properties of the barrier (Meisenberg and Simmons, 1983). The neurotransmitter actions of these peptides and of cholecystokinin (CCK, below) are not within the purview of this chapter. Peripheral injections of vasopressin and oxytocin often facilitate learning (e.g., Dietrich and Allen, 1997), but several investigators have hypothesized that this occurs through interactions with stress hormones (Sahgal, 1984; McGaugh, 1989). Therefore, a direct role for oxytocin and vasopressin as hormones, as opposed to neurotransmitters, in learning and memory has not been clearly established.

3.25.4.2 Cholecystokinin

Like oxytocin and vasopressin, CCK is released both centrally as a neurotransmitter and peripherally as a hormone. However, peripheral CCK, which is released from the duodenum of the small intestine during eating, does modulate learning and memory. In fact, posttraining eating itself enhances memory consolidation (Huston et al., 1974; Flood et al., 1987). Similar to epinephrine (*See* Chapter 3.26), CCK interacts with its receptors on the vagus nerve, which synapses on the brainstem nucleus of the solitary tract. Both vagotomy (Flood et al., 1987) and lesions of the stria terminalis (Flood et al., 1995), which projects from the nucleus of the solitary tract to the amygdala, abolish CCK effects on memory.

Other gastrointestinal hormones, including bombesin and gastrin-releasing peptide, also enhance memory consolidation, but these effects appear to be mediated by CCK release, as an antagonist for peripheral CCK receptors blocks these effects (Morley et al., 1994).

3.25.5 Summary and Conclusions

Hormone effects on memory function range from mild to potent, but hormonal milieu always plays a role, even when it is not experimentally manipulated. The majority of studies directly examining hormone effects on cognition have looked at ovarian and adrenal steroids.

The effects of estrogen resulting from the rat estrous cycle or when replaced following removal of the ovaries vary from memory enhancement, to impairment, to lack of effect. Attempts at a unifying explanation for such wide-ranging results continue to be refined, but as yet no single construct accounts for all the data. Important factors have been hypothesized to include the degree of hippocampal involvement in a task, whether a task requires working or reference memory, the involvement of relational representations, task stressfulness, and the breadth of cue selection available. Estrogenic effects on cognition are paralleled by anatomical and physiological changes, but behavioral findings are sometimes dissociated from these other measures.

Data on ovarian hormone effects on learning and memory in aged females are also variable but differ in several ways from findings with young adults. Thus, it is important to use aged subjects when modeling aging.

Human studies tend to find positive effects of estrogen on verbal and fine motor tasks, but effects on spatial cognition are more variable across studies. Postmenopausal hormone replacement has been correlated with positive effects, but results from controlled studies suggest that replacement may only be beneficial when initiated close to the onset of menopause and can be harmful if initiated past the age of 65.

Glucocorticoid effects on cognition are powerful but, like estrogen effects, also variable. Glucocorticoid effects can be enhancing or impairing, depending on the amount of acute exposure, and interact with a number of other variables including the timing of exposure in relation to different stages of learning, memory consolidation and recall, whether stress or glucocorticoid exposure is chronic, memory system(s) involved, and both the sex and age of subjects. All of these factors can alter and in some cases reverse the direction of glucocorticoid effects in experimental animals and in humans.

Many other hormones have been investigated with respect to cognition, including testosterone and the peptide hormones oxytocin, vasopressin, and CCK. Mnemonic effects of all these hormones are often positive when they are seen. Nevertheless, the effects of these and other peripheral hormones are also complex and interact with other variables including other hormone systems.

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3.26 Memory Modulation

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3.26.1 Introduction

Brain systems have many tasks to perform in enabling the formation of memories. New information must be encoded and stored in ways that enable the information subsequently to be retrieved and expressed in behavior. Understanding the cellular mechanisms and brain systems responsible for enabling and orchestrating these various complex tasks is the aim of research on the neurobiology of learning and memory. A key role in such orchestration is played by systems that modulate the consolidation of memories of recent experiences. Our memories are not all created equally strong: Some experiences are well remembered, while others are remembered poorly, if at all. Understanding the neurobiological processes and systems that contribute to such differences in the strength of our memories is a special quest of research on memory modulation. Not only do modulatory systems influence neurobiological processes underlying the consolidation of new information, but more recent evidence indicates that these systems also affect other mnemonic processes, including memory extinction, memory recall, and working memory.

Research on memory modulation was stimulated by findings reported a little over half a century ago that, in rats, retention of a recently learned response was impaired by administration of electroconvulsive shock (ECS) (Duncan, 1949; Gerard, 1949). These findings were the first to provide compelling evidence supporting the hypothesis proposed half a century earlier (Müller and Pilzecker, 1900) that neural memory traces activated by new experiences perseverate in a fragile state and gradually become consolidated. Subsequent studies using ECS and other treatments to impair brain functioning shortly after training provided extensive evidence that such treatments impair memory by interfering with time-dependent processes involved in memory consolidation (McGaugh, 1966; McGaugh and Herz, 1972). The findings of such studies also revealed that susceptibility to posttraining modulating influences

is a common feature of animal memory: Posttraining treatments affect memory in mollusks, fish, insects, and birds, as well as rodents and primates (Cherkin, 1969; Agranoff, 1980; Menzel, 1983; Kandel, 2001).

In providing evidence that new memories remain fragile for a while before becoming consolidated, the findings of Duncan and Gerard suggested the possibility that memory consolidation might also be enhanced by stimulating brain functioning. In support of this implication, many studies subsequently reported that, in rats, retention is enhanced by injections of stimulant drugs, including strychnine, the GABAergic antagonists picrotoxin and bicuculline, pentylenetetrazole, and amphetamine, administered shortly after training, and that such treatments are generally ineffective when administered several hours after learning (Breen and McGaugh, 1961; McGaugh and Petrinovich, 1965; McGaugh, 1966, 1973; Doty and Doty, 1966; Evangelista and Izquierdo, 1971; McGaugh and Herz, 1972; Grecksch and Matthies, 1981; Carr and White, 1984; Brioni and McGaugh, 1988; Castellano and Pavone, 1988). Such a time-dependent susceptibility thus clearly suggests that the drugs affected memory by modulating the consolidation of recently acquired information.

In studies of the effects of treatments modulating learning and memory it is essential to distinguish the effects of the treatments on memory from the effects of the treatments on, e.g., attentional, motivational and motor processes that may directly affect the behavior used to make inferences about memory. The use of posttraining treatments to alter brain functioning shortly after training has provided an effective technique for excluding such performance effects in investigating the effects of modulatory treatments on memory consolidation (McGaugh, 1966, 1989; McGaugh and Herz, 1972).

3.26.2 Endogenous Modulation of Consolidation

Memory consolidation appears to be a highly adaptive function because, as noted above, evidence of consolidation is found in a wide variety of animal species. But why do our long-term memories and those of other animals consolidate slowly? There seems to be no *a priori* reason to assume that neurobiological mechanisms are not capable of consolidating memory quickly. Considerable evidence suggests that the slow consolidation of memories may serve a highly important adaptive function by enabling endogenous processes activated by an experience, and thus occurring shortly after the event, to modulate memory strength. In a paper published shortly after those reporting that posttraining drug administration can enhance memory consolidation (e.g., Breen and McGaugh, 1961; McGaugh, 1966), Livingston suggested that stimulation of the limbic system and brainstem reticular formation might promote the storage of recently activated brain events by initiating a "neurohormonal influence (favoring) future repetitions of the same neural activities" (Livingston, 1967, p. 576). Kety subsequently offered the more specific suggestion that adrenergic catecholamines released in emotional states may serve "to reinforce and consolidate new and significant sensory patterns in the neocortex" (Kety, 1972, p. 73). Although the specific details of current findings and theoretical interpretations differ in many ways from those early views offered by Livingston and Kety, recent findings are consistent with their general hypotheses.

3.26.3 Modulating Influences of Adrenal Stress Hormones

Emotionally arousing experiences are generally well remembered (Christianson, 1992; McGaugh, 2003). As William James (1890) noted, "An experience may be so exciting emotionally as to almost leave a scar on the cerebral tissue" (James, 1890, p. 670). The susceptibility of memory consolidation processes to modulating influences induced after learning provides the opportunity for neurobiological processes activated by emotional arousal to regulate the strength of memory traces representing important experiences (McGaugh, 1983; McGaugh and Gold, 1989). Extensive evidence indicates that stress hormones released by the adrenal glands, epinephrine and cortisol (corticosterone in rodents), by emotionally arousing experiences modulate memory consolidation (McGaugh and Roozendaal, 2002). It is well established that, in rats and mice, hormones of the adrenal medulla and adrenal cortex are released during and immediately after stressful stimulation of the kinds used in aversively motivated learning tasks (McCarty and Gold, 1981; McGaugh and Gold, 1989; Aguilar-Valles et al., 2005), and that removal of these stress hormones by adrenalectomy generally results in memory impairment (Borrell et al., 1983, 1984; Oitzl and de Kloet, 1992; Roozendaal et al., 1996b; Roozendaal, 2000).

3.26.3.1 Epinephrine

Gold and van Buskirk (1975, 1978) were the first to report that, in adrenally intact rats, systemic posttraining injections of the adrenomedullary hormone epinephrine enhance long-term retention of inhibitory avoidance. As found in previous studies of the memory-enhancing effects of stimulant drugs, the epinephrine effects were dose dependent and time dependent. Moderate doses of posttraining epinephrine enhanced retention performance, whereas lower doses or higher doses were less effective. Furthermore, as was found with stimulant drugs, memory enhancement was greatest when epinephrine was administered shortly after training (**Figure 1**). Comparable effects were obtained in subsequent experiments using many different types of training tasks commonly used in experiments with rats and mice, including inhibitory avoidance, active avoidance, discrimination learning, and appetitively motivated tasks (Izquierdo and Dias, 1985; Sternberg et al., 1985; Introini-Collison and McGaugh, 1986; Liang et al., 1986; Costa-Miserachs et al., 1994). Additionally, numerous studies have shown that peripherally administered amphetamine, which increases the release of epinephrine from the adrenal medulla, also enhances memory consolidation when given shortly after training (Martinez et al., 1980).

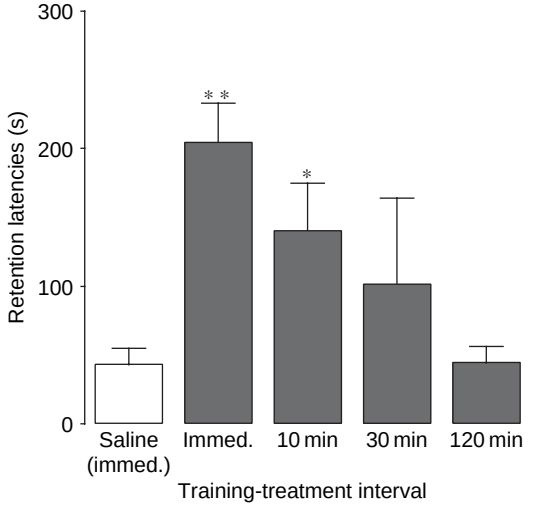

Figure 1 Posttraining systemic injection of epinephrine induces time-dependent memory enhancement. Epinephrine (0.1 mg/kg, ip) enhanced 24-h retention performance on an inhibitory avoidance task when injected either immediately or 10 min after training but was ineffective when given 30 or 120 min after training. Results represent retention latencies (mean + SEM) in seconds. *, $p < .05$; **, $p < .01$ as compared with the saline group. From Gold PE and van Buskirk R (1975) Facilitation of time-dependent memory processes with posttrial epinephrine injections. *Behav. Biol.* 13: 145–153.

Epinephrine effects on memory consolidation appear to be initiated, at least in part, by the activation of β-adrenoceptors located in the periphery, as this hormone does not readily cross the blood–brain barrier (Weil-Malherbe et al., 1959). Sotalol, a β-adrenoceptor antagonist that does not readily enter the brain, blocks the enhancing effects of peripherally administered epinephrine on memory for inhibitory avoidance training (Introini-Collison et al., 1992). Epinephrine effects are most likely mediated by activation of β-adrenoceptors located on vagal afferents that project to the nucleus of the solitary tract (NTS) in the brain stem (Schreurs et al., 1986), which sends noradrenergic projections to forebrain regions involved in memory consolidation, including the amygdala (Ricardo and Koh, 1978). Furthermore, the NTS regulates noradrenergic activity of the forebrain via indirect projections to noradrenergic cell groups in the locus coeruleus (Williams and Clayton, 2001). Vagotomy attenuates the memory-enhancing effects induced by systemic administration of 4-OH-amphetamine, a peripherally acting derivative of amphetamine that induces epinephrine release (Williams and Jensen, 1991). The evidence that inactivation of the NTS with the sodium channel blocker lidocaine prevents epinephrine effects on memory consolidation, as well as the finding that the β-adrenoceptor agonist clenbuterol infused into the NTS posttraining enhances memory, very strongly suggests that epinephrine effects on memory consolidation are mediated via activation of the NTS (Williams and McGaugh, 1993). Additionally, the finding that, in rats as well as human subjects, posttraining electrical stimulation of vagal afferents enhances memory consolidation provides further evidence that projections mediated by the ascending vagus are involved in regulating memory consolidation (Clark et al., 1995, 1999; Ghacibeh et al., 2006).

Thus, the NTS appears to be an interface between peripheral adrenergic activation and brain processes regulating memory consolidation. However, posttraining peripheral administration of β-adrenoceptor agonists that are able to enter the brain, including dipivefrin and clenbuterol, also enhance memory consolidation. The memory enhancement induced by dipivefrin and clenbuterol is blocked by the β-adrenoceptor antagonist propranolol, which readily

enters the brain, but not by the peripherally acting antagonist sotalol (Introini-Collison et al., 1992). Considered together, these findings indicate that the modulatory effects of epinephrine on memory consolidation are initiated by activation of peripheral β-adrenoceptors, but that memory consolidation is also modulated by direct activation of β-adrenoceptors within the brain. As discussed below, noradrenergic activation of the basolateral region of the amygdala (BLA), arising from noradrenergic cell groups in the NTS and locus coeruleus, is critically involved in mediating the effects of epinephrine, as well as those of many other neuromodulatory systems, on memory consolidation (Roozendaal, 2007).

Other findings suggest that epinephrine may also influence memory consolidation by enhancing glycogenolysis in the liver (Messier and White, 1984, 1987; Gold, 1995). Posttraining peripheral administration of glucose produces dose- and time-dependent effects on memory comparable to those produced by epinephrine (Gold, 1986). Additionally, doses of epinephrine and glucose that are optimal for enhancing retention induce comparable levels of plasma glucose (Hall and Gold, 1986). β-Adrenoceptor antagonists do not block glucose effects on memory (Gold et al., 1986). Glucose readily enters the brain and, thus, can directly influence brain glucoreceptors (Oomura et al., 1988). The finding that intracerebroventricular injections of glucose produce dose- and time-dependent enhancement of memory consolidation clearly suggests that peripherally administered glucose may affect memory by directly altering brain functioning (Lee et al., 1988). However, the finding that memory is also influenced by peripherally administered fructose, a sugar that has little influence on the brain, suggests that this sugar, as well as glucose, may also act, at least in part, at peripheral sites in influencing memory (Messier and White, 1987). In support of this view, Talley et al. (2002) showed that vagotomy blocks the memory-enhancing effects of peripherally administered L-glucose, an enantiomer of glucose that does not cross the blood–brain barrier.

3.26.3.2 Glucocorticoids

There is also extensive evidence that adrenocortical hormones are involved in modulating memory consolidation (for reviews, see de Kloet, 1991; Bohus, 1994; McEwen and Sapolsky, 1995; Lupien and McEwen, 1997; Roozendaal, 2000). As with epinephrine, posttraining injections of glucocorticoids produce dose- and time-dependent enhancement of memory (Cottrell and Nakajima, 1977; Sandi and Rose, 1994; Roozendaal and McGaugh, 1996a; Zorawski and Killcross, 2002; Okuda et al., 2004). However, in contrast to epinephrine, glucocorticoids are highly lipophylic and, thus, readily enter the brain and bind directly to mineralocorticoid receptors (MRs) and glucocorticoid receptors (GRs) (McEwen et al., 1968; de Kloet, 1991). These two receptor types differ in their affinity for corticosterone and synthetic ligands. MRs have a high affinity for the natural steroids corticosterone and aldosterone, whereas GRs have a high affinity for synthetic ligands such as dexamethasone and the GR agonist RU 28362 (Reul and de Kloet, 1985; Sutanto and de Kloet, 1987; Reul et al., 1990). As a consequence, MRs are mostly saturated during basal levels of corticosterone, whereas GRs become occupied by higher levels of corticosterone induced by stressful stimulation.

The memory-modulating effects of glucocorticoids released following arousing stimulation appear to involve the selective activation of the low-affinity GRs (Oitzl and de Kloet, 1992; Roozendaal et al., 1996b; Lupien and McEwen, 1997), as blockade of GRs, but not MRs, shortly before or immediately after training impairs long-term memory. Such findings provide strong support for the hypothesis that endogenously released glucocorticoids enhance memory consolidation. Glucocorticoids are known to act through intracellular and intranuclear receptors and can affect gene transcription either by direct binding of receptor homodimers to DNA (Beato et al., 1995; Datson et al., 2001) or via protein–protein interactions with other transcription factors such as Jun or Fos (Heck et al., 1994). However, as discussed later, glucocorticoids may also act more rapidly by interacting with membrane receptors and/or potentiating the efficacy of the norepinephrine signal cascade via an interaction with G-protein-mediated actions (Roozendaal et al., 2002b).

3.26.3.3 Adrenergic-Glucocorticoid Interactions

Evidence from several kinds of studies indicates that catecholamines and glucocorticoids released from the adrenal glands interact in influencing memory consolidation. Glucocorticoids alter the sensitivity of epinephrine in influencing memory consolidation

in adrenalectomized rats (Borrell et al., 1983, 1984). Further, in adrenally intact rats, administration of meytrapone, a corticosterone-synthesis inhibitor that reduces the elevation of circulating corticosterone induced by aversive stimulation, attenuates the memory-enhancing effects of epinephrine administered posttraining (Roozendaal et al., 1996a). Such findings suggest that synergistic actions of epinephrine and corticosterone may be essential in mediating stress effects on memory enhancement.

The studies cited above used emotionally arousing footshock training, conditions that induce the release of both corticosterone and epinephrine. Studies using an object recognition task investigated whether adrenergic activation induced by emotional arousal is essential in enabling corticosterone effects on memory consolidation (Okuda et al., 2004). Rats were given either extensive habituation to an apparatus or no prior habituation and were then allowed to explore objects in the apparatus. Placing rats in a novel testing apparatus evokes novelty-induced arousal, and habituation of rats to the apparatus is known to reduce this arousal response (de Boer et al., 1990). Corticosterone administered immediately posttraining to nonhabituated (i.e., emotionally aroused) rats enhanced their 24-h retention performance. In contrast, posttraining corticosterone did not enhance retention of object recognition in habituated rats (Okuda et al., 2004), providing evidence that training-associated emotional arousal may be essential for enabling glucocorticoid effects on memory consolidation. Other findings indicated that training-induced adrenergic activation is a critical component of emotional arousal in enabling glucocorticoid effects on memory consolidation. As is shown in **Figure 2**, the β-adrenoceptor antagonist propranolol, coadministered with the corticosterone immediately after the object recognition training, blocked the corticosterone-induced memory enhancement (Roozendaal et al., 2006b). To investigate whether a pharmacologically induced increase in adrenergic activity enables glucocorticoid effects on memory consolidation, a low dose of the α_2-adrenoceptor antagonist yohimbine was administered to well-habituated (i.e., nonaroused) rats immediately after object recognition training. Corticosterone administered together with yohimbine induced dose-dependent enhancement of memory consolidation (Roozendaal et al., 2006b). Posttraining injections of corticosterone and yohimbine separated by a 4-h delay did not enhance memory consolidation. These findings are thus consistent with the hypothesis that adrenergic activation is essential in enabling glucocorticoid enhancement of memory consolidation. The nature of this interaction is discussed in more detail later.

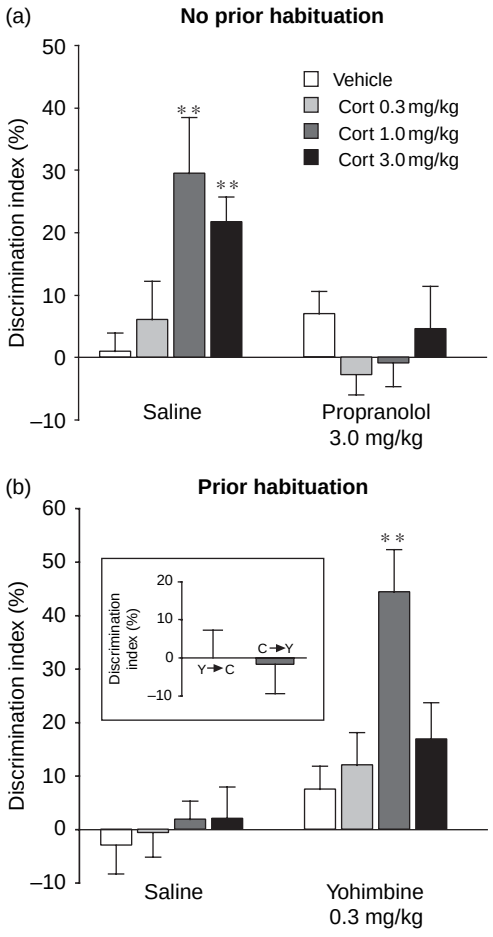

Figure 2 Glucocorticoid effects on memory consolidation for object recognition training require adrenergic activation. (a) Immediate posttraining administration of the β-adrenoceptor antagonist propranolol (3.0 mg/kg, sc) blocked the corticosterone-induced enhancement of object recognition memory in naïve rats. (b) The α_2-adrenoceptor antagonist yohimbine (0.3 mg/kg, sc) enabled a corticosterone effect on object recognition memory in habituated rats. Inset: Posttraining injections of yohimbine (0.3 mg/kg, sc) and corticosterone (1.0 mg/kg, sc) separated by a 4-h delay did not induce memory enhancement. Y → C; Yohimbine administered immediately after training and corticosterone 4 h later; C → Y; corticosterone administered immediately after training and yohimbine 4 h later. Results represent discrimination index (mean ± SEM) in percentage on a 24-h retention trial. **, $p < .01$, as compared with the corresponding vehicle group. From Roozendaal B, Okuda S, Van der Zee EA, and McGaugh JL (2006b) Glucocorticoid enhancement of memory requires arousal-induced noradrenergic activation in the basolateral amygdala. *Proc. Natl. Acad. Sci. USA* 103: 6741–6746.

3.26.3.4 Other Neuromodulatory Systems

As discussed earlier in this chapter, drugs affecting many other neuromodulatory and transmitter systems also influence memory consolidation. The dose- and time-dependent enhancement of memory induced by the stimulant drugs known to act via GABA (picrotoxin, bicuculline) and catecholamines (amphetamine, clenbuterol) has also been obtained in studies investigating the effects of opiate receptor antagonists (Messing et al., 1979; Introini and Baratti, 1984) and muscarinic cholinergic receptor agonists (Stratton and Petrinovich, 1963; Flood et al., 1981; Baratti et al., 1984; Introini-Collison and McGaugh, 1988; Power et al., 2003b), as well as drugs and hormones affecting several other systems, including corticotropin-releasing hormone (Roozendaal et al., 2002a), adrenocorticotropin (Gold and van Buskirk, 1976), vasopressin (de Wied, 1984), oxytocin (Bohus, 1980), substance P (Huston and Staubli, 1981; Schlesinger et al., 1986), histamine (Passani et al., 2001; da Silva et al., 2006), and cholecystokinin (Flood et al., 1987). Additionally, many studies have investigated interactions of these systems in modulating memory consolidation (McGaugh, 1989; McGaugh and Gold, 1989).

As is discussed below, the effects of many neuromodulatory systems are mediated by interactions with noradrenergic and muscarinic cholinergic systems within the amygdala. The initial research investigating such interactions investigated the effects of peripherally administered drugs and hormones (McGaugh and Cahill, 1997). Considerable evidence indicates that opiate and GABAergic influences on memory consolidation are mediated via adrenergic influences. The finding that the β-adrenoceptor antagonist propranolol blocks the memory-enhancing effects of the opiate receptor antagonist naloxone (Izquierdo and Graudenz, 1980) is consistent with evidence that opiates regulate the release of norepinephrine in the brain (Arbilla and Langer, 1978; Nakamura et al., 1982). Further, the β-adrenoceptor agonist clenbuterol blocks the memory impairment induced by the GABAergic agonist muscimol (Introini-Collison et al., 1994). As discussed below, such findings are consistent with the hypothesis that opioids and GABA impair memory by decreasing norepinephrine release in the brain (Quirarte et al., 1998; Hatfield et al., 1999). Thus, noradrenergic activation appears to be critical for opioid peptidergic and GABAergic influences on memory consolidation.

In contrast to the effects of opioid peptidergic and GABAergic drugs, cholinergic effects do not appear to be mediated by adrenergic activation. However, there is extensive evidence that muscarinic activity is a requirement for norepinephrine-induced memory enhancement. Systemic injections of the muscarinic cholinergic receptor antagonist atropine attenuate the memory-enhancing effects of the β-adrenoceptor agonist clenbuterol as well as that of epinephrine (Introini-Collison and McGaugh, 1988; Introini-Collison and Baratti, 1992). Thus, cholinergic activation appears to provide modulatory influences on memory consolidation that are downstream from adrenergic activation.

3.26.4 Involvement of the Amygdala in Modulating Memory Consolidation

Goddard's (1964) finding that electrical stimulation of the amygdala administered shortly after rats were trained on an aversively motivated task impaired their memory of the training was the first to suggest that the amygdala plays a role in influencing memory consolidation. The conclusion that the amygdala stimulation disrupted the consolidation of the memory of the training was confirmed by many subsequent findings from other laboratories (Kesner and Wilburn, 1974; McGaugh and Gold, 1976). One possible interpretation of these findings is that the stimulation disrupted the consolidation of memory processes occurring within the amygdala. However, many subsequent findings have indicated that amygdala stimulation modulates memory consolidation via influences mediated by amygdala efferent projections to other brain regions. The finding that posttraining electrical stimulation of the amygdala can either enhance or impair memory, depending on the stimulation intensity and the training conditions (Gold et al., 1975), clearly indicates that the effects are modulatory and not simply memory impairing. Further, the evidence that lesions of the stria terminalis (a major amygdala pathway) block the memory-impairing effects of posttraining electrical stimulation (Liang and McGaugh, 1983) strongly suggested that the modulation involves amygdala projections to other brain regions (see following).

3.26.4.1 Noradrenergic Influences in the BLA

Experiments by Kesner and Ellis (Ellis and Kesner, 1981; Kesner and Ellis, 1983) and Gallagher et al.

(1981) were the first to use posttraining drug infusions to investigate the involvement of neuromodulatory systems in the amygdala in memory consolidation. ß-Adrenoceptor antagonists infused into the amygdala impaired rats' retention of inhibitory avoidance, and concurrent infusion of norepinephrine blocked the memory impairment (Gallagher et al., 1981). These investigators also found that, as is found with systemic administration (Messing et al., 1979; Izquierdo and Graudenz, 1980), posttraining intraamygdala infusions of opioid peptidergic agonists and antagonists impaired and enhanced memory, respectively. The findings of more recent studies indicate that the BLA is selectively involved in such amygdala memory-modulatory influences. The adjacent central nucleus does not appear to play a significant role, if any, in modulating memory consolidation (Tomaz et al., 1992; Parent and McGaugh, 1994; Roozendaal and McGaugh, 1996a, 1997a; DaCunha et al., 1999; McGaugh et al., 2000). Thus, the effects of relatively large intraamygdala drug infusion volumes typically used in most early studies as well as many recent studies are likely the result of selective influences on BLA activity. Moreover, findings of more recent studies indicating that posttraining intraamygdala infusions of drugs influence retention performance tested 24 h or longer after the training but do not affect performance tested within a few hours after training, provide strong evidence that the treatments selectively affect the consolidation of long-term memory (Bianchin et al., 1999; Schafe and LeDoux, 2000; Barros et al., 2002).

Other early findings also implicated the amygdala in adrenergic influences on memory consolidation. Adrenal demedullation or posttraining administration of epinephrine alter the memory-modulating effects of electrical stimulation of the amygdala (Liang et al., 1985), and lesions of either the amygdala or the stria terminalis block epinephrine effects on memory consolidation (Cahill and McGaugh, 1991; Liang and McGaugh, 1983). Although earlier studies reported evidence suggesting that epinephrine induces the release of norepinephrine in the brain (Gold and van Buskirk, 1978), the finding that posttraining intraamygdala infusions of the β-adrenoceptor antagonist propranolol block epinephrine effects on memory consolidation (Liang et al., 1986) provided the first evidence suggesting that epinephrine effects on memory are mediated by noradrenergic activation within the amygdala. In support of this implication, many subsequent studies reported that posttraining infusions of norepinephrine or the β-adrenoceptor agonist clenbuterol into the amygdala (or selectively into the BLA) produce dose-dependent enhancement of memory consolidation (Liang et al., 1986, 1990, 1995; Introini-Collison et al., 1991, 1996; Izquierdo et al., 1992; Bianchin et al., 1999; Ferry and McGaugh, 1999; Hatfield and McGaugh, 1999; LaLumiere et al., 2003, 2005; Huff et al., 2005). Furthermore, posttraining intraamygdala infusions of β-adrenoceptor antagonists impair retention and block the memory-enhancing effects of norepinephrine coadministered (Liang et al., 1986, 1995; Salinas and McGaugh, 1995).

In addition to β-adrenoceptor influences, α-adrenoceptor activation in the BLA also modulates memory consolidation. Intra-BLA infusions of the α_1-adrenoceptor antagonist prazosin impair inhibitory avoidance memory, whereas infusions of the nonselective α-adrenoceptor agonist phenylephrine, administered together with yohimbine, an α_2-adrenoceptor antagonist, enhance retention (Ferry et al., 1999a). The α_1-adrenoceptor-induced memory enhancement most likely involves an interaction with ß-adrenoceptors, as posttraining intra-BLA infusions of the β-adrenoceptor antagonist atenolol block the memory enhancement produced by activation of α_1-adrenoceptors. The finding that posttraining intraamygdala infusions of the synthetic cyclic adenosine monophosphate (cAMP) analog 8-bromo-cAMP enhance retention (Liang et al., 1995) is consistent with the hypothesis that activation of ß-adrenoceptors modulates memory via a direct coupling to adenylate cyclase. Thus, the finding that intra-BLA infusions of the α_1-adrenoceptor antagonist prazosin do not prevent the memory enhancement induced by concurrently infused 8-bromo-cAMP suggests that the memory-enhancing effects of α_1-adrenoceptor activation are mediated by an interaction with ß-adrenoceptors upstream from cAMP, probably at the G-protein level (Ferry et al., 1999b).

There is extensive evidence indicating that noradrenergic activity within the BLA also plays an important role in mediating the modulatory effects of other hormones and neurotransmitters on memory consolidation (Roozendaal, 2007). Many studies have reported that, as with peripherally administered drugs, intra-BLA infusions of the GABAergic receptor antagonists bicuculline and picrotoxin enhance memory consolidation and that GABAergic receptor agonists impair memory (e.g., Brioni et al., 1989; Izquierdo et al., 1992; Bianchin et al., 1999; Wilensky et al., 2000; Huff et al., 2005). Similarly, the opioid peptidergic antagonist naloxone enhances memory

when infused into the amygdala posttraining, whereas opioid peptidergic agonists impair memory consolidation (McGaugh et al., 1988; Introini-Collison et al., 1989). Consistent with the evidence from studies using peripheral drug administration, β-adrenoceptor antagonists infused into the amygdala block the memory-enhancing effects of bicuculline or naloxone infused concurrently (McGaugh et al., 1988, 1990; Introini-Collison et al., 1989). In contrast, intraamygdala injections of α_1- or α_2-adrenoceptor antagonists do not block naloxone effects on memory consolidation (McGaugh et al., 1988). Thus, as was found with peripherally administered drugs, GABAergic and opioid peptidergic influences within the BLA appear to modulate memory consolidation by influencing β-adrenoceptor activation via influences on the release of norepinephrine. Ragozino and Gold (1994) reported that posttraining intraamygdala infusions of glucose block the memory impairment induced by the opiate drug morphine. However, such glucose infusions do not attenuate the memory impairment induced by propranolol (Lennartz et al., 1996; McNay and Gold, 1998). Thus, glucose effects do not appear to act via adrenergic activation within the amygdala.

Other recent studies reported that β-adrenoceptor activation within the BLA is also required for mediating the memory-modulatory effects of corticotropin-releasing hormone (CRH) and orphanin FQ/nociceptin (OFQ/N), a recently discovered opioid-like peptide. Retention is enhanced by posttraining intra-BLA infusions of CRH (Liang and Lee, 1988) and impaired by a CRH receptor antagonist (Roozendaal et al., 2002a). Unlike CRH, posttraining intra-BLA infusions of OFQ/N impair retention, and an OFQ/N receptor antagonist enhances retention (Roozendaal et al., 2007). Atenolol infused into the BLA blocks the memory-enhancing effect of CRH and the OFQ/N antagonist, whereas it potentiates the memory-impairing effect of OFQ/N administered concurrently (Roozendaal et al., 2007). These findings thus indicate that, as with many other neuromodulatory systems, endogenously released CRH and OFQ/N interact with noradrenergic activity within the BLA in modulating memory consolidation. Similar findings were obtained in studies of the effects of dopamine. Posttraining intra-BLA infusions of dopamine induce dose-dependent memory enhancement that is blocked by coinfusion of a β-adrenoceptor antagonist as well as D1 or D2 receptor antagonists (LaLumiere et al., 2004). However, noradrenergic activation within the BLA affecting memory also appears to require concurrent interaction with dopamine receptors, as dopamine receptor antagonists block the memory-enhancing effects of posttraining intra-BLA infusions of clenbuterol (LaLumiere et al., 2004).

The extensive evidence indicating that adrenoceptor activation within the amygdala is critical for the modulation of memory consolidation suggests that emotionally arousing learning experiences should induce the release of norepinephrine within the amygdala and that drugs and hormones that enhance memory consolidation should increase the release. Findings of studies using *in vivo* microdialysis and high-performance liquid chromatography to measure ongoing changes in norepinephrine levels in the amygdala strongly support these implications. As is shown in **Table 1**, footshock comparable to that typically used in inhibitory avoidance training significantly increases amygdala norepinephrine levels (Galvez et al., 1996; Quirarte et al., 1998). Moreover, drugs and hormones that enhance memory consolidation (e.g., epinephrine, picrotoxin, and naloxone) potentiate footshock-induced increases in norepinephrine levels in the

Table 1 Treatment effects on memory and amygdala norepinephrine levels

Treatment	*Effect on memory*	*Effect on amygdala norepinephrine levels*	*Reference*
Footshock	Varies directly with footshock intensity	Varies with footshock intensity	Quirarte et al. 1998
Epinephrine	Enhances	Increases	Williams et al. 1998
Corticosterone	Enhances	Increases	McIntyre et al. 2004
Muscimol	Impairs	Decreases	Hatfield et al. 1999
Picrotoxin	Enhances	Increases	Hatfield et al. 1999
β-endorphin	Impairs	Decreases	Quirarte et al. 1998
Naloxone	Enhances	Increases	Quirarte et al. 1998
Orphanin FQ/nociceptin	Impairs	Decreases	Kawahara et al. 2004

amygdala, and drugs that impair consolidation (e.g., muscimol, β-endorphin, and OFQ/N) decrease amygdala norepinephrine levels (Quirarte et al., 1998; Williams et al., 1998; Hatfield et al. 1999; Kawahara et al., 2004). Additionally, stimulation of the vagus nerve or the NTS increases norepinephrine levels in the amygdala and enhances memory consolidation (Clayton and Williams, 2000; Hassert et al., 2004). McIntyre et al. (2002) investigated norepinephrine levels in the amygdala induced by inhibitory avoidance training. Consistent with findings of previous studies using footshock (Galvez et al., 1996; Quirarte et al., 1998; Hatfield et al., 1999), norepinephrine levels increased following the training. However, the duration of the increased norepinephrine levels seen after training was greater than that previously found with footshock stimulation alone (i.e., without training). Additionally, and importantly, the increase in norepinephrine levels assessed in individual animals over an interval of 90 min after training correlated highly with their subsequent retention performance, tested the following day (**Figure 3**).

3.26.4.2 Glucocorticoid Influences in the BLA

There is extensive evidence that glucocorticoids affect memory consolidation through influences involving the BLA. The findings of studies of the effects of glucocorticoids on memory for inhibitory avoidance training are similar to those of studies of the effects of epinephrine. Lesions of the BLA or stria terminalis block the memory-enhancing effects of posttraining systemic injections of the synthetic glucocorticoid dexamethasone (Roozendaal and McGaugh, 1996a,b). Furthermore, memory is modulated by either systemic or intra-BLA infusions of glucocorticoids (Roozendaal and McGaugh, 1996a, 1997a), and like the effects of epinephrine, such modulation requires noradrenergic activation within the amygdala. Intra-BLA infusions of a ß-adrenoceptor antagonist block the memory-enhancing effects of systemic injections of dexamethasone or corticosterone, as well as the effects of the GR agonist RU 28362 infused into the BLA concurrently (Quirarte et al., 1997; Roozendaal et al., 2002b, 2006a,b).

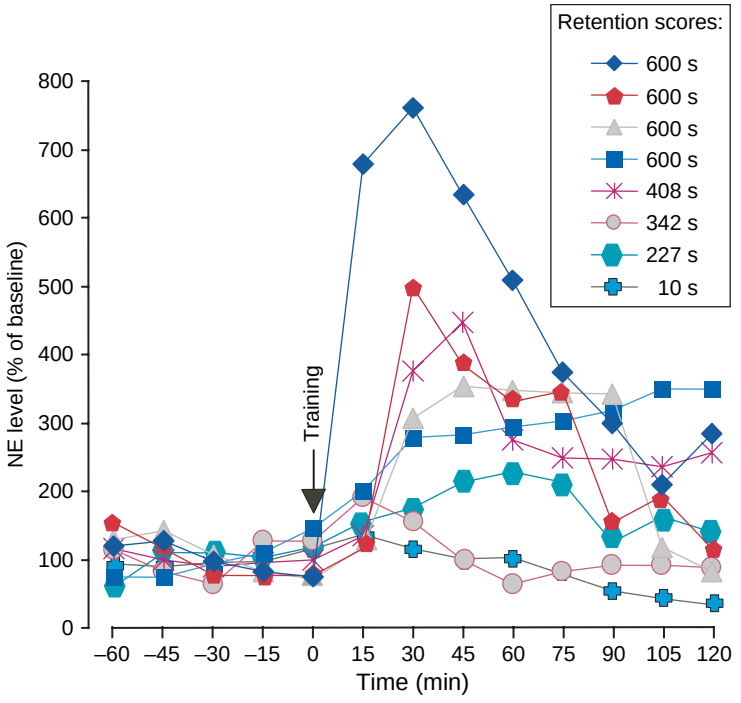

Figure 3 Norepinephrine levels in the amygdala in individual animals following inhibitory avoidance training. Percent of baseline norepinephrine following inhibitory avoidance training is graphed for each individual rat. The key notes retention score on the following day. Amygdala norepinephrine levels correlate with 24-h retention performance. Correlation values for the first five posttraining samples varied from +0.75 to +0.92. From McIntyre CK, Hatfield T, and McGaugh JL (2002) Amygdala norepinephrine levels after training predict inhibitory avoidance retention performance in rats. *Eur. J. Neurosci.* 16: 1223–1226.

As discussed above, studies investigating the effects of glucocorticoids administered systemically after object recognition training found that, in naïve (i.e., emotionally aroused) rats, propranolol blocked the memory enhancement induced by corticosterone and that, in habituated (i.e., emotionally less aroused) rats, corticosterone enhanced memory only when norepinephrine release was stimulated by yohimbine (Roozendaal et al., 2006b). A subsequent experiment (Roozendaal et al., 2006b) found that, in naive rats, intra-BLA infusions of a β-adrenoceptor antagonist blocked the effects of systemically administered corticosterone on object recognition memory. Further, in habituated rats, corticosterone activated BLA neurons, as assessed by phosphorylated cAMP response-element binding (pCREB) immunoreactivity levels, only in animals also given yohimbine. Considered together, these findings provide strong evidence that the BLA is a critical locus of the synergistic actions of glucocorticoids and emotional arousal-induced noradrenergic activation in influencing memory consolidation.

Findings of studies investigating the mechanism of glucocorticoid interactions with the noradrenergic system suggest that activation of GRs in the BLA may facilitate memory consolidation by potentiating the norepinephrine signaling cascade through an interaction with G-protein-mediated effects. The enhancement of memory for inhibitory avoidance training induced by posttraining intra-BLA infusions of the GR agonist RU 28362 is blocked by concurrent infusion of Rp-cAMPS, a drug that inhibits protein kinase A activity and thus blocks the norepinephrine signaling cascade (Roozendaal et al., 2002b). Moreover, intra-BLA infusions of the GR antagonist RU 38486 attenuate the memory-enhancing effects of the ß-adrenoceptor agonist clenbuterol infused concurrently such that a much higher dose of clenbuterol (100 ng vs. 1 ng) is required to induce memory enhancement (Roozendaal et al., 2002b).

As was found with epinephrine, glucocorticoid effects on memory consolidation also appear to involve brain stem nuclei, including the NTS, that send noradrenergic projections to the BLA. A GR antagonist infused into the NTS attenuates the memory-enhancing effects of systemically administered dexamethasone (Roozendaal et al., 1999b). Moreover, the finding that posttraining infusions of RU 28362 into the NTS enhance inhibitory avoidance retention and that intra-BLA infusions of a ß-adrenoceptor antagonist block the enhancement (Roozendaal et al., 1999b) provides additional evidence that the NTS influence on memory consolidation involves noradrenergic activation of the BLA (Williams et al., 1998, 2000; Clayton and Williams, 2000; Miyashita and Williams 2002).

3.26.4.3 Cholinergic Influences in the BLA

The finding that stria terminalis lesions block the memory-enhancing effect of systemically administered cholinergic drugs (Introini-Collison et al., 1989) provided the first evidence suggesting that muscarinic cholinergic influences within the amygdala may be involved in regulating memory consolidation. Subsequent studies have provided extensive evidence that posttraining intraamygdala infusions of muscarinic cholinergic receptor agonists and antagonists enhance and impair, respectively, memory for many kinds of training, including inhibitory avoidance, Pavlovian fear conditioning, conditioned place preference, and change in reward magnitude (Introini-Collison et al., 1996; Vazdarjanova and McGaugh, 1999; Salinas et al., 1997; Passani et al., 2001; Power and McGaugh, 2002; Schroeder and Packard, 2002; Power et al., 2003a,b; LaLumiere et al., 2004). Results of experiments using posttraining infusions of the muscarinic cholinergic receptor agonist oxotremorine administered together with selective antagonists indicate that both M1 and M2 muscarinic cholinergic receptor types are involved in the memory-enhancing effects of cholinergic activation (Power et al., 2003a). The finding that lesions of the nucleus basalis, the major source of cholinergic innervation of the BLA, impair inhibitory avoidance retention and that posttraining intra-BLA infusions of either oxotremorine or the acetylcholinesterase inhibitor physostigmine attenuate the memory impairment provided additional evidence that cholinergic activation within the BLA regulates memory consolidation (Power and McGaugh, 2002).

As noted above, in contrast to the effects of GABAergic and opioid peptidergic drugs, cholinergic effects on memory consolidation do not require concurrent noradrenergic activation. A ß-adrenoceptor antagonist does not block the memory-enhancing effect of intraamygdala infusions of oxotremorine. However, a low and otherwise ineffective dose of the muscarinic cholinergic receptor antagonist atropine blocks the memory enhancement induced by intraamygdala infusions of clenbuterol (Dalmaz et al., 1993; Salinas et al., 1997). Thus, cholinergic activation in the BLA appears to act downstream

from adrenergic activation in modulating memory consolidation. Other findings indicate that cholinergic activation within the BLA is critical for enabling glucocorticoid as well as dopamine enhancement of memory consolidation. Atropine infused into the BLA blocks the memory-enhancing effects of RU 28362 or dopamine infused concurrently as well as the effects of systemically administered dexamethasone (Power et al., 2000; LaLumiere et al., 2004). Conversely, cholinergic activation within the BLA affecting memory also appears to require concurrent interaction with dopamine, as dopamine receptor antagonists block the memory-enhancing effects of posttraining intra-BLA infusions of oxotremorine (LaLumiere et al., 2004).

Studies of the effects of histamine receptor antagonists and agonists infused into the BLA provide additional evidence of a role of acetylcholine in the BLA in modulating consolidation (Passini et al., 2001). H_3 receptor antagonists (ciproxifan, clobenprobit, or thioperamide) infused into the BLA decrease acetylcholine release, as assessed by *in vivo* microdialysis, and concurrent infusions of the H_2 receptor agonist cimetidine block the decreased acetylcholine release. Moreover, posttraining intra-BLA infusions of H_3 receptor antagonists, administered in doses found to decrease acetylcholine release, impair memory for contextual fear conditioning (Passani et al., 2001). As other studies have reported that posttraining systemically administered H_3 receptor antagonists enhance memory and block memory impairment induced by a muscarinic cholinergic antagonist (Bernaerts et al., 2004), it seems likely that the central and peripheral actions of H_3 receptor antagonists involve different actions. There is also evidence that acetylcholine is released within the amygdala during training. Studies using *in vivo* microdialysis have shown that acetylcholine levels in the amygdala increase while rats perform a spontaneous alternation task and that the increase is correlated with performance on the task (Gold, 2003; McIntyre et al., 2003). As this task is known to involve hippocampal functioning, these findings are consistent with other evidence, discussed below, suggesting that the amygdala influences memory processing that involves the hippocampus. **Figure 4** summarizes some of the neuromodulatory interactions within the BLA involved in regulating memory consolidation.

3.26.4.4 Other Neuromodulatory Influences in the BLA

The BLA is also involved in mediating the modulatory effects of other hormones and neurotransmitters on memory consolidation. For example, a recent study reported not only that the reproductive hormone relaxin binds to specific receptors in hypothalamic regions to regulate reproductive behaviors but also that posttraining intra-BLA infusions of relaxin also induce dose-dependent impairment of inhibitory avoidance memory (Ma et al., 2005). Other recent studies implicated the amygdala in the memory-modulatory effects of bombesin (gastrin-releasing peptide). Posttraining intra-BLA infusions of the bombesin receptor antagonist RC-3095 impair memory of inhibitory avoidance (Roesler et al., 2004b), whereas temporary inactivation of the BLA blocked the memory-modulatory effects of systemically administered bombesin or its antagonist (Rashidy-Pour and Razvani, 1998; Roesler et al., 2004b). Furthermore, the finding that bombesin infused into the NTS also modulates memory consolidation (Williams and McGaugh, 1994) and that inactivation of the NTS blocks the effects of peripherally administered bombesin (Rashidy-Pour and Razvani, 1998) suggests that noradrenergic activity may be essential in mediating the effects of bombesin on memory consolidation. This suggestion is supported by the evidence that the bombesin receptor antagonist selectively impaired memory consolidation of aversively motivated inhibitory avoidance training and not that of emotionally less arousing object recognition training (Roesler et al., 2004a), which, as discussed above, induces less noradrenergic activation of the BLA.

3.26.5 Involvement of the Amygdala in Modulating Memory Extinction

Several studies have investigated whether extinction learning (i.e., learning that cues that previously predicted aversive or appetitive consequences no longer predict such consequences) is regulated by the same neuromodulatory systems that regulated the original learning. An early study found that posttraining peripheral administration of picrotoxin enhances the extinction of cued fear conditioning (McGaugh et al., 1990). Experiments using intra-BLA infusions have found that the GABAergic antagonist bicuculline and norepinephrine administered posttraining enhance extinction of contextual fear conditioning (Berlau and McGaugh, 2006). If such extinction involves the same processes engaged by fear conditioning, the effects of bicuculline would be expected to require β-adrenoceptor activation. Consistent with

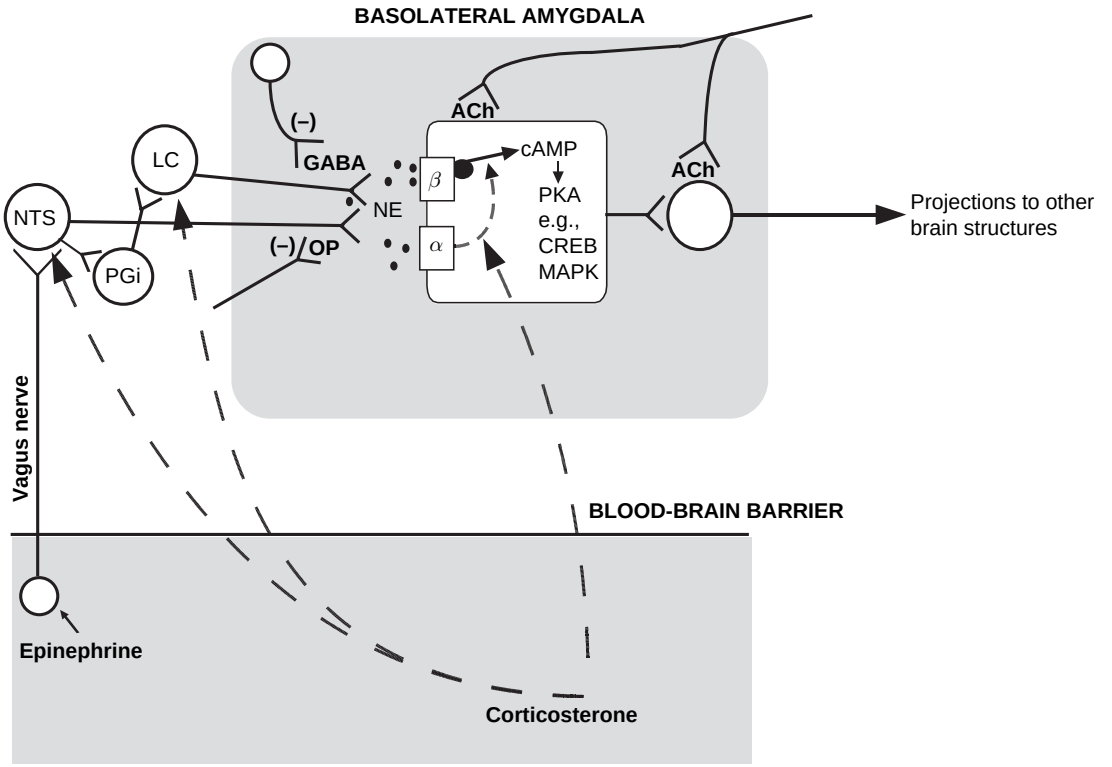

Figure 4 Schematic summarizing the role of the noradrenergic system of the basolateral amygdala in memory consolidation. Norepinephrine (NE) is released in the basolateral amygdala following training in aversively motivated tasks and binds to both β-adrenoceptors and α_1-adrenoceptors at postsynaptic sites. The β-adrenoceptor is coupled directly to adenylate cyclase to stimulate cAMP formation. The α_1-adrenoceptor modulates the response induced by β-adrenoceptor stimulation. Intracellular cAMP can initiate a cascade of molecular events in the basolateral amygdala. The memory-modulatory effects of several other neuromodulatory influences, including that of epinephrine, glucocorticoid, opioid peptidergic, and GABAergic systems, are mediated by converging influences on the noradrenergic system of the basolateral amygdala. Drug interactions with the noradrenergic system can occur at both presynaptic and postsynaptic loci. These noradrenergic effects in the basolateral amygdala are required for regulating memory consolidation in other brain regions. α, α_1-adrenoceptor; ACh, acetylcholine; β, β-adrenoceptor; cAMP, adenosine 3′,5′-cyclic monophosphate; CREB, cAMP-response element-binding protein; GABA, gamma-aminobutyric acid; LC, locus coeruleus; MAPK, mitogen-activated protein kinase, NTS, nucleus of the solitary tract; OP, opioid peptide; Pgi, nucleus paragigantocellularis; PKA, protein kinase A. From McGaugh JL (2000) Memory: A century of consolidation. *Science* 287: 248–251, and Roozendaal B (2000) Glucocorticoids and the regulation of memory consolidation. *Psychoneuroendocrinology* 25: 213–238.

that implication, posttraining infusions of bicuculline did not enhance extinction memory consolidation when infused into the BLA together with a low and otherwise ineffective dose of propranolol. Additionally, the GABAergic agonist muscimol coinfused into the BLA with norepinephrine posttraining did not block the memory enhancement induced by norepinephrine. These findings are consistent with the evidence discussed above indicating that norepinephrine effects in the BLA act downstream from GABAergic influences (Introini-Collison et al., 1994; McGaugh and Cahill, 1997). Other studies have reported that extinction of fear conditioning is enhanced by pre- or postextinction infusions of the *N*-methyl-D-aspartate (NMDA) partial agonist d-cycloserine (Walker et al., 2002; Ledgerwood et al., 2003, 2005). Furthermore, intra-BLA infusions of d-cycloserine block the impairing effects of concurrent administration of the GABAergic agonist muscimol on the consolidation of extinction memory (Akirav, 2007). Consistent with extensive evidence of glucococorticoid enhancement of consolidation of memory of fear-based training, dexamethasone administered systemically after extinction training or intra-amygdally prior to extinction also enhanced extinction of fear-potentiated startle (Yang et al., 2006). Furthermore, corticosterone given systemically immediately after retention testing on a

contextual fear conditioning task facilitated extinction of this memory (Cai et al., 2006). Other studies have reported that the BLA modulates the extinction of conditioned place preference. Glucose or oxotremorine infused into the BLA immediately after extinction training enhanced the extinction of amphetamine-induced place preference (Schroeder and Packard, 2003, 2004).

3.26.6 Amygdala Interactions with Other Brain Systems in Modulating Memory

Although many of the experiments investigating BLA involvement in memory consolidation have used inhibitory avoidance training and testing (Parent and McGaugh, 1994; Izquierdo et al., 1997; McGaugh et al., 2000; McGaugh and Izquierdo, 2000; Wilensky et al., 2000), comparable effects of posttraining amygdala treatments have been obtained in experiments using many different kinds of training tasks, including contextual fear conditioning (Sacchetti et al., 1999; Vazdarjanova and McGaugh, 1999; LaLumiere et al., 2003), cued fear conditioning (Sacchetti et al., 1999; Schafe and LeDoux, 2000; Roozendaal et al., 2006a), Y-maze discrimination training (McGaugh et al., 1988), change in reward magnitude (Salinas et al., 1997), conditioned place preference (Hsu et al., 2002; Schroeder and Packard, 2003, 2004), radial-arm maze appetitive training (Packard and Chen, 1999), water-maze spatial and cued training (Packard et al., 1994; Packard and Teather, 1998), conditioned taste aversion (Miranda et al., 2003), olfactory training (Kilpatrick and Cahill, 2003a), object recognition (Roozendaal et al., 2006b), extinction of contextual fear conditioning (Berlau and McGaugh, 2006), and extinction of conditioned reward (Schroeder and Packard, 2003). Thus, although there is abundant evidence that the BLA is involved in modulating memory of aversively motivated training, such as footshock training used in inhibitory avoidance and Pavlovian fear conditioning, the evidence also clearly indicates that the BLA modulates the consolidation of memory for many different kinds of training experiences (McGaugh, 2002; Packard and Cahill, 2001), and as these different training experiences are known to engage different brain systems both during training and during the consolidation occurring after training (Quillfeldt et al., 1996; Zanatta et al., 1996; Izquierdo et al., 1997; Packard and Knowlton, 2002; Gold, 2004), the BLA-induced modulation no doubt involves influences on processing occurring in these other brain regions.

As discussed above, there is considerable evidence that neuromodulatory interactions occurring within the amygdala influence memory consolidation. Such influences may be, at least in part, a result of influences on neuroplasticity within the amygdala. However, several kinds of evidence suggest that alterations in amygdala functioning affect memory consolidation through amygdala influences on other brain regions involved in memory consolidation. The BLA sends projections to many other brain regions, some via the stria terminalis (Young, 1993; Pitkänen, 2000; Petrovich et al., 2001; Price, 2003; Sah et al., 2003). The evidence, discussed above, that stria terminalis lesions block the memory-modulating effects of electrical stimulation and intraamygdala drug infusions strongly suggests that modulation within the amygdala is not sufficient to affect memory: efferent projections seem required. The evidence that posttraining BLA treatments affect memory for many kinds of training clearly suggests that processing in different brain regions is required. This implication is supported by the finding that training known to involve the amygdala (e.g., Pavlovian fear conditioning) induces the expression of several transcriptionally regulated genes implicated in synaptic plasticity in many brain areas, including the hippocampus, striatum, and cortex, as well as the amygdala (Ressler et al., 2002). These effects appear to be involved in memory consolidation and not simply a result of nonspecific effects of stress or arousal, as they were found only when the stimuli used in the training induced learning. The findings of the many types of studies discussed later provide compelling evidence that the amygdala interacts with other brain regions in modulating the consolidation of memory for different kinds of training.

3.26.6.1 BLA Interactions with the Caudate Nucleus, Hippocampus, and Nucleus Accumbens

The amygdala projects directly to the caudate nucleus (via the stria terminalis) and both directly and indirectly to the hippocampus (Pitkänen, 2000; Petrovich et al., 2001). The evidence that stria terminalis lesions block the memory-enhancing effects of oxotremorine that was infused posttraining into the caudate nucleus suggests that efferents from the amygdala influence memory processing involving

the caudate nucleus (Packard et al., 1996). There is considerable evidence that the caudate nucleus and hippocampus are involved in different kinds of learning (e.g., Packard and McGaugh, 1992, 1996; McDonald and White, 1993; Packard and Cahill, 2001). In studies of rats given water-maze training, Packard and colleagues (Packard et al., 1994; Packard and Teather, 1998) found that amphetamine that was infused posttraining into the caudate nucleus selectively enhanced memory of visually cued training, whereas infusions administered into the dorsal hippocampus selectively enhanced memory of spatial training. In contrast, amphetamine infused into the amygdala posttraining enhanced memory for both types of training. Additionally, inactivation of the hippocampus (with lidocaine) prior to testing blocked retention of the spatial training, and inactivation of the caudate nucleus blocked retention of the visually cued training. But importantly, inactivation of the amygdala prior to retention testing did not block memory of either kind of training. Thus, although the amygdala modulates the consolidation of both caudate nucleus-dependent and hippocampus-dependent tasks, such findings suggest that it is not a locus of memory for either type of training. Additionally, in rats trained in a radial-arm maze spatial task, lidocaine infused into the amygdala blocked the memory enhancement induced by posttraining intrahippocampal infusions of glutamate (Packard and Chen, 1999). Such findings are consistent with extensive evidence that the hippocampus is involved in the learning of contextual information (Hirsh, 1974; Rudy and Sutherland, 1989; Phillips and LeDoux, 1992; Eichenbaum et al., 1996; McNish et al., 1997; Matus-Amat et al., 2004).

In fear conditioning tasks, including inhibitory avoidance, that are typically used in memory modulation studies, the rats learn that footshock occurs in a specific context. Further, that information can be learned if rats are first exposed to the context and then, on a subsequent day, given a brief footshock. As is shown in **Figure 5**, infusions of oxotremorine administered into the hippocampus after context exposure enhanced the subsequent conditioning, but infusions administered after the footshock training were ineffective (Malin and McGaugh, 2006). In contrast, oxotremorine infused into the rostral anterior cingulate cortex selectively enhanced memory when administered after the footshock. Oxotremorine infused into the BLA enhanced retention when administered after either the context or footshock training, consistent with extensive evidence that BLA activity modulates memory for many different kinds of experiences. Other findings of studies of the effects of posttraining intraamygdala infusions of a GR agonist provide additional evidence of BLA–hippocampus interactions in memory consolidation. As is shown in **Figure 6**, unilateral posttraining intrahippocampal infusions of the specific GR agonist RU 28362 enhanced rats' retention of inhibitory avoidance training, and the retention enhancement was blocked selectively by ipsilateral infusions of a ß-adrenoceptor antagonist into the BLA (Roozendaal et al., 1999a). The memory enhancement induced by GR activation in the hippocampus is also blocked by lesions of the BLA, stria terminalis, or nucleus accumbens (Roozendaal and McGaugh, 1997b; Roozendaal et al., 2001).

The BLA projects to the nucleus accumbens primarily via the stria terminalis (Kelley et al., 1982; Wright et al., 1996). The possible involvement of the BLA–stria terminalis–nucleus accumbens pathway in modulating memory consolidation was suggested by the finding that lesions of the nucleus accumbens, like lesions of the BLA, block the memory-enhancing effects of systemically administered dexamethasone (Roozendaal and McGaugh, 1996a; Setlow et al., 2000). Furthermore, the finding that unilateral lesions of the BLA combined with contralateral (unilateral) lesions of the nucleus accumbens also blocked the dexamethasone effect strongly indicates that these two structures interact via the stria terminalis in influencing memory consolidation (Setlow et al., 2000). Further evidence of BLA–nucleus accumbens interactions in modulating memory consolidation is provided by the finding (LaLumiere et al., 2005) that memory enhancement induced by intra-BLA infusions of dopamine is blocked by infusions of a dopamine receptor antagonist into the nucleus accumbens shell (but not the core). Conversely, a dopamine receptor antagonist infused into the BLA blocks the memory enhancement induced by infusions of dopamine infused into the nucleus accumbens shell posttraining (**Figure 7**).

As the hippocampus is known to project to the nucleus accumbens, that region may be a critical locus of converging BLA and hippocampal modulatory influences on memory consolidation (Mulder et al., 1998). The finding that inactivation of the nucleus accumbens with infusions of bupivacaine prior to training blocks the acquisition of contextual fear conditioning provides evidence consistent with this hypothesis (Haralambous and Westbrook, 1999). It is not known whether the BLA–nucleus accumbens

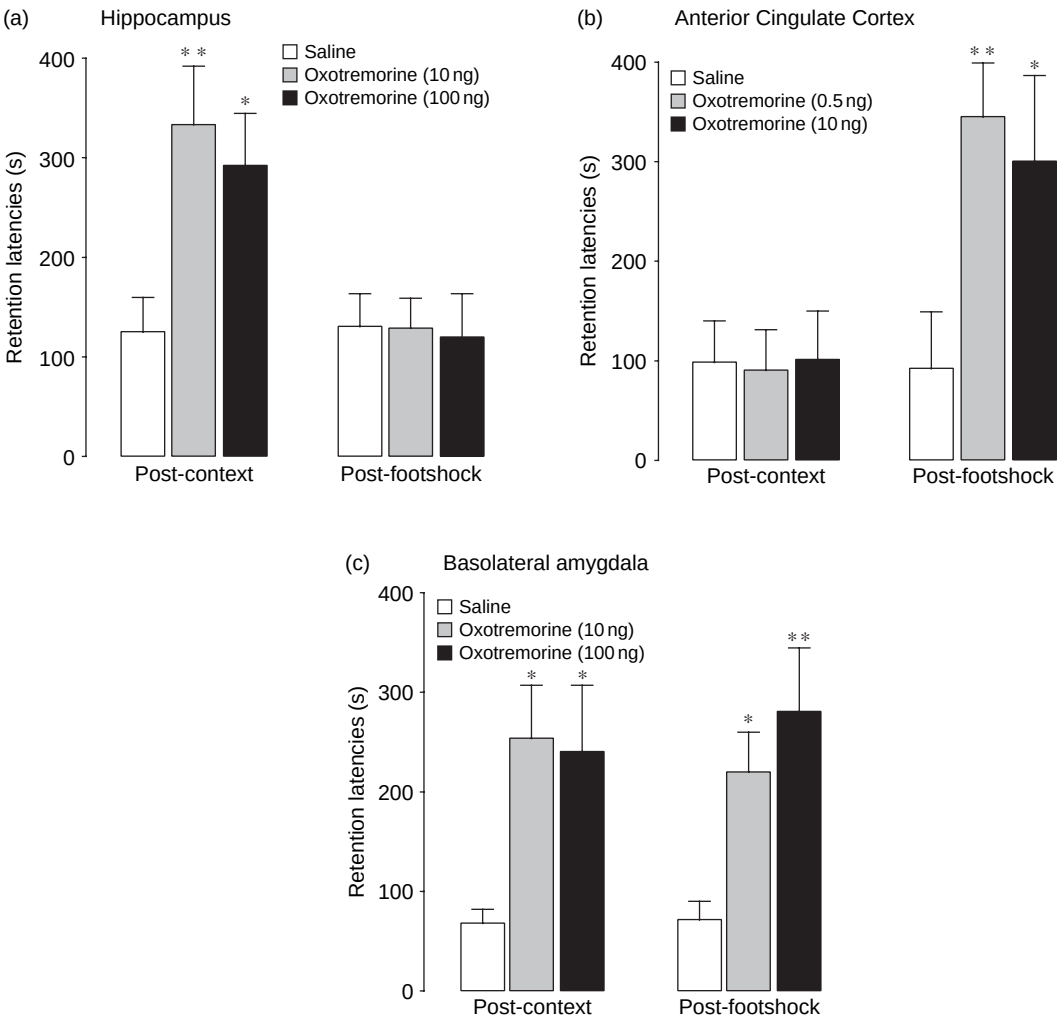

Figure 5 Differential involvement of the hippocampus, anterior cingulate cortex, and basolateral amygdala in memory for context and footshock. (a) Posttraining infusions of the muscarinic cholinergic receptor agonist oxotremorine (10 or 100 ng in 0.5 μl) into the dorsal hippocampus enhanced 48-h inhibitory avoidance retention latencies when administered after context exposure but not after the shock exposure given 24 h later. (b) Posttraining infusions of oxotremorine (0.5 or 10 ng in 0.5 μl) into the anterior cingulate cortex selectively enhanced 48-h inhibitory avoidance retention latencies when administered after the shock experience but not after the context exposure. (c) Posttraining infusions of oxotremorine (10 or 100 ng in 0.2 μl) into the basolateral amygdala enhanced 48-h inhibitory avoidance retention latencies when administered after either the context exposure or the shock experience. Results represent retention latencies (mean + SEM) in seconds. $*$, $p < .05$; $**$, $p < .01$ compared with the corresponding saline group. From Malin EL and McGaugh JL (2006) Differential involvement of the hippocampus, anterior cingulate cortex and basolateral amygdala in memory for context and footshock. *Proc. Natl. Acad. Sci. USA* 103: 1959–1963.

and hippocampus–nucleus accumbens pathways are also involved in mediating other BLA neuromodulatory influences on memory consolidation.

Other findings indicate that noradrenergic stimulation of the BLA that enhances memory consolidation also increases dorsal hippocampal levels of activity-regulated cytoskeletal protein (Arc) (McIntyre et al., 2005), an immediate-early gene implicated in hippocampal synaptic plasticity and memory consolidation processes (Guzowski et al., 2000). Additionally, inactivation of the BLA with infusions of lidocaine impairs memory consolidation and decreases Arc protein levels in the dorsal hippocampus (McIntyre et al., 2005). Further, the finding that intra-BLA infusions of muscimol attenuate the increase in Arc mRNA induced by contextual fear conditioning provides

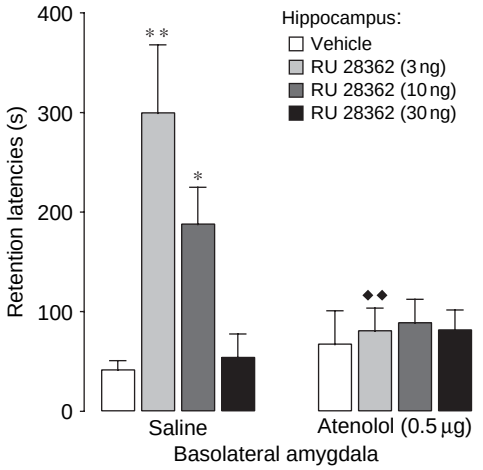

Figure 6 Glucocorticoid effects in the hippocampus on memory consolidation require noradrenergic activity of the basolateral amygdala. Immediate posttraining unilateral infusions of the glucocorticoid receptor agonist RU 28362 (3, 10, or 30 ng in 0.5 µl) induced dose-dependent enhancement of 48-h inhibitory avoidance retention latencies in rats given saline infusions into the basolateral amygdala concurrently. Ipsilateral infusions of the β-adrenoceptor antagonist atenolol (0.5 µg in 0.2 µl) into the basolateral blocked the memory enhancement induced by the glucocorticoid receptor agonist. Results represent retention latencies (mean + SEM) in seconds. *, $p < .05$; **, $p < .01$ compared with the corresponding vehicle group. ◆◆, $p < .01$ compared with the corresponding saline group. From Roozendaal B, Nguyen BT, Power A, and McGaugh JL (1999a) Basolateral amygdala noradrenergic influence enables enhancement of memory consolidation induced by hippocampal glucocorticoid receptor activation. *Proc. Natl. Acad. Sci. USA* 96: 11642–11647.

further evidence that the BLA modulates memory consolidation via regulation of Arc expression in the hippocampus (Huff et al., 2006).

Studies of BLA influences on hippocampal neuroplasticity provide additional important evidence of amygdala–hippocampal interactions (Abe, 2001). Electrical stimulation of the BLA enhances the induction of long-term potentiation (LTP) in the dentate gyrus of the hippocampus (Ikegaya et al., 1995b; Akirav and Richter-Levin, 1999; Frey et al., 2001; Almaguer-Melian et al., 2003). Also, selective lesions of the BLA or infusions of a ß-adrenoceptor antagonist into the BLA block the induction of LTP in the dentate gyrus (Ikegaya et al., 1994, 1995a, 1997). Consistent with the findings of BLA modulation of memory, norepinephrine and corticosterone both influence the effects of BLA stimulation on dentate gyrus LTP (Akirav and Richter-Levin, 2002). Recent findings indicate that electrical stimulation of the BLA also enhances LTP at cortical synapses onto striatal neurons (Popescu et al., 2007). Such findings fit well with the evidence that posttraining amygdala activation enhances consolidation of striatal-dependent memory (Packard et al., 1994; Packard and Teather, 1998).

The recent findings that Pavlovian fear conditioning induces an increase in synchronization of theta-frequency activity in the lateral amygdala and CA1 region of the hippocampus strongly suggest that activation of an amygdala–hippocampus circuit is involved in fear-based learning (Pape et al., 2005). More generally, studies of synchronized oscillatory activity occurring within the BLA suggest that such activity may facilitate the temporal lobe as well as neocortical processes involved in consolidating explicit or declarative memory (Paré, 2003; Pelletier and Paré, 2004). Importantly, there is also evidence that the firing of cells in the BLA of cats is increased greatly by a single footshock and that the increased firing lasts for at least 2 h (Pelletier et al., 2005). Such increased firing may serve to modulate memory processing in efferent brain regions, including the entorhinal cortex and hippocampus (Paré et al., 2002; Pelletier and Paré, 2004; McGaugh, 2005). This view is supported by additional physiological evidence that, in cats, during the early stages of reward-based learning, training-induced BLA activity increases the impulse transmission from perirhinal to entorhinal cortical regions (Paz et al., 2006).

3.26.6.2 BLA–Cortical Interactions in Memory Consolidation

It is now well established that posttraining infusions of drugs into various cortical regions can impair or enhance the consolidation of memory for several kinds of training (Ardenghi et al., 1997; Izquierdo et al., 1997; Baldi et al., 1999; Sacchetti et al., 1999; Malin and McGaugh, 2006). The findings of several recent studies indicate that the BLA modulates cortical functioning involved in memory consolidation. Neurons within the BLA project directly to the entorhinal cortex (Paré et al., 1995; Paré and Gaudreau, 1996; Pikkarainen et al., 1999; Petrovich et al., 2001). The memory enhancement induced by posttraining drug infusions administered into the entorhinal cortex (Izquierdo and Medina, 1997) requires a functioning BLA, as lesions of the BLA prevent the memory enhancement induced by 8-bromo-cAMP infused posttraining into the

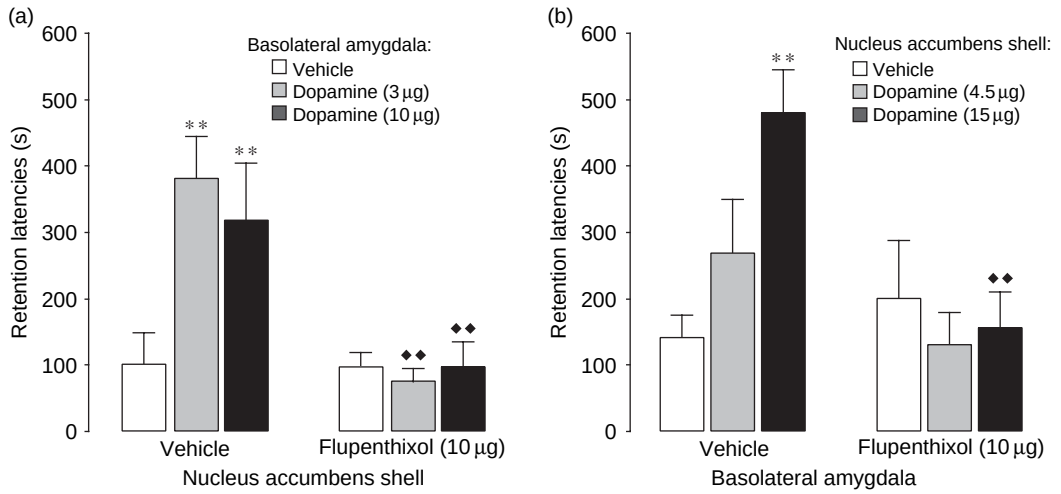

Figure 7 Modulation of memory consolidation by the basolateral amygdala or nucleus accumbens shell requires concurrent dopamine receptor activation in both brain regions. (a) Intrabasolateral amygdala infusions of dopamine (3 or 10 μg in 0.2 μl) immediately after inhibitory avoidance training produced enhancement of 48-h retention performance in rats receiving vehicle into the nucleus accumbens shell. Infusion of the general dopamine receptor antagonist *cis*-flupenthixol (10 μg in 0.3 μl) into the nucleus accumbens shell blocked the memory enhancement induced by dopamine infusions into the basolateral amygdala. (b) Intranucleus accumbens shell infusions of dopamine (4.5 or 15 μg in 0.3 μl) also induced memory enhancement, and this effect was blocked by concurrent infusions of *cis*-flupenthixol (10 μg in 0.2 μl) into the basolateral amygdala. Results represent retention latencies (mean + SEM) in seconds. **, $p < .01$ compared with the vehicle group; ◆◆, $p < .01$ compared with the corresponding vehicle group. From LaLumiere RT, Nawar EM, and McGaugh JL (2005) Modulation of memory consolidation by the basolateral amygdala or nucleus accumbens shell requires concurrent dopamine receptor activation in both brain regions. *Learn. Mem.* 12: 296–301.

entorhinal cortex (Roesler et al., 2002). Other recent studies have reported that BLA lesions or blocking of β-adrenoceptors in the BLA also block the memory-enhancing effects of 8-bromo-cAMP infused post-training into the insular cortex (Miranda and McGaugh, 2004) and of oxotremorine infused into the rostral anterior cingulate cortex (Malin et al., 2007). Additionally, the finding that lesions of the rostral anterior cingular cortex block the memory-enhancing effects of oxotremorine infused posttraining into the BLA indicates that cortical functioning is essential for BLA memory-modulatory effects (Malin et al., 2007). However, other evidence indicates that the rostral anterior cingulate cortex and the BLA serve quite different functions in memory. As discussed earlier, the anterior cingulate cortex appears to play a somewhat selective role in memory for nociceptive information, whereas extensive evidence indicates that the BLA is not dedicated to the modulation of any specific kinds of information (Packard et al., 1994; Malin and McGaugh, 2006). The BLA appears to be promiscuous in its modulation of emotionally influenced memory consolidation (McGaugh, 2002).

Other recent studies reported evidence indicating interactions between the BLA and the medial prefrontal cortex in regulating memory consolidation. Inactivation of the medial prefrontal cortex with the AMPA-receptor antagonist CNQX impairs consolidation of inhibitory avoidance memory (Liang et al., 1996). In contrast, activation of noradrenergic and dopaminergic mechanisms in the medial prefrontal cortex enhances consolidation of inhibitory avoidance and trace fear conditioning (Liang, 2001; Runyan and Dash, 2004). A GR agonist infused into the medial prefrontal cortex induces similar memory enhancement. However, and importantly, lesions of the BLA block this GR agonist–induced memory enhancement. Furthermore, consistent with the evidence of reciprocal inhibitory influences between both brain regions (McDonald, 1991; Perez-Jaranay and Vives, 1991; Rosenkranz and Grace, 2002), infusions of RU 28362 into the medial prefrontal cortex after inhibitory avoidance training increases BLA activity, as assessed with phosphorylation of extracellular-regulated kinase (ERK), a member of the mitogen-activated protein kinase family (Roozendaal et al., unpublished findings). Further,

blockade of this increase in phosphorylated ERK levels in the BLA with the MEK inhibitor PD98059 blocks the memory enhancement induced by medial prefrontal cortex GR agonist infusions. Interestingly, infusions of a GR agonist into the BLA induce a similar increase in phosphorylated ERK activity in the medial prefrontal cortex, suggesting mutual interactions between both brain regions in regulating memory consolidation.

It is likely that the BLA also influences cortical functioning, at least in part, via its projection through the stria terminalis (Price, 1981) to the nucleus basalis, which provides cholinergic activation of the cortex. Studies have reported findings suggesting that the nucleus basalis–cortical projections may be essential for learning-induced cortical plasticity (Miasnikov et al., 2001, 2006; Weinberger, 2003). Stimulation of the BLA activates the cortex, as indicated by EEG desynchronization, and potentiates nucleus basalis influences on cortical activation. Moreover, inactivation of the nucleus basalis with lidocaine blocks the BLA effects on cortical activation (Dringenberg and Vanderwolf, 1996; Dringenberg et al., 2001). Thus, the BLA may influence cortical functioning in memory consolidation, at least in part, through its effects on the nucleus basalis and consequent cholinergic activation of the cortex. In support of this suggestion, Power et al. (2002) reported that selective lesions of cortical nucleus basalis corticopetal cholinergic projections induced by 192-IgG saporin blocked the dose-dependent enhancement of inhibitory avoidance induced by posttraining intra-BLA infusions of norepinephrine (**Figure 8**). Thus, it is clear that cortical cholinergic activity is required for BLA influences on memory consolidation. **Figure 9** summarizes the interaction of the BLA with other systems in regulating memory consolidation.

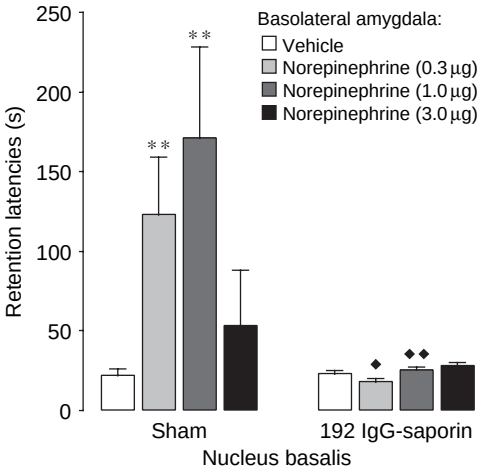

Figure 8 Lesions of nucleus basalis cholinergic neurons with 192 IgG-saporin block the memory enhancement induced by posttraining infusions of norepinephrine into the basolateral amygdala. Intrabasolateral amygdala infusions of norepinephrine (0.3, 1.0, or 3.0 ng in 0.2 µl) immediately after inhibitory avoidance training produced a dose-dependent enhancement of 48-h retention performance in sham-operated rats. Rats with 192 IgG-saporin lesions did not show memory enhancement with norepinephrine infusions. Results represent retention latencies (mean + SEM) in seconds. **, $p < .01$ compared with the saline group; ◆, $p < .05$; ◆◆, $p < .01$ compared with the corresponding sham lesion group. From Power AE, Thal LJ, and McGaugh JL (2002) Lesions of the nucleus basalis magnocellularis induced by 192 IgG-saporin block memory enhancement with posttraining norepinephrine in the basolateral amygdala. *Proc. Natl. Acad. Sci. USA* 99: 2315–2319.

3.26.7 Amygdala Activity and Modulation of Human Memory Consolidation

As discussed above, the findings of animal experiments very clearly provide extensive evidence that stress hormones released by emotional experiences influence memory consolidation and that the influence is mediated by activation of the BLA. The findings of many human studies of effects of emotional arousal, stress hormones, and amygdala activation on memory are consistent with those of animal studies (Cahill and McGaugh, 1998, 2000; Cahill, 2000; Dolan, 2000; Buchanan and Adolphs, 2004; LaBar and Cabeza, 2006). Cortisol administered to subjects prior to presentations of words or pictures enhanced subsequent recall (Buchanan and Lovallo, 2001; Abercrombie et al., 2003, 2006; Kuhlmann and Wolf, 2006). Amphetamine administered to human subjects, either before or after they learned lists of words, also enhanced long-term memory (Soetens et al., 1993, 1995). Administration of propranolol to subjects prior to their viewing an emotionally arousing slide presentation blocked the enhancing effects of emotional arousal on long-term memory (Cahill et al., 1994). Propranolol also blocks the memory enhancement produced by stress-released epinephrine (Nielson and Jensen, 1994). Further, epinephrine or cold pressor stress (which stimulates the release of adrenal stress hormones) administered to subjects after they viewed emotionally arousing slides enhanced the subjects' long-term memory of the slides (Cahill and Alkire, 2003; Cahill et al., 2003). Similar effects were produced by administration of the α_2-adrenoceptor

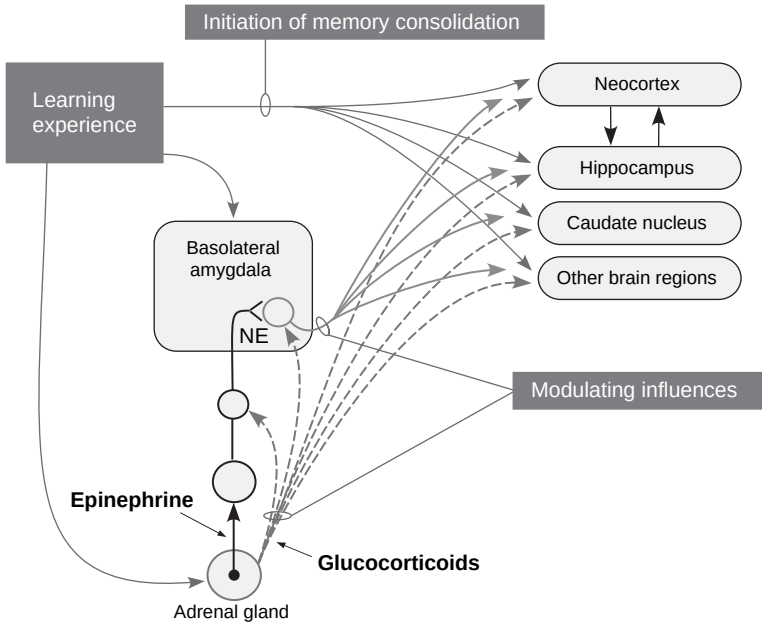

Figure 9 Schematic summarizing interactions of the basolateral amygdala with other brain regions in mediating emotional arousal–induced modulation of memory consolidation. Experiences initiate memory consolidation in many brain regions involved in the forms of memory represented. Emotionally arousing experiences also release adrenal epinephrine and glucocorticoids and activate the release of norepinephrine in the basolateral amygdala. The basolateral amygdala modulates memory consolidation by influencing neuroplasticity in other brain regions. From McGaugh JL (2000) Memory: A century of consolidation. *Science* 287: 248–251.

antagonist yohimbine, which stimulates norepinephrine release (O'Carroll et al., 1999; Southwick et al., 2002). These findings of studies of memory in human subjects are, thus, consistent with those of the animal experiments discussed above in providing evidence of the central roles of emotional activation and stress hormones in modulating memory consolidation (*See* Chapter 3.24).

Recent human studies have also provided extensive evidence that amygdala activation is involved in enabling the enhanced memory induced by emotional arousal. Memory for emotionally arousing material is not enhanced in human subjects with selective bilateral lesions of the amygdala, as it is in normal subjects (Cahill et al., 1995; Adolphs et al., 1997). Studies using positron emission tomography (PET) and functional magnetic resonance imaging (fMRI) brain imaging have provided additional evidence that the influence of emotional arousal on human memory involves amygdala activation. In the first study of the relationship between amygdala activity during encoding and subsequent memory, Cahill et al. (1996) reported that amygdala activity assessed by PET imaging as subjects viewed emotionally arousing films correlated highly (+0.93) with the subjects' recall of the films assessed in a surprise memory test 3 weeks later. Importantly, the degree of emotional arousal rather than the valence of the emotionally arousing material appears to be critical in influencing memory. In a subsequent study using PET imaging, Hamann et al. (1999, 2002) reported that amygdala activity induced by viewing either pleasant or unpleasant slides correlated highly with memory for the slides assessed 1 month later. Studies using fMRI have obtained highly similar findings. Canli et al. (2000) found that subjects' memory for a series of scenes tested 3 weeks after brain scanning correlated highly with amygdala activity induced by viewing the scenes. Furthermore, and importantly, the relationship between amygdala activity during encoding and subsequent memory was greatest for the scenes that the subjects had rated as being the most emotionally intense.

Human memory studies have provided additional evidence of the importance of noradrenergic acti vation of the amygdala. When assessed during encoding, PET imaging of amygdala activity that is assessed over many minutes of arousal as well as event-related fMRI of amygdala activity induced by single items both predict long-term memory of the

arousing stimuli (Cahill et al., 1996; Canli et al., 2000). And, importantly, β-adrenoceptor antagonists (e.g., propranolol) block the increase in amygdala activity and enhanced retention induced by emotional stimuli obtained in fMRI studies (Strange and Dolan, 2004; van Stegeren et al., 2005). Thus, β-adrenergic activation of the amygdala appears to be essential for the short-latency modulation induced by brief and mild emotional arousal used in fMRI studies as well as the effects found in human and animal studies, with longer intervals of time between learning and stress hormone activation or administration.

An additional finding of human brain imaging studies of memory studies is that, with both PET and fMRI experiments, activity of the right amygdala is related to enhanced memory in men, whereas activity of the left amygdala is correlated with enhanced memory in women (Cahill et al., 2001, 2004; Canli et al., 2001). Understanding the bases of such sex differences may provide further insights into mechanisms of emotional arousal underlying influences on memory consolidation.

Other findings based on an analysis of PET and fMRI scans provide evidence, consistent with that from many animal studies, indicating that amygdala activation influences memory processing in other brain regions. The activity of the amygdala and hippocampal/parahippocampal regions are correlated during emotional arousal (Hamann et al., 1999), and such activation is correlated with subsequent retention (Dolcos et al., 2004). The results of a 'path analysis' (structural equation modeling) study (Kilpatrick and Cahill, 2003b) of amygdala activity scanned, using PET, while subjects viewed neutral or emotionally arousing films (Cahill et al., 1996) suggest that emotional arousal increased amygdala influences on activity of the ipsilateral parahippocampal gyrus and ventrolateral prefrontal cortex. Such findings provide additional evidence that amygdala influences on activity of other brain regions are critical in creating lasting memories.

3.26.8 Involvement of the Amygdala in Modulating Memory Retrieval and Working Memory

Most studies investigating neuromodulatory influences on memory have focused on the neurobiological mechanisms underlying the consolidation of recent experiences. However, there is also much evidence that neuromodulatory systems influence memory retrieval and working memory. Most of such studies have investigated the effects of either peripherally administered hormones and neurotransmitters or the effects of direct infusions of a variety of drugs into the hippocampus and medial prefrontal cortex, brain regions that are critically involved in regulating memory retrieval and working memory. However, consistent with its role in memory consolidation, recent findings indicate that the BLA, via its projections to these brain regions, also plays an important modulatory role in regulating drug effects on these memory functions.

3.26.8.1 Memory Retrieval

Several studies investigated the effects of peripherally administered hormones and drugs on memory retrieval in rats. Stress exposure or the glucocorticoid corticosterone administered systemically shortly before testing for memory of training on inhibitory avoidance or water-maze spatial tasks (24 h earlier) produces temporary impairment of retention performance (Bohus, 1973; de Quervain et al., 1998; Yang et al., 2003; Roozendaal et al., 2004a; Pakdel and Rashidy-Pour, 2006; Sajadi et al., 2006). As the same treatments administered shortly before training do not affect either acquisition or retention performance assessed immediately after acquisition, such findings indicate that glucocorticoids impair retention by influencing memory retrieval. These findings are consistent with those indicating that stress exposure or glucocorticoids administered immediately after a learning session also impair retention performance tested 30–60 min after the session, that is, at a time when the memory trace has not yet been consolidated into long-term memory (Diamond et al., 1999; Woodson et al., 2003; Okuda et al., 2004). Similarly, as is found with memory consolidation, glucocorticoid effects on memory retrieval depend on concurrent activation of noradrenergic mechanisms. The β-adrenoceptor antagonist propranolol administered systemically 30 min before inhibitory avoidance retention testing blocks the memory retrieval impairment induced by concurrent injections of corticosterone (Roozendaal et al., 2004a). As stimulation of β_1-adrenoceptors with systemic injections of the selective agonist xamoterol induces a memory retrieval impairment comparable to that seen after corticosterone administration (Roozendaal et al., 2004b), the findings suggest that glucocorticoid effects on memory retrieval impairment involve activation of noradrenergic mechanisms. Norepinephrine

effects on memory retrieval are most likely dose dependent, as other studies reported that under other conditions, norepinephrine or noradrenergic stimulation can also enhance memory retrieval (Sara and Devauges, 1989; Devauges and Sara, 1991; Barros et al., 2001; Murchison et al., 2004). Peripheral administration of the opioid peptidergic antagonist naloxone or D2, but not D1, dopamine receptor antagonists also block the impairing effect of concurrently administered corticosterone or dexamethasone on memory retrieval (Rashidy-Pour et al., 2004; Pakdel and Rashidy-Pour, 2006). Other studies have reported that memory retrieval is also influenced by systemic administration of drugs affecting several other modulatory systems, including epinephrine, adrenocorticotropin, β-endorphin, vasopressin, acetylcholine, and serotonin (e.g., Altman et al., 1987; Izquierdo et al., 2002; Sato et al., 2004). In investigating drug effects on learning and memory, including memory retrieval, it is critically important to distinguish the effects of the drugs on memory retrieval from those on other processes that may affect the behavior used to assess memory. Not all of the studies cited above adequately controlled for such performance effects.

Many studies have reported evidence indicating that the hippocampus is involved in memory retrieval (Hirsch, 1974; Squire et al., 2001). Inactivation of the hippocampus with local infusions of the glutamatergic AMPA/kainate receptor antagonist LY326325 or the GABAergic agonist muscimol impairs memory retrieval of water-maze spatial and contextual fear conditioning tasks (Holt and Maren, 1999; Riedel et al., 1999). As the GR agonist RU 28362 administered into the hippocampus shortly before retention testing also impairs retrieval of spatial memory (Roozendaal et al., 2003, 2004b), such findings indicate that glucocorticoid-induced memory retrieval impairment depends, in part, on GR activation in the hippocampus. Consistent with the findings of experiments of peripherally administered drugs, a β-adrenoceptor antagonist infused into the hippocampus prevents the retrieval-impairing effect of a GR agonist administered concurrently (Roozendaal et al., 2004b). Other studies have shown that the effect of novelty stress on memory retrieval is blocked by intrahippocampal infusions of the AMPA receptor antagonist CNQX and the metabotropic glutamate receptor antagonist MCPG, as well as the cAMP blocker Rp-cAMPs (Izquierdo et al., 2000). In contrast, infusions of the protein synthesis inhibitor anisomycin do not block corticosterone effects on memory retrieval (Sajadi et al., 2006), suggesting that stress and corticosterone may influence memory retrieval through a rapid, protein synthesis–independent mechanism.

Retrieval of memory of emotionally arousing information also induces activation of the BLA (Hall et al., 2001; Boujabit et al., 2003). Furthermore, intra-BLA infusions of norepinephrine or CNQX affect memory retrieval of inhibitory avoidance training (Liang et al., 1996; Barros et al., 2001). In contrast, intra-BLA infusions of a GR agonist do not appear to affect memory retrieval (Roozendaal et al., 2003). However, the BLA interacts with the hippocampus in mediating glucocorticoid effects on memory retrieval. Lesions of the BLA or infusions of a β-adrenoceptor antagonist into the BLA block the impairing effect of a GR agonist infused into the hippocampus on memory retrieval (Roozendaal et al., 2003, 2004b). These findings are thus consistent with those described earlier on memory consolidation (e.g., Roozendaal and McGaugh, 1997b; Roozendaal et al., 1999a) and indicate that the BLA regulates memory retrieval via interactions with other brain regions.

The findings of studies examining stress hormone effects on memory retrieval in humans are consistent with those of animal experiments and indicate that glucocorticoids impair memory retrieval via an interaction with noradrenergic mechanisms. Stress-level cortisol or cortisone administration to human subjects impairs delayed, but not immediate, recall on episodic tasks (de Quervain et al., 2000; Wolf et al., 2001; Buchanan and Adolphs, 2004; Buss et al., 2004; Het et al., 2005; Kuhlmann et al., 2005a,b, 2006), and the β-adrenoceptor antagonist propranolol given orally blocks the impairing effect of glucocorticoids on memory retrieval (de Quervain et al., 2007). Recent findings from an $H_2^{15}O$-PET study indicate that glucocorticoid effects on memory retrieval in human subjects are also mediated, at least in part, by actions in the hippocampus (de Quervain et al., 2003). However, other findings of human imaging studies indicate that the amygdala is also activated during the retrieval of previously learned emotionally arousing material and that the effect is independent of the valence of the emotional material (Dolan, 2000). Further, recent findings of human brain imaging studies are consistent with findings of animal studies in indicating that the amygdala and hippocampus interact during the retrieval of emotionally arousing information (Dolcos et al., 2005; Greenberg et al., 2005; Smith et al., 2006).

3.26.8.2 Working Memory

Evidence from lesion, pharmacological, imaging, and clinical studies indicates that working memory, a dynamic process whereby information is updated continuously, depends on the integrity of the medial prefrontal cortex (Brito et al., 1982; Fuster, 1991; Taylor et al., 1999; Rowe et al., 2000; Levy and Fallow, 2001; Stern et al., 2001; Lee and Kesner, 2003). Decrements in prefrontal cortical function are induced by local depletion of norepinephrine and dopamine, suggesting that these monoamines regulate prefrontal cortical function (Brozoski et al., 1979; Bubser and Schmidt, 1990; Cai et al., 1993). The importance of endogenous norepinephrine and dopamine stimulation in the medial prefrontal cortex is indicated by studies in which local infusion of either noradrenergic α_2 (Li et al., 1999) or dopaminergic D1 antagonists (Sawaguchi and Goldman-Rakic, 1991; Seamans et al., 1998) administered into the medial prefrontal cortex impairs performance on working memory tasks. In contrast, activation of α_2-adrenoceptors with guanfacine improves working memory functions in rats and monkeys. Together, these data suggest that norepinephrine and dopamine are necessary for optimal medial prefrontal cortical function.

Excessive levels of norepinephrine or dopamine, however, impair working memory. The impairing effects of high doses of norepinephrine are mediated by activation of the α_1- and β-adrenoceptor (Arnsten and Jentsch, 1997; Arnsten et al., 1999; Birnbaum et al., 1999; Ramos et al., 2005). In contrast, α_2-adrenoceptor activation enhances working memory (Taylor et al., 1999). Electrophysiological studies have shown increased medial prefrontal cortical activity in the delay period during which the information needs to be retained (Fuster, 1991). Adrenergic agents that enhance working memory increase this neuronal activity during the delay period, whereas adrenergic drugs that impair working memory decrease such neuronal activity (Li et al., 1999). Dopaminergic D1 receptor agonists influence working memory following an inverted-U-shaped dose–response relationship. Too little or too much D1 receptor stimulation impairs prefrontal cortical activity and working memory in mice, rats, and monkeys (Cai and Arnsten, 1997; Zahrt et al., 1997; Li et al., 1999; Lidow et al., 2003).

Working memory deficits are also observed following exposure to stress (Arnsten and Goldman-Rakic, 1998). Mild uncontrollable stress impairs performance on a delayed alternation task but does not impair performance on nonmnemonic control tasks that have similar motivational and motor demands (Murphy et al., 1996). Mild stress, such as noise or exposure to the predator odor TMT, increases norepinephrine and dopamine turnover in the medial prefrontal cortex (Finlay et al., 1995; Morrow et al., 2000). The medial prefrontal cortex response to stress is blocked by anxiolytic benzodiazepine drugs and mimicked by the anxiogenic benzodiazepine inverse agonist FG7142 (Tam and Roth, 1985; Murphy et al., 1996; Birnbaum et al., 1999). Also, like stress, glucocorticoid administration impairs working memory. Basal levels of endogenous glucocorticoids are required to maintain prefrontal cortical function (Mizoguchi et al., 2004), but systemic injections of stress doses of corticosterone or intramedial prefrontal cortical administration of the GR agonist RU 28362 impair working memory, as assessed by delayed alternation performance, in rats (Roozendaal et al., 2004c). Additionally, stress-level cortisol treatment impairs prefrontal cortex–dependent inhibitory control of behaviors in squirrel monkeys (Lyons et al., 2000), as well as working memory performance in human subjects (Lupien et al., 1999; Young et al., 1999; Wolf et al., 2001). Glucocorticoids appear to interact with noradrenergic mechanisms in inducing working memory impairment, as systemic administration of the β-adrenoceptor antagonist propranolol blocks the working memory impairment of corticosterone administered concurrently (Roozendaal et al., 2004c). Such findings suggest that corticosterone effects on working memory impairment may involve a facilitation of noradrenergic mechanisms in the medial prefrontal cortex. This hypothesis is supported by findings of an *in vivo* microdialysis study indicating that systemic administration of corticosterone increases levels of norepinephrine in the medial prefrontal cortex (Thomas et al., 1994).

Glucocorticoid-induced working memory impairment also depends on interactions of the medial prefrontal cortex with the BLA. As discussed earlier, the BLA both sends projections to and receives projections from the medial prefrontal cortex (McDonald, 1991; Perez-Jaranay and Vives, 1991; Rosenkranz and Grace, 2002). The BLA itself does not appear to play a significant role in working memory (Wan et al., 1994; Bianchin et al., 1999; Roozendaal et al., 2004c), but lesions of the BLA block the impairment induced by either systemic administration of corticosterone or infusions of a GR agonist into the medial prefrontal cortex (Roozendaal et al., 2004c) (**Figure 10**). These findings thus indicate that the BLA interacts with the medial prefrontal cortex in regulating stress hormone effects on working memory.

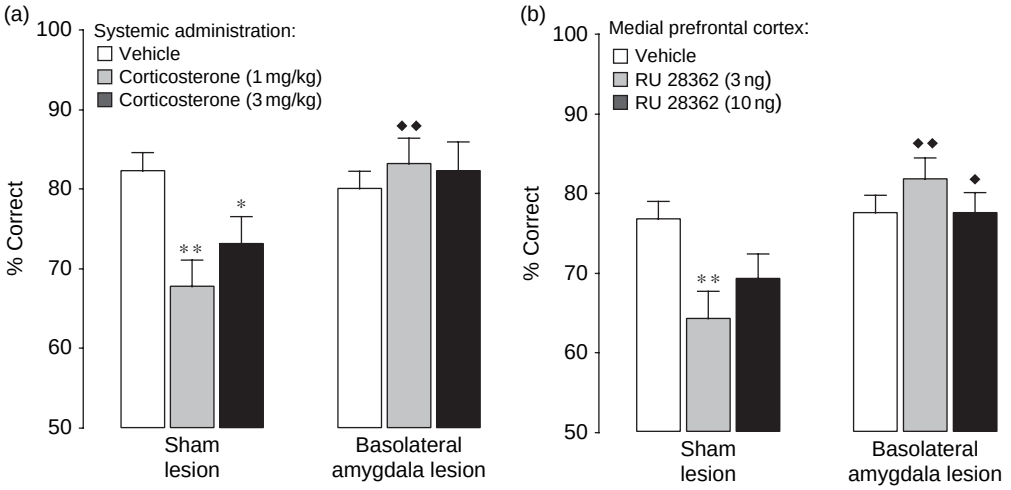

Figure 10 The basolateral amygdala interacts with the medial prefrontal cortex in mediating glucocorticoid effects on working memory. Systemic injections of corticosterone (1.0 or 3.0 mg/kg, ip) (a) or infusions of the specific glucocorticoid receptor agonist RU 28362 (3.0 or 10.0 ng in 0.5 μl) into the medial prefrontal cortex (b) impaired delayed alternation performance in sham-lesioned rats. Lesions of the basolateral amygdala blocked working memory impairment induced by either corticosterone or RU 28362. Results represent percent correct choices (mean + SEM). *, $p < .05$; **, $p < .01$ compared with the corresponding vehicle group; ◆, $p < .05$; ◆◆, $p < .01$ compared with the corresponding sham-lesion group. From Roozendaal B, McReynolds JR, and McGaugh JL (2004c) The basolateral amygdala interacts with the medial prefrontal cortex in regulating glucocorticoid effects on working memory impairment. *J. Neurosci.* 24: 1385–1392.

3.26.9 Concluding Comments

Research investigating memory modulation evolved from many sources. Key, of course, was Müller and Pilzecker's (1900) perseveration–consolidation hypothesis proposed over a century ago. The subsequent findings that treatments such as electroconvulsive shock administered after training induces retrograde amnesia (Duncan, 1949; McGaugh and Herz, 1972) provided critical evidence supporting that hypothesis. Those findings also suggested the possibility that posttraining treatments that stimulate brain activity might enhance memory consolidation. This implication was confirmed by evidence that posttraining administration of stimulant drugs enhances long-term memory (Breen and McGaugh, 1961; McGaugh, 1966, 1973). Livingston's (1967) hypothesis that activation of the limbic system might promote the storage of recently activated brain events, as well as Kety's (1972) proposal that adrenergic catecholamines released by emotional states may serve to influence consolidation, suggested physiological processes that might mediate the posttraining modulation of memory consolidation induced by stimulant drugs. Although the findings obtained in studies of memory modulation fit perhaps in a general way with these early ideas, there are many more parts to this memory modulatory system, and they interact in complex ways that were not anticipated. The early studies of adrenal stress hormone influences on memory consolidation (e.g., Gold and van Buskirk, 1975, 1976; McGaugh, 1989; McGaugh and Gold, 1989; Roozendaal, 2007) provided compelling evidence that peripheral hormones released by emotional arousals play an important role. The findings (e.g., Liang et al., 1986) suggesting that stress hormones as well as drugs affect memory by noradrenergic activation of the amygdala (i.e., the BLA) provided strong evidence suggesting that the BLA is an essential part of a memory-modulatory system. This suggestion has now been confirmed by the extensive findings that the BLA interacts with many other brain regions in modulating memory consolidation (McGaugh, 2000, 2002, 2004). The findings indicating that stress hormones modulate memory retrieval and working memory via noradrenergic influences and the BLA provide yet other chapter to the story of memory modulation (Roozendaal, 2002; Roozendaal et al., 2003, 2004a). Of course, our understanding of memory-modulatory systems is incomplete. Much more needs to be discovered about how the BLA interacts with other brain systems and how it acts to influence neuroplasticity in other brain regions involved in consolidating newly acquired information of many different kinds. But in the past several decades, research on memory modulation has made significant

progress in understanding how emotional arousal influences the consolidation and retrieval of significant experiences. Research findings have revealed at least some understanding of why it is, as William James (1890) noted, that emotional experiences may leave scars on the cerebral tissue.

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3.27 Memory-Enhancing Drugs

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3.27.1 Background

3.27.1.1 Introduction

The formation of new memories can be impaired in many ways, from lesioning brain regions to blocking neurotransmitter actions to interfering with activation of transcription factors. Thus, the ability to interfere with memory processing is readily demonstrated. Remarkably, there are also treatments that can enhance memory processing. Like studies that impair memory, evidence for enhancement of memory offers insights into possible mechanisms responsible for forming new memories. Development of drugs that enhance memory – cognitive enhancers, 'smart pills,' anti-aging drugs – also address a more romantic vision of ways to make us smarter and to keep us that way as we age. Descriptions of tests of drug enhancement of memory during aging will be included in the discussions of some of the drugs; a more complete and recent discussion of drug enhancement in aging and Alzheimer's disease can be found in Disterhoft and Oh (2006). The ability to enhance memory and other cognitive functions raises a host of ethical issues about whether and when we should use these treatments; these issues are also covered elsewhere (cf. Rose, 2002; Farah et al., 2004).

3.27.1.2 Early Studies of Drug Enhancement of Learning and Memory

Pharmacological treatments that produce amnesia receive much of the attention as tools with which to investigate the neurobiological bases of learning and memory. Many of these treatments impair memory when administered near the time of training. In particular, the findings that some treatments impair memory when given after training (i.e., the treatments produce retrograde amnesia) provide much of the evidence for memory consolidation, the view that memory formation takes time to reach completion.

There is also a significant literature showing that some drugs can enhance memory. What is perhaps the first demonstration that a drug could improve memory was provided about 90 years ago by Karl

Lashley (Lashley, 1917), who showed that injections of low doses of strychnine increased the rate of food-motivated maze learning. The current era of tests of drug enhancement of memory can be traced to McGaugh and Petrinovich (1959), who extended Lashley's findings and reported enhanced maze learning in rats that received strychnine 10 min prior to training on each of 5 days. Subsequent experiments showed that strychnine, and also pentylenetetrazol, enhanced memory in rats, mice, rabbits, and cats. Enhanced memory was evident for a wide range of tasks in addition to food-motivated mazes, including discrimination learning, avoidance learning, and classical conditioning (Kelemen and Bovet, 1961; McGaugh and Thomson, 1962; Hunt and Krivanek, 1966; Benevento and Kandel, 1967; Cholewiak et al., 1968).

3.27.1.3 The Posttraining Design

These early findings were very encouraging, but the designs of the experiments left open many interpretations of the effects beyond specific actions on learning and memory processing. For example, strychnine might alter exploration in a manner that fostered better learning (Whishaw and Cooper, 1970). This is just one of many performance variables – others include sensory acuity, motivation to find food, attention, and retrieval mechanisms – that could lead to more rapid learning by mechanisms other than effects on memory. Considerations such as these led to the use of an experimental design shared with studies of retrograde amnesia. These are demonstrations that treatments administered after training can retroactively impair or enhance memory (Duncan, 1949; McGaugh, 1966).

Retrograde effects on memory provide much of the support for studies of memory consolidation (McGaugh, 1966, 2000; Gold and McGaugh, 1975; Gold, 2006). In addition to addressing issues regarding memory consolidation, the use of the posttraining design is very important for distinguishing between drug enhancement of memory formation and modification of performance variables (Gold and McGaugh, 1978; Gold, 1986; McGaugh, 1989). With a posttraining design, all subjects – control and experimental alike – are trained in the absence of drug treatment. Because the drug is administered after training, there can be no differences in performance variables such as motivation or locomotor activity during training that can explain later improvements on tests of memory. The tests of memory are generally administered 24 h or more after training and drug treatment, at a time when the direct effects of the drug have dissipated. The additional demonstration of a retrograde enhancement gradient, in which the enhancement of memory decreases as the time between training and treatment increases, is an important procedure to ensure that the drug administered after training is not having residual effects on memory tests given later, typically 1 or more days after the training-treatment procedure. For example, consider findings that a drug enhances memory at 24 h after training if the drug is administered within a few minutes of training but not if administered 4 h later. Restated, the findings show that the drug-induced enhancement of memory is evident when the drug is administered 24 h before testing but not 20 h before testing. Findings such as these do not support interpretations of the enhanced memory as the result of lingering effects of the drug, but instead convincingly demonstrate the absence of a proactive effect on memory processing during testing.

3.27.1.4 Posttraining Drug Enhancement of Memory

The posttraining design has been used to great benefit in the era of drug enhancement of memory that began in the 1960s and continues now. McGaugh and colleagues led the studies that examined the efficacy of a wide range of drugs in enhancing memory for learning in many tasks in rodents (McGaugh, 1966, 1989, 2000). In most of these experiments, rats were trained daily and received the drug treatment at variable delays after training. The major overall findings were that (1) posttraining administration of any of several drugs could enhance memory, (2) the dose-response was an inverted-U or more complex function, and (3) the effects on memory decreased with time between training and testing. Most of the drugs found to enhance memory were stimulants, including strychnine, pentylenetetrazol, caffeine, and amphetamine, among others. There were also differences reported in the dose-response functions across rat and mouse strains, across ages, and across tasks. Some of these variables will be discussed again later in considering hormonal enhancement of memory. Of interest, memory enhancement was evident not only for appetitive and aversive tasks, but also for latent memory, for example, learning about a spatial maze on 'pretraining' trials administered without reward or punishment (McGaugh, 1989). In addition, the posttraining design has been used effectively in

demonstrating enhancement of memory in humans with drugs and other treatments (Parker et al., 1980; Manning et al., 1992; Soetens et al., 1993; Nielson et al., 1996; Bruce et al., 1999; Scholey and Fowles, 2002; Cahill and Alkire, 2003).

3.27.1.5 Memory Consolidation versus Memory Modulation

One of the striking features that came from studies of enhancement of memory with drugs, as well as impairments of memory with many treatments, was that the time courses of retrograde enhancement and retrograde amnesia were very different in different experimental conditions (Gold and McGaugh, 1975; Gold, 2006). Across amnestic treatments, retrograde amnesia and enhancement gradients might be as short as 500 ms or as long as weeks. The variability in the time windows during which memory is susceptible to modification has important implications for the goals of studies of memory consolidation, whether using treatments that enhance or impair memory. One of the key goals of memory consolidation experiments has been the identification of the time needed to form new memories, a time often based on the temporal properties obtained in retrograde amnesia or enhancement studies. One set of temporal properties comes from retrograde amnesia and enhancement gradients. Because these are highly variable, it is difficult to use any one temporal gradient to discuss the time needed for memory formation. A second set of temporal properties is often identified through amnesia studies that test the time after training to the onset of amnesia. These studies also yield a wide range of time parameters, from minutes to many hours (cf. Gold, 2006). Thus, it seems very difficult to fit these times, with poor temporal constraints, into a framework of memory consolidation, a practice nonetheless prevalent even today in investigations into cellular and molecular mechanisms of memory.

If memory consolidation studies do not yield a time constant for the formation of memory, then the issue is why do memories remain susceptible to modification during the time soon after training? It was thinking such as this that led to the development of an alternative way to think about drugs that enhance memory. Perhaps if retrograde amnesia and enhancement gradients do not reflect the time needed for memory formation, the gradients reflect the ability of some endogenous responses to experience to modulate later memory for that experience. Simply put, events that lead to high arousal, and particularly the neurochemical and hormonal consequences of the arousal, will modulate later memory (Gold and McGaugh, 1975; Gold, 1987; Cahill and McGaugh, 1998). And it is clear in many contexts that experiences with high arousal and affect are those best remembered.

The special role of postexperiential modulation of memory is well illustrated for learning about events that are unexpected and rather quick. Two examples are experiencing a near-accident while driving through an intersection or receiving a footshock when entering a dark compartment. In both instances, the neuroendocrine responses to the incidents largely come after, not before, the experience. The idea of modulation of memory is that the formation of new memories retains the capacity to be regulated by the ensuing neurobiological components of emotion and arousal. In general terms, the implication is that organismic responses that evolved to respond physiologically to potentially dangerous situations, including both endocrine and brain responses, were adopted by brain processes involved in the formation of memory as mechanisms through which to select from all experiences those that should be best remembered.

A major implication of this view is that memory for a mild experience will be made stronger by administration of treatments that activate some of the biological responses associated with emotion and arousal. In particular, this thinking predicts that treatments that activate or mimic hormonal and neurochemical functions related to emotion and arousal will enhance memory. As described next, these are precisely the results obtained in more recent studies that examine drugs aimed at hormones and neuromodulators.

3.27.2 Peripheral Factors

3.27.2.1 Epinephrine

Epinephrine is perhaps the hormone best studied and best understood as an enhancer of memory formation processes. In early experiments (Gold and van Buskirk, 1975), rats were trained in a one-trial inhibitory avoidance task using a footshock of low intensity. Soon after training, the rats received an injection of epinephrine. When tested for memory 24 h later, those rats that received the epinephrine had higher avoidance latencies (**Figure 1**, left graph). The effects were time-dependent; enhancement of memory decreased as the time after training increased. In addition, the

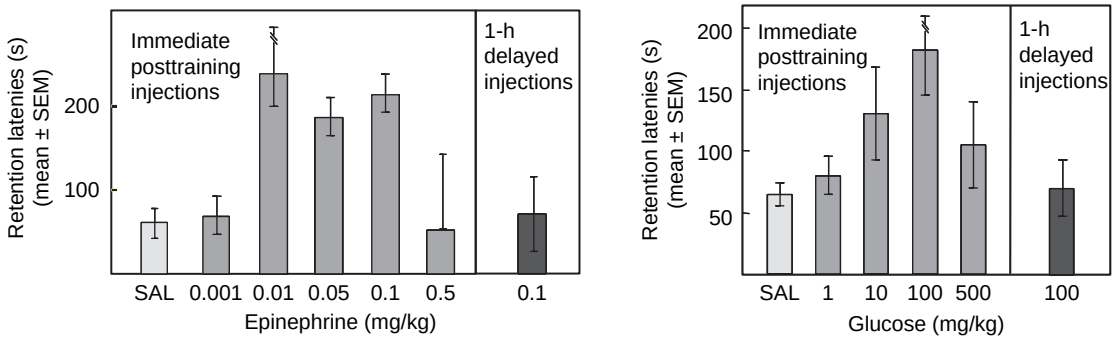

Figure 1 Dose-response function for epinephrine and glucose effects on memory in rats. Rats were trained in a one-trial inhibitory avoidance task; received injections of saline (SAL), epinephrine, or glucose immediately after training; and were tested 24 h later. Note the inverted-U dose-response curve for enhancement of memory seen on the test trial. Note also that injections of epinephrine or glucose 1 h after training did not significantly enhance memory on tests 24 h later. Left from Gold PE and van Buskirk RB (1975) Facilitation of time dependent memory processes with posttrial epinephrine injections. *Behav. Biol.* 13: 145–15; used with permission; right from Gold PE (1986) Glucose modulation of memory storage processing. *Behav. Neural Biol.* 45: 342–349; used with permission.

effects were dose-dependent, following an inverted-U dose-response curve. When circulating levels of epinephrine were measured after training and epinephrine injections, the findings indicated that the epinephrine dose optimal for enhancing memory resulted in peak circulating levels comparable to those seen after training with more intense footshock (Gold and McCarty, 1981; McCarty and Gold, 1981). Thus, epinephrine apparently mimicked an endogenous response to training, and the mimicry promoted the formation of new memory much as would a higher footshock level.

Later experiments showed that epinephrine, like strychnine and analeptics studied earlier, enhanced memory for a wide range of tasks, including not only avoidance tasks but also food- and water-motivated mazes, unrewarded spontaneous alternation measures of spatial working memory, and habituation and extinction (Gold, 1995). In humans, epinephrine enhances verbal memory for emotional information (Cahill and Alkire, 2003).

The proximal mechanisms by which epinephrine enhances memory have received considerable attention. Circulating epinephrine is largely excluded from the brain (Axelrod et al., 1959), indicating that it is likely that the hormone acts directly on peripheral targets in a manner that leads to regulation of brain functions. There are two main positions, which are not mutually exclusive, regarding the nature of the peripheral target. One position is that epinephrine acts on β-adrenergic receptors on the vagus nerve, with vagal afferents carrying to the brain information about the epinephrine signal (Williams and Clayton, 2003). The principal evidence for this view is that epinephrine effects on memory are blocked by inactivation of the vagus terminal field in the nucleus tractus solitarius (Clayton and Williams, 2000). Furthermore, posttraining vagal stimulation enhances memory in both rats and humans (Clark et al., 1998, 1999) and results in release of norepinephrine in the amygdala (Hassert et al., 2004). Together, the findings suggest that epinephrine may regulate brain memory processes via actions on vagal afferents. More direct tests (e.g., blockade of the effects of epinephrine on memory in vagotomized rats, neurophysiological measures of vagal activity, and neurochemical measures in the vagal terminal field, the nucleus tractus solitarius) have not yet been performed.

The effects of epinephrine on memory are also blocked by inactivation of the locus coeruleus or amygdala, as well as by blockade of β-adrenergic receptors in the amygdala, the latter presumably blocking central norepinephrine actions and not direct actions in the amygdala of circulating epinephrine. In addition, epinephrine injections result in release of central norepinephrine throughout the brain, including the amygdala (Gold and van Buskirk, 1978a,b; Williams et al., 1998). A second proximal mechanism proposed to mediate epinephrine enhancement of memory is based on a classic physiological response to epinephrine release from the adrenal medulla, an increase in blood glucose levels mediated in large part by liberation of hepatic glucose stores (McNay and Gold, 2002). These findings comprise the next section.

3.27.2.2 Glucose

Posttraining injections of glucose enhance memory in time- and dose-dependent manner similar to that of epinephrine (**Figure 1**, right graph). The dose-response function has an inverted-U form, with maximal enhancement of memory obtained at doses that lead to blood glucose levels similar to those attained after epinephrine doses that enhance memory (Gold, 1986; Hall and Gold, 1986). In addition, although enhancement of memory with epinephrine is blocked by peripheral coadministration of adrenergic receptor antagonists (Gold and van Buskirk, 1978b), enhancement of memory by glucose remains intact in the presence of adrenergic antagonists (Gold, 1986). In addition, modest food restriction, to deplete hepatic glucose stores, reduces the efficacy with which epinephrine but not glucose enhances memory (Talley et al., 2000); enhancement of memory with glucose is also intact after vagotomy (Talley et al., 2002) These findings suggest that increases in blood glucose levels subsequent to epinephrine release contribute importantly to the effects of the hormone on memory.

Like epinephrine, glucose enhances memory after training on a wide range of aversive tasks, including inhibitory avoidance (Gold, 1986; Kopf et al., 2001; Rashidy-Pour, 2001), conditioned emotional response (Messier and White, 1984), and swim (Oomura et al., 1993; Li et al., 1998) tasks. Although tests of glucose, like those of epinephrine, grew from considerations of stress and arousal responses and therefore initially involved aversive tasks, glucose also enhances memory for nonaversive tasks, including spatial memory in a radial arm maze and nonmatching to sample task (Winocur and Gagnon, 1998), spatial working memory in spontaneous alternation tasks (Ragozzino et al., 1996; McNay and Gold, 2002), operant bar pressing (Messier et al., 1990), and habituation of exploratory behavior (Kopf and Baratti, 1996). Moreover, glucose also enhances verbal memory in humans, including in healthy young adult and aged populations, as well as in individuals with Down syndrome and Alzheimer's disease (Manning et al., 1993; for reviews, see Korol, 2002; McNay and Gold, 2002; Messier, 2004; Gold, 2005). In individuals with Alzheimer's disease, the effects of glucose were particularly pronounced both in terms of percent improvement and breadth of cognitive domains (**Figure 2**). Of interest, the dose of glucose that enhances memory in humans results in increases in circulating levels comparable to those related to glucose and epinephrine enhancement of memory in rodents. The pervasive enhancement of memory by glucose across multiple classes of memory and species suggests that glucose may play a key role in regulating neural plasticities involved in the formation of memory in multiple neural systems. Because of this, the mechanisms by which glucose mediates its effects on memory are important for understanding basic properties of memory formation. In addition, development of drugs targeting these mechanisms has the potential to enhance memory in human populations, particularly those with cognitive dysfunctions.

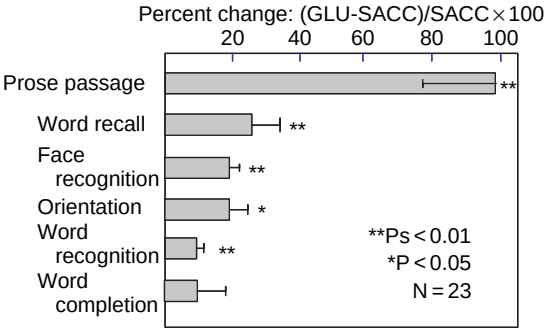

Figure 2 Glucose enhancement of cognitive functions in individuals with Alzheimer's disease. The patients were each tested on two occasions, once soon after ingesting a fruit drink sweetened with saccharin (SACC, control) and once after ingesting a fruit drink sweetened with glucose (GLU). On the glucose treatment day compared to the saccharin treatment day, the patients performed better when tested for memory of new information, on a narrative prose passage and word recall tests, as well as retrieval of old information included in the face recognition and orientation tests. From Manning CA, Ragozzino M, and Gold PE (1993) Glucose enhancement of memory in patients with Alzheimer's disease. *Neurobiol. Aging* 14: 523–528; used with permission.

In contrast to circulating epinephrine, which is excluded from the brain, glucose has ready access to brain targets via a facilitated transport system (Choeiri et al., 2005; McNay and Gold, 2002). The direct access to the brain opens the possibility that glucose effects on memory are a result of direct brain actions. Consistent with this possibility, microinjections of glucose into the lateral ventricles (Lee et al., 1988) or into any of several neural sites also enhance memory. For example, glucose injections into the medial septum (Ragozzino et al., 1995; Stefani and Gold, 1998; Talley et al, 1999), hippocampus (Ragozzino et al., 1998; Krebs and Parent, 2005), and amygdala (Ragozzino et al., 1994; Lennartz et al., 1996; McNay and Gold, 1998) all enhance memory for some tasks, presumably by upregulating the contributions of these systems to memory.

During the past decade, it has become clear that different brain systems are especially important for processing different attributes of memory (cf. White and McDonald, 2002; Poldrack and Packard, 2003; Gold, 2004; Korol, 2004). Consistent with this view, microinjections of glucose into different brain regions are generally effective at enhancing memory in those tasks typically associated with each memory system. The roles of multiple memory systems are, however, more complex than just separation of functions. In many instances, damage to or inactivation of one system can enhance learning associated with a different system. These demonstrations are taken as evidence that memory systems at times compete for control over learning and memory functions. If damage of a brain region can enhance learning and memory for a task best associated with another neural system, then enhancement of processing in one brain area might impair learning and memory for a noncanonical task. Like hippocampal damage or inactivation, glucose injections into the striatum impaired learning of a spatial task, suggesting that glucose upregulated striatal processing in a manner that interfered with hippocampal functions (Pych et al., 2006). Related to these issues, glucose injections into the hippocampus or striatum alter the preference for selection of place and response strategies (Canal et al., 2005) in a T-maze that can be solved using either strategy (Tolman et al., 1946; Packard and McGaugh, 1996).

These findings raise significant issues important for developing drugs to enhance learning and memory. If multiple memory systems compete with each other for control over learning, systemically administered drugs might enhance the memory processing of brain systems that are positively and negatively associated with learning the task. Yet, the findings suggest otherwise. As noted earlier, most drugs that enhance memory do so for a wide range of tasks. Assessments of fluxes in extracellular glucose levels during learning as well as measures of glucose effects on neurotransmitter functions offer clues that might reconcile this apparent paradox. Glucose levels in the brain appear to respond to training in a region specific manner. Specifically, extracellular glucose levels are depleted in the hippocampus but not the striatum when rats are engaged in a spatial working memory task (McNay et al., 2001). Systemic injections of glucose block that depletion but, in the absence of depletion, do not appear to increase extracellular glucose levels. Thus, there may be regional specificity that links delivery of glucose to those brain areas that are engaged by the task.

Similar relationships appear in examinations of release of acetylcholine in the hippocampus while rats are tested on a spatial working memory task and receive glucose to enhance memory. In this experiment (Ragozzino et al., 1996), glucose augmented the testing-related increase in release of acetylcholine in the hippocampus. Importantly, however, glucose did not lead to an increase in acetylcholine release in control animals resting in a holding cage. Thus, the neurochemical effects of glucose were restricted to conditions under which the brain area was activated. In showing that glucose delivery and neurochemical consequences were sensitive to cognitive functions, the findings suggest that other systemic drug treatments might similarly be amenable to functional targeting of action. This possibility has important implications for development of memory-enhancing drug treatments and needs more experimental investigation.

At a mechanistic level, one promising candidate for glucose effects on memory is that glucose may act by modulating the conductance of central adenosine triphosphate-sensitive potassium (K-ATP) channels. These channels are best characterized for their significant contribution to the resting baseline membrane potential of pancreatic β-cells and thereby regulate of insulin secretion (Ashcroft, 2005; Hansen, 2006). When blood glucose levels rise, intracellular ATP levels increase. The high ATP levels inhibit the K-ATP channels and depolarize the cells, activating voltage-sensitive Ca^{++} channels with an influx of Ca^{++} triggering release of insulin. Because of the significance of these channels to regulation of blood glucose levels, including in patients with diabetes, there is a relatively large repertoire of drugs aimed at these channels, particularly including drugs that act at a sulfonylurea regulatory subunit of the K-ATP channels to open and close the channels. K-ATP channel conductance is decreased by increases in intracellular ATP levels and by sulfonylurea-class drugs such as glibenclamide and is increased by decreases in intracellular ATP concentration and by drugs such as lemakalim.

Of potential interest to understanding glucose effects on brain function, K-ATP channels exist in the brain and are widely distributed (cf. Liss and Roeper, 2001). In the ventromedial hypothalamus, the channels appear to be important in regulating food intake, apparently sensing extracellular glucose levels in manner much like that seen in pancreatic β-cells. The wide brain distribution of the channels points to other functions as

well, in particular linking cellular excitability and metabolic states. With findings showing dynamic changes in extracellular glucose levels in the context of memory tests, fluctuations in extracellular glucose may modulate cell excitability. According to this view, decreases in K-ATP channel conductance would increase cellular sensitivity to depolarizing stimuli and increase the likelihood of stimulus-evoked neurotransmitter release, a possibility for which there is a good deal of evidence (e.g., Amoroso et al., 1990; Fellows et al., 1993; Panten et al., 1996). Additionally, there may be significant contributions of K-ATP channels to regulation of excessive glutamate release and subsequent excitotoxity after hypoxia and other brain insults, as well as involvement in neurodegenerative conditions like Alzheimer's disease and Parkinson's disease (Haddad and Jiang, 1994; Dirnagl et al., 1999; Yamada et al., 2001; cf. Liss and Roeper, 2001).

With respect to learning and memory, injections of glibenclamide, a drug that closes K-ATP channels, attenuated impairments of inhibitory (passive) avoidance memory produced by intraventricular injections of various K-ATP channel openers (Ghelardini et al., 1998). Several experiments have examined the effects on memory of direct brain injections of drugs that act at the K-ATP channel. The findings support the view that glucose may modulate learning and memory processes by acting on central K-ATP channels. Injections of glibenclamide into either the medial septum or the hippocampus result in enhanced memory (Stefani and Gold, 1998, 2001; Stefani et al., 1999). Conversely, injections of lemakalim or galanin, drugs that open the K-ATP channel, impair alternation performance; these impairments can be reversed by concomitant glucose administration (**Figure 3**). With systemic injections as well, drugs that open and close K-ATP channels block and potentiate, respectively, glucose enhancement of memory (Rashidy-Pour, 2001). Moreover, a recent paper suggests that the K-ATP channel blocker, glibenclamide, can override central nervous system deficits observed in humans under experimental hypoglycemia (Bingham et al., 2003). Subjects made hypoglycemic via glucose/insulin clamps exhibited impaired performance on a four-choice reaction time test. The impairments were reversed by pretreatment with glibenclamide. These findings add to the evidence that K-ATP channels may be a step subsequent to glucose actions in regulating brain and cognitive functions.

An elegant approach to understanding the mechanism by which glucose enhances memory is found in a recent paper that examined possible molecular pathways that might be regulated by glucose (Dash et al., 2006). The experiments examined possible glucose activation of the tuberous sclerosis complex-mammalian target of rapamycin (TSC-mTOR) pathway. The selection for investigation of the TSC-mTOR cascade was based on a convergence of signaling pathways important for mediating the effects of growth factors in promoting protein synthesis important for memory formation with the pathways involved in nutrient-mediated growth processes. Rats were weakly trained on the hidden platform version of the swim task. The rats received posttraining injections of glucose or of treatments that impair the TSC-mTOR pathway. The findings indicate that glucose activated the pathway and enhanced memory, whereas treatments that interfered with the pathway impaired memory (**Figure 4**). Moreover, glucose was unable to enhance memory in the presence of inhibition of the TSC-mTOR pathway. In addition to supporting a specific molecular basis for glucose enhancement of memory, this experiment also opens a new avenue of investigation in which drugs long known to enhance memory can be used to identify the molecular bases of memory formation. Moreover, the findings open the possibility of future drug development targeting, from a multitude of potential actions of glucose, to those cellular and molecular responses specifically related to memory formation.

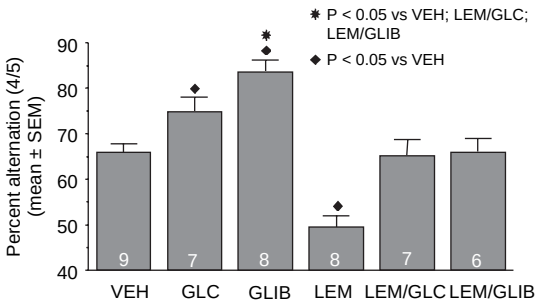

Figure 3 Effects of K^+-ATP channel modulators on memory in young rats. Drugs were injected into the hippocampus prior to spontaneous alternation testing on a 4-arm plus-shaped maze. Like glucose (GLC), the channel opening drug glibenclamide (GLIB) enhanced alternation scores, while the channel closing drug lemakalim (LEM) impaired alternation scores. Combination treatments were additive. VEH, vehicle. From Stefani MR and Gold PE (2001) Intra-hippocampal infusions of K-ATP channel modulators influence spontaneous alternation performance: Relationships to acetylcholine release in the hippocampus. *J. Neurosci.* 21: 609–614; used with permission.

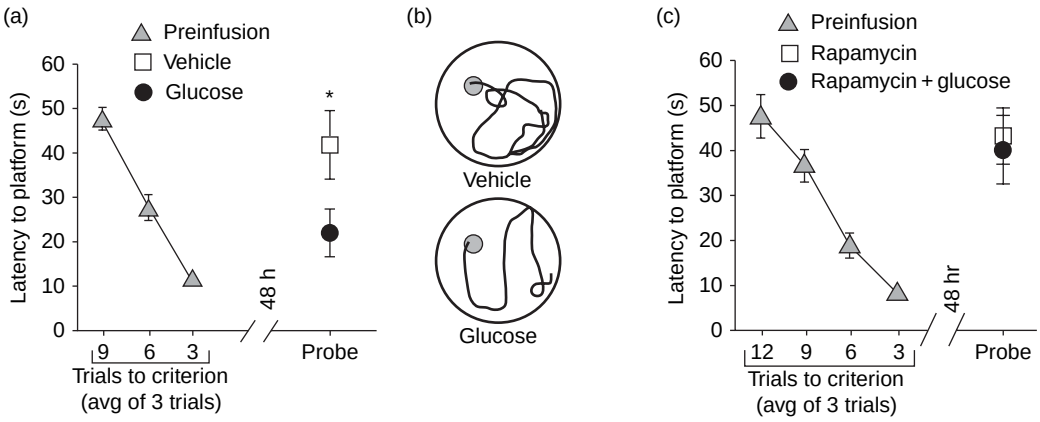

Figure 4 (a) Posttraining infusion of glucose augments long-term spatial memory. Animals were trained in the hidden platform version of the Morris water maze by using a soft criterion and then were infused with either vehicle ($n = 9$) or 20 μg/hippocampus glucose ($n = 10$). Training and probe trial performance (latency) are shown. (b) Representative probe trial traces of a vehicle-infused and a glucose-infused animal showing the path taken before the first platform crossing. Glucose-infused animals had a reduced mean distance traveled and an increased number of platform crossings when compared with vehicle-infused rats (not shown) (c) Posttraining administration of glucose did not enhance memory in the presence of the mTOR inhibitor rapamycin. Animals were infused with either 0.9 ng of rapamycin/hippocampus or 0.9 ng of rapamycin plus 20 μg of glucose/hippocampus. Error bars indicate SEM. From Dash PK, Orsi SA, and Moore AN (2006) Spatial memory formation and memory-enhancing effect of glucose involves activation of the tuberous sclerosis complex–mammalian target of rapamycin pathway. *J. Neurosci.* 26: 8048–8056; used with permission.

Pharmacological enhancement of memory by epinephrine and glucose has yielded interesting relationships with age-related impairments of memory (Gold, 2004, 2006). Aged rats and mice exhibit rapid forgetting across tasks, including inhibitory avoidance tasks. A hypothesis that decreases in release of epinephrine from the adrenals into blood during training might be diminished in aged rats was incorrect. Contrary to the hypothesis, epinephrine release after simple handling or after footshock was substantially greater in aged (2-year-old) rats than in young adult rats (Mabry et al., 1995a,c). Under conditions of greater stress such as immersion in cold water as in the swim task, aged rats produced extraordinarily high circulating levels of epinephrine as compared to young rats (Mabry et al., 1995b).

An explanation for the surprising result that aging was accompanied by increased rather than decreased release of a key memory-enhancing hormone comes from studies of differences in increases in blood glucose in young and old rats after stress. Aged rats exhibit increases in glucose levels that are absent at low levels of stress or doses of injected epinephrine and diminished at high levels of stress or epinephrine dose (Mabry et al., 1995a). Thus, the findings open the possibility that there is an impairment of the mechanisms by which epinephrine leads to glucose liberation from hepatic stores. Related to this view is evidence that glucose enhances memory in aged rats (McNay and Gold, 2001). Moreover, when engaged in a spatial working memory task sensitive to manipulations of the hippocampus, extracellular levels decline in both young and aged rats, but do so to much greater magnitude and duration in aged rats. Injections of glucose that enhance memory in aged rats block this considerable depletion of extracellular glucose levels in the hippocampus. Whether these endocrine, neurochemical, and behavior results are related to glucose effects on neurotransmitters like acetylcholine or are based on other actions of glucose requires additional attention.

3.27.2.3 ACTH and Glucocorticoids

In addition to enhancement of memory by epinephrine and glucose, there is extensive evidence showing that other humoral factors also enhance memory. A spate of papers in the 1970s and 1980s examined the effects of adrenocorticotropic hormone (ACTH) on memory (cf. de Wied, 1990). As with other drugs that enhance memory, ACTH did so with an inverted-U dose-response curve and in a retrograde time-dependent manner (Gold and van Buskirk, 1976). One of the most intriguing aspects of

that work was the identification of the site on the ACTH molecule most important for enhancing memory (de Wied, 1999). The 39 amino acids that comprise ACTH are cleaved from the precursor protein proopiomelanocortin. Most of the memory-enhancing properties were retained with the small peptide $ACTH_{4-9}$. Importantly, although the small peptides had effects on memory formation, they did not lead to the release of corticosterone from the adrenal cortex. Thus, the peptides had actions on memory that did not depend on the classic hormonal response to ACTH. Perhaps because of limitations on the analytical and pharmacological methods of the time, these studies were not continued in parallel with those of epinephrine and glucose.

Of more intense current interest is the contribution of glucocorticoids to memory formation. Although not necessary for the effects on memory of the small ACTH-based peptides, corticosterone itself also enhances memory for many tasks, with characteristics again including inverted-U dose-response curves and retrograde temporal gradients (cf., McEwen, 2001; Luine, 2002; Roozendaal, 2002; Wolf, 2003; Diamond et al., 2004; Sandi, 2004; Korol and Gold, 2007). The inverted-U dose-response curve may result from differential binding at low and high concentrations to mineralocorticoid and glucocorticoid receptors (Oitzl and de Kloet, 1992; Pavlides et al., 1996; Ahmed et al., 2006).

As is true for many modulators, the amygdala appears to be important for mediating the effects of corticosterone on memory (Roozendaal, 2002, 2003). Lesions of the stria terminalis or injections of β-adrenergic antagonists into the amygdala block the memory enhancement produced by systemic corticosterone administration.

Glucocorticoid enhancement of memory may be accomplished by interactions with norepinephrine modulation of memory. In particular, enhancement of memory after intra-amygdala injections of a glucocorticoid agonist was blocked by coadministration of either a β-adrenergic receptor antagonist or a protein kinase A inhibitor (Roozendaal et al., 2002). In the converse experiment, a glucocorticoid antagonist attenuated memory enhancement produced by a β-adrenergic receptor agonist, but not by an analog of adenosine 3′,5′-cyclic monophosphate (cAMP), a presumed downstream product of norepinephrine actions important for the enhancement of memory. Thus, the interaction of glucocorticoids and norepinephrine to enhance memory may come at a cell locus between norepinephrine activation of postsynaptic β-receptors and the initiation of a molecular cascade through cAMP. A schematic of possible interactions of glucocorticoids with neurotransmitters and cAMP is shown in **Figure 5**.

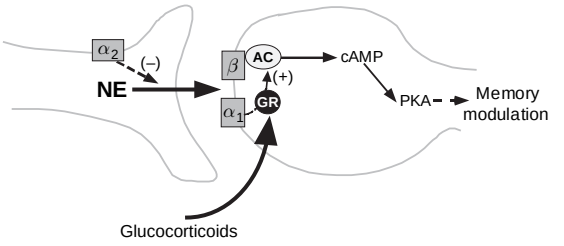

Figure 5 Schematic summarizing glucocorticoid effects on the β-adrenoceptor-AMP/protein kinase A (PKA) signaling pathway in the basolateral nucleus of the amygdala in influencing memory consolidation. Norepinephrine (NE) is released following training in aversively motivated tasks and binds to both β-adrenoceptors and α_1-adrenoceptors at postsynaptic sites. The β-adrenoceptor is coupled directly to adenylate cyclase to stimulate cAMP formation. The α_1-adrenoceptor modulates the response induced by β-adrenoceptor stimulation. Glucocorticoids may facilitate the β-adrenoceptor-cAMP system via a coupling with α_1-adrenoceptors. Other studies have demonstrated that cAMP may initiate a cascade of intracellular events involving activation of cAMP-dependent protein kinase (PKA). Our findings suggest that these effects in the basolateral amygdala are required for regulating memory consolidation in other brain regions. Abbreviations: α_1, α_1-adrenoceptor; α_2, α_2-adrenoceptor; AC, adenylate cyclase; β, β-adrenoceptor; GR, glucocorticoid receptor; cAMP, adenosine 3′,5′-cyclic monophosphate. From Roozendaal B, Quirarte GL, and McGaugh JL (2002) Glucocorticoids interact with the basolateral amygdala b-adrenoreceptor-cAMP/PKA system in influencing memory consolidation. *Eur. J. Neurosci*. 15: 553–560; used with permission.

3.27.2.4 Estrogen

Estrogen appears to act both to enhance memory and to regulate the cognitive strategy used to solve learning tasks (cf. Dohanich, 2002; Korol, 2004; Korol and Gold, 2007). Posttraining injections of estrogen enhance memory in rodents trained in the swim task (Packard and Teather, 1997a,b; Gresack and Frick, 2006), inhibitory and active avoidance tasks (Farr et al., 2000; Rhodes and Frye, 2006), and object recognition task (Gresack and Frick, 2006). Estrogen regulation of acetylcholine release at the time of training may contribute to the effects on memory (Marriott and Korol, 2003; Gibbs et al., 2004).

In contrast to other drugs noted earlier, estrogen appears to enhance learning and memory for some tasks and to impair learning and memory for others (Korol, 2004). The differences might be mediated by differential actions of estrogen on multiple memory systems (White and McDonald, 2002; Gold, 2004; Kesner and Rogers, 2004; White, 2004). Direct comparisons of estrogen effects across different versions of a food-motivated maze reveal that estrogen, administered for 2 days prior to training, enhances memory for hippocampus-sensitive place learning but impairs memory for striatum-sensitive response learning (**Figure 6**; Korol and Kolo, 2002). Estrogen status is also related to whether rats select place or response solutions to a T-maze that can be solved equally well using either strategy. Naturally cycling female rats preferentially exhibit place response at high estrogen and response strategies at low estrogen phases of their estrus cycle (Korol et al., 2004). Also, ovariectomized rats given estrogen choose place strategies, whereas those not given estrogen show response strategies. These findings suggest that estrogen can be viewed at once as a memory-enhancing and a memory-impairing treatment, depending on the specific task attributes. These differential effects seem likely to have their bases in actions of estrogen on different memory systems. Consistent with this view, estrogen administered directly to the hippocampus or striatum reveals enhanced memory for spatial tasks and for response tasks, respectively (Zurkovsky et al., 2007).

One aspect of the interest in estrogen and memory is that decreases in estrogen levels might lead to cognitive changes at the time of menopause. In addition to findings obtained in rodents, as earlier, support for this view came from several experiments that examined the effects of hormone replacement therapies on women, finding evidence for enhancement of memory in several experimental settings (for reviews see: Sherwin, 2006; Maki, 2006). In contrast, the findings of the large randomized controlled trials in the Women's Health Initiative Memory (WHIM) study failed to support the idea that hormone replacement therapy protected against cognitive decline in postmenopausal women (Espeland et al.,

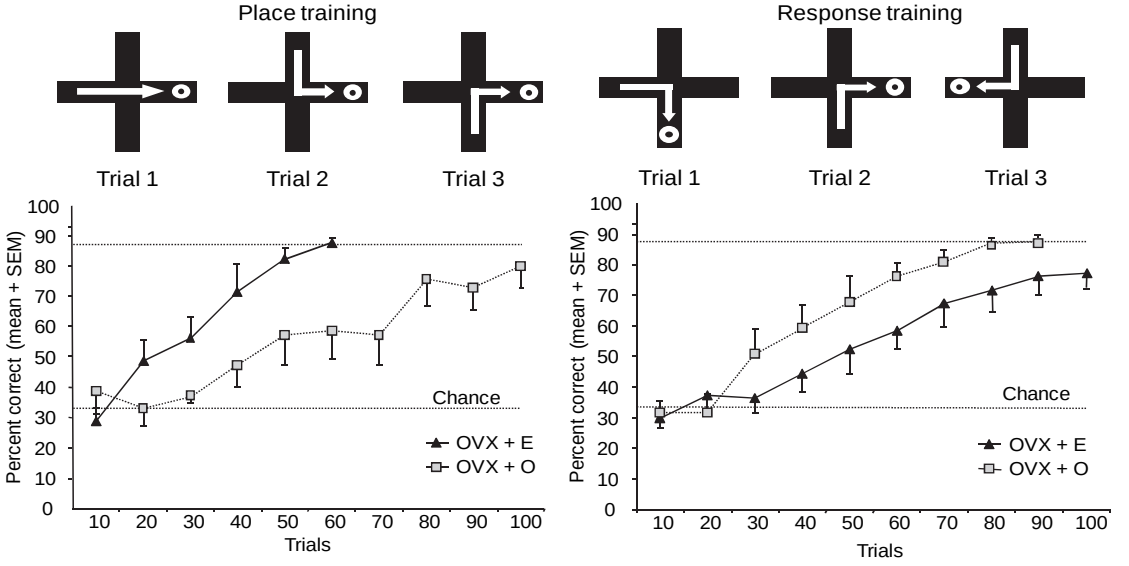

Figure 6 Effects of estrogen on maze learning in ovariectomized rats. Top figures show graphical representation of training protocols for place and response tasks in a plus-shaped maze. Place training required rats to locate food reward in an arm that maintained its position relative to the room cues throughout training. Start arms were randomly assigned across the other three arms. Response training required rats to locate food by making a right or left (not shown) turn. The goal arm was varied across training but maintained its position relative to the start arm with respect to the correct turn. Bottom graphs show learning curves, with trials groups into 10-trial blocks, in place and response versions of the maze for ovariectomized rats trained after treatment with oil (O) or estradiol (E). For place training, E-treated rats had steeper learning curves than did O-treated rats, suggesting faster learning. For response learning, E-treated rats were slower to acquire the task than were O-treated rats. Note that data reflect training until all rats reached criterion. OVX, ovariectomy. From Korol DL and Kolo LL (2002) Estrogen-induced changes in place and response learning in young adult female rats. *Behav. Neurosci.* 116: 411–420; used with permission.

2004; Shumaker et al., 2004). Of several variables, it appears that time since menopause before the start of a hormone regimen may be especially important. In the WHIM experiments, hormone therapy began in women at approximately 72 years of age, whereas those in smaller prior studies had tested women at or close to the time of menopause. This difference has led to suggestions that there is a time window after decline in ovarian function during which the hormones are most effective at enhancing cognition (Gibbs and Gabor, 2003; Maki, 2006; Sherwin, 2006). The time window has direct support in studies of both rodents and nonhuman primates (e.g., Lacreuse et al., 2002; Daniel et al., 2006). There is also evidence that fitness and exercise may interact with hormone treatments in postmenopausal women, augmenting the positive effects and blunting negative effects of the hormones on both brain and cognitive measures (Erickson et al., 2007).

3.27.3 Neurotransmitters

3.27.3.1 Overview

Drugs targeting many neurotransmitters can enhance memory. For most neurotransmitters, or at least for specific receptors, the effects on memory of agonists and antagonists oppose each other. For example, systemic and central administration of norepinephrine and glutamate receptor agonists enhances memory, whereas administration of their antagonists impairs memory; gamma-aminobutyric acid (GABA) and opioid agonists impair memory, and antagonists enhance memory. With central injections, intrahippocampal injections of many drugs with these targets of action similarly enhance and impair memory (Izquierdo et al., 1992; Packard, 1999; Farr et al., 2000; Roesler et al., 2003). Particularly when injected systemically, these effects are often additive (Gold, 1995). Combined subthreshold doses of two memory-enhancing drugs often enhance memory, and coadministration of a memory-enhancing and -impairing drug often cancels their respective effects on memory.

However, injections into specific brain regions are not always additive across drugs, suggesting hierarchical arrangements that cannot always be explained by simple interactions across neurochemical systems and instead suggesting serial processing, at least in part, of neurotransmitter functions in modulation of memory. Additivity might explain findings that intra-amygdala injections of clenbuterol (β-noradrenergic agonist) enhance memory and that the enhancement is blocked by atropine (muscarinic antagonist) (Introini-Collison et al., 1996); injections of cholinergic antagonists can themselves impair memory (Izquierdo et al., 1993). However, memory enhancement with the cholinergic muscarinic receptor agonist, oxotremorine, injected into the amygdala is not blocked by propranolol (Introini-Collison et al., 1996). Studies examining the interactions between neurotransmitter-related drugs have provided insights into the organization of neurochemical substrates of memory modulation (McGaugh et al., 1993; Gold, 1995; McIntyre et al., 2003b; Power et al., 2003), although the full nature of these interactions awaits further clarification.

Nonetheless, the general pharmacological picture seems quite clear, especially for systemically administered drugs. When administered near the time of training on many tasks, drugs that activate or mimic norepinephrine, acetylcholine, or glutamate enhance memory, and drugs that activate or mimic GABA or opioids impair memory. The converse examples exist for drugs that interfere with the functions of the respective neurochemical systems.

The roles of many of these neurotransmitter systems are often characterized in different manners. The roles of acetylcholine and norepinephrine are described in terms of modulating memory formation (Gold, 2003; Power et al., 2003; McIntyre et al., 2003b). In contrast, the role ascribed to glutamate is generally one of being intrinsic to memory formation, a direct component of the processes that make memories rather than of processes that regulate or modulate memory formation (Riedle et al., 2003). Drugs that act at the *N*-methyl-D-aspartate (NMDA) receptor for glutamate are thought to be permissive in enabling permanent changes in synaptic efficacy, as seen in several forms of long-term potentiation. However, some of these functions are modulatory in nature (i.e., amplifying the main tetanus- or experience-induced changes in synaptic efficacy). The designations of modulator and mediator become quite murky as the pharmacological evidence grows.

3.27.3.2 Acetylcholine

Research in the early 1980s provided evidence that postmortem examinations of the brains of individuals with Alzheimer's disease revealed dramatic loss of forebrain cholinergic markers (Bartus et al., 1982; Whitehouse et al., 1982; Coyle et al., 1983; Winkler

et al., 1998). These findings led to successful experiments in rats and mice revealing enhancement of memory and attention in young and aged rats and mice, as well as in rodent models of Alzheimer's disease, using drugs that augment cholinergic functions (Dutar et al., 1995; Everitt and Robbins, 1997; Iversen, 1998; Woolf, 1998; Disterhoft et al., 1999; Hasselmo, 1999; Sarter et al., 1999; van der Zee and Luiten, 1999; Sarter and Bruno, 2000). The promise of these agents led directly to the development of cholinesterase inhibitors that block the breakdown of acetylcholine to treat cognitive decline in Alzheimer's disease. Currently prescribed drugs include tacrine (Cognex), donepizil (Aricept), rivastigmine (Exelon), and galantamine (Reminyl) (cf. Disterhoft and Oh, 2006).

The drugs most often tested include agents that augment cholinergic functions indirectly or by direct receptor actions. Many studies examine the effects of acetylcholinesterase inhibitors, such as physostigmine, on memory in rodents. By inhibiting the degradation of acetylcholine after it is released from terminals, physostigmine increases the local concentration and the duration of the neurochemical actions of acetylcholine. Acetylcholinesterase inhibitors enhance learning and memory in rodents for tasks including inhibitory avoidance (Riekkinen et al., 1991), place and object recognition (Lamirault et al., 2003), and discrimination training (Marighetto et al., 2000). Acetylcholinesterase inhibitors are also very effective at reversing the impairing effects on memory of many drugs and brain lesions that impair memory (cf. Iversen, 1998; Gold and Stone, 1988). Drugs acting at either muscarinic or nicotinic cholinergic receptors also enhance learning, memory, and neural plasticity in rats and mice in many contexts and support the view that the neurotransmitter has important functions in regulating memory processing (e.g., Power et al., 2003; Gold, 2004; Hasselmo, 2006; Levin et al., 2006).

Acetylcholine appears to have many functions relevant to enhancement of memory. Release of acetylcholine may contribute to the memory-enhancing effects of glucose (**Figure 7**). Although neither peripheral nor intrahippocampal injections of glucose themselves increase the release of acetylcholine in the hippocampus, the injections augment acetylcholine release in the hippocampus while rats are engaged in a hippocampus-sensitive task (Ragozzino et al., 1996, 1998). In general, the magnitude of the increase in release is associated with enhancement of memory by glucose.

Another function of acetylcholine may be to balance the relative contributions of different memory systems to memory (Gold, 2003), perhaps regulating

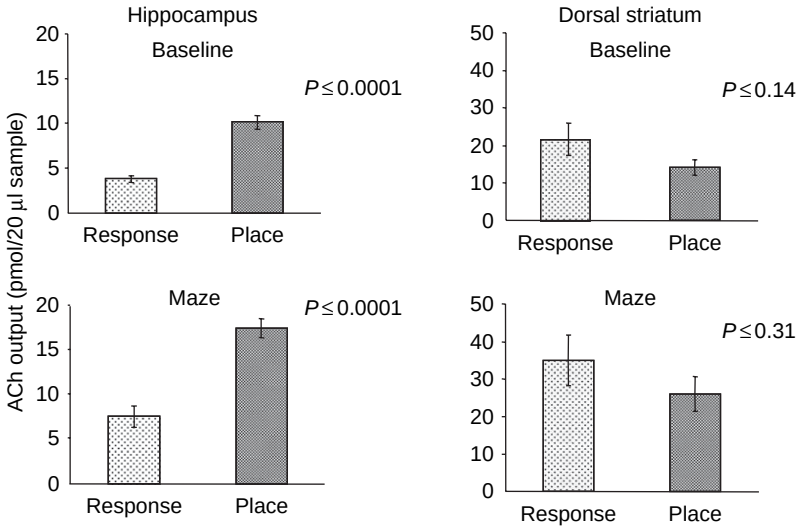

Figure 7 Acetylcholine (ACh) content in microdialysis samples collected concurrently from the hippocampus and dorsal striatum. Note the difference in *y*-axes for the two brain areas. Extracellular concentrations of ACh were greater in the striatum than in the hippocampus. Within the hippocampus, ACh release was significantly greater in rats that used a spatial strategy than in rats that used a response strategy. This was evident both prior to and during training. Although the scores were not significantly different at baseline or during training, the relationship between ACh release in the striatum of rats in the two groups was in the direction opposite that seen in the hippocampus. From McIntyre CK, Marriott LK, and Gold PE (2003a) Patterns of brain acetylcholine release predict individual differences in preferred learning strategies in rats. *Neurobiol. Learn. Mem.* 79: 177–183; used with permission.

the content of memory by modulating the level of activation of multiple memory systems. In one example, rats trained on a dual-solution T-maze solved equally well using either place (hippocampus-sensitive) or response (striatum-sensitive) solutions. The ratio of release of acetylcholine in the hippocampus versus dorsolateral striatum, measured just before training, predicted whether individual rats would show a preference for learning using a place or response solution (McIntyre et al., 2003a). These shifts may be related to evidence showing that acetylcholine shifts the cognitive function from attention at low levels of release to memory consolidation at higher levels of release (Hasselmo and McGaughy, 2004). These findings fit a broader context of evidence that acetylcholine regulates signal-to-noise ratios at the time of learning, regulates theta rhythms in the hippocampus, and modulates neuronal excitability (cf. Gold, 2003). More generally, the results suggest that acetylcholine enhances memory processing in multiple memory systems. By upregulating some systems more than others, the consequence would be that acetylcholine may alter the quality of memories as well as the strength of memories.

Acetylcholine also promotes neural plasticity assessed anatomically in the brains of honeybees (**Figure 8**; Ismail et al., 2006). New foragers exhibit substantial growth in the size of the mushroom body neuropil after extensive foraging experience. However, less experience is needed before neuropil growth is evident if a bee's foraging experience is coupled with administration of pilocarpine, a muscarinic agonist. Acetylcholine appears to promote neuroplasticity in somatosensory cortex (Dykes, 1997) and permit neurophysiological plasticity in auditory cortex during classical conditioning to tones (Weinberger, 2003, 2004).

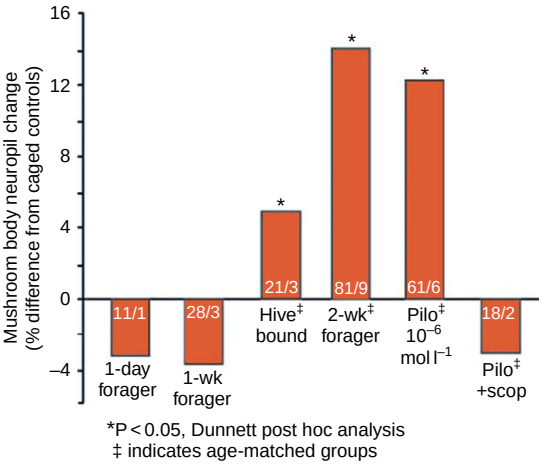

Figure 8 Effects of foraging experience with and without muscarinic agonist on the mushroom bodies. Estimates of the volume of the mushroom body neuropil were made for a total of 309 individuals, across nine experiments, conducted over three field seasons. To facilitate comparisons across all treatments, the % difference in neuropil volume relative to the caged control group in each experiment was calculated. Statistical analysis of these data used a mixed analysis of variance model ($F = 39.00$; $P < 0.0001$) and Dunnett *post hoc* tests (groups showing significant differences from the caged control group are indicated with the % difference). Key experimental groups are shown with the % difference indicated. Sample sizes are given in each bar (number of brains/number of trials). From Ismail N, Robinson GE, and Fahrbach SE (2006) Stimulation of muscarinic receptors mimics experience-dependent plasticity in the honey bee brain. *Proc. Natl. Acad. Sci. USA* 103: 207–211; used with permission.

3.27.3.3 Norepinephrine

Considerable evidence indicates that release of norepinephrine, particularly in the amygdala, may be a key brain component mediating enhancement of memory by a wide range of drugs (McIntyre et al., 2003; McGaugh, 2004). To some extent, interest in the role of norepinephrine in memory grew from the apparent involvement of the neurotransmitter in mediating the effects of epinephrine on memory. Indirect measures of norepinephrine release after epinephrine injections under conditions that enhance memory revealed release of norepinephrine throughout the brain (Gold and van Buskirk, 1978a,b); intermediate and high levels of release were associated with memory enhancement and impairment, respectively. More recent experiments, using *in vivo* microdialysis to study more directly release of norepinephrine, have found that peripheral epinephrine injections result in release of epinephrine within the amygdala (Williams et al., 1998). In addition, the magnitude of posttraining release of norepinephrine in the amygdala is positively correlated with later memory after inhibitory avoidance training. Posttraining injections of norepinephrine or β-adrenergic agonists directly into the amygdala enhance memory in an inverted-U dose-response manner (Liang et al., 1990; Hatfield and McGaugh, 1999).

The amygdala, and particularly the basolateral nucleus of the amygdala, may be a central mediator of many memory-enhancing treatments. For example, interference with noradrenergic functions in the amygdala, by lesions or by injections of β-adrenergic

antagonists such as propranolol, block enhancement of memory by epinephrine, corticosterone, and GABA and opioid antagonists (McGaugh et al., 1988; Introini-Collison et al., 1989; Roozendaal et al., 1999). Moreover, there are considerable interactions between norepinephrine and acetylcholine within the amygdala. Often, the effects of cholinergic drugs on memory are not blocked by interference with β-noradrenergic receptors, in contrast to interactions of the β-noradrenergic receptors with other memory-enhancing treatments, results suggesting convergence of cholinergic and noradrenergic mechanisms within the amygdala to enhance memory (cf. McIntyre et al., 2003b).

Thus, neurochemical mechanisms in the amygdala may be a core mechanism common to the enhancement of memory with a wide range of treatments. Identifying the neurochemical bases of these effects may offer a plan for rational development of therapeutic agents targeted directly at mechanisms underlying memory enhancement by many treatments.

3.27.3.4 Glutamate

As the major excitatory neurotransmitter in the brain, it is not surprising that drugs that interfere with glutamate functions impair memory formation (cf. Riedel et al., 2003, for comprehensive review). A focus on memory-enhancing glutamatergic drugs yields far fewer examples, although there is clear evidence that glutamate itself enhances memory when injected directly into memory systems important for the learning task. Glutamate injections directly into the amygdala, administered immediately after presentation of a weak taste as the conditioned stimulus, enhance acquisition of a conditioned taste aversion to that taste (Miranda et al., 2002). In the same experiment, the investigators found that presentation of an unconditioned stimulus, lithium chloride, resulted in large increases in glutamate release measured with *in vivo* microdialysis. Other examples of glutamate enhancement of memory include enhancement of inhibitory avoidance training with posttraining injections of glutamate into the locus coeruleus (Clayton and Williams, 2000). Because the locus coeruleus is the site of cell bodies of neurons that distribute norepinephrine to the forebrain, the enhancement may be mediated by norepinephrine release at sites distal to the site of injection. Glutamate may also enhance memory processing at sites proximal to the injection. Using a dual-solution maze in which a rat could find food reward using either place or response solutions, glutamate infusions into the hippocampus or striatum differentially enhance place and response learning assessed on later probe trials (Packard, 1999).

Glutamate acts at several receptors with quite different functions, including both ligand-gated ion channels (ionotropic receptors) and G-protein-coupled (metabotropic) receptors. One receptor that has received attention is the metabotropic glutamate receptor subtype 5 (Simonyi et al., 2005). Posttraining injections of a drug, 3,3′-difluorobenzaldezine, that enhances these receptors facilitate later memory for spatial memory (Balschun et al., 2006). There is also evidence that ampakines, drugs that enhance alpha-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA)-type glutamate receptors, enhance learning and long-term potentiation (Lynch, 2006). AMPA receptors are responsible for fast excitatory neurotransmission mediated by glutamate, and the receptor modulation may reflect general increases in neural excitability as a mechanism of action. Enhancement of memory in a delayed matching to sample task has been seen in sleep-deprived monkeys (Porrino et al., 2005), although studies using post-training designs and unimpaired versus impaired memory have not been assessed. Of interest, parallel brain imaging studies show that the ampakines led to normalization of changes in metabolic activity in those brain areas where alterations were seen after sleep deprivation and not in other areas. These findings are reminiscent of the effects, described earlier, of glucose on acetylcholine release in the hippocampus when rats were engaged in training but not when rats were at rest (Ragozzino et al., 1996). The situational selectivity of such drug effects deserves considerable attention in developing drugs effective in enhancing multiple forms of memory.

3.27.4 Intracellular Factors

3.27.4.1 Calcium Channel Blockers

Considerable evidence suggests that dysfunctions of calcium regulation can impair memory and that such dysfunctions might contribute to those impairments during aging (cf. Disterhoft et al., 1996, 2004; Foster and Kumar, 2002; Foster, 2006). Disterhoft and colleagues have provided extensive evidence suggesting that drugs that attenuate age-related increases in

calcium during activation of hippocampal (CA1) pyramidal neurons can enhance learning and memory in aged rabbits and rats. The task involves following the presentation of a tone with an air puff to the cornea, after a short delay, to establish an eyeblink classical conditioning response. Acquisition of this learned response is slowed or absent in aged rabbits and rats. Neurophysiological recordings from CA1 neurons reveal that there are age-related increases in the afterhyperpolarization (AHP) evident after a burst of action potentials. The AHP is characterized by an outward potassium current mediated by an influx of calcium that occurs with the action potential and serves to limit additional action potentials during its occurrence.

Of particular relevance to aging and memory, the AHPs are greatly enhanced in hippocampal pyramidal neurons of aged versus young animals, suggesting that the pyramidal neurons are less excitable and, perhaps, less able to participate in or to initiate mechanisms of learning and memory. Administration of nimodipine, an L-type calcium channel antagonist, reversed the age-related impairments in conditioning and in the AHPs (Deyo, et al., 1989; Disterhoft et al., 1996; cf. Disterhoft et al., 2004). Of additional interest, drugs that augment cholinergic functions, including cholinesterase inhibitors and muscarinic receptor agonists, also enhanced learning and memory in this system and attenuated the AHP in aged animals (cf. Disterhoft and Oh, 2006). Findings such as these offer an excellent opportunity to address the mechanisms in common across treatment domains.

3.27.4.2 Intracellular Molecular Targets

As discussed earlier, considerable evidence quite consistently identifies classes of drugs, particularly those acting on specific neurotransmitters and receptors, that enhance memory. However, the cellular mechanisms by which the drugs act are less clear. Also unclear is whether deeper understanding of the most effective drugs to enhance memory will come from more specific identification of neurotransmitter receptors that mediate enhancement of memory, from examinations of combination drug protocols that act on multiple receptors (e.g., memory enhancing drug cocktails) or from drugs that act on putative intracellular mechanisms that might mediate memory enhancement by one or several neurotransmitters.

Of possible intracellular mechanisms that might contribute to enhancement of memory, cAMP response element-binding protein (CREB) has received considerable attention as an important regulator of memory formation (Silva et al., 1998; Izquierdo et al., 2002; Barco et al., 2003; Tully et al., 2003; Carlezon et al., 2005; Josselyn and Nguyen, 2005). In particular, CREB is often thought to facilitate the conversion of early to late stages of memory. Phosphorylation of CREB is correlated with memory formation (Izquierdo et al., 2002), and interference with CREB functions impairs memory formation (Guzowski and McGaugh, 1997). Although there have been significant attempts to identify pharmacological agents that might enhance CREB functions and thereby enhance memory (Barco et al., 2003; Tully et al., 2003), explicit tests of such drugs are not yet readily found. Also missing, for CREB as well as for many other putative components of the molecular biology of memory formation, are tests of whether these factors are the mediators of enhancement of memory by neurotransmitter actions or whether the factors elicit changes in neurotransmission related to modulation of memory. It seems unlikely that CREB is essential for memory. Epinephrine enhances memory in CREB-knockout mice (Frankland et al., 2004), and memory seems normalized by spaced versus massed training trials (Kogan et al., 1997). These findings suggest that activation of CREB is not necessary for memory formation or for modulation of memory, though it may be a step that precedes or interacts with the mechanisms by which systemic epinephrine injections modulate memory.

The absence of more information examining interrelationships between intracellular molecules implicated in memory processing and memory-enhancing treatments appears to be based on two elements. First, molecular cascades important to memory have primarily been identified with drugs that block specific components of these cascades. Complementary drugs that activate these cascades often do not exist or have not received sufficient attention in this regard. Second, the paucity of studies examining molecular cascades in memory enhancement may reflect the presumption in such studies that investigations are examining the mechanisms of memory formation rather than the mechanisms that modulate memory formation. At this point, either modulation or mechanism of memory formation seems to provide a plausible explanation of the findings obtained with studies of molecular cascades of memory, though the view that

the molecules make new memories is dominant in research efforts.

A recent study explicitly related memory enhancement to an intracellular molecular target (McIntyre et al., 2005). Memory-enhancing injections of clenbuterol, a β-adrenergic agonist, were administered immediately after inhibitory avoidance training. Consistent with past findings, the treatment enhanced later memory. This treatment also resulted in increased expression of Arc protein. Arc is localized to dendritic regions near points of synaptic stimulation and appears to be associated with synaptic plasticity and memory (Guzowski et al., 2005). This is an early study in what promises to be a very useful approach of coupling memory-enhancing treatments to gene and protein responses important for the enhancement of memory.

3.27.5 Conclusions

Even over 50 years after the main demonstrations that drugs can enhance memory, the findings still seem quite remarkable in several respects. Although it is rather easy to imagine that interference with neural activity can impair memory, it is more surprising, at least to this writer, that drug perturbation of neural activity can also improve memory formation. Many of the drugs that enhance memory are related to neurotransmitters. For example, amphetamine augments catecholamine functions by blocking reuptake mechanisms. β-adrenergic, nicotinic, and glutamatergic receptor agonists act largely on postsynaptic receptors to mimic the action of the neurotransmitters norepinephrine, acetylcholine and glutamate, respectively. Although these neurotransmitters all appear to have a positive role in memory formation, the drugs that mimic them do not match the temporal and spatial patterning that appears to be a key element of neural processing generally, and in the formation of memory in particular. The apparent illogic may be addressed by findings that many neurotransmitters, notably including norepinephrine, acetylcholine, glutamate, and others, may have important extrasynaptic functions with release sites for neurotransmitters that are not always associated with close postsynaptic appositions (Descarries and Mechawar, 2000; Bach-y-rita, 2001; Descarries et al., 2004; Carmignoto and Fellin, 2006; Vizi and Mike, 2006). Findings such as these suggest that the temporal and spatial properties of neural activity may not apply to all neurotransmitters in all brain areas and may instead function much like local hormones, changing the functional state of the areas they bathe. One of those functional states may be readiness to alter connectivity in response to information flow, a function consistent with the idea of modulation of memory. Another explanation is based on the neurophysiological responses to some neurotransmitters involved in enhancement of memory. An example here is that the iontophoretic application of low levels of norepinephrine, serotonin, or acetylcholine can enhance both the postsynaptic excitation and inhibition produced by glutamate and GABA, respectively. In this way, the neuromodulators may enhance the impact of information flowing through traditional synapses, augmenting the signal-to-noise ratio and thereby enhancing memory formation.

It is also possible to move beyond these system-level views of how memory-enhancing drugs might work to the cellular and molecular bases by which they work. Mechanisms at this level might be the consequence of receptor binding leading to biochemical cascades important to memory or might be, similarly but less directly, the consequence of amplification of signals handled by other neurotransmitters, as discussed earlier. In contrast to the investigations of drugs that impair memory, far less attention has been given to the cellular and molecular bases by which well-studied drugs enhance memory. This is a research area ripe for future investigations.

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3.28 Episodic Memory Decline and Healthy Aging

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Aging is accompanied by continuing degradation of the anatomy and function of our brain. While our brain shrinks and its functions decline, cognitive processes also slow down and begin to falter. Among the most prevalent cognitive problems in older adults are deficits in memory function. Difficulties in learning and memory can be found to a certain degree in all older adults. Understanding age-associated memory decline is important for two reasons. First, in view of the mounting number of older adults in today's society, cognitive aging is increasingly becoming a problem in our health care system, and therapeutic intervention methods can only be developed on the basis of knowledge obtained through basic research. Second, there is a subgroup of elderly whose memory impairments are more severe, preventing normal functioning in their environment. In these persons, such impairments can be the earliest manifestation of pathological age-related conditions, such as Alzheimer's disease (AD). Particularly in the early stages of this disease, the differentiation from normal age-related memory impairments is very difficult. Thus, it is important to delineate which memory impairments can be regarded as correlates of normal aging and which impairments are associated with age-related pathology.

One type of memory that is particularly affected by the aging process is our memory for personally experienced past events, or episodic memory (EM) (Tulving, 1983; Gabrieli, 1998). Clinical studies have shown that EM is primarily dependent on the integrity of the medial temporal lobe (MTL) memory system

(Milner, 1972; Squire et al., 2001). However, the prefrontal cortex (PFC) also plays an important role in EM (Stuss and Alexander, 2000; Fletcher and Henson, 2001; Maril et al., 2003). As a result of increased accessibility of brain imaging techniques, such as positron emission tomography (PET) and functional magnetic resonance imaging (fMRI), aging research has now started to focus on the relationship between age-related changes in memory performance and changes in brain function. Functional neuroimaging (PET and fMRI) provides an ideal method to study patterns of neurocognitive decline, because changes in brain activity can be directly related to the effects of aging on behavioral measures, providing a link between cerebral aging and cognitive aging. One popular neurocognitive view is that the decline in EM associated with healthy aging results from a selective deterioration of PFC regions, whereas degradation of other brain regions, such as the MTL, is a hallmark of pathological age-related conditions (West, 1996; Buckner, 2004; Hedden and Gabrieli, 2004). However, there is now substantial evidence that MTL decline contributes to EM deficits in healthy older adults, and hence, it is no longer possible to attribute these deficits exclusively to PFC decline. In this chapter, we review recent PET and fMRI studies of healthy aging and EM with a focus on age-related changes in PFC and MTL regions.

In relation to these findings, we address two major factors thought to underlie age-related memory decline and strongly linked to PFC and MTL function, namely, deficits in executive function and deficits in binding processes. The term 'executive function' describes a set of cognitive abilities that control and regulate other abilities and behaviors. With respect to EM, executive functions are necessary to keep information available online so that it can be encoded in, or retrieved from, EM. The PFC is generally thought to be the key brain region underlying executive functions (Miller and Cohen, 2001). Binding refers to our capacity to bind into one coherent representation the individual elements that together make up an episode in memory, such as sensory inputs, thoughts, and emotions. According to relational memory theory, different subregions of MTL are differentially involved in memory for relations between items and memory for individual items (Cohen and Eichenbaum, 1993; Eichenbaum et al., 1994). In particular, the hippocampal formation is more involved in binding or relational memory operations, whereas the surrounding parahippocampal cortex is more involved in individual item memory.

This chapter has four main sections. In the first section, we begin with a brief overview of behavioral evidence indicating how EM performance changes as we age. In the second section, we discuss the anatomical and physiological changes in MTL and PFC that accompany the aging process. In the third section, we focus on functional neuroimaging studies of EM that reveal age-related changes in MTL and/or PFC regions. In the final section, we discuss different interpretations of age-related memory decline that have emerged from these findings, and how they relate to deficits in executive function and binding capacity.

3.28.1 Effects of Aging on Memory Performance: Executive Functions Versus Binding Deficits

Age-related deficits in EM may reflect difficulties during the formation of new episodic memory traces (encoding) and/or during the recovery of stored memory traces (retrieval). Two main theories of cognitive aging have been put forward to account for age-related deficits in EM encoding and retrieval. According to the resource deficit hypothesis (Craik, 1986), age-related cognitive impairments, including EM deficits, are the result of a general reduction in attentional resources. As a result, older adults have greater difficulties with cognitive tasks that provide less environmental support and, hence, require greater self-initiated processing. According to the binding deficit hypothesis (Johnson et al., 1993; Naveh-Benjamin, 2000), older adults are impaired in forming and remembering associations between individual items and between items and their context. As a result, age-related EM impairments are more pronounced on tasks that require binding between study items. In separate sections below, we discuss some examples of behavioral studies of encoding, retrieval, and aging in relation to these two theories of cognitive aging. Within each of these sections, we group studies according to whether they manipulated factors affecting mainly encoding or retrieval. However, it is important to keep in mind that behavioral studies cannot make a clear distinction between these two phases of episodic memory.

3.28.1.1 Resource Deficit Hypothesis

3.28.1.1.1 Encoding

In terms of encoding, the resource deficit hypothesis is supported by evidence that age-related deficits are

most pronounced when there is little environmental support to help encoding. For instance, age-related differences tend to be larger when young and older adults intentionally try to encode a list of study items than when a deep semantic processing task is used to guide encoding (Craik and Simon, 1980; Burke and Light, 1981). As an example, in a recent study (Troyer et al., 2006), older adults showed significantly impaired recognition memory performance following intentional encoding of a list of proper names, but they performed equally well as young adults when encoding was incidental and involved making semantic associations to the names.

Likewise, age differences in encoding can be reduced by providing additional semantic context with the study items. For example, in one study (Craik et al., 1987), young and older adults encoded a list of words either with or without a short descriptive phrase. Age-related deficits were much smaller when the encoded words were accompanied by the descriptive phrases. Also, age-related deficits in encoding become greater when there is no apparent link between the elements. Smith et al. (1998) found that older adults' difficulties in recalling associations between two pictures were attenuated when the two pictures were linked by preexistent relationships. The authors interpreted these findings in terms of age-related differences in self-initiated processing.

3.28.1.1.2 Retrieval

Similar to encoding, retrieval studies of aging have shown that age-related deficits in retrieval become larger on tasks that put a greater demand on executive function. Older adults experience more difficulties on recall and context memory tasks, which are more dependent on self-initiated search processes, than on recognition tasks, in which candidate targets are provided by the experimenter and the participant only needs to decide whether or not they were part of the study list (Rabinowitz, 1984; Rabinowitz and Craik, 1986; Craik and McDowd, 1987). For example, in a study by Craik and McDowd, young and older adults performed cued recall and recognition tasks, both of which also included a secondary reaction time task. As expected, older adults performed less well than the young adults on the recall task, but not on the recognition task (see **Figure 1**). Moreover, relative to recognition, older adults were much more impaired during recall on the secondary task than were the young adults. These findings are in line with the idea that recall requires more resources than

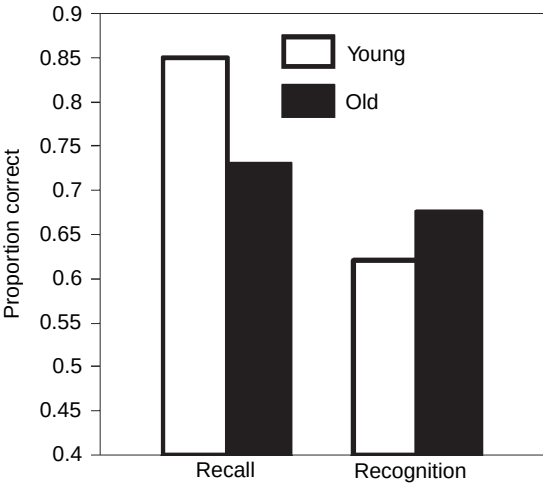

Figure 1 Reduced memory performance in older adults compared with young adults during cued recall, but not during recognition. Adapted from Craik FIM and McDowd JM (1987) Age differences in recall and recognition. *J. Exp. Psychol. Learn. Mem. Cogn.* 13: 474–479.

recognition and that these resources are reduced in older adults.

One explanation for the greater age-related decline in recall than in recognition is that older adults do not spontaneously produce retrieval cues to guide the search process. In line with this idea, several studies have found that age-related deficits are reduced when cues are provided (Park and Shaw, 1992; Naveh-Benjamin and Craik, 1995). For example, age differences in EM become smaller when the encoding context is reinstated during retrieval. In a study by Naveh-Benjamin and Craik, young and older adults encoded words that were either shown in one of two different fonts or spoken by a female or a male voice. Age differences in recognition performance were much smaller when the font or voice was reinstated during recognition. In other words, older adults benefit when provided with environmental support at retrieval.

3.28.1.2 Binding Deficit Hypothesis

3.28.1.2.1 Encoding

In addition to deficits in self-initiated processing, several studies have shown that older adults consistently exhibit age-related deficits in encoding tasks that require binding different pieces of information together, even when memory for individual features is intact (Schacter et al., 1994; Chalfonte and Johnson, 1996; Mitchell et al., 2000b; Naveh-Benjamin, 2000). Chalfonte and Johnson (1996) tested age-related differences in encoding of

both color and item information across three experiments. While no effect of age was found for individual features, older adults exhibited significant deficits in recognition for combined features compared with younger adults. Furthermore, age-related differences in memory for bound features persisted across various encoding instructions. Similar results were reported by Mitchell et al. (2000b), who also interpreted results as an age-related deficit in binding features in memory.

Recent studies have indicated that this associative deficit is not limited to arbitrary associations (e.g., word–color, item–location) but is also found in more ecologically valid pairings (Naveh-Benjamin, 2000; Naveh-Benjamin et al., 2004). For instance, in a study by Naveh-Benjamin and colleagues (2004), young and older adults studied a list of face–name pairs. Memory for individual faces and names was tested using forced-choice recognition tests in which the faces and names were paired with other names and faces not seen at study. Associative memory for the face–name pairs was tested in a separate test that included only studied faces and names. Participants paired a face or name with the corresponding name or face shown at study by choosing from two alternatives. Although older adults performed similarly to the young adults on individual face and name recognition, they were considerably impaired on the associative recognition test. These age-related differences persisted even when the young adults encoded information under divided attention (see **Figure 2**). These results indicate that binding deficits in older adults cannot be explained solely by a reduction in attentional resources. Thus, available evidence suggests that, even though older adults show deficits in general memory performance, this deficit is greater for associative tasks.

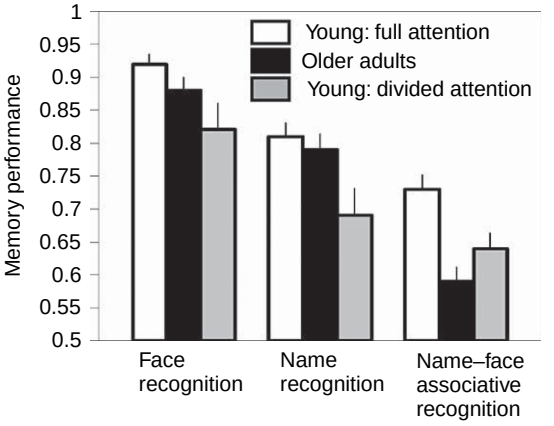

Figure 2 Reduced memory performance in older adults compared with young adults during associative recognition, but not during recognition of single faces and names; binding deficits in older adults cannot be explained solely by a reduction in attentional resources. Adapted from Naveh-Benjamin M, Guez J, and Shulman S (2004) Older adults' associative deficit in episodic memory: Assessing the role of decline in attentional resources. *Psychon. Bull. Rev.* 11: 1067–1073.

3.28.1.2.2 Retrieval

Age-related associative deficits are present during retrieval as well. According to dual-process models, recognition memory can be based on the recovery of specific contextual details (recollection) or on the mere feeling that an event is old or new in the absence of confirmatory contextual information (familiarity). Older adults are more impaired in recollection than in familiarity, which has been demonstrated using the Remember/Know (R/K) paradigm (Parkin and Walter, 1992; Mantyla, 1993; Java, 1996; Davidson and Glisky, 2002; Bastin and Van der Linden, 2003), ROC curves (Howard et al., 2006), and the process-dissociation procedure (Jennings and Jacoby, 1993). For example, Parkin and Walter (1992) used the R/K paradigm in which participants indicate whether their recognition judgment was based on recollection (R) or familiarity (K). They found that older adults made fewer R responses and more K responses than younger adults. Given that recollection involves the retrieval of associations between core and contextual elements of an episode, this evidence is consistent with the view that binding deficits are an important factor in age-related EM decline (Naveh-Benjamin, 2000).

In line with the aforementioned deficits in recollection, older adults are also less accurate in recalling information associated with the context in which an item was encoded, including the color, case, or font of words (Park and Puglisi, 1985); modality of presentation (e.g., auditory or visual) (Light et al., 1992); and speaker gender (Kausler and Puckett, 1981). For example, when given a list of made-up facts (e.g., Bob Hope's father was a fireman) and tested on them 1 week later, both younger and older adults could successfully recall the items, but older adults were impaired at knowing where they had first learned the information (McIntyre and Craik, 1987). Similarly, older adults are impaired at knowing where on a computer screen an item was presented, though they have little problem in recognition of items themselves (Parkin et al., 1995). Thus, impaired memory for context appears to exceed memory impairments for individual items. A prevailing

theory accounting for these recollection and context memory deficits involves the binding deficit hypothesis.

3.28.1.3 Summary

To summarize, behavioral evidence of EM encoding and retrieval has provided support for both resource and binding deficit hypotheses. In support of the resource deficit hypothesis, age-related deficits in encoding and retrieval are greater on EM tasks with little environmental support, which require more self-initiated processing. The resource deficit hypothesis is in line with the idea that a general reduction in executive function plays an important role in age-related memory decline. In support of the binding deficit hypothesis, age deficits are more pronounced when encoding and retrieval tasks require the formation or recollection of item–item or item–context associations. The binding deficit hypothesis supports the idea that a decline in relational memory functions plays an important role in age-related EM deficits.

3.28.1.4 Assumptions Regarding Brain Regions Underlying Resource and Binding Deficits

Adding brain assumptions to the resource and binding deficit hypotheses is straightforward, because different neural substrates have been proposed for executive and binding operations. As noted in the introduction, the PFC is considered the key brain region underlying executive functions and the managing of attentional resources (Miller and Cohen, 2001). Based on this generally accepted idea, Craik (1983) proposed that older adults' deficits in executive processing are related to a reduction in the efficiency of PFC functioning.

The strong relation between PFC function and executive processes is exemplified by patient, animal, and functional neuroimaging studies using the well-known Wisconsin Card Sorting Test (WCST) – a task that draws heavily on top-down attentional and executive processes (Miller and Cohen, 2001). In this task, participants sort a set of cards according to number, color, and symbol without being told the correct sorting rule, and this rule changes periodically. The experimenter only indicates whether a response was correct or not. Thus, any given card can be associated with several possible actions, and the correct one is determined by whichever rule is currently in effect. Several researchers have found that humans with PFC damage show clear deficits in the WCST. They are able to acquire the initial rule but are unable to adapt their behavior when the rule changes (Milner, 1963). Similar findings have been reported in animal studies. For instance, Dias et al. found that monkeys with experimental lesions to the PFC show very similar deficits in analog task of the WCST (Dias et al., 1996b, 1997). Finally, functional neuroimaging studies have also indicated the importance of PFC in the WCST. For example, a recent fMRI study found that different PFC subregions mediate different types of executive operations during WCST performance. Whereas ventrolateral PFC activity in the WCST was related to simple working memory operations, dorsolateral PFC activity was associated with more complex/manipulative working memory operations (Lie et al., 2006).

Similar to the strong relation between PFC and executive function, there is substantial evidence that the MTL is critical for binding. Recent evidence indicates that within MTL, binding is specifically associated with the function of the hippocampus, whereas the surrounding cortical regions (e.g., perirhinal cortex) are more involved in individual item memory (Brown and Aggleton, 2001; Eichenbaum, 2006).

The selective role of the hippocampus in binding operations can be exemplified with patient, animal, and functional neuroimaging studies that capitalized on the distinction between recollection-based (item–context binding) and familiarity-based (individual item memory) EM retrieval. Regarding patient data, using several different measures including ROC curves, Yonelinas and colleagues (2002) found that hypoxic patients, who typically have greater hippocampal than parahippocampal damage, showed a decline in recollection measures but normal familiarity. This was also reflected in their ROC curves, which were less asymmetrical (recollection) and more curvilinear (familiarity). These findings suggest that an intact hippocampus is necessary for recollection but not for familiarity. Regarding animal data, Fortin et al. (2004) also examined the contribution of recollection and familiarity processes using ROC curves in an odor recognition memory test designed for rodents. Normal rats showed ROC curves that had asymmetrical and curvilinear components, indicating the existence of both recollection and familiarity. However, following selective damage to the hippocampus, the ROC curve was not asymmetrical

(recollection) but completely curvilinear (familiarity), supporting the view that the hippocampus specifically mediates the capacity for recollection. Finally, regarding functional neuroimaging data, a recent study by Daselaar et al. (2006a) used confidence ratings to distinguish between recollection and familiarity. Confirming patient and animal findings, they found that the hippocampus showed selective activity for high confidence retrieval (recollection), whereas parahippocampal and rhinal activity showed a gradual function (familiarity) with confidence.

Thus, based on patient, animal, and functional neuroimaging evidence, we can infer that resource and binding deficits are tied to age-related changes in PFC and MTL (hippocampus), respectively. In the remainder of this chapter, we focus on how the structure and function of PFC and MTL change as a result of the aging process, and in the final section, we discuss a possible link between these behavioral and neurobiological changes.

3.28.2 Effects of Aging on PFC and MTL Anatomy and Physiology

The effects of aging on the brain occur at many levels from genes to gross anatomy. Reviewing this large research domain is beyond the scope of this chapter. Here, we focus on PFC and MTL and mention only three examples of cerebral aging measures that have been directly related to cognitive decline in aging humans: brain atrophy, declining white matter integrity, and dopamine deficits.

3.28.2.1 Brain Atrophy

In postmortem and *in vivo* studies, the brains of older adults tend to have lower volumes of gray matter than young adult brains (Resnick et al., 2003). These volume declines are not always related to a loss of cells but can also be ascribed to lower synaptic densities in older adults (Terry, 2000). Cross-sectional studies suggest that the volume of gray matter declines linearly with age, whereas white matter volume increases during childhood, plateaus during young adulthood and middle age, and declines during old age, an inverted U function (Raz et al., 2005).

Apart from differential age effects on gray and white matter volume, the relation between age and brain volume is also not uniform across different brain regions. The region most affected is the PFC, whereas other regions, such as the occipital cortex, are relatively unaffected by the aging process (Raz et al., 1997). With an average decline rate of between 0.9% and 1.5% per year, the frontal lobes show the steepest rate of atrophy (Pfefferbaum et al., 1998; Resnick et al., 2003; Raz et al., 2005).

The disproportionate effect of aging on PFC volume, together with the finding that age-related differences tend to be larger on tasks assumed to depend on PFC function, has led to the proposal that age-related deficits are primarily the result of frontal decline (for a review, see West, 1996). Indeed, frontal atrophy has been shown to correlate with cognitive deficits mediated by frontal regions. For example, Gunning-Dixon and Raz (2003) found that in a large group of older adults, perseveration errors on the WCST negatively correlated with prefrontal volume. However, other brain regions, including the basal ganglia (Bugiani et al., 1978; Schwartz et al., 1985) and the thalamus (Petersen et al., 2000; Gallo et al., 2004), also show a pronounced decline in brain volume with increasing age. In fact, in the last decades of normal life, volumetric changes in PFC do not differ from those in other neocortical areas (Resnick et al., 2000; Salat et al., 1999).

MTL volume also declines with age, though not all subregions (e.g., entorhinal cortex, hippocampus, parahippocampal gyrus) are equally affected. For example, as shown in **Figure 3**, a recent longitudinal study found that in healthy older adults, the hippocampus showed substantial atrophy, whereas the entorhinal cortex did not (Raz et al., 2005). Furthermore, studies have shown that the rate of hippocampal atrophy increases with age (Scahill et al., 2003; Raz, 2004). In one study, for example, this rate was an average of 0.86% per year in the whole sample (26–82 years) but 1.18% when considering only individuals over 50 years of age (Raz, 2004). A review of 12 studies estimated that after the age of 70 this rate may be as high as 1.85% per year (see Raz et al., 2005). The differential effects of aging on hippocampus and entorhinal cortex is very interesting, because the entorhinal cortex is one of the regions first affected by AD (Braak et al., 1993). As discussed later, together with recent fMRI evidence of dissociations between hippocampal and rhinal functions in aging (Daselaar et al., 2006b), these findings have implications for the early diagnosis of AD.

Several studies have suggested that the decline in hippocampal volume contributes to age-related deficits in EM (Golomb et al., 1993, 1994; Lupien et al., 1998; Sperling et al., 2003). For instance, Golomb et al. (1993)

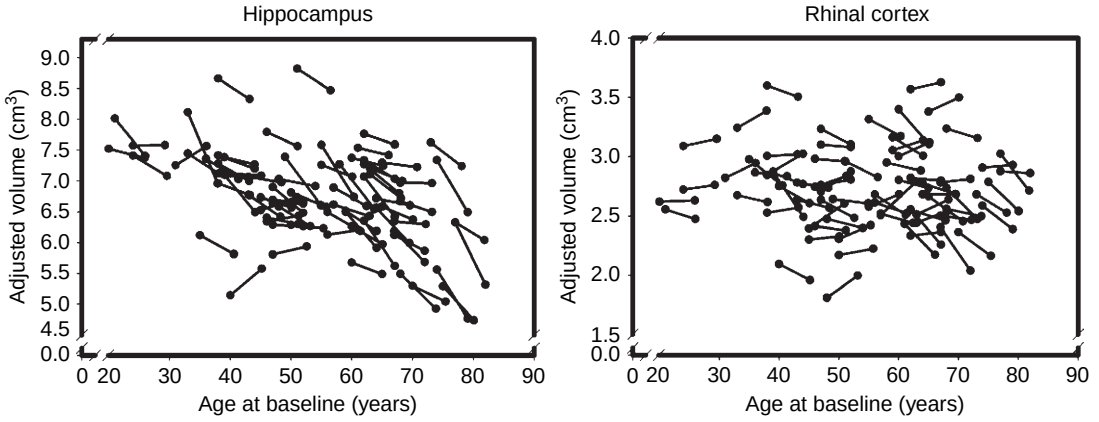

Figure 3 Longitudinal changes in volumes of hippocampus and rhinal cortex as a function of baseline age. From Raz N, Lindenberger U, Rodrigue KM, et al. (2005) Regional brain changes in aging healthy adults: general trends, individual differences and modifiers. *Cereb. Cortex* 15: 1676–1689.

investigated the link between hippocampal atrophy and memory performance in a group of healthy older adults. They found that, after controlling for such factors as age, education, and vocabulary skills, individuals with hippocampal atrophy performed less well on memory tests compared with those with no decline. Furthermore, as part of another longitudinal study on memory function, Golomb et al. (1996) found a significant correlation between hippocampal atrophy across a 3.8-year span and decline in memory performance in a group of older adults (mean age, 68.4 years). Additionally, Persson and colleagues (2005) found reduced hippocampal volume in a group of older adults whose episodic memory performance declined across a decade compared with that of a group whose memory performance remained stable.

3.28.2.2 Declining White Matter Integrity

In addition to declining gray matter, aging is also accompanied by reduced white matter integrity. White matter is composed of myelinated axons, which are essential for efficient neural transmission. When we age, myelin undergoes a number of significant changes including splitting of the myelin layers, increase in myelin volume resulting in redundant myelin sheets around axons, and finally, a breakdown of myelin (Wozniak and Lim, 2006).

A method to study the integrity of white matter *in vivo* is an MRI technique termed diffusion tensor imaging (DTI). This technique measures the magnitude and directionality of water diffusion within the brain. Without barriers, the movement of water is uniform in all directions. In the presence of barriers such as cell membranes, fibers, and myelin, the diffusion is greater in a certain direction and is termed anisotropic. Using DTI, the fractional anisotropy (FA) can be computed, indicating the degree of anisotropy in a particular area. Since degradation of white matter structure will result in a lower FA value, this measure can be used as an index of white matter integrity (Sullivan and Pfefferbaum, 2006).

Several DTI studies have shown that the integrity of white matter deteriorates with advancing age (Salat et al., 2005; Sullivan et al., 2006). For instance, Salat and colleagues calculated whole-brain maps of FA to determine whether particular fiber systems of the brain are preferentially vulnerable to white matter degeneration. The results showed significant age-related decline in FA in frontal white matter, the internal capsule, and the genu of the corpus callosum. However, FA in temporal and posterior regions was relatively preserved (**Figure 4**). These findings indicate that fiber tracts in PFC are more vulnerable to age-related degeneration than those within MTL regions. Similarly, Sullivan and colleagues (2006) found that frontocallosal fibers showed a much steeper rate of age-related decline than fibers in more posterior regions of the brain.

Reductions in FA have also been linked to a decline in cognitive abilities in older adults (Sullivan et al., 2001; Madden et al., 2004; Charlton et al., 2006). For example, a recent study by Charlton et al. (2006) investigated the relationship between white matter structure and cognition in 106 healthy middle-aged and elderly adults. They calculated correlations between DTI measures and performance on a test battery assessing executive function, working

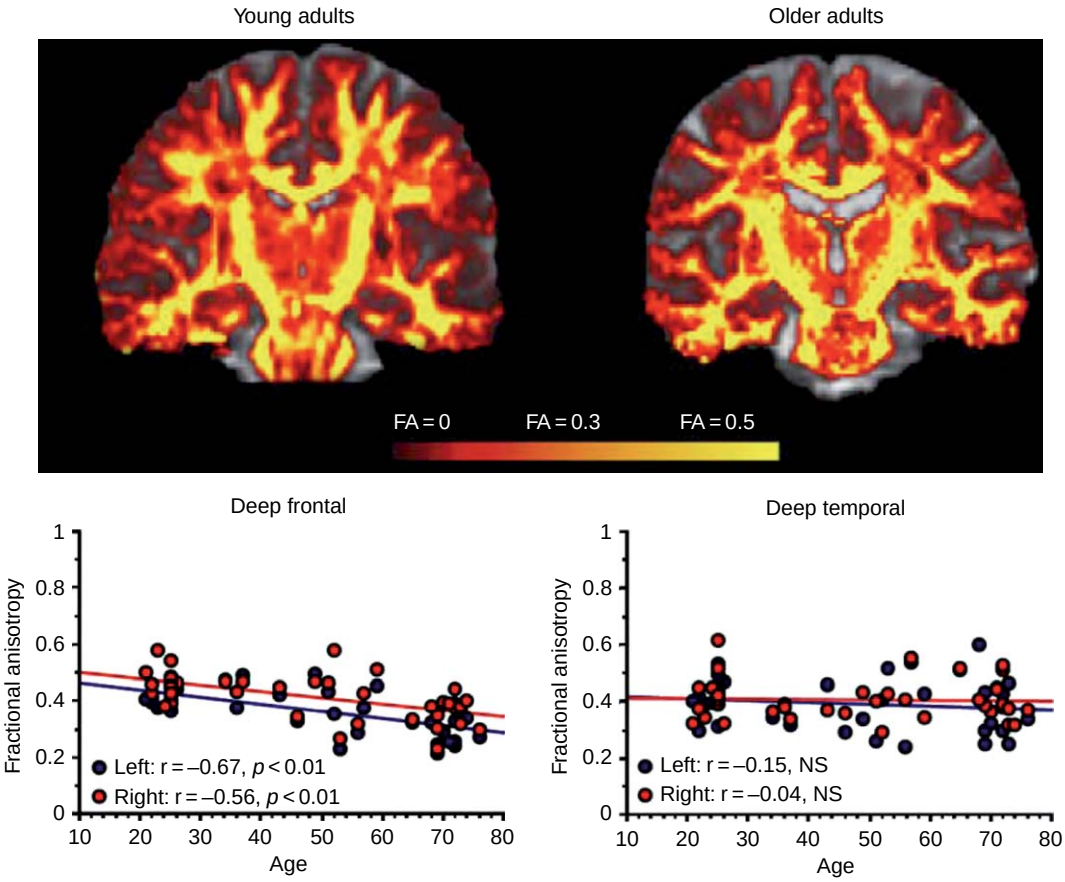

Figure 4 Steeper age-related decline in fractional anisotropy (FA) in frontal than in temporal white matter tracts. Adapted from Salat DH, Tuch DS, Greve DN, et al. (2005) Age-related alterations in white matter microstructure measured by diffusion tensor imaging. *Neurobiol. Aging* 26: 1215–1227.

memory, and information-processing speed. The results indicated a reduction in FA with age, which correlated with reduced performance in all three cognitive domains. However, after controlling for age, FA parameters correlated with working memory but not with the other two cognitive domains. These results indicate that white matter damage is an important factor in age-related cognitive decline and that working memory operations are particularly vulnerable to this type of cerebral aging.

3.28.2.3 Dopamine Deficits

Aging affects not only brain anatomy but also brain physiology, including the function of neurotransmitter systems, such as serotonin, acetylcholine, and dopamine (Strong, 1998). Associated with volume decrements in PFC are decreases in dopamine (DA) concentration and transporter availability (Volkow et al., 2000). Additionally, dopamine D_2 receptor density declines at a rate of 8% per decade, beginning in the 40s. There is abundant evidence that DA systems play an important role not only in motor operations but also in higher-order cognitive processes. DA function can be measured *in vivo* using PET (Bäckman and Farde, 2005).

There is strong evidence of age-related losses in pre- and postsynaptic DA markers, which may reflect decreases in the number of neurons, the number of synapses per neuron, and/or the expression of receptor proteins in each neuron. D_1 and D_2 receptor binding declines from early adulthood at a rate of 4–10% per decade, and this decline is correlated with the decline of dopamine transporter, possibly reflecting a common causal mechanism. DA loss with aging has been observed in frontal, temporal, and occipital cortices as well as in hippocampus and thalamus (Kaasinen et al., 2000; Inoue et al., 2001). The magnitude of extrastriatal DA decline mirrors that observed within the striatum itself. Given the

cognitive role of frontostriatal loops, age-related striatal DA deficits could also account for age-related cognitive deficits associated with PFC dysfunction. Moreover, age-related deficits in DA binding have been observed in PFC, as well as in posterior cortical and hippocampal regions. Evidence is mixed as to whether these declines are linear (see Reeves et al., 2002) or exponential (Bannon and Whitty, 1997; Rinne et al., 1998; Ghilardi et al., 2000) across adulthood.

The relationship between age-related changes in DA and age-related cognitive differences has been examined in only a small number of studies. Despite the paucity of data, findings are remarkably consistent. Age deficits in striatal DA have been associated with reduction in episodic memory (Bäckman et al., 2000; Erixon-Lindroth et al., 2005), executive function (Volkow et al., 1998; Mozley et al., 2001; Erixon-Lindroth et al., 2005), and motor performance (Wang et al., 1998; Mozley et al., 2001). Furthermore, several studies have also found that striatal DA markers serve as a significant predictor of cognitive performance, after controlling for the effects of age (Bäckman et al., 2000; Volkow et al., 1998), as well as that age-related cognitive deficits are mediated by reductions in striatal DA functioning (Erixon-Lindroth et al., 2005).

3.28.2.4 Summary

In sum, results indicate that the brain undergoes significant structural changes with age, but age-related atrophy differs across and within regions. Regarding volumetric measures of gray matter, studies have shown that the frontal lobes exhibit the highest rate of decline, and posterior regions the most moderate decline. The MTL also shows substantial atrophy in healthy aging, but the rate of decline differs for different subregions. For instance, whereas the hippocampus shows a marked decline, the rhinal cortex is relatively preserved in healthy aging. Measures of white matter volume and integrity (i.e., FA) also show differential aging effects throughout the brain. Similar to gray matter decline, age-related white matter degradation and DA dysfunction are greater in anterior compared with posterior regions. Correlations between these measures and cognitive function emphasize the importance that these changes have on cognitive functions. Finally, age-related decline in both pre- and postsynaptic DA markers also tends to follow this anterior–posterior gradient of decline. These patterns coincide with behavioral findings that show greater age-related performance decrements in cognitive functions mediated by frontal functioning (see West, 1996). In general, age-related decline in brain anatomy and physiology is most pronounced within PFC but is also present in MTL regions, particularly in the hippocampus. Based on existing evidence that PFC and MTL play key roles in executive and binding functions, these changes can readily account for the deficits reported in behavioral EM studies. The next section discusses functional neuroimaging studies, which provide a bridge between behavioral and anatomical findings by directly measuring age-related differences in brain activity during the performance of cognitive tasks.

3.28.3 Effects of Aging on PFC and MTL Activity

Before reviewing the findings of functional neuroimaging studies of EM, it is useful to first describe two patterns of age-related differences in brain activity that are consistently found in PFC and MTL. In the case of PFC, the most consistent finding has been an age-related reduction in lateralization. This evidence has been conceptualized in a model called Hemispheric Asymmetry Reduction in Older Adults (HAROLD), which states that, under similar conditions, PFC activity tends to be less lateralized in older than in younger adults (Cabeza et al., 2002). This model is supported by functional neuroimaging, electrophysiological, and behavioral evidence in the domains of episodic memory, semantic memory, working memory, perception, and inhibitory control (Cabeza, 2002).

In the case of MTL, the most consistent finding has been an age-related reduction in activity. However, recent studies suggest that not all MTL regions show reduced activity in older adults. Indeed, some MTL regions show preserved or increased activity in older adults, possibly reflecting differential age effects on various EM processes. In this section, we first review the effects of aging on PFC activation in encoding and retrieval tasks. Then we turn to the effects of aging on MTL activation as it relates to these cognitive processes. Although the number of studies is still too limited to identify clear patterns in the data, and a considerable amount of variability still remains unexplained, we try to emphasize the most consistent findings across studies.

3.28.3.1 PFC

3.28.3.1.1 Encoding

Functional imaging studies of encoding and aging have investigated a wide variety of stimuli, including words, word pairs, faces, and scenes. Despite this variety of stimuli, PFC findings have been quite consistent: older adults typically show reduced left PFC activity during encoding compared with younger adults. However, there is some evidence suggesting that this effect may be modulated by whether encoding is intentional or incidental. In intentional encoding conditions, participants are asked to learn information for a subsequent memory test, while in incidental encoding conditions, a memory test is not mentioned and participants are only asked to perform a certain task (semantic or nonsemantic). In terms of the resource deficit hypothesis, intentional encoding tasks provide less environmental support than incidental encoding tasks and, thus, are more dependent on PFC-mediated executive functions. Following, we consider first intentional encoding studies, then incidental encoding studies, and finally, studies comparing intentional vs. incidental encoding.

3.28.3.1.1.(i) Intentional encoding studies In a PET study by Grady et al. (1995), young and older adults intentionally encoded pictures of faces followed by a recognition test. Regarding encoding-related PFC activity, they found that older adults showed less activity in the left PFC than younger adults.

In another PET study by Cabeza et al. (1997), young and older adults intentionally encoded a list of word pairs for subsequent cued recall and recognition tests. During encoding, older adults showed increased activity in several regions, including the insula, but reduced activity in occipitotemporal and left prefrontal regions. Importantly, Cabeza and colleagues noted that younger adults selectively recruited the left PFC, whereas older adults showed equivalent activity in left and right PFC (i.e., HAROLD). Since younger adults and older adults had similar memory scores on both cued recall and recognition tasks, they interpreted the additional recruitment of right PFC by older adults as compensatory.

In another PET study of encoding and aging, Anderson et al. (2000) assessed intentional encoding of moderately associated word-pairs under conditions of full and divided attention. During full attention, they found a pattern similar to the one observed in the PET study by Cabeza and colleagues (1997). Whereas the older adults showed reduced activity in left PFC, they showed increased activity in right PFC, leading to bilateral frontal activity in older adults.

3.28.3.1.1.(ii) Incidental encoding studies Age-related differences in PFC activation have also been shown when investigating levels of processing at encoding. When comparing deep and shallow encoding of words, Stebbins et al. (2002) reported greater activity in both younger adults and older adults for the deep relative to the shallow encoding condition. However, the older adults showed decreased activation in left PFC. Furthermore, decreased performance on neuropsychological tests correlated with reduced PFC activity. As a result of the reduced left PFC activity, PFC activity in older adults was more symmetric than in younger adults, again in accord with the HAROLD model.

Rosen et al. (2002) also studied deep and shallow encoding of words in younger adults and older adults. However, they distinguished between older adults with high and low memory scores based on a neuropsychological test battery. They reported equivalent left PFC activity but greater right PFC activity in the old-high memory group relative to younger adults. In contrast, the old-low memory group showed reduced activity in both left and right PFC. As a result, the old-high group showed a more bilateral pattern of PFC activity than younger adults (HAROLD).

Similarly, using a verbal encoding/recognition task, Daselaar et al. (2003b) compared groups of high- and low-performing older adults, divided *post hoc* based on their memory scores. During the semantic encoding task (pleasant/unpleasant decisions), all groups showed left lateralized activations patterns, but PFC activity was slightly less lateralized in the low-performing elderly, and even less so in the high-performing elderly. Consistent with the results of Cabeza et al. (2002), these findings support the compensatory interpretation of HAROLD.

Morcom et al. (2003) used event-related fMRI to study subsequent memory for semantically encoded words. Recognition memory for these words was tested after a short and a longer delay. At the short delay, performance in older adults was equal to that of younger adults at the long delay. Under these conditions, activity in left inferior PFC was greater for subsequently recognized than forgotten words and was equivalent in both age groups. However,

older adults showed greater right PFC activity than younger adults, again resulting in a more bilateral pattern of frontal activity (HAROLD).

Gutchess et al. (2005) studied subsequent picture memory using a deep processing task. While young and older adults showed equivalent activity in right PFC, the older adults showed increased activity in the left PFC. Since picture encoding in younger adults was associated with bilateral PFC activity, these findings suggest a selective recruitment of left PFC, which may be compensatory.

A recent study by Dennis et al. (2006) used hybrid blocked/event-related analyses to distinguish between transient and sustained subsequent memory effects during deep incidental encoding of words. Subsequent memory was defined as parametric increases in encoding activity as a function of a combined subsequent memory/confidence scale. This parametric response was measured in each trial (transient activity) and in blocks of eight trials (sustained activity). Similar to the study conducted by Gutchess et al., subsequent memory analyses of transient activity showed age-related increases in left PFC. At the same time, subsequent memory analyses of sustained activity showed age-related reductions in right PFC. Contrary to Stebbins et al.'s study (2002), these findings suggest that during semantic classification, older adults can show even greater PFC-mediated semantic processing than young adults. Additionally, the decline in sustained subsequent memory activity in PFC may involve age-related deficits in sustained attention that impact encoding processes. The results underline the importance of investigating aging effects on both transient and sustained neural activity.

Finally, Persson and colleagues (2006) used longitudinal behavioral data to identify two groups of older adults that differed with regard to whether their performance on tests of episodic memory remained stable or declined over a decade. During incidental encoding of words, both groups showed equivalent activation in left PFC, but the elderly subjects with the greatest decline in memory performance showed additional right PFC activity. Moreover, mean DTI measures (FA) in the anterior corpus callosum correlated negatively with activation in right PFC. These results demonstrate that cognitive decline is associated with differences in the structure as well as function of the aging brain and suggest that contralateral PFC recruitment is either caused by structural disruption or is a compensatory response to such disruption.

3.28.3.1.1.(iii) Intentional versus incidental encoding Grady and colleagues (1999) compared intentional vs. incidental encoding conditions across various stimuli (e.g., words, pictures). They scanned participants during shallow (uppercase/lowercase, picture size), deep (living/nonliving), and intentional encoding conditions. Overall, picture encoding resulted in greater activity in visual and MTL regions, while word encoding yielded greater activity in left PFC and left lateral temporal cortex. However, deep encoding produced greater left PFC activity, while intentional encoding yielded greater right PFC activity. Though older adults showed the same patterns, the overall level of activity was reduced. Interestingly, researchers did not find a difference in deep vs. intentional encoding of pictures, indicating that age-related differences were greater for words than for pictures.

The same research group conducted another study comparing intentional and incidental encoding conditions, this time using faces as study items (Grady et al., 2002). Convergent with their earlier study investigating intentional face encoding (Grady et al., 1995), older adults showed decreased activity in left PFC compared with younger adults and diminished connectivity between frontal and MTL areas during both encoding conditions.

Despite the lack of activation differences across encoding instructions in the studies reported by Grady et al., a more recent study did find age-related frontal differences for intentional encoding instructions. Logan et al. (2002) reported that during self-initiated, intentional encoding instructions, older adults compared with younger adults showed less activity in left PFC but greater activity in right PFC, resulting in a more bilateral activity pattern (HAROLD). Results were similar for intentional encoding of both verbal and nonverbal material. Interestingly, further exploratory analyses revealed that this pattern was present in a group of old-older adults (mean age, 80), but not in a group of young-older adults (mean age, 67), suggesting that contralateral recruitment is associated with more pronounced age-related cognitive decline. At the same time, the decrease in left PFC was not present in older adults during incidental encoding instructions, suggesting that frontal reductions can be remediated by providing environmental support during encoding (**Figure 5**).

3.28.3.1.1.(iv) Summary To summarize encoding studies, the most consistent finding was an age-related reduction in left PFC activity. This

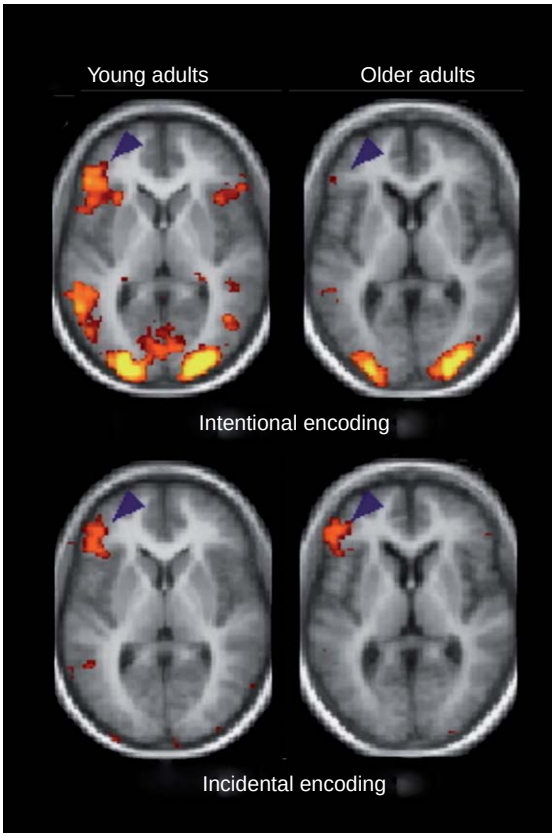

Figure 5 Reduced left PFC activity in older compared with younger adults during intentional encoding, but similar activity during incidental encoding. Adapted from Logan JM, Sanders AL, Snyder AZ, Morris JC, and Buckner RL (2002) Under-recruitment and nonselective recruitment: Dissociable neural mechanisms associated with aging. *Neuron* 33: 827–840.

finding was more frequent for intentional than for incidental encoding studies, suggesting that, in line with the resource deficit hypothesis, the environmental support provided by a deep semantic encoding task may attenuate the age-related decrease in left PFC activity. This effect was found within subjects in the study by Logan et al. (2002). The difference between intentional vs. incidental encoding conditions suggests an important strategic component in age-related memory decline. The reduction in left PFC activity was often coupled with an increase in right PFC activity, leading to a bilateral pattern of PFC activity in older adults (HAROLD). Importantly, extending to encoding a finding originally reported for retrieval (Cabeza et al., 2002), two studies that divided older adults into high and low performers found the HAROLD pattern only in the high-performing group (Rosen et al., 2002; Daselaar et al., 2003b). These findings provide direct support for the compensation account of HAROLD.

3.28.3.1.2 Retrieval

In line with the resource deficit hypothesis, age-related deficits in episodic retrieval tend to be more pronounced for recall and context memory tasks than for recognition tasks (Spencer and Raz, 1995). However, considerable differences in activity have also been observed during simple recognition tasks. We first review studies looking at recognition processes and then examine those that focus on recall and different forms of context memory.

3.28.3.1.2.(i) Recognition memory The face encoding study by Grady et al. (1995) also included a face recognition test. During this task, older adults showed reduced activity in parietal and occipital regions but equivalent activity to young adults in right PFC. This last finding contrasts with the age-related reduction in left PFC activity found in the same study during face encoding. Based on these results, the authors suggested that age effects are more pronounced on encoding than on retrieval. As noted below, however, many subsequent PET and fMRI studies have found reliable age-related changes in PFC activity during episodic retrieval.

The word-pair encoding study by Cabeza et al. (1997) also included a word-pair recognition task. During this task, older adults showed reduced activity in right PFC but increased activity in other brain regions, such as the precuneus. The age-related reduction in right PFC contrasts with the lack of age effects in this region's activity in Grady et al.'s (1995) study. This inconsistency could reflect differences in stimuli (faces vs. words) or retrieval processes (recognition of items vs. recognition of pairs).

The age-related reduction in right PFC activity during recognition was replicated by Madden et al. (1999), using a single-word recognition task. Additionally, this study found an age-related increase in left PFC. This age-related increase extended to recognition a finding previously reported for recall by Cabeza et al. (1997), which is reviewed here. As in the previous recall study, the age-related increase in left PFC led to a more bilateral pattern in older adults (i.e., HAROLD). In a subsequent study, Madden et al. (1999) reanalyzed the recognition data using a stepwise regression method that distinguished between exponential (tau) and

Gaussian (mu) components of RT distributions. Young adults showed a correlation between mu and right PFC activity, whereas the older adults showed correlations in left and right PFC regions related to both mu and tau. Since tau is associated with task-specific decision processes, and mu with residual sensory coding and response processes, the authors concluded that attentional demands were greater for older adults, leading to the recruitment of additional regions. These findings suggest that the retrieval network is more widely distributed in older adults.

Daselaar et al. (2003b) used event-related fMRI to study recognition of words in younger adults and older adults. Based on recognition performance in the scanner, older adults were divided into old-high and old-low groups. During recognition, compared with baseline, the old-low group showed much increased activity throughout the brain relative to the other groups. In addition, the old-low group and younger adults showed bilateral PFC activity, whereas the old-high group showed a more left lateralized pattern of frontal activity. In other words, the old-low group showed a nonselective increase in global brain activity, whereas the old-high group showed a selective recruitment of left PFC. The authors interpreted these findings in terms of strategic retrieval differences. Interestingly, when correctly recognized old words were compared with correctly rejected new words, these group differences disappeared. The difference in activity between these two trial types is generally considered to be a correlate of retrieval success. Hence, these findings suggest that age-related differences in episodic recognition primarily reflect strategic search deficits.

Velanova et al. (2006) also investigated differences between young and older adults during word retrieval in an easy (15 study repetitions) and a difficult (one study repetition) recognition task. Although many correlates of retrieval were similar between the groups, including medial and lateral parietal responses to successful recognition, older adults showed increased recruitment of PFC regions relative to young adults during the difficult condition. This effect was not significant during the easy condition. Moreover, the timing of increased recruitment in older adults occurred at relatively late stages of the retrieval event. These findings suggest that older adults fail to engage appropriate PFC-mediated executive processes at early stages of retrieval, and as a result, PFC involvement is extended at late stages to compensate.

3.28.3.1.2.(ii) Recall and context memory The aforementioned word-pair study by Cabeza et al. (1997) included not only a recognition test but also a cued-recall test. During recall, older adults showed weaker activity in the anterior cingulate and left temporal cortex. In addition, older adults showed weaker activations in right PFC than the younger adults. Conversely, older adults showed greater activity than younger adults in left PFC. The net result was that PFC activity during recall was right lateralized in younger adults but bilateral in older adults. The authors noted this change in hemispheric asymmetry and interpreted it as compensatory. This was the first study identifying the HAROLD pattern and the first one suggesting the compensatory interpretation of this finding. As noted, this study also compared age-related changes in activity during recall and recognition. These changes were more pronounced during recall than during recognition, consistent with behavioral evidence that recall is more sensitive to aging.

Bäckman et al. (1997) found a result similar to Cabeza et al. (1997), using word-stem cued recall instead of word-pair cued recall: younger adults activated right PFC, whereas older adults activated both left and right PFC (HAROLD). Also using word pairs, Anderson et al. (2000) investigated the effects of divided attention on cued recall. They reported negligible effects of divided attention in both groups. However, under full attention conditions, older adults showed weaker activations primarily in right PFC but stronger activations primarily in left PFC, suggesting an attenuation of the right-lateralized pattern shown by younger adults (HAROLD).

Cabeza et al. (2000) investigated item and temporal-order memory tasks. In the item task, a word pair was presented consisting of one studied word and one new word, and participants indicated which word was studied. In the temporal-order task, both words were studied, and participants indicated which of the two words appeared later in the study list. They reported that younger adults showed increased activation in right PFC for temporal-order compared with item memory, whereas older adults did not. In contrast, the activations during item memory were relatively unaffected by age. These findings are in line with the resource deficit hypothesis, indicating that memory deficits in older adults are a result of PFC dysfunction and that context memory is more heavily dependent on the frontal lobes than item memory is.

In another study of context memory by Cabeza and colleagues (2002), younger adults, high-performing

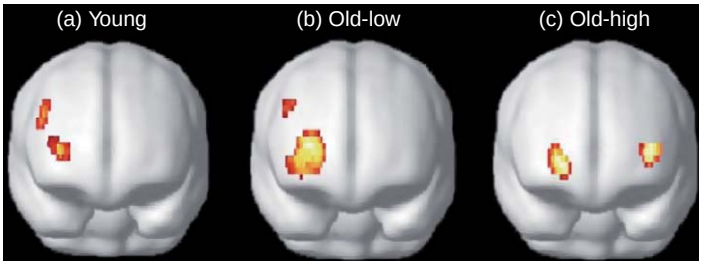

Figure 6 PFC activity during EM retrieval was right-lateralized in young (a) and low-old (b) participants, but bilateral in old-high (c) subjects. From Cabeza R, Anderson ND, Locantore JK, and McIntosh AR (2002) Aging gracefully: Compensatory brain activity in high-performing older adults. *Neuroimage* 17: 1394–1402.

older adults (old-high), and low-performing older adults (old-low) studied words presented auditorily or visually. During scanning, they were presented with words visually and made either old/new decisions (item memory) or heard/seen decisions (context memory). Consistent with their previous results, younger adults showed right PFC activity for context trials, whereas older adults showed bilateral PFC activity (HAROLD). Importantly, however, this pattern was only seen for the old-high adults, supporting a compensation account of the HAROLD pattern (**Figure 6**).

3.28.3.1.2.(iii) Summary Summarizing the studies on PFC and retrieval, the HAROLD pattern has been found more frequently in studies using tasks with little environmental support, including recall and context memory tasks than during simple item recognition. This was exemplified in the study by Cabeza et al. (1997), which included both recall and recognition tasks. These findings suggest a three-way interaction between age, executive demand, and frontal laterality. Importantly, distinguishing between old-high and old-low adults, the study by Cabeza et al. (2002) provided direct evidence for the compensation account of HAROLD.

3.28.3.2 MTL

Frontal activations in aging showed both reductions and increases across aging, as well as shifts in lateralization of activation. On the other hand, activation within the MTL generally shows age-related decreases compared with that seen in younger adults. However, some studies show a shift in the foci of activation from the hippocampus proper to more parahippocampal regions in aging. Evidence for such a shift is presented later, where appropriate, and is discussed further in the conclusions.

3.28.3.2.1 Encoding

Though the binding deficit hypothesis would predict age-related reductions in MTL activity, particularly during associative encoding, reductions have been found during encoding of individual items as well. We first discuss studies focusing on individual item encoding and then discuss the results of the only study reporting MTL differences using an associative encoding task.

3.28.3.2.1.(i) Individual item encoding In their study examining face encoding, Grady et al. (1995) found that older adults showed less activity not only in the left PFC but also in MTL compared with younger adults. Furthermore, they found a highly significant correlation between hippocampus and left PFC activity in younger adults, but not in older adults. Based on these results, they concluded that encoding in older adults is accompanied by reduced neural activity and diminished connectivity between PFC and MTL areas.

Daselaar et al. (2003a) investigated levels of processing in aging using a deep (living/nonliving) vs. shallow (uppercase/lowercase) encoding task. Despite seeing common activation of regions involved in a semantic network across both age groups, activation differences were seen when comparing levels of processing. Older adults revealed significantly less activation in left anterior hippocampus during deep relative to shallow classification. The researchers concluded that under-recruitment of MTL regions contributes, at least in part, to age-related impairments in encoding.

Similarly, the same group showed decreased activity in the MTL for poor-performing older adults compared with young and high-performing elderly. Despite similar PFC activation, Daselaar et al. (2003b) found that the older adults showed decreased activity in the left hippocampus during successful

encoding of words. Based on these findings, they concluded that MTL dysfunction during encoding is an important factor in age-related memory decline.

Similar to Daselaar et al. (2003b), Gutchess et al.'s (2005) study of subsequent picture memory observed reduced activity in the MTL for subsequently remembered items, even when older adults were not divided into high- and low-memory groups. Additionally, older adults exhibited a significant negative correlation between inferior frontal and parahippocampal activity, whereas younger adults did not. These results suggest that those older adults exhibiting the least involvement of the parahippocampal region conversely activated PFC areas the most.

Similar results were reported in the study by Dennis et al. (2006), which distinguished between transient and sustained subsequent memory for words. In line with Gutchess et al., they found that older adults showed a reduced transient subsequent memory effect in the hippocampus coupled with an increased effect in left PFC. These data suggest that PFC regions could be activated in a compensatory manner to offset declines in MTL activations in older adults.

3.28.3.2.1.(ii) Associative encoding Mitchell et al. (2000a) conducted the only fMRI study reporting MTL differences in an associative encoding task. In each trial, participants were presented with an object in a particular screen location and had to hold in working memory the object, its location, or both (combination trials). Combination trials can be assumed to involve the binding of different information into an integrated memory trace. Older adults showed a deficit in accuracy in the combination condition but not in the object or location conditions. Two regions were differentially involved in the combination condition in younger adults but not in older adults: a left anterior hippocampal region and an anteromedial PFC region (right BA 10). According to the authors, disruption of a hippocampal–PFC circuit may underlie binding deficits in older adults.

3.28.3.2.1.(iii) Summary In line with the binding deficit hypothesis, Mitchell and collegues found age-related reductions in MTL activity specifically during associative encoding. Yet several other studies also found age-related MTL reductions during encoding of individual items (Daselaar et al., 2003a,b; Gutchess et al., 2005; Dennis et al., 2006). Moreover, the study by Daselaar et al. (2003b) directly linked reduced MTL activity during single-word encoding to impaired performance on a subsequent recognition test. These findings suggest that age-related binding deficits play a role not only in complex associative memory tasks but also in simpler item memory tasks. One explanation for these results is that, in general, item memory tasks also have an associative component. In fact, the deep processing tasks used in these tasks are specifically designed to invoke semantic associations in relation to the study items. As discussed in the next section, recollection of these associations can be used as confirmatory evidence during EM retrieval. The results from two studies suggest that PFC regions might be activated in a compensatory manner to offset declines in MTL activations in older adults (Gutchess et al., 2005; Dennis et al., 2006). In particular, using correlational analyses, Gutchess et al. (2005) found that the older adults who showed the least MTL activity also showed the greatest PFC activity. These findings suggest that older adults may try to compensate for deficits in binding mediated by MTL by recruiting additional executive processes mediated by PFC.

3.28.3.2.2 Retrieval

In contrast to encoding, MTL differences during retrieval involve not only decreases but also age-related increases. This pattern has been observed during recognition memory tasks as well as cued recall and autobiographical retrieval tasks.

3.28.3.2.2.(i) Recognition memory Cabeza et al. (2004) investigated the effects of aging on several cognitive tasks including a verbal recognition task. Within the medial temporal lobes, they found a dissociation between a hippocampal region, which showed weaker activity in older adults than in younger adults, and a parahippocampal region, which showed the converse pattern. Given evidence that hippocampal and parahippocampal regions are, respectively, more involved in recollection vs. familiarity (Aggleton and Brown, 1999; Yonelinas, 2002), this finding is consistent with the notion that older adults are more impaired in recollection than in familiarity (e.g., Parkin and Walter, 1992; Jennings and Jacoby, 1993). Indeed, the age-related increase in parahippocampal cortex suggests that older adults may be compensating for recollection deficits by relying more on familiarity. Supporting this idea, older adults had a larger number of K responses than younger adults, and these responses were positively correlated with the parahippocampal activation.

A recent follow-up study by the same group showed a similar pattern of results (Prince et al., 2005). Young and older adults made old/new

judgments about previously studied words, followed by a confidence judgment from low to high. On the basis of previous research (Yonelinas, 2001), recollection was measured as an exponential change in brain activity as a function of confidence and familiarity, and as a linear change. The results revealed a clear double dissociation within MTL: Whereas recollection-related activity in the hippocampus was reduced by aging, familiarity-related activity in rhinal cortex was increased by aging (see **Figure 7(a)**). These results suggested that older adults compensated for deficits in recollection processes mediated by the hippocampus by relying more on familiarity processes mediated by rhinal cortex. Supporting this interpretation, within-participants regression analyses based on single-trial activity showed that recognition accuracy was determined by only hippocampal activity in young adults but by both hippocampal and rhinal activity in older adults. Also consistent with the notion of compensation, functional connectivity analyses showed that correlations between the hippocampus and posterior regions associated with recollection were greater in younger adults, whereas correlations between rhinal cortex and bilateral PFC regions were greater in older adults (see **Figure 7(b)**). The latter effect suggests a top-down modulation of PFC on rhinal activity in older adults. The finding of preserved rhinal function in healthy older adults has important clinical implications, because this region is impaired early in AD (Killiany et al., 2000; Pennanen et al., 2004).

3.28.3.2.2.(ii) Cued recall, autobiographical retrieval, and context memory In addition to increases in left PFC during word-stem cued recall, Bäckman and colleagues (1997) found increased MTL activation in older adults. Just as the increased left PFC activation resulted in more bilateral frontal activation for older adults, increased activation in left MTL had the same effect: Compared with younger adults, older adults show more bilateral MTL activity. It should be noted that this bilateral activation was not accompanied by increased performance, as older adults recalled only about half as many words as did younger adults.

This HAROLD pattern within MTL was also observed in a recent study using event-related fMRI. Maguire and Frith (2003) investigated the recall of autobiographical events gathered in a pre-scan interview. Although the groups activated largely the same regions, they observed a striking difference in the MTL. Younger adults showed left lateralized hippocampal activity, whereas older adults showed bilateral activity in the MTL. These findings suggest that HAROLD extends beyond the PFC not only to other cortical regions (as shown by several studies) but also to subcortical areas.

3.28.3.2.2.(iii) Summary In sum, retrieval studies have found both increases and decreases in MTL activity. The findings by Daselaar and colleagues suggest that at least some of these increases reflect a shift from recollection-based (hippocampus) to familiarity-based (rhinal cortex) retrieval. Furthermore, their functional connectivity findings suggest that the greater reliance on familiarity processes in older adults may be mediated by a top-down frontal modulation.

3.28.3.2.3 Linking cognitive theories to age-related changes in PFC and MTL

In the first part of this chapter, we discussed behavioral evidence indicating how EM encoding and retrieval performance changes as a function of aging. We also discussed two important cognitive hypotheses that have been put forward to account for age-related deficits in EM – the resource deficit hypothesis and the binding deficit hypothesis. In the following sections, we discussed how aging affects the anatomy and function of two brain regions thought to be critical to EM function, PFC and MTL. Here, we connect these behavioral and neurobiological findings by linking the resource and binding deficit hypotheses to PFC and MTL function in older adults. Finally, returning to the beginning of the chapter, we discuss the relevance of these findings in terms of the clinical distinction between healthy and pathological deficits in EM.

3.28.3.3 Resource Deficit Hypothesis and PFC Function

As described in the behavioral section of this chapter, the resource deficit hypothesis postulates that aging reduces attentional resources, and as a result, older adults have greater difficulties with cognitive tasks, including EM tasks, that require greater self-initiated processing. This hypothesis predicts that age-related differences should be smaller when the task provides a supportive environment that reduces attentional demands. Among other findings, the resource deficit hypothesis is supported by evidence that when attentional resources are reduced in younger adults, they tend to show EM deficits that resemble those of older adults (Jennings and Jacoby, 1993; Anderson et al., 1998).

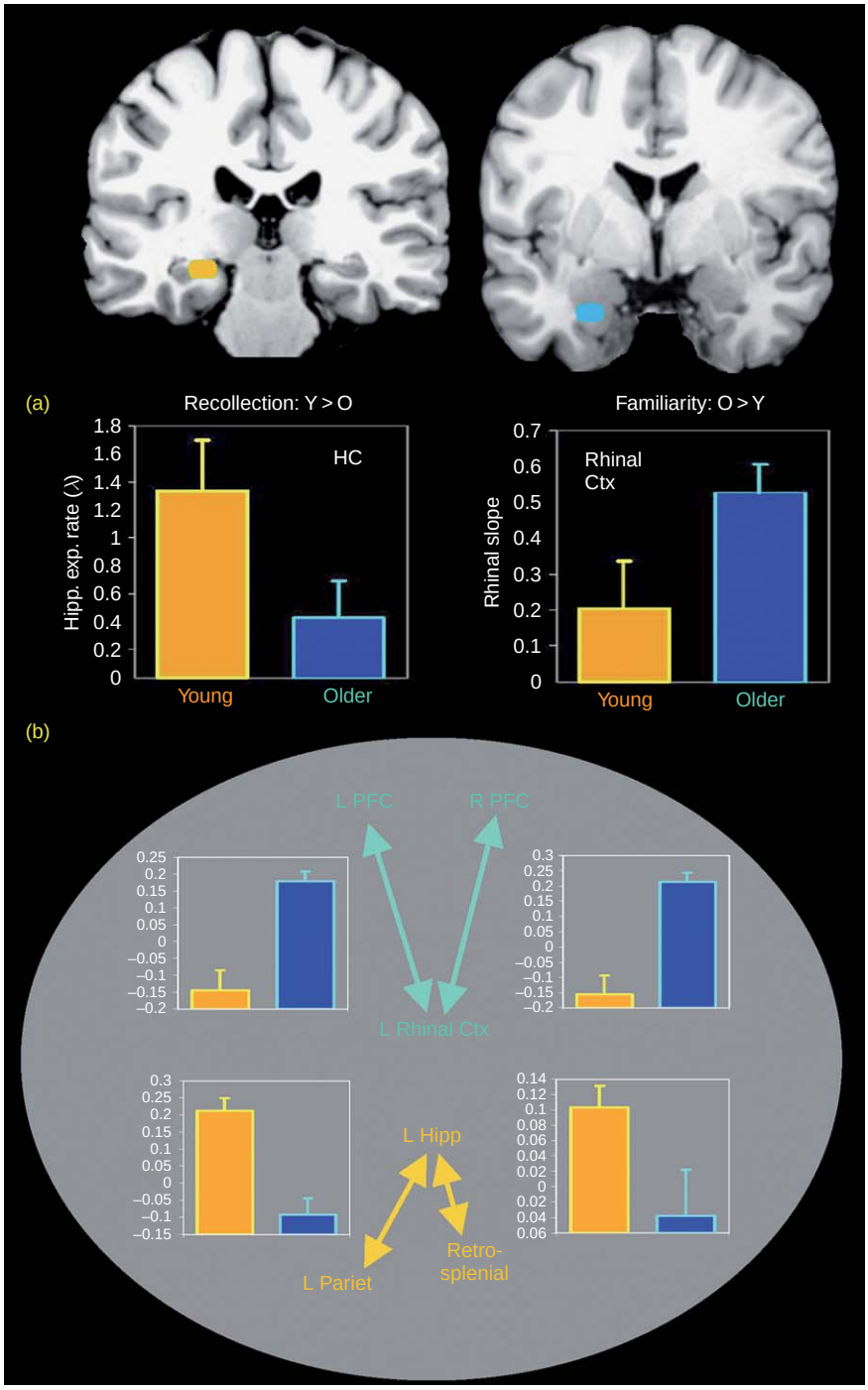

Figure 7 (a) The effects of aging yielded a double dissociations between two MTL subregions: Whereas recollection-related activity (exponential increase) in the hippocampus was attenuated by aging, familiarity-related activity (linear decrease) in the rhinal cortex was enhanced by aging. The hippocampal exponential rate parameter (λ) provides a measure of the sharpness of the exponential increase of the perceived oldness function in the hippocampus. The rhinal slope parameter provides a measure of the steepness of the perceived oldness function in the rhinal cortex. (b) Younger adults showed greater connectivity between the hippocampus and posterior regions associated with recollection, whereas older adults showed greater correlations between rhinal cortex and bilateral PFC regions. The latter effect suggests a top-down modulation of PFC on rhinal activity in older adults. Adapted from Daselaar SM, Fleck MS, Dobbins IG, Madden DJ, and Cabeza R (2006b) Effects of healthy aging on hippocampal and rhinal memory functions: An event-related fMRI study. *Cereb. Cortex* 16: 1771–1782.

Regarding neural correlates, Craik (1983) proposed that older adults' deficits in processing are related to a reduction in the efficiency of PFC functioning. As discussed in this chapter, there is much evidence in support of this idea. First, anatomical and physiological studies indicate that PFC is one of the brain regions most affected by the aging process. This region shows the most prominent gray matter atrophy and the most widespread degeneration of white matter integrity, as well as a substantial loss of DA modulation. Moreover, functional neuroimaging studies have found age-related changes in PFC activity that are generally in line with the resource deficit hypothesis.

Given the critical role of PFC in managing attentional resources, the resource deficit hypothesis predicts that age-related changes in PFC activity will be larger for tasks involving greater self-initiated processing and/or less environmental support. The results of functional neuroimaging studies are generally consistent with this prediction. During EM encoding, age-related decreases in left PFC activation were found frequently during intentional encoding conditions (which provide less environmental support) but rarely during incidental encoding conditions (which provide greater environmental support). Similarly, during EM retrieval, age-related differences in PFC activity were usually larger for recall and context memory tasks (which require greater cognitive resources) than for recognition memory tasks (which require fewer cognitive resources). Thus, in general, age effects on PFC activity tend to increase as a function of the demands placed on cognitive resources.

However, not all age-related changes in PFC activity suggested decline; on the contrary, many studies found age-related increases in PFC that suggested compensatory mechanisms in the aging brain. In particular, several encoding and retrieval studies found activations in contralateral PFC regions in older adults that were not seen in young adults. Importantly, experimental comparisons between high- and low-performing older adults (Cabeza et al., 2002; Rosen et al., 2002) demonstrated the beneficial contribution of contralateral PFC recruitment to memory performance in older adults. Moreover, a recent study using transcranial magnetic stimulation (TMS) found that in younger adults, episodic retrieval performance was impaired by TMS of right PFC but not of left PFC, whereas in older adults it was impaired by either right or left PFC stimulation (Rossi et al., 2004). This result indicates that the left PFC was less critical for younger adults and was used more by older adults, consistent with the compensation hypothesis.

It is important to note that resource-deficit and compensatory interpretations are not incompatible. In fact, it is reasonable to assume that the recruitment of additional brain regions (e.g., in the contralateral PFC hemisphere) reflects an attempt to compensate for reduced cognitive resources. One way in which older adults could counteract deficits in the particular pool of cognitive resources required by a cognitive task is to tap into other pools of cognitive resources. If one task is particularly dependent on cognitive processes mediated by one hemisphere, the other hemisphere represents an alternative pool of cognitive resources. Thus, in the case of PFC-mediated cognitive resources, if older adults have deficits in PFC activity in one hemisphere, they may compensate for these deficits by recruiting contralateral PFC regions. Moreover, age-related decreases suggestive of resource-deficits and age-related increases suggestive of compensation have often been found in the same conditions. For example, intentional encoding studies have shown age-related decreases in left PFC activity coupled with age-related increases in right PFC, leading to a dramatic reduction in hemispheric asymmetry in older adults (ie., HAROLD).

3.28.3.4 Binding Deficit Hypothesis and MTL Function

As noted in the first part of the chapter, the binding deficit hypothesis postulates that age-related memory deficits are primarily the result of difficulties in encoding and retrieving novel associations between items. This hypothesis predicts that older adults are particularly impaired in EM tasks that involve relations between individual items or between items and their context. Given that relational memory has been strongly associated with the hippocampus (Eichenbaum et al., 1994), this hypothesis also predicts that older adults will show decreased hippocampal activity during memory tasks, particularly when they involve relational information.

As noted in the preceding parts of the chapter, anatomical, physiological, and functional neuroimaging studies have identified considerable age-related changes not only in PFC but also in MTL regions. For instance, the MTL also shows substantial atrophy in aging. Yet the rate of decline differs for different subregions. Whereas the hippocampus shows a marked decline, the rhinal cortex is relatively preserved in healthy aging (**Figure 3**). This finding is in line with the idea that age-related memory deficits are

particularly pronounced during relational memory tasks, which depend on the hippocampus.

In line with anatomical findings, functional neuroimaging studies have found substantial age-related changes in MTL activity during both encoding and retrieval. Several studies have found age-related decreases in both hippocampal and parahippocampal regions. During encoding, however, declines in hippocampal activation are also seen for encoding of individual features in healthy older adults (Grady et al., 1995; Schiavetto et al., 2002; Daselaar et al., 2003a,b; Gutchess et al., 2005). Finally, during retrieval, some studies found decreases in hippocampal activity (Cabeza et al., 2004, 2005), but also greater activity in older than younger adults in parahippocampal (Cabeza et al., 2004), rhinal (Cabeza et al., 2005), or contralateral MTL (Bäckman et al., 1997; Maguire and Frith, 2003) regions, which may be compensatory.

In general, age-related changes in MTL activity are consistent with the binding deficit hypothesis. Consistent with this hypothesis, age-related reductions in hippocampal activity were found during the encoding of complex scenes, which involve associations among picture elements (Gutchess et al., 2005), and during deep encoding of words, which involves identification of semantic associations (Daselaar et al., 2003a,b; Dennis et al., 2006). Finally, a recent study specifically associated age-related reductions in hippocampal activity with recollection, which involves recovery of item-context associations (Daselaar et al., 2006b). Yet it should be noted that age-related changes in MTL activity were often accompanied by concomitant changes in PFC activity. Hence, in these cases, it is unclear whether such changes signal MTL dysfunction or whether they are the result of a decline in executive processes mediated by PFC regions. However, studies using incidental encoding tasks with minimal self-initiated processing requirements have also identified age-related differences in MTL activity without significant changes in PFC activity (Daselaar et al., 2003a,b).

As in the case of PFC, not all age-related changes in MTL activity suggest decline; several findings suggest compensation. First, similar to the bilateral pattern frequently observed in PFC, older adults have also demonstrated bilateral hippocampal recruitment while performing memory retrieval tasks (Maguire and Frith, 2003). Second, during retrieval, older adults have been found to show reduced activity in the hippocampus but increased activity in other brain regions such as the parahippocampal gyrus (Cabeza et al., 2004) and the rhinal cortex (Daselaar et al., 2006b). These results were interpreted as a recruitment of familiarity processes mediated by parahippocampal regions in order to compensate for the decline of recollection processes that are dependent on the hippocampus proper. These results fit well with the relational memory view (Cohen and Eichenbaum, 1993; Eichenbaum et al., 1994), which states that the hippocampus is involved in binding an item with its context (recollection), whereas the surrounding parahippocampal cortex mediates item-specific memory processes (familiarity). As noted in the behavioral section of the chapter, this distinction is supported not only by functional neuroimaging data in healthy young adults (Daselaar et al., 2006a) but also by lesion data from both humans (Yonelinas et al., 2002) and animals (Fortin et al., 2004).

3.28.3.5 Healthy versus Pathological Aging

As mentioned at the beginning of this chapter, one of the biggest challenges in cognitive aging research is to isolate the effects of healthy aging from those of pathological aging. A general review of the structural neuroimaging literature suggests that healthy aging is accompanied by greater declines in frontal regions compared with MTL (Raz et al., 2005). In contrast, pathological aging is characterized by greater decline in MTL than in frontal regions (Braak et al., 1993; Kemper, 1994). In fact, functional neuroimaging evidence suggests that prefrontal activity tends to be maintained or even increased in early AD (Grady, 2005). Thus, these findings suggest that memory decline in healthy aging is more dependent on frontal than MTL deficits, whereas the opposite pattern is more characteristic of pathological aging (see West, 1996; Buckner, 2004, for reviews). In view of these findings, clinical studies aimed at an early diagnosis of age-related pathology have mainly targeted changes in MTL (Nestor et al., 2004). Yet the studies reviewed in this chapter clearly indicate that healthy older adults are also prone to MTL decline. Hence, rather than focusing on MTL deficits alone, diagnosis of age-related pathology may be improved by employing some type of composite score reflecting the ratio between MTL and frontal decline.

In terms of MTL dysfunction in healthy and pathological aging, it is also critical to assess the specific type or loci of MTL dysfunction. Critically, a decline in hippocampal function can be seen in both healthy aging and AD. Thus, even though hippocampal volume decline is an excellent marker of concurrent AD (Scheltens et al., 2002), it is not a reliable measure for distinguishing normal aging from early stages of

the disease (Raz et al., 2005). In contrast, changes in the entorhinal cortex are not apparent in healthy aging (**Figure 3**), but they are present in early AD patients with only mild impairments (Dickerson et al., 2004). In a discriminant analysis, Pennanen and colleagues (2004) showed that, although hippocampal volume is indeed the best marker to discriminate AD patients from normal controls, the volume of the entorhinal cortex is much better in distinguishing between incipient AD (mild cognitive impairment, MCI) and healthy aging. Finally, it should be noted that, despite the rigorous screening procedures typical of functional neuroimaging studies of healthy aging, it remains possible that early symptoms of age-related pathology went undetected in some of the studies reviewed in this chapter.

3.28.3.6 Summary

In this chapter, we reviewed behavioral, anatomical, and functional neuroimaging evidence highlighting the role of PFC and MTL regions in age-related decline in EM function. The chapter focused on two major factors thought to underlie age-related memory decline and strongly linked to PFC and MTL function, namely, deficits in executive function and deficits in binding processes. In line with a reduction in PFC and MTL function, we discussed behavioral studies indicating that age deficits are most pronounced on tasks that put a great demand on executive and binding operations, respectively. We also discussed anatomical studies indicating a general decline with age in the anatomy and physiology of PFC and MTL. Linking these behavioral and anatomical findings, we discussed functional neuroimaging studies that generally showed age-related decreases in PFC and MTL activity during both EM encoding and retrieval. Yet some of these studies also found preserved or increased levels of PFC or MTL activity in older adults, which may be compensatory. Regarding PFC, several EM studies have found an age-related increase in contralateral PFC activity leading to an overall reduction in frontal asymmetry in older adults (HAROLD). As discussed, studies that divided older adults into high and low performers provided strong support for the idea that HAROLD reflects a successful compensatory mechanism. Regarding MTL, several EM studies reported age-related decreases in MTL activity, particularly during EM encoding. Yet studies of EM retrieval have also found age-related increases in MTL activity. Recent findings suggest that at least some of these increases reflect a compensatory shift from hippocampal-based recollection processes to parahippocampal-based familiarity processes. In sum, in view of the substantial changes in PFC and MTL that take place when we grow older, a reduction in EM function seems inevitable. Yet our review also suggests that some older adults may cope well with this reduction by shifting to alternative brain resources within PFC and MTL that can compensate for the general deficits in executive and binding operations underlying age-related EM decline.

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3.29 Alzheimer's Disease: Neurostructures

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3.29.1 Introduction

3.29.1.1 Early Alzheimer's Disease and Mild Cognitive Impairment

Alzheimer's disease (AD) is a neurodegenerative disorder that primarily affects elderly individuals and is characterized by a progressive and gradual decline in cognitive, functional, and behavioral abilities. Despite relatively good characterization of the clinical progression of AD, its underlying pathophysiological mechanisms are still poorly understood. The diagnostic criteria for AD require the presence of a primary memory disorder with at least one other affected cognitive domain and an associated deficit in activities of daily living (McKhann et al., 1984). It is now well recognized, however, that by the time an individual meets these frank diagnostic criteria, the pathologic progression of the disease has reached a more moderate severity. As a result, investigators are increasingly attempting to diagnose AD in its

'preclinical' stage, which has led to a relatively new diagnostic entity termed 'mild cognitive impairment' (MCI). The MCI classification comprises individuals who are at elevated risk for developing dementia because they have subjective cognitive complaints and have objective evidence of suboptimal cognitive function (Petersen and Morris, 2005).

Despite recent refinements in the characterization of the phenotype of AD, there has been an emphasis on the development of several important disease-related biological measurements. Currently, the sensitivity and specificity for separating early AD and controls by neuropsychological assessment (sensitivity 95%, specificity 89%) (Salmon et al., 2002) and magnetic resonance imaging (MRI) (Laakso et al., 1998) (hippocampal volume sensitivity 82%, specificity 97%, see also Matsuda et al., 2007) are similar. The development of objective biological markers for AD pathology and disease severity, in conjunction with neuropsychological evaluation, is crucial for several reasons. First, pharmacological interventions for disease modification may only be effective for individuals who are at risk for developing AD or who are at the earliest stages of the disease. Second, biomarkers that reflect disease pathology are needed to evaluate the effectiveness of interventions in reversing the disease process. Finally, biological measurement can increase specificity of the AD diagnosis by differentiating processes that are due to AD pathology from other neurodegenerative dementias and cognitive changes due to normal or average aging.

3.29.1.2 Structural Brain Imaging

Recent advances in the acquisition and analysis of MRI data afford the remarkable capability of visualizing and quantifying age-associated changes in brain morphology. Structural MRI protocols can be used to examine macrostructural changes – gross differences in tissue volume that reflect parenchymal atrophy – or microstructural changes – fiber tract integrity and pathology that can be altered due to subtle changes in myelin-associated pathology. In the current chapter, we explore the role of structural MRI as a potential diagnostic tool and its utility as a disease biomarker in the characterization of Alzheimer's patients. In conjunction with other imaging modalities and with detailed clinical data, structural MRI has emerged as a promising biological tool that has received a large amount of study in the context of AD characterization.

Before considering the role of MRI in characterizing macro- and microstructural changes seen in AD, it is important to examine what we have learned about the normal aging process from MRI. A fundamental problem of diagnosing AD at its earliest stages lies in the ability to disentangle clinical or biological changes that are attributable to normal aging from those that are due to a neurodegenerative process. Here, we discuss the patterns of neuromorphological changes that appear in normal aging captured by MRI and evidence that age-associated neuropsychological changes are at least somewhat attributable to the observed brain structural changes.

3.29.2 Normal Aging and Brain Volume Loss

3.29.2.1 Total Brain Volume across the Life Span

Over the past 20 years, investigators have used structural MRI to examine the macrostructural changes across the healthy life span. While there has been variability in findings across studies, perhaps due to discrepancies in methodology and sample characteristics, some consistent patterns have emerged. In terms of total brain volume, one large cross-sectional study that quantified total paranchymal volume across the entire life span demonstrated that the brain volume of individuals aged 71–80 years was smaller than that of healthy 2- and 3-year-old participants (Courchesne et al., 2000). Age-associated total volume loss has been shown in both research samples (Resnick et al., 2000; Good et al., 2001) and in population studies (DeCarli et al., 2005). Total brain volume may begin to decline as early as age 30, continue linearly until about age 60, and then decline at a more precipitous rate. In one of the few longitudinal examinations of total brain volume, Resnick and colleagues showed an annual decline of about $5.4 \pm 0.3\,cm^3$ in healthy individuals above age 60. The ventricular system, which may provide an index of global brain atrophy (**Figure 1**), is reported to increase at a rate of about 3% annually (Raz and Rodrigue, 2006).

3.29.2.2 Total Brain Gray versus White Matter Macrostructural Loss

There is some debate in the literature regarding the relative prominence of gray versus white matter structural changes associated with normal aging (Peters and Rosene, 2003). Age-associated white matter loss may

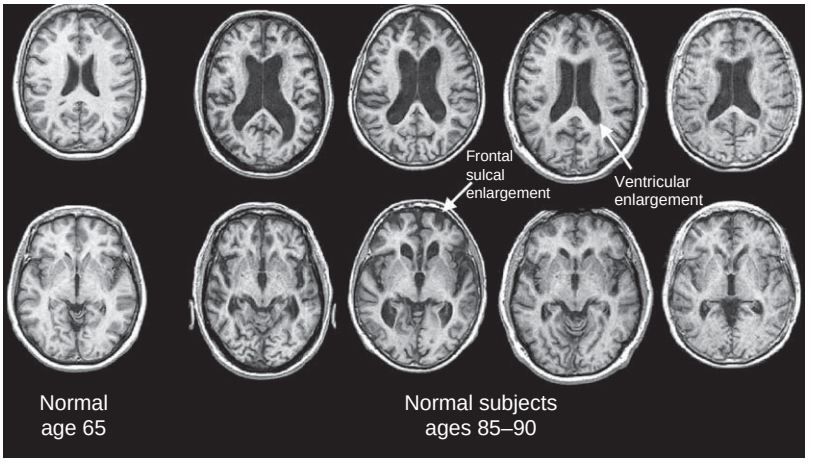

Figure 1 MRI in normal elderly and normal very old subjects. Note ventricular enlargement, frontal sulcal enlargement, and greater prominence of sulcal markings in very old subjects ages 85–90.

be a manifestation of myelin degeneration (Peters, 2002) and consistent with the idea that the aging brain is characterized by deficient neuronal connectivity (Albert, 1993). Indeed, several studies have demonstrated either a relatively greater age-associated decline in white matter than gray matter or white matter decline in the absence of gray matter decline (Guttmann et al., 1998; Franklin et al., 2002; Bartzokis et al., 2003; Resnick et al., 2003). However, the opposite pattern has also been observed: age-associated gray matter changes have been observed in the absence of white matter changes (Blatter et al., 1995; Thompson et al., 2003; Sullivan et al., 2004). Discrepancies may be due to a number of factors. First, white matter may increase up to age 45–55 and then begin to decrease (Bartzokis et al., 2001) so that white matter loss may only appear in quite old samples (**Figure 2**). Second, and similarly, approaches to segmentation (e.g., probabilistic, Bayesian, pixel intensity driven) may differ across laboratories both in terms of segmentation algorithm and intensity thresholds. Finally, white matter changes may be subtle and heterogeneous and could best be captured through examination of white matter microstructure or white matter pathology.

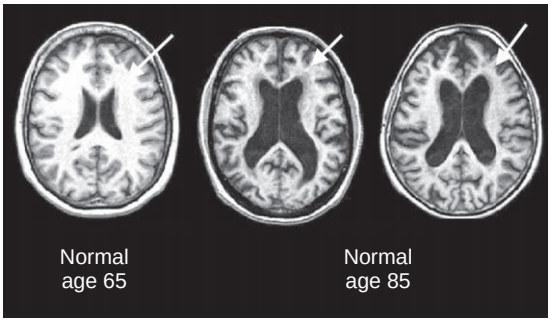

Figure 2 Reduced white matter in over-85-year-old normal individuals.

3.29.2.3 Total White Matter Microstructural Changes

In addition to white matter volume, which can be appreciated with T1-weighted high-resolution anatomical MR images, there has been a recent emphasis on the quantification of anisotropy and white matter hyperintensities (WMH) on diffusion-weighted and FLAIR-weighted or T2-weighted imaging, respectively. The most commonly derived measurement from diffusion tensor imaging (DTI) is fractional anisotropy (FA). Because white matter fibers are mostly highly organized in parallel orientation, water motion is restricted, causing FA measurements derived from white matter to be relatively more anisotropic than gray matter or cerebrospinal fluid (CSF). Anisotropy refers to the orientation and coherence of white matter fiber tracts and may be a more sensitive marker of the integrity of myelin sheaths than examination of bulk volume. For example, studies have been inconsistent in demonstrating bulk volume white matter change as a function of normal aging, but diffusion tensor studies have been much more unified in revealing anisotropic changes across the life span (Nusbaum et al., 2001; O'Sullivan et al., 2001; Pfefferbaum and Sullivan, 2003; Head et al., 2004; Sullivan and Pfefferbaum, 2006), which may be linear beginning at about age 20 (Pfefferbaum

and Sullivan, 2003). In terms of regional distribution of age-associated anisotropic changes, DTI studies have generally mirrored positive volumetry studies in demonstrating an anterior-to-posterior gradient of effects (Head et al., 2004; Pfefferbaum et al., 2005; Salat et al., 2005).

WMH are areas of increased lucency appearing on FLAIR- or T2-weighted images. They can be discrete, or punctate, or may appear more confluent with the lateral ventricles (**Figure 3**). Until recently, WMH were considered clinically irrelevant, but a culmination of sample- and population-based research has demonstrated their functional significance (Malloy et al., 2007). With the exception of those that appear as smooth 'rims' or 'caps' along the surface of the lateral ventricles, WMH are thought to be ischemic in nature and reflect rarefaction of myelin, breakdown of vessel endothelium, and microvascular disease (Fazekas et al., 1993). The presence of WMH is common among normal elderly adults, and chronological age appears to be the most salient predictor of severity (Jernigan et al., 1991; de Leeuw et al., 2001). Vascular risk factors, such as hypertension, also account for much variability in severity of WMH (de Leeuw et al., 2001).

3.29.2.4 Regional Differences in Brain Volume Loss with Age

3.29.2.4.1 Lack of uniformity of brain volume loss

Although consideration of total brain volume across the life span is important in establishing gross anatomical changes, a closer examination of regional effects can help identify specific neurobiologic systems that selectively decline with age. Normal aging is associated with a 'posteriorization' of the pattern of preserved tissue. In cortical areas, there appears to be a consistent anterior-posterior gradient in degree of atrophy across the adult life span, with greater frontal than posterior atrophy and indication from functional imaging that cognitive functions may be mediated by more posterior locations with advancing age (e.g., Hazlett et al., 1998). Comprehensive findings of age-associated regional decline have been reviewed in great detail elsewhere (Raz, 2000, 2004; Raz and Rodrigue, 2006). In this chapter we will focus on the frontal lobe, the area of greatest decline, and the temporal lobe, the area most altered in AD.

3.29.2.4.2 Frontal lobe volume loss and aging

Most studies that have examined regional cortical effects with aging report that the greatest amount of age-associated volumetric loss occurs in frontal cortical regions (DeCarli et al., 1994; Raz et al., 1997; Salat et al., 1999; Tisserand et al., 2002; Resnick et al., 2003), followed by the temporal lobes (Cowell et al., 1994; Sullivan et al., 1995), with relative sparing of the occipital lobes and primary sensory regions (Bartzokis et al., 2001; Good et al., 2001). A recent review by Raz and Rodrigue (2006) nicely contrasts cross-sectional structural MRI studies and longitudinal studies, and a review by Hedden (Hedden and Gabrieli, 2005) emphasizes frontal-striatal aging with more posterior sparing.

Although MRI studies have shown consistent age-associated reductions in frontal lobe volume, it is unclear whether the changes are due to selective reduction of tissue type within specific frontal subregions. To address this issue, we examined age-related relative gray and white matter volume changes in four discrete prefrontal brain regions (Brickman et al., 2005). Seventy neurologically and cognitively healthy adults were recruited, with 10

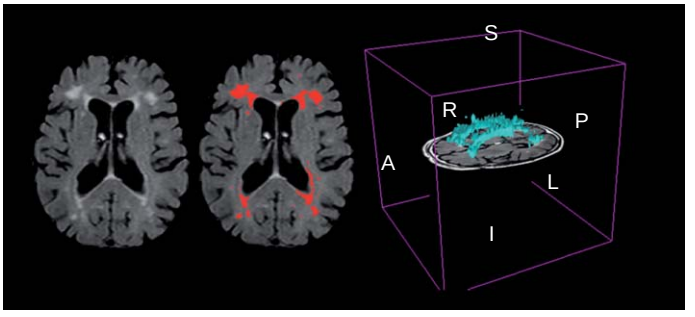

Figure 3 White matter hyperintensity surrounding ventricular cavity and extending into prefrontal region in patient with AD. Left: skull-stripped FLAIR image. Middle: Computer algorithm identifies hyperintensity regions. Right: Hyperintensities represented in three dimensions in cyan. Note shape similar to ventricular cavity.

subjects represented in each age decade from 20s to 80s, and scanned with high-resolution structural MRI. Prefrontal regions were segmented into orbital (Brodmann Areas [BA]: 11, 12, 47), medial/cingulate (BA: 24, 25, 32), dorsolateral (BA: 44, 45, 46), and lateral (BA: 8, 9, 10) gray and white matter using a stereotaxic atlas method (**Figure 4**). Total frontal lobe volume declined linearly across the seven decades, with the lateral and cingulate frontal areas showing the greatest amount of decline (**Figure 5**). In general, gray matter showed a gradual decline across the decades, whereas white matter remained

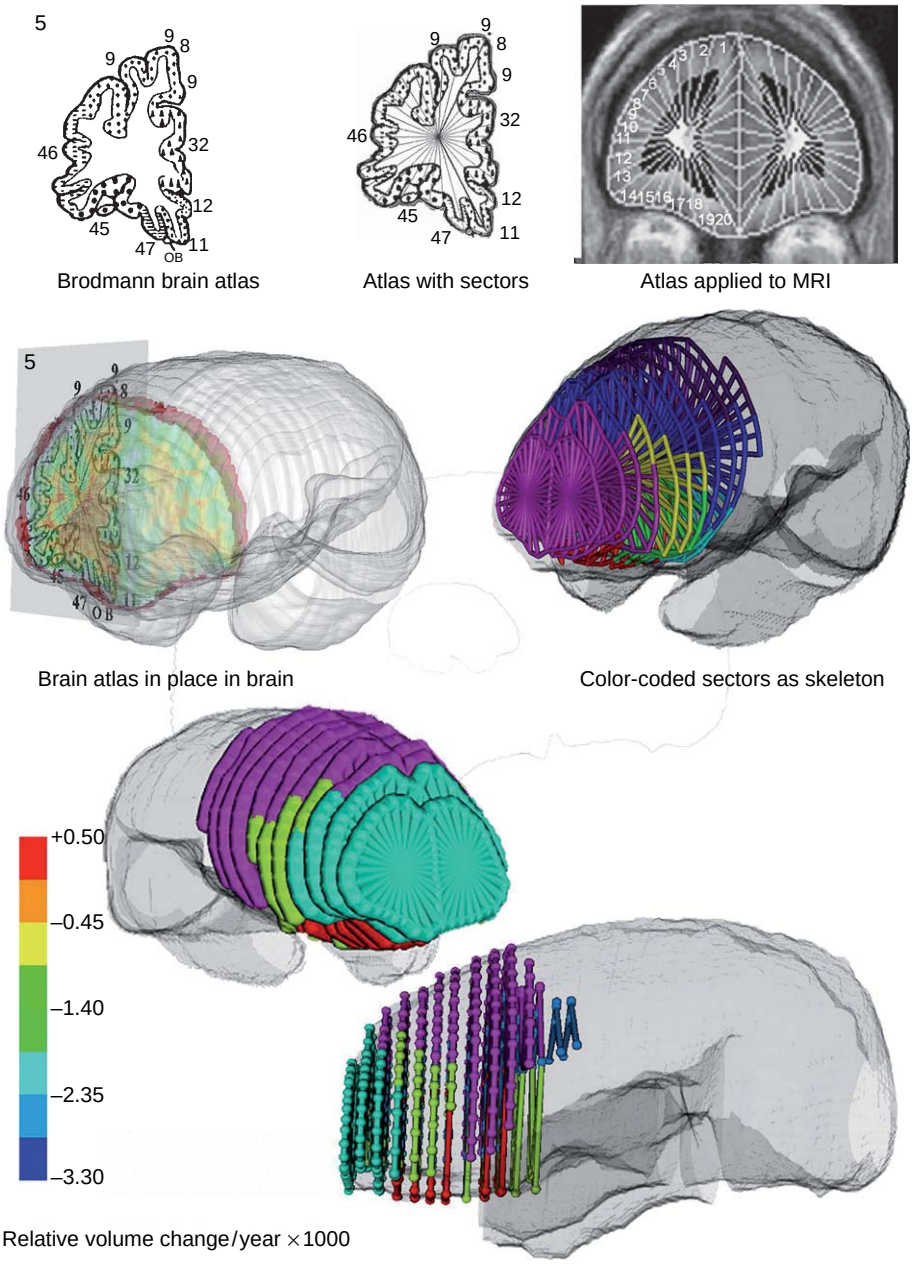

Figure 4 Brodmann area analysis. Top row: (left) Perry atlas showing Brodmann areas drawn from microscopic examination of human brain. (middle) Perry atlas with computer drawn sectors and (right) matching coronal MRI with computer-drawn sectors. Middle row: (left) Perry atlas in 3D position within MRI brain surface and (right) computer-drawn sectors as skeleton. Bottom row: Change in age per year for each of the Brodmann areas of the brain: color scale shows relative change per year × 1000.

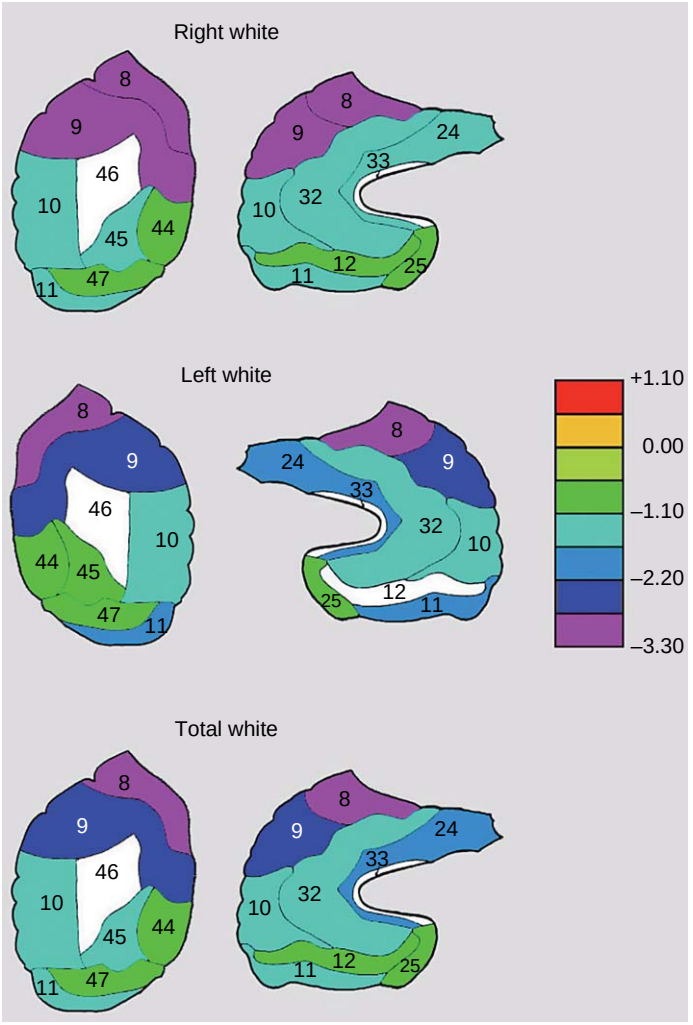

Figure 5 Rate of change of relative gray matter volumes in prefrontal cortex of 70 normal controls ages 20–90. Greatest rate of volume loss is in dorsolateral areas 8 and 9 and, in the medial frontal lobe, area 32.

relatively stable until the last two decades, particularly in lateral, dorsolateral, and orbital regions. These findings are consistent with others that have suggested that white matter decline may be more prominent among the very old (Salat et al., 1999; Raz et al., 2005).

Findings of subcortical gray matter structures that are involved in frontal circuitry have been less consistent. Most (Krishnan et al., 1990; Jernigan et al., 1991; McDonald et al., 1991; Murphy et al., 1992; Raz et al., 1995; Gunning-Dixon et al., 1998; Zimmerman et al., 2006) but not all (Raz et al., 2000) studies that have examined the caudate nucleus have shown an age-associated volumetric loss. Those examining the putamen have been less consistent; some have demonstrated that increased age is associated with decreased volume (McDonald et al., 1991; Gunning-Dixon et al., 1998; Raz et al., 2000), and others have shown no significant difference in the putamens of older subjects relative to younger (Murphy et al., 1992). In our own study (Brickman et al., 2003), increased age was associated with a decrease in the absolute size of the caudate and no change in absolute putamen size. However, when considering the relative size of the two structures, increased age was associated with an increase in putamen size and no differences in caudate size, indicating a relative age-associated preservation of these structures. Findings regarding the thalamus have been similarly discrepant (Raz and Rodrigue, 2006).

3.29.2.4.3 Temporal lobe volume loss and aging

In detailed studies comparing the frontal, temporal, parietal, and occipital lobe volumes, Allen et al. (2005) found temporal lobe volume was relatively stable from age 20 to 60 but then decreased and was best fit with a cubic function. In contrast, frontal lobe gray declined in a linear trend across the life span.

Some investigators have reported age-associated decline in hippocampal volume (**Figure 6**) (Murphy et al., 1996; Mu et al., 1999; Pruessner et al., 2001; Raz et al., 2004), while others have not (Sullivan et al., 1995; Good et al., 2001). Longitudinal analyses of hippocampal volume have suggested a 1.5% volume loss per year in healthy individuals ranging in age from 18 to 42 years (Pruessner et al., 2001), though another longitudinal study observed a similar rate of decline but only among individuals over 50 (Raz et al., 2004).

Differences in hippocampus findings across studies may be due to quantification protocols. For example, Raz et al. (2004) explicitly compared age effects in the entorhinal cortex to the hippocampus proper and found a selective age-associated decline in the hippocampus only. Good et al. (2001), who did not show a cross-sectional association between age and hippocampal density, used voxel-based morphometry (VBM), which involves nonlinear spatial normalization of the MR images that may distort small structures.

3.29.2.4.4 Multivariate approaches to regional brain loss with age

Past examinations of neuromorphological changes across the healthy adult life span have primarily relied on region-of-interest or voxel-based approaches. While these cross-sectional and longitudinal efforts have illuminated a tremendous amount of information about structural changes, they do not consider the relationship among brain regions. We recently conducted the first study that has applied a multivariate 'covariance' approach to understanding distributed patterns of age-associated morphological change (Brickman et al., 2007). Using a multivariate approach based on principal components analysis and the subprofile scaling model, which captures sources of between- and within-group variation, patterns of gray and white matter were identified that could reliably discriminate between younger and older participants. Consistent with previous efforts, these patterns included anterior cortical regions, but they also included widespread posterior cortical and subcortical regions. Forward application of the patterns to an independent sample (Brickman et al., in press) showed good stability of the identified gray matter pattern, but not white matter. The findings were similar to a recent study by Alexander and colleagues, who also used a principal components approach (Alexander et al., 2006).

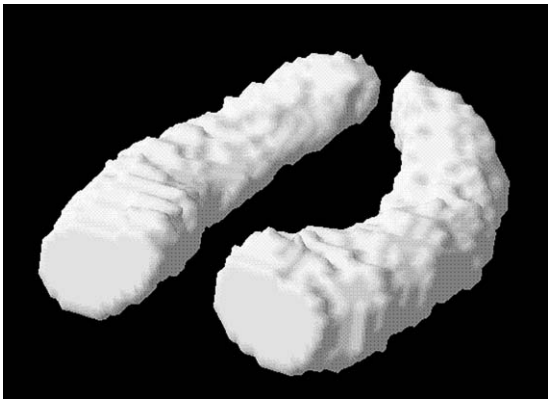

Figure 6 Three-dimensional representation of hippocampus based on tracing of 70 coronal slices. Hippocampus is viewed from the back of the head looking down and forward.

3.29.3 Cognition and Brain Volume Loss across the Normal Adult Life Span

3.29.3.1 Executive Function and Frontal Lobe Aging

The exact pattern of changes in neuropsychological function across the adult life span remains somewhat elusive. One compelling, though controversial (Greenwood, 2000; Band et al., 2002), theory, termed 'Frontal Aging Hypothesis' (West, 1996, 2000) proposes that cognitive functions mediated by the frontal lobes are most vulnerable to the effects of age and selectively decline in normal, healthy aging. Although cognitive functions supported by more posterior regions clearly decline in normal aging (Greenwood, 2000; Small, 2001), the theory argues that there is greater magnitude of decline for processes mediated by the frontal lobes.

Executive function refers to a set of cognitive processes involved in complex goal-directed behavior, adaptation, planning, cognitive flexibility, and allocation of attentional resources (Loring, 1999). Although the construct is somewhat heterogeneous, comprised of several components, there is good consensus in the neuropsychological literature for its standard

assessment (Lezak, 1995). Executive function is thought to be mediated by the frontal lobes (Elliott, 2003) and by frontal-striatal circuitry (Alexander et al., 1986; Alexander and Crutcher, 1990; Chow and Cummings, 1999; Lichter and Cummings, 2001). Several lines of research indeed demonstrate particular age-associated decline in executive functioning (Whelihan and Lesher, 1985; Ardila and Roselli, 1989; Daigneault et al., 1992; MacPherson et al., 2002). In our own study, cluster analysis was applied to performance on a battery of neuropsychological tests to define three cognitive profiles among healthy older adults (Gunstad et al., 2006). Relative to younger adults, older participants clustered in to one of three neuropsychologically defined groups: one with relatively poor performance across all cognitive domains, one with selective executive impairment alone, and one with executive impairment and psychomotor slowing. Despite the heterogeneity in neuropsychological profiles, all groups evidenced a consistent reduction on performance of executive tests relative to younger adults.

3.29.3.2 Normal Age-Associated Cognitive Decline Is Partially Attributable to Morphological Change

A critical question is whether the morphological changes observed with normal aging account for some of the cognitive changes that occur across the adult life span. On a face-valid level, normal age-associated cognitive changes are characterized primarily by a decline in executive abilities, and structural changes show a general pattern of anterior-to-posterior age-associated atrophy or anisotropic changes and increases in WMH, suggesting a link between the two. More recent work has addressed this issue by comparing regional morphology to performance on batteries of neuropsychological tests. Indeed, several reports suggest that age-associated morphological changes mediate normal age-associated cognitive decline. Gunning-Dixon and Raz (2003) reported age-related variability on a task of executive functioning that was mediated by prefrontal cortex volumes. Raz and colleagues (Raz et al., 1998) also examined the relationship between several cortical volumes and performance on cognitive tests of executive functioning, working memory, explicit memory, and priming in healthy adults across the life span. Findings indicated that a decrease in dorsolateral prefrontal gray matter volume mediated age-related cognitive deficits on a test of executive functioning. Further support for a role of the prefrontal cortex in age-associated cognitive decline was provided in a longitudinal voxel-based morphometry study (Tisserand et al., 2004), where age-related cognitive decline was associated with decreased gray matter density in prefrontal and medial temporal lobes. We showed that chronological age interacts with the gray matter volume of the lateral frontal lobe in predicting a summary score of executive test performance, suggesting a stronger relationship between lateral frontal lobe volume and executive functioning among older healthy adults (Zimmerman et al., 2006). Similarly, we demonstrated that relative frontal lobe white matter volume mediated the association between chronological age and performance on tasks of executive ability and declarative memory (Brickman et al., 2006). Using a multivariate approach, we demonstrated that the degree to which older adults evidenced a regional pattern of age-associated grey and white matter atrophy was associated with performance on tests of executive function and memory (Brickman et al., 2007).

3.29.3.3 Hippocampal Aging and Memory Function

A particularly important consideration when discussing normal aging in the context of AD is the specific role of the hippocampus. While it is arguable that many cortical and subcortical areas are involved with several aspects of cognition, it has long been known that medial temporal lobe structures, particularly the hippocampus and its anatomically related structures, play a specialized role in learning and memory (Scoville and Milner, 1957; Rempel-Clower et al., 1996). However, attempts to understand the relationship between the size of the hippocampus and memory function have been remarkably mixed (Van Petten, 2004). A recent meta-analysis reported that, on average, the association between hippocampal volume and memory test performance was negative among studies that included children and young adults and positive among studies that included older adults (Van Petten, 2004). Notably, the consistency of the observed relationship among older adults was weak, and the average magnitude of the correlation coefficients between hippocampal volume and memory test performance among older adults was small. This important observation highlights the heterogeneity in reported hippocampal volume effects on cognition among healthy older adults and suggests that the volume of the

hippocampus may be positively related to cognition only in the face of age-associated pathology.

3.29.3.4 White Matter Microstructure and Cognition

Relatively few studies have examined the impact of age-related differences in anisotropy and their impact on cognition. One study showed that decreases in anisotropy in the anterior limb of the internal capsule were associated with poorer performance on a visual target detection task (Madden et al., 2004). A recent study by Grieve and colleagues demonstrated significant age-associated decreases in anisotropy in prefrontal and parietal regions, which were associated with performance on neuropsychological tests of executive function (Grieve et al., 2007).

The severity of WMH is also associated with poorer cognitive abilities among older adults (Gunning-Dixon and Raz, 2000). Cook and colleagues (Cook et al., 2002) identified an association between subcortical WMH volume and cognitive functioning in older adults who performed within normal limits on neuropsychological tests. A recent report from the Framingham study showed that older individuals with greater WMH volumes performed significantly worse on visuospatial, visual scanning, speed, and learning tasks (Au et al., 2006).

In summary, a long history of examination of cognitive changes with normal aging and their neuroanatomical correlates reveals a general pattern with a large amount of heterogeneity. The 'frontal aging hypothesis' is one convenient approach to summarizing the changes observed with normal aging; cognitive abilities, such as executive functions, which are mediated by frontal lobe circuitry, appear to decline with age at the greatest rate. Structural changes parallel these findings, with an anterior-posterior gradient of age-associated atrophic changes. Findings regarding the relative age-associated differences in white and gray matter bulk volume are still inconsistent, but advances in white matter imaging with diffusion- and FLAIR-weighted sequences suggest that white matter changes may be largely microstructural in nature. Finally, whether normal age-associated changes in hippocampal volume impact cognitive abilities is still unclear; one reasonable possibility for discrepant findings is that hippocampal structure impacts cognition only in the face of pathology.

3.29.4 Alzheimer's Disease and MCI as Cognitive Disorders

AD is a cognitive disorder that can only be definitively diagnosed through microscopic examination of brain tissue. Most antemortem diagnostic schemes (McKhann et al., 1984; American Psychiatric Association, 1994) require a significant decline in memory function accompanied by an impact on functional abilities. It is therefore not surprising that neuropsychological measures of memory ability, particularly delayed recall on list-learning tests, have been repeatedly demonstrated to discriminate between those meeting diagnostic criteria for AD and otherwise similar elderly control subjects (Welsh et al., 1991), even among very mildly impaired individuals (Salmon et al., 2002). Although memory impairment is the defining cognitive feature of AD, differences between mildly affected AD patients and neurologically healthy controls or individuals with other types of dementia have been reported in other cognitive domains such as global cognitive status (Salmon et al., 2002), verbal fluency (Monsch et al., 1992; Salmon et al., 2002), and executive function (Lafleche and Albert, 1995). As, by definition, the cognitive deficits in AD are progressive, patients ultimately evidence severe deficits across domains, and in later stages of the disease, the neuropsychological profile is defined by severe global impairment.

Although neuropsychological evaluation can detect AD with a high degree of sensitivity and specificity when individuals meet diagnostic criteria (Salmon et al., 2002), it is now clear that the disease pathology is present up to years before individuals reach the point of frank cognitive impairment (Morris et al., 2001). To deal with this issue, the term 'mild cognitive impairment' has been applied to describe individuals who may be at particular risk for future dementia because their cognitive abilities fall between what would be expected for normal aging and the degree of severity seen in frank dementia (Petersen et al., 1999). A major thrust in defining the construct has been the assumption that secondary prevention is more effective than tertiary prevention (Petersen, 2007), underlying the necessity for the identification of the disease at its earliest possible point. Current algorithms for diagnosis of MCI draw a distinction between 'amnestic' subtypes and 'nonamnestic' subtypes, based on the whether memory is the primary cognitive domain evidencing abnormal ability in the

context of essentially normal functional activities (Petersen, 2004). Ostensibly, it is the amnestic MCI subtype that is most specifically associated with future development of AD. It is important to note that the concept of MCI would be largely irrelevant if there were objective clinical tools that could identify Alzheimer's pathology at its earliest manifestations in the brain. Nonetheless, the development and subsequent refinement of the MCI concept have increased diagnostic sensitivity and specificity and have stimulated the need for the identification of biomarkers. In the following section we explore the structural changes associated with AD and MCI that have been identified with MRI.

3.29.5 Brain Volume Changes in Alzheimer's Disease

3.29.5.1 Total Brain Volume

One of the more consistent findings in the structural neuroimaging literature involving AD is that markers of total brain atrophy, including total brain volume or relative volume to total intracranial space, are accentuated, and ventricular size or amount of sulcal cerebral spinal fluid is reduced compared to controls. These measures may provide information about the overall health of the brain or may provide relative markers for overall stage of the disease, but tell us little about specific neurobiological underpinnings. Nonetheless, both cross-sectional (Seab et al., 1988; Murphy et al., 1993; Lehericy et al., 1994; Ohnishi et al., 2001) and longitudinal (Cuénod et al., 1993; Fox et al., 1996, 1999; Tanabe et al., 1997; Chan et al., 2001a, 2003; Du et al., 2001; Thompson et al., 2003) data show marked reduction of total brain volume or expansion of the ventricular system, including increases in the AD brain, that is related to the severity of the disease (Seab et al., 1988; Murphy et al., 1993; Fox et al., 1996, 1999). Rates of total brain volume decline per year have been reported to range from less than 1% to greater than 12%, with an average reported *per annum* percentage loss of total volume loss of about 3.6% (Ramani et al., 2006). While the rate of total brain atrophy is greater in AD and in individuals who eventually develop AD than in matched controls, markers of total brain atrophy are not specific to the AD diagnosis. Thus, measures of total brain volume may be useful in staging severity of the disease but may have very little differential diagnostic clinical utility.

3.29.5.2 Regional Brain Changes

The earliest clinical application of structural neuroimaging to dementia by de Leon (de Leon et al., 1979), Tsai (Tsai and Tsuang, 1979), and others alerted researchers to the potential of the approach. An early application to AD was for the purpose of ruling out other brain pathologies, such as cortical infarction or diffuse cerebral vascular disease, that may account for the cognitive changes observed in the patient (McKhann et al., 1984). However, researchers quickly began to recognize the potential utility of MRI for the appreciation of more subtle aspects of the disease. The majority of structural MRI studies of AD have asked the question, "Are there aspects of morphology that can reliably differentiate patients with AD from otherwise similar older adults?" In more recent years, this question has been refined to parallel developments in the clinical nosology of AD. For example, newer studies focus on morphological differences among individuals with MCI, AD, and matched controls. Furthermore, improvement in the resolution of acquired structural MRI scans has advanced our ability to quantify smaller brain structures with good reliability, and recent efforts have been informed by pathological studies of AD in identifying regions of interest that are affected at the earliest stages of the disease. As reviewed in the sections that follow, more posterior regions of the brain tend to show abnormalities in AD, while frontal lobe change (reviewed in the section titled 'Frontal lobe volume loss and aging') has been characteristic of normal aging (**Figure 7**).

Longitudinal analysis of changes in brain structure and the use of baseline analysis of morphology to predict future decline have been other recent methodological advancements in the understanding of brain structure in AD. Finally, although not the focus of this review, the combination of structural

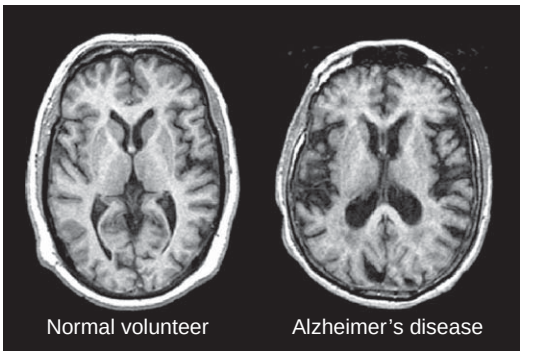

Figure 7 Typical MRI in AD.

imaging protocols and functional brain analysis might ultimately be the most powerful approach to *in vivo* characterization of the AD brain.

3.29.5.2.1 Frontal and parietal lobes

Despite the marked frontal lobe changes observed in normal aging, relatively few investigators have examined gross lobar volumetric decreases in AD outside of the temporal lobe findings reviewed in the next section. One report suggested that AD patients in mild stages of the disease have reduced left, but not right, frontal lobe volumes compared to controls (Laakso et al., 1998); others have reported bilateral frontal lobe volumetric reduction in more advanced stages (Rusinek et al., 1991; DeCarli et al., 1995). AD-related volumetric loss has also been reported in the parietal lobes (Foundas et al., 1997; Kidron et al., 1997) and occipital lobes (Rusinek et al., 1991). Effect sizes for frontal lobe volume in AD versus normal volunteers and frontotemporal dementia versus normal volunteers were 0.63 (our computation) and 1.86, respectively. In this study, due to very large standard deviation in anterior temporal lobe size in AD (mean 28.8, SD 30.5), temporal lobe effects size for AD versus normal volunteers was only 0.21 (our computation).

3.29.5.2.2 Temporal lobe structures

One early study that quantified total hippocampal volume showed about a 40% reduction among individuals in early to moderate stages of dementia severity (Seab et al., 1988). This finding is generally consistent with other reports of bilateral hippocampal proper volume loss, ranging from about 10% (Killiany et al., 2002) up to about 60% (Bobinski et al., 1995; reviewed in Ramani et al., 2006). In fact, most studies that have quantified total hippocampal volume have indeed shown significant volume reduction in AD patients compared to matched controls (Krasuski et al., 1998; Bobinski et al., 1999; Csernansky et al., 2000; Du et al., 2001). Thus, the culmination of evidence suggests reliable and marked volume loss in the hippocampus proper in mild, moderate, and severe AD.

Pathological studies confirm, however, that the earliest deposition of the AD-defining β-amyloid plaque occurs in the entorhinal cortex (Hyman et al., 1984, 1990; Arnold et al., 1991; Braak et al., 1993; Gómez-Isla et al., 1996). Recent advances in visualizing the boundaries of medial temporal lobe structures and more reliable region-of-interest (ROI) protocols have allowed for the quantification of the entorhinal cortex *in vivo*. These advances have led to the effort to increase the specificity of volumetric studies in AD. Juottonen and colleagues (Juottonen et al., 1998) used a histologically based protocol to identify and measure the entorhinal, perihinal, and temporopolar cortices of patients with AD versus controls. The authors found a 40% volume reduction that was specific to the entorhinal cortex. Volume reductions in the entorhinal cortex discriminated between patients and controls with 92% accuracy. While this finding is reminiscent of the observed 40% reduction in the hippocampus proper in AD (Seab et al., 1988), authors that have specifically contrasted entorhinal cortex and hippocampus volume within the same subjects have observed an important dissociation. For example, Killiany and colleagues (Killiany et al., 2002) observed about a 60% reduction in entorhinal cortex volume but only a 10% reduction in hippocampus volumes among patients with AD compared to controls. Other reports have shown more subtle differences, but usually in the same direction, with relatively greater entorhinal cortex volume reduction than hippocampus volume reduction in AD (Bobinski et al., 1999; Du et al., 2001). Further, the degree of entorhinal cortex atrophy in AD is correlated with the distribution of neurofibrillary tangles, one of the defining pathological markers of AD (Bobinski et al., 1999).

While hippocampus and entorhinal cortex volume analysis appears to reliably differentiate those with AD from matched controls, discrimination from other age-associated neurodegenerative diseases based on these measures alone is poor (de Leon et al., 2007). For example, global atrophy rates are similar between patients diagnosed with AD and those diagnosed with Lewy body dementia (Hashimoto et al., 1998; Barber et al., 1999; O'Brien et al., 2001; Minoshima et al., 2002), and entorhinal cortex and hippocampus volume are similarly affected in individuals with frontotemporal dementia (Frisoni et al., 1999; de Leon et al., 2007).

Generalized atrophy in the temporal lobe has also been reported (Cuénod et al., 1993; Killiany et al., 1993; Kidron et al., 1997; Juottonen et al., 1998), although one study that examined the volume of the superior temporal gyrus showed no difference between AD patients and controls (Bobinski et al., 1999).

Although morphometric studies of the hippocampus and entorhinal cortex in AD are important in establishing the ability to use structural MRI to visualize pathologically related volumetric loss, the more

critical issue is whether MRI can be used to detect AD-related changes at earlier stages of the disease. Thus, many authors have cross-sectionally compared individuals with MCI to matched controls. As noted, MCI can be thought of as a 'preclinical' state of AD, and so-called 'conversion rates' to meeting full diagnostic criteria for AD have been reported to be remarkably high by some research groups (Petersen, 2004, 2007), although 'MCI' is not necessarily synonymous with 'early AD,' and several individuals meeting criteria for MCI do not ultimately convert (Petersen et al., 2001; Ritchie et al., 2001; Larrieu et al., 2002). Studies that have used MRI to compare MCI patients to controls have demonstrated statistically reliable increased amounts of medial temporal lobe atrophy in the former group (Kaye et al., 1997; Jack et al., 1999; Visser et al., 1999; Karas et al., 2004; reviewed in Mosconi et al., 2007). Not surprisingly, within the medial temporal lobe, significant volume reduction in the hippocampus and entorhinal cortex has been reported among patients with MCI or very early AD compared to healthy controls, with values falling in the intermediate range between controls and more-moderately demented AD patients (Juottonen et al., 1998; Bobinski et al., 1999; De Toledo-Morrell et al., 2000; Xu et al., 2000; Chan et al., 2001b; Dickerson et al., 2001; Du et al., 2001; Pennanen et al., 2004; Wolf et al., 2004; Bell-McGinty et al., 2005; Apostolova et al., 2006; Devanand et al., 2007).

In addition to AD- and MCI-associated atrophic changes seen in hippocampus and entorhinal cortex, several studies have attempted to identify other disease-associated structural changes (Atiya et al., 2003; Ramani et al., 2006). These areas have generally paralleled the distribution of AD pathology and will be reviewed briefly here. Significant AD-related atrophy has been observed in other medial temporal lobe structures besides the entorhinal cortex and hippocampus, including parahippocampal gyrus (Kesslak et al., 1991; Ikeda et al., 1994; Jack et al., 1997; Callen et al., 2001; Suzuki et al., 2003) and amygdala (Scott et al., 1991; Lehericy et al., 1994; Jack et al., 1997; Krasuski et al., 1998). Effect sizes for the difference between controls and AD patients were larger for the amygdala (1.28) than hippocampus (0.99), indicating the salience of amygdala change (Basso et al., 2006).

3.29.5.2.3 Corpus callosum

Similar to studies of normal aging, there has been a recent interest in white matter volume and white matter markers of pathology among patients with AD. Janowsky and colleagues (Janowsky et al., 1996) compared the size of the corpus callosum in patients with AD versus controls and found significant reductions in the former. Teipel and colleagues (Teipel et al., 2002) studied the longitudinal progression of corpus callosum volume in AD and found an average annual rate of decline in the splenium of about 12% among patients relative to 1.5% in controls, and the rate of corpus callosum change was significantly related to the rate of clinical decline among the patient group. The splenium, isthmus, the body, and the rostral portion of the genu of the corpus callosum were significantly reduced in volume in AD (Chaim et al., 2007). Further, the size of the corpus callosum was related to the severity of scores on a test of memory.

3.29.5.2.4 Fornix and mamillary bodies

Significantly smaller volumes were observed in the fornix and mammillary bodies in patients with AD as compared with healthy controls and MCI participants (Copenhaver et al., 2006).

3.29.5.3 Longitudinal Analyses

Cross-sectional studies suggest that volumes of structures within the medial temporal lobe can be used to dissociate among groups of patients and roughly map onto the severity of the associated cognitive symptoms, as MCI and AD diagnoses are primarily based on severity of cognitive symptoms. More powerful approaches to understanding the structural underpinnings of the cognitive loss seen in MCI and AD include longitudinal analyses, structural analysis of baseline characteristics in the prediction of future cognitive decline, and individual differences studies comparing structural volumes to specific cognitive symptoms. Indeed, longitudinal analyses of AD patients compared to controls consistently show a greater rate of general atrophy among the patient group (Fotenos et al., 2005), which can be observed even within a 3- to 6-month longitudinal follow-up period (Bradley et al., 2002). Using boundary shift methods, Fox and colleagues have shown that individuals carrying the familial AD gene are dissociable from matched controls at baseline and in terms of greater atrophy rates over time (Ridha et al., 2006), as well as an increased likelihood of classification as AD among those with sporadic forms of the disease (Ridha et al., 2006). Boundary shift methodology as well as other anatomical mapping techniques allow for increased reliability in analysis of longitudinal

MRI data (Thompson et al., 2004). But traditional ROI approaches have also reliably dissociated longitudinal rates of change in AD from normal aging. For example, Weiner and colleagues, using manual tracing ROI approaches, demonstrated that the rate of volumetric loss of the entorhinal cortex among patients with AD was five times greater than that of controls and that the rate of volumetric loss was greater in entorhinal cortex relative to areas of cortex (Ezekiel et al., 2004). The pattern of results has generally paralleled that seen in cross-sectional studies of AD. That is, *per annum,* volume loss in AD patients ranges from about 3–4% in AD patients and about 0.4–1.5% in controls for the hippocampus proper, and 6–7% in patients and about 1.5% in controls for the entorhinal cortex (Jack et al., 1998, 2000; Du et al., 2001; Schott et al., 2003; reviewed in Ramani et al., 2006).

Most investigators agree that there are significant atrophic changes in medial temporal lobe structures among patients with MCI and AD that are much more marked than those observed in normal aging. A critical question is whether aspects of brain morphology at one point in time can predict future decline from one state to another (i.e., normal to MCI, MCI to AD). One study measured hippocampus volumes in 80 patients meeting diagnostic criteria for MCI and followed them longitudinally for an average of 32 months (Jack et al., 1999). Of the 80 participants, 27 converted to AD; baseline hippocampal volume significantly predicted future conversion to AD (relative risk = 0.69), even after controlling for relevant variables, such as neuropsychological test scores and age. Results from the same research group suggested that among MCI patients who cognitively decline, the rate of atrophy within the hippocampus is greater than in those who remain cognitively stable (Jack et al., 2000). Other studies have supported these general findings using visual rating scales or manual tracing hippocampus ROI protocols (Visser et al., 2002), although both classification accuracy and sensitivity appear to benefit from analysis of both hippocampus volume and other medial temporal lobe structures (Convit, 2000). Much like the cross-sectional data, volume of the entorhinal cortex best discriminates between those destined to decline cognitively and those who remain relatively stable (Killiany et al., 2000, 2002; Dickerson et al., 2001; Devanand et al., 2007). Volume decrease in the fornix and mamillary bodies has been suggested to become apparent at the point of conversion from MCI to AD (Copenhaver et al., 2006).

3.29.5.4 White Matter

3.29.5.4.1 White matter macrostructure

Bulk volume reduction of white matter has been a focus of recent investigation, with particular emphasis on the corpus callosum. Studies have been reliable in demonstrating volume reduction in this region (Janowsky et al., 1996; Hampel et al., 1998; Pantel et al., 1999; Black et al., 2000). Consistent with the crossing of temperoparietal fibers in the body of the corpus callosum, correlations between neuropsychological test scores and corpus callosum volume have been observed (Janowsky et al., 1996; Black et al., 2000; Chaim et al., 2007).

3.29.5.4.2 White matter microstructure

Several diffusion-based indices have been used to understand microstructural abnormalities associated with AD and MCI (Ramani et al., 2006). Among the more common is diffusion tensor imaging, which has been used by some research groups to lend support to a 'myelin-centered model' of pathological aging (Bartzokis, 2004; Bartzokis et al., 2004a,b). The distribution of anisotropic abnormalities in AD follows the observed volumetric changes and the known distribution of AD pathology (**Figure 8**). One study that compared measures of anisotropy among younger healthy adults, older healthy adults, and patients with AD found that older adults had significantly reduced measures of anisotropy in anterior regions, but that AD evidenced a reduction in anisotropy in more posterior regions without additional change in anterior regions (Head et al., 2004). Similarly, fractional anisotropy was reduced in white matter of the splenium of the corpus callosum but not the genu (Teipel et al., 2002; Naggara et al., 2006; Stahl et al., 2007). In an interesting analysis of FA using a multivariate approach, of 21 voxel regions with positive peak loadings consistent with decreased FA in AD, 16 were posterior to the anterior commissure (Teipel et al., 2002). The findings suggest that AD can be differentiated from normal aging by more posterior involvement of white matter myelin-associated pathology. Not all studies have shown this pattern consistently, however; anistropic changes in AD have been reported throughout most cortical and subcortical regions (Ramani et al., 2006). For example, Bozzali and colleagues showed widespread decreases in fractional anisotropy, including areas of frontal, temporal, and parietal lobes, with relative sparing of posterior cortex among patients with AD compared to matched controls (Bozzali et al., 2002). Others have

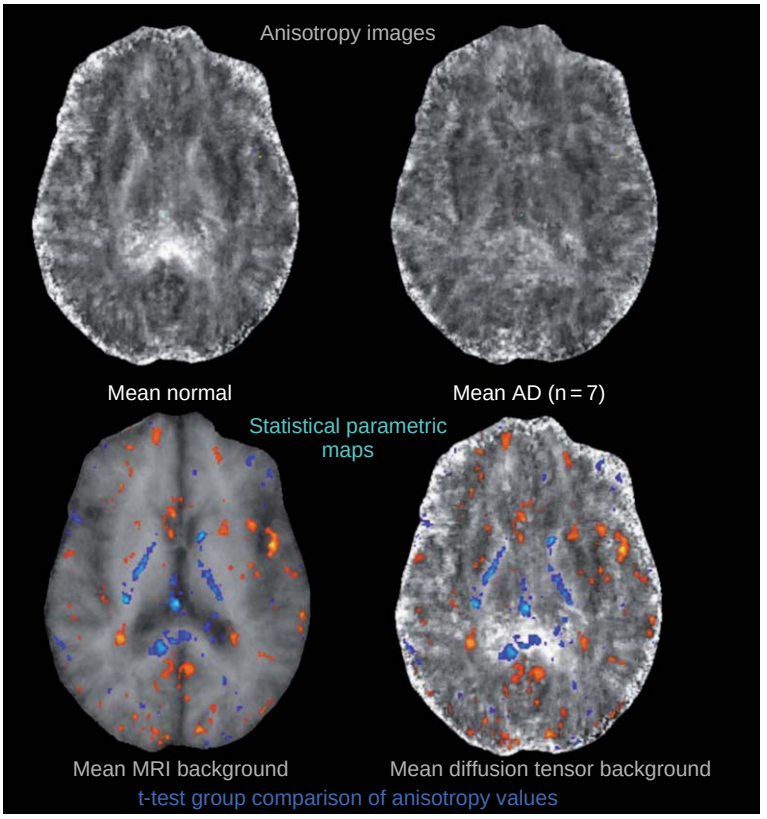

Figure 8 Comparison of seven normal individuals and seven patients with AD. Blue areas are $p < .05$, relative anisotropy in patients lower than normal controls. Note blue areas are posterior, in posterior limb of internal capsule and splenium but not genu of internal capsule.

shown relative sparing of occipital lobe white matter anisotropy in the context of more anterior DTI deficits among patients with AD (Takahashi et al., 2002). Marked anisotropic changes in AD may be evident later in the disease course, as one study that used DTI with MCI patients noted only reductions in diffusivity but not in fractional anisotropy (Fellgiebel et al., 2004).

Observations of increased WMH on FLAIR- or T2-weighted images in individuals with MCI and AD have partially suggested that cerebrovascular disease somehow interacts with AD pathology (Skoog, 2000; Launer, 2002; Kalaria, 2003; Malloy et al., 2007). For example, WMH are more prevalent and severe in AD patients compared to nondemented but demographically similar older adults (Scheltens et al., 1992), and older adults who are not demented but who have increased WMH burden are at higher risk for the development of AD (Vermeer et al., 2003; Prins et al., 2004). Van der Flier and colleagues (van der Flier et al., 2004) found that volume of the medial temporal lobe and severity of WMH independently predicted AD diagnosis, but that the two measures interacted, suggesting a synergistic effect. However, Bigler and colleagues found that, after controlling for total brain volume, WMH burden did not account for additional variance in performance on a test of global cognitive function (Bigler et al., 2002). This finding is consistent with other reports that measures of regional and global atrophy are more strongly associated with cognition than WMH volume alone (Mungas et al., 2001).

3.29.6 Summary

In summary, structural neuroimaging studies have revealed morphological patterns in MCI and AD that are defined primarily by medial temporal lobe atrophy, especially the entorhinal cortex and hippocampus. As the severity of the disease progresses, more regions become atrophic, and moderate to severe stages of dementia are associated with profound global atrophy. The rate and degree of regional

and total brain atrophy are associated with the severity of cognitive symptoms, risk of future cognitive decline, and rate of cognitive decline. Micro- and macrostructural white matter changes are prevalent in patients with AD and may interact with degree of atrophy in their contribution to cognitive function, although atrophy appears to account for relatively more cognitive deficit.

The pattern of structural changes seen in AD in the context of normal aging may suggest that AD is simply 'accelerated' aging. However, a careful consideration of the extant literature would lend greater support for a 'multiple factor framework' of aging and dementia (Buckner, 2004). Normal aging is characterized primarily by a decline in anterior brain systems. This is evident from the structural imaging studies that have demonstrated a relatively greater normal age-associated volume loss, together with white matter microstructural changes, in frontal and frontal-subcortical circuitry and from neuropsychological studies that show a relatively greater decline in frontal lobe–mediated cognitive tasks. AD impacts the temporal lobes, with the earliest involvement in the entorhinal cortex, and its defining cognitive symptom is a loss of hippocampus-mediated memory. It is important to note, however, that AD occurs in the context of aging, so that the pathological changes associated with AD are embedded, and perhaps interacting, with the normal age-associated changes. As the cognitive and neurobiological changes seen with normal aging are in fact quite heterogeneous, it is very difficult to disentangle 'normal' from 'pathological' changes in elderly individuals. Ultimately, multiple functional and structural imaging modalities combined with detailed neuropsychological and neurological evaluation may provide the most diagnostic and disease monitoring specificity and sensitivity.

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LEARNING AND MEMORY: A COMPREHENSIVE REFERENCE

Volume 4
MOLECULAR MECHANISMS OF MEMORY

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4.01 Introduction and Overview

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4.01.1 Introduction

This fourth volume of the *Comprehensive Handbook of Learning and Memory* delves deeply into the cellular and molecular mechanisms mediating lasting changes in behavior, changes that occur in response to environmental signals. This volume is indeed the most comprehensive description in existence concerning the genetics, biochemistry, and cell biology of memory formation. Cumulatively, the reviews in this volume describe an impressive range of processes underlying memory, from atom-level resolution in some paradigms to cell circuit-level mechanisms in others, and essentially all points in between. Moreover, the phylogenetic range of the contents is equally impressive, with descriptions of memory processes in animal systems on a continuum from one of the simplest, *Caenorhabditis elegans*, to the most complex, humans.

It is striking that while the chapters in this volume deal with specific cellular and molecular mechanisms, there are in most chapters strong and direct tie-ins to behavior in the living animal. This appealing aspect of these studies arises in large part from three historical developments, in my opinion. First, many of the invertebrate systems were specifically chosen because the animal lent itself to bridging from behavior to cells to molecules. Thus tying the molecules and cells to the behavior was built into the experimental design of these studies from the outset. A second development was the advent of the technical capacity to genetically engineer mice through means of homologous gene recombination. This allowed those interested in the molecular basis of vertebrate memory to bridge from molecule to behavior within a single animal and ushered in a new era in neurobiology. The final development is more sociological than technical. Many of the leading investigators in the area of molecular and cellular mechanisms of memory actually started out as behavioral psychologists. Therefore, tying molecular and cellular mechanisms directly back to the behaving animal always was an intellectual emphasis for them. This established a culture within the subdiscipline that placed a priority on interdisciplinary studies bridging from molecules to behavior.

In the current era, we also are compelled to always consider the relevance of our basic neurobiological studies to the human condition. Advances over the last 20 years in our understanding of the basic molecular and cellular biology of memory have laid the foundation for a capacity to develop new treatments for human diseases of learning and memory. This is not an abstract principle but rather a declarative statement concerning important recent advances in the field. Descriptions of these recent advances are contained in many chapters, and sections of chapters, in this volume. These descriptions are not split out

into a separate translational or disease section of the volume, but rather are distributed throughout in places appropriate to their specific intellectual milieu. I find this a more satisfying concept for organization of the volume, and an accurate representation of the ongoing cross talk between human and basic science studies of learning and memory.

A final comment is that in many ways this volume is a snapshot of the state of scientific understanding of the neurobiology of learning and memory at the beginning of the twenty-first century. Thus, besides being a valuable resource for contemporary scientists, I believe this volume will provide a useful historical reference point as well. I am only emboldened to make this statement because of the very many outstanding scholars and scientists who have contributed their individual chapters to this work and because of the exceptionally important discoveries by the many scientists that they cite therein.

4.01.2 Organization

The organization of the volume is fairly straightforward considering the complexity of the topic at hand (see **Figure 1**). Part 1, the introduction and overview component, is this chapter and Chapter 4.02. I am very grateful to Eric Kandel, Craig H. Bailey, Angel Barco, and Robert D. Hawkins for their contribution in writing Chapter 4.02. I commissioned Eric and his colleagues to write a personal overview of contemporary discoveries and approaches in the learning and memory field. I expected that they would deliver a very readable and interesting review concerning the overall topic of the molecular and cellular biology of memory formation, describing a diverse set of model systems and approaches. They certainly hit the mark in this regard. Unbeknownst to them, I also anticipated that they might write an excellent bridge chapter that would serve to help guide a knowledgeable reader into the much more detailed following chapters in the book. When I received and read their chapter I was delighted that they had achieved this as well. I strongly encourage anyone looking for a conceptual starting point for considering the detailed molecular and cellular basis of memory, as described in this overall volume, to begin by reading Chapter 4.02.

Part 2 of the volume, comprising Chapters 4.03–4.19, covers model systems-level and cellular-level approaches to investigating learning and memory. This part can be broadly subdivided into five sections (more specific descriptions of the contents of each section will be given): Section 1 deals with nonassociative learning mechanisms. Section 2 discusses associative learning and memory, as investigated in invertebrate model organisms. Section 3 makes an important transition and describes studies of associative and spatial memory in vertebrates. Section 4 reviews topics related to memory disruption, and section 5 describes types and forms of plasticity at the cellular and synaptic level that underlie memory formation and storage.

The last section of Part 2, describing cellular mechanisms and synaptic plasticity, also serves as a foundation for and transition to Part 3 of the volume. Part 3 covers molecular-level approaches and emerging areas of discovery. This second half of the book has five sections as well: Section 1 deals with the *N*-methyl-D-aspartate (NMDA) receptor and its immediate biochemical targets. Section 2 describes genomic and postgenomic signaling. Section 3 covers the important areas of synaptic structure and signaling, and section 4 covers plasticity of cell structure and retrograde synaptic signaling. Section 5, the final section, is admittedly based on a subjective assessment on my part. This section highlights what I consider to be several emerging areas of investigation that will have a large impact on our thinking about cellular and molecular mechanisms of memory formation in the near future.

Having delivered this brief overview, in the remainder of this chapter I will describe more specifically the contents of Parts 2 and 3 of the volume (see **Figure 1**).

4.01.2.1 Part 2A: Systems-Level Approaches

4.01.2.1.1 Nonassociative learning

In this first major section of the book, we will start by covering the simplest forms of learning, that is, nonassociative forms of learning and memory. Studies of these simple forms of learning and memory have yielded great insights into the enormous complexity of information acquisition and storage in the nervous system, when approached at the cellular and molecular levels. In Chapter 4.03, one of the pioneers of this type of work, Jack Byrne, provides a review of studies of sensitization and habituation using invertebrate model systems.

This overview chapter is then followed by a description of sensitization and habituation in the *C. elegans* model system by Cathy Rankin, who pioneered the adaptation of this organism for use in

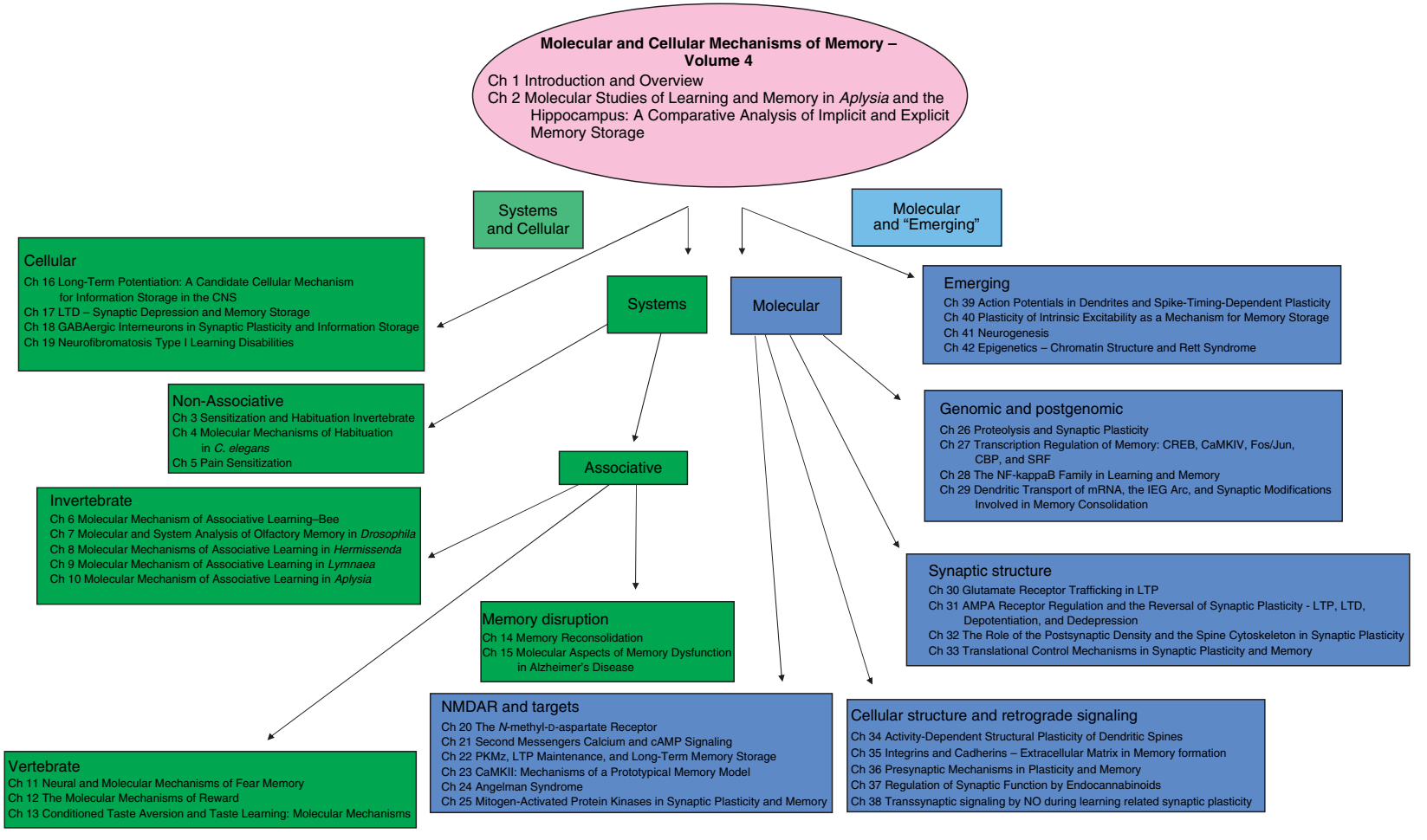

Figure 1 Contents and organization of Volume 4 of the *Comprehensive Handbook of Learning and Memory*. This volume covers molecular and cellular mechanisms of memory formation. This figure describes the overall organization and major topic areas of the volume.

behavioral studies. Studies of this sort in *C. elegans* have a particularly important context, for this organism stands apart from all others in that its entire anatomical structure and developmental program have been determined at the cellular level. Thus behavioral studies in *C. elegans* have the potential to bring an unprecedented level of certainty to understanding the neural circuitry underlying specific behaviors. In addition, *C. elegans* is a powerful genetic model system in its own right, allowing the potential to combine anatomy, genetics, and behavior in a unique fashion, as is illustrated by the studies in Chapter 4.04.

Nonassociative learning in vertebrates, especially humans, tends to not receive the same level of attention among learning and memory neurobiologists that higher-order forms of learning receive. However, sensitization and habituation are also of great relevance in humans in the clinical setting. Chapter 4.05 describes pain sensitization in vertebrate model systems, highlighting interesting parallels between molecular mechanisms of vertebrate associative learning (that will be described later in the volume) and mechanisms of nonassociative learning in these same animals. In addition, this chapter reminds us of the great significance of studying nonassociative learning and memory in the modern context of translational biomedical research.

4.01.2.1.2 Associative learning and memories of contingency

The next major section transitions us to more complex forms of learning and memory, that is, associative forms wherein an animal learns a predictive or contingent relationship between two environmental signals. Of course, this associative relationship must be represented at the molecular level in some fashion in the nervous system, and the memory for the relationship must be stored there as well. Thus, the associative learning section explores the fascinating question of how contingencies and associations are represented at the molecular and cellular level in the nervous system, and how unique molecular events produced by associative stimuli trigger lasting cellular changes manifest as memory.

4.01.2.1.3 Associative learning in invertebrate models

We first explore these questions in a series of chapters describing studies of associative conditioning in invertebrates. Each of five chapters gives an overview of a particular organismal system, describing the animal, its ethologically relevant behaviors and capacity for forming associations, the underlying circuitry, and the known cellular and molecular mechanisms contributing to altered behavior in response to associative environmental stimuli. These chapters thus provide a useful overview of the different organisms and approaches, and more detailed information about cellular and molecular mechanisms.

We are fortunate to have leading investigators for each of five unique model systems contributing to this section. In Chapter 4.06, Uli Mueller and his colleagues describe the honeybee system. Chapter 4.07 provides us with a review of learning and memory studies in the granddaddy of all genetic model systems, *Drosophila*, from Thomas Preat and his group. In Chapter 4.08, Terry Crow and his collaborators review the *Hermissenda* system, and in Chapter 4.09 George Kemenes introduces us to *Lymnaea.* Finally in this section, in Chapter 4.10 Jack Byrne describes *Aplysia* associative conditioning and reviews the unique molecular and cellular components of associative learning and memory in this system.

In editing and reviewing these chapters, I noted several interesting commonalities across these systems, which I would like to comment on in passing. First, these are interesting systems to read about, because I suspect that many readers will be introduced to these systems for the first time through this volume. Second, considerable cleverness is evident on the part of the scientists using these systems, in adapting ethologically relevant behaviors in the animal to the laboratory setting, and in undertaking very detailed molecular and cellular studies in conjunction with that approach. Finally, the types of associations these relatively simple animals are capable of making is astonishing in many instances. Many of you reading about these organisms for the first time will be surprised at the complex environmental contingencies that slugs and bugs are capable of learning.

4.01.2.1.4 Associative learning in vertebrate models

The next section moves up the phylogenetic tree to explore associative learning and memory in vertebrate systems. As this volume focuses on molecular and cellular mechanisms, all three of these chapters describe studies utilizing rodents. In Chapter 4.11, Glenn Schafe and Joe LeDoux lend their synaptic selves to the effort of describing rodent fear conditioning and its underlying cellular and molecular basis.

Their chapter describes the impressive body of literature that has been able to take studies of this particular behavior into the realm of specific cellular and molecular mechanisms, in the anatomically complex vertebrate central nervous system (CNS). These studies have allowed the delineation of mechanisms that encode a complex environmental contingency and allow the manifestation of appropriate behavioral change. In Chapter 4.12, Eric Nestler and Catharine Winstanley describe studies of the flip side of behavioral conditioning, reward-reinforced behaviors. They present an overview of reward systems in the vertebrate CNS, and describe how these systems can be co-opted to reinforce addictive behaviors. Once again, the level of understanding of the cellular and molecular mechanisms at work in these processes is quite impressive. Finally, in Chapter 4.13 in this section Kobi Rosenblum describes taste learning and conditioned taste aversion in rodents. The cortically based systems at work here, as well as the quasi-declarative nature of taste memorization, make this a fascinating system for study. Indeed, it is likely that taste learning and memory are among the most sophisticated types of information processing that rodents are capable of.

4.01.2.1.5 Memory disruption

The robustness and long-lived nature of memory are two of the most impressive aspects of the phenomenon, and indeed these attributes compelled many neuroscientists to be interested in learning and memory in the first place. Against this backdrop, the possibility of loss of previously established memories, and the loss of the capacity for new memory formation, are particularly compelling for many of us to consider. In Chapter 4.14, Cristina Alberini and Stephen Taubenfeld discuss the need for memories that have already been formed to undergo a complex process of re-establishment after every instance of recall, a phenomenon referred to as memory reconsolidation.

In Chapter 4.15, Lennart Mucke, Jeannie Chin, and Eric Roberson describe molecular mechanisms underlying the most debilitating disease of memory in existence, Alzheimer's disease (AD). This is one of the most prominent areas of biomedical research extant in the United States and Europe at present. I also would like to highlight this chapter by Mucke and colleagues for another reason. Their chapter sits at a position in this volume immediately preceding roughly 20 subsequent chapters that detail the cellular and molecular underpinnings of memory. A striking attribute of their chapter is the large number of cellular and molecular processes that are disrupted in AD, processes that are subsequently discussed in a basic science context in further chapters of this volume. I note this for two reasons. First, it is impressive and somewhat discouraging that so many of the molecular mechanisms implicated in normal memory formation are potentially disrupted in AD. Second, on a more practical side Chapter 4.15 serves as a valuable cross-reference for the biomedical relevance of understanding the detailed cellular and molecular processes described in the final sections of this volume.

4.01.2.2 Part 2B: Cellular-Level Approaches

In Chapter 4.16 of the volume, we transition from larger systems-level analyses involving whole organisms to descriptions of more specific cellular and molecular processes involved in learning and memory. I chose to make this transition with a chapter describing long-term potentiation (LTP), perhaps the cellular keystone of higher-order vertebrate memory formation. Directly indicative of that fact, and of the seminal contribution made by Tim Bliss and Terje Lomo in their discovery of LTP, every chapter after this transition point makes some reference to LTP, to a greater or lesser extent depending on the specific topic under review. Thus Chapter 4.16 provides a foundation for all subsequent chapters in this volume. More significantly, Bliss and Lomo's discovery of LTP provides an essential foundation for the modern understanding of the molecular and cellular basis of vertebrate memory formation.

The undoubted importance of LTP notwithstanding, synaptic long-term depression (LTD) plays crucial roles in vertebrate CNS function and memory formation as well. In Chapter 4.17, Christian Hansel and Mark Bear describe LTD, the conceptual mirror image of LTP. They also provide a nice coverage of the functional and behavioral significance of LTD and highlight that the phenomenon is not simply a cellular antagonist of potentiation, but rather a mnemonic mechanism in its own right.

The importance of plasticity of inhibitory synapses and cells has historically been under-appreciated. In Chapter 4.18, Chris McBain and his colleagues describe both the plasticity of inhibitory cells and synapses, and the importance of plasticity of inhibitory circuits in memory and cognitive function. To reinforce this message, in Chapter 4.19 Alcino

Silva and his collaborators describe learning and memory deficiencies associated with neurofibromatosis type 1 and describe their intriguing series of studies that identified disruption of inhibitory synapse function as a basis for human learning and memory deficits in this disorder. These two complementary chapters close out the systems and cellular section of the volume and set the stage for the detailed molecular descriptions of the final part of the volume.

4.01.2.3 Part 3A: Molecular-Level Approaches

Starting with Chapter 4.20, we begin to dissect the complex molecular systems that subserve learning, memory, and memory storage. The molecular complexity of memory is indeed awe-inspiring and not to be taken lightly. One must remember that the molecular machinery of vertebrate memory may underlie the most complex biological process in existence, at least that is concretely identifiable at this point. Chapters 4.20–4.38 describe individual components of this machinery in a comprehensive and organized fashion.

These chapters proceed in an order roughly approximating the order of molecular information flow in a neuron participating in the formation of a memory. Thus we start with a description of the NMDA subtype of glutamate receptor and its immediate molecular targets in the cell in Chapters 4.20–4.25. The next section, Chapters 4.26–4.29, describes signaling to the nucleus and the genome contained therein and reviews transcriptional and posttranscriptional mechanisms involved in memory formation. Both the immediate targets of the NMDA receptor and the altered transcriptional readout impinge upon the synapse to effect altered cell function and signaling, a critical step in the plasticity that underlies memory. Chapters 4.30–4.33 describe the translational and posttranslational mechanisms that underlie altered synaptic function in memory. Finally, it is clear that transcriptional, translational, and posttranslational mechanisms all ultimately mediate altered cell structure and communication in memory formation. These alterations involve both the pre- and postsynaptic compartments, and encompass the physical and molecular structure of both the neuron and the extracellular space. These components of the memory machinery are described in Chapters 4.34–4.38.

4.01.2.3.1 The NMDA receptor and its immediate targets

What better place to start a description of the molecular basis of memory than with the NMDA receptor itself? After all, it is both a molecular coincidence detector and a known key component of vertebrate memory formation. Chapter 4.20 describes NMDA structure and function and highlights that the NMDA receptor is in fact a huge molecular machine in its own right. In Chapter 4.21, Dan Storm and Kristin Eckel-Mahan describe two of the immediate effectors of the NMDA receptor, as well as other plasticity-related receptors: calcium and cyclic adenosine monophosphate (cAMP).

The next three chapters then discuss two memory molecules that are capable of converting a transient signal into a lasting effect in the cell – protein kinase C (PKC) and calcium/calmodulin-dependent protein kinase II (CaMKII). Chapter 4.22, by Todd Sacktor, describes the roles of PKC and especially of the PKMzeta isoform in synaptic plasticity and memory. Chapter 4.23 brings us a description by Roger Colbran of the ways and means by which CaMKII can be regulated and persistently activated to serve as an information storage device. Chapter 4.24 by Ed Weeber and his colleagues highlights the clinical relevance of studies of CaMKII regulation, by focusing on Angelman mental retardation syndrome and disruptions of hippocampal CaMKII regulation in this human learning and memory disorder.

Finally in this section, Ray Kelleher describes the mitogen-activated protein kinase (MAPK) pathways and their roles in synaptic plasticity and memory formation. Chapter 4.25 covers one of the essential signal integration pathways in plasticity and memory formation, a pathway that also is a prototype regulator of gene transcription and protein translation. The description of this pathway helps us transition to the next section of the volume, dealing with transcriptional regulation and mRNA trafficking.

4.01.2.3.2 Genomic and postgenomic signaling

Genomic signaling in the context of synaptic plasticity and memory formation involves two basic components: Getting a signal to the transcription regulatory machinery to alter expression of the appropriate gene targets, and getting the newly transcribed mRNAs to the right places in the cell. Chapters 4.26–4.30 deal with various fascinating aspects of these processes.

Chapter 4.26 by Ashok Hegde describes a critical gene target in *Aplysia* sensitization and synaptic

facilitation, a ubiquitin C-terminal hydrolase. Ashok's chapter describes an interesting molecular system that demonstrates an important role for protein degradation in plasticity and memory, and also involves an interesting interplay of transcriptional and posttranslational regulation in order to generate a lasting signal in a neuron.

The next two chapters describe important transcription factors in plasticity and memory, those proteins that help directly translate a genome-level signal into the appropriate direct transcriptional change. In Chapter 4.27, Sheena Josselyn and Christy Cole describe transcription regulation by cAMP response element binding protein (CREB) and CREB-associated pathways, highlighting the first transcription factor to be clearly linked to synaptic plasticity and memory formation. In Chapter 4.28, Molly Meffert describes a pathway recently introduced to the plasticity and memory field, nuclear factor kappa B (NFκB). While NFκB signaling is fairly new material for most neurobiologists, in fact this pathway has long been one of the premier pathways for study in immune system transcriptional regulation.

Once changes in gene expression are triggered in the nucleus, the products of those changes must find the right site within the neuron for their residence. In Chapter 4.29, Oz Steward discusses the prototype marker for neuronal mRNA trafficking in the mammalian CNS, Arc. Studies of Arc trafficking have shed important new light on the existence and mechanisms of specific mRNA targeting in neurons, especially in the context of activity-dependent cellular plasticity and the mechanisms that tell targeted molecules where to go in the neuron, a process referred to as synaptic tagging.

Targeted molecules end up in specific sections of the neuron and at specific synapses. Thus we have completed a conceptual loop through the neuron – from the NMDA receptor at a synapse, to the nucleus, and back to a targeted synapse. Therefore in the next section we return to a discussion of synaptic mechanisms and receptors. However, it is important to remember that plasticity at synapses occurs not only (nor even predominantly) in response to genome-originating signals, but also in response to locally generated signals. Therefore, the next section deals with the synapse and its receptors not only as targets of transcriptional regulation, but also as targets of posttranslational modification and localized alterations in protein synthesis.

4.01.2.3.3 Synaptic structure and signaling

This is a substantial section that describes several of the most popularly studied and clearly important mechanisms in synaptic plasticity and memory. Indeed, in the mammalian CNS the processes described in this section are of paramount relevance. This is because the postsynaptic compartment and its environs are both the quintessential functional compartment for receiving externally generated signals, and a known locus for change at the molecular level in plasticity and behavioral memory.

In Chapter 4.30, Michael Browning and his colleagues describe mechanisms of glutamate receptor trafficking and regulation, focusing on those mechanisms operating during synaptic plasticity and memory. Rick Huganir and Hey-Kyoung Lee follow in Chapter 4.31 by reviewing a fascinating set of mechanisms controlling bidirectional regulation of glutamate receptor function during synaptic potentiation and depression. These two chapters provide a nice overview of the mechanism for dynamic regulation of glutamate receptor function in synaptic plasticity and memory.

Of course, glutamate receptors and their many associated signaling molecules sit within a specialized synaptic machine – the postsynaptic density (PSD). In Chapter 4.32, Mary Kennedy reviews the structure and function of the PSD and brings to bear sophisticated kinetic modeling approaches to help us understand how this machine achieves its effects. In Chapter 4.33, Eric Klann and Nahum Sonnenberg describe the necessity of protein synthesis for synaptic plasticity and memory, with an emphasis on localized dendritic protein synthesis being involved in these processes.

4.01.2.3.4 Plasticity of cellular structure and retrograde signaling

Molecular changes associated with memory formation are not limited to a single postsynaptic compartment, but rather encompass associated presynaptic and structural changes as well. Moreover, even in the case where a single postsynaptic site might undergo alteration, its presynaptic partner can change in concert. In Chapter 4.34, Lucas Pozzo-Miller and Christopher Chapleau describe activity-dependent structural changes in dendritic spines as an example of neuronal structural change in plasticity and memory. In Chapter 4.35, Ron Davis reviews the role of the extracellular matrix in the maintenance of synaptic structural change, focusing

specifically on integrins and cadherins. Chapter 4.36 by Craig Powell and Pablo Castillo describes presynaptic mechanisms in plasticity, covering both plasticity of the presynaptic terminal and evidence that changes at this locus are involved in various forms of memory. In considering how signals may get from the postsynaptic compartment to the presynaptic compartment, David Lovinger enlightens us with Chapter 4.37, describing regulation of synaptic function by endocannabinoids. Finally for this section, Bob Hawkins reprises the retrograde signaling theme by reviewing the role of nitric oxide in synaptic plasticity (Chapter 4.38).

4.01.2.4 Part 3B: Emerging Areas

The final section of the volume covers a somewhat disparate set of topics that nevertheless have two things in common. First, the topics of this section, in my opinion, are emerging areas of emphasis in the plasticity and memory field, topics that have the potential to give us fundamentally new perspectives on several aspects of CNS and neuronal function underlying behavioral change. Second, these mechanisms have in common that they affect or involve the entire neuron.

In Chapter 4.39, Jack Waters and Fritjof Helmchen describe back-propagating action potentials in dendrites and their capacity to regulate both the magnitude and direction of synaptic plasticity. This is followed by Chapter 4.40, where Jack Byrne and Ricccardo Mozzachiodi describe plasticity of intrinsic cellular properties, such as altered excitability, as a locus of plastic change. Neurogenesis has emerged as a viable mechanism contributing to memory formation in the adult, in complete contrast to the dogma of only a few years ago. In Chapter 4.41, Rusty Gage, Sebastian Jessberger, and James Aimone review adult neurogenesis and its mechanisms of control and the implications of this system for a potentially new cellular basis of memory. Finally, in Chapter 4.42 Jonathan Levenson and Marcelo Wood describe recent discoveries implicating epigenetic mechanisms of long-term regulation of gene expression in plasticity and memory formation.

These provocative topics may force us to consider that the entire neuron may be a locus of the engram and a site of information storage in the CNS. Thus, while synapse specificity is a powerful attribute in terms of specifying connections in a behavior-mediating circuit, synapse-specific changes may be embedded within a neuron that has had its basic properties changed in a cell-wide fashion as well. Both processes may act in concert to enable learning and memory, and the four chapters of this final section presage this potential paradigm shift in our thinking concerning vertebrate memory formation.

Acknowledgements and Thank-Yous

The Editors at Elsevier Publishing have been great to work with on this project. Executing a book series of the scope and magnitude of the *Comprehensive Handbook of Learning and Memory* is no small feat. In this vein, I would like to thank the Elsevier publishing group for deciding to invest in this project and for giving me and the other editors the opportunity to participate. I in particular wish to thank Johannes Menzel for his support and encouragement, and Joanna DeSouza for the immense amount of work she put in on organizing the series. I also am very appreciative to Vicki Hixon, who works with me at the McKnight Brain Institute at UAB, for all the tremendous help she gave me, and always with a smile. My wife, Kim Strifert, supported me throughout, from London to Houston to Memory Lane (see **Figure 2**).

I also am very grateful to Jack Byrne for inviting me to participate as a volume editor and contributor to the series. The series is both visionary and timely, and Jack has provided great leadership as editor. I sincerely appreciate the opportunity to participate. Jack, Randolph Menzel, Howard Eichenbaum, and Roddy Roediger are all not only outstanding scientists but also fine colleagues, and I thank them all for their many suggestions and for the valuable

Figure 2 Memory Lane is a state of mind, a fascinating topic of scientific inquiry, and a small road outside of Trussville, Alabama, USA. The editor of this volume dwells on all three.

brainstorming that they did not only for the overall series but for my volume as well.

Most important is recognizing the tremendous debt that I owe to the authors of the individual chapters in this volume. I have been consistently and thoroughly impressed at the quality of the chapters that the authors provided for this book. I thank them to begin with for even agreeing to provide a chapter at all. I thank them tremendously and in every instance for the superb quality of the material that they produced – a reflection of their professionalism, integrity, and scholarship.

4.02 Molecular Studies of Learning and Memory in *Aplysia* and the Hippocampus: A Comparative Analysis of Implicit and Explicit Memory Storage

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4.02.1 Introduction

Modern behavioral and biological studies have revealed that memory is not a unitary faculty of the mind but consists of distinct families of mental processes that can be grouped into at least two general categories, each with its own rules (Polster et al., 1991; Squire and Zola-Morgan, 1991). Explicit or declarative memory is the conscious recall of knowledge about people, places, and things and is particularly well developed in the vertebrate brain. Implicit or nondeclarative memory is memory for motor and perceptual skills as well as other tasks and is expressed through performance, without conscious recall of past experience. Implicit memory includes simple associative forms of memory, such as classical and operant conditioning, and nonassociative forms, such as sensitization and habituation. Explicit and implicit memory have been localized to different neural systems within the brain (Milner, 1985; Polster et al., 1991; Squire, 1992). As first shown by Brenda Milner in her neuropsychological studies of the patient H.M., explicit memory is critically dependent on structures in the medial temporal lobe of the cerebral cortex, including the hippocampal formation. Implicit memory is a family of different processes that are represented in a number of brain systems including

the cerebellum, the striatum, the amygdala, and in the simplest cases, the sensory and motor pathways recruited during the learning process for particular perceptual or motor skills. As a result, implicit memory can be studied in a variety of simple reflex systems, including those of higher invertebrates, whereas explicit memory is best studied in mammals.

Two experimental model systems have been extensively studied as representative examples of these two forms of memory storage: sensitization in the marine snail *Aplysia californica* as an example of implicit memory, and spatial memory formation in rodents as an example of explicit memory. We use them here as points of comparison to consider similarities and differences in implicit and explicit memory storage.

4.02.2 Short-Term, Intermediate-Term, and Long-Term Forms of Storage Mechanisms

Recent studies of simple forms of implicit memory in higher invertebrates and more complex forms of explicit memory in mammals suggest that changes in the strength and structure of synaptic connections underlie these diverse forms of memory storage (Kandel, 2001). For both implicit and explicit memory, two general types of storage mechanisms have been described: short-term memory lasting minutes and long-term memory lasting days, weeks, or longer. This temporal distinction is reflected in specific mechanisms for the synaptic plasticity that underlie each form of behavioral memory as well as specific molecular requirements for each of these two forms of synaptic plasticity. The short-term forms involve the covalent modifications of preexisting proteins by a variety of kinases and are expressed as alterations in the effectiveness of preexisting connections. In contrast, the long-term forms require *de novo* gene expression and the synthesis of new mRNAs and proteins. Moreover, the long-term forms often are associated with the growth of new synaptic connections. For both implicit and explicit memory storage, the synaptic growth is thought to represent the final and self-sustaining change that stabilizes the long-term process. In addition to short- and long-term memory, there are, for most types of learning, a family of intermediate processes that last one or more hours and often require translation but not transcription. These can be produced by various behavioral training protocols or in simplified neuronal systems using repeated or prolonged stimulation.

In this chapter, we discuss and compare critical synaptic sites and the underlying cellular and molecular mechanisms of short-term, intermediate-term (**Figure 1**), and long-term (**Figure 2**) memory storage that have been identified by neurobiological studies of elementary forms of implicit memory in *Aplysia* and explicit memory storage in rodents.

4.02.2.1 Implicit Memory: Sensitization and Classical Conditioning of the Gill-Withdrawal Reflex in *Aplysia*

The central nervous system (CNS) of *Aplysia* contains only approximately 20,000 large and frequently identifiable nerve cells, clustered into nine major ganglia. The ability to identify many of the individual neurons of this nervous system and record their activity has made it possible to define the major components of the neuronal circuits of specific behaviors and to delineate the critical sites and underlying mechanisms used to store memory-related representations.

The cellular and molecular mechanisms contributing to implicit memory storage have been most extensively studied for the gill- and siphon-withdrawal reflex of *Aplysia* (Carew and Sahley, 1986; Byrne and Kandel, 1996; Kandel, 2001). As is true for other types of defensive reflexes, the gill- and siphon-withdrawal reflex can be modified by several different forms of implicit learning. We begin by focusing on *sensitization,* a form of learned fear, evident as an elementary form of nonassociative learning of this defensive behavior. When a light touch is applied to the siphon of *Aplysia,* the animal responds by withdrawing its siphon and gill. This response can be enhanced or sensitized when the animal is presented with a noxious (fear-inducing) stimulus such as a tail shock. As is the case with other forms of memory, the memory for sensitization of the withdrawal reflex is graded as a function of training: A single tail shock produces short-term sensitization that lasts for minutes, whereas repeated tail shocks given at spaced intervals produce long-term sensitization that can last for several weeks (Castellucci et al., 1986). The reflex also exhibits classical conditioning, an associative form of learning. Here the siphon stimulus is presented in a paired fashion just before the tail shock so that the animal learns about the predictive relationship between the two stimuli. Enhancement of the withdrawal reflex is greater and longer lasting with paired training (classical conditioning), compared with unpaired training or training with the tail shock alone (sensitization) (Carew et al., 1981, 1983; Antonov et al., 2001).

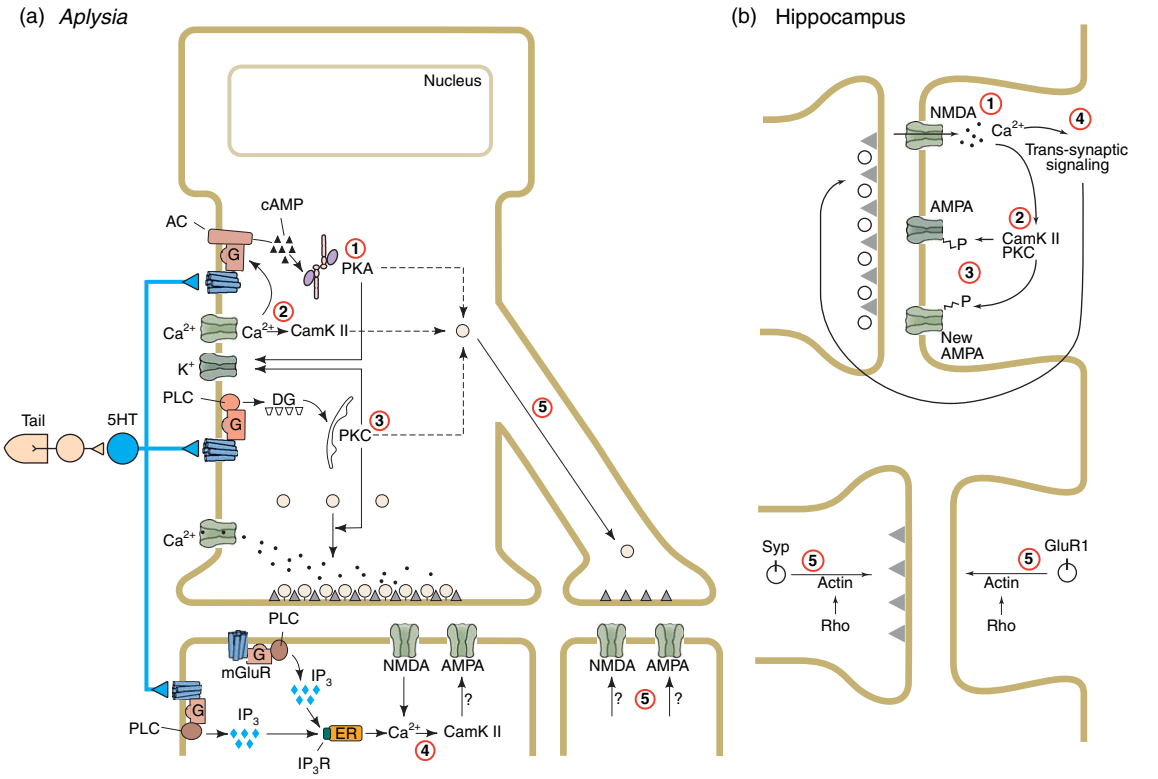

Figure 1 Mechanisms of short- and intermediate-term memory formation in *Aplysia* and hippocampus. (a) *Aplysia.* Different forms of short- and intermediate-term synaptic plasticity contributing to learning and memory in *Aplysia* involve different combinations of pre- and postsynaptic molecules including (1) presynaptic cyclic adenosine monophosphate–dependent protein kinase, (2) presynaptic Ca^{2+} and CamKII, (3) presynaptic protein kinase C, (4) postsynaptic Ca^{2+} and CamKII, and (5) recruitment of pre- and possibly postsynaptic molecules to seed potential new synaptic sites. (b) Hippocampus. Early-phase long-term potentiation in the CA1 region of hippocampus involves (1) Ca^{2+} influx through postsynaptic *N*-methyl-D-aspartate (NMDA) receptor channels, (2) activation of protein kinases including CamKII and PKC, (3) increased conductance of existing adenosine monophosphate (AMPA) receptor channels and membrane insertion of new AMPA receptors, (4) the engagement of trans-synaptic signaling, which can enhance presynaptic transmitter release, and (5) recruitment of pre- and postsynaptic molecules to seed potential new synaptic sites. See the text for details.

The relative simplicity of the neuronal circuit underlying these behavioral modifications – including direct monosynaptic connections between identified mechanoreceptor sensory neurons and their follower cells (Castellucci et al., 1970) – has allowed reduction of the analysis of short- and long-term memory for sensitization and classical conditioning to the cellular and molecular level. This monosynaptic sensory to motor neuron connection, which is thought to be glutamatergic (Dale and Kandel, 1993; Trudeau and Castellucci, 1993; Conrad et al., 1999), can be reconstituted in dissociated cell culture. Here the tail shocks are replaced with brief applications of serotonin (5-HT), a modulatory transmitter normally released by sensitizing stimuli in the intact animal (Glanzman et al., 1989; Mackey et al., 1989; Marinesco and Carew, 2002). This simplified *in vitro* model system reproduces what is observed during behavioral training. A single brief application of 5-HT produces a short-term change in synaptic effectiveness (short-term facilitation, or STF), whereas repeated and spaced applications of 5-HT produce changes in synaptic strength that can last as long as the cells survive in culture (long-term facilitation, or LTF) (Montarolo et al., 1986). Facilitation is also larger and longer lasting if the presynaptic sensory neuron fires action potentials just before the application of 5-HT, analogous to classical conditioning (Eliot et al., 1994a; Schacher et al., 1997; Bao et al., 1998).

4.02.2.2 Explicit Memory: Spatial Memory in Rodents

Mice have a well-developed capability for certain types of explicit memory. In particular, the brain of rodents is

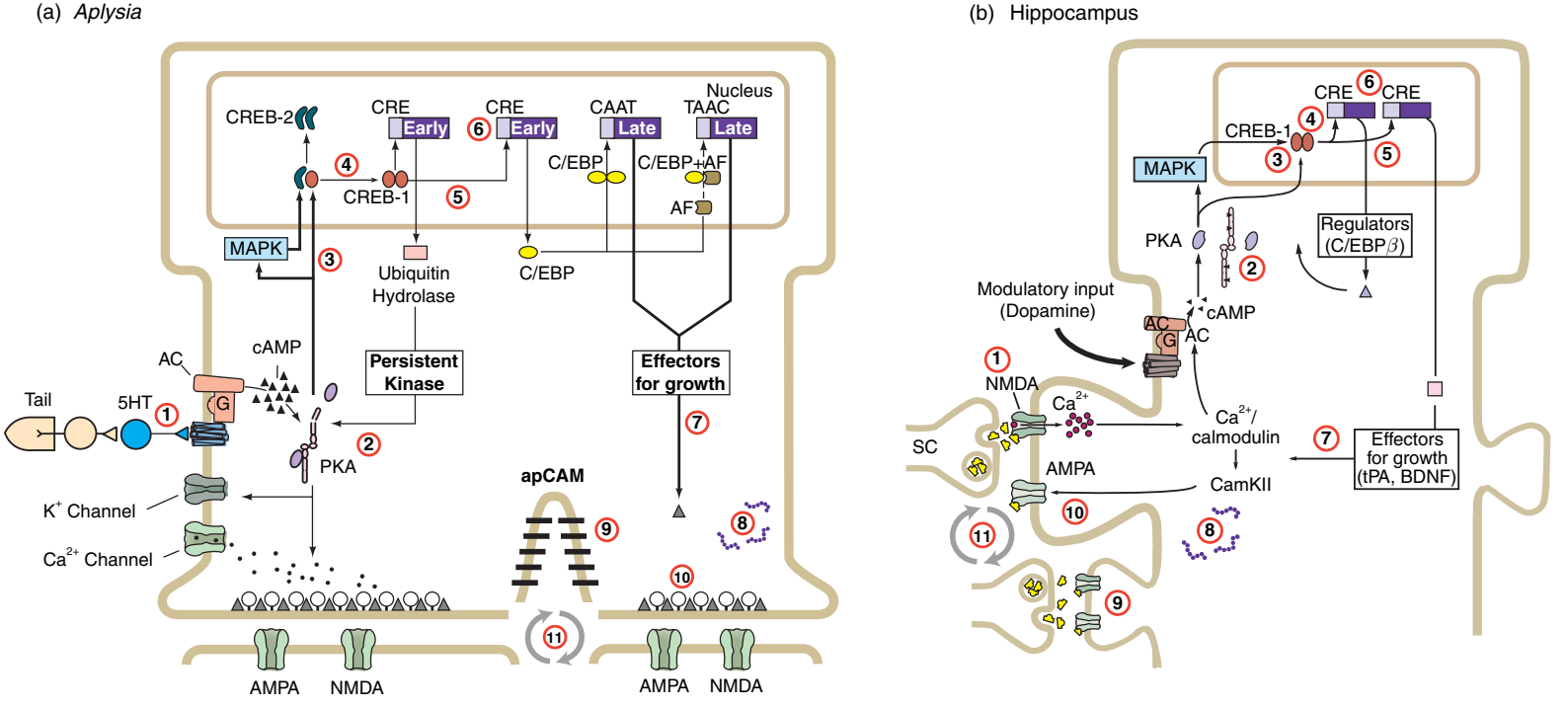

Figure 2 Mechanisms of long-term memory formation. Long-term synaptic plasticity contributing to learning and memory in both *Aplysia* (a) and hippocampus (b) involves a sequence of cellular and molecular mechanisms including (1) neurotransmitter release and short-term strengthening of synaptic connections, (2) equilibrium between kinase and phosphatase activities at the synapse, (3) retrograde transport from the synapse to the nucleus, (4) activation of nuclear transcription factors, (5) activity-dependent induction of gene expression, (6) chromatin alteration and epigenetic changes in gene expression, (7) synaptic capture of newly synthesized gene products, (8) local protein synthesis at active synapses, (9) synaptic growth and the formation of new synapses, (10) activation of preexisting silent synapses, and (11) self-perpetuating mechanisms and the molecular basis of memory persistence. The location of these events, which may act in part to stabilize some of the changes that occur during short- and intermediate term plasticity, moves from the synapse (1–2) to the nucleus (3–6) and then back to the synpase (7–11). Molecular details are discussed in the text.

highly adapted for navigating complex spatial environments. The hippocampus, a key subcortical structure in the mammalian brain, is particularly important for spatial learning and memory and represents a significant percentage of the forebrain in rodents. A remarkable feature of the hippocampus is its regularity along its cross-sectional axis, presenting large arrays of neurons aligned in distinct layers, namely, the CA1, CA3, and dentate gyrus fields. Four major pathways connect these three regions and the entorhinal cortex. This highly organized cellular anatomy has facilitated the electrophysiological analysis of these pathways both *in vivo* and *in vitro*. The phenomenon of long-term potentiation (LTP), currently thought to be the cellular correlate or, at least, a requirement for explicit memory formation in the hippocampus, was first described in the perforant pathway of the hippocampus of rabbits by Bliss and Lomo (1973) over 30 years ago. At about this time it was also appreciated that the hippocampal pyramidal neurons of the CA1 region, which undergo LTP in response to stimulation of the Schaffer collateral pathways, encode space: they are 'place cells.' These cells fire when the animal moves to a specific spatial location, suggesting that this brain region may contain the cellular substrate for the formation of spatial maps (O'Keefe and Dostrovsky, 1971). A large number of lesion, pharmacological, and genetic experiments have confirmed that the Schaffer collaterals, the connections between CA3 and CA1 pyramidal neurons, play a major role in the encoding and storage of spatial memories. As with *Aplysia,* it is possible to distinguish two stages during LTP in the Schaffer collateral pathway: an early, short-term stage (E-LTP), which lasts minutes and can be induced by stimulating the hippocampal slice with a single 100-Hz train of 1 s duration, and a later, long-term stage (L-LTP), which lasts much longer and can be induced by four or more repeated 100-Hz trains (Huang and Kandel, 1994; Martin et al., 2000).

4.02.3 Cellular and Molecular Mechanisms Underlying Short- and Intermediate-Term Forms of Implicit and Explicit Memory Storage

4.02.3.1 Short-Term Memory Involves Covalent Modifications of Preexisting Proteins and Short-Term Enhancement of Preexisting Synaptic Connections

These mechanisms were first explored in *Aplysia* (Schwartz et al., 1971; Brunelli et al., 1976; Kandel and Schwartz, 1982). 5-HT released *in vivo* during sensitization, or applied directly to cultured *Aplysia* neurons, binds to cell surface receptors on the sensory neurons and promotes the production of the diffusible second messenger cyclic adenosine monophosphate (cAMP) by activating the enzyme adenylyl cyclase (AC). This increase in internal concentration of cAMP results in a short-term increase in synaptic strength of the sensory-to-motor-neuron connection (STF). This facilitation is partially a result of the enhanced release of the transmitter glutamate by the sensory neuron onto its follower cells and is accompanied by an increase in the excitability of the sensory neuron attributable to the depression of specific sets of potassium channels, a broadening of the action potential, and an increase in Ca^{2+} influx in the presynaptic terminal (Klein et al., 1982; Castellucci et al., 1986; Dale et al., 1988; Eliot et al., 1993; Byrne and Kandel, 1996). In addition, the changes in cAMP and Ca^{2+} levels triggered by the activation of 5-HT receptors and ion channels regulate the activity of different kinases and phosphatases that control the duration and strength of the changes in synaptic efficiency, as we discuss later.

Many aspects of the basic cellular mechanisms that trigger LTP formation in the hippocampus of mammals recapitulate those found in *Aplysia,* suggesting a basic similarity in the cellular underpinnings for explicit and implicit memory. The primary excitatory neurotransmitter in this case is also glutamate. Again, second messengers are activated by synaptic stimulation, and several of these are similar in both systems. In hippocampal pyramidal neurons, synaptic release of glutamate triggers Ca^{2+} influx through *N*-methyl-D-aspartate receptors (NMDAR) and activation of several kinases including Ca^{2+}/camodulin-dependent protein kinase (CamKII), protein kinase C (PKC), and mitogen-activated protein kinase (MAPK). Spaced trains of high-frequency stimulation can also activate cAMP-dependent protein kinase (PKA) through the Ca^{2+}/calmodulin-mediated stimulation of postsynaptic adenylyl cyclase (Blitzer et al., 1995). In *Aplysia,* these second messengers mediate the transient reinforcement of synaptic connections by covalent modifications of channel closure and the enhancement of neurotransmitter release at presynaptic terminals (Martin et al., 2000; Kandel, 2001). In the hippocampus, expression of the early phase of LTP (E-LTP) involves both an increase in the number of postsynaptic alpha-amino-3-hydroxyl-5-methyl-4-isoxazolepropionate receptors (AMPARs) in the plasma membrane and the phosphorylation of specific subunits

and consequent modification of the biophysical properties of the channel (Malenka and Bear, 2004). E-LTP can, with certain stimulation parameters, also involve an increase in presynaptic transmitter release (Voronin and Cherubini, 2003).

4.02.3.2 Different Protocols Engage Different Combinations of Second Messenger Mechanisms

Studies of facilitation of the sensory-to-motor-neuron synaptic connection in *Aplysia* first demonstrated that even slightly different variations in the learning paradigm or in the pattern of synaptic stimulation recruit different second messenger mechanisms alone or in combination. This insight first emerged in a comparison of sensitization and dishabituation, two related forms of nonassociative learning that differ depending on the state (rested or depressed) of the reflex (Byrne and Kandel, 1996; Antonov et al., 2005). Sensitization results in the strengthening of a reflex response. On the synaptic level, this leads to the facilitation of rested synapses, which can be mimicked by a brief exposure to 5-HT. This involves activation of adenylyl cyclase and PKA in the sensory neuron, leading to reduced K^+ current, increased action potential duration, increased Ca^{2+} influx, and increased transmitter release, as described earlier. A longer exposure to 5-HT recruits, in addition, activation of PKC, which can also further increase the duration of action potentials in the presynaptic sensory neuron. Dishabituation, the strengthening of a previously habituated reflex, is reflected in the facilitation at depressed synapses. This involves PKC as well, but in this case it acts through a mechanism that is independent of spike broadening and is thought to involve vesicle mobilization. The spike broadening–independent component of facilitation may also involve Ca^{2+}/CamKII (Nakanishi et al., 1997).

In addition, as first appreciated by Ghirardi et al. (1995; see also Sutton and Carew, 2000; Sharma et al., 2003b), longer exposure to 5-HT can induce intermediate-term facilitation, which requires protein synthesis and involves MAP kinase as well as PKA or (under some circumstances) PKC.

The ability of different mechanisms to be recruited depending on experimental variables such as the pathway (Zalutsky and Nicoll, 1990), induction protocol (Grover and Teyler, 1990), saline composition (Larkman et al., 1992), and stage of development (Grosshans et al., 2002; Jensen et al., 2003) also applies to hippocampal LTP. For example, LTP in neonatal animals primarily involves PKA, whereas in more mature animals it involves CamKII (Yasuda et al., 2003).

4.02.3.3 Many Protocols Involve Pre- and Postsynaptic Mechanisms

Although the short-term mechanisms described for facilitation in *Aplysia* following sensitization or for depression following habituation have been found to be presynaptic, intermediate-term facilitation can also involve postsynaptic mechanisms including intracellular Ca^{2+} release from inositol-1,4,5-trisphosphate (IP3)-sensitive stores, activation of CamKII or PKC, and AMPA receptor insertion (Chitwood et al., 2001; Roberts and Glanzman, 2003; Jin et al., 2004, 2005; Li et al., 2005). To investigate the precise roles of pre- and postsynaptic mechanisms with intermediate protocols, Jin et al. (2004, 2005) examined facilitation induced by either a single, brief 5-HT exposure typically used to produce short-term facilitation (1 min, 50 $\mu mol\ L^{-1}$) or a more prolonged (10 min, 20 $\mu mol\ L^{-1}$) 5-HT exposure following a single pretest (rested) at sensory-motor neuron synapses in isolated cell culture. The facilitation with either protocol lasted more than 30 min compared with test alone controls, but the 10-min exposure to 5-HT produced larger facilitation than a 1-min exposure. With a 1-min exposure, bath application of an inhibitor of PKA (KT5720) or injection of a peptide inhibitor of PKA into the presynaptic sensory neuron reduced the facilitation, as did bath application of an inhibitor of CamKII (KN93) or presynaptic injection of a peptide inhibitor of CamKII (CamKII 281-309). By contrast, bath application of an inhibitor of PKC (Go6983) or injection of 1,2-bis(2-aminophenoxy)ethane-*N,N,N′,N′*-tetraacetic acid (BAPTA) into the postsynaptic motor neuron had no significant effects. None of the bath-applied inhibitors affected homosynaptic depression or basal synaptic transmission. These results suggest that, with a short application of 5-HT that produces short-term facilitation, presynaptic PKA and CamKII play critical roles, and that PKC and postsynaptic Ca^{2+} are not involved.

With the 10-min 5-HT protocol that is thought to produce intermediate-term facilitation, bath application of an inhibitor of PKA (KT5720) or presynaptic injection of the PKA inhibitor did not have a significant effect, but bath application of inhibitors of either PKC (Go6983) or CamKII (KN93) reduced the facilitation. The facilitation is mediated in part by presynaptic mechanisms, since injection of a peptide

inhibitor of PKC (PKC 19-31) into the presynaptic sensory neuron also reduced the facilitation by 10-min exposure to 5-HT (although presynaptic injection of a peptide inhibitor of CamKII did not.) However, it also is mediated in part by postsynaptic mechanisms, since injection of either BAPTA or CamKII 281-309 into the postsynaptic motor neuron also reduced facilitation. By contrast, postsynaptic injection of PKC 19-31 did not reduce the facilitation. These results indicate that with a longer 5-HT application presynaptic PKC plays an important role (as it does with dishabituation), and postsynaptic Ca^{2+} and CamKII are also important, suggesting that not only the specific kinases involved but also their site of action may depend on the duration of 5-HT exposure. Thus, whereas facilitation with a short application of 5-HT is presynaptic, facilitation with a longer 5-HT exposure involves both presynaptic (PKC) and postsynaptic (Ca^{2+} and CamKII) mechanisms.

These findings are not restricted to dissociated cell culture but are also seen in a reduced preparation of the behaving animal. Facilitation of sensory-motor neuron excitatory postsynaptic potentials (EPSPs) in a semi-intact preparation during actual behavioral sensitization also involves different mechanisms and sites depending on the training protocol (Antonov et al., 2005, 2006). Short-term sensitization produced by a single tail shock leads to synaptic facilitation, which involves presynaptic PKA and CamKII that produce transient spike broadening as well as some longer-lasting mechanisms of facilitation. Intermediate-term sensitization produced by a train of four shocks also involves presynaptic PKA and CamKII and, in addition, involves postsynaptic Ca^{2+} and CamKII, which are recruited with a slight delay after the shock. After that delay, the pre- and postsynaptic mechanisms both contribute and are more than additive.

Classical conditioning (like intermediate-term facilitation induced by 5-HT) involves not only pre- but also postsynaptic mechanisms. Thus, facilitation of sensory-motor neuron EPSPs during classical conditioning in a semi-intact preparation can be blocked either by injecting a peptide inhibitor of PKA into the sensory neuron or by injecting BAPTA into the motor neuron (Antonov et al., 2003), providing the strongest evidence to date that either activity-dependent facilitation or Hebbian LTP contributes to synaptic plasticity underlying behavioral learning. In addition, conditioning is accompanied by increases in evoked firing and membrane resistance of the sensory neuron, and those presynaptic effects are also blocked either by injecting an inhibitor of PKA into the sensory neuron or by injecting BAPTA into the postsynaptic motor neuron. These results suggest that the pre- and postsynaptic mechanisms are not independent but, rather, interact through retrograde signaling.

At least under some conditions pre- as well as postsynaptic mechanisms have also been found to contribute to early-phase LTP in hippocampus (Arancio et al., 1995, 1996, 2001; Malgaroli et al., 1995; Ryan et al., 1996; Zakharenko et al., 2003; Ninan and Arancio, 2004; Wang et al., 2005; Lu and Hawkins, 2006; Ninan et al., 2006). As in *Aplysia,* in several cases inhibitors injected into the pre- and postsynaptic neurons have more than additive effects (Arancio et al., 2001; Wang et al., 2005; Lu and Hawkins, 2006), suggesting that the pre- and postsynaptic mechanisms of LTP are not independent but, rather, act synergistically.

The sensory-to-motor-neuron synapses of the gill- and siphon-withdrawal reflex of *Aplysia* also exhibit plasticity that underlies another elementary form of nonassociative learning – habituation. Habituation is probably the most ubiquitous form of learning in animals, including man. It is a process whereby an animal learns through repeated exposure that the consequences of a weak stimulus are neither noxious nor rewarding. As a result, and in contrast to sensitization, the animal learns to ignore the stimulus. For example, *Aplysia* will initially respond to a weak tactile stimulus to the siphon with a brisk withdrawal of the gill and siphon. But with repeated stimulation, the animal learns to ignore the stimulus and exhibits progressively smaller reflex responses.

As is the case with sensitization, the memory for habituation can exist in both a short-term and long-term form. A single training session of 10 stimuli produces a memory that lasts for 10 or 15 min (Pinsker et al., 1970). In contrast, four repeated training sessions of 10 stimuli each produce a memory that persists for at least 3 weeks (Carew et al., 1972). Both the short-term and long-term behavioral modifications are reflected by a decrease in the strength of the sensory-to-motor-neuron connection. The synaptic depression resulting from habituation is homosynaptic; it results, as does the behavior, from a change in activity in the same pathway that is excited by the stimulus that elicits the reflex. At the cellular level, the homosynaptic depression that underlies short-term habituation results from a decrease in the number of transmitter quanta released per action potential from the presynaptic terminals of the sensory neurons (Castellucci and

Kandel, 1974), and this is the result of a reduced Ca^{2+} influx (Klein and Kandel, 1980).

During homosynaptic depression, the reduction in transmitter release produced by a single stimulus is already evident in response to the second stimulus and can persist for 10–15 min. This suggests that the presynaptic molecular events responsible for the decrease in transmitter release are set in motion by a single stimulus and have been completed by the time the second stimulus is given. To explain how this decrease in transmitter release might occur, a modeling study by Gingrich and Byrne (1985) predicted that the pool of releasable transmitter quanta might be depleted by habituation. This hypothesis was tested directly by Bailey and Chen (1988c), using the electron microscope to visualize the presynaptic terminals of sensory neurons that had been altered by short-term habituation. They found that short-term habituation did not alter the number of sensory neuron presynaptic terminals, the number of transmitter release sites (active zones) within the presynaptic terminals, or the size of active zones. Nor did it alter the total number of synaptic vesicles in a presynaptic terminal. Rather, there was a dramatic reduction in the number of vesicles that were docked at release sites within the active zones, and thus there were fewer packets of transmitter ready to be released. Subsequent experiments further indicated that, in addition to the reduction in the number of synaptic vesicles docked at the active zone, habituation might also interfere with a mechanism directly coupled to the release process (Eliot et al., 1994b; Armitage and Siegelbaum, 1998). Combined, these studies suggested that habituation leads to the selective depletion of synaptic vesicles from the active zone and a consequent failure to mobilize overlying vesicles in the presynaptic terminal. To overcome habituation, therefore, a dishabituating stimulus would first have to mobilize vesicles into sensory neuron active zones (Hochner et al., 1986).

4.02.3.4 Redistribution of Synaptic Components and Early Microstructural Modifications

Recent imaging studies have revealed that even the early phase of hippocampal LTP can also be accompanied by concomitant pre- and postsynaptic alterations in the structure of the synapse. Tens of minutes after the induction of LTP, there is an outgrowth of new pre- and postsynaptic processes (Engert and Bonhoeffer, 1999; Maletic-Savatic et al., 1999; Ninonenko et al., 2003), and even earlier (minutes), there are increases in spine size (Matsuzaki at al., 2004), clusters of postsynaptic glutamate receptors (Shi et al., 1999), and clusters of presynaptic vesicle-associated proteins and sites where the pre- and postsynaptic clusters colocalize (Antonova et al., 2001). Any of these early structural changes could also be a 'tag' that must be stabilized by protein synthesis for more enduring plasticity (see section 4.02.4.4).

Similar to the clustering of synaptic proteins during LTP, intermediate-term facilitation in *Aplysia* is accompanied by the enrichment of empty sensory neuron varicosities with synaptic vesicles, leading to the rapid presynaptic activation of silent synapses (Kim et al., 2003). However, these intermediate-term changes do not persist for 24 h unless they are stabilized by additional molecular events (including the machinery for translational activation) recruited during the long-term process. The early (hours) stages of LTF are also accompanied by clustering of postsynaptic proteins including the *Aplysia* homologs of NMDA and AMPA receptors (Li et al., 2004). It is not yet known whether intermediate-term facilitation is accompanied by similar postsynaptic changes, but it seems likely that it is. However, presynaptic microstructural changes can occur during even earlier phases of learning-related synaptic plasticity in *Aplysia:* homosynaptic potentiation induced by moderate tetanic stimulation of the presynaptic neuron is accompanied by rapid (less than 10 min) aggregation of the vesicle-associated protein synaptophysin into new clusters or puncta (Jin et al., 2003), as occurs during early-phase LTP in hippocampal neurons and intermediate-term facilitation by 5-HT in *Aplysia.*

The rapid increases in clusters or puncta of presynaptic (synaptophysin) and postsynaptic (GluR1) proteins at the onset of LTP in hippocampal neurons are dependent on NMDA receptor activation and actin polymerization (Antonova et al., 2001). Maintenance of the increases for 30 min does not require protein synthesis, but maintenance for 3 h does (Antonova et al., 2001, 2005). Time-lapse imaging of synaptophysin–green fluorescent protein (GFP) revealed that the puncta are formed by aggregation of material from a more diffuse background level, as is also thought to occur for GluR1 (Shi et al., 1999). That aggregation may involve two types of molecules that can regulate actin: RhoA and VASP. For example, the rapid increases in puncta of both pre- and postsynaptic proteins are blocked by two inhibitors of RhoA, toxin B and Y27632, and immunocytochemical studies have shown that VASP is located at synapses and is phosphorylated and activated both presynaptically and

postsynaptically during potentiation (Wang et al., 2005). Thus, the redistribution of synaptic proteins appears to involve similar molecular pathways (regulation of actin by RhoA and perhaps also VASP) on both sides of the synapse.

Activity-dependent remodeling of the actin network is also involved in the formation of new varicosities and the enrichment of presynaptic proteins during the early stages of LTF in *Aplysia* (Hatada et al., 2000; Udo et al., 2005). In that case, actin is regulated by a different Rho GTPase, Cdc42. In addition, homosynaptic potentiation in *Aplysia* is blocked by presynaptic (but not postsynaptic) injection of phalloidin, which binds actin (Jin et al., 2003), suggesting that actin could also be involved in the rapid increase in puncta of synaptophysin during the potentiation.

Collectively, these results suggest that even the early phases of learning-related synaptic plasticity can already engage a coordinated sequence of pre- and postsynaptic functional and structural changes that may ultimately lead to the formation of new synapses, as occurs during synapse formation in development (Sanes and Lichtman, 1999; Cohen-Cory, 2002). Like synapse formation during development, these processes probably involve a variety of transsynaptic messengers. Recent evidence suggests that nitric oxide (NO) is one of several messengers involved in both the functional and structural changes during the early phases of hippocampal LTP.

4.02.4 Cellular and Molecular Mechanisms Underlying Long-Term Forms of Memory Storage

As mentioned above, the inhibition of transcription or translation does not affect short-term memory but blocks the formation of long-term memory in a variety of model systems, suggesting that the stabilization of memory traces depends on *de novo* gene expression (Kandel, 2001).

4.02.4.1 Gating Signals at the Synapse: A Balance between the Activities of Protein Kinases and Phosphatases

Synaptic stimulation of *Aplysia* sensory neurons leads to a local increase in cAMP and the activation of PKA by causing the catalytic subunits of this enzyme to dissociate from the regulatory subunits. The catalytic subunits can then phosphorylate different substrates in the synaptic terminals, such as potassium channels and proteins involved in exocytosis, leading to enhanced transmitter release during short-term memory. When synaptic stimulation reaches a given threshold or is repeated a number of times, it causes a persistent increase in the level of cAMP and leads to longer-lasting forms of synaptic plasticity. At the molecular level, this more robust pattern of stimulation causes the catalytic subunit of PKA to recruit p42 MAPK. Both then move to the nucleus, where they phosphorylate nuclear targets including other kinases that, in turn, can phosphorylate transcription factors and activate gene expression required for the induction of long-term memory (Bacskai et al., 1993; Martin et al., 1997b; Purcell et al., 2003).

In rodents, inhibition of PKA and MAPK does not affect E-LTP at Schaffer collateral synapses, but these kinases are required for L-LTP (Frey et al., 1993; English and Sweatt, 1996, 1997; Abel et al., 1997). The role of PKA seems to be different in hippocampal neurons than during LTF formation in *Aplysia* sensory neurons. In the hippocampus, PKA does not translocate to the nucleus and plays only a synaptic role: It can phosphorylate different targets, such as the GluR1 subunit of AMPAR (Lee et al., 2000), and it favors the induction of LTP by counteracting the activity of protein phosphatases (Abel et al., 1997; Winder et al., 1998). Finally, it also tags the synapse enabling the consolidation of the long-term process (Barco et al., 2002). In contrast, the role of MAPK appears to be more conserved, and its activation and translocation to the nucleus are also required, at least for forskolin- or brain-derived neurotrophic factor (BDNF)-mediated L-LTP (Martin et al., 1997b; Patterson et al., 2001).

In addition to protein kinases, synaptic protein phosphatases also play a key role in regulating the initiation of long-term synaptic changes. Various protein phosphatases, such as PP1 and calcineurin, oppose the local activity of PKA and act as inhibitory constraints on memory formation. Thus, an increase in calcineurin activity causes defects in long-term memory and L-LTP (Mansuy et al., 1998; Winder et al., 1998), whereas a reduction has the opposite effect (Malleret et al., 2001). Similarly, a reduction in PP1 activity also improves memory in mice (Genoux et al., 2002). Recent experiments in cultured *Aplysia* neurons indicate that calcineurin may also act in this organism as a memory suppressor for sensitization (Sharma et al., 2003a). Therefore, in both systems a

balance between phosphatase and kinase activities at a given synapse gates the synaptic signals that eventually reach the nucleus and can regulate both memory storage and retrieval (Abel et al., 1998).

4.02.4.2 Gating Signals at the Nucleus: Triggering *de Novo* Gene Expression

One of the features that fundamentally distinguish the storage of long-term memory from short-term cellular changes is the requirement for the activation of gene expression. Recently, Thompson et al. (2004) have found that, in the *Aplysia* sensory-motor neuron culture preparation, 5-HT stimulation that produces LTF triggers the nuclear translocation of importins, proteins involved in carrying cargos through nuclear pore complexes. Similarly, in hippocampal neurons, NMDA activation or LTP induction, but not depolarization, leads to translocation of importin (Thompson et al., 2004). The future identification of the molecular cargoes of importin and its signaling role in the nucleus are likely to increase our understanding of how transcription-dependent memory is regulated.

Studies in *Aplysia* revealed the participation of the cAMP/PKA-signaling pathway and the transcription factor, cAMP response element binding protein (CREB), in transcriptional activation by synaptic stimulation. During LTF in *Aplysia* sensory neurons, PKA activates gene expression via an *Aplysia* CREB1 (ApCREB1). In 1990, Dash et al. first demonstrated a role for CREB in LTF by microinjecting CRE oligonucleotides into sensory neurons cocultured with motor neurons (Dash et al. 1990). These decoy oligonucleotides inhibit the function of ApCREB1 by directly binding to this protein, thereby preventing its binding to CRE sites in regulatory regions that actvate expression of cAMP-responsive genes. Whereas injection of the CRE oligonucleotide had no effect on STF, it selectively blocked LTF. Studies by a number of laboratories have now revealed that different members of the CREB family of transcription factors participate in the molecular switch that regulates LTF formation (Bartsch et al., 1995, 1998; Lonze and Ginty, 2002; Barco et al., 2003). Both the CREB activator ApCREB1 and the repressor ApCREB2 contribute to this process. The formation of LTF requires the activation of ApCREB1 by PKA and the concomitant downregulation of ApCREB2 by MAPK (Guan et al., 2003). Injection of anti-ApCREB2 antibodies into *Aplysia* sensory neurons causes a single pulse of 5-HT, which normally induces STF lasting minutes, to evoke LTF that lasts several days (Bartsch et al., 1995). Conversely, injection of pApCREB1 phosphorylated by PKA can by itself trigger facilitation lasting 24 h, and this facilitation can be stabilized by a single pulse of 5-HT (Bartsch et al., 1998; Casadio et al., 1999).

These studies revealed that the transition from STF to LTF requires the simultaneous removal of transcriptional repressors and activation of transcriptional activators. Transcriptional repressors and activators can interact with each other both physically and functionally. Guan et al. (2002) used chromatin immunoprecipitation techniques to examine directly the role of CREB-mediated responses in long-term synaptic integration in the nucleus of *Aplysia* sensory neurons. They found that both facilitatory and inhibitory modulatory transmitters alter promoter occupancy by activator or repressor CREB isoforms and subsequently affect nucleosome structure bidirectionally through acetylation and deacetylation of histone residues in chromatin.

The complete set of genes regulated by a transcription factor in a specific cell type is still not known. In *Aplysia* sensory neurons, the activity of ApCREB1 leads to the expression of several immediate-response genes, such as ubiquitin hydrolase, that stabilizes STF (Hegde et al., 1997) and the transcription factor CCAAT-box-enhanced binding-protein (C/EPB), whose induction has been shown to be critical for LTF (Alberini et al., 1994). This induced transcription factor (in concert with other constitutively expressed molecules such as ApAF [Bartsch et al., 2000]) activate a second wave of downstream genes that can ultimately lead to the growth of new synaptic connections. These genes represent only a few of the family of gene products generated by CREB activity.

The participation of the cAMP/CREB pathway appears to be a general feature of long-term memory formation throughout the animal kingdom. The first genetic screenings designed to identify learning mutants in *Drosophila* revealed two interesting mutants, *dunce* and *rutabaga,* with specific defects in memory formation (Dudai et al., 1976; Duerr and Quinn, 1982) that were subsequently shown to affect genes in the cAMP signaling pathway (Byers et al., 1981; Waddell and Quinn, 2001). Experiments in transgenic flies have confirmed that the balance between CREB activator and repressor isoforms is critical for long-term behavioral memory. Thus, overexpression of an inhibitory form of CREB (dCREB-2b) blocked long-term olfactory memory but did not alter short-term memory (Yin et al., 1994; Perazzona et al., 2004). Indeed, most of the upstream signaling cascade leading to CREB activation

appears to be conserved through evolution, and many aspects of the role of CREB in synaptic plasticity described in invertebrates have also been observed in the mammalian brain. However, the role of CREB in explicit forms of memory appears to be more complex than in implicit forms of memory in invertebrates (for reviews, see Lonze and Ginty, 2002; Barco et al., 2003).

In mammals, CREB has been shown to regulate the expression of more than 100 genes, but it is still not clear how many of these putative downstream genes are actually regulated during learning and required for memory storage (Mayr and Montminy, 2001; Lonze and Ginty, 2002). The current list of target genes is heterogeneous and includes genes with very diverse functions, from regulation of transcription and metabolism to genes affecting cell structure or signaling. Many CREB targets, such as c-fos, EGR-1, or C/EBP, are themselves transcription factors, whose induction may trigger a second wave of gene expression. The availability of the complete mouse and human genome, the availability of the *Aplysia* neuronal transcriptome (Moroz et al., 2006), the development of new bioinformatics tools for their analysis, and the recent application of new unbiased, genome-wide screening approaches has begun to reveal the gene profiles regulated by CREB under different physiological conditions (Conkright et al., 2003; Euskirchen et al., 2004; Impey et al., 2004; Barco et al., 2005; Zhang et al., 2005). Although we have focused on CREB-dependent gene expression because of its conserved role in memory formation through evolution, other transcription factors, such as ApAF and C/EBP in *Aplysia* and SRF, C/EBP, c-fos, EGR-1, or NF-$\kappa\beta$ in mice (Tischmeyer and Grimm, 1999; Albensi and Mattson, 2000; Izquierdo and Cammarota, 2004; Ramanan et al., 2005) are also likely to contribute to the transcriptional regulation that accompanies long-lasting forms of synaptic plasticity.

The epigenetic marking of chromatin, by histone modifications, chromatin methylation, and the activity of retrotransposons, may have long-term consequences on transcriptional regulation of specific gene *loci* involved in long-term synaptic changes, and thus adds a new layer of complexity to our view of how nuclear function and synaptic activity affect one another (Guan et al., 2002; Hsieh and Gage, 2005; Levenson and Sweatt, 2005). The contribution of histone tail acetylation, a modification that favors transcription and is associated with active *loci*, was first revealed for LTF formation by Guan et al. (2002) in *Aplysia*. In addition to finding that facilitatory and inhibitory stimuli alter, bidirectionally, the acetylation stage and structure of promoters driven by the expression of genes involved in the maintenance of LTF, such as C/EBP, this study also demonstrated that enhancing histone acetylation with deacetylase (HDAC) inhibitors facilitates the induction of LTF. HDAC inhibitors have now been shown to enhance L-LTP in the Schaffer collateral pathway of mammals and memory formation in hippocampus-dependent tasks (Alarcon et al., 2004; Korzus et al., 2004; Yeh et al., 2004; Levenson et al., 2005). Conversely, mice with reduced histone acetyltransferase activity have deficits in both long-lasting forms of memory and LTP (Bourtchouladze et al., 2003; Alarcon et al., 2004; Korzus et al., 2004; Wood et al., 2005). These results indicate that critical chromatin remodeling occurs during the formation of long-term memory, and that these nuclear changes are required for the stable maintenance of memory storage.

4.02.4.3 Local Protein Synthesis

In addition to transcription in the nucleus and protein synthesis in the cell body, long-term memory also requires a second site of local protein synthesis at the synapse. A number of distinct mRNAs have been localized in the axons of *Aplysia* and in the dendrites of rodent hippocampal neurons (for review, see Steward and Schuman, 2001, 2003). The molecular mechanisms that target these mRNAs to the synapse are largely unknown, but some are carried by the kinesin motors, the key anterograde transport machinery (Puthanveettil and Kandel, 2006). Some of these mRNAs are thought to involve the recognition of *cis*-acting elements in their 3′ untranslated region by specific RNA-binding proteins that interact with the cytoskeleton. Once transported to the dendritic compartments, these mRNAs are translated only after docking at active synaptic sites, a process frequently referred to as synaptic or local protein synthesis. Recent studies suggest that regulation of local protein synthesis plays a major role in the control of synaptic strength at the sensory-to-motor-neuron connection in *Aplysia* and during L-LTP in the hippocampus. For example, Casadio et al. (1999) found that long-term, synapse-specific facilitation induced by 5-HT requires local protein synthesis for the stable maintenance of learning-induced synaptic growth. In the hippocampus, the induction of LTP in the Schaffer collateral pathway is accompanied by the transport of polysomes from dendritic shafts to active spines of CA1 neurons, suggesting a critical role for local protein synthesis in the morphological changes associated with LTP

(Ostroff et al., 2002), and local inhibition of protein synthesis blocks L-LTP in the Schaffer collateral pathway (Bradshaw et al., 2003; Cracco et al., 2005). The control of translation at the synapse is likely to be complex and involve several different mechanisms, including different types of mRNA transport and docking, cytoplasmic poly-adenylation, mTOR (which is the target of the selective protein synthesis inhibitor rapamycin; Cammalleri et al., 2003; Purcell et al., 2003), and the phosphorylation of different translation factors (see recent review by Sutton and Schuman, 2005). Many of the molecules contributing to the regulation of this process are required for both LTF in *Aplysia* and L-LTP in the hippocampus, including BDNF (which promotes local protein synthesis; Aakalu et al., 2001; Purcell et al., 2003), mTOR, and the cytoplasmic polyadenylation element binding protein (CPEB), which activates dormant mRNAs (Huang et al., 2002; Si et al., 2003a). The role of local protein synthesis in the capture and stabilization of synapse-specific forms of long-term plasticity is discussed in the following section.

4.02.4.4 Moving Back to the Synapse: Capture of Activity-Induced Gene Products

Following the sending of a retrograde signal to the nucleus and the subsequent transcriptional activation, newly synthesized gene products, both mRNAs and proteins, have to be delivered by kinesin-mediated fast axonal transport (Puthanveettil and Kandel, 2006) specifically to the synapses whose activation originally triggered the wave of gene expression. To explain how this specificity can be achieved in a biologically economical way given the massive number of synapses in a single neuron, Martin et al. (1997a) and Frey and Morris (1997) proposed the synaptic capture hypothesis. This hypothesis, also referred to as synaptic tagging, proposes that the products of gene expression are delivered throughout the cell but are only functionally incorporated in those specific synapses that have been tagged by previous synaptic activity. The 'synaptic tag' model has now been supported by a number of studies in both the rodent hippocampus (Frey and Morris, 1997, 1998; Barco et al., 2002; Dudek and Fields, 2002) and *Aplysia* (Martin et al., 1997a; Casadio et al., 1999).

Studies of synaptic capture at the synapses between the sensory and motor neurons of the gill-withdrawal reflex in *Aplysia* have demonstrated that the production of CRE-driven gene products in the nucleus is not sufficient to achieve synapse-specific LTF. One also needs a PKA-mediated covalent signal to mark the stimulated synapses, and consequent local protein synthesis to stabilize that mark (Martin et al., 1997a; Casadio et al., 1999). Thus, injection into the cell body of phosphorylated CREB-1 gives rise to LTF at all the synapses of the sensory neuron, but this facilitation is not maintained beyond 24–48 h unless one of the synapses is also marked by triggering the short-term process with a single pulse of 5-HT (Casadio et al., 1999). Once marked, that synapse and only that synapse shows maintained facilitation and growth.

Experiments in the rat hippocampus by Frey and Morris have demonstrated, in turn, that once transcription-dependent LTP has been induced at one pathway, the long-term process can be 'captured' at a second pathway receiving a single train that would normally produce only E-LTP. The stimulus for the short-term process causes a transient potentiation and, in addition, marks the synaptic terminals, enabling the capture of the newly expressed gene products. The properties of synaptic capture observed for intracompartmental capture in hippocampal CA1 neurons are similar to those described in the bifurcated sensory neurons of *Aplysia* (Martin et al., 1997a). However, in mammals, where there are two dendritic compartments – apical and basal – the tag appears to be restricted to specific dendritic compartments, and additional mechanisms are required to capture across compartments (Alarcon et al., 2006). Most of the molecular details underlying these processes are still unknown, but experiments with a line of transgenic mice expressing a constitutively active CREB protein have demonstrated that PKA activity is part of the tagging signal, and that the synaptic capture of CRE-driven transcription may be sufficient to support L-LTP (Barco et al., 2002). More recent experiments in these mutants and in BDNF-deficient mice support a role for presynaptically released BDNF in the tagging of the synapse for subsequent capture of L-LTP (Barco et al., 2005). Local synthesis of proteins, which we have discussed in the previous section, has also been proposed to be involved in synaptic capture of long-term forms in both *Aplysia* and rodents (Martin et al., 1997a; Barco et al., 2002). Martin et al. (1997a) investigated the role of local protein synthesis in an *Aplysia* culture system in which a single bifurcated sensory neuron was plated in contact with two spatially separated gill motor neurons. In this system, repeated application of 5-HT to one synapse produces a CREB-mediated, synapse-specific LTF that can be blocked by the local application

of inhibitors of translation, suggesting that local protein synthesis at the synapse is also required as part of the retrograde signaling cascade for the initiation of synapse-specific LTF.

4.02.4.5 The Stable Strengthening of Synaptic Connections: Synaptic Growth, Silent Synapses, and Self-Maintenance Mechanisms

Although a number of molecular components that underlie the functional changes associated with the storage of long-term memory have been characterized, little is known about how these are regulated by and coupled to the signaling pathways that give rise to the synaptic structural changes (Bailey et al., 2004).

Activity-induced remodeling of preexisting synapses and the growth of new synapses have been found to accompany various forms of long-term memory, a phenomenon particularly well documented in *Aplysia* sensory neurons (Bailey and Kandel, 1993). Long-term sensitization has been extensively studied in this respect because it is associated with a robust growth of new synaptic connections between the sensory neurons and their postsynaptic target cells (Bailey and Chen, 1983, 1988a,b, 1989). Studies of long-term sensitization have revealed that it is accompanied by two forms of learning-related structural plasticity: (1) the remodeling of preexisting synapses, resulting in an increase in the number, size, and vesicle content of presynaptic transmitter release sites (active zones) (Bailey and Chen, 1983), and (2) a growth process that appears similar to synaptogenesis during development and leads to a pronounced increase in the total number of presynaptic varicosities per sensory neuron (Bailey and Chen, 1988a). Sensory neurons from long-term sensitized animals exhibit a twofold increase in the total number of synaptic varicosities, as well as an enlargement in the size of each neuron's axonal arbor. The increase in the size and synaptic vesicle complement of sensory neuron active zones was found to be relatively transient, whereas the changes in varicosity and active zone number were much more stable and persisted for at least 3 weeks after training, similar to the duration of the behavioral change (Bailey and Chen, 1989). These findings demonstrated, for the first time, that learning-induced structural changes could be detected at the level of identified synaptic connections known to be critically involved in behavioral modification and suggested that the growth of new sensory neuron synapses is likely to represent the final and perhaps most stable phase of long-term memory storage in *Aplysia*.

More recently, Kim et al. (2003) have examined the contribution of each class of presynaptic structural change – remodeling of preexisting synapses and the growth of new synapses – to the different time-dependent phases of LTF. They monitored both functional and structural synaptic changes continuously using time-lapse confocal imaging of presynaptic varicosities of sensory neurons labeled with three different fluorescent markers: the whole-cell marker *Alexa-594* and two presynaptic marker proteins, *synaptophysin-eGFP* to monitor changes in the distribution of synaptic vesicles within individual varicosities and *synapto-PHluorin* to monitor active transmitter release sites (Miesenbock et al., 1998). Repeated pulses of 5-HT induced two temporally, morphologically, and molecularly distinct classes of presynaptic changes: (1) relatively rapid activation of silent presynaptic terminals through the filling of preexisting empty varicosities with synaptic vesicles, which requires translation but not transcription, and (2) generation of new synaptic varicosities, which occurs more slowly and requires both transcription and translation. In addition to its role in LTF, the rapid (hours) activation of silent presynaptic terminals may also contribute to the intermediate phase of synaptic plasticity and memory storage (Ghirardi et al., 1995; Mauelshagen et al., 1996; Sutton et al., 2001). These findings, the first to be made on individually identified presynaptic varicosities, suggest that long-term changes in synaptic effectiveness in *Aplysia* may involve the differential regulation of two fundamentally disparate forms of presynaptic compartment: (1) nascent (empty) silent varicosities that can be rapidly and reversibly remodeled into active transmitter release sites and (2) mature, more stable and functionally competent varicosities that may undergo a process of fission to form new stable synaptic contacts (Bailey et al., 2005).

Activation of silent synapses also plays a major role in LTP in mammals. In this case, the term *silent synapse* refers to excitatory glutamatergic synapses whose postsynaptic membrane contains NMDARs but no AMPARs (Malinow et al., 2000; Malinow and Malenka, 2002). The lack of AMPAR-mediated signaling renders these synapses inactive, or 'silent,' under normal conditions. Synaptic stimulation activates these silent synapses through the insertion of AMPARs into the postsynaptic membrane, a phenomenon sometimes referred to as AMPA-fication. Calcium/CamKII plays a critical role in this process.

Once this kinase is activated by high-frequency stimulation, it phosphorylates AMPARs or associated proteins, triggering their insertion into the postsynaptic membrane. The synapse is then no longer silent, and postsynaptic responses are, by consequence, enhanced. Conversely, synapses can be made to be silent, for example, after LTD induction, by removing AMPARs from the postsynaptic membrane (Malinow and Malenka, 2002).

In addition to changes in synaptic function, activity-induced growth and/or remodeling of synaptic connections are also important in the storage of explicit memory. However, their specific role is not as clear as in *Aplysia,* because the functional contribution of individual synapses to memory processes in the large and complex neuronal circuits of the mammalian brain is not yet well defined (see reviews by Lamprecht and LeDoux, 2004; Hayashi and Majewska, 2005; Segal, 2005). The generation and enlargement of dendritic spines has been associated with the production of LTP and synaptic activity in organotypic hippocampal slices (Matsuzaki et al., 2004; Nagerl et al., 2004), or acute slices of neonatal animals (Zhou et al., 2004), whereas these structural changes are much more subtle in the adult brain (Lang et al., 2004). In adults there is only a modest production of new spines (Zuo et al., 2005), and learning-related plasticity seems to rely more on subcellular changes than on anatomical changes. Thus, neuronal activity regulates the diffusion of molecules across the neck of dendritic spines (Bloodgood and Sabatini, 2005) and the transport of polysomes from dendritic shafts to active spines (Ostroff et al., 2002), as well as the trafficking of neurotransmitter receptors (Malinow and Malenka, 2002).

Biological molecules have a relatively short half-life (hours to days) compared with the duration of memory (days, weeks, even years). How then, in the absence of frank anatomical changes, can the altered molecular composition of a synapse be maintained for such a long time? Most answers to this elusive question rely on some type of self-sustained alteration that can somehow modulate synaptic strength. For example, Malinow and colleagues have proposed that two regulatory pathways control the insertion and removal of AMPA receptors at the synapse: The maintenance pathway is always on and controls the constant turnover of receptor subunits, whereas the constructive pathway is only turned on during LTP induction (Malinow et al., 2000; Malinow and Malenka, 2002). The activation of the constructive pathway and insertion of new AMPARs would cause the growth and/or maturation of postsynaptic densities, enabling the formation of new memories, whereas the maintenance pathway would be responsible for their stabilization (Hayashi et al., 2000; Lisman and Zhabotinsky, 2001). Another interesting model for long-term memory storage was suggested by Crick more than 20 years ago (Crick, 1984). Crick proposed that autocatalytic kinases might provide the molecular mechanism for long-lasting, self-maintained changes in synaptic function. John Lisman further developed this idea based on the autocatalytic properties of the calcium/CamKII (Lisman and Zhabotinsky, 2001).

More recently, Kandel and Si have proposed a model based on the prion-like properties of the *Aplysia* neuronal CPEB (Si et al., 2003b). *Aplysia* CPEB has two conformational states: one is inactive or acts as a repressor, and the other is active. In a naive synapse, the basal level of CPEB expression is low, and its state is inactive or repressive. However, if a given threshold is reached, CPEB switches to the prion-like state, which activates the translation of dormant mRNAs through the elongation of their poly-A tail (Si et al., 2003a). Once the prion state is established at an activated synapse, dormant mRNAs, made in the cell body and distributed cell-wide, would be translated only at the activated synapses. Because the activated CPEB can be self-perpetuating, it could contribute to a self-sustaining, synapse-specific long-term molecular change. Interestingly, a structurally similar neuronal isoform of CPEB, CPEB-3, has been found in mouse hippocampal neurons, where it is induced by the neurotransmitter dopamine (Theis et al., 2003).

These molecular mechanisms are not mutually exclusive: The synaptic translation of CamKII mRNAs can be regulated by CPEB, and the synthesis and traffic of new AMPAR subunits may require CamKII activity as well as enhanced protein synthesis (Burgin et al., 1990; Ouyang et al., 1999; Huang et al., 2002) .

4.02.5 Concluding Remarks

Cell biological and molecular studies of both implicit and explicit memory processes have revealed two major forms of storage mechanisms. The storage of long-term memory is associated with altered gene expression, as well as the synthesis of new mRNAs and proteins, and is often accompanied by changes in both the number and structure of synaptic connections. In contrast, short-term (up to about 1 h) memory and the early phases of long-term synaptic

storage in *Aplysia* and hippocampus involve covalent modification of preexisting proteins in either the presynaptic (*Aplysia*) or postsynaptic (hippocampus) neuron, leading to an alteration in the strength of preexisting synaptic connections.

The discovery of intermediate (2–3 h) forms of synaptic plasticity that involve elements of the short-term and long-term molecular mechanisms in both *Aplysia* and hippocampus have demonstrated that distinct phases that overlap these processes can be revealed by specific protocols. These new intermediate phases may also form a bridge to the morphological changes that occur during long-term plasticity: Recent imaging studies of living synapses in hippocampus and *Aplysia* have shown that even the early phases of synaptic plasticity are accompanied by microstructural alterations. These early structural changes may seed the formation of new functional synapses, which may then be further elaborated and stabilized by additional long-term training. This emerging view is likely to supplement the more established idea that early-phase plasticity exclusively involves either pre- or postsynaptic covalent modifications and suggests that these early phases of synaptic plasticity also may already involve microstructural alterations capable of contributing importantly to the transition between short-term and long-term memory storage.

Finally, as we have outlined in this chapter, one of the key unifying findings emerging from the molecular study of implicit and explicit memory processes is the unexpected realization that these distinct forms of memory, which differ not only in the neural systems involved but also in the nature of the information stored and in the role of attention in that storage, nevertheless share a common set of molecular mechanisms for their long-term representation. Thus, whereas animals and humans are capable of a wide variety of learning processes that use a number of different second messenger and signaling cascades, they may recruit the same restricted set of molecular logic for the storage of long-term memory.

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4.03 Sensitization and Habituation: Invertebrate

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4.03.1 Introduction

Survival of animals is dependent on their capacity to adapt to their environment by modifying their behavior. The experience-induced modification of behavior is a manifestation of learning, whereas memory is the retention of a learned behavior over time. A conceptual scheme that has driven the investigation of learning for the largest part of the twentieth century rests on the distinction between associative and nonassociative forms of learning. Nonassociative learning is best exemplified by habituation and sensitization. Habituation is defined as the gradual waning of a behavioral response to a weak or moderate stimulus that is presented repeatedly. Following habituation, the response may be restored to its initial state either passively with time (i.e., spontaneous recovery), or with the presentation of a novel stimulus (i.e., dishabituation). Sensitization is defined as the enhancement of a behavioral response elicited by a weak stimulus following another, usually noxious stimulus. Sensitization can also develop in response to a moderate stimulus that is presented repeatedly at relatively short intervals.

Associative learning refers to the formation of an association either between two stimuli (i.e., classical conditioning), or between a behavior and a stimulus (i.e., operant conditioning). In classical conditioning, a novel or weak stimulus (conditioned stimulus; CS) is paired with a stimulus that generally elicits a reflexive response (unconditioned stimulus and response, respectively; US and UR). After sufficient training with contingent CS-US presentations (which may be a single trial), the CS comes to elicit a learned response (conditioned response; CR), which often resembles the UR (or some aspect of it). Operant conditioning is an experimental procedure in which the behavior of an animal may be followed by either a desirable or an aversive stimulus, arranged by the

experimenter. The desirable stimulus (e.g., food) will typically increase the future occurrence of this behavior (a process called positive reinforcement). An aversive stimulus (e.g., a noxious electric shock) will tend to decrease the future probability of this behavior (a process called punishment). A behavior can also be reinforced when it becomes contingent with the removal of an aversive stimulus from the animal's environment (i.e., negative reinforcement). Thus, through the processes of operant conditioning an animal learns the consequences of its behavior.

Humans and other animals are capable of displaying more complex forms of learning than the four types described above. However, these four types are likely to constitute the building blocks for more complex forms of learning. Thus, a major goal of neurobiologists is to explain the anatomical, biophysical, and molecular processes of the nervous system that underlie simple forms of learning and memory. Specifically, what parts of the nervous system are critical for learning? How is information about a learned event acquired and encoded in neuronal terms? How is information stored, and, once stored, how is it retrieved? Most neuroscientists believe that the answers to these questions lie in understanding the ways in which the properties of individual nerve cells in general, and synaptic connections, in particular, change when learning occurs. To that end, the investigation of neuronal mechanisms of learning and memory in invertebrates has been very fruitful over the past 40 years. This chapter will focus on mechanisms of habituation and sensitization in *Aplysia* and other invertebrates. A detailed discussion of mechanisms of associative learning in *Aplysia* and other invertebrates can be found in subsequent chapters (*See* Chapters 4.02, 4.04, 4.06, 4.07, 4.08, 4.09, 4.10).

4.03.2 Habituation and Sensitization in *Aplysia*

4.03.2.1 Aplysia Withdrawal Reflexes and Underlying Neural Circuits

One animal that is well suited for the examination of the molecular, cellular, morphological, and network mechanisms underlying neuronal plasticity and learning and memory is the marine mollusc *Aplysia*. This animal has a relatively simple nervous system with large, identifiable neurons that are accessible for detailed anatomical, biophysical, and biochemical studies. Neurons and neural circuits that mediate many behaviors in *Aplysia* have been identified. In several cases, these behaviors can be modified by learning. Moreover, specific loci within neural circuits at which modifications occur during learning have been identified and aspects of the cellular mechanisms underlying these modifications have been analyzed.

Two withdrawal reflexes of *Aplysia* have been used extensively to analyze the neuronal mechanisms contributing to nonassociative and associative learning (for reviews see Hawkins and Kandel, 1984; Carew and Sahley, 1986; Byrne, 1987; Byrne et al., 1991, 1993). The first behavior is the siphon–gill withdrawal reflex. Within the mantle cavity is the respiratory organ of the animal, the gill, and protruding from the mantle cavity is the siphon (**Figure 1**). The

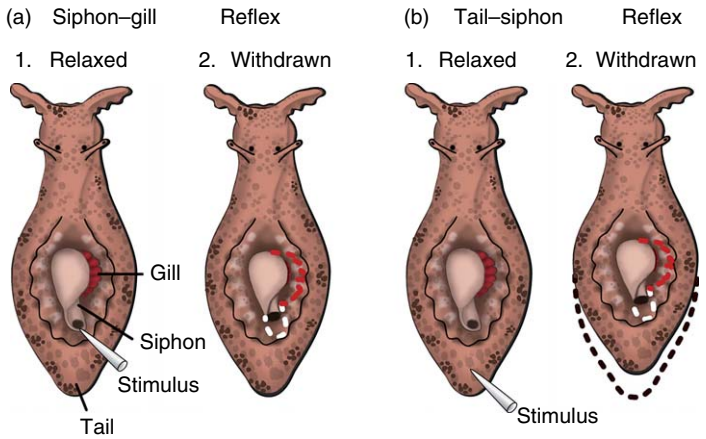

Figure 1 Siphon–gill and tail–siphon withdrawal reflexes of *Aplysia*. (a) Siphon–gill withdrawal. Dorsal view of *Aplysia* (1) Relaxed position. (2) A stimulus (e.g., a water jet, brief touch, or weak electric shock) applied to the siphon causes the siphon and the gill to withdraw into the mantle cavity. (b) Tail–siphon withdrawal reflex. (1) Relaxed position. (2) A stimulus applied to the tail elicits a reflex withdrawal of the tail and siphon.

siphon–gill withdrawal reflex is elicited when a tactile or electrical stimulus is delivered to the siphon and results in withdrawal of the siphon and gill (**Figure 1(a)**). A second behavior that has been examined extensively is the tail–siphon withdrawal reflex. Tactile or electrical stimulation of the tail elicits a coordinated set of defensive responses, two components of which are a reflex withdrawal of the tail and the siphon (**Figure 1(b)**).

A prerequisite for the analysis of the neural and molecular basis of learning is an understanding of the neural circuit that controls the behavior. The afferent limb of the siphon–gill withdrawal reflex consists of sensory neurons with somata in the abdominal ganglion. The siphon sensory neurons (SN) monosynaptically excite gill and siphon motor neurons (MN) that are also located in the abdominal ganglion (**Figure 2**). Activation of the gill and siphon motor neurons leads to contraction of the gill and siphon. Excitatory, inhibitory, and modulatory interneurons (IN) in the withdrawal circuit have also been identified, although only excitatory interneurons are illustrated in **Figure 2**. The afferent limb of the tail–siphon withdrawal reflex consists of a bilaterally symmetric cluster of sensory neurons that are located in the left and right pleural ganglia (Walters et al., 1983a). These sensory neurons make monosynaptic excitatory connections with motor neurons in the adjacent pedal ganglion, which produce withdrawal of the tail (**Figure 2**). In addition, the tail sensory neurons form synapses with various identified excitatory and inhibitory interneurons (Buonomano et al., 1992; Cleary and Byrne, 1993; Xu et al., 1994). Some of these interneurons activate motor neurons in the abdominal ganglion, which control reflex withdrawal of the siphon. Moreover, several additional neurons modulate the tail–siphon withdrawal reflex (Raymond and Byrne, 1994; Cleary et al., 1995) (**Figure 3(a1)**).

The sensory neurons for both the siphon–gill and tail–siphon withdrawal reflexes are similar and appear to be important plastic elements in the neural circuits. Changes in their membrane properties and the strength of their synaptic connections (synaptic efficacy) are associated with learning and memory. Moreover, the properties of these neurons are modulated by *in vitro* analogs of behavioral training.

4.03.2.2 Habituation

Habituation, perhaps the simplest form of nonassociative learning, refers to the gradual waning of the responses elicited by a repeatedly presented stimulus. Repeated presentation of a relatively weak stimulus will most probably lead to habituation, whereas repeated presentation of a relatively strong stimulus

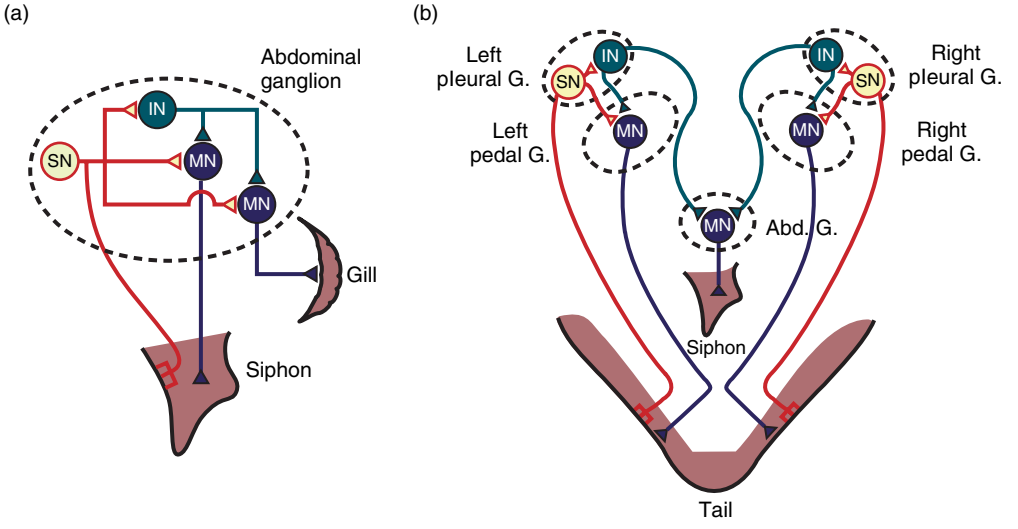

Figure 2 Simplified circuit diagrams of the siphon–gill (a) and tail–siphon (b) withdrawal reflexes. Stimuli activate the afferent terminals of mechanoreceptor sensory neurons (SN) whose somata are located in central ganglia. The sensory neurons make excitatory synaptic connections (triangles) with interneurons (IN) and motor neurons (MN). The excitatory interneurons provide a parallel pathway for excitation of the motor neurons. Action potentials elicited in the motor neurons, triggered by the combined input from the SNs and INs, propagate out peripheral nerves to activate muscle cells and produce the subsequent reflex withdrawal of the organs. Modulatory neurons (not shown here but see **Figure 3(a1)**), such as those containing serotonin (5-HT), regulate the properties of the circuit elements, and, consequently, the strength of the behavioral responses.

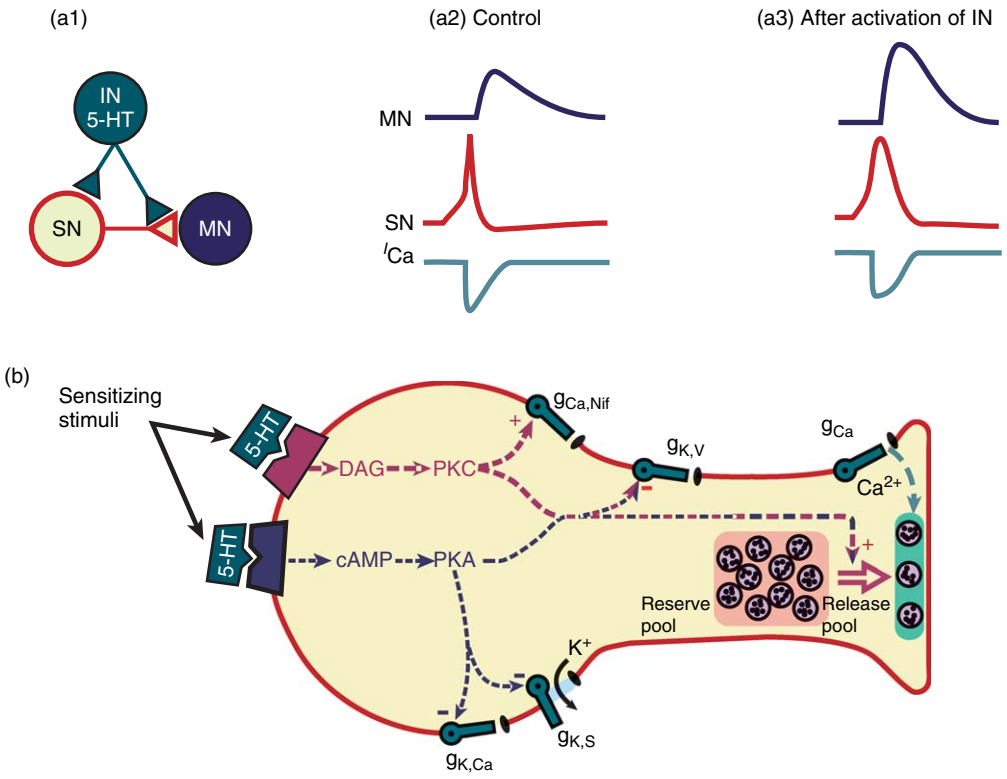

Figure 3 Model of heterosynaptic facilitation of the sensorimotor connection that contributes to short-term sensitization in *Aplysia*. (a1) Sensitizing stimuli activate facilitatory interneurons (IN) that release modulatory transmitters, one of which is 5-HT. The modulator leads to an alteration of the properties of the sensory neuron (SN). (a2, a3) An action potential in a SN after the sensitizing stimulus results in greater transmitter release and hence a larger postsynaptic potential in the motor neuron (MN) than an action potential prior to the sensitizing stimulus. For short-term sensitization the enhancement of transmitter release is due, at least in part, to broadening of the action potential and an enhanced flow of Ca^{2+} into the sensory neuron. (b) Molecular events in the sensory neuron. 5-HT released from the facilitatory interneuron (Part a1) binds to at least two distinct classes of receptors on the outer surface of the membrane of the sensory neuron, which leads to the transient activation of two intracellular second messengers: DAG and cAMP. These second messengers, acting though their respective protein kinases, affect multiple cellular processes, the combined effects of which lead to enhanced transmitter release when a subsequent action potential is fired in the sensory neuron. See section 4.03.2.3.1 for abbreviation definitions.

may lead to sensitization, which is discussed in the next section of the chapter. Habituation is generally distinguished from simple fatigue or sensory adaptation because responsiveness can be rapidly restored (dishabituated) by the presentation of a novel stimulus to the animal. The parametric features of habituation have been previously described in detail by Thompson and Spencer (1966).

Habituation shares some features with more complex forms of learning. First, habituation has a temporal gradient. Similar to most forms of learning, each trial has only a transient effect, which necessitates the presentation of multiple trials. Second, the interval at which training trials are presented is critical. Massing many trials together may lead to faster, albeit only short-lived habituation. In contrast, spacing trials too far apart may lead to little or no habituation. Therefore, an optimal intertrial interval exists, which is determined by the stimulus features and the response system. Third, the effects of habituation training are reversible. As mentioned above, they can be reversed spontaneously with the passage of time (spontaneous recovery), or they may be reversed by the presentation of a novel stimulus (dishabituation). Fourth, habituation learning is stimulus-specific. Although habituation can generalize to novel stimuli, this generalization is limited and depends on the degree of physical similarity between the trained and novel stimuli. Habituation is an indispensable form of learning. It is probably the earliest manifestation of the ability of all animals to store and retrieve the memory of a stimulus, as well as the

ability to filter out stimuli that are inconsequential. The latter is a necessary element of selective attention, which places behavior under the dynamic control of stimuli that carry important behavioral contingencies (more on attention can be found in subsequent chapters; *See* Chapters 1.13, 2.02).

A major step in the understanding of neural mechanisms of habituation was made in the early 1970s (Pinsker et al., 1970; Carew and Kandel, 1973). In a group of three seminal articles, it was reported that the siphon–gill withdrawal reflex of *Aplysia* can display habituation, that habituation was accompanied by a decrease in the spike activity of gill motor neurons in response to tactile stimulation of the siphon, and that an activity-induced decrease in the efficacy of sensorimotor synapses could be responsible for the reduced responsiveness of motor neurons and for behavioral habituation (Castellucci et al., 1970; Kupfermann et al., 1970; Pinsker et al., 1970). Starting with those early reports, behavioral habituation of withdrawal reflexes became tightly linked to homosynaptic depression of sensorimotor synapses. Since then, the vast majority of research studies that aimed at understanding the mechanisms of habituation focused on understanding the mechanisms of synaptic depression, which, similar to habituation, can appear in both short- and long-term forms.

4.03.2.2.1 Short-term depression of Aplysia sensorimotor synapses

Quantal analysis of sensorimotor synapses suggested that short-term homosynaptic depression involves primarily a decrease in presynaptic transmitter release (Castellucci and Kandel, 1974). The presynaptic nature of synaptic depression was also supported by a more recent report that repeated application of exogenous glutamate to the postsynaptic neuron did not result in depression, that blockade of postsynaptic glutamate receptors did not block depression, and that synaptic depression did not correlate with changes in the amplitude of miniature excitatory postsynaptic potentials (mEPSPs) (Armitage and Siegelbaum, 1998). To account for the depressive effect of repeated activity of sensory neurons on transmitter release, an early model of depression relied on cumulative inactivation of calcium channels and decline in the calcium entering the presynaptic terminal and triggering release (Klein et al., 1980).

Extensive parametric analysis of the kinetics of depression and recovery from depression as a function of the stimulation frequency revealed that the mechanisms must be more complex than just a decrease in calcium influx or a depletion of presynaptic vesicles (Byrne, 1982). A quantitative model of transmission at the sensorimotor synapse suggested that the inactivation kinetics of presynaptic calcium channels cannot account for the kinetics of depression; neither does simple depletion of releasable vesicles (Gingrich and Byrne, 1985). Rather, the model suggested the existence of dynamic interactions between use-dependent depletion of readily releasable vesicles and calcium-dependent mobilization of stored vesicles to supply the releasable ones. This model of synaptic depression was supported by morphological studies of the sensorimotor synapse, which indicated that the fraction of readily releasable vesicles decreased with activity, parallel to synaptic depression (Bailey and Chen, 1988b).

A subsequent study of spontaneous release from cultured sensory neurons revealed that synaptic depression was accompanied by a decrease in the frequency of mEPSPs (Eliot et al., 1994). However, the change in mini EPSP frequency did not parallel the synaptic depression in magnitude or in duration. This finding argued against the depletion of presynaptic terminals with releasable vesicles as the sole mechanism of depression, and suggested that depression may be due to a change in excitation–secretion coupling as well. However, this decrease in excitation–secretion coupling does not appear to be due to the decrease in calcium influx that had been previously suggested (Klein et al., 1980): Calcium imaging of cultured sensory neurons revealed that the calcium transients are unaffected by repetitive activity (Armitage and Siegelbaum, 1998).

Based on theoretical and statistical analyses of transmission at the sensorimotor synapse, another model of synaptic depression put forth the activity-dependent inactivation of individual release sites, proposing the transient switching off of presynaptic release machinery following an action potential (Royer et al., 2000). A similar model of synaptic depression arising from inactivation of release sites was suggested by Gover et al. (2002). Finally, the comparison of transmitter release from cultured sensory neurons stimulated by hypertonic solutions versus electrical activity revealed that both types of stimuli draw transmitter from the same presynaptic pool, and suggested that depression is mediated by both depletion of releasable transmitter and a change in excitation–secretion coupling (Zhao and Klein, 2002). Thus, the most recently proposed model of depression of sensorimotor synapses relies again on activity-dependent depletion of releasable vesicles,

acknowledging though that there must be at least one other process that contributes as well.

Despite their differences, the studies outlined in this section have two common elements. First, they all supported the presynaptic nature of depression. Second, they all employed repetitive stimulation of the synapse at intervals at least as long as 1 s. However, when the sensorimotor synapse is stimulated at 10 Hz (100-ms interval), but not at 1 Hz, depression of evoked excitatory postsynaptic potentials (EPSPs) partly results from desensitization of the postsynaptic receptors (Antzoulatos et al., 2003). Therefore, both the kinetics of synaptic depression (Byrne, 1982; Eliot et al., 1994) and the mechanisms underlying it depend on the stimulation regime.

Another form of short-term depression of *Aplysia* sensorimotor synapses can be elicited by brief exposure to the neuropeptide Phe-Met-Arg-Phe-NH_2 (FMRFa). Because activation of a third type of synapse, a modulatory one, and release of a neuromodulator are required, this form of plasticity is termed heterosynaptic. In contrast, depression arising exclusively from intrinsic activity is termed homosynaptic depression. FMRFa-immunoreactive inhibitory interneurons have been identified that innervate tail and siphon sensory neurons (Mackey et al., 1987; Small et al., 1992; Xu et al., 1994). These interneurons are activated by shock to nerves that innervate the tail, and stimulation of these neurons inhibits sensorimotor synapses. However, the extent to which activation of these FMRFa-immunoreactive neurons contributes to habituation has not been determined.

Applying FMRFa to sensory neurons leads to a hyperpolarization of the membrane potential, a decrease in the duration of the action potential, and inhibition of synaptic transmission via the modulation of potassium conductances (Abrams et al., 1984; Ocorr and Byrne, 1985; Belardetti et al., 1987; Critz et al., 1991; Pieroni and Byrne, 1992). Moreover, FMRFa directly affects presynaptic Ca^{2+} currents (Blumenfeld et al., 1990; Edmonds et al., 1990) and the release machinery itself, as indicated by a decrease in the frequency of mEPSPs (Dale and Kandel, 1990). The second messenger mediating the actions of FMRFa seems to be arachidonic acid (AA) produced by phospholipid metabolism (Piomelli et al., 1987) and its downstream metabolite 12-hydroperoxyeicosatetraenoic acid (12-HPETE) (Buttner et al., 1989). Recently, FMRFa was found to inhibit one member of the MAP kinase family, extracellular signal-regulated protein kinase (ERK), but activate another, p38 mitogen-activated protein kinase (p38 MAPK) (Guan et al., 2003; Fioravante et al., 2006). The latter probably activates a phospholipase A2 molecule, which in turn can release AA from phospholipids (Piomelli, 1991). FMRFa also engages protein phosphatases in regulating the outward potassium currents (Ichinose and Byrne, 1991), in particular protein phosphatase 1 (PP1). In other systems, p38 MAPK can activate PP1 (Westermarck et al., 2001), raising the interesting possibility that FMRFa exercises its actions on sensory neuron conductances through a p38 MAPK-PP1 pathway.

4.03.2.2.2 Long-term depression of Aplysia sensorimotor synapses

Repetitive stimulation of *Aplysia* withdrawal reflexes can lead to both short- and long-term habituation (Pinsker et al., 1970; Carew and Kandel, 1973; Stopfer et al., 1996). Short- and long-term habituation share aspects of a common mechanism, synaptic depression. However, whereas short-term synaptic depression arises primarily from transient changes in release, long-term depression has been attributed to persistent structural changes in sensory neurons (Bailey and Chen, 1988a). Extensive morphological analyses of sensory neurons from habituated animals have revealed that the number of synaptic contacts is reduced compared to controls. Moreover, the structure of presynaptic terminals is affected, with fewer synaptic vesicles and reduced size of active zones (the sites of transmitter release).

In addition, activation of sensory neurons at 2 Hz for 15 min induces prolonged (at least 80 min) homosynaptic depression (long-term depression; LTD) of isolated *Aplysia* sensorimotor synapses in cell culture (Lin and Glanzman, 1996). This form of depression relies on activation of postsynaptic *N*-methyl-D-aspartate (NMDA)-like receptors and is sensitive to postsynaptic Ca^{2+}, because infusion of the calcium chelator BAPTA into the postsynaptic motor neuron blocks induction of LTD, but not short-term synaptic depression. Similarly, prolonged habituation of the siphon-elicited gill withdrawal reflex in reduced preparations was recently shown to depend on activity of postsynaptic glutamate receptors both of the NMDA and non-NMDA type (Ezzeddine and Glanzman, 2003).

A more extensively studied form of long-term synaptic depression in *Aplysia* is elicited by repeated application of the neuropeptide FMRFa (Montarolo

et al., 1988; Guan et al., 2003). FMRFa-induced LTD requires transcription, translation (Montarolo et al., 1988; Bailey et al., 1992), but also gene silencing (Guan et al., 2002). Inducing events in FMRFa-mediated LTD include activation of p38 MAPK and recruitment of the transcription repressor CREB2 (cAMP response element binding protein) to the promoter region of genes such as *c/ebp* (Guan et al., 2003; Fioravante et al., 2006).

Little is known about the mechanisms underlying consolidation of LTD. Two genes that are regulated by FMRFa and could be important in the consolidation of LTD are sensorin (Sun et al., 2001) and *Aplysia* cell adhesion molecule (Schacher et al., 2000), even though the requirement of their regulation for LTD has not been demonstrated. Finally, expression of heterosynaptic LTD is accompanied by morphological changes, including loss of presynaptic varicosities and retraction of neurites (Schacher and Montarolo, 1991).

4.03.2.3 Sensitization

Sensitization refers to the augmentation of the behavioral response elicited by a test stimulus. Sensitization to a test stimulus can be induced in one of two ways. First, it can be induced by presentation of another, usually strong stimulus. An example of such sensitization is pseudoconditioning, where an increase in responsiveness to the CS in a classical conditioning procedure may not be due to associative learning (as in classical conditioning), but instead due to the sensitization induced by the strong US. Second, sensitization can be induced by the mere repetition of the test stimulus. As mentioned above, the repetition of a weak stimulus will lead to habituation of the behavioral response, whereas repetition of a moderate to strong stimulus may lead to sensitization. This form of sensitization can sometimes appear as a transient rise in response magnitude before habituation eventually ensues.

Both forms of sensitization have been studied in *Aplysia*, with major emphasis on the former one described above. Similar to habituation, sensitization was also attributed early on to plasticity of the sensorimotor synapse (see next section). Although habituation was attributed to homosynaptic depression of the synapse, sensitization was attributed to heterosynaptic facilitation, induced by the diffuse release of neuromodulators, such as serotonin. Also similar to habituation, sensitization can appear both in short- and long-term forms, which have been extensively studied in their neuronal analogs, short and long-term synaptic facilitation.

4.03.2.3.1 *Short-term sensitization*

In *Aplysia*, sensitization of withdrawal reflexes can be induced by electric shocks to the tail or the lateral body wall of the animal (Carew et al., 1971). Peripheral electric shock has been shown to modulate transmission at the sensorimotor synapse through heterosynaptic facilitation (Carew et al., 1971; Walters et al., 1983a,b).

Several lines of evidence suggest that serotonin (5-HT) is the neurotransmitter involved in heterosynaptic facilitation. First, 5-HT is present in *Aplysia* hemolymph, and its concentration increases in sensitized animals (Levenson et al., 1999). Recent studies also indicated that 5-HT concentration increases in several regions of the *Aplysia* CNS in response to nerve stimulation (Marinesco and Carew, 2002). Second, serotonergic cells are present (Hawkins, 1989; Nolen and Carew, 1994) and serotonergic fibers are in close proximity to sensory neurons (SNs) (Zhang et al., 1991; Marinesco and Carew, 2002; Zhang et al., 2003). Third, depletion of endogenous 5-HT by addition of a neurotoxin (5,7-DHT) blocks the ability of tail stimuli to sensitize the gill-withdrawal reflex (Glanzman et al., 1989). Along the same lines, application of the 5-HT receptor antagonist cyproheptadine blocks facilitation induced by nerve stimulation (Mercer et al., 1991). Finally, exogenously applied 5-HT mimics the actions of tail stimulation both in facilitating the strength of synaptic connections and in increasing the strength of reflex responses (Brunelli et al., 1976; Walters et al., 1983a, b; Abrams et al., 1984; Zhang et al., 1997), and nerve shock-induced 5-HT release correlates with synaptic plasticity (Marinesco et al., 2006).

The conclusions drawn from studies conducted in the 1970s and 1980s led to the formulation of a model for short-term sensitization, according to which sensitizing stimuli activate serotonergic facilitatory interneurons, releasing 5-HT and activating a serial cascade of events in the sensory neurons. The binding of 5-HT to one class of receptors on the outer surface of the membrane of the sensory neurons leads to the activation of adenylyl cyclase, which in turn, leads to an elevation of the intracellular level of the second messenger adenosine-3′,5′-monophosphate (cyclic AMP, cAMP) in sensory neurons. When cAMP binds to the regulatory subunit of cAMP-dependent protein kinase (protein kinase A, PKA), the catalytic subunit is freed and can now add phosphate groups to specific substrate proteins and, hence, alter their functional

properties (Bernier et al., 1982; Ocorr and Byrne, 1985; Pollock et al., 1985; Ocorr and Byrne, 1986; Ocorr et al., 1986; Sweatt et al., 1989). One effect of activated PKA is phosphorylation of a class of membrane channels (S-K^+ channels, named for their ability to be modulated by serotonin) and reduction of the S-K^+ conductance ($G_{K,S}$) (Klein and Kandel, 1980; Klein et al., 1982; Siegelbaum et al., 1982). Consequently, a test stimulus triggers a greater number of action potentials in the sensory neuron after sensitization. Each of these spikes is broader, leading to increased Ca^{2+} influx and enhanced transmitter release. As a result, the follower motor neuron is more intensely activated, and the behavioral response is enhanced (i.e., sensitized). Thus, it was previously believed that serotonin (5-HT) exerted all of its actions in the sensory neurons via the cAMP-mediated reduction of $G_{K,S}$.

Later studies, however, indicated that the effects of 5-HT are more complex than originally suggested. Not only $G_{K,S}$ is modulated by 5-HT, but also at least three other conductances: 5-HT increases a dihydropyridine-sensitive Ca^{2+} current ($G_{Ca,Nif}$) (Braha et al., 1990; Edmonds et al., 1990), decreases a component of the Ca^{2+}-activated K^+ current ($G_{K,Ca}$) (Walsh and Byrne, 1989), and modulates a voltage-dependent K^+ current ($G_{K,V}$) (Baxter and Byrne, 1989, 1990a; Goldsmith and Abrams, 1992; Hochner and Kandel, 1992; Sugita et al., 1994; White et al., 1994). The effects of channel modulation appear to be synergistic, favoring increased sensory neuron excitability or transmitter release. Spike broadening, which has a major impact on transmitter release, is probably due to modulation of $G_{K,V}$ rather than $G_{K,S}$, whereas $G_{K,S}$ and $G_{K,Ca}$ appear to be critical for regulating membrane excitability, with modest effects on spike duration (Baxter and Byrne, 1990a, b). The 5-HT-induced increase in $G_{Ca,Nif}$ does not appear to contribute to enhanced transmitter release, as this conductance is not directly responsible for triggering exocytosis of synaptic vesicles, although it may contribute to accumulation of presynaptic calcium during intense activity (Edmonds et al., 1990).

Two of the three 5-HT-induced effects on K^+ conductances (modulation of $G_{K,S}$ and $G_{K,Ca}$) are mediated exclusively by PKA. The effects of 5-HT on $G_{K,V}$ appear to be caused by activation of two second messenger pathways, only one of which is the cAMP pathway mentioned above (Hochner and Kandel, 1992). Serotonin also appears to act through another class of receptors to increase the level of the second messenger diacylglycerol (DAG). DAG activates protein kinase C (PKC), leading to its translocation (Sossin, 2007). PKC, like PKA, is involved in the spike-duration-dependent process of facilitation (Sugita et al., 1992, 1994). In addition, a nifedipine-sensitive Ca^{2+} conductance ($G_{Ca,Nif}$) and the delayed K^+ conductance ($G_{K,V}$) are regulated by PKC. The modulation of $G_{K,V}$ contributes importantly to the increase in duration of the action potential (**Figure 3(a3)**). Because of its small magnitude, the modulation of $G_{Ca,Nif}$ appears to play a minor role in the facilitatory process.

The role of ionic conductances in modulation of synaptic transmission is relatively well understood. Less well understood is a second process that has a profound effect on synaptic transmission, but which is independent of spike duration (spike-duration independent process; SDI). The existence of the second process was first postulated based on a mathematical model of a sensory neuron (Gingrich and Byrne, 1985, 1987). Experimental studies have provided support for the SDI process (Hochner et al., 1986; Braha et al., 1990; Pieroni and Byrne, 1992; Klein, 1993, 1994). Although the mechanism is poorly understood, the SDI process is likely to include mobilization of vesicles into a readily releasable pool. This process appears to be particularly important when the sensory neuron is depressed by previous low frequency (ISI = 10 s) stimulation (Braha et al., 1990; Ghirardi et al., 1992; Pieroni and Byrne, 1992; Klein, 1993; Sugita et al., 1997), making the SDI process an attractive candidate for dishabituation mechanisms and for maintaining synaptic strength during high levels of release (see below). The relative contribution of PKA and PKC to facilitation of previously depressed synapses varies as a function of the extent of preexisting depression. In nondepressed synapses, 5-HT produces short-term facilitation that can be blocked completely by inhibitors of PKA but is not affected by H7, an inhibitor of PKC. In contrast, as synapses become more depressed, the inhibitor of PKC becomes progressively more effective in blocking 5-HT-induced short-term facilitation (Braha et al., 1990; Ghirardi et al., 1992; Sugita et al., 1997).

Nevertheless, it has only recently become clear that, apart from dishabituation, sensitization may also involve facilitation of depressed synapses, because a moderate stimulus does not trigger a single spike in the sensory neuron, but a burst of spikes (Phares et al., 2003). By the end of this burst, the motor neuron responses depress substantially, regardless of the state of the first response. Enhancement of these depressed

responses by sensitization is likely to involve the SDI process.

Progress has been made in understanding the second-messenger cascades involved in the SDI process and identified synaptic terminal proteins as downstream targets. These targets include synapsin and SNAP-25, highly conserved synaptic proteins that appear to regulate homosynaptic depression and short-term heterosynaptic facilitation. Synapsin is localized in presynaptic nerve terminals, where it interacts with synaptic vesicles, actin and spectrin (Jovanovic et al., 1996; Matsubara et al., 1996; Hosaka et al., 1999; Zimmer et al., 2000). Because the interaction of synapsin with actin and synaptic vesicles is regulated by phosphorylation, synapsin is believed to reversibly tether synaptic vesicles in a reserve pool, thereby regulating vesicle availability and mobilization (Hilfiker et al., 1998, 1999). The *Aplysia* isoform of synapsin (apSyn) contains the same domain arrangement as other vertebrate and invertebrate synapsins (Angers et al., 2002). Several potential regulatory sites have been identified throughout the sequence of apSyn. In addition to the PKA/CAMK I consensus phosphorylation site in the A-domain, two potential MAPK sites and several PKC sites are detected. In ganglia and in cultured cells, synapsin localizes in presynaptic varicosities and forms distinct puncta, presumably due to the aggregation of protein and its interaction with vesicle membranes (Angers et al., 2002).

ApSyn is phosphorylated following application of 5-HT, which results in short-term facilitation of the sensorimotor synapse. This phosphorylation requires PKA and MAPK. Also, 5-HT results in a reduction in the number of apSyn puncta, which represents the dissociation of the protein from synaptic vesicles (and probably the cytoskeleton) upon phosphorylation. The reduction of apSyn puncta after 5-HT is dynamic and reversible, and it requires PKA and MAPK activity (Angers et al., 2002). Finally, recent results from apSyn overexpression experiments indicated that synapsin regulates basal synaptic strength, homosynaptic depression and 5-HT-induced recovery from depression (Fioravante et al., 2007).

Based on the results described above, the following model has been proposed (Angers et al., 2002): At rest, most vesicles are clustered in a filamentous protein network forming the reserve pool, and 5-HT can modulate the function of apSyn by altering its phosphorylation state via PKA and MAPK. Upon phosphorylation, apSyn molecules dissociate from the vesicles and the cytoskeleton, allowing vesicles to be mobilized to release sites when they become depleted.

Another highly conserved synaptic protein, SNAP-25, has recently been implicated in the regulation of short-term facilitation, especially in previously depressed synapses. In these synapses, PKC, rather than PKA, predominantly mediates 5-HT-induced dedepression (Ghirardi et al., 1992; Dumitriu et al., 2006) through phosphorylation of SNAP-25 (Houeland et al., 2007) and probably other, yet unidentified synaptic targets.

4.03.2.3.2 Long-term sensitization

Whereas short-term sensitization can be induced by a single brief stimulus, the induction of long-term sensitization, whose memory can persist for days to weeks, requires a more extensive training regime (e.g., repeating the sensitizing stimuli over a 1.5-h period). Compared with short-term sensitization, less is known about the cellular mechanisms underlying long-term sensitization. One simplifying hypothesis is that the mechanisms underlying the expression of long-term sensitization are the same as those of short-term sensitization, but extended in time. Some evidence supports this hypothesis. For example, similar to short-term sensitization, K^+ conductances and excitability of sensory neurons are also modified by long-term sensitization (Scholz and Byrne, 1987; Cleary et al., 1998).

Biophysical properties of neurons mediating the *Aplysia* withdrawal reflexes have been examined following long-term sensitization induced by a single 1.5-h-long training session (1-day protocol), or by four such sessions repeated at 24-h intervals (4-day protocol). Twenty-four hours following the 1-day protocol of long-term sensitization training, three biophysical properties of tail sensory neurons are altered: Neuronal excitability, the after-depolarization following long current pulses, and the after-depolarization following short current pulses (Cleary et al., 1998). In addition to the biophysical properties of sensory neurons, 1-day training affects two properties of motor neurons: The resting membrane potential is increased and the spike threshold is decreased (Cleary et al., 1998). Long-term sensitization also correlates with facilitation of the sensorimotor synapses, both after 1-day training and after 4-day training (Frost et al., 1985; Cleary et al., 1998; Wainwright et al., 2004; Antzoulatos and Byrne, 2007). Surprisingly, although short-term sensitization is associated with spike broadening in sensory neurons (see above), long-term sensitization is associated with spike narrowing in sensory neurons (Antzoulatos and Byrne, 2007). The functional effects of the spike narrowing are not clear,

but narrowing of the spike may be related to a decrease in spike propagation failures that occurs in response to high-frequency peripheral stimulation after long-term sensitization.

Another branch of the research on long-term sensitization has focused on the morphological effects of sensitization training on sensory neurons. One-day training for long-term sensitization does not induce gross structural changes in sensory neuron morphology, even though it does induce long-term changes in excitability and synaptic strength (Cleary et al., 1998). In contrast to 1-day training, 4-day training is more effective at inducing outgrowth when compared to untrained controls (Bailey and Chen, 1983; Wainwright et al., 2002;, 2004). This outgrowth includes an increase in the total arborization length of sensory neuron branches, in the number of sensory neuron branch points and varicosities, and in the number of synaptic contacts between sensory and motor neurons (Wainwright et al., 2002, 2004).

Biochemical correlates of long-term sensitization have also been examined. Most of these studies have focused on the induction phase, identifying changes in levels of the second messenger cAMP and regulation of several proteins (Barzilai et al., 1989; Eskin et al., 1989; Muller and Carew, 1998; Zwartjes et al., 1998). Progress has been made in identifying biochemical changes related to the consolidation or expression of long-term sensitization. Recently, long-term training was observed to produce enhanced uptake of glutamate (Levenson et al., 2000), the putative transmitter of sensory neurons (Antzoulatos and Byrne, 2004). This increase in uptake occurred in sensory neurons and appeared to be caused by an increased number of glutamate transporters. Although the functional role of this enhanced uptake is presently unclear, it indicates that clearance of glutamate from the cleft may be an important factor in the regulation of synaptic efficacy (Chin et al., 2002b). A change in glutamate uptake could potentially exert a significant effect on synaptic efficacy by regulating the amount of transmitter available for release, the rate of clearance from the cleft, and thereby the duration of the EPSP and the degree of receptor desensitization (Antzoulatos et al., 2003).

A substantial amount of data indicates that the induction of both short- and long-term sensitization partly share common cellular pathways (**Figure 4**). For example, both forms of sensitization activate the cAMP/PKA cascade. In the long-term form, however, activation is prolonged and sufficient to induce gene transcription and new protein synthesis (Castellucci et al., 1989; Levenson et al., 1999). This finding is consistent with the relatively long duration of the training period required for inducing long-term sensitization and the lasting duration of the effects. cAMP presumably exerts its major effects by activation of PKA (Schacher et al., 1988; Scholz and Byrne, 1988; O'Leary et al., 1995; Muller and Carew, 1998). Activated PKA translocates to the nucleus,

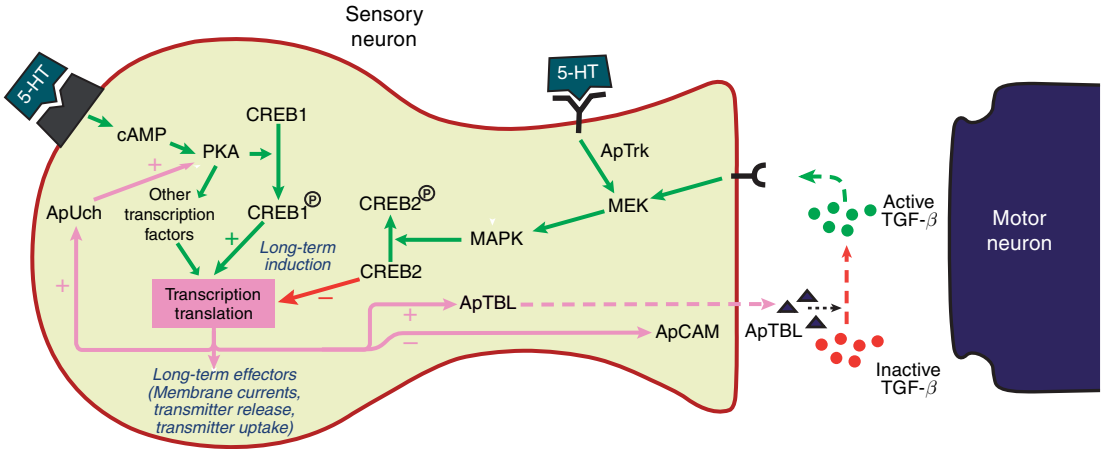

Figure 4 Simplified scheme of the mechanisms in sensory neurons that contribute to long-term sensitization. Sensitization training leads to release of 5-HT, which activates the cAMP/PKA cascade and the ERK MAPK cascade. PKA phosphorylates and activates CREB1, whereas ERK phosphorylates and inhibits CREB2. CREB1 acts as an initiator of gene transcription and CREB2 acts as a repressor of gene transcription. The combined effects of activation of CREB1 and inhibition of CREB2 lead to regulation of the synthesis of at least ten proteins, only three of which (apTBL, apUCH, apCAM) are shown. ApTBL is believed to activate latent forms of TGF-β, which can then bind to receptors on the sensory neuron and further activate MAPK. See section 4.03.2.3.2 for abbreviation definitions.

where it phosphorylates and activates the transcription factor CREB1. Activated CREB1, which is necessary for long-term facilitation (LTF), binds to the promoter region of responsive genes, and induces their expression (Dash et al., 1990, 1991; Bartsch et al., 1998; Guan et al., 2002). A prolonged increase in cAMP also leads to the induction and subsequent expression of a gene encoding the protein ubiquitin C-terminal hydrolase (Ap-Uch). This neuron-specific enzyme enhances the degradation of certain proteins including the regulatory subunits of PKA (Hegde et al., 1997). With fewer regulatory subunits of PKA to bind to catalytic subunits, the catalytic subunits are persistently active and may contribute to long-term facilitation of transmitter release (Muller and Carew, 1998).

In addition to the cAMP/PKA cascade, sensitization training and prolonged 5-HT application also activate MAPK (Sacktor and Schwartz, 1990; Sossin and Schwartz, 1992, 1993; Sossin et al., 1994; Sossin and Schwartz, 1994; Martin et al., 1997a; Sharma et al., 2003; Sharma and Carew, 2004). Prolonged application of 5-HT results in persistent phosphorylation (and subsequent activation) of MAPK though activation of a tyrosine receptor kinase-like molecule (ApTrk) (Ormond et al., 2004), cAMP (Martin et al., 1997b; Michael et al., 1998) (but see Dyer et al., 2003), and/or the neuropeptide sensorin (Hu et al., 2004b). Activated MAPK translocates to the nucleus (Martin et al., 1997b) where it may regulate gene transcription, possibly through inhibition of the transcription factor CREB2 (Bartsch et al., 1995). Since under basal conditions CREB2 acts as a repressor of gene transcription, its inhibition may lead to derepression and net gene expression in concert with CREB1 (**Figure 4**). The involvement of additional transcription factors such as ApAF (*Aplysia* activating factor) (Bartsch et al., 2000; Lee et al., 2006) and ApLLP (*Aplysia* LAPS18-like protein) (Kim et al., 2003a, 2006) in learning-induced gene expression is currently being investigated.

Recently, it has become clear that the role of transcription factors in long-term memory formation is not limited to the induction phase but may also extend to the consolidation phase. For example, prolonged treatment with 5-HT leads to the binding of CREB1 to the promoter of its own gene and induces CREB1 synthesis (Mohamed et al., 2005). The newly synthesized CREB1 appears to be necessary for LTF (Liu et al., 2008). This observation agrees well with earlier findings that the requirement for gene expression is not limited to the induction phase. The necessity of prolonged transcription and translation for LTF observed at 24 h persists for at least 7–9 h after induction (Alberini et al., 1994; O'Leary et al., 1995). These results suggest that CREB1 can regulate its own level of expression, giving rise to a CREB1 positive feedback loop that is necessary for memory consolidation.

In addition to CREB1, several other proteins are regulated during LTF. One of the newly synthesized proteins, intermediate filament protein (IFP) (Noel et al., 1993), is thought to contribute to the new growth observed after prolonged treatment with 5-HT. Increased synthesis of calmodulin (CaM) (Zwartjes et al., 1998) also occurs, but the functional significance of this effect has not been determined. The neuropeptide sensorin is also upregulated by 5-HT and is thought to contribute to the formation and stabilization of new synapses (Hu et al., 2004a,b).

Aplysia tolloid/BMP-like protein (apTBL-1) (Liu et al., 1997) is also synthesized in response to increases in cAMP. Tolloid and the related molecule BMP-1 appear to function as secreted Zn^{2+} proteases. A signal sequence at the amino terminal indicates that apTBL-1 is secreted to the extracellular space where one of its actions may be to activate members of the TGF-β family of growth factors (**Figure 4**). Indeed, in sensory neurons, TGF-β mimics the effects of 5-HT in that it produces long-term increases in synaptic strength and excitability of the sensory neurons (Zhang et al., 1997; Farr et al., 1999). Interestingly, TGF-β activates the MEK/MAPK pathway in the sensory neurons and induces MAPK translocation to the nucleus (Chin et al., 2002a, 2006), where it phosphorylates CREB1 (Chin et al., 2006). This activation could yield another round of protein synthesis to further consolidate long-term sensitization.

LTF involves not only increased synthesis but also downregulation of proteins such as the regulatory subunit of PKA (discussed earlier in this section) and a homolog of neuronal cell adhesion molecule (NCAM). Downregulation of NCAM alters the interaction of the neuron with other cells and allows the restructuring of the axon arbor (Mayford et al., 1992; Bailey et al., 1997). The sensory neuron could then form additional connections with the same postsynaptic target or make new connections with other cells.

4.03.2.3.3 Other temporal domains for the memory of sensitization

Operationally, memory has frequently been divided into two temporal domains, short-term and long-term. It has become increasingly clear from studies of a number of memory systems that this distinction

is overly restrictive. For example, in *Aplysia*, Carew and his colleagues (Sutton et al., 2001) and Kandel and his colleagues (Ghirardi et al., 1995) discovered an intermediate phase of memory that has distinctive temporal characteristics and a unique molecular signature. The intermediate-phase memory (ITM) for sensitization is expressed approximately 30 min to 3 h after the beginning of training. It declines completely prior to the onset of long-term memory. Like long-term sensitization, its induction requires protein synthesis, but unlike long-term memory, it does not require mRNA synthesis. The expression of the intermediate-phase memory requires the persistent activation of PKA (Muller and Carew, 1998; Sutton and Carew, 2000; Sutton et al., 2001).

An intermediate-term facilitation (ITF) of the sensorimotor synapse, which is produced by application of five pulses of 5-HT (an analog of sensitization training), corresponds to the ITM as it displays similar temporal dynamics and requires protein synthesis but not RNA synthesis (Ghirardi et al., 1995; Sutton and Carew, 2000). This latter feature of ITF distinguishes it from short-term facilitation, which requires neither protein nor RNA synthesis, and long-term facilitation, which requires both (see above). Depending on the induction protocol, ITF may require intermediate-term activation of PKA or PKC (Sossin et al., 1994; Sutton and Carew, 2000; Pepio et al., 2002; Lim and Sossin, 2006). These kinases can be activated for hours following prolonged treatment with 5-HT (Muller and Carew, 1998; Sutton and Carew, 2000). Finally, activation of previously silent release sites has also been implicated in ITF and could be a mechanism for memory consolidation (Kim et al., 2003b).

In addition to the intermediate-phase memory, it is likely that *Aplysia* has different phases of long-term memory. For example, at 24 h after sensitization training there is increased synthesis of a number of proteins, some of which are different from those whose synthesis is increased during and immediately after training (Noel et al., 1993). These results suggest that the memory for sensitization that persists for more than 24 h may be dependent on the synthesis of proteins occurring at 24 h and may have a different molecular signature than the 24-h memory.

Based on the experimental results reviewed above, a synthesis of the sensitization mechanisms in *Aplysia* can now be attempted. A brief sensitizing experience can affect the animal transiently (i.e., short-term sensitization), through an increase in the excitability of sensory neurons and in the efficacy of sensory neuron synapses. Short-term facilitation is achieved through spike-broadening-mediated and spike-duration-independent increases in transmitter release. These modifications, lasting up to several minutes, are mediated by phosphorylation of K^+ channels and other effector molecules, such as synapsin. More prolonged training, which typically involves multiple, appropriately timed stages of sensitization, can lead to modifications lasting 24 h or more. A single day of sensitization training leads to persistent increases in the efficacy of sensory neuron synapses and in the excitability of sensory neurons. Long-term facilitation after a single day of training does not involve gross structural changes of sensory neurons or changes in the number of synaptic varicosities. With 4 days of training, long-term sensitization is still accompanied by synaptic facilitation, but changes in sensory neuron excitability are not as prominent as they are after short-term training or after a single session of long-term training. After 4 days of training, long-term synaptic facilitation is achieved through an increase in the number of synaptic contacts. Collectively, these results indicate that facilitation of sensory neuron synapses is a ubiquitous feature of sensitization. However, the mechanisms that support the facilitation vary over time and with the extent of training. The conversion of one type of long-term expression mechanism to another is interesting, as it presumably reflects the engagement of a distinct set of genes that are part of an overall program for the expression of particularly enduring forms of long-term memory.

4.03.3 Habituation and Sensitization in Other Invertebrates

4.03.3.1 Gastropod Molluscs

4.03.3.1.1 Tritonia

To escape a noxious stimulus, the opisthobranch *Tritonia diomedea* initiates stereotypical oscillatory swimming. This escape swim can be dissected into several components, including number of cycles per swim, latency to swim onset, and swim cycle period. The various swim components can exhibit habituation, dishabituation, and/or sensitization (Frost et al., 1996). In particular, the escape swim undergoes habituation and dishabituation of the number of cycles per swim (Mongeluzi and Frost, 2000). Swimming probability can also decrease as a result of habituation (Brown,

1998). This habituation is accompanied by sensitization of the latency to swim onset (Frost et al., 1998).

The neural circuit underlying swim consists of sensory neurons, precentral pattern generating (CPG) neurons, and motor neurons. This circuit can be studied in the isolated perfused brain of *Tritonia*, where electrical stimulation of a nerve can elicit fictive swimming patterns (Dorsett et al., 1973). Habituation of fictive swimming correlates with a decrease in the cycle number and cycle period of swim motor programs (Frost et al., 1996; Brown, 1997) and appears to involve plasticity at multiple loci, including decrement at the first afferent synapse. Sensitization appears to involve enhanced excitability and synaptic strength in one of the CPG interneurons. Modulation of interneurons can be mediated by 5-HT, which has diverse effects on multiple loci of the circuit (Sakurai et al., 2006).

4.03.3.1.2 *Land snail* (Helix)

Land snails withdraw in response to weak tactile stimulation. The withdrawal behavior is mediated by a neuronal circuit involving four groups of nerve cells: Sensory neurons, motor neurons, modulatory neurons, and command neurons (Balaban, 2002). This withdrawal can be habituated or sensitized, depending on the intensity of stimulation. Habituation of the withdrawal behavior emerges from depletion of neurotransmitter at sensory cell synapses as well as heterosynaptic inhibition mediated by FMRFa-containing neurons (Balaban et al., 1991). Sensitization appears to be mediated by serotonergic modulatory cells whose spiking frequency increases following noxious stimulation (Balaban, 2002). These serotonergic cells are electrically coupled so that they get recruited and fire synchronously in response to strong excitatory input. One gene that is upregulated by external noxious input is the *Helix* Command Specific #2 (HCS2) (Balaban et al., 2001). The *HCS2* gene encodes a precursor protein whose processed products may function as neuromodulators or neurotransmitters mediating the withdrawal reactions of the snail (Korshunova et al., 2006). Application of neurotransmitters and second messengers known to be involved in withdrawal behavior result in upregulation of *HCS2* gene (Balaban, 2002).

The mechanisms underlying habituation and sensitization in the *Helix* can be further investigated by reconstructing behaviorally relevant synapses in culture. Using this approach, mechanosensory neuron-withdrawal interneuron synapses were found to display several forms of short-term synaptic plasticity such as facilitation, augmentation, and posttetanic potentiation (Fiumara et al., 2005).

4.03.3.2 Arthropods

4.03.3.2.1 *Crayfish* (Procambarus clarkii)

A crayfish escapes from noxious stimuli by flipping its tail. A key component of the tail-flip circuit is a pair of large neurons called the lateral giants (LGs), which run the length of the animal's nerve cord. The LGs are the decision and command cells for the tail-flip. The crayfish tail-flip response exhibits habituation (Wine et al., 1975) and sensitization (Krasne and Glanzman, 1986). Plastic changes induced during learning involve modulation of the strength of synaptic input driving the LGs (Edwards et al., 1999). A diminution of transmitter release with repeated activation of afferents is thought to underlie habituation (Krasne and Roberts, 1967; Zucker, 1972). An inhibitory pathway was also identified that can tonically inhibit the LGs (Krasne and Wine, 1975; Vu and Krasne, 1992, 1993; Vu et al., 1993). This putatively GABAergic (Vu and Krasne, 1993) (but see Heitler et al., 2001) inhibitory pathway also plays a major role in habituation (Krasne and Teshiba, 1995). In addition to the regulation of synaptic strength, habituation also results in decreased excitability of LGs (Araki and Nagayama, 2005). Bath application of the endogenous neuromodulators 5-HT and octopamine decrease the rate of LG habituation to repetitive sensory stimulation (Araki et al., 2005). Octopamine is also thought to at least partly mediate sensitization, because it mimics the sensitizing effects of strong stimulation on the tail-flip (Glanzman and Krasne, 1986; Krasne and Glanzman, 1986).

4.03.3.2.2 *Honeybee* (Apis mellifera)

Honeybees, like other insects, are superb at learning. For example, classical conditioning of feeding behavior can be produced by pairing a visual or olfactory stimulus with sugar solution to the antennae. Numerous studies described the molecular mechanisms underlying memory formation, which involve upregulation of the cAMP pathway and activation of PKA resulting in CREB-mediated transcription of downstream genes (Menzel, 2001) (*See* Chapter 4.06). Nonassociative learning has also been studied in the honeybee, albeit to a lesser extent. Habituation of the proboscis extension reflex can be elicited by repeatedly touching one antenna with a droplet of sugar water (Braun and Bicker, 1992) and lasts for at least 10 min (Bicker and Hahnlein, 1994). Following habituation, the proboscis

extension response can be restored spontaneously with time (spontaneous recovery) (Bicker and Hahnlein, 1994) or by stimulating the contralateral antenna (dishabituation) (Braun and Bicker, 1992). Application of tyramine, a metabolic precursor of the endogenous neuromodulator octopamine, accelerates the rate of habituation of the reflex (Braun and Bicker, 1992). Recently, activation of PKA was implicated in habituation of the reflex, but not dishabituation or spontaneous recovery, suggesting that the cellular mechanisms mediating habituation, dishabituation, and spontaneous recovery are distinct (Muller and Hildebrandt, 2002). With repeated training sessions over 2 days, long-term memory for habituation lasting for 24 h can be demonstrated (Bicker and Hahnlein, 1994). Finally, sensitization of the antenna reflex can be produced as a result of presenting gustatory stimuli to the antennae (Mauelshagen, 1993; Menzel et al., 1999).

4.03.3.2.3 Drosophila melanogaster

Because the neural circuitry in the fruit fly is both complex and inaccessible, the fly might seem to be an unpromising subject for studying the neural basis of learning. However, the ease with which genetic studies are performed compensates for the difficulty in performing electrophysiological studies (DeZazzo and Tully, 1995). A multitude of behaviors have been used as a model system to study nonassociative learning in *Drosophila*, including proboscis extension (Duerr and Quinn, 1982), thoracic bristle-elicited cleaning reflex (Corfas and Dudai, 1989), landing response (Rees and Spatz, 1989; Asztalos et al., 1993), jump-and-flight escape response (Engel and Wu, 1996), and odor-elicited startle response (Cho et al., 2004). Habituation has been demonstrated in all of these behaviors and molecular pathways underlying this form of learning have been identified and include the cAMP/PKA pathway (dunce and rutabaga mutants) (Duerr and Quinn, 1982; Corfas and Dudai, 1989; Engel and Wu, 1996; Cho et al., 2004), protein phosphatase 1 (Asztalos et al., 1993), and cGMP-dependent protein kinase (PKG) (Engel et al., 2000). In flies carrying the rutabaga mutation, which leads to diminished cAMP synthesis, habituation of the cleaning reflex is abnormally short-lived but dishabituation is unaffected (Corfas and Dudai, 1989). Moreover, in flies carrying the dunce mutation, which results in elevated cAMP levels, habituation rates of the jump-and-flight reflex are moderately increased but spontaneous recovery and dishabituation are not affected (Engel and Wu, 1996). These results reinforce the idea that the processes underlying habituation, dishabituation, and spontaneous recovery are distinct (also see the section titled 'Honeybee (*Apis mellifera*)'). Finally, in the dunce and rutabaga flies, sensitization of the proboscis-extension reflex dissipates more rapidly compared to wild-type controls (Duerr and Quinn, 1982).

4.03.3.3 Annelids

4.03.3.3.1 Leech

In the leech *Hirudo medicinalis*, nonassociative learning has been studied in several well characterized behaviors: Movements in response to light and water currents (Ratner, 1972), bending (Lockery and Kristan, 1991), shortening reflex to repeated light (Lockery et al., 1985) or tactile stimulation (Belardetti et al., 1982; Boulis and Sahley, 1988; Sahley et al., 1994), and swimming (Catarsi et al., 1993; Zaccardi et al., 2001).

In the shortening reflex of the leech, the neuronal changes underlying habituation and sensitization occur in the pathway from mechanosensory neurons to electrically coupled neurons, the S cells (Bagnoli and Magni, 1975; Sahley et al., 1994). Habituation of this reflex can reach asymptotic levels after 20 training trials and correlates with decreased S-cell excitability (Burrell et al., 2001). The reflex can be restored following application of a single noxious stimulus (dishabituation) (Boulis and Sahley, 1988). The potentiation of the shortening reflex observed during sensitization requires the S neurons, as their ablation disrupts sensitization (Sahley et al., 1994). This potentiation is mediated by 5-HT through an increase of cAMP (Belardetti et al., 1982), which also increases S-cell excitability (Burrell et al., 2001). Depletion of 5-HT disrupts sensitization (Sahley et al., 1994). Interestingly, ablation of the S cells only partly disrupts dishabituation, indicating that separate processes contribute to dishabituation and sensitization (Ehrlich et al., 1992; Sahley et al., 1994).

An additional mechanism that could potentially contribute to habituation of the shortening reflex involves depression of the synapses of touch (T) sensory neurons onto their follower target neurons. This synaptic depression has been associated with an increase in the amplitude of the T-cell after-hyperpolarizing potential (AHP) that follows their discharge (Brunelli et al., 1997; Scuri et al., 2002). The lasting increase in AHP amplitude, following low-frequency stimulation of T cells, has been attributed, in turn, to increased activity of the electrogenic Na^+ pump, and requires activation of phospholipase A2 and the downstream arachidonic acid metabolites (Scuri et al., 2005).

4.03.3.4 Nematoda

4.03.3.4.1 Caenorhabditis elegans

C. elegans is a valuable model system for cellular and molecular studies of learning. Its principal advantages are threefold. First, its nervous system is extremely simple. It has a total of 302 neurons, the anatomical connectivity of which has been described at the electron microscopy level. Second, the developmental lineage of each neuron is completely specified. Third, its entire genome has been sequenced, making it highly amenable to a number of genetic and molecular manipulations. *C. elegans* responds to a vibratory stimulus applied to the medium in which they locomote by swimming backwards. This reaction, known as the tap withdrawal reflex, exhibits habituation, dishabituation, sensitization, and long-term (24-h) retention of habituation training (Rankin et al., 1990). Laser ablation studies have been used to elucidate the neural circuitry supporting the tap withdrawal reflex and to identify likely sites of plasticity within the network. Plastic changes during habituation appear to occur at the chemical synapses between presynaptic sensory neurons and postsynaptic command interneurons (Wicks and Rankin, 1997). Analysis of several *C. elegans* mutants has revealed that synapses at the locus of plasticity in the network may be glutamatergic (Rose and Rankin, 2001). Mutation of the gene coding for the brain-specific inorganic phosphate transporter *eat-4* results in more rapid habituation compared to wild-type worms and slower recovery (Rankin and Wicks, 2000). The protein coded by *eat-4* is involved in the regulation of glutamatergic transmission and is homologous to the mammalian vesicular glutamate transporter VGLUT1 (Bellocchio et al., 2000). *Eat-4* worms also do not display dishabituation, suggesting that neurotransmitter regulation plays a role in habituation and dishabituation (Rankin and Wicks, 2000). Moreover, worms that carry a mutation in glr-1, an excitatory glutamate receptor expressed in postsynaptic command interneurons, do not display long-term memory for habituation (Rose et al., 2003). In general, the study of behavioral genetics in the worm has provided significant insights into the ways in which genes regulate behavior (Rankin, 2002).

4.03.4 Emerging Principles

As a result of research on several invertebrate model systems, some general principles have emerged. A list of these principles might include the following: (1) short-term and long-term forms of learning and memory require changes in existing neural circuits, (2) these changes may involve multiple cellular mechanisms within single neurons, (3) second messenger systems play a role in mediating cellular changes, (4) changes in the properties of membrane channels are commonly correlated with learning and memory, (5) changes in intrinsic excitability (*See* Chapter 4.40) and synaptic efficacy are correlated with short- and long-term memory, and (6) long-term memory requires new protein synthesis and growth, whereas short-term memory does not.

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4.04 Molecular Mechanisms of Habituation in *C. elegans*

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4.04.1 Introduction to Habituation

The most basic form of learning is nonassociative learning, which involves alterations in response to a single (sometimes repeated) stimulus. Habituation, dishabituation, and sensitization are the three main forms of nonassociative learning. Habituation is the simplest form of nonassociative learning and has been defined as a decrease in response to repeated or long-lasting stimulation (Groves and Thompson, 1970). This form of learning has been found in all organisms studied, from protozoa to humans. If a strong, novel stimulus is presented after the organism has been habituated to the initial stimuli, the organism will immediately recover its habituated response. This phenomenon is known as dishabituation and has been used to distinguish habituation from sensory adaptation or fatigue. Although a great deal is known about the characteristics of habituation, very little is known about the molecular mechanisms that underlie it.

Habituation allows an organism to ignore irrelevant stimuli; thus it is the basis for selective attention. If organisms did not habituate, then they would give equal attention to all stimuli in the environment and could not attend to stimuli important for survival. Further, the behavioral rules that govern habituation described by Groves and Thompson (1970) are followed in all species and systems studied. For these reasons, the mechanisms underlying this simple form of learning are likely to be highly conserved throughout evolution. To accurately study such a simple form of learning, it becomes increasingly important to reduce any other factors that could confound such study. Because of this, organisms that exhibit very simple behaviors and that can easily be studied at the cellular and genetic levels provide good model systems to discover the molecular mechanisms involved in habituation.

4.04.2 *Caenorhabditis elegans* as a Model System

Caenorhabditis elegans is a very powerful and useful model system in which to study molecular mechanisms of simple forms of learning and memory. Rankin et al. (1990) first showed that *C. elegans* are capable of a variety of simple behaviors including habituation, dishabituation, and long-term memory lasting for at least 24 h. This nematode has a small nervous system that is

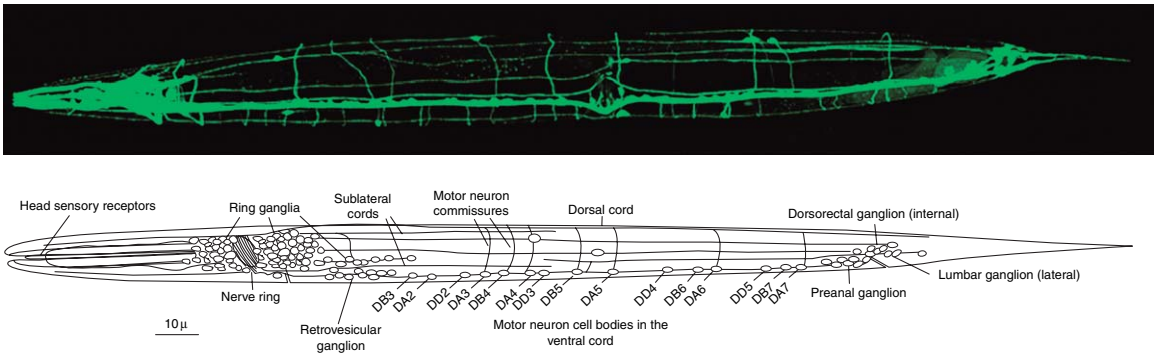

Figure 1 The nervous system of *C. elegans*. Top: *C. elegans* nervous system labeled with a pan-neuronal GFP (photo courtesy of W. Materi and D. Pilgrim). Worm is 1 mm in length. Bottom: Anatomical drawing of the nervous system of *C. elegans*. Drawing courtesy of Richard Durbin.

composed of only 302 neurons, which allows for the unique ability to map behaviors to specific identified neurons and sites of plasticity (**Figure 1**). The connectivity of the nervous system has been resolved at the electron microscope level and has produced a complete wiring diagram indicating 5000 chemical synapses and 3000 electrical synapses (White et al., 1986). Further, the entire cell lineage has been investigated and determined. Combining this knowledge, researchers have been able ablate single identified neurons and investigate the behavioral outcome (Chalfie et al., 1985). Also, since the cuticle of this nematode is transparent, the use of laser ablation of single, identified neurons has made circuit analysis possible, and the use of genetic markers such as green fluorescent protein (GFP) has allowed the analysis of changes in expression patterns of specific gene products *in vivo* (Chalfie et al., 1994). The worm's genome has been mapped and sequenced, which has led to the identification of numerous genes and gene products (Wood, 1988; Riddle et al., 1997). Also, the genetics of *C. elegans* is easy to manipulate. The use of both forward and backward genetic screens and, more recently, RNA interference techniques has led to the identification of a large number of genes that play a role in mediating various behaviors. Another major advantage of this model system is that it is relatively simple to manipulate gene expression spatially or temporally, allowing for the investigation of the roles of specific genes in targeted tissues and/or at specified time points.

4.04.3 Olfactory Habituation

The presentation of an olfactory stimulus can lead to a chemotaxic response, which is the migration toward or away from that stimulus. *C. elegans* will perform both of these behaviors; it is attracted to various ions, amines, and some volatile substances (ketones, esters, etc.), and it is repelled by acidic pH, D-tryptophan, and various volatile substances (benzaldehyde, octanol, etc.; Bargmann and Mori, 1997). Continuous or repeated presentation of such compounds can result in a decrement of the chemotaxic response (Colbert and Bargmann, 1995). Bernhard and Van der Kooy (2000) showed that this decrease in behavioral response could be mediated by two forms of olfactory plasticity: adaptation and habituation. Although adaptation can be considered a form of plasticity, it is not considered to be a form of learning because the response decrement is mediated by sensory fatigue, and the response will return to baseline levels only after sufficient time is allowed for the sensory system to recover. On the other hand, habituation to olfactory stimuli is considered a learning process because, although a similar response decrement occurs as with adaptation, when a novel or noxious stimulus is administered, dishabituation will occur.

Using solubilized Na^+ (an attractant ion), Wen et al. (1997) showed that olfactory habituation can occur in *C. elegans*. They showed that, when exposed to an attractant (75 $mmol^{-1}$ $NaCH_3COO$) for a prolonged time, the chemotaxic response to migrate toward that attractant diminished. But when worms were exposed to a much higher concentration of $NaCH_3COO$ (300 $mmol^{-1}$) for a brief period of time and then given the same behavioral assay, the habituated response returned to near baseline levels, indicating that dishabituation had occurred and that this behavioral decrement was habituation and not adaptation.

To investigate the differences between the processes of habituation and adaptation, Bernhard and Van der Kooy (2000) varied preexposure

concentrations of the volatile odorant diacetyl (DA) within a single paradigm. They found that preexposing and testing worms in high concentrations of DA induced a nonreversible decrement in chemotaxic response despite the introduction of a strong, novel stimulus. When preexposed to an intermediate concentration of DA, no decrement of response was observed. Interestingly, at very low concentrations of preexposure and testing, worms exhibited a decrement in response that could be dishabituated. Taken together, these data suggest that the processes of olfactory plasticity can be dissociated from habituation by the concentration of DA used, with adaptation requiring high concentrations of DA and habituation requiring low concentrations of DA.

Although a number of genes important for adaptation have been identified, little is known about the genetics of olfactory habituation. Wen et al. (1997) showed that mutant worms, *lrn-1* and *lrn-2,* had deficits in classical conditioning associative learning paradigms but showed no deficits in nonassociative habituation. However, more recently, Morrison and van der Kooy (2001) showed that a mutation in an alpha-amino-3-hydroxyl-5-methyl-4-isoxazolepropionate (AMPA)-type glutamate receptor subunit, *glr-1,* impaired both olfactory associative learning and habituation. These data suggest that the mechanisms involved in associative learning and habituation may be dissociable at the level of the *lrn-1* and *lrn-2* genes but share a common pathway that involves the *glr-1* gene.

4.04.4 Mechanosensory Habituation

4.04.4.1 Mechanosensation in *C. elegans*

Mechanosensation, which is the transduction of mechanical force into intracellular signals, allows organisms to sense touch, vibration, and other tactile stimuli. In *C. elegans,* mechanosensation has been studied in two major ways: first, touch to the body from gentle stimulation with a small hair and, second, vibration felt through the surrounding environment resulting from the administration of a mechanical tap delivered to the side of a Petri dish (Garcia-Anoveros and Corey, 1997). Most of the research on habituation has been done using the tap stimulus. The advantage of using the tap stimulus as opposed to the body touch when studying mechanosensory behavior is that the strength of stimulation can be controlled using a machine, whereas there is uncontrollable variation when delivering touch using a handheld device. In response to a tap stimulus, a worm will respond by swimming backward (a reversal), which has been termed the tap-withdrawal response.

4.04.4.2 Habituation

Using the tap-withdrawal response, Rankin et al. (1990) were the first to show that *C. elegans* is capable of nonassociative learning. When they administered a tap stimulus to the side of the Petri plate holding the worm, they observed that the distance that the worms reversed in response to the tap decreased when the stimulus was repeated at regular intervals. The response returned to baseline levels (spontaneous recovery) a few minutes following the last tap stimulus. To ensure that this response decrement was habituation and not sensory fatigue, they followed habituation training with a brief electrical shock in order to dishabituate the worms. Following shock, their response to tap increased significantly above the habituated level, indicating that the electrical shock had induced dishabituation, and the original response decrement observed was habituation and not adaptation or fatigue.

4.04.4.3 Behavioral Analyses of Short-Term Habituation

Thompson and Spencer (1966) and Groves and Thompson (1970) laid out the behavioral characteristics of habituation. These same criteria are used today to define habituation, and so far, all species studied show these same characteristics. Having a behavior well characterized is an asset when trying to determine underlying cellular mechanisms. It is important for researchers to make constant comparisons between behavior and the hypothesized cellular mechanisms in order to develop a greater understanding of the factors that govern habituation.

In their descriptions of habituation, Thompson and Spencer (1966) and Groves and Thompson (1970) missed one aspect of habituation that is common in all systems studied and can be used to find clues about possible molecular mechanisms of habituation. These early papers stated that habituation is sensitive to frequency, with high-frequency stimuli producing more rapid habituation than low-frequency stimuli, and that habituation recovers spontaneously. Both of these are correct; however, in all species studied, frequency also affects the rate of spontaneous recovery, with high-frequency

stimulation leading to more rapid spontaneous recovery than low-frequency stimulation (Rankin and Broster, 1992; **Figure 2**(**a**)). Rankin and Broster (1992) showed that in *C. elegans* this relationship of spontaneous recovery to frequency of stimulation held, regardless of the number of stimuli delivered (as long as decrement had reached asymptotic levels) and regardless of the level of habituation reached (when levels of habituation were matched between worms habituated with high and low frequencies, rate of recovery was still dependent on frequency of the habituation). This is important for two reasons. The first is that this difference is the opposite of what would be predicted by fatigue or adaptation in that, in both of those cases, the more complete the decrement the longer the recovery. With high frequency the decrement is rapid and often complete, but recovery is very rapid, while with low frequency the decrement is not complete and yet recovery takes much longer than for high-frequency stimulation. Thus, the sensitivity of spontaneous recovery to the frequency of stimulation is a second way, in addition to dishabituation, to distinguish whether a behavioral decrement is the result of habituation, or the result of sensory adaptation or motor fatigue. The second reason that the sensitivity of recovery to frequency of stimulation is important is the deductions one can draw from this about molecular

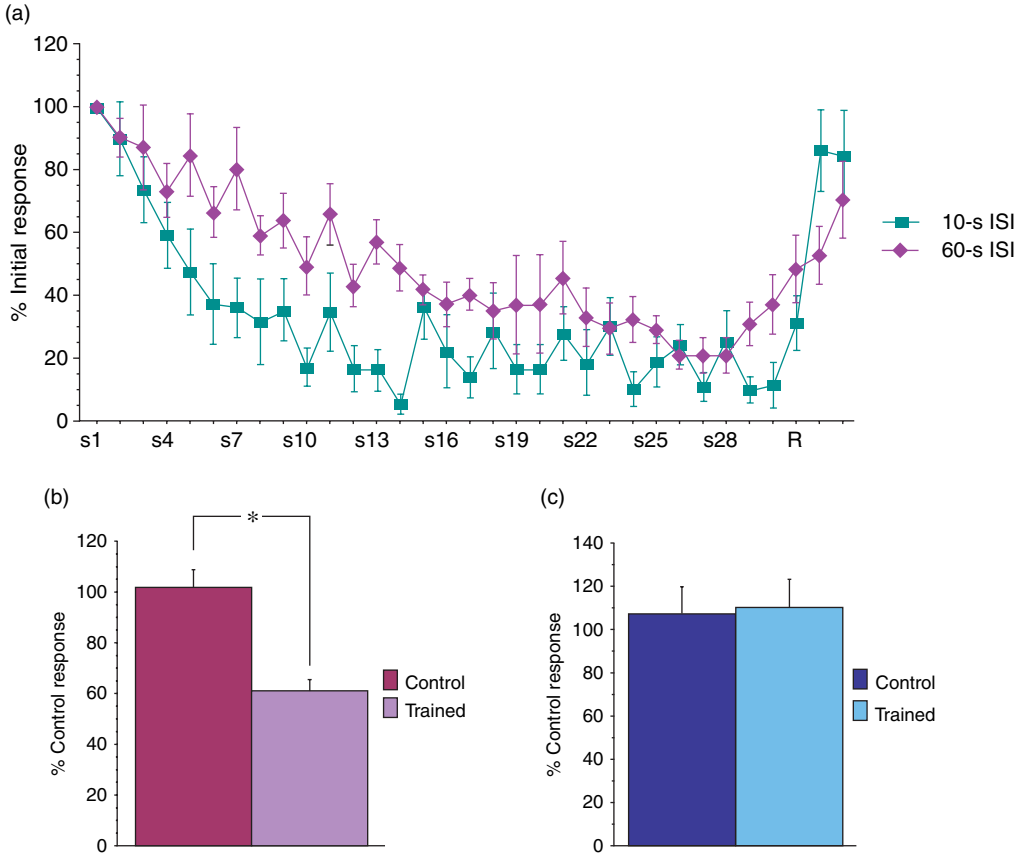

Figure 2 (a) Reversal responses of wild-type worms shown as the mean percent initial response across 30 tap stimuli with three recovery taps at 30 s, 5 min, and 10 min after habituation training. The 10-s interstimulus interval (ISI) group shows more rapid habituation and a lower asymptotic level when compared to the 60-s ISI group. The 10-s ISI group also shows faster and more rapid recovery following habituation training than the 60-s ISI group. (b) Mean percentage of control response magnitude for long-term memory for five test stimuli 24 h following habituation training given at a 60-s ISI. The significantly lower level of responding in the trained group compared to the control group indicates the retention of memory for habituation training. (c) Mean percentage of control response magnitude for long-term memory for five test taps 24 h following habituation training given at a 10-s ISI. The lack of memory observed when training is given at a 10-s ISI suggests either that 60-s ISI training selectively recruits molecular mechanisms needed to induce the formation of long-term memory for habituation training, or that 10-s ISI training recruits molecular mechanisms that block the formation of long-term memory (Butterfield and Rankin, unpublished results, 2006).

mechanisms of habituation. If an animal that has habituated to stimuli at a high frequency (i.e., short interstimulus intervals, ISIs) recovers rapidly, while an animal habituated to the same level to a low frequency recovers more slowly, this indicates that the two habituated animals are not the same, and some different processes have been activated in the neurons of the two animals to regulate recovery differently. From this observation Rankin and Broster (1992) hypothesized that habituation was not mediated by a single molecular mechanism, but that stimulation at different frequencies recruited different cellular mechanisms. The observation that long ISIs can be used to produce long-term memory for habituation, while short ISIs cannot, provides further support for this hypothesis (Beck and Rankin, 1997; **Figures 2**(**b**) and **2**(**c**)). Broster and Rankin (1994) hypothesized that, if different ISIs recruited different molecular mechanisms, then studies of genes involved in habituation should lead, at the very least, to the discovery of genes that play a role in habituation to all frequencies, genes that play a role in habituation to high frequencies, and genes that play a role in habituation to low frequencies. We have recently found support for this hypothesis and identified two genes that are involved in short-term habituation. One specifically affects habituation at high frequencies; the other specifically affects habituation at low frequencies (Rankin, unpublished data, 2006).

4.04.5 Neural Circuit

4.04.5.1 Identifying Neurons Involved in Habituation

Once the behavior was well characterized, the next step in discovering the molecular mechanisms involved in habituation to tap stimuli was to identify the neural circuit responsible for this behavior. The neural circuit underlying the behaviors of backward swimming in response to head touch and forward swimming in response to tail touch was characterized by Chalfie et al. (1985). Wicks and Rankin (1995) investigated the neural circuit underlying the tap-withdrawal response by studying the effects of laser ablating cells in the head and tail touch circuits on the response to tap and found that the response to tap involves integration of sensory input from both the head and tail. The neural circuit for the response to tap consists of five mechanosensory cells, two bilaterally paired PLM neurons that transduce tail touch, and 2 bilaterally paired ALM neurons and a single AVM neuron that transduce head touch; these sensory neurons synapse onto four pairs of command interneurons that mediate forward (AVAs and AVDs) and backward movement (AVBs and PVCs; **Figure 3**(**a**)). Ablation of all sensory cells completely abolished the response to tap (Wicks and Rankin, 1995). Ablation of only the head touch neurons (ALMs and AVM) resulted in consistent forward swimming in response to tap (termed an acceleration) in contrast to the consistent reversal responses seen in intact worms. Similarly, backward swimming was always seen in response to tap in PLM ablated (PLM–) worms. These reversals were larger than the reversals of intact animals, suggesting that in the intact worms the effect of stimulation of the tail cells by the tap competes with the effect of stimulation of the head touch cells and moderates the response size. Interestingly, ablation of AVM only resulted in a decrease in reversal frequency and magnitude of reversals and an increase in acceleration (forward swimming) frequency. In a study of the response to tap across development, it was observed that, at younger stages, worms responded by both reversing and accelerating at equal frequencies, similar to what is observed in AVM– worms (Chiba and Rankin, 1990). Since AVM is not present at hatching and only becomes fully functional in young adults, Chiba and Rankin (1990) suggested that the shift in adult worms to predominantly reversing response to tap may be mediated by the development of AVM. All these results combined indicated that the response to tap was mediated by the integration of inputs from two competing neural circuits, one driving forward movement and one driving backward movement.

4.04.5.2 Roles of Identified Neurons in Habituation

To identify the sites of changes in the pathway(s) underlying habituation of the tap-withdrawal response, Wicks and Rankin (1996b) laser ablated specific touch cells and observed any changes in habituation rate or asymptotic level. Laser ablation of the PLM sensory neurons results in consistent backward movement in response to tap. When given the short-term habituation training at a 10-s and 60-s ISI, PLM– worms showed habituation at both ISIs. The initial slope of the PLM– group was smaller than that of the intact group (**Figures 3**(**b**) and **3**(**c**)). When Wicks and Rankin (1996b) investigated the role of the touch cells of the anterior mechanosensory field (AVM and ALM), they found

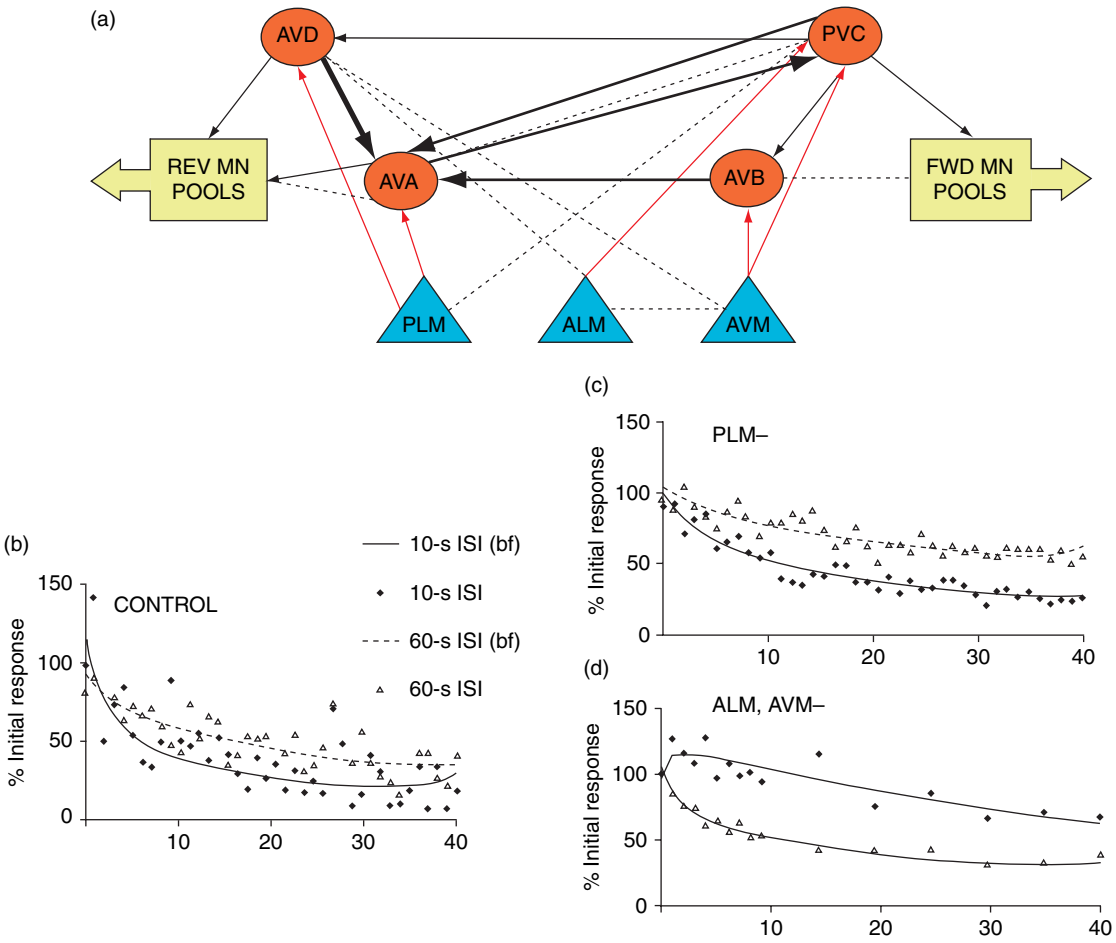

Figure 3 (a) Simplified neural circuit underlying tap withdrawal. The circuit consists of five mechanosensory neurons (*triangles*), eight command interneurons (*circles*), and two motor neuron pools (*squares*). All cells represent bilateral classes of cells except ALM, which is a single cell. The arrows and dotted lines represent chemical synapses and gap junctions, respectively. The number of synaptic contacts is proportional to the width of the arrows. The red-colored arrows indicate the synaptic connections that have been hypothesized to be the sites of plasticity that mediate habituation (Wicks and Rankin, 1997; Kitamura et al., 2001). (b, c) Scatter plot graphs fitted with best fit lines (bf) demonstrating the effect of ISI (10 s vs. 60 s) on the kinetics of habituation of the reversal response in intact animals (control) and PLM-ablated animals (PLM–). (d) Scatter plot graph showing the effects of ablation of ALM and AVM on the kinetics if habituation of acceleration responses. Wicks and Rankin (1996b) hypothesized that the responses of both the PLM– and AVM, ALM– groups combine to make up the behavior we see in the intact animal. Figure adapted from Wicks SR and Rankin CH (1996b). The integration of antagonistic reflexes revealed by laser ablation of identified neurons determines habituation kinetics of the *Caenorhabditis elegans* tap withdrawal response. *J. Comp. Physiol. A* 179: 675–685, with permission from Springer-Verlag.

results that were quite different from those of the PLM– group. Worms with ablations in the head sensory neurons accelerate forward in response to tap. They found that both ALM– and ALM, AVM– groups habituated slower at a 10-s ISI than at a 60-s ISI, and they showed an initial response facilitation prior to the decrement that was especially strong when habituated at a 10-s ISI (**Figure 3(d)**). Further, the ALM, AVM– group had a significantly higher asymptotic level at a 10-s ISI as opposed to the 60-s ISI. Finally, ablation of one of the pairs of command interneurons (PVCs), thought to modulate forward movement, resulted in worms that reversed 100% of the time; however, unlike the PLM– worms, the reversals seen in PVC– worms were not significantly different in magnitude from control worms. The results support the hypothesis of Chalfie et al. (1985) that the PLM cells make inhibitory chemical connections to the head touch circuit interneurons (AVD and AVA); these connections remain intact

following PVC ablation and continue to compete with the head sensory neuron stimulation. Taken together, the results of ablation of cells important in both the forward and backward motion pathways have shown that reversals and accelerations habituate at different rates; thus their relative contribution to the intact response varies over the course of habituation. For example, at a 10-s ISI the initial facilitation of the acceleration response competes more strongly with the reversals and the reversals seen in intact worms decrease in amplitude very quickly. This suggests that to understand the habituation of behavior it is not sufficient to study the mechanisms underlying the decrement in a single cell, but it may be necessary to understand the effect of repeated stimulation on all aspects of the neural circuits underlying the behavior.

4.04.5.3 Localizing the Site of Plasticity in the Neural Circuit

Two behaviors that share command interneurons and motor neuron pools with the tap-withdrawal response are thermal sensation and spontaneous reversing. All of these behaviors differ from tap at the level of sensory input. Wicks and Rankin (1997) hypothesized that, if the site of plasticity that mediates habituation is located at the sensory input level, then habituation to tap should not alter baseline levels of both the response to a heat probe and spontaneous reversing. On the other hand, if the site of plasticity is at the level of the interneurons and motor neurons, then these behaviors should be altered by habituation training in response to tap. Wicks and Rankin (1997) found that habituation to tap did not affect other behaviors, thus providing data to support the hypothesis that the site of plasticity lies in the touch cells and/or the synaptic connections they make onto the interneurons.

Investigation into habituation of anterior body touch has also led to increased knowledge of how habituation occurs in the neural circuit. Kitamura et al. (2001) performed gentle body touch using a hair at a 15-s ISI, using intact worms, and observed the expected kinetics of habituation with an initial, rapid decrement of reversal magnitude followed by an asymptotic level of that response. Through systematic ablations of combinations of neurons involved in the response to anterior body touch (ALM, AVM, AVD, and PVC), they found that laser ablation of both AVD interneurons resulted in significantly more rapid habituation than observed in intact animals. The ablation of any of the other interneurons had no effect on the rate of habituation, suggesting that the AVD interneurons play a critical role in the habituation to anterior body touch. Kitamura et al. (2001) also found that coablating the right ALM and the AVM neurons resulted in rapid habituation, suggesting that in these animals the chemical synapse that the left ALM makes with the right AVD and PVC interneurons is responsible for the behavior observed. Further investigation led to the conclusion that the chemical synapses between the left ALM sensory neurons and the PVC interneurons mediate the rapid habituation of the coablated worms. The fact that more rapid habituation can be attributed to at least two sites in the neural circuit suggests that each synapse in the circuit may have the potential to be the site of plasticity.

Taken together, the studies performed by both Wicks and Rankin (1995) and Kitamura et al. (2001) show that habituation to mechanosensory stimuli in intact animals involves the integration of a variety of inputs. However, the most likely site of plasticity appears to be situated at the level of the chemical synapses between the sensory neurons and their target interneurons.

4.04.6 Genetic Dissection of Short-Term Habituation

4.04.6.1 Role of Genes Involved in Glutamate Neurotransmission

A number of genes involved in glutamate neurotransmission are expressed in the touch cells and the command interneurons, suggesting that glutamatergic transmission plays a major role in the response to tap. Presynaptically, in the sensory neurons (ALM, AVM, and PLM) a homologue of a mammalian glutamate vesicular transporter, known as EAT-4, is expressed (Lee et al., 1999). Postsynaptically, in the command interneurons (AVA, AVB, AVD, and PVC) homologues of both the mammalian AMPA/Kainate-type and n-methyl-D-aspartate (NMDA) glutamatergic receptors, GLR-1 and NMR-1, respectively, are expressed (Hart et al., 1995; Maricq et al., 1995; Brockie et al., 2001). If glutamatergic transmission plays a critical role in habituation in *C. elegans,* then worms that lack, or have mutations in, one or more of these genes should have altered patterns of habituation.

Rankin and Wicks (2000) first examined *eat-4* mutants using the short-term habituation paradigm. Initially, they found that, when compared to

wild-type worms, there was no difference in the initial response to the tap stimulus. When given repeated stimulation, at both 10- and 60-s ISIs, *eat-4* worms habituated more rapidly and reached a lower asymptotic level than wild-type worms. Similarly, *eat-4* worms showed much slower spontaneous recovery following habituation training; however, the dependence on ISI was still present in both habituation and recovery kinetics (faster decrement and faster recovery from short ISI training as compared to long ISI training). This result suggests that the absence of EAT-4 disrupts one or more cellular mechanisms of habituation but leaves others (ISI dependent processes) intact. Interestingly, *eat-4* is also the first gene that has been shown to play a role in dishabituation. When given a dishabituating stimulus (an electric shock), the *eat-4* mutants did not show facilitation of the response above the habituated level, indicating that they did not dishabituate. Since *eat-4* worms still show ISI-dependent spontaneous recovery, the decrement seen in these worms is habituation and not fatigue or adaptation. Because we do not know the relationship between the molecular mechanisms of habituation and the molecular mechanisms of dishabituation, these results illustrate the importance of having more than a single way to distinguish habituation from fatigue. Using a transgenic rescue strain (DA1242) produced by Lee et al. (1999), Rankin and Wicks (2000) showed that wild-type habituation and dishabituation behaviors were also rescued. These studies with *eat-4* worms support the hypothesis that glutamatergic transmission plays an important role in habituation of the tap-withdrawal response and that the glutamate vesicular transporter is essential for dishabituation.

Because *eat-4* worms did not respond differently from wild-type in response to the initial tap stimuli but differed only after repeated stimuli, Rankin and Wicks (2000) concluded that EAT-4 (a glutamate vesicular transporter) is not required for glutamatergic transmission but, rather, is required for sustained synaptic activity. The hypothesis is that the touch sensory neurons of *eat-4* worms have fewer glutamate-filled vesicles than those of wild-type worms, and so they are quickly exhausted in response to repeated stimulation. The importance of neurotransmitter vesicles in habituation is supported by work in *Aplysia* that has shown that there are fewer synaptic vesicles in the active zones of sensory neurons from habituated animals than from the terminals of nonhabituated animals (Bailey and Chen, 1988). The regulation of the amount of glutamate in vesicles in the terminals of the sensory neurons and/or the regulation of vesicular release are the most likely mechanisms underlying the behavioral changes observed during habituation.

Since the deficits in presynaptic release of glutamate seen in *eat-4* worms alter the kinetics of habituation to tap, it was suggested that postsynaptic glutamate receptors should also play a significant role in the same behavior. Genes for four ionotropic glutamate receptors are expressed on the command interneurons: *glr-1, glr-2, nmr-1*, and *nmr-2* (Hart et al., 1995; Maricq et al., 1995; Brockie et al., 2001). *glr-1* and *glr-2* have been shown to form heteromeric receptors (Chang and Rongo, 2005), and *nmr-1* and *nmr-2* are thought to form heteromeric receptors as well. Studies to date have focused mainly on mutations in *glr-1* and *nmr-1*. When given habituation training, *glr-1* worms showed smaller initial responses to tap than wild-type animals but showed relatively normal short-term habituation to 10- and 60-s ISIs when compared to wild-type worms (Rose et al., 2002). *nmr-1* worms showed normal short-term habituation, indistinguishable from wild-type. The results thus far indicate that none of the glutamate receptor genes tested alone gives the same pattern as EAT-4–deficient worms, which suggests that habituation may be mediated postsynaptically by the activation of an, as yet, unidentified glutamate receptor or by the simultaneous activation of multiple glutamate receptors.

4.04.6.2 Other Identified Components of Habituation

Sanyal et al. (2004) showed that dopamine, which plays an important role in behavioral plasticity in many mammalian systems, also may play an important role in modulating *C. elegans* habituation. Sanyal et al. (2004) studied mutations in two genes involved in dopamine regulation and neurotransmission; *dop-1* mutants, which do not express a dopamine receptor on neurons involved in the mechanosensory circuit, show altered patterns of habituation compared to wild-type worms, as do *cat-2* mutants, which lack an enzyme that is required for dopamine synthesis. Sanyal et al. found that *dop-1* mutants and *cat-2* mutants had a more rapid decrement in reversal frequency (the number of worms that respond to each tap) than wild-type worms. However, when measuring reversal length (the dependent variable in all previous studies mentioned), there was no difference among all three groups. This result

suggests two alternative hypotheses: the first is that different mechanisms regulate the decrease in the probability of a reversal response and the size of the response; the second is that dopamine may not be involved in habituation to tap, but instead may modulate the integration of sensory stimuli from the head and tail touch circuits.

Xu et al. (2002) performed a forward genetic screen on habituation to tap and isolated *hab-1,* a mutant which habituated more slowly and responded at a higher asymptotic level than wild-type worms when tested at both 2- and 10-s ISIs. Further, the *hab-1* mutants responded like wild-type worms to the initial tap and also responded normally to a dishabituating stimulus. Unfortunately, the gene product of *hab-1* has yet to be identified. However, the observed pattern of slower habituation is opposite to the results seen with both *eat-4* and *dop-1,* suggesting that the gene product of *hab-1* may play an antagonistic role to the molecular mechanisms of glutamatergic and dopamine signaling that is hypothesized to underlie short-term habituation.

4.04.7 Analyses of Long-Term Habituation

4.04.7.1 Dependence on Protocol

Analysis of long-term memory for habituation to the tap-withdrawal response has led to increased knowledge and further understanding of the mechanisms that govern habituation behavior. Rankin et al. (1990) were the first to report long-term memory habituation to tap. Using a modified protocol from experiments with *Aplysia* (Carew et al., 1972), they were able to show that if worms were given distributed habituation training they were able to retain memory for that training for at least 24 h. To investigate the behavioral parameters that reliably produce long-term memory for habituation, Beck and Rankin (1997) examined ISI and type of training. They replicated the finding of Rankin et al. (1990) that long-term memory could be produced by distributed training, and they found that, if they used a massed training protocol where all stimuli were delivered without break periods instead of a distributed training protocol, long-term memory was not observed. In addition, they found that long-term memory could not be reliably produced from training at a 10-s ISI, whereas it was produced using a 60-s ISI. This result supports the hypothesis of Rankin and Broster (1992) that there may be specific cellular mechanisms, which are recruited by training with a 60-s ISI or inhibited by training with a 10-s ISI, to induce memory formation during habituation training (**Figures 2(b)** and **2(c)**). Taken together, these data show that long-term memory of habituation training is most reliably produced when stimuli are administered at longer ISIs in a distributed or spaced manner. This parallels observations made in studies that found that distributed training is superior to massed training for the induction of long-term memory in many species, including *Aplysia, Drosophila,* and humans (Ebbinghaus, 1885; Carew *et al.*, 1972; Tully and Quinn, 1985).

4.04.7.2 Molecular Correlates of Memory for Habituation Training

Since distributed training was essential for the induction of long-term memory for habituation training, it was hypothesized by Beck and Rankin (1995) that the mechanisms that are responsible for consolidation of the memory were most likely occurring during the interblock intervals. To assess this, Beck and Rankin (1995) used heat shock (32 °C) to block protein synthesis and disrupt these cellular mechanisms. The cellular response to heat shock that was first observed in *Drosophila* has been shown to be same in every organism studied (Schlesinger et al., 1982). The response is the termination of protein synthesis of all proteins other than a class of proteins called heat shock proteins, the production of which is significantly increased. To determine the timing of memory consolidation of habituation training, heat shock was delivered before, during, or after training. Beck and Rankin (1995) found that disruption of memory consolidation by heat shock occurred during but not before or after habituation training, which supported their hypothesis that some of mechanisms that mediate the induction of long-term memory are occurring during the interblock periods. As well, they found that neither the kinetics of habituation nor the initial response to tap was affected by heat shock treatment. These data suggest that repeated tap stimulation with intervals between blocks triggers molecular mechanisms that involve protein synthesis.

Since the research with *eat-4* mutants suggested that glutamatergic neurotransmission plays a pivotal role in short-term habituation, Rose et al. (2002) hypothesized it might also play a role in long-term habituation. To investigate this, Rose et al. (2002) used the *eat-4* mutants to test for the presence of long-term memory for habituation training using the distributed training protocol. Interestingly, even though *eat-4* mutants habituate faster and more

completely than wild-type worms, they did not retain memory for this training 24 h later. This suggests that the sustained glutamate release that is important in short-term habituation is also critical to the formation of long-term memory. Rose et al. used the *eat-4* rescue strain, DA1242, and found that DA1242 worms showed normal memory 24 h after training; this supports the hypothesis that presynaptic glutamate release is essential for the formation of long-term memory.

Because release of presynaptic glutamate appeared to be essential for the induction of long-term memory, Rose et al. (2002) hypothesized that postsynaptic glutamatergic receptors might be involved in aspects of long-term memory for habituation as well. To examine this hypothesis Rose et al. (2003) tested worms with mutations in *glr-1,* a homologue of mammalian AMPA/Kainate glutamate receptor subunit (GluR1) and worms with a mutation in *nmr-1,* a homologue of mammalian NMDA-type glutamate receptor subunit (NR1). They found that worms with a mutation in *nmr-1* showed normal long-term memory for habituation. In contrast, *glr-1* worms showed no long-term memory for habituation. To confirm the importance of glutamate receptors in the formation of long-term memory, wild-type worms were treated with DNQX, a competitive non-NMDA glutamate receptor antagonist, during training. Treatment with DNQX had no effect on short-term habituation, but it did block the formation of long-term memory (Rose et al., 2003). These data suggest that *glr-1* plays a critical role in the induction of long-term memory but is not an essential component for short-term habituation.

The role of glutamate receptors in synaptic plasticity and memory formation has been extensively studied in mammalian systems. The most prominent and well-characterized forms of synaptic plasticity that may underlie mammalian memory formation are long-term potentiation (LTP) and long-term depression (LTD). Both LTP and LTD involve changes in synaptic expression levels of GluR1-containing AMPA-type glutamate receptors and trafficking of the receptors to and from the postsynaptic membrane (Malinow and Malenka, 2002). To examine whether similar processes were involved in the induction of memory for habituation training Rose et al. (2003) tested whether habituation training affected the expression pattern of the GluR1 homologue, GLR-1. Using worms carrying chimeric receptors made up of GLR-1 tagged with GFP (GLR-1::GFP), they were able to visualize changes in punctate *glr-1* expression (Rongo and Kaplan, 1999) along the ventral nerve cord (an area that corresponds to important synaptic sites reported in electron microscopy studies; White et al., 1986). They found that, following distributed habituation training, the number of GLR-1::GFP puncta along the ventral nerve cord did not change, but the size of the puncta in trained worms was significantly smaller than in control worms (**Figures 4(a)** and **4(b)**). This result suggests that the distributed habituation training did not alter the number of synapses along the nerve cord but rather reduced the number of receptors expressed per synapse on the interneurons. Further, blocking protein synthesis by applying heat shock during training blocked the downregulation of GLR-1::GFP expression. These data suggest that, although *glr-1* does not appear to play an important role in short-term habituation, its regulation critically mediates long-term memory for habituation.

4.04.8 Summary

The evidence discussed has shown that *C. elegans* can habituate to both olfactory stimuli and mechanosensory stimuli. Little is known about olfactory habituation other than that it appears to require low doses of the chemical stimuli and is dependent on the *glr-1* gene. On the other hand, mechanosensory habituation has been studied at the level of behavior, neural components, and genes involved. Using the tap-withdrawal paradigm, the dependence upon ISI appears to mediate a large number of aspects of habituation, including the rate of habituation, the level of habituation, and spontaneous recovery from habituation. The neural circuit that mediates habituation to tap has been thoroughly investigated, and this has led to the hypothesis that the most likely sites of plasticity are the synaptic connections between the sensory neurons and the command interneurons. Thus far, the molecular components that affect habituation include presynaptic glutamate release and dopaminergic neurotransmission, and are independent of the activation of *glr-1.* However, distributed habituation training that induces long-term memory recruits other cellular processes. These processes have been shown to be protein synthesis dependent and rely heavily upon the alterations in the expression of *glr-1.*

Taken together, these data suggest that habituation is not a simple or singular process but, rather, is mediated by a complex set of events that incorporates a variety of molecular mechanisms. These events

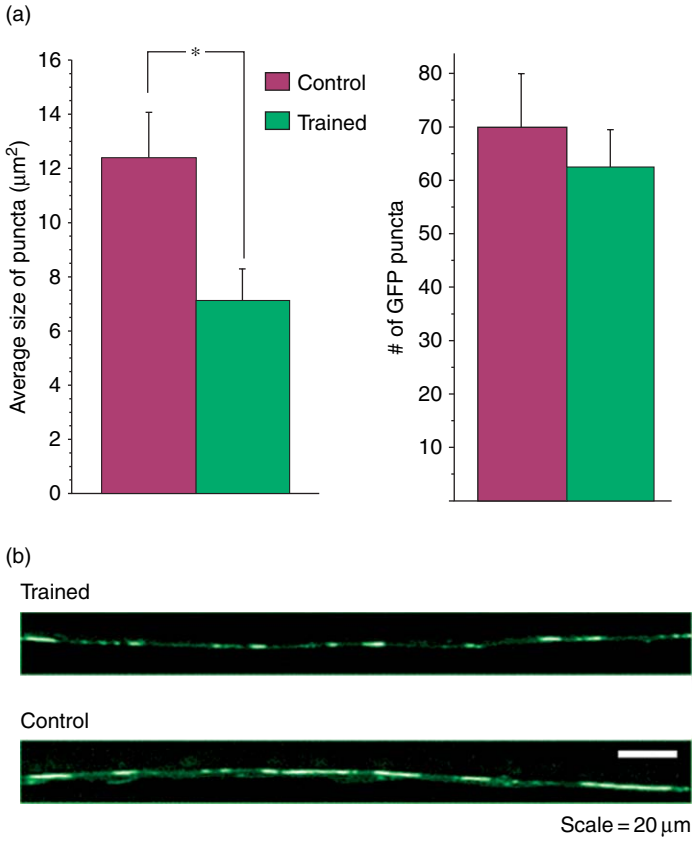

Figure 4 (a) Long-term memory for habituation training correlates with changes in GLR-1::GFP expression in the posterior ventral nerve cord 24 h after distributed habituation training. There were significantly smaller GFP puncta in the trained worms than in control worms; however, the number of puncta was not different between the two groups, suggesting that memory was reflected in a change in the average size of synapses and not a change in the number of synapses (Rose et al., 2003). (b) Confocal images of GLR-1::GFP expression in trained and control worms taken 24 h after distributed training.

integrate multiple neurotransmitters, multiple receptors, and multiple subcircuits, each mediating different aspects of habituation. Rather than there being a single mechanism of habituation, the data from *C. elegans* lead to the hypothesis that there are multiple mechanisms that can underlie this seemingly simple response decrement. Because of its well-studied genetics, physiology, and behavior, *C. elegans* is now, and will continue to be, a very powerful model system in which to elucidate the cellular events and processes that underlie key components involved in learning and memory.

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4.05 Pain Sensitization

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4.05.1 Introduction

Over 10 years ago, Allan Basbaum published a review article entitled 'Memories of pain' (Basbaum, 1996). This article highlighted the role of central nervous system (CNS) plasticity in mediating pain sensitization, and since that time research into the molecular mechanisms mediating pain sensitization has exploded, leading to a new appreciation for the complexity of pain and its sensitization. Here we will introduce the neurobiology of pain and discuss the many mechanisms of sensitization in the pain neuraxis, as well as their relation to mechanisms of learning and memory.

4.05.1.1 Defining Pain: Acute Versus Chronic Pain

Pain is an unpleasant sensory and emotional experience associated with actual or potential tissue damage or described in terms of such damage (Merskey, 1979). Physiological pain serves a vital survival function as it alerts organisms to the presence of damaging or potentially damaging stimuli. The importance of the protective role of pain is exemplified by the detrimental outcomes of numerous pathological conditions that are characterized by the lack of pain sensation. A common group with significant morbidity due to the lack of pain perception is diabetics with peripheral neuropathy. In these patients, unnoticed repetitive injuries to the joints can lead to permanent joint deformity. Diabetic patients with peripheral neuropathy can also develop ulcers caused by undetected excessive pressure, by rubbing against the skin on the foot, or by stepping on a sharp object (Boulton, 2004). These ulcers can erode to the bone and lead to serious infections with resultant sepsis or need for amputations. Another rare, but yet very dramatic, example of the

protective role of pain is seen in patients with congenital insensitivity to pain. In these patients, pain and temperature insensitivity is manifested in childhood and results in the occurrence of painless fractures, ulcers, burns, and self-mutilation that can lead to death at a young age (Nagasako et al., 2003).

Under normal physiological conditions, pain results from trauma, inflammation, or nerve injury, thus acting as an early warning system that protects the body from further injury. Physiological pain usually subsides as the injury heals and can be frequently diagnosed and treated. Chronic pain, on the other hand, is considered a pathological condition with little or no beneficial function. It can last for a prolonged period of time, outlast the initial pathology, and it is often accompanied by maladaptive behaviors, irritability, depression, and disruption of work and social relationships (American Pain Foundation, 2002). Chronic pain may result from ongoing causes of pain with a known pathology such as in arthritis and cancer. Alternatively, chronic pain may be caused by either an unknown pathology or an associated injury, making their treatment even more challenging.

A major but also distinct component of pain is nociception, which is the sensory process by which a noxious stimulus, such as one induced by tissue damage, is transduced to neurophysiological signals that are then transmitted to the CNS. Nociception can occur without pain perception and pain perception can occur without nociception. An example of pain in the absence of nociception is seen in the phantom limb syndrome, when patients with amputated limbs report feeling pain in the missing limb.

4.05.1.2 Chronic Pain and Synaptic Plasticity

The understanding of the neural mechanisms underlying nociception and pain perception has significantly increased in the last 2 decades. A key finding for this advancement was the realization that responses of the sensory system to a given input are not fixed, but rather change, as a result of previous neuronal activity. This means that the responses of the sensory system are highly dependent on the neural memory of the system. This neuronal plasticity, also referred to as sensitization, has been observed and studied in different parts of the pain neuraxis such as peripheral nociceptors, spinal dorsal horn neurons, rostroventral medulla, anterior cingulate cortex, and amygdala.

Maladaptive and persistent neural changes in the sensory system can occur in response to trauma, inflammation, or nerve injury and are thought to underlie chronic pain. A classic example of sensitization is seen in the dorsal horn of the spinal cord (**Figure 1**). Following tissue injury, spinal cord dorsal horn neurons, which are the first CNS processing station for pain signal from the periphery, show a decreased threshold for action potential firing, increased responsiveness to a given stimulus, and receptive field enlargement (Woolf, 1983; Woolf and Wall, 1986; Cook et al., 1987). The increased responsiveness of

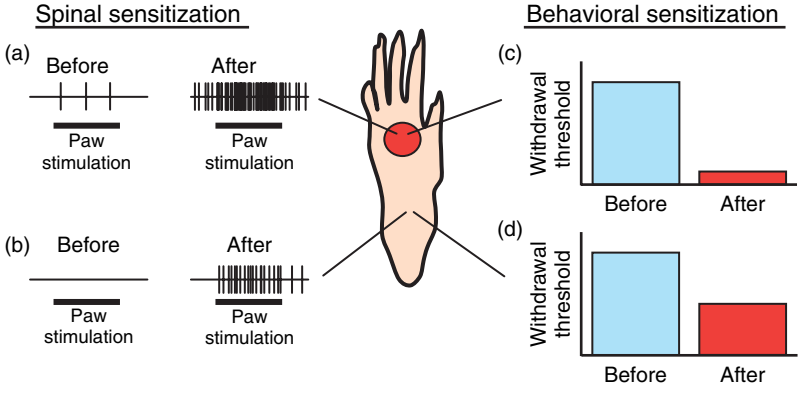

Figure 1 Diagrammatic representation of injury-induced spinal and behavioral sensitization. (a, b) Responses of a spinal cord dorsal horn neuron to paw stimulation before and after tissue injury. (a) Responses to stimulation in the injured area (red circle). After tissue injury, dorsal horn neurons exhibit increased responsiveness to a given stimulus. (b) Responses to stimulation in the noninjured area. Before tissue injury, the receptive field of the neuron did not include the stimulated area. After tissue injury, the neuron responds to stimulation in the noninjured area, demonstrating an enlargement of the receptive field (c, d) Paw withdrawal thresholds in response to mechanical stimulation before and after tissue injury. After tissue injury, withdrawal thresholds in response to mechanical stimulation of the injured (c) and noninjured areas (d) decrease.

dorsal horn neurons to a given stimulus correlates with the behavioral sensitization commonly observed after tissue injury (**Figure 1**). This pain sensitization is characterized by increased pain responses to normally noxious stimuli (hyperalgesia) and/or by pain responses to previously innocuous stimuli (allodynia). Hyperalgesia and allodynia after tissue injury can be experienced both in the injured area (primary hyperalgesia or allodynia) and in adjacent noninjured areas (secondary hyperalgesia or allodynia). Receptive field enlargement of dorsal horn neurons could therefore explain, at least in part, the phenomenon of secondary allodynia and hyperalgesia.

For years, researchers have been trying to identify neural processes that specifically mediate pathological but not physiological pain. The end goal is to develop novel and more efficient approaches for treating chronic pain conditions by decreasing maladaptive pain without affecting physiological pain, which is a vital adaptive response. Numerous studies have revealed striking similarities between the neural mechanisms underlying pain sensitization and those underlying learning and memory (Ji et al., 2003). These similarities have made the development of novel analgesic therapies even more difficult by adding yet another level of potential undesired side effects. At the same time, realization of this relationship has led to the development of a cognitive-behavioral approach to managing chronic pain that has been shown to be highly effective in helping to manage chronic pain. In this chapter, we will start by briefly discussing the advantages and limitations associated with the use of animal models for the study of pain and by summarizing basic concepts of the physiology of pain. We will then discuss the different forms of sensitization that have been reported in different areas of the pain neuraxis, including their anatomical and cellular substrates and their association with behavioral hypersensitivity that is induced by trauma, inflammation, or nerve injury. The chapter will end by comparing the neural mechanisms of pain with those of learning and memory and by discussing the implications for pain management.

4.05.2 Animal Models for the Study of Pain

The goal of pain research is to acquire new knowledge on the mechanisms, pathogenesis, diagnosis, and treatment of pain. The use of animal models of pain is essential to achieve this goal but, because of the negative nature of pain, there are a number of concerns as well as ethical, moral, and legal issues associated with the use of animals to study pain. Ethical guidelines for the investigation of experimental pain in animals have been established by the International Association for the Study of Pain (IASP) (Zimmermann, 1983). These guidelines primarily aim at ensuring that the animal is exposed to the minimal pain necessary for the purposes of the experiment. Whenever possible, experiments should be carried out in anesthetized animals utilizing transient stimuli and avoiding tissue damage. Many experiments to investigate the neural basis of pain can be pursued using these methods. However, to evaluate the mechanisms underlying persistent pain-related behaviors after tissue injury, the use of conscious animals and their exposure to tissue injury are imperative. To minimize discomfort and pain, the duration of all pain experiments should be as short as possible and the number of animals involved should be kept to a minimum. If possible, the investigator should expose the pain stimulus to himself to ensure that the experimental animal is not exposed to pain greater than humans could tolerate. Finally, to minimize discomfort, most experiments utilize techniques in which the animal has control over the intensity and duration of the pain stimulus by measuring the latency or threshold for withdrawal responses to the pain stimulus.

By definition, pain is a subjective perception of a given stimulus. Therefore, the main limitation of the use of animal models to study pain is that the investigator cannot determine with certainty the animal's perception. Animal models of pain are designed to mimic human pain conditions and employ characteristic animal behavioral responses to pain such as licking, withdrawal, and vocalization. Although not perfect, these methods allow the investigator to infer that the animal is experiencing pain. Changes in these stereotypical behaviors have long been used to measure the efficacy of experimental manipulations and pharmacological agents in reducing pain. Many of the drugs currently used to treat clinical pain have been shown to decrease nociceptive behavioral responses using animal models (Negus et al., 2006). Furthermore, neural mechanisms thought to underlie chronic pain have been observed both in human and in animal models of pain (Ji et al., 2003; Klein et al., 2004). It can therefore be concluded that animal models of pain are indeed useful for studying pathological pain.

4.05.3 Physiology of Pain

Pain processing starts with the conversion of noxious stimuli to neurophysiological signals (**Figure 2**). These neural signals are transmitted from the site of injury to the spinal cord and the brain where information about the intensity, quality, and location of the stimuli is processed. This processing is not unidirectional, however; descending pathways from the brain can significantly affect nociceptive transmission in the spinal cord, thus affecting pain perception. In this section, we will discuss the physiological basis of pain. The processes of transduction, transmission, modulation, and perception of pain will be discussed.

4.05.3.1 Pain Transduction

As in all sensory systems, the nociceptive system starts with the transduction of an external stimulus, in this case a noxious stimulus (thermal, mechanical, or chemical), to a neurophysiological signal. The transduction process occurs in nociceptor terminals that are located in the skin, muscles, and other end-organ tissues. Nociceptors are peripheral nerve endings of primary sensory neurons that selectively respond to noxious stimuli. The cell bodies of these primary sensory neurons are located in the dorsal root ganglia (DRG) and in the trigeminal ganglia. Dorsal root and trigeminal ganglion neurons are pseudo-unipolar cells. This means that these neurons have one axon that splits into two processes, with each process functioning as an axon. One of these processes conveys information from the periphery to the soma while the other one transmits information from the soma to neurons in the dorsal horn of the spinal cord or in the trigeminal nucleus caudalis of the brainstem. Two major classes of nociceptors have been described: Aδ- and C-nociceptors (Raja et al., 1999). Aδ-nociceptors are small-diameter, thinly myelinated fibers that conduct at 5–30 m/s and respond to either heat (thermal nociceptors) or intensive pressure (mechanical nociceptors), whereas C-nociceptors are small-diameter unmyelinated fibers that conduct slowly (<1 m/s) and respond to high-intensity mechanical, thermal, or chemical stimuli.

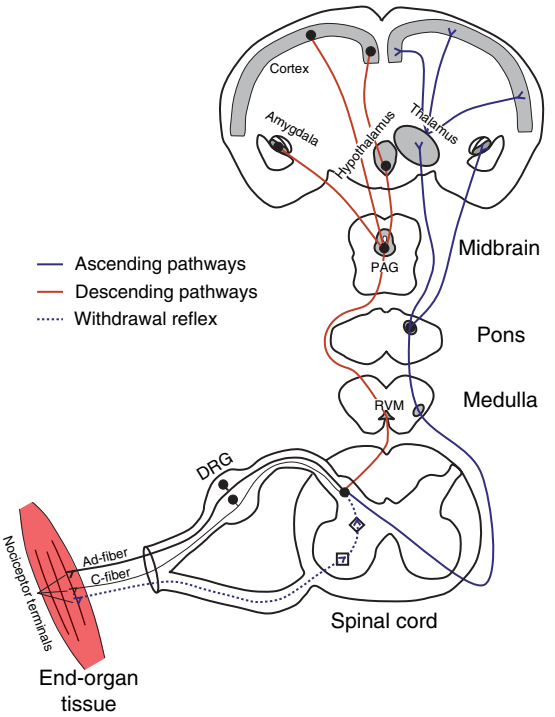

Figure 2 Illustration of anatomical pain pathways. Nociceptive information is transduced in nociceptor terminals located in the skin, muscle, and other end-organ tissues. Small-diameter unmyelinated C-fibers transmit the nociceptive information to neurons in the dorsal horn of the spinal cord, which in turn project to various brain regions via ascending pathways (solid blue line) that relay in the medulla, pons, and thalamus. Activation of motor neurons (□) by excitatory interneurons (◇) mediates the pain withdrawal reflex (dotted blue line). Descending pathways from the brain (red line), that relay in the periaqueductal gray matter (PAG) and rostroventromedial medulla (RVM), modulate nociceptive transmission in the dorsal horn. DRG, dorsal root ganglia.

The exact mechanism by which noxious stimuli are translated to neurophysiological signals in the peripheral nerve endings is not completely understood, but it is thought to involve the activation of proteins designed to convert noxious stimuli to depolarizing electric potentials. A family of receptors that has received increasing attention in recent years as major sensory transducers is the transient receptor potential (TRP) ion channel family. TRP channels are expressed in nociceptor terminals and transduce a wide range of thermal, mechanical, and chemical stimuli (Wang and Woolf, 2005; Story and Gereau, 2006). The TRPV1 receptor is activated by noxious heat ($\geq$43 °C), protons, or capsaicin (the pungent compound found in hot chili peppers); TRPV2 by noxious heat ($\geq$52 °C); TRPV4 by mechanical stimuli or protons; TRPM8 by cold ($\leq$23–28 °C), menthol, and icilin (a super cooling agent); and TRPA1 by noxious cold ($\leq$18 °C), icilin, mustard, cinnamon, or mechanical stimuli. Since TRP channels are activated within a range of noxious temperatures, and also by protons, chemicals, and intensive pressure, it has been hypothesized that these receptors have a prominent role in thermal, chemical, and mechanical nociception. Other receptors and ion channels such as the metabotropic glutamate receptors, the TREK-1 potassium channel, and the acid-sensing ion channels (ASIC) and dorsal root acid-sensing ion channels (DRASIC) have also been identified as potential sensory transducers in nociceptor terminals. The ultimate function of these channels is to provide a transduction current that depolarizes the nociceptor terminals and thereby initiate action potential firing in the nociceptor.

4.05.3.2 Pain Transmission

Nerve impulses generated in the periphery by noxious stimuli propagate via axons of DRG neurons to the dorsal horn of the spinal cord (**Figure 2**). The dorsal horn of the spinal cord is subdivided into six layers or laminae based on cytoarchitectural characteristics. C nociceptive afferents terminate mainly in laminae I and II, whereas Aδ afferents terminate mainly in laminae I and V (Basbaum and Jessell, 2000). Following DRG activation by peripheral noxious stimuli, neurotransmitters and peptides are released from DRG afferent terminals into the dorsal horn of the spinal cord. In the dorsal horn, these neurotransmitters and peptides act on postsynaptic receptors expressed by three types of dorsal horn neurons: excitatory interneurons, inhibitory interneurons, and projection neurons. Excitatory and inhibitory interneurons are part of the dorsal horn local circuitry; these neurons synapse on and modulate the firing of projection neurons, motor neurons, and other interneurons (**Figure 2**). Activation of motor neurons by excitatory interneurons mediates the classic pain withdrawal reflexes. Inhibitory interneurons can also exert presynaptic inhibition of nociceptive primary afferents, indirectly regulating the excitability of dorsal horn neurons by decreasing primary afferent input. Projection neurons, on the other hand, are the dorsal horn output neurons. These second-order neurons convey nociceptive information from the dorsal horn of the spinal cord to the thalamus, hypothalamus, and different brainstem structures via five major ascending pain pathways: the spinothalamic, spinohypothalamic, spinoparabrachial, spinoreticular, and spinomesencephalic tracts (Basbaum and Jessell, 2000; Almeida et al., 2004). These ascending pathways in turn project to various cortical and limbic structures such as the somatosensory cortex, cingulate cortex, insular cortex, prefrontal cortex, and amygdala (**Figure 2**). Sensory discriminative information about the localization, quality, and intensity of the noxious stimulus as well as the attentional, emotional, and cognitive components of pain are processed in these cortical and limbic structures (Almeida et al., 2004).

4.05.3.3 Pain Modulation

As discussed in the previous section, nociceptive transmission and synaptic efficacy in both primary afferent endings and dorsal horn neurons can be modulated by local circuits in the spinal cord. But this is not the only modulatory mechanism; the excitability of dorsal horn projection neurons and primary afferent endings can also be modulated by descending pathways from the brain that originate in the periaqueductal gray matter (PAG), project to the rostroventromedial medulla (RVM), and then terminate in the dorsal horn of the spinal cord (**Figure 2**).

Earlier studies focused on descending inhibition of spinal nociceptive processing, but now we know that descending modulatory pathways can be both inhibitory and facilitatory (Fields, 2000). Early in the 1990s, pioneering work by Howard Fields and colleagues identified three types of neurons in the RVM: on-cells, which facilitate nociceptive transmission; off-cells, which have a net inhibitory effect on nociceptive transmission; and neutral cells that show no nociception-related change in activity (Fields et al., 1983, 1991). *In vivo* electrophysiological recordings in the RVM revealed that on-cells discharge just prior to

withdrawal from a noxious stimulus, whereas off-cells pause during withdrawal from a noxious stimulus (Fields et al., 1983). Both types of cells are activated by electrical stimulation of the PAG and they both project directly to laminae I, II, and V of the dorsal horn, where nociceptive-specific neurons are located (Fields et al., 1995; Fields, 2000). Descending modulation of dorsal horn projection neurons and primary afferent endings seems to be mediated via activation or inhibition of excitatory and inhibitory local circuit interneurons in the dorsal horn. Physiologically, activation of descending inhibitory control upon peripheral noxious stimuli seems to provide a negative feedback mechanism that decreases nociceptive transmission at the level of the spinal cord, whereas activation of descending facilitatory pathways has been proposed to contribute to the development and maintenance of hyperalgesia and allodynia (Helmstetter and Tershner, 1994; Fields, 2000; Gebhart, 2004).

4.05.3.4 Pain Perception

The mechanisms of transduction, transmission, and modulation of noxious sensory stimuli described in the previous sections offer an explanation for how noxious sensory stimuli are translated to neurophysiological signals and how these signals are then propagated to and modulated by the CNS. However, these neural processes do not explain how certain stimuli are ultimately perceived as painful. The perception of pain is more than just direct processing of sensory stimuli. Clinical studies have demonstrated that the processes underlying pain perception comprise complex behavioral, psychological, and emotional factors (Turk, 2003; Lewandowski, 2004). Social and environmental factors influence the perception of pain, as do past experience and culture. Consequently, an apparently similar stimulus can generate enormous individual differences in pain perception. Several questions thus arise. How does pain perception occur? What are the neural mechanisms underlying how we perceive pain?

Projections from ascending nociceptive pathways to various cortical and limbic structures such as the cingulate cortex, insular cortex, prefrontal cortex, and amygdala have been described (Almeida et al., 2004) (**Figure 2**). Because of the long-standing role of these cortical and limbic structures in cognitive, emotional, and motivational processes, it has been proposed that their activation upon noxious stimuli is involved in mediating the negative emotional reactions to pain that can result in activity avoidance and escape behaviors (Coleman-Mesches and McGaugh, 1995; Johansen et al., 2001; Gao et al., 2004; Johansen and Fields, 2004; Lei et al., 2004; Tang et al., 2005). Much progress has been made in recent years in identifying brain regions and potential neural mechanisms underlying the cognitive and affective components of pain. This progress has been limited, however, by the methodological and conceptual difficulties associated with studying a subjective process such as pain perception, which becomes even more technically challenging when using animal models. The emergence of novel brain imaging techniques with better image quality and resolution has allowed researchers to perform studies that examine the neuroanatomical basis of different cognitive and emotional components of pain in humans.

4.05.4 Pain Sensitization

Under normal physiological conditions, pain acts as an early warning system to prevent injury. Many acute pain responses are simple reflexes (**Figure 2**). A classic example is the reflexive withdrawal experienced by individuals when they accidentally touch a hot surface. That immediate reflexive withdrawal from the heat source protects the body from burning. This physiological experience encompasses all of the pain components described in the previous section. First, the heat stimulus is translated to a neurophysiological signal in peripheral nerve endings, probably via the activation of one of the TRP ion channels. The nociceptive information is then transmitted to the dorsal horn of the spinal cord, where several processes take place:

1. Interneurons in the dorsal horn activate motor neurons in the ventral horn that mediate the withdrawal reflex.
2. Projection neurons send the nociceptive information to the somatosensory cortex, where information about the location, intensity, and quality of the stimulus is processed.
3. Projection neurons in the dorsal horn convey the nociceptive information to different cortical and limbic structures such as the anterior cingulate cortex, the insular cortex, and the amygdala, where pain awareness and associative learning occur.

The neural processes described in this example occur under normal physiological conditions, in uninjured tissue, when pain acts as a warning system to prevent

injury. When injury does occur, however, pain serves a slightly different but still physiologically adaptive role. After injury, hypersensitivity to both noxious and previously innocuous stimuli develops. Hypersensitivity after injury can be seen as a memory of the injury that engages a heightened protective system developed to prevent further injury, which could interfere with the healing process. A good example of this type of pain is the hypersensitivity experienced when a bone is broken. In this case, normally innocuous movements or stimuli such as light touch become painful. Movements that would otherwise be normal would interfere with the healing process of a broken bone; thus pain, in this case, serves a protective role to support the healing process.

After an injury, the responses of the sensory system to a given stimulus also increase. These increases can be seen at both the peripheral and central levels and are thought to underlie pain hypersensitivity. Heightened neuronal responses of the sensory system are commonly referred to as sensitization. Pain sensitization is characterized by a decreased threshold for action potential firing, increased responsiveness to a given stimulus, and receptive field enlargement. Different types of sensitization have been described in the peripheral and central nervous systems. Sensitization can occur as a consequence of an injury such as in the broken bone example; in this case, sensitization is an adaptive process and should subside as the injury heals. However, there are many instances in which sensitization can be maladaptive, outlasting the initial injury or with an unknown pathology. This pathological sensitization is thought to underlie chronic pain conditions. The properties, characteristics, and potential mechanisms of these various kinds of sensitization will be discussed in the following sections.

4.05.4.1 Peripheral Sensitization

Following tissue injury, multiple inflammatory mediators, such as substance P, prostaglandin E_2, bradykinin, nerve growth factor, tumor necrosis factor, and glutamate, are released by mast cells, macrophages, immune cells, and injured cells at the site of injury (Bhave and Gereau, 2004). Release of these inflammatory mediators initiates a cascade of events that leads to increased responsiveness of nociceptors, a phenomenon called peripheral sensitization (**Figure 3**).

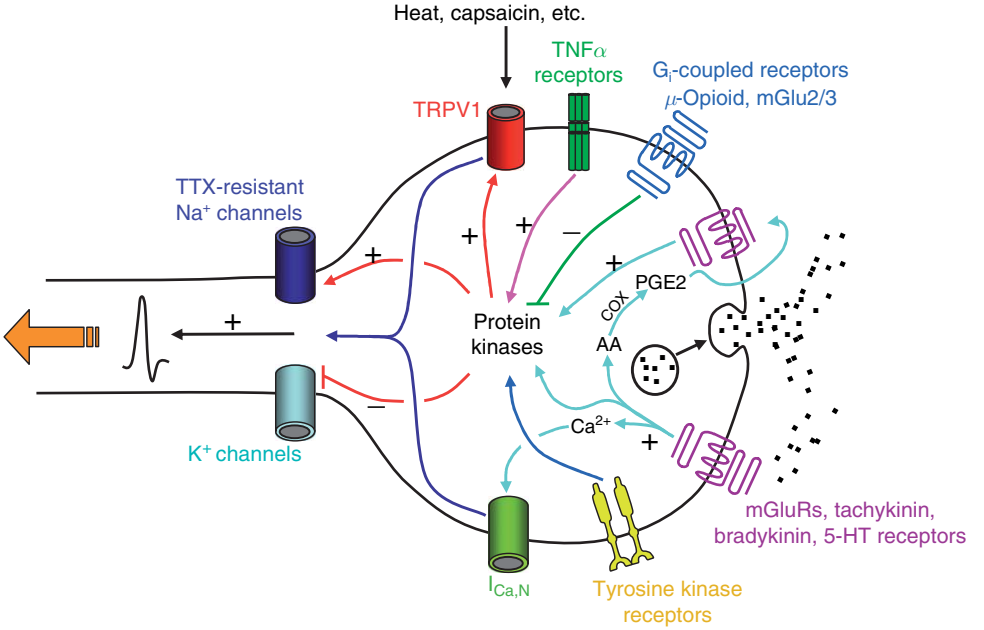

Figure 3 Potential mechanisms underlying peripheral sensitization. Following tissue injury, multiple inflammatory mediators are released at the site of injury and activate several membrane receptors. Subsequent activation of protein kinases and the phosphorylation of ion channels increase the excitability of dorsal root ganglia (DRG) neurons and thus contribute to peripheral sensitization. Sensitization of DRG responses is thought to underlie the reduced threshold that is commonly observed after tissue injury. 5-HT, serotonin; AA, arachidonic acid; Ca^{2+}, calcium; COX, cyclooxygenase; $I_{Ca,N}$, calcium-activated nonselective cation currents; K^+, potassium; mGluR, metabotropic glutamate receptor; PGE2, prostaglandin E2; TNFα, tumor necrosis factor alpha; TRPV1, transient receptor potential V1; TTX, tetrodotoxin.

Peripheral sensitization could result from a change in the cell-surface expression of ion channels that contribute to sensory transduction or action potential generation or from posttranslational modifications of ion channels that modulate the channel properties without necessarily affecting their cell-surface expression. Inflammatory mediators can directly activate ion channel proteins or indirectly activate intracellular pathways via the activation of G-protein-coupled receptors or receptor tyrosine kinases. Activation of intracellular pathways can result in acute modification of ion channels or in the activation of transcription factors, with a resultant increase in transcription and translation of new proteins. Transcriptional, translational, and posttranslational changes can therefore underlie the phenomenon of peripheral sensitization.

4.05.4.1.1 Acute modification of primary sensory neurons

Increased responsiveness of nociceptors to a given stimulus can be observed fairly quickly after tissue injury. Given that transcriptional and translational processes take longer to occur than the development of peripheral sensitization, it has been proposed that the initial stages of peripheral sensitization involve posttranslational modifications of ion channels or transducer molecules and not transcriptional or translational processes. On the other hand, maintenance of peripheral sensitization is thought to involve all three of these processes; that is, gene transcription, protein translation, and posttranslational modifications.

Posttranslational modifications such as phosphorylation can alter the biophysical properties of ion channels that are already inserted in the membrane at the time of injury. Many posttranslational modifications of this type are known to occur and to modulate the excitability of DRG neurons (Bhave and Gereau, 2004). An example of a posttranslational modification that occurs in DRG neurons and that sensitizes the responses of the neuron to a given stimulus is the phosphorylation of the sensory transducer ion channel TRPV1. Phosphorylation of TRPV1 by protein kinase A (PKA), protein kinase C (PKC), and calcium/calmodulin-dependent protein kinase II (CaMKII) results in sensitization of the channel's responses to capsaicin, heat, or pH and also in blockade of desensitization. These alterations in the biophysical properties of the channel could therefore contribute to the reduced threshold for noxious stimuli commonly observed after tissue injury *in vivo*. A caveat of most of the studies that have examined posttranslational modifications in DRG neurons is that the experiments have been conducted in *in vitro* preparations. It is still unknown whether these posttranslational modifications occur after tissue injury and if they contribute to pain hypersensitivity. The development of antibodies specific for modified proteins (i.e., phosphorylated TRPV1) would help answer this type of question.

As in many neurons in the CNS, it is also possible that after injury, posttranslational modifications of ion channels in DRG neurons promote the insertion of ion channels into the membrane. Increased availability of ion channels for activation would increase neuronal depolarizing responses to a given stimulus without necessarily altering the biophysical properties of the individual channels. Although not studied in DRG neurons thus far, trafficking of ion channels is a phenomenon that has received a lot of attention in recent years as one of the major mechanisms used by neurons to control synaptic responses. It is therefore very likely that ion channel trafficking in DRG neurons also underlies a component of peripheral sensitization.

In order for posttranslational modifications to occur, intracellular signaling molecules such as protein kinases need to be activated in DRG neurons in response to tissue injury (**Figure 3**). Activation of receptor tyrosine kinases and G-protein-coupled receptors by inflammatory mediators, such as nerve growth factor (NGF) or glutamate, trigger the activation of intracellular biochemical cascades that are initiated by the production or release of second messengers (Crawford et al., 1997; Bhave and Gereau, 2004). Examples of these second messengers are cyclic adenosine monophosphate (cAMP), diacylglycerol (DAG), inositol 1,4,5-triphosphate (IP_3), calcium (Ca^{2+}), and arachidonic acid (AA). Each of these second messengers either activates protein kinases that directly phosphorylate target proteins such as ion channels or produce other second messengers that can also directly bind to and modulate target proteins. AA is a second messenger system that has been extensively studied in DRG neurons (**Figure 3**). AA is released inside the cell in response to the activation of phospholipase A_2 or phospholipase C, which are enzymes that are activated by G-protein-coupled receptors. AA is oxidized by cyclooxygenases to produce prostaglandins and thromboxanes. Prostaglandins diffuse out of the cell and act on nearby prostanoid receptors. Activation of prostanoid receptors triggers biochemical cascades that mediate peripheral sensitization. The mechanism of action of nonsteroidal

anti-inflammatory drugs commonly used to treat inflammatory pain is to inhibit cyclooxygenase and subsequently reduce prostaglandin synthesis. It has been demonstrated that administration of these anti-inflammatory drugs prior to injury (i.e., before a surgical procedure) is more effective at decreasing inflammatory pain than administration after injury. Administration of these anti-inflammatory drugs prior to injury presumably prevents the development of peripheral sensitization.

4.05.4.1.2 Long-term modifications of primary sensory neurons

In addition to acutely modulating the biophysical properties of ion channels via posttranslational modifications, activation of intracellular signaling cascades after injury can also result in the activation of transcription factors that regulate gene expression and can subsequently result in the synthesis of new proteins such as ion channels, sensory transducers, and/or neuromodulators.

Changes in protein expression after injury can last for days, weeks, or even longer. For this reason, increases in protein expression have been proposed as one of the main mechanisms involved in the maintenance of persistent pain. For example, following persistent inflammation, there is an increase in mRNA levels of the tetrodotoxin (TTX)-resistant sodium channel Nav1.8 in small-diameter DRG neurons (Tanaka et al., 1998). This increase in mRNA levels is accompanied by an increase in the amplitude and density of TTX-resistant sodium currents, suggesting that Nav1.8 protein levels are also increased. Increased sodium currents could in turn increase the excitability of DRG neurons by decreasing the action potential threshold, thus producing the increased response to a fixed stimulus that typifies peripheral sensitization.

Translational changes independent of changes in transcription have also been observed in DRG neurons after inflammation and are thought to contribute to hypersensitivity (Ji et al., 2002b). Increased levels of the sensory transducer TRPV1 are observed at the protein level, but not at the mRNA level, in DRG neurons after persistent inflammation. These inflammation-induced increases in TRPV1 are dependent on NGF-induced activation of p38 mitogen-activated protein kinase (MAPK) and are thought to be mediated via downstream activation of a translational regulator such as eIF4E. In general, transcription and translational processes in DRG neurons after injury are thought to be initiated by the activation of the same receptors and second messenger pathways that mediate posttranslational modifications, but it is thought to require repeated stimulation and/or prolonged action of these second messenger systems.

As discussed in an earlier section of this chapter, nociceptive information is conveyed to the dorsal horn of the spinal cord via Aδ- and C-nociceptive fibers, whereas nonnoxious tactile information is transmitted to the dorsal horn by large-diameter Aβ-sensory fibers. Pain sensitization can be mediated by cellular and molecular changes that occur in peripheral nociceptive fibers that lead to increased responsiveness of nociceptors. Enhanced nociceptor responses in turn lead to increased release of neurotransmitters and neuromodulators from central DRG terminals, subsequently increasing dorsal horn excitability. In addition to these plastic phenomena, a phenotypic switch in Aβ-sensory fibers has also been observed after injury (Neumann et al., 1996). Increased excitability of dorsal horn neurons after injury is thought to be partly mediated by an elevation in the release of substance P by primary nociceptive afferents in the dorsal horn. Under normal conditions, substance P is exclusively expressed in nociceptive afferents; however, part of the phenotypic switch of Aβ-sensory fibers includes that they start synthesizing substance P, which is a peptide not normally expressed by these neurons. Thus, both *de novo* substance P protein synthesis in Aβ-sensory fibers and increases in the translation of this peptide in nociceptive fibers are thought to contribute to the maintenance of pain sensitization by increasing the excitability of dorsal horn neurons. Recruitment of Aβ-sensory fibers is also thought to mediate mechanical allodynia, which is pain to previously innocuous tactile stimulation (Neumann et al., 1996).

4.05.4.2 Sensitization in the Dorsal Horn of the Spinal Cord

Activity-dependent increases in excitability and synaptic transmission occur in the dorsal horn of the spinal cord in response to a short burst of repeated nociceptive input. This spinal sensitization is traditionally referred to as central sensitization to distinguish it from peripheral sensitization. Windup, spinal long-term potentiation (LTP), and classic central sensitization are three different forms of pain-induced sensitization that have been described in the dorsal horn and that are thought to underlie persistent pain. Although these three types of sensitization are considered three different phenomena, their

cellular and molecular mechanisms share many common properties. All these forms of sensitization are blocked or decreased by ablation of neurokinin 1 (NK1) receptor-expressing projection neurons in the dorsal horn, and they all depend on glutamatergic synaptic transmission. Some of these forms of sensitization are restricted to the activated synapse (homosynaptic), whereas others spread to adjacent synapses (heterosynaptic). The properties, characteristics, and potential mechanisms of windup, spinal LTP, and classic central sensitization will be discussed in the following sections.

4.05.4.2.1 Windup: Short-term sensitization of dorsal horn neurons

Normal healthy individuals report higher pain intensity ratings to electrical, mechanical, or thermal stimuli of constant intensity as the frequency of the stimulus delivered increases (Herrero et al., 2000). This change in reported pain is considered a behavioral correlate of windup, which is a progressive and frequency-dependent increase in the excitability of dorsal horn neurons that is evoked by a short train of repeated stimulation of nociceptive afferents. Windup can therefore be considered a cellular memory of nociceptor input and one form of sensitization of dorsal horn neurons. Experimentally, windup can be induced in the dorsal horn by stimulating nociceptive afferents at frequencies between 0.2 and 20 Hz, with the greatest degree of windup generated by 1- to 2-Hz stimulations (Herrero et al., 2000). Stimulations lower than 0.2 Hz fail to induce windup, whereas stimulations higher than 20 Hz induce habituation of the response, or winddown. A characteristic property of windup is that, unlike the other forms of central sensitization, it is only manifested during the course of the stimulus (Ji et al., 2003).

C-fiber stimulation generates slow excitatory synaptic potentials in the dorsal horn. Windup is thought to result from the temporal summation of these slow excitatory synaptic potentials (Herrero et al., 2000; Ji et al., 2003). The current view is that windup is mediated by cumulative depolarization of the neuron induced by the activation of alpha-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA) receptors after repeated presynaptic stimulation. AMPA-mediated depolarization triggers the removal of the voltage-dependent Mg^{2+} block of *N*-methyl-D-aspartate (NMDA) receptors, resulting in further increase of temporal summation. In addition, activation of voltage-gated Ca^{2+} channels by neuronal depolarization has been proposed to generate plateau potentials that also contribute to the expression of windup (Herrero et al., 2000; Ji et al., 2003).

As discussed in previous sections, nociceptor stimulation results in presynaptic release of neurotransmitters and peptides in the dorsal horn of the spinal cord. Glutamate is the primary excitatory neurotransmitter that mediates synaptic transmission between peripheral afferents and the dorsal horn, and its release has been shown to be necessary for the development of windup. Presynaptic release of neuropeptides such as substance P and calcitonin gene-related peptide (CGRP) are also crucial for the expression of windup (Herrero et al., 2000). At the postsynaptic level, windup seems to depend on the activation of G-protein-coupled receptors and NMDA receptor activation, the latter being important for both the induction and the maintenance of windup (Herrero et al., 2000; Ji et al., 2003). Windup can be recorded in acute spinal cord slice preparations, demonstrating that spinal intersegmental and supraspinal modulation are not necessary for its development. At the same time, infusion of opioids or serotonin into the spinal cord inhibits the generation of windup, suggesting that inputs from supraspinal structures could potentially modulate windup (Herrero et al., 2000).

Under normal physiological conditions, windup is expressed as a homosynaptic form of sensitization; thus it can be induced by stimulation of C-fibers but not by A-fiber stimulation (Thompson et al., 1994; Ji et al., 2003). After inflammation, several plastic changes modulate the induction of windup (Thompson et al., 1994; Herrero et al., 2000) (**Figure 4**). Thus, stimulation of C-fibers that innervate the inflamed area produces enhanced windup, stimulation of afferent fibers that innervate uninflamed areas adjacent to the inflamed area or of Aβ-fibers that normally fails to induce windup can now induce windup, and a general reduction in the threshold for windup is observed. The physiological significance of windup is a matter of debate. The high-frequency synchronized stimulation used to induce windup does not naturally occur in nociceptor afferents in response to noxious stimulation; therefore, the physiological relevance of windup has been questioned. At the same time, because a windup-like phenomenon has been observed clinically, it is still possible that some form of windup occurs in response to repeated noxious stimulation of constant intensity, contributing to hypersensitivity.

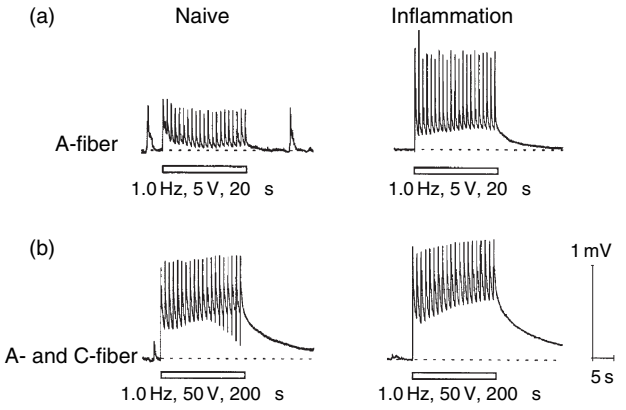

Figure 4 Effects of tissue injury on windup. Summated ventral root potentials (VRP) following low-frequency (1-Hz) repetitive stimulation of A-fibers or both A- and C-fibers recorded in spinal cords from naive or ultraviolet (UV)-injured animals. The horizontal bar indicates the stimulation period. (a) Stimulation of A-fibers (5 V, 20 μs) in naive animals failed to sustain a summated depolarization (left panel). An identical stimulation in spinal cords from UV-injured animals induced a robust and sustained depolarization (right panel) indicating that, after injury, windup can be induced by stimulation of A-fibers that fail to induce windup under normal conditions. (b) Stimulation of both A- and C-fibers (50 V, 200 μs) induced a robust and sustained depolarization in spinal cords from naive animals (left panel). An identical stimulation in spinal cords from UV-injured animals increased the amplitude of the sustained depolarization (right panel) indicating that, after injury, windup is enhanced. Adapted from Thompson SW, Dray A, and Urban L (1994) Injury-induced plasticity of spinal reflex activity: NK1 neurokinin receptor activation and enhanced A- and C-fiber mediated responses in the rat spinal cord in vitro. *J. Neurosci.* 14: 3672–3687.

4.05.4.2.2 Spinal long-term potentiation

Activity-dependent synaptic LTP and long-term depression (LTD) of excitatory postsynaptic potentials (EPSPs) can both be evoked in dorsal horn neurons following brief high-frequency repeated stimulation of presynaptic central DRG afferents (Randic et al., 1993). The development of LTP or LTD seems to depend on the postsynaptic membrane potential, with hyperpolarized potentials (−85 mV) favoring the development of LTD and more depolarized potentials (−70 mV) favoring the development of LTP. Both LTP and LTD are homosynaptic forms of sensitization; thus, potentiation or depression of EPSPs is normally restricted to the activated synapse and not other adjacent synapses. LTP or an LTP-like phenomenon can be induced in animals and in humans and has been strongly correlated with pain behavior. Synchronized high-frequency stimulation of peripheral afferents in intact animals or in acute spinal cord slice preparations can result in long-lasting increases in evoked postsynaptic responses of dorsal horn neurons (Randic et al., 1993; Sandkuhler and Liu, 1998). Similarly, in humans, high-frequency electrical stimulation of cutaneous afferents, using stimulation protocols that resemble the ones used to induce experimental LTP in animals, has been shown to induce long-term increases in reported pain sensitivity (Klein et al., 2004). The cellular and intracellular signaling mechanisms that underlie dorsal horn LTP and those that underlie injury-induced pain hypersensitivity are strikingly similar. For this reason, dorsal horn LTP has been commonly used as a cellular model of afferent-induced hyperalgesia.

LTP induction in the dorsal horn is dependent on a rise in free intracellular calcium in the postsynaptic cell as well as on postsynaptic activation of AMPA, NMDA, metabotropic glutamate receptors (mGluRs), and low-threshold T-type voltage-gated calcium channels. Activation of various intracellular signaling molecules such as PKA, PKC, extracellular signal-regulated protein kinase (ERK), Src tyrosine kinase (Src), and CaMKII in dorsal horn neurons is also necessary for the induction of LTP (Sandkuhler, 2000; Sandkuhler et al., 2000; Woolf and Salter, 2000; Ikeda et al., 2003). The activation of these intracellular molecules leads to posttranslational changes of target proteins such as ion channels, membrane receptors, and/or accessory subunits. Posttranslational modifications modulate the cell-surface expression and function of these proteins, ultimately increasing synaptic efficacy in the dorsal horn. Activation and posttranslational modifications of neuropeptide receptors such as NK1 and tachykinin receptors in the dorsal horn are also necessary for the induction of LTP (Sandkuhler et al., 2000). An interesting feature of dorsal horn LTP is that it can only be induced in a subpopulation of dorsal horn neurons.

LTP is preferentially induced in dorsal horn projection neurons that express the NK1 receptor but not in dorsal horn neurons that do not express this receptor (Ikeda et al., 2003). These findings, together with behavioral studies that show that ablation of NK1-expressing neurons in the dorsal horn attenuates the development of inflammation-induced hypersensitivity (Mantyh et al., 1997), suggest that NK1-expressing projection neurons are an important site of nociceptive plasticity in the dorsal horn.

Despite the cellular and molecular similarities between dorsal horn LTP and injury-induced pain hypersensitivity, the biological significance of dorsal horn LTP has long been questioned. High-frequency synchronized stimulation like the one used to induce experimental LTP does not resemble the low-frequency unsynchronized afferent barrage induced physiologically by natural noxious stimuli. In addition, although LTP has been shown to occur in the dorsal horn *in vivo* in response to natural noxious stimulation, this naturally occurring LTP can be induced in spinalized animals but not in intact animals. A recent study by Ikeda and colleagues put an end to this debate (Ikeda et al., 2006) (**Figure 5**). In their elegant study, Ikeda and colleagues showed that low-frequency stimulation (LFS) of C-fibers, at a frequency that resembles the afferent barrage that occurs during inflammation, induces a robust synaptic LTP of evoked EPSCs in NK1-expressing dorsal horn neurons that project to the periaqueductal grey (PAG). Ikeda et al. further demonstrated that LTP can be induced *in vivo*, in intact animals, by a natural low-frequency afferent barrage that is induced by peripheral inflammation. The mechanisms underlying the induction of LFS-induced LTP in the dorsal horn resemble those of injury-induced hyperalgesia (Ikeda et al., 2006), further supporting the idea that LTP of synaptic strength in the dorsal horn underlies inflammation-induced pain hypersensitivity.

4.05.4.2.3 Classic central sensitization

Following tissue injury and under pathological pain conditions, second-order nociceptive-specific neurons in the dorsal horn of the spinal cord show a decreased threshold for action potential firing, increased responsiveness to a given stimulus, and receptive field enlargement (Woolf, 1983; Woolf and Wall, 1986; Cook et al., 1987; Ji et al., 2003) (**Figure 1**). These electrophysiological changes are collectively referred to as central sensitization and, as peripheral sensitization, are thought to underlie the development of chronic pain. Unlike windup and dorsal horn LTP, central sensitization is considered a heterosynaptic form of sensitization, as these electrophysiological changes occur in both the activated synapse and also in adjacent synapses. Sensitization and recruitment of synapses adjacent to the activated synapses are thought to contribute to the receptive field enlargement that is characteristic of central sensitization and are thought to underlie the pain hypersensitivity that is experienced in noninjured areas following an associated tissue injury (secondary allodynia).

The molecular mechanisms underlying central sensitization and dorsal horn LTP are very similar. As is the case for dorsal horn LTP, central sensitization selectively occurs in NK1-expressing neurons in the dorsal horn and depends on the activation of AMPA, NMDA, and mGluRs. Multiple intracellular signaling pathways, such as ERK, PKA, PKC, CaMKII, and Src, are activated in dorsal horn neurons downstream of the activation of these receptors (Ji et al., 2003). Activation of an intracellular signaling pathway can exert both short-term and long-term neuronal modifications. Acute posttranslational modifications of ion channels are known to occur fairly quickly after the activation of intracellular molecules and are thought to mediate the early component of central sensitization, whereas sustained activation of intracellular molecules commonly results in their translocation to the nucleus, where they can activate different transcription factors. Transcription and translation of new proteins, in combination with sustained posttranslational modifications of ion channels, are thought to contribute to the maintenance of central sensitization.

The ERK signaling cascade is a good example of an intracellular signaling pathway that exerts both short-term and long-term neuronal modifications that contribute to central sensitization (**Figure 6**). ERK is activated in dorsal horn neurons shortly after tissue injury, downstream of the activation of a diversity of membrane receptors (Ji et al., 1999; Karim et al., 2001; Pezet et al., 2002; Adwanikar et al., 2004; Kawasaki et al., 2004; Slack et al., 2004; Kawasaki et al., 2006; Svensson et al., 2006). ERK directly phosphorylates the potassium channel Kv4.2, decreasing Kv4.2-mediated potassium currents, thereby increasing neuronal excitability (Hu and Gereau, 2003; Hu et al., 2003, 2006). ERK also regulates phosphorylation and the activity of the NR1 NMDA receptor subunit (Slack et al., 2004). In addition to these acute modifications of ion channels, ERK can also translocate to the nucleus, where it phosphorylates and activates the cAMP response element binding protein (CREB), which in turn promotes the transcription of immediate early genes such as c-fos

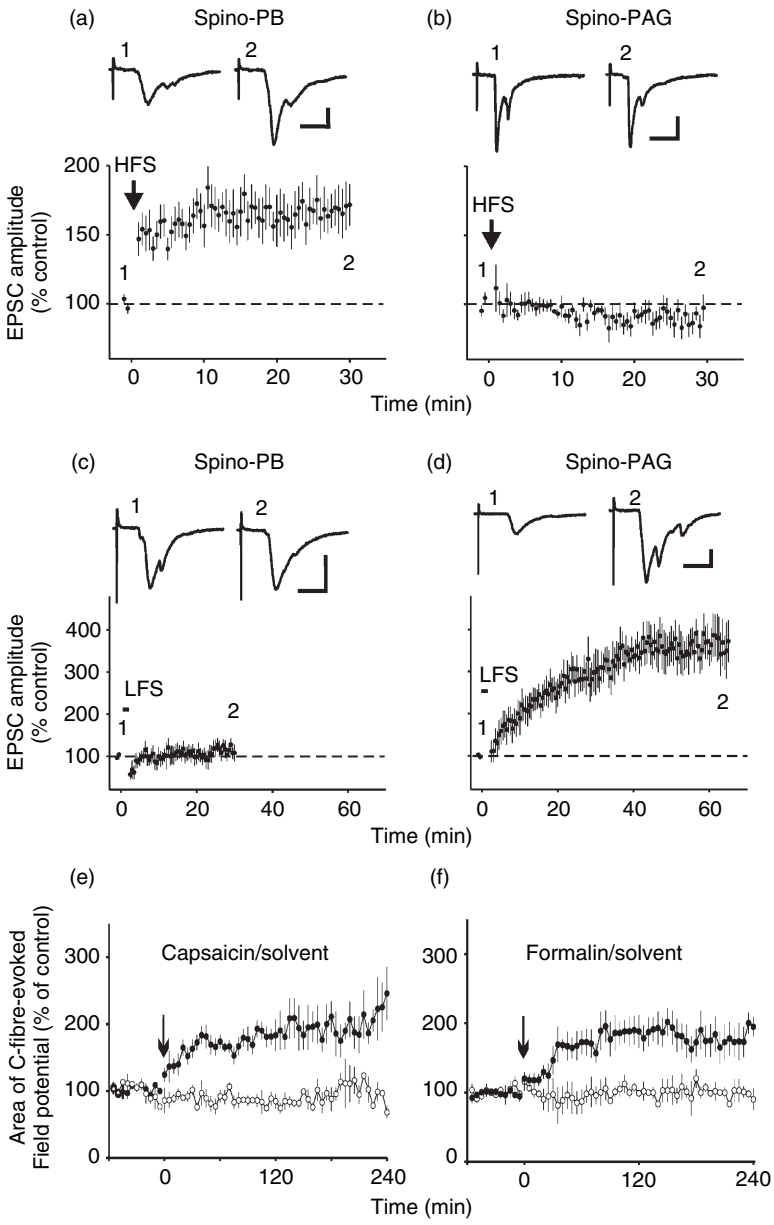

Figure 5 Spinal LTP can be induced by low-frequency afferent barrage evoked by electrical stimulation in vitro or inflammation of peripheral tissue in vivo. (a, b) High-frequency stimulation (HFS) of C-fibers selectively induced LTP in parabrachial (PB)-projecting but not in periaqueductal gray (PAG)-projecting lamina I neurons. (c, d) Low-frequency stimulation of C-fibers selectively induced LTP in PAG-projecting but not in PB-projecting lamina I neurons. Insets: Representative C-fiber-evoked excitatory postsynaptic currents (EPSCs) before and after C-fiber high frequency (a, b) or low-frequency (c, d) stimulation. Calibration bars: 20 ms/200 pA. (e, f) Inflammation induces LTP in the dorsal horn of the spinal cord *in vivo*. Extracellular recordings in the superficial dorsal horn following electrical stimulation of the sciatic nerve in vivo in anesthetized animals. Intraplantar injection (indicated by the arrow) of capsaicin (e) or formalin (f) induced spinal LTP *in vivo* (solid circles). Intraplantar injection of vehicle (open circles) failed to induce spinal LTP. Adapted from Ikeda H, Stark J, Fischer H, et al. (2006) Synaptic amplifier of inflammatory pain in the spinal dorsal horn. *Science* 312: 1659–1662.

and of other late-expressing genes such as prodynorphin and NK1 (Ji et al., 2002a; Kominato et al., 2003; Kawasaki et al., 2004; Song et al., 2005). Blockade of ERK activation in the dorsal horn decreases injury-induced hypersensitivity, NR1 phosphorylation, mGluR5-mediated increases in excitability, and CREB-mediated transcriptional regulation of c-fos, NK1, and prodynorphin.

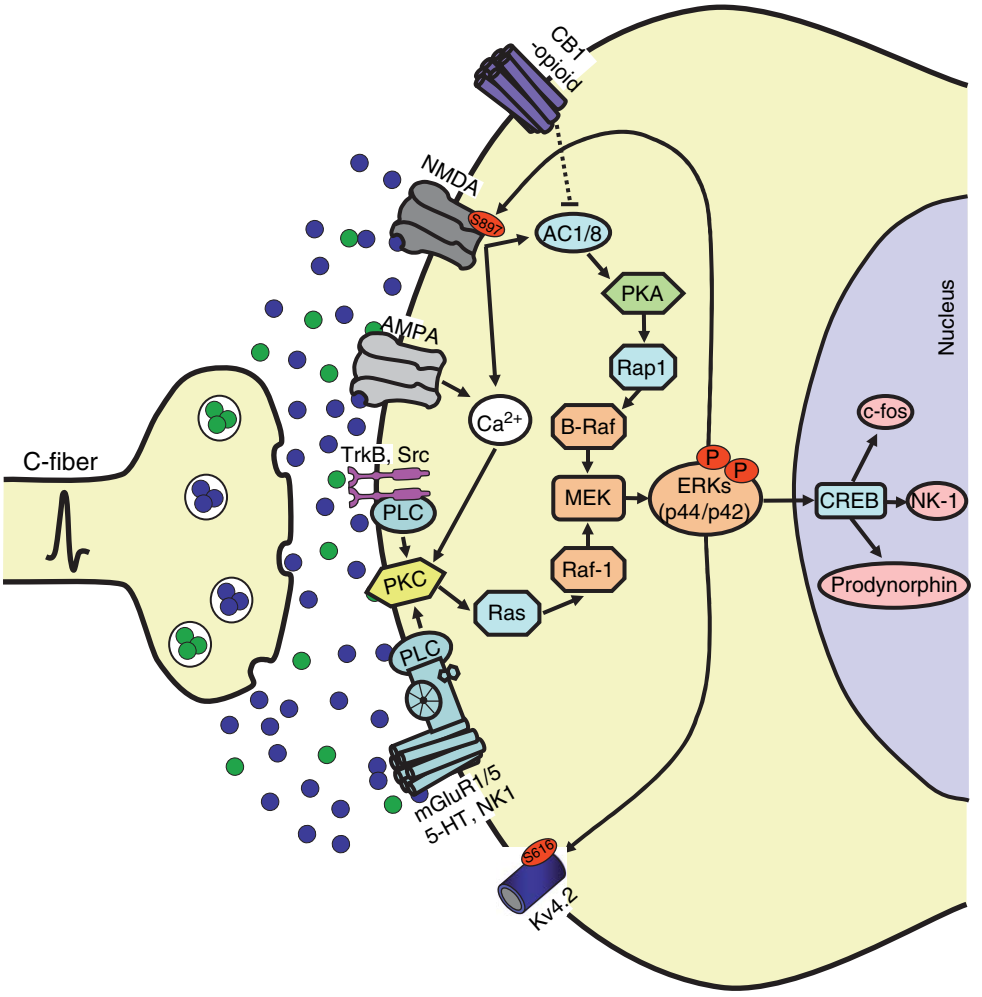

Figure 6 The extracellular signal-regulated protein kinase (ERK) signaling cascade in the dorsal horn contributes to central sensitization. Numerous cell-surface signals can activate the ERK signaling cascade in dorsal horn neurons in response to tissue injury. ERK directly phosphorylates the potassium channel Kv4.2 at Serine-616 (S616) and regulates phosphorylation of the NMDA receptor subunit NR1 at Serine-897 (S897). ERK can also translocate to the nucleus and activate CREB-dependent transcription of c-fos, NK1, and prodynorphin. 5-HT, 5-hydroxytryptamine (serotonin receptor); AC1/8, adenylyl cyclases 1 and 8; AMPA, alpha-amino-3-hydroxy-5-methyl-4-isoxazole-propionic acid receptor; B-Raf, B-Raf kinase; Ca^{2+}, calcium; CB1, cannabinoid receptor 1; CREB, cAMP-response element binding protein; MEK, mitogen-activated protein kinase/ERK; mGluR1/5, metabotropic glutamate receptors 1 and 5; NK1, neurokinin 1 receptor; NMDA, *N*-methyl-D-aspartate receptor; PKA, protein kinase A; PKC, protein kinase C; PLC, phospholipase C; Raf1, Raf-1 kinase; Rap1, repressor activated protein 1; Ras, Ras GTPase; Src, Src tyrosine kinase; TrkB, tyrosine kinase receptor B.

In addition to the increased excitatory transmission observed in the dorsal horn during central sensitization, a decrease in inhibitory tone also contributes to the development and maintenance of central sensitization. Decreased synaptic efficacy in local inhibitory circuits seems to result from decreased gamma-aminobutyric acid (GABA) release, decreased receptor expression and function, and, although a controversial hypothesis, by a loss of inhibitory interneurons. Decreases in depolarization-induced GABA release are observed in the dorsal horn in a nerve injury model (Lever et al., 2003). Reduction in primary afferent-evoked inhibitory transmission and a decrease in the expression of GABA receptors and the GABA-synthesizing enzyme glutamic acid decarboxylase (GAD) are also observed after tissue or nerve injury (Castro-Lopes et al., 1995; Moore et al., 2002). In addition, intrathecal administration of GABA receptor antagonists has been shown to mimic central sensitization, further supporting

the hypothesis that decreased GABAergic tone contributes to the development of central sensitization (Sivilotti and Woolf, 1994). The dramatic loss of GABA and GAD immunostaining that is observed in the dorsal horn after nerve injury has led to the hypothesis that excitotoxic death of GABAergic neurons might contribute to decreased inhibitory tone after tissue injury. This is a controversial hypothesis, however, because stereological analysis of neuronal packing density and staining with apoptotic markers have failed to demonstrate a significant loss of neurons after tissue or nerve injury (Polgar et al., 2003, 2004, 2005). At the same time, the lack of changes in stereological studies is also debatable (Scholz et al., 2005). Despite this debate, it is well accepted that a decrease in GABAergic tone contributes to central sensitization; therefore, increasing GABAergic transmission in the dorsal horn could offer an alternative to alleviate pain. Preclinical studies in rodents have indeed demonstrated that transplanting neuronal cells bioengineered to synthesize GABA into the lumbar subarachnoid space decreases hypersensitivity in animal models of chronic neuropathic pain (Eaton et al., 2007).

4.05.4.3 Sensitization in Supraspinal Structures

Plastic changes in neuronal responses to noxious stimuli after tissue injury have also been observed and studied in supraspinal structures. Descending modulatory pathways are activated after tissue injury and can inhibit or facilitate the excitability of dorsal horn neurons. Descending inhibition or facilitation of pain transmission seems to depend on the nature of the stimulus, the environment in which the stimulus is applied, and the behavioral state of the animal. Supraspinal structures thus provide yet another level of modulation of pain processing. Pain-induced sensitization of the rostroventral medulla (RVM), the anterior cingulate cortex (ACC), and the amygdala has been described. The mechanisms of sensitization in these three supraspinal structures will be discussed in this section.

4.05.4.3.1 Rostroventral medulla

The RVM is a major source of descending modulation to the spinal dorsal horn. Realization that stimulation of the RVM could produce hyperalgesia led to the hypothesis that descending facilitation of nociceptive transmission could underlie injury-induced hypersensitivity (Zhuo and Gebhart, 1990). Many studies have followed up this observation and there is now substantial evidence that supports that sensitization of the RVM indeed contributes to the maintenance of injury-induced hypersensitivity (Porreca et al., 2002). Three types of neurons have been identified in the RVM. On-cells discharge just prior to withdrawal from a noxious stimulus, off-cells pause during withdrawal from a noxious stimulus, and neutral-cells show no nociception-related change in activity (Fields et al., 1983). Activation of on-cells has been proposed to facilitate nociceptive transmission in the dorsal horn. Consistent with this hypothesis, inflammation-induced persistent nociceptive input that is associated with hypersensitivity has been shown to increase the activity of on-cells in the RVM in an NMDA-dependent manner (Xu et al., 2007). Furthermore, chemical inactivation of the RVM or infusion of NMDA receptor antagonist into the RVM reduces injury-induced hypersensitivity (Urban et al., 1999; Wei and Pertovaara, 1999; Porreca et al., 2002). Interestingly, after inflammation, neutral-cells in the RVM that were initially unresponsive to noxious stimuli undergo a phenotypic switch, exhibiting response profiles that are characteristic of either on- or off-cells (Miki et al., 2002). Recruitment of neutral-cells might therefore contribute to RVM sensitization after inflammation. RVM sensitization has also been linked to the hypersensitivity that is commonly observed following opioid withdrawal. Opioid withdrawal-induced hypersensitivity is correlated with increased activity of on-cells in the RVM, and chemical inactivation of the RVM decreases opioid withdrawal-induced hypersensitivity (Bederson et al., 1990; Kaplan and Fields, 1991). Release of various neurotransmitters and neuropeptides such as glutamate, neurotensin, and cholecystokinin in the RVM have been shown to contribute to pain-induced RVM sensitization (Urban et al., 1996).

RVM modulation of injury-induced hypersensitivity seems to result, at least in part, from the activation of a spino-bulbo-spinal positive feedback loop (Suzuki et al., 2002). Following tissue injury, NK1-expressing projection neurons in the dorsal horn activate serotonergic neurons in the RVM, which in turn, via the activation of serotonergic descending facilitatory pathways, increase the excitability of dorsal horn neurons. Ablation of NK1-expressing dorsal horn neurons, which decreases injury-induced hypersensitivity, also decreases injury-induced activation of serotonergic RVM neurons. Furthermore, blockade of serotonin receptors in the dorsal horn reduces injury-induced

hypersensitivity, reproducing the effects of ablation of NK1-expressing dorsal horn neurons. *In vitro*, in an acute spinal cord slice preparation, serotonin application transforms silent synapses in the dorsal horn into functional ones, thus providing another potential mechanism for the RVM-mediated facilitation of nociceptive transmission in the dorsal horn (Li and Zhuo, 1998). In summary, activation of the spino-bulbo-spinal circuit in response to sustained nociceptive input generates a positive feedback loop that seems to perpetuate central sensitization, contributing in this way to the maintenance of injury-induced hypersensitivity.

4.05.4.3.2 Anterior cingulate cortex

Imaging studies in humans have repeatedly demonstrated increased neural activity in the ACC of healthy individuals as well as of chronic pain patients in response to noxious stimulation (Apkarian et al., 2005). Noxious stimulus-induced neuronal activity in the ACC positively correlates with the reported unpleasantness of the noxious stimuli (Rainville et al., 1997). For this reason, the ACC has been proposed to be a neural center that mediates the negative emotional component of pain. Consistent with this hypothesis, frontal lobotomies or cingulotomies in chronic pain patients have been shown to decrease the reported unpleasantness of pain without affecting nociception, or the sensory component of pain (Romanelli et al., 2004). Similarly, electrolytic lesions of the ACC have been shown to decrease injury-induced hypersensitivity in animal models of inflammatory and neuropathic pain (Donahue et al., 2001).

Zhuo and colleagues have extensively studied the cellular and molecular mechanisms that underlie ACC modulation of pain. They have shown that under normal physiological conditions, ACC neurons respond to peripheral noxious stimuli and can exhibit both LTP and LTD of synaptic transmission (Zhuo, 2005). Under chronic pain conditions, or after amputation of a digit, ACC neurons exhibit increased excitability, a long-lasting potentiation of noxious stimulus-induced synaptic responses, and lose the capability to undergo synaptic LTD (Wei et al., 1999; Wei and Zhuo, 2001; Gao et al., 2006) (**Figure 7**). Potentiation of synaptic responses to noxious stimuli and loss of LTD have therefore been proposed to mediate ACC modulation of nociception. Both ACC modulation of nociception and LTP are dependent on the activation of adenylyl cyclases 1 and 8 (AC1 and AC8) and on the expression levels of the NMDA receptor subunit NR2B (Wei et al., 2001, 2002; Wu et al., 2005). Thus, genetic elimination of AC1 and AC8 decreases injury-induced hypersensitivity and local activation of adenylyl cyclases in the ACC of these animals rescues the injury-induced hypersensitivity phenotype (Wei et al., 2002). On the other hand, genetic overexpression of NR2B in forebrain regions, including the ACC, increases inflammation-induced nociceptive behavior. Moreover, NR2B expression has been shown to be upregulated in the ACC after injury, and pharmacological blockade of these receptors in the ACC decreases injury-induced hypersensitivity. After tissue injury, activation of NMDA, AC1, and AC8 in the ACC results in long-lasting activation of the transcription factor CREB and in a subsequent increase in the transcription of immediate early genes such as c-fos (Wei et al., 2001, 2002). Transcriptional changes in the ACC are therefore thought to mediate pain-induced long-lasting sensitization of the ACC.

As mentioned, the ACC has been proposed to mediate an emotional component of pain. Multiple studies in animals support this hypothesis. ACC neurons have been shown to be activated during pain avoidance behaviors (Koyama et al., 2001). In addition, lesions of the ACC decrease both inflammation-induced conditioned place avoidance and noxious stimulus-induced escape-avoidance behavior after nerve injury (Johansen et al., 2001; Gao et al., 2004; LaGraize et al., 2004) (**Figure 8**). Furthermore, glutamatergic activation of the ACC during conditioning produces avoidance learning in the absence of peripheral noxious stimulation, suggesting that activation of the ACC is sufficient to induce pain unpleasantness (Johansen and Fields, 2004) (**Figure 8**). In support of this hypothesis, human neuroimaging studies have shown a reduction in ACC activity when the unpleasantness of pain is removed from the pain experience by performing hypnotic suggestions that affect pain unpleasantness without altering the perceived intensity of pain (Rainville et al., 1997).

4.05.4.3.3 Amygdala

The amygdala is part of the spino-ponto-amygdaloid pathway, a major ascending pronociceptive pathway that originates in lamina I of the spinal cord and medullary dorsal horn and relays in the parabrachial (PB) area to terminate in the central nucleus of the amygdala (CeA) (Bernard et al., 1989; Bernard and Besson, 1990; Jasmin et al., 1997). The amygdala also

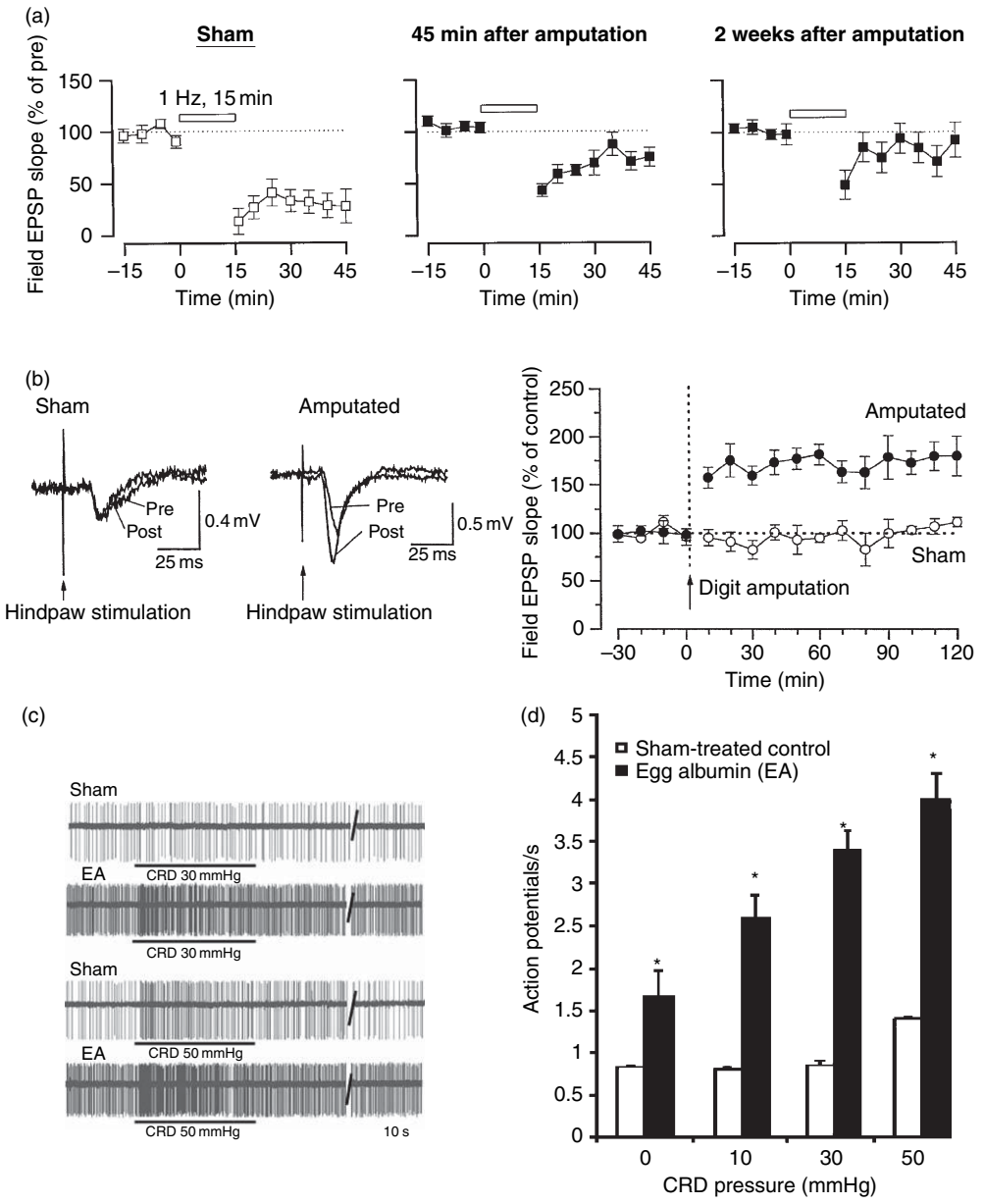

Figure 7 Injury induces loss of long-term depression (LTD), potentiation of synaptic transmission, and increased excitability in the anterior cingulate cortex (ACC). (a) Long-term potentiation (LTP) was recorded in ACC slices from sham animals or animals that had a single hindpaw digit amputated. Low-frequency stimulation, indicated by the horizontal bar, induced a robust LTD in the ACC of sham animals (left panel). An identical stimulation failed to induce LTD in the ACC of amputated animals 45 min (middle panel) and 2 weeks (right panel) after the amputation. (b) Amputation of a single hindpaw digit potentiates synaptic transmission in the ACC. Left panel shows representative traces of hindpaw-stimulation-evoked EPSPs in the ACC before (Pre) and after (Post) sham treatment or amputation. Right panel shows the time-course of hindpaw-stimulation-evoked EPSPs in the ACC. Amputation, but not sham treatment, induced a long-lasting potentiation of ACC responses to hindpaw stimulation. (c) Increased excitability in the ACC of viscerally hypersensitive rats. Representative single-unit recordings in the ACC in response to colorectal distention (CRD) in sham-treated and viscerally hypersensitive (EA) animals. Viscerally hypersensitive animals show increased background activity and CRD-evoked responses in the ACC when compared to sham-treated animals. (d) Frequency of firing in the ACC in response to CRD increases in a stimulus-intensity-dependent manner in viscerally hypersensitive animals (EA) when compared to sham-treated animals. Adapted from (a) Wei F, Li P, and Zhuo M (1999) Loss of synaptic depression in mammalian anterior cingulate cortex after amputation. *J. Neurosci.* 19: 9346–9354; (b) Wei F and Zhuo M (2001) Potentiation of sensory responses in the anterior cingulate cortex following digit amputation in the anaesthetised rat. *J. Physiol.* 532: 823–833; and (c, d) Gao J, Wu X, Owyang C, and Li Y (2006) Enhanced responses of the anterior cingulate cortex neurones to colonic distension in viscerally hypersensitive rats. *J. Physiol.* 570: 169–183.

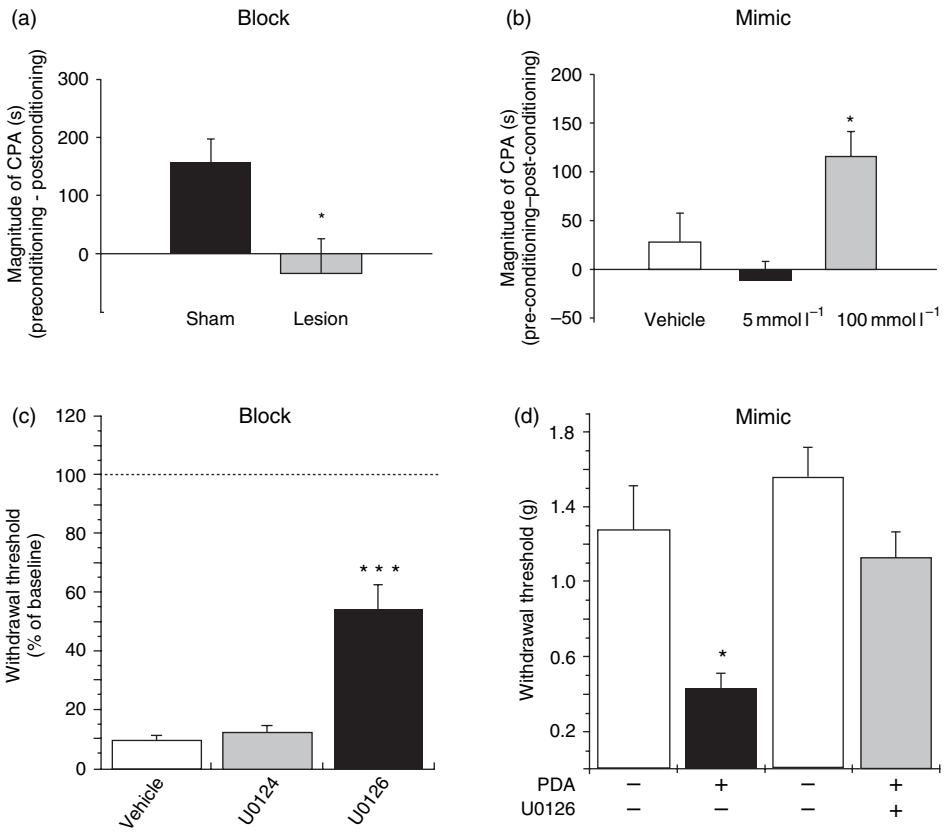

Figure 8 The anterior cingulate cortex (ACC) and the amygdala are important sites of modulation of inflammation-induced behavioral sensitization. (a) Inflammation-induced conditioned place avoidance (CPA) is blocked by lesions of the ACC. Animals with sham treatment show a higher magnitude of CPA scores than animals with ACC lesions. (b) Glutamatergic stimulation of the ACC mimics inflammation-induced conditioned place avoidance in the absence of inflammation. Animals were intra-ACC infused with vehicle, 5 mmol l^{-1} or 100 mmol l^{-1} of the glutamate agonist homocysteic acid (HCA). Infusion of HCA into the ACC induced a robust CPA in a dose-dependent manner. (c) Pharmacological blockade of ERK activation in the amygdala decreases inflammation-induced mechanical hypersensitivity. Animals were injected with 5% formalin into the hindpaw to induce inflammation. The MEK inhibitor U0126, the structural analog control compound U0124, or vehicle was infused into the amygdala and the effects of these treatments on mechanical thresholds were analyzed. Infusion of U0126 into the amygdala decreased inflammation-induced hypersensitivity when compared to vehicle- or U0124-treated animals. (d) Pharmacological activation of ERK in the amygdala mimics inflammation-induced mechanical hypersensitivity in the absence of inflammation. Mice received an intra-amygdala infusion of the phorbol ester PDA (120 pmol), which results in robust ERK activation, or a co-infusion of PDA (120 pmol) plus U0126 (1.5 nmol), and mechanical withdrawal thresholds were measured. Infusion of PDA into the amygdala decreased paw-withdrawal thresholds in response to mechanical stimulation when compared to baseline thresholds. PDA effects were blocked by co-infusion with U0126. Adapted from (a) Johansen JP, Fields HL, and Manning BH (2001) The affective component of pain in rodents: Direct evidence for a contribution of the anterior cingulate cortex. *Proc. Natl. Acad. Sci. USA* 98: 8077–8082; (b) Johansen JP and Fields HL (2004) Glutamatergic activation of anterior cingulate cortex produces an aversive teaching signal. *Nat. Neurosci.* 7: 398–403; and (c, d) Carrasquillo Y and Gereau RW IV (2007) Activation of the extracellular signal-regulated kinase in the amygdala modulates pain perception. *J. Neurosci.* 27: 1543–1551.

receives a direct projection from the spinal cord, with most of this tract originating in lamina V of the dorsal horn where nociceptive-specific neurons are located (Burstein and Potrebic, 1993). In addition to being part of ascending pain pathways, the amygdala has also been proposed to be part of a descending modulatory pain pathway (Hopkins and Holstege, 1978; Helmstetter et al., 1998; McGaraughty and Heinricher, 2002; Shane et al., 2003). Projections from the amygdala to the PAG have been identified using anatomical retrograde tracers (Hopkins and Holstege, 1978); these projections are thought to feed into the PAG-RVM-dorsal horn descending modulatory pain pathway (**Figure 2**).

In vivo electrophysiological recordings have mapped amygdala neurons that respond to peripheral

noxious stimuli (Bernard et al., 1992; Neugebauer and Li, 2002, 2003) (**Figure 9**). The majority of these neurons are located in the CeA, which is the target of the spino-ponto-amygdaloid ascending pain pathway (Bernard et al., 1989; Bernard and Besson, 1990; Jasmin et al., 1997). Under normal physiological conditions, CeA neurons have been shown to be preferentially excited or inhibited by cutaneous noxious stimuli or by noxious stimulation of deep tissue in a stimulus intensity-dependent manner, with receptive fields that included most areas of the body (four limbs, tail, trunk, face, cornea, tongue, and intraoral region). Moreover, in chronic pain conditions, CeA neurons have been shown to undergo neuroplastic changes, suggesting that plasticity in the amygdala might underlie pain hypersensitivity (Neugebauer and Li, 2003) (**Figure 9**). For example, induction of arthritis has been shown to result in increased responsiveness of CeA neurons to nociceptive afferent input, reduction of response threshold, and receptive field enlargement (Neugebauer and Li, 2003) (**Figure 9**). This arthritis-induced amygdala sensitization is dependent on the activation of mGluR1, both NMDA and non-NMDA ionotropic glutamate receptors, and the CGRP1 receptor (Li and Neugebauer, 2004a, 2004b; Han et al., 2005b).

Sensitization of amygdala neurons is preserved in slices obtained from arthritic animals (**Figure 9**). This has allowed for further examination of the mechanisms of amygdala sensitization at a cellular level. Whole-cell voltage-clamp recordings of CeA neurons from brain slices revealed enhanced synaptic transmission and increased excitability in CeA neurons after the induction of arthritis, visceral pain, or neuropathic pain (Neugebauer et al., 2003; Han et al., 2004, 2005b; Ikeda et al., 2007). Interestingly, in the neuropathic pain model, the degree of amygdala potentiation positively correlates with the degree of behavioral hypersensitivity (Ikeda et al., 2007). Consistent with the *in vivo* recording, the changes in synaptic transmission in slices from arthritic and visceral pain animals are dependent on the activation of mGluR1, NMDA receptors, and the CGRP1 receptor. Surprisingly, amygdala potentiation after visceral pain is not dependent on NMDA receptor activation, suggesting that different types of pain activate different molecular pathways in the amygdala. Persistent pain-induced amygdala sensitization correlates with upregulation of mGluR1 protein expression levels and with increased phosphorylation of the NR1 subunit of the NMDA receptor in the amygdala (Neugebauer et al., 2003; Bird et al., 2005). Finally, enhancement of group III mGluR-mediated inhibition of CeA synaptic transmission (Han et al., 2004) and increases in PKA-mediated NMDA receptor function (Bird et al., 2005) have also been observed after the induction of arthritis.

Despite all the progress made in characterizing the responses of amygdala neurons to noxious stimuli under conditions of persistent pain, very little is known about the contribution of the amygdala to the behavioral expression of injury-induced persistent hypersensitivity. Numerous studies have repeatedly demonstrated that lesions or chemical inactivation of the amygdala do not affect baseline nociception. Surprisingly, at present, the effect of lesions or chemical inactivation of the amygdala on injury-induced persistent hypersensitivity has not been evaluated. However, ERK activation is observed in noxious-responsive amygdala neurons during inflammation, and pharmacological blockade of ERK activation in the amygdala reduces inflammation-induced hypersensitivity (Carrasquillo and Gereau, 2007) (**Figures 8 and 9**). In addition, blockade of mGluR1 or the CGRP1 receptor in the amygdala has been shown to reduce arthritis-induced hypersensitivity, demonstrating that neuroplastic changes in the amygdala indeed contribute to behavioral hypersensitivity (Han et al., 2005b; Han and Neugebauer, 2005). Moreover, pharmacological activation of ERK in the amygdala has been shown to induce behavioral hypersensitivity that mimics inflammation-induced hypersensitivity but occurs in the absence of peripheral tissue injury, suggesting that ERK activation in the amygdala is sufficient to induce pain hypersensitivity (Carrasquillo and Gereau, 2007).

Similar to the ACC, the amygdala has also been proposed to mediate an emotional component of pain. Consistent with this, lesions of the amygdala significantly reduce conditioned place avoidance induced by paw inflammation, electric foot shock, or visceral pain in rats (Tanimoto et al., 2003; Gao et al., 2004). In addition, blockade of mGluR1, mGluR5, or the CGRP1 receptor in the amygdala decreases vocalization after noxious stimulation of an arthritic knee, a behavioral response that has been proposed to represent an affective component of pain (Han et al., 2005a, 2005b; Han and Neugebauer, 2005).

4.05.5 Cognitive Component of Pain

Many psychobehavioral studies have demonstrated that pain-related neural activity, and concurrently,

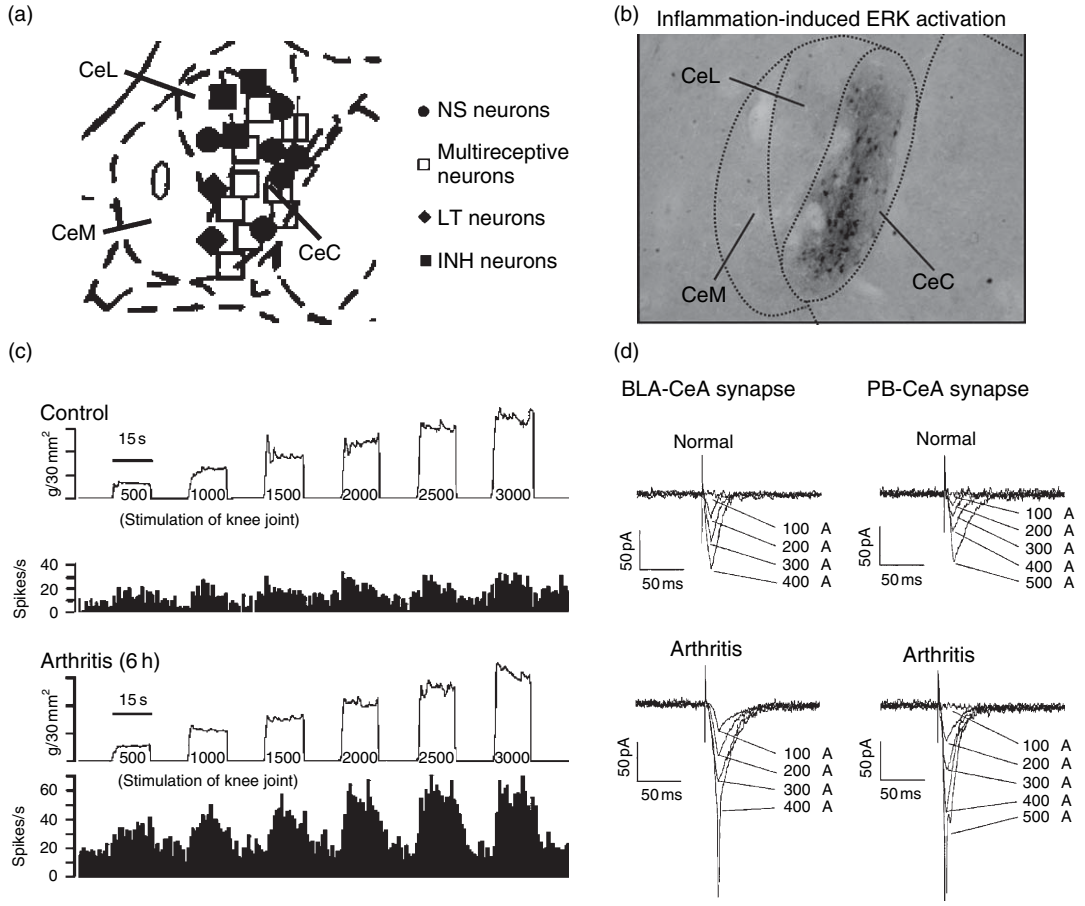

Figure 9 Neuroplastic changes in the amygdala occur after tissue injury. (a, b) Diagrams depicting the subdivisions of the central nucleus of the amygdala (CeA) in a rodent coronal brain section. CeM, medial subdivision; CeL, lateral subdivision; CeC, lateral capsular subdivision. (a) Amygdala neurons with knee-joint input are localized to the lateral capsular subdivision of the CeA. NS, nociceptive specific; LT, low threshold; INH, inhibited. (b) Immunohistochemistry for phospho-ERK in the amygdala after formalin-induced inflammation of one hindpaw. Inflammation-induced ERK activation is localized to the lateral capsular subdivision of the CeA. (c) Responses of amygdala neurons to deep tissue stimulation increase after the induction of arthritis. Representative single-unit recordings in the amygdala in response to graded stimulation of the knee joint in control animals and after the induction of arthritis. Arthritic animals show increased background activity and deep tissue stimulation-evoked responses in the amygdala when compared to control animals. (d) Synaptic transmission in the amygdala is enhanced after the induction of arthritis. Lower thresholds for evoked EPSCs and orthodromic spike generation were observed in slices from arthritic animals after stimulation of either the basolateral amygdala-central amygdala (BLA-CeA) or the parabrachial area-central amygdala (PB-CeA) when compared to slices from control animals. Adapted from (a) Neugebauer V and Li W (2002) Processing of nociceptive mechanical and thermal information in central amygdala neurons with knee-joint input. *J. Neurophysiol.* 87: 103–112; (b) Carrasquillo Y and Gereau RW IV (2007) Activation of the extracellular signal-regulated kinase in the amygdala modulates pain perception. *J. Neurosci.* 27: 1543–1551; (c) Neugebauer V and Li W (2003) Differential sensitization of amygdala neurons to afferent inputs in a model of arthritic pain. *J. Neurophysiol.* 89: 716–727; and (d) Neugebauer V, Li W, Bird GC, Bhave G, and Gereau RW IV (2003) Synaptic plasticity in the amygdala in a model of arthritic pain: Differential roles of metabotropic glutamate receptors 1 and 5. *J. Neurosci.* 23: 52–63.

the sensation of pain, can be strongly modulated by emotions and cognitive awareness (Turk, 2003; Lewandowski, 2004). For many years, anecdotal evidence has supported the idea that attention and distraction can influence pain perception. Distraction has thus been commonly used by parents and pediatricians to perform procedures that produce minor discomfort and pain in children such as injections, cleaning of wounds, and tooth extraction. Scientific studies in both humans and nonhuman primates also support the hypothesis that attention and distraction influence pain perception (Villemure

and Bushnell, 2002). These studies show that increased attention to a painful stimulus increases both pain intensity ratings and neuronal activity in certain brain regions such as the ACC, thalamus, and the prefrontal cortex and that, conversely, distraction or diverted attention from the pain stimulus decreases pain intensity ratings, reported unpleasantness of pain, and neuronal activity (Villemure and Bushnell, 2002). Although much progress has been made in elucidating the neural mechanisms of modulation of pain perception by attention and distraction, the results of these studies are hard to interpret because of the difficulty in controlling and measuring the degree of attention between subjects and because other factors such as vigilance, mood, and anxiety can also contribute to the reported pain and the unpleasantness ratings.

Anticipation or expectation can also strongly influence the perception of pain (Bushnell et al., 2004; Vase et al., 2004). This works in both directions; thus, anticipation or expectation of a painful stimulus increases the intensity of perceived pain, and expectation of pain relief decreases the intensity of perceived pain. The latter is classically exemplified by the phenomenon of placebo analgesia. Placebo analgesia is accompanied by decreases in neuronal activity of pain-related brain regions such as the ACC, insular cortex, and thalamus (Vase et al., 2004). Conversely, anticipation and expectation of a painful stimulus activates brain regions associated with pain processing such as the ACC, prefrontal cortex, insular cortex, and PAG (Bushnell et al., 2004). Interestingly, ACC activity in response to painful stimulation is very similar to the activation induced by anticipation of a painful stimulus even when the anticipation is not followed by painful stimulation. These results suggest that cognitive processes such as expectation can strongly influence the brain's responses to certain stimuli and ultimately affect the perception of pain. The power of cognitive awareness in pain perception was very well exemplified in a recent study by deCharms and colleagues at Stanford University, which showed that both healthy volunteers and chronic pain patients can learn to control their pain-induced neural activity in the ACC by using various cognitive strategy guidelines and real-time neuroimaging feedback (deCharms et al., 2005). This study specifically showed that modulation of ACC activity is accompanied by an associated change in reported pain perception.

4.05.5.1 Implications for Pain Management

An interesting yet counterintuitive fact is that injury is neither necessary nor sufficient to induce pain (Fields, 2000). In many instances, pain is not necessarily the manifestation of an injury but rather the manifestation of pathophysiological neural changes in the pain neuraxis. In support of this hypothesis, studies in both animals and humans have shown that electrical stimulation of certain brain regions can elicit pain in the absence of injury (Fields, 2000). Furthermore, as mentioned in previous sections of this chapter, pharmacological manipulations in the amygdala or the ACC can mimic inflammation-induced pain hypersensitivity in the absence of tissue injury or can result in avoidance learning in the absence of peripheral noxious stimulation, respectively (Johansen and Fields, 2004; Carrasquillo and Gereau, 2007) (**Figure 8**). A classic clinical example of pain without injury is seen in the phantom limb syndrome, when patients with amputated limbs report feelings of pain in the missing limb.

The limbically augmented pain syndrome (LAPS) theory could offer an explanation for situations in which pain is not accompanied by any obvious injury. The LAPS theory states that stress and exposure to emotionally traumatic events lead to a sensitized corticolimbic state that is analogous to central sensitization in the spinal cord and brainstem, but occurs in supraspinal structures that subserve both nociceptive processing and affective regulation (Rome and Rome, 2000). This sensitized corticolimbic state can in turn increase the perception of pain.

The scientific evidence demonstrating that emotions and cognitive factors strongly influence pain perception and the data suggesting that neuronal modifications in different regions of the pain neuraxis could explain these psychological modulations changed the clinical view of pain and consequently the approaches that are typically used for pain management. In addition to the more traditional pharmacological, physical, and surgical treatments for chronic pain, now a cognitive-behavioral approach is also commonly included in standard pain management practices. Cognitive-behavioral therapy (CBT) is designed to offer patients strategies to cope with their pain by identifying beliefs and fears that interfere with their functioning and well-being (Osborne et al., 2006). CBT has been shown to improve not only the reported pain intensity but also other non-pain-related variables such as mood, depression, pain interference, disability, return to work, and health care utilization (Kerns et al., 2006).

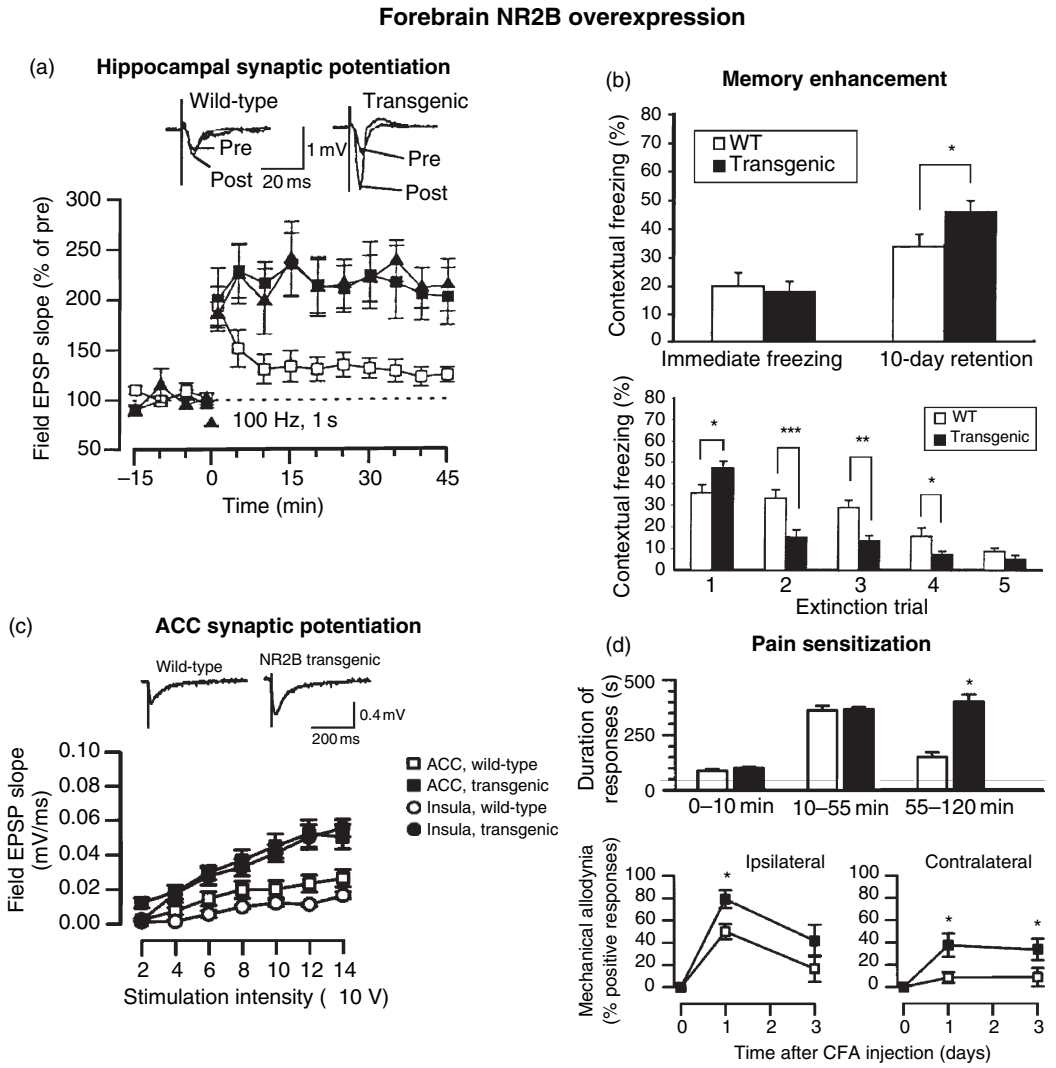

Figure 10 Overexpression of *N*-methyl-D-aspartate-type glutamate receptor subunit (NR2B) in the forebrain facilitates synaptic potentiation in the hippocampus and the ACC and enhances learning and memory and pain hypersensitivity in mice. (a) Synaptic potentiation in the hippocampus of NR2B-overexpressing mice. Time course of evoked excitatory postsynaptic potentials (EPSCs) in slices from wild-type (WT) and transgenic animals. Tg-1, filled squares; Tg-2, filled triangles; WT, open squares. Tetanic stimulation-evoked EPSPs are potentiated in NR2B overexpressing mice when compared to WT. Inset: Representative evoked EPSP before (pre-) and after (post-) tetanic stimulation in WT (left) and transgenic (right) slices. (b) Enhancement of contextual fear memory (top panel) and faster fear extinction (bottom panel) in NR2B-overexpressing mice. Top panel: Animals received a single conditioned stimulus/unconditioned stimulus (CS/US) pairing training. Retention test was performed 10 days after training. Percentage of freezing rate was increased in NR2B-overexpressing mice when compared to WT. Bottom panel: Animals received a single CS/US pairing training and were then subjected to five extinction trials. Percentage of freezing decreased faster in NR2B-overexpressing mice than in WT mice. (c) NR2B overexpression enhances NMDA receptor-mediated synaptic responses in the ACC. The slope of the field EPSP in response to graded stimulation intensities is higher in slices from NR2B-overexpressing mice than in slices from WT animals. Inset: Representative field EPSPs recorded from ACC slices of WT and NR2B-overexpressing animals. (d) Behavioral responses to intraplantar injection of formalin (top panel) or complete Freund's adjuvant (CFA) (bottom panel) are increased in NR2B-overexpressing mice. Top panel: Total time that WT (□) and NR2B-overexpressing (■) mice spent engaging nociceptive behavior in each phase of the formalin test. NR2B-overexpressing mice spent more time engaging in nociceptive behavior in the third phase of the formalin test when compared to WT mice. Bottom panel: The percentage of positive responses to mechanical stimulation was measured before (time 0) and 1 and 3 days after intraplantar injection of CFA. Increased percentage of responses after CFA was observed in NR2B-overexpressing mice (■) when compared to wild-type mice (□). Adapted from (a, b) Tang YP, Shimizu E, Dube GR, et al. (1999) Genetic enhancement of learning and memory in mice. *Nature* 401: 63–69, with permission from Nature Publishing Group; (c, d) Wei F, Wang GD, Kerchner GA, et al. (2001) Genetic enhancement of inflammatory pain by forebrain NR2B overexpression. *Nat. Neurosci.* 4: 164–169, with permission from Nature Publishing Group.

4.05.6 Learning and Memory Versus Chronic Pain

As we have discussed in this chapter, neural changes occur at different sites in the pain neuraxis in response to injury. This plasticity is thought to underlie the development and maintenance of chronic pain. Similar changes in excitability and synaptic strength occur in the hippocampus, cortex, and amygdala and are thought to underlie learning and memory. The similarities in the cellular mechanisms underlying pain and learning and memory are striking; many cellular processes and signaling molecules that are known to be important mediators of pain are also important regulators of learning and memory (Ji et al., 2003). The overlapping function of these cellular processes in pain and learning and memory was evident in genetically modified mice that overexpress NR2B in the forebrain (Tang et al., 1999; Wei et al., 2001) (**Figure 10**). Overexpression of NR2B in the forebrain resulted in facilitation of synaptic potentiation in the hippocampus and in superior performance in learning and memory tasks (Tang et al., 1999). A separate study showed that, unfortunately, these NR2B-overexpressing mice are smarter at the expense of also being more susceptible to pain (Wei et al., 2001). As in the hippocampus, enhanced synaptic responses were also observed in NR2B-overexpressing mice in the ACC and insular cortex, two key structures for pain processing. Accordingly, these mice also exhibited enhanced nociceptive responses after inflammation. The similarities in the neural mechanisms underlying pain and learning and memory have made the development of novel analgesic therapies even more challenging. At the same time, as discussed in the previous section of this chapter, the tight relationship that exists between pain and cognition has led to the development of a cognitive-behavioral approach to managing chronic pain that has been shown to be highly effective in helping patients cope with their pain.

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4.06 Molecular Mechanism of Associative Learning in the Bee

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4.06.1 Associative Olfactory Learning in Bees

To cope with the highly variable environment, as well as individual and social tasks, honeybees perform a variety of behaviors including orientation, foraging, and social communication. When searching bees discover flowers providing food, they learn to associate the visual and olfactory signals with the reward (nectar and pollen) and quickly optimize their foraging strategy based on nectar flow and sucrose concentration. For optimal learning, stimuli of food sources like odor and color (conditioned stimuli, CS) that predict reward have to be perceived before the bees actually experience the sucrose reward (unconditioned stimuli, US) (reviewed in Menzel and Müller, 1996). This associative learning under natural conditions reveals many characteristics of associative learning in mammals. Studying the honeybee's learning behavior in the laboratory was a major breakthrough and provided the opportunity to study associative conditioning of the proboscis extension reflex (PER) at the behavioral, the cellular, and the molecular level under controlled conditions (**Figure 1**). The PER that is elicited by an appetitive stimulus (sucrose) is conditioned by pairing an odor stimulus (CS, conditioned stimulus) with a sucrose reward (US, unconditioned stimulus). A single pairing that lasts only a few seconds induces an associative memory that is initially at a high level but decays over the following days (Menzel and Müller, 1996; Menzel, 1999). As in other model systems, repetitions of conditioning trials induce a stable memory (**Figure 2(a)**). In the honeybee, three successive conditioning trials, given within a time window of a few minutes, induce a long-lasting memory that is stable for many days (>7 days) and which shows all properties of a long-term memory (LTM) (Menzel and Müller, 1996). Memories induced by a single or by repeated conditioning trials differ in their sensitivity to amnestic treatments like cooling. While cooling immediately after a single-trial conditioning impairs memory formation, the same treatment after multiple-trial conditioning does not (Menzel et al., 1974; Erber et al., 1980; Müller, 1996). Thus, repetition of conditioning trials accelerates the transfer into amnesia-resistant memory.

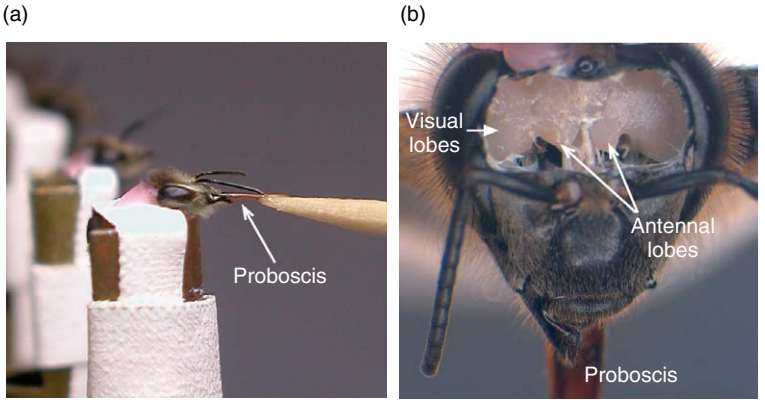

Figure 1 Honeybees fixed in metal tubes for olfactory conditioning of the proboscis extension reflex (PER). (a) Bees mounted in the metal tubes can freely move their proboscis and antennae. Stimulation of the antennae or the proboscis with sucrose solution (toothpick) elicits the PER. (b) Dorsal view of a honeybee head with open head capsule. The direct access to neuronal networks like the antennal lobes allows monitoring of neuronal activity and manipulation of signaling cascades during learning *in vivo*.

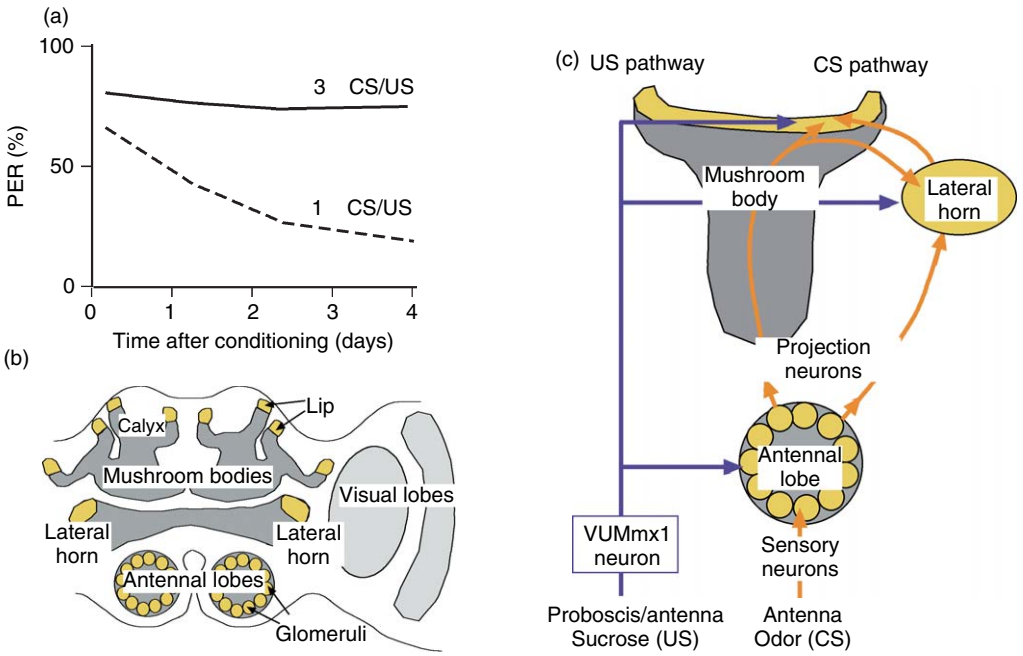

Figure 2 Associative olfactory learning in honeybee and the underlying neuronal circuits. (a) Associative conditioning of the PER consists of the pairing of an odor, the conditioned stimulus (CS), with a following sucrose reward, the unconditioned stimulus (US). A single pairing (1×CS/US) that lasts a few seconds leads to formation of a memory that decays over days. Multiple conditioning trials (3×CS/US with an intertrial interval of 2 min) induce a robust long-term memory that provides the basis for the analysis of the molecular signaling cascades underlying learning and memory formation. (b) The schematic section through the honeybee brain shows the major neuronal circuits implicated in processing olfactory and visual information. While the antennal lobes and the visual lobes are primary sensory centers, the mushroom bodies are sites that process different sensory modalities. The antennal lobes with their glomerular structure, the lip of the mushroom body calyces, and the lateral horn are areas implicated in olfactory information processing. (c) The neuronal circuits that mediate CS and the US information are well characterized in honeybees. CS pathway: the antennal lobes are the primary processing site of odor information and receive their input from sensory neuron in the antenna. CS information leaves the antennal lobes via projection neurons that transmit the information to the calyces of the mushroom bodies and the lateral horn. The antennal lobes, the calyces of the mushroom bodies, and the lateral horn are convergence sites of the CS and the US pathways. These brain areas are innervated by the ventral unpaired median (VUM) mx1 neuron that can substitute the US function in associative learning. The neuronal circuits connecting the output sites of these brain areas to the motor circuitry mediating the PER are unknown.

4.06.2 Neural Circuits Mediating Associative Olfactory Learning

The brain areas and neuronal circuits mediating odor information (CS pathway) and reward information (US pathway) in associative learning in the honeybee have been analyzed using a variety of techniques and approaches. While histological and immunohistological techniques provided the basic information concerning morphological features of the neuronal pathways, studies using electrophysiological and optical recording approaches in intact animals added important functional aspects to our knowledge of the neuronal circuits involved in CS and US processing (**Figures 2(b)** and **2(c)**).

4.06.2.1 CS Pathway

Olfactory information from the chemosensory receptors on the antennae is relayed via the antennal lobes (AL) to the calyces of the mushroom bodies (MB) and the lateral horn (LH) (**Figure (2c)**). In each AL, the 160 glomeruli comprise sites of dense synaptic connections between sensory neurons (≈60 000), local interneurons (4000) and projection neurons (PNs) (≈800) (Galizia and Menzel, 2000). A considerable fraction of the local interneurons connecting the glomeruli within an AL exhibit gamma-aminobutyric acid (GABA) immunoreactivity (Schäfer and Bicker, 1986). This GABAergic network modulates the overall activity in the ALs and, together with a second glomerular-specific inhibitory network, contributes to the sharpening of the odor representation at the level of output neurons that transmit the information to higher-order neuropils (Sachse and Galizia, 2002). Blocking of the inhibitory network in the ALs impairs odor discrimination but not learning (Stopfer et al., 1997).

Different types of PNs transmit odor information from the AL to the lip region of the MB calyces and the LH in the lateral protocerebrum (**Figure 2(c)**). While PNs in the median antennocerebral tract show odor-specific activity profiles for different odors and conduct the information with a delayed code, the PNs in the lateral antennocerebral tract have rather unspecific broadband activity profiles for different odors and transmit the information without delay (Müller et al., 2002). This points to a dual coding of odor information by extracting and transmitting different features in the time domain by different sets of PNs. Immunohistochemical studies demonstrate the presence of acetylcholinesterase and acetylcholine receptors in the PNs and their target area, the lip region of the MB. Thus, the major CS input into the MBs seems to be cholinergic (Kreissl and Bicker, 1989).

The MBs, which consist of densely packed Kenyon cells (KC) are prominent, well-characterized brain structures and play a central role in learning and memory formation in several species (*See* Chapters 1.28, 4.07). The olfactory input by the PNs is confined to the lip region of the calyces. Optical recording techniques demonstrate that odors evoke combinatorial activity patterns in both the glomeruli of the ALs and the lip region of the MBs. While these patterns are prominent at the level of the ALs, odors generate only brief and sparse responses at stimulus onset in the KC of the MBs (Szyszka et al., 2005). The inhibitory recurrent neurons connecting output areas of MBs with input in the lip region of the MBs are probably involved in this process (Ganeshina and Menzel, 2001).

4.06.2.2 US Pathway

Appetitive chemosensory pathways from the antennae and the proboscis project to the suboesophageal ganglion and terminate near motor neurons involved in proboscis extension. Ventral unpaired median (VUM) neurons receive chemosensory input, and the single identified VUMmx1 neuron can substitute for the US function in associative learning, as demonstrated by depolarization of VUMmx1 shortly after a CS presentation (Hammer, 1993). The VUMmx1 innervates the ALs, the MBs, and both LHs and thus converges with brain areas that process odor information (**Figure 2(c)**). Injections of octopamine, the putative transmitter of VUMmx1 (Kreissl et al., 1994), either into the ALs or the MBs substitute for the US function (Hammer and Menzel, 1995, 1998), supporting the role of VUMmx1 in reward processing in these two brain areas. Pairing of CS stimulation with octopamine injection into either the AL or the MB leads to a conditioned response as tested after 20 min, supporting independent contributions of the AL and MB in memory formation. This function in memory formation, however, differs between the AL and the MB, since only CS presentation followed by octopamine injections into the AL leads to a normal detectable acquisition. Such different roles of the ALs and the MBs have initially been proposed by experiments using local cooling of AL and MB as amnestic treatment to interfere with associative learning (Menzel et al., 1974; Erber et al., 1980).

4.06.3 The Molecular Cascades Mediating CS and US Pathways in Olfactory Learning

The robust learning paradigm in combination with the accessibility and the size of the honeybee brain provides the unique opportunity to apply biochemical techniques to monitor *in vivo* induced activities of signaling cascades (Hildebrandt and Müller, 1995a,b). Rapid termination (<0.5 s) of biochemical reactions by liquid nitrogen freezing of the whole animal, followed by freeze-drying, tissue dissection, and optimized biochemical assays, allows the determination of learning-induced activation of signaling cascades in brain areas like the ALs and the MBs at defined times after *in vivo* stimulation.

The search for the signaling cascade contributing to US processing revealed a specific role of the cyclic adenosine monophosphate (cAMP)-dependent protein kinase A (PKA). Stimulation of the antenna with sucrose, the US, induces a fast transient activation of PKA in the ALs (Hildebrandt and Müller, 1995b) that is back to baseline within a few seconds. US-induced PKA activation in the ALs is stimulus specific, since odor stimulation (CS) or mechanical stimulation of the antennae does not affect PKA activity (Hildebrandt and Müller, 1995b). The exclusively US-induced PKA activation in the ALs is sensitive to the monoamine reuptake blocker reserpine and thus depends on the monoamines present in the AL. One of the latter is octopamine, the putative transmitter of the VUMmx1 neuron that densely innervates the ALs. Thus, octopamine is responsible for linking US stimulation to transient PKA activation (Hildebrandt and Müller, 1995a).

The PKA immunostaining is to its largest extent detected in the local interneurons that connect the glomeruli within the AL (Müller, 1997a). This, together with the biochemical measurements and the dense innervation of the ALs by the VUMmx1 neuron, suggests a global modulatory function of US-induced processes mediated via the octopamine–cAMP/PKA cascade in the AL. The observation that silencing expression of the octopamine receptor in the honeybee specifically impairs olfactory learning but not odor discrimination (Farooqui et al., 2003), together with the specific requirement of octopamine in *Drosophila* appetitive, but not aversive, olfactory learning (Schwaerzel et al., 2003), points to a conserved role of octopamine in insect appetitive learning.

In addition to the specific activation of the cAMP/PKA pathway, US stimulation also triggers Ca^{2+}-regulated signaling cascades like the Ca^{2+}-phospholipid-dependent protein kinase C (PKC) in the AL (Grünbaum and Müller, 1998). In contrast to the US-specific activation of the cAMP/PKA pathway, PKC is activated by both US and CS stimulation (Grünbaum and Müller, 1998). Inhibition of the transient CS- or US-induced PKC activation does not affect associative learning, suggesting that PKC-mediated processes may contribute to chemosensory information processing rather than to learning and memory formation.

In addition to the ALs, the VUMmx1 neuron innervates the lip region of the MB calyces that receives olfactory input from the ALs (**Figure 2(c)**). In contrast to the ALs, however, US stimulation does not activate the cAMP/PKA cascade in the MBs *in vivo*. This, together with the observation that octopamine stimulates the cAMP/PKA cascade in cultured KC from the MBs (Müller, 1997a), points to a different coupling of the octopamine receptors in the MBs: most likely, US stimulation activates octopamine receptors that trigger Ca^{2+}-regulated signaling cascades in the MBs (Balfanz et al., 2005). The observed differences in the expression of voltage-sensitive and Ca^{2+}-dependent currents in neurons of the ALs and the MBs (Grunewald, 2003) support the idea that information in olfactory learning is processed differently in ALs and MBs.

4.06.4 Associative Conditioning: Induction of Molecular Processes Underlying Memory Formation

During associative conditioning, parameters of the CS and US stimulus (strength, duration) and the temporal relations between stimuli are deciding factors for the process of memory formation. Depending on these parameters, different molecular processes are triggered during the short training phase, and depending on the triggered molecular processes, distinct forms of memory are developed. At least three memory phases can be identified in the various model systems: a short-term memory (STM) in the range of minutes, a mid-term memory (MTM) in the range of hours, and a stable LTM which lasts for days and weeks. In honeybees, as in many other systems, the LTM can be pharmacologically divided into a translation-dependent early phase (eLTM, 1–2 days)

and a transcription-dependent late phase (lLTM, ≥3 days) (Menzel, 1999; Müller, 2002). The defined period of associative conditioning (seconds to minutes) and the experimental accessibility of the convergence sites of CS and US processing in honeybees provided the ideal situation to determine the learning-induced dynamic activation of molecular signaling cascades and their contribution to formation of distinct memory phases *in vivo*.

4.06.5 Induction of LTM: The Critical Role of the cAMP/PKA Cascade

The requirement of cAMP/PKA-dependent processes in LTM formation and processes of long-lasting neural plasticity is observed in all systems investigated so far and seems to be conserved throughout the animal kingdom (*See* Chapters 4.07, 4.10, 4.21). Blocking of PKA during and immediately after the training phase leads to a selective loss of LTM, while STM and MTM are unaffected (Müller, 2000). Direct measurements show that the temporal dynamics of US-triggered PKA activation in the ALs is modified by the temporal sequence of CS and US stimuli and by the number of successive conditioning trials (**Figure 3**). A single CS/US forward-pairing, which induces a weak olfactory memory, leads to a transient increase in PKA activity that returns to basal levels 60 s after the conditioning trial. Repetition of CS/US forward-pairings (3× CS/US with an intertrial interval of 2 min) that induce LTM prolongs PKA activity in the ALs up to more than 3 min. US stimulation alone, as well as single or repeated US/CS backward-pairings, which do not induce appetitive memory, lead to a significantly shorter but similar pattern of PKA activation. This first *in vivo* evidence for a link between the sequence

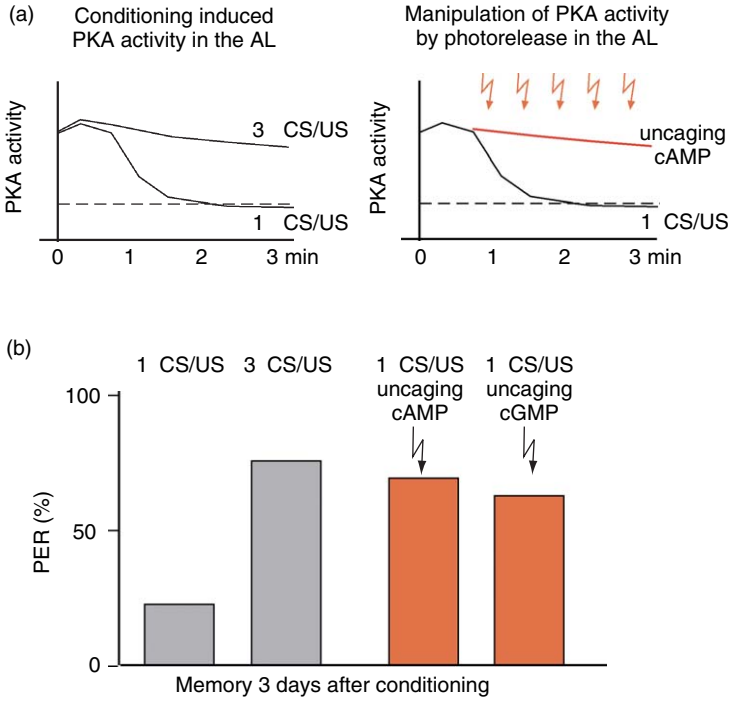

Figure 3 Induction of LTM requires a prolonged PKA activation in the antennal lobes. (a) The activation pattern of PKA in the antennal lobes depends on the sequence and the number of successive conditioning trials. While a single CS/US pairing induces a transient activation of about 1 min, repeated CS/US pairings (2-min intertrial interval) that induce an LTM lead to a prolongation of PKA activity up to more than 3 min. Local uncaging of cAMP in the antennal lobes enables the imitation of the prolonged PKA activation *in vivo,* while the animal receives a single CS/US pairing. (b) Honeybees are trained under conditions allowing access to the brain to photorelease cAMP or cyclic guanosine monophosphate (cGMP) during conditioning. Under these conditions honeybees form an LTM as tested 3 days after 3×CS/US pairings, but not after a single CS/US pairing. A single CS/US pairing together with the imitation of the prolonged PKA activation by uncaging cAMP in the AL induces LTM. Since the prolonged PKA activation is mediated via the nitric oxide/cGMP cascade, single CS/US pairing combined with uncaging of cGMP in the AL also induce LTM.

and number of CS and US stimulation and temporal dynamics of PKA activation in the ALs suggested that prolonged PKA activation in the AL is involved in the formation of associative LTM.

This hypothesis was tested by photolytic release of caged cAMP in the ALs (**Figure 1(b)**) to artificially prolong PKA activation during olfactory conditioning. A single conditioning trial combined with the local replay of the prolonged PKA activation in the ALs *in vivo* is sufficient to induce long-lasting memory (**Figures 3** and **4**). These experiments provided direct functional evidence for a link between conditioning parameters, temporal patterns of PKA activation in the ALs, and formation of LTM in intact animals.

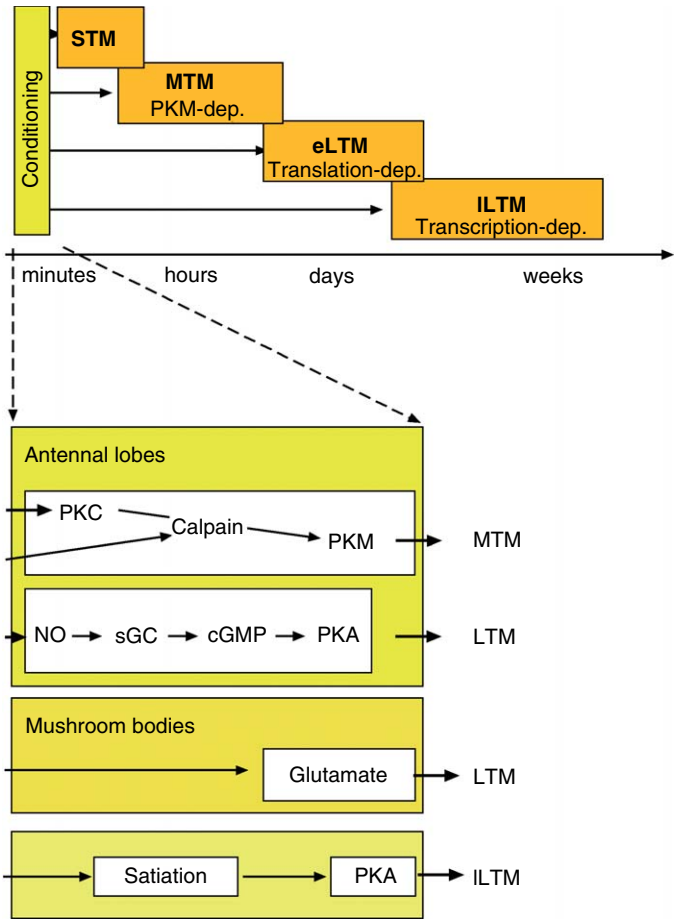

Figure 4 Scheme of the molecular mechanisms underlying the induction and maintenance of different memory phases in honeybees. As in other species, different memory phases are mechanistically distinguishable in the honeybee. While short-term memory (STM) and mid-term memory (MTM) can be formed by existing proteins, long-term memory (LTM) requires protein and RNA synthesis. LTM can be pharmacologically dissected into an early phase (eLTM) that requires protein synthesis and a late phase (lLTM) that, in addition, requires RNA synthesis. STM, MTM, and LTM are induced independently and by parallel processes. Two independent processes involved in maintenance of MTM and induction of LTM are localized in the antennal lobes. MTM requires the formation of a constitutive protein kinase M (PKM) for its maintenance. PKM is formed in the antennal lobes by cleaving protein kinase C (PKC) by the protease calpain. Also located in the antennal lobes is the molecular process underlying the prolonged activation of the protein kinase A (PKA) that is required for LTM induction. This prolonged PKA activation is mediated by activation of the soluble guanylate cyclase (sGC) by nitric oxide (NO) and induces subsequent processes of LTM formation. In addition to the antennal lobes, processes located in the mushroom bodies also contribute to LTM formation. Photolytic release of glutamate in the mushroom bodies immediately after conditioning facilitates LTM formation. The satiation status during conditioning modulates learning and memory formation. The formation of the late LTM can be specifically rescued via a PKA-dependent process, indicating that eLTM and lLTM are induced by two parallel PKA-dependent mechanisms. The molecular targets triggered by these early events in the ALs and the MBs are presently unknown.

However, the molecular processes triggered by the prolonged PKA activation in the ALs that finally lead to LTM are unknown.

Artificial prolongation of PKA activation in the ALs, in conjunction with single-trial conditioning, does not reach the level of conditioned responses that is achieved after multiple-trial conditioning. Thus, it is possible that other molecular processes or neural circuits (e.g., in the MBs) also contribute in parallel to LTM induction.

4.06.6 Induction of LTM: The Nitric Oxide/cGMP System Acts as a Signal Integrator during Associative Conditioning

In the nervous system, nitric oxide (NO) has been implicated in various physiological functions including chemosensory information processing and learning (Müller and Hildebrandt 1995; Müller, 1996, 1997b). Inhibition of NO synthase (NOS) in honeybees that is mainly localized in brain areas like the ALs and the lip of the MB calyces impair both LTM formation (Müller, 1996) and prolonged PKA activation (Müller, 2000). The finding that blocking of the soluble guanylate cyclase, the major target of NO in neuronal tissues, also impairs multiple-trial induced prolonged PKA activation and LTM formation provided first evidence for a crosstalk between cyclic guanosine monophosphate (cGMP) and cAMP signaling in this system. Theoretically, cGMP can interact with the cAMP cascade via cGMP-dependent protein kinase, cGMP-regulated phosphodiesterases, and cyclic nucleotide-gated channels. Although the target of cGMP has not yet been identified *in vivo*, the synergistic activation of honeybee PKA by cAMP and cGMP (Leboulle and Müller, 2004) points to a direct function of cGMP in the prolonged increase in PKA activity in the AL.

This systemic analysis does not allow for localizing the contributing brain areas. Again the uncaging technique served as valuable approach to verify that the prolonged PKA activation in the ALs is mediated via NO and cGMP *in vivo* (**Figures 3** and **4**). As demonstrated with uncaging cAMP, a single conditioning trial followed by photorelease of cGMP leads to formation of a long-lasting memory (**Figure 3**). A single conditioning trial in combination with uncaging NO in the ALs impairs memory formation. Although the reason for this is unknown, photolyzing NO in the ALs may interfere with other functions of NO in signal processing in the ALs. It has been demonstrated that blocking NO in the AL impairs odor discrimination (Hosler et al., 2000). Due to the localization and Ca^{2+}-dependence of NOS, the odor-specific changes in Ca^{2+}-concentrations in subsets of glomeruli during odor processing (Joerges et al., 1997) may induce an odor-specific release of NO required for odor discrimination. The artificial release of NO within the whole ALs would interfere with such a process. Moreover, the NO/cGMP system in the ALs is also implicated in integrative processing of appetitive signals during habituation (Müller and Hildebrandt, 1995). Characterizing the temporal aspects of NO and cGMP function using the uncaging technique *in vivo* revealed clear differences between NO/cGMP function in associative and nonassociative learning. Both the cellular network mediating the NO/cGMP function and the temporal parameters of the stimulus-induced activation of the NO/cGMP system differ between associative learning and habituation (Müller and Hildebrandt, 2002). Irrespective of their differences, however, both NO/cGMP-mediated processes contribute to integrative signal processing during application of stimuli. In case of associative learning, the function of the NO/cGMP system in a very narrow time window during conditioning is critical for induction of LTM and thus for processes that become evident days later (**Figure 4**).

4.06.7 Glutamate-Mediated Signaling Cascades in the Mushroom Bodies Are Involved in Memory Formation

Glutamate is the major excitatory transmitter in the mammalian brain and plays a central role in neuronal plasticity in vertebrates (*See* Chapters 4.16, 4.20). Although the function of glutamate as main transmitter at insect neuromuscular junctions is well studied, the role of glutamate in the insect brain is poorly understood. The existence of glutamate-like immunoreactivity (Bicker et al., 1988), glutamate-induced ion currents (Barbara et al., 2005), glutamate transporters, and glutamate receptors (Kucharski et al., 2000; Funada et al., 2004; Zannat et al., 2006) provides considerable evidence for a potential role of glutamate in the honeybee brain.

Systemic application of drugs affecting reuptake of glutamate before repeated training trials impairs memory tested after 24 h but does not affect acquisition or memory tested at 1 h (Maleszka et al., 2000).

A similar effect is observed by injection of MK-801, a noncompetitive *N*-methyl-D-aspartate (NMDA) receptor antagonist (Si et al., 2004). Although interpretation of the function of glutamate transmission based on pharmacological studies is difficult, a molecular approach used in *Drosophila* (Xia et al., 2005) supports the idea of an implication of glutamate in insect learning. Stable and transient knockdown of NMDA receptors in the whole *Drosophila* brain disrupts aversive olfactory learning and impairs long-term memory. However, as in the honeybee, the particular brain site at which glutamate influences memory remains unclear. Photolytic uncaging of glutamate in the honeybee brain revealed direct evidence for a defined spatial and temporal contribution of glutamate in LTM formation (Locatelli et al., 2005) and, in addition, overcame problems caused by the insufficient pharmacological characterization of the drugs used on insect glutamate receptors and reuptake systems.

Uncaging glutamate in the MBs immediately after a weak training protocol (single-trial training) improves the formation of a long-lasting memory (2 days) and thus mimics the effect of a strong training protocol (**Figure 4**). The effect of glutamate seems to be confined to a time window after associative training, since release of glutamate immediately before training has no effect. This post-conditioning effect of glutamate release on late memory formation allows two interpretations: Either it mimics a situation that is usually induced by repeated conditioning trials and thus facilitates learning, or post-conditioning release of glutamate directly contributes to early processes of memory formation. The function of glutamate is restricted to the MBs circuitry, since glutamate release in the ALs does not affect processes of learning or memory formation. The demonstrated role of glutamate in memory formation strengthens the notion that glutamatergic neurotransmission is a conserved key element in neural plasticity across species.

4.06.8 Induction and Maintenance of MTM: The Ca^{2+}-Dependent Cleavage of PKC by Calpain

Calcium-mediated signaling cascades play a fundamental role in the regulation of cellular processes. The signaling cascade that involves PKC is implicated in synaptic plasticity in various systems (*See* Chapter 4.22). Initiated by the finding that both US and CS stimulation induce a transient PKC activation in the ALs, the function of the signaling cascade that involves PKC has been analyzed in detail (Grünbaum and Müller, 1998). The temporal dynamics of PKC activation in the ALs are independent of the sequence of CS and US stimulation and of the number of successive conditioning trials. Inhibition experiments reveal evidence that these transient PKC modulations during the conditioning phase do not directly contribute to learning or memory formation. In maintenance of a specific memory phase, however, a function of the signaling cascade involving PKC has been demonstrated.

Repeated conditioning trials that induce LTM lead to an increase in PKC activity in the ALs beginning 1 h after conditioning and lasting up to 3 days (Grünbaum and Müller, 1998). This long-lasting elevation of PKC activity is not induced by a single conditioning trial. Two independent and mechanistically distinct mechanisms contribute to this training-induced increase in PKC activity. In the early phase, ranging from 1 to 16 h, the increased PKC activity can be attributed to a constitutively active PKC, a form of PKC known as PKM. PKM is formed by cleavage of the activated PKC by the Ca^{2+}-dependent protease calpain. Inhibition of calpain activity during conditioning eliminates the early phase of elevated PKC activity from 1–16 h after training. This supports the idea that training activates PKC and calpain. Only activated PKC is susceptible to calpain action resulting in the production of PKM. The inhibition of calpain during conditioning impairs memory in a time window between 1 and 16 h after learning, suggesting that learning-triggered PKM production is required for maintaining the MTM (**Figure 4**). This is supported by the observation that blocking calpain does not affect acquisition, the early memory phase up to 30 min, and memory after 1 day.

The late phase of training-induced elevation of PKC activity observed 1–3 days after learning is eliminated by injection of blockers of protein and RNA synthesis in a narrow time window during and after conditioning. Thus, induction of the two mechanistically different processes expressing the early and late phase of elevated PKC activity occur in parallel during and/or shortly after conditioning. The mechanism underlying the elevated PKC activity in the late phase, as well as its function in memory formation, is unclear. It is probably one of several parallel mechanisms occurring in different neuronal circuits required for the formation of the late phase of long-term memory.

4.06.9 Influence of Satiation Reveals a Parallel Function of the cAMP Cascade in the Process of Memory Formation

In addition to parameters concerning the relation and number of US and CS stimulation during associative learning, factors like stress, satiation, etc., influence learning and memory formation. Although the latter parameters are usually strictly controlled in behavioral experiments, the manipulation of satiation level during associative learning in honeybee uncovered new features of the molecular machinery leading to LTM formation (Friedrich et al., 2004).

As in other paradigms using appetitive associative conditioning, a reliable induction of LTM in the honeybee requires training of hungry animals. Only multiple-trial conditioning of animals starved for about 18 h leads to a robust acquisition and induction of MTM and the two LTM phases, eLTM and lLTM. An additional feeding 4 h before multiple-trial conditioning is sufficient to impair acquisition and all subsequent memory phases. Such an additional feeding also impairs memory induced by a single-trial conditioning. This is of particular importance, since presently only manipulations of satiation during learning or amnestic treatment by cooling immediately after learning have been shown to interfere with memory induced by a single conditioning trial. The restriction of the different satiation levels to the time window of conditioning and the impairment of both LTM phases that require the cAMP/PKA cascade during conditioning (Müller, 2000; Friedrich et al., 2004) pointed to a contribution of the cAMP/PKA cascade in satiation-dependent impairment of memory formation. Determination of the basal PKA levels in honeybee brains at different satiation levels revealed a characteristic difference: hungry bees starved for 18 h show higher basal PKA activity in the brain than bees fed 4 h before. Elevating the low basal PKA activity levels in animals fed 4 h before conditioning specifically rescues the transcription-dependent lLTM (**Figure 4**). This is remarkable, since acquisition, MTM, and eLTM are still impaired. Since in all model systems, both eLTM and lLTM require PKA for their induction, the findings in honeybees support the existence of two parallel cAMP/PKA pathways implicated in LTM formation: one triggers molecular cascades leading to translation-dependent eLTM, and the other is involved in triggering cascades required for transcription-dependent lLTM. The specific rescue of lLTM by elevating basal PKA activity in fed animals suggests that other molecular pathways involved in learning and memory formation also contribute to the network of molecular interactions between satiation and learning processes.

4.06.10 Summary

The accessibility of the honeybee brain provides the basis for the analysis of learning-induced changes of signaling cascades in defined brain areas. The conditioning-induced transient activation of different signaling cascades occurs in a narrow time window of a few minutes during and after conditioning and is highly dependent on the training parameters, which are critical for induction of distinct memories. The temporal action of signaling cascades can be imitated by local uncaging of substances activating these cascades. Thus, combination of behavioral and biochemical techniques reveals important insights into the organization of the molecular network underlying memory formation *in vivo*.

The temporal analysis of the conditioning-induced activation of PKA, which plays a conserved role in LTM formation in all model systems, demonstrated a critical function for a prolonged PKA activation in the ALs in LTM formation. This prolonged PKA activation is mediated by the NO/cGMP system and is necessary to induce molecular processes underlying eLTM and lLTM. An independent, PKA-mediated process specifically implicated in lLTM formation could be identified by altering the bee's satiation level during conditioning. The network of molecular processes implicated in LTM formation also includes processes localized in the MBs. Here, signaling cascades activated by the neurotransmitter glutamate are involved in processes that facilitate LTM formation, an observation that supports the idea that the function of glutamate in synaptic plasticity is conserved. The molecular processes involved in induction and maintenance of MTM are independent of those underlying induction of LTM. In the ALs, conditioning triggers the cleavage of PKC by calpain that results in production of PKM. The autonomously active PKM is specifically required to maintain MTM in the range of a few hours.

Taken together, the temporal and spatial analysis of signaling cascades triggered by associative learning in honeybees reveals a parallel organization of the molecular network contributing to memory formation. Such a parallel-organized network is perfect for the fine-tuning of the multiphasic process of memory formation by training parameters and other factors influencing learning like satiation, stress, and circadian rhythm.

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4.07 Molecular and System Analysis of Olfactory Memory in *Drosophila*

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4.07.1 Introduction: Three Types of Olfactory Memories and a Memory Center

Drosophila melanogaster has been used as a model system in investigating many cellular and developmental processes. The *Drosophila* central nervous system is composed of neurons and glia that operate on the same fundamental principles as their mammalian counterparts. Even though the *Drosophila* brain has only 100 000 cells (Ito et al., 2003), it produces complex behaviors and sustains various forms of learning and memory, which are highly amenable to analysis by current genetic methods. Moreover, *Drosophila* studies make it possible to investigate different concepts of memory that may eventually be generalized to other species, because roughly 50% of human genes have a *Drosophila* ortholog (Rubin et al., 2000).

4.07.1.1 Olfactory Aversive Conditioning

We will focus on the most commonly used paradigm to assess *Drosophila* memory, olfactory aversive conditioning (*See* also Chapter 1.28). This paradigm was instrumental in the discovery of most learning and memory mutants, and still contributes to our increasing comprehension of molecular and cellular processes that underlie associative memory. Importantly, the characterization of specific anatomical and memory mutants has allowed us to partially decipher the dynamics of memory phases in this system.

The principle, originally developed by Quinn et al. (1974) and improved by Tully and Quinn (1985), is as follows: for training, a single associative-learning trial consisting of an odor (the conditioned stimulus, CS^+) is accompanied by electric shocks (the unconditioned stimulus, US); and after a short rest period, a second odor (the conditioned stimulus, CS^-) is presented in

absence of shock. To test memory, flies are placed in an apparatus where they can choose between two compartments that are presented simultaneously: one containing the CS^+ odor, the other one the odor CS^-. After 2 min of test, flies from each compartment are isolated and counted to calculate a memory performance index (PI) that typically ranges from 0 (no memory) to 1 (perfect score). This protocol generates immediate memory scores of around 0.85, indicating that the majority of flies are able to learn and remember. To increase the efficiency of this process, our laboratory has developed an automated conditioning setup that allows parallel training of six groups of flies by means of a barrel (for schematic representation, see Pascual and Preat, 2001).

If flies are submitted to one training cycle (i.e., odor 1 paired with electric shock reinforcement for 1 min, hereafter called the short protocol), 1-day memory retention is weak. Multiple massed repetitions (hereafter called the massed protocol) of this single cycle generate memory that can be measured for a few days. Multiple spaced repetitions with a rest period of 15 min between each cycle (hereafter called the long protocol) generate a stabilized memory, called long-term memory (LTM), which can be measured for 1 week (Tully et al., 1994).

In addition to these quantitative variations, an important question is whether the different protocols induce distinct types of memory. Several general treatments have been used to disrupt specific memory components in wild-type flies. After the short protocol, 3-h memory retention is disrupted by submitting the flies to a cold shock immediately after training, but becomes progressively more resistant when this treatment is administrated at later times (Quinn and Dudai, 1976; Tully et al., 1994). This observation characterizes two memory phases, one anesthesia-sensitive memory (ASM, or labile memory) and one anesthesia-resistant memory (ARM, or consolidated memory). ASM and ARM are also generated after the massed protocol. Flies fed with an inhibitor of protein synthesis, cycloheximide, show a disrupted LTM but normal ARM (Tully et al., 1994) and ASM (Dudai, 1977), suggesting that unlike ARM and ASM, LTM depends on *de novo* protein synthesis (cf **Table 1**). These results suggest that flies can form three different types of olfactory memory, and the search for mutants that affect these phases differently is a major goal of *Drosophila* memory research.

4.07.1.2 The Mushroom Bodies Are the Center of Olfactory Memory

Olfactory receptor neurons, located in the antennae and maxillary palps, project their axons into the antennal lobes (ALs). From the ALs, projection neurons convey the olfactory information via the antennoglomerular tract in part to mushroom bodies (MBs) (**Figure 1**), a pair of prominent and characteristically shaped neural centers. In the 1970s and 1980s, the function of the MBs was assessed in different insect species with classical interventionism approaches, cooling or ablation (Menzel et al., 1974; Erber et al., 1980). In the cockroach, MBs are a specialized neuropile involved in processing and storing multimodal sensory information (Li and Strausfeld, 1997). Over the years, MBs have been implicated in olfactory learning and memory (Davis, 2005), and in a variety of complex functions including courtship learning (Mehren et al., 2004), context generalization in visual learning (Liu et al., 1998), walking activity (Martin et al., 1998), and sleep (Joiner et al., 2006; Pitman

Table 1 Synopsis of different memory phases, the protocols that generate them, and their properties

	Anesthesia-sensitive memory	*Anesthesia-resistant memory*		*Long-term memory*
Duration	Short-lasting memories: Short-term memory (STM) lasting a few minutes and middle-term memory (MTM) lasting a few hours	Semi-stabilized memory lasting a few days		Stabilized memory lasting more than 1 week
Protocol	All protocols	Short protocol	Massed protocol	Long protocol
Anesthesia treatment	Sensitive	Resistant	Resistant ?	Resistant ?
Protein-dependent synthesis	No	No		Yes

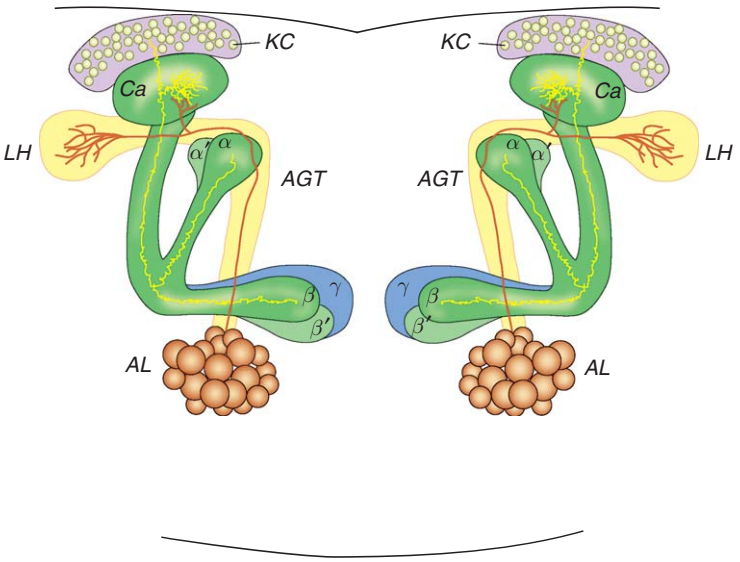

Figure 1 *Drosophila* olfactory system. Olfactory sensory neurons project to the antennal lobes (AL). From there, projection neurons project through the antennoglomerular tract (AGT) and connect mushroom body (MB) dendrites localized in the calyx (Ca), as well as the lateral horn (LH). Each MB is composed of about 2500 neurons, the Kenyon cells (KC). Three types of KC project in five lobes: α/β, α'/β', and γ.

et al., 2006). In *Drosophila*, MBs are composed of three main classes of neurons whose axons divide to form two vertical lobes (α and α') and three median ones (β, β', and γ) (Crittenden et al., 1998) (**Figure 1**).

Brain mutants with MB structural defects were first isolated in the 1980s. Unlimited numbers of animals with the same anatomical defect could be assessed using mutants, without interventionism. This helped to highlight the role of MBs in olfactory learning and memory (Heisenberg et al., 1985). However, one caveat of this approach is that the anatomical defects are generally not specific to the MBs (de Belle and Heisenberg, 1994). An alternative approach was used to generate flies without MBs (de Belle and Heisenberg, 1994). Hydroxyurea, an anti-mitotic agent, was fed to newly hatched wild-type larvae. At this early developmental stage, only five neuroblasts – the neurons' precursor cells – are mitotically active within each brain hemisphere: the four that generate the MBs, and one in the antennal lobe. Thus, hydroxyurea treatment leads to viable adults flies with almost no MBs. These flies turned out to show no olfactory memory (de Belle and Heisenberg, 1994), although abilities to mediate olfactory memory (shock reactivity, olfaction, and locomotor behavior) were intact. Together, these data strongly implicate the MBs in associative olfactory memory.

The essential role of MBs in olfactory memory is further outlined by the high expression in the MBs of many proteins involved in learning and memory, as will be seen.

4.07.2 Short-Lasting Memories

4.07.2.1 The cAMP Pathway Plays a Key Role in Associative Memory

We do not intend to review here in detail all *Drosophila* learning and memory genes, or the various conditioning protocols (for review see Waddell and Quinn, 2001; Dubnau et al., 2003b; Heisenberg, 2003; Davis, 2005; McGuire et al., 2005; Skoulakis and Grammenoudi, 2006). Rather, we will describe the different approaches that have allowed the analysis of *Drosophila* associative memory at the molecular, cellular, network, and systemic levels.

The strength of *Drosophila* molecular genetic tools underlies the discovery and the dissection of new molecular pathways involved in memory. The use of mutants, and in particular the recently designed conditional mutants, is a powerful means to link the molecular, cellular, network, and system levels. The global experimental plan is to generate random single-gene mutations and to select mutants that show a specific learning or memory defect. In the second

step, the aim is to identify the mutated gene and to understand its function in memory (Benzer, 1973). The first screens for *Drosophila* learning and memory mutants were produced by Seymour Benzer and colleagues more than 30 years ago. Four thousand fly stocks carrying ethyl methane sulfonate-induced mutations were tested for their ability to learn in an olfactory conditioning assay (Quinn et al., 1974; Dudai et al., 1976; Quinn et al., 1979). The first *Drosophila* learning and/or memory mutants characterized were *dunce* (*dnc*) and *rutabaga* (*rut*). Biochemical approaches showed that *dnc* flies were deficient in one form of cyclic adenosine monophosphate (cAMP) phosphodiesterase activity (Byers et al., 1981; Davis and Kiger, 1981), and that *rut* flies were deficient in the activity of a Ca^{2+}/calmodulin-sensitive adenylyl cyclase (Livingstone et al., 1984). The current view is that Rut adenylyl cyclase could act during learning as a coincidence detector for the US and CS, because of the ability of adenylyl cyclase to respond to two different types of signals, namely extracellular monoamines and intracellular calcium levels (Livingstone et al., 1984; McGuire et al., 2003).

However, EMS-induced mutations are often difficult to localize in genome, and therefore the main problem with these first learning and/or memory mutants was to identify the mutated gene. The first step consists of genetically mapping the mutation by complementation tests, using *Drosophila*'s deletion collection. This, however, only defines a broad area covering many other genes (Dudai et al., 1976; Livingstone et al., 1984). Thus, the cloning of the responsible gene took years and was facilitated by the use of new mutant alleles induced by the insertion of a transposable P-element. Description of P-element insertions within 200 nucleotides of the *rut* transcription start site identified *rut* as the gene for the Ca^{2+}/calmodulin-responsive adenylyl cyclase (Han et al., 1992; Levin et al., 1992). On the other hand, new EMS-induced *dnc* mutants came from a screen for female sterility mutants (Mohler, 1977). The *dnc* gene was identified by recombinational mapping of *dnc* mutations with restriction site polymorphisms as genetic markers (Davis and Davidson, 1984) and by identification of homologous cDNA clones to known phosphodiesterases (Chen et al., 1986). *dnc* encodes a cAMP-specific-phosphodiesterase II. Another mutant, *amnesiac* (*amn*) mutant, was identified in a screen for flies with affected memory (Quinn et al., 1979). The *amn* gene was later identified thanks to a P-element-induced allele, which acts as a second site suppressor of the *dnc* female sterility phenotype, and has been repeatedly isolated since (Feany and Quinn, 1995; Moore et al., 1998; Toba et al., 1999). The Amn peptide shows some similarity to a putative adenylyl cyclase activating peptide (PACAP). The biochemical activity of Amn is unknown, but both the genetic and molecular evidence suggests that Amn acts through adenylyl cyclase to increase the concentration of cAMP (Feany and Quinn, 1995).

Discoveries of these proteins displaying major roles in memory processes suggested important roles for intracellular cAMP and cell signaling cascade in olfactory learning. Protein kinase A (PKA) being a major effector for cAMP, Drain et al. tested the hypothesis that PKA is involved in memory processes (Drain et al., 1991). PKA is a tetramer composed of two catalytic (C) and two regulatory (R) subunits. In the absence of cAMP, regulatory subunits bind to the catalytic subunits and inhibit their activity, and conversely, at elevated levels, cAMP binds to the regulatory subunits, which then release the catalytic subunits to phosphorylate target proteins. Protein kinase inhibitor (PKI) is a PKA inhibitory peptide that binds the PKA catalytic subunit and prevents its release in response to cAMP. Ubiquitous PKI overexpression disrupts olfactory memory (Drain et al., 1991).

Another approach identified a new mutant of the catalytic subunit gene (DCO) of PKA. Enhancer detection screening is a combination of histological screening and P-element mutagenesis. The modified P-element carries a reporter gene and is randomly inserted in genome by classical techniques of mutagenesis. Screening is based upon the fact that expression of the reporter gene mimics the expression of the endogenous gene where this reporter gene was inserted (**Figure 2**). The aim is to first find by histology lines whose reporter expression pattern includes the MBs. The integrated P-element may disrupt expression of the MB-expressed gene; therefore testing memory performance of the mutant lines identifies new proteins involved in this process. Mutants in the catalytic subunit gene of PKA were isolated by this approach (Skoulakis et al., 1993). Reduction of 80% PKA activity in heteroallelic mutants of DCO generated a memory disruption. The R-type I subunit of PKA is also crucial for associative learning (Goodwin et al., 1997).

An additional study outlining the important role of cAMP signaling in learning and memory came from a study of mutations in neurofibromatosis type 1 (*Nf1*) *Drosophila* homolog. Mutations in the human *NF1* gene cause benign and malignant tumors of the nervous system and generate learning deficits in humans (Ferner et al., 1996; North et al., 1997; Gutmann, 1999). Mice heterozygous for *Nf1*

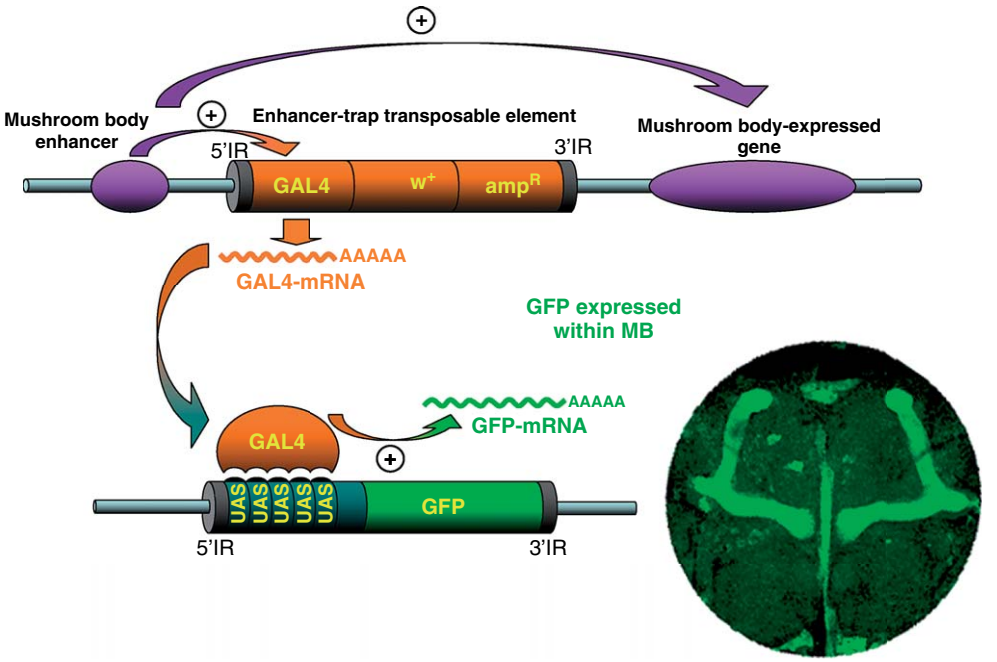

Figure 2 The GAL4/upstream activating sequence (UAS) system. When an enhancer-trap transposable element is inserted near transcription enhancers that control expression in a given structure, the GAL4 gene that is contained in the P-element is expressed in the same structure. If this fly also contains a reporter gene downstream of the UAS sequences, GAL4 will drive transcription of the reporter. GFP, green fluorescent protein; MB, mushroom body.

mutations have an increased predisposition to learning impairment (Silva et al., 1997). The *Drosophila* NF1 protein shows a high degree of conservation with the human protein (60%). Flies lacking NF1 at the adult stage show an olfactory memory defect, suggesting that the human homolog is involved in learning and memory, and memory impairment is not due to developmental defect (Guo et al., 2000). Genetic and biochemical analysis have revealed that NF1 inhibits Ras activity (Cichowski and Jacks, 2001; Zhu and Parada, 2002). Interestingly, NF1 also participates in the activation of adenylyl cyclase, increasing cAMP levels via at least two distinct pathways: by an NF1/Ras-dependent mechanism stimulated by growth factors and by an NF1/Gαs-dependent pathway, acting through Rut (Hannan et al., 2006). By overexpressing a constitutively active PKA catalytic subunit in an *NF1* mutant background, Guo et al. rescued the NF1 memory defect, suggesting the biochemical deficiency in the *NF1* mutants must reside upstream of PKA induction in the cAMP pathway (Guo et al., 2000).

In conclusion, the cAMP is particularly important to support olfactory associative memory. Both a lack (in *rut, DCO, amn, NF1*) or an excess (in *dunce*) of cAMP pathway activity can cause memory impairment, and the learning score of double mutant *dnc*, *rut* is reduced when compared with either single mutants (Tully and Quinn, 1985), which suggests that cAMP levels have to be finely regulated during memory formation to control behavioral plasticity. Alternatively, Dnc and Rut may act in different subsets of neurons.

4.07.2.2 Mushroom Bodies and the cAMP Pathway

Drosophila MBs are required for olfactory learning and memory, and insights from mutants have indicated that the cAMP pathway plays a key role in memory establishment. Nevertheless, until recently it remained to be proved that cAMP metabolism is strictly required in the MBs for learning and memory. A powerful tool for spatial control of gene expression is the GAL4/UAS enhancer-trap technique (Bellen et al., 1989; Wilson et al., 1989; Brand and Perrimon, 1993) (**Figure 2**), which enables selective activation of any cloned gene in a wide variety of tissues and cells. With this system (Brand and Perrimon, 1993), Connolly and colleagues (1996) ectopically stimulated MB cAMP signaling by expressing a constitutively activated Gα_s-protein (Gα_s^*). Permanent adenylyl cyclase activation

specifically in the MBs leads to an impaired associative memory.

That memory is impaired after ablation or functional disruption of a brain structure does not necessarily imply that the memory trace itself is localized within this structure. To localize short-term memory (STM), it was necessary to rescue the memory abilities of a mutant by expressing the corresponding protein in a specific brain structure. As seen earlier (**Figure 1**), MBs are composed of three main classes of neurons. One class of neurons bifurcates to generate one vertical branch in the α lobe and one horizontal branch in the β lobe; another generates one branch in another vertical lobe, the α' lobe, and one in the horizontal β' lobe; and a third class of neurons generates a single projection into the horizontal γ lobe (Crittenden et al., 1998). Zars and colleagues showed that expression of Rut Ca^{2+}/calmodulin adenylyl cyclase (Rut-AC) in γ lobes of MBs, but not in α/β lobes, partially restores a normal learning capacity in *rut* flies (Zars et al., 2000), whereas McGuire et al. reported that Rut-AC has to be expressed simultaneously in γ and α/β lobes to rescue the memory defect due to *rut*. A new recent study indicates that marginal *rut* rescue is observed when cyclase is expressed only in some α/β neurons (depending on the odor pair used for conditioning), an intermediate rescue is observed with γ expression, while a full rescue is obtained with α/β and γ expression (Akalal et al., 2006).

However, in these experiments, Rut was expressed not only in the MBs at the adult stage, but also during development. Thus, the behavioral rescue might have been due to the correction of an MB developmental defect, an indirect cause of the learning defect. To circumvent this hypothesis, techniques have been developed to control spatial and temporal expression of a protein. Using the TARGET and SWITCH methods (**Figure 3**), Ron Davis and his team showed that the presence of Rut in adult $\alpha/\beta/\gamma$ lobes of MBs alone was sufficient to rescue *rut* memory defect (McGuire et al., 2003; Mao et al., 2004).

These discoveries raise a new set of questions. For example, at which step of information processing are the MBs involved: acquisition, consolidation, or retrieval? Do the MBs carry a memory trace or are they a relay? These challenging problems are being solved with new genetic and imaging techniques.

4.07.2.3 Mushroom Bodies Anatomic-Functional Maps

A sophisticated approach to disturb neuronal circuits, based on a rapid and reversible blockage of synaptic

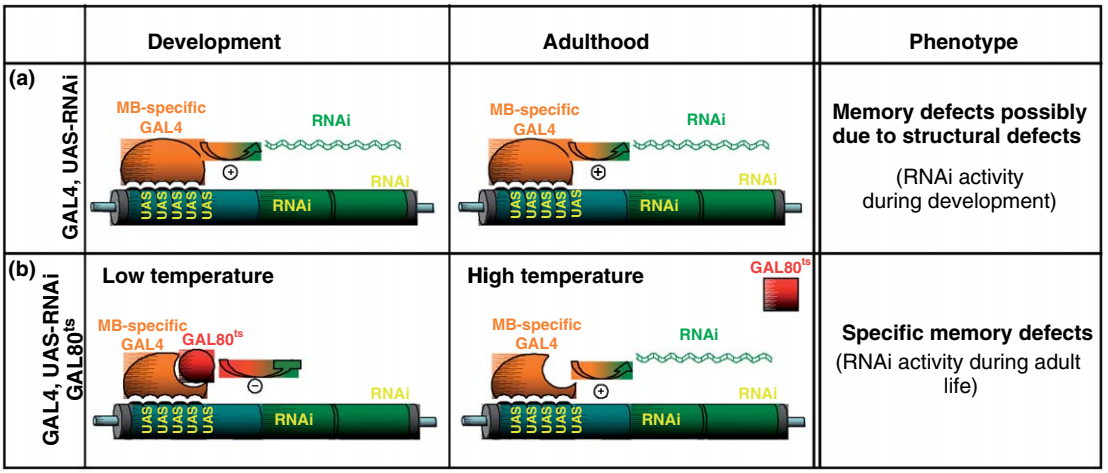

Figure 3 The TARGET technique helps us to discriminate between developmental and adult physiological effects. A sequence encoding an inverted repeat RNA (RNAi) is placed under UAS control. (a) GAL4 activates RNAi expression during development and adulthood. A memory defect could be caused indirectly by structural defects during development. (b) The TARGET system solves this problem. Flies are maintained at low temperature during their developmental stages. At this temperature, $GAL80^{ts}$ inhibits the transcription activity of GAL4, so that no RNAi is expressed. When adults emerge, they are transferred at permissive temperature (30 °C); the $GAL80^{ts}$ protein becomes ineffective and GAL4 activates the transcription of the RNAi in adult MBs. The putative memory defects are due to the decreased concentration of the mRNA targeted by the RNAi. In another use of the TARGET system, Rut expression in adult *rut* mutant background was sufficient to rescue *rut* memory defect (McGuire et al., 2003).

transmission, was developed by Kitamoto (2001). The *shibire* (*shi*) gene encodes a microtubule-associated guanosine triphosphatase (GTPase), dynamin, which is involved in endocytosis and is essential for synaptic vesicle recycling and maintenance of the readily releasable pool of synaptic vesicles. The temperature-sensitive allele, shi^{ts1}, is defective in vesicle recycling at the restrictive temperature (>29 °C), resulting in a rapid blockade of synaptic transmission. The shi^{ts1} mutation has a dominant effect, blocking chemical synapses even in the presence of a normal shi^{+} allele. Expression of the shi^{ts1} tool can therefore be used within the GAL4/UAS system. The GAL4/UAS-shi^{ts1} approach is very powerful, as it allows us to inhibit particular brain circuits at precise times. To determine whether the MBs are required during the acquisition, consolidation or retrieval phases, the temperature-sensitive Shi^{ts1} protein was specifically expressed in MB neurons in order to transiently disrupt synaptic neurotransmission (Dubnau et al., 2001; McGuire et al., 2001; Schwaerzel et al., 2002). It was shown that the synaptic outputs of MB neurons are required during retrieval of the STM but not during acquisition or consolidation. Which of the MB lobe outputs are required for STM retrieval? Two groups implicated mainly α/β and γ neurons (Dubnau et al., 2001; Schwaerzel et al., 2003), while another study suggested that only the α/β neurons were required (McGuire et al., 2001). Using a γ-specific Gal4 driver, we showed that γ neuron output is indeed required for STM and middle-term memory (MTM) retrieval (Isabel et al., 2004). All together, these initial studies indicate that a STM/MTM trace is localized at α/β and γ output synapses or upstream of these synapses. Interestingly, it was shown recently that α'/β' lobes are involved in acquisition and consolidation but not in memory retrieval (Krashes et al., 2007), showing that the MB lobes play very distinct roles.

Taken together, these experiments suggest that associative memory requires the sequential involvement of different subsets of MB neurons: α'/β' lobes for acquisition and consolidation, and $\alpha/\beta/\gamma$ for memory retrieval. However, as seen above, Rut-AC is thought to act as the major temporal integrator of associative learning (Livingstone et al., 1984), and the learning defect of *rut* flies can be totally rescued by expression of Rut-AC only in the $\alpha/\beta/\gamma$ lobes (McGuire et al., 2003; Akalal et al., 2006). This observation is paradoxical with the shi^{ts1}approach, which shows that α'/β' lobes (Krashes et al., 2007), but not $\alpha/\beta/\gamma$ lobes, are required for acquisition. It is possible that α'/β' neurons stimulate α/β or γ neurons. Perhaps the coincidence detection model is too simple, and further studies will be necessary to elucidate this discrepancy.

4.07.2.4 Imaging Mushroom Bodies and Antennal Lobes

One major caveat of *Drosophila* central brain studies is that direct electrophysiological analysis is scarce (Wilson et al., 2004), due to the small size of neuron cell bodies (less than 5 μm in diameter). To circumvent this difficulty, two partially alternative approaches have been followed: analysis of learning and memory mutants at the neuromuscular junction (Zhong and Wu, 1991; Renger et al., 2000), which avoids the complexity of brain physiology, as well as the analysis of isolated MB neurons in culture (Wright and Zhong, 1995). These experimental systems can provide interesting molecular and cellular information, but they are inadequate for assessing neuronal function at the level necessary for a global understanding of memory systems.

Odor processing occurs in a complex tissue environment, and the identification of the repertoire of brain cell assemblies involved in olfactory memory requires visualization of their network activity at high spatial and temporal resolution, in relatively intact preparations. Optical neural activity recordings allow the study of brain activity with micrometer spatial resolution, and activity-sensitive fluorescent probes have been recently used in *Drosophila*. Those sensors are proteins, and their expression can therefore be restricted to specific subsets of neurons with the GAL4/UAS system. Several sensors have been successfully brought to *Drosophila*, including those that monitor the local change of pH that accompanies neurotransmitter release (Yu et al., 2004) or changes in the intracellular calcium concentration that provide a valuable indicator of electrical activity (Aequorin (Rosay et al., 2001); Cameleon (Fiala et al., 2002); Camgaroo (Yu et al., 2003); and G-CaMP (Wang et al., 2003, 2004b)). Using the G-CaMP reporter and two-photon microscopy, stereotyped odor-evoked patterns have been observed in the antennal lobe glomeruli (Wang et al., 2003) and in the MBs (Wang et al., 2004b).

The ultimate goal of future imaging studies is to build a functional map of cell assemblies encoding memory in different regions of *Drosophila* brain, by comparing the activity of trained and naive animals, in normal flies or memory mutants. A first step, achieved as a transient change in the spatial code, was

observed in the antennal lobe of wild-type flies 3 min after olfactory associative conditioning (Yu et al., 2004). What happens to MB calcium concentrations during memory processes? The G-CaMP reporter was driven in α/β lobes to assess whether these lobes, which are functionally active during memory retrieval (McGuire et al., 2001; Isabel et al., 2004; Akalal et al., 2006), present an odor-evoked calcium signal dependent on associative short-lasting memory (Yu et al., 2006). Surprisingly, although calcium responses to odor and electric shock can readily be detected in the α/β neurons, one pairing of odor and electric shock did not alter odor-evoked calcium concentration in these neurons. Yu et al. suggest two alternative explanations. Firstly, short-lasting memory traces are not formed in α/β neurons but in other types of MB neurons such as α'/β' lobes, a hypothesis which fits with *shi*^{ts1}experiments driven in α'/β' neurons (Krashes et al., 2007), and/or γ lobes (Yu et al., 2006). Secondly, short-lasting memory traces might be produced by calcium-independent cellular mechanisms (Yu et al., 2006).

4.07.2.5 Around the Mushroom Bodies

MBs are innervated by two groups of well-characterized amnesiac and dopaminergic neurons that are involved in olfactory associative memory: dopaminergic neurons (DNs) and the dorsal paired median (DPM) neurons that express the Amn peptide.

4.07.2.5.1 Dopaminergic neurons are required for aversive conditioning

Dopamine (DA) is a neuromodulator that is involved in appetitive reinforcement in mammals (Mirenowicz and Schultz, 1996) and in *Aplysia* (Brembs et al., 2002). It has been known for a while that *Drosophila* synthesizes DA (Hirsh and Davidson, 1981; Livingstone and Tempel, 1983; Wright, 1987; Neckameyer and Quinn, 1989). The recent genetic and imaging tools developed in *Drosophila* allow us to assess the roles of DA in learning and memory. Based on the fact that tyrosine-hydroxylase (TH) is the rate-limiting step in DA biosynthesis, a Gal4-driver whose expression mimics that of TH was tested (Friggi-Grelin et al., 2003). This tool has allowed the identification of six neuronal dopaminergic clusters that project in particular to MBs and to the central complex (Friggi-Grelin et al., 2003), confirming earlier TH and DA immunolabeling studies (Nassel and Elekes, 1992). By combining the TH-GAL4 driver and UAS-Shi^{ts1}, Schwaerzel et al. showed that blocking DNs only during acquisition disrupts STM (Schwaerzel et al., 2003). Thus DNs are required for aversive conditioning, and could convey US (in this case, electric shock) information (Schwaerzel et al., 2003). Another study has shown that in naive flies, electric shock generates a strong activity in the DNs, whereas the odor generates a weak signal. However, after several pairings between the odor and the shock, odor-evoked activity is significantly prolonged. In agreement with the behavioral approach (Schwaerzel et al., 2003), *in vivo* imaging therefore suggests that DNs play a role in aversive conditioning in *Drosophila* (Riemensperger et al., 2005). To study if the activation of DNs is sufficient to mediate the aversive cue, Schroll et al. have developed a technique to remotely stimulate DNs. By expressing a light-activated cation channel (channelrhodopsin-2), in larvae, DNs can be stimulated specifically by illuminating the larvae with blue light. The authors used a discriminatory learning paradigm: one odor (CS^+) is associated with a reinforcing salt stimulus as the aversive US, while another odor (CS^-) is presented without salt (Gerber and Hendel, 2006). Interestingly, the reinforcing salt stimulus could be replaced functionally by DN activation with blue light. DNs are therefore sufficient to convey the US for aversive conditioning (Schroll et al., 2006).

4.07.2.5.2 Dorsal paired median neurons are required for consolidation

Although *amn* is an MTM mutant (Quinn et al., 1979) putatively involved in cAMP metabolism (Feany and Quinn, 1995), Amn is not expressed in the MB but in the DPM neurons which project onto all the MB lobes (Waddell et al., 2000). In the *amn* mutant, expression of wild-type Amn in the DPM neurons re-establishes normal MTM (Waddell et al., 2000). Conversely, when Shi^{ts1} is expressed at restrictive temperature into DPM, MTM is affected (Waddell et al., 2000). Blocking the DPM during the consolidation phase only (Keene et al., 2004), and more precisely, 30 min after conditioning (Keene et al., 2006), phenocopies the *amn* mutant memory defect (Waddell et al., 2000). Scott Waddell's group has used an elegant approach to determine the site of action of DPM neurons projecting onto the MBs. By driving Dscam expression, a protein involved in axonal guidance (Wang et al., 2002), in the DPM neurons during development, DPM innervation of the $\alpha/\beta/\gamma$ lobes is disrupted. However, those flies show normal memory, indicating that MTM is stabilized in α'/β' lobes in response to DPM. Co-expression of Dscam

and Shi^{ts1} abolishes memory (Keene et al., 2006) and confirms the role of the α'/β' lobes during memory consolidation. Also, as mentioned above, Amn might activate an adenylyl cyclase. Thus, Amn could activate Rut via a G-protein-coupled receptor, in order to extend MTM. However, *rut* MTM is rescued when Rut is specifically expressed in the $\alpha/\beta/\gamma$ lobes of the MBs, even though Amn stimulates the α'/β' lobes. It is therefore difficult to imagine that Amn is directly involved in Rut activation, unless Amn diffuses into the $\alpha/\beta/\gamma$ lobes. Since it is likely that DPM neurons also synthesize acetylcholine (Keene et al., 2004), it is possible that this transmitter is directly involved in the stimulating MBs.

Yu et al. took an imaging approach to study the function of the Amn-expressing DPM neurons in memory. DPM neurons respond to both the shock (the US) and to the odor (the CS), and pairing the CS and the US increases odor-evoked calcium signals in the same time window during which DPM neuron synaptic transmission is required for normal memory (Yu et al., 2005). Interestingly, the delayed olfactory memory trace of DPM neuron processes that innervate the vertical lobes is branch-specific (Yu et al., 2005). Conclusions from the behavioral experiments using Shi^{ts1} (Waddell et al., 2000; Keene et al., 2004, 2006) are therefore strengthened by imaging. One hypothesis is that the α'/β' neurons and the DPM neurons form a mutually reinforcing loop that is necessary for this consolidation (Krashes et al., 2007).

4.07.3 Anatomical, Molecular, and Systemic Analysis of Consolidated Memories: Anesthesia-Resistant Memory and Long-Term Memory

4.07.3.1 Anesthesia-Resistant Memory

As described earlier, ARM is a semi-stabilized memory that can be generated either by a single training cycle or massed training (ARM is a subset of total memory, whose proportion increases with time after training, conversely to ASM).

4.07.3.1.1 Anesthesia-resistant memory is localized in mushroom bodies

MBs are involved in acquisition, consolidation, and retrieval of short-lasting memory, but does ARM depend on MBs? To assess the anatomical location of ARM, flies whose different MB neurons were blocked by Shi^{ts} were trained for one cycle, submitted 1 h afterward to a cold shock to disrupt ASM, and tested 1 h after that to measure ARM. When α/β neurons are blocked, ARM is almost totally abolished, whereas γ neuron blockade does not disrupt ARM. Thus we showed that ARM generated after one training cycle relies mainly on α/β neurons MBs (Isabel et al., 2004).

4.07.3.1.2 Molecular pathways

4.07.3.1.2.(i) Radish *Radish* (*rsh*) is a mutant whose residual memory is erased by cold-shock anesthesia (Folkers et al., 1993). Thus *rsh* is specifically deficient in ARM, and it is the only currently known mutant that presents this characteristic. The *rsh* gene was localized within a 180-kb interval in the 11D-E region of the X chromosome, and several candidate genes were identified (Folkers et al., 1993). Recently, Josh Dubnau's group reported that the gene responsible for the *rsh* phenotype was a phospholipase A2 (PLA2), whose expression could rescue the mutant defect (Chiang et al., 2004). However, this rescue experiment could not be reproduced, and it turned out that PLA2 maps 95 kb outside the behaviorally determined deletion interval (Folkers et al., 2006). Instead a second team has reported that *rsh* encodes a novel protein, corresponding to the predicted gene CG15720, and containing possible nuclear localization motifs and 23 predicted PKA and 14 predicted PKC phosphorylation sequences (Folkers et al., 2006). Expression of this gene under the control of an inducible heat-shock promotor in a *rsh* background restores normal ARM (Folkers et al., 2006). The Rsh protein is highly expressed in the MBs (Folkers et al., 2006), which corroborates the behavioral localization of ARM (Isabel et al., 2004).

4.07.3.1.2.(ii) Atypical protein kinase M The atypical protein kinase M (aPKM) is a persistently active truncated isoform of atypical protein kinase C (aPKC). Overexpression of either a mouse or *Drosophila* aPKM transgene enhances memory after massed conditioning, but not after spaced training (Drier et al., 2002). It is therefore conceivable that aPKC is a molecular component of ARM. Because this effect is not blocked in a *rsh* background, the authors proposed that PKM acts downstream of *rsh*. In support of this, inhibition of aPKM disrupts consolidated memory after massed conditioning (Drier et al., 2002).

4.07.3.2 Long-Term Memory

4.07.3.2.1 Long-term memory is localized in vertical lobes of mushroom bodies

MB lobes are known to be required to form short-lasting memory: α'/β' lobes for acquisition and consolidation and $\alpha/\beta/\gamma$ for retrieval. Are MBs involved in LTM? We have identified in our lab the *alpha-lobes absent* (*ala*) mutant, which shows abnormal MB anatomy (Boquet et al., 2000). This mutant shows a particularly unusual MB phenotype: 10% of *ala* individuals possess all five MB lobes, 36% lack the horizontal β and β' lobes, and 4.5% lack vertical α and α' lobes (the remaining subpopulations present different MB phenotypes in the left and the right hemispheres) (Pascual and Preat, 2001). The *ala* mutant flies were trained according to the three following procedures: the short protocol, massed protocol, and long protocol. We analyzed separately the brains of flies that had made the correct and the wrong choice during the memory test, to calculate the memory score of each class of *ala* mutants. The *ala* flies lacking α/α' lobes display a normal STM and ARM, but no LTM at 24 h (Pascual and Preat, 2001) or 5 h (Isabel et al., 2004) after spaced conditioning. Thus, MBs, and more precisely, the α/α' vertical lobes, are necessary to form LTM (Pascual and Preat, 2001). By expressing Shi^{ts1} in α/β lobes, it was further shown that α lobe outputs are required during LTM retrieval (Isabel et al., 2004). Is there an LTM trace in the α vertical lobes?

The G-CaMP reporter was driven in α/β neurons to analyze whether these lobes present an odor-evoked calcium level dependent on associative long-lasting memory (Yu et al., 2006). Whereas α/β neurons presented no increase in calcium during STM, α branches, but not β branches (which belong to the same group of neurons), present an increase in response to conditioned odor after spaced conditioning. This result corroborates the behavioral data (Pascual and Preat, 2001; Isabel et al., 2004). Moreover, Ron Davis's group has shown that this increased calcium signal is blocked after inhibition of protein synthesis, and also by the expression of the inhibitory form of the transcription factor Creb (see next section for details), which blocks LTM (Yu et al., 2006). The authors confirmed that *amn* is required to form LTM, as the mutant fails to increase calcium activity after LTM conditioning (Yu et al., 2006). The hypothesis that DPM neurons participate in LTM consolidation is supported by the observation of a delayed memory trace in the DPM neurons that innervate vertical lobes (Yu et al., 2005, 2006).

4.07.3.2.2 Molecular pathways

4.07.3.2.2.(i) Transcriptional regulation

4.07.3.2.2.(i).a Creb

PKA, in response to increased cAMP, is believed to activate a subset of Creb family proteins in the nucleus (Bacskai et al., 1993) that could in turn activate gene expression required for LTM. The *Drosophila* Creb family gene produces seven alternatively spliced isoforms, among which is a PKA-responsive transcriptional factor (dCreb-a) and another that is an antagonist of the PKA-responsive transcription (dCreb2-b) (Yin et al., 1995). LTM is disrupted by dCreb2-b repressor overexpression (Yin et al., 1994; Perazzona et al., 2004), likely due to inhibition of the gene expression required to establish LTM. Yin and colleagues reported that flies overexpressing the dCreb2-a activator generated LTM after a single training cycle (Yin et al., 1995). However, this result could not be replicated, and it was shown that the original dCreb2-a transgenic flies carried an accidental mutation that produced a truncated protein with no DNA binding domain (Perazzona et al., 2004). Moreover, ubiquitous adult induction of the correct Creb2-a isoform led to lethality (Perazzona et al., 2004), likely because of ectopic expression of downstream proteins. The exact role of Creb in LTM formation therefore remains to be elucidated.

4.07.3.2.2.(i).b Notch

Notch is a signaling receptor controlling cell fate determination and pattern formation in development (Artavanis-Tsakonas et al., 1999). This transmembrane protein is cleaved in response to ligands such as Delta. Its cytoplasmic part can enter the nucleus to promote regulation of gene expression (Kidd et al., 1998; Schroeter et al., 1998; Struhl and Adachi, 1998). Besides its involvement in development, Notch is required for the regulation of neurite outgrowth in the adult mammalian brain (Sestan et al., 1999). Because of this role in neural ultrastructure regulation, and thus potentially in neuronal plasticity (Wang et al., 2004a) and memory (Costa et al., 2003) in mammals (for review, see Costa et al., 2005), its role in associative memory in *Drosophila* was assessed independently by two teams (Ge et al., 2004; Presente et al., 2004). To circumvent developmental roles of Notch, inducible Notch

manipulations were performed at the adult stage. A temperature-sensitive Notch mutant allele and a dominant-negative Notch mutant displayed intact STM and ARM and impaired LTM when adult flies were submitted to the nonpermissive temperature for 2 days (Ge et al., 2004; Presente et al., 2004). Because these experiments do not demonstrate where Notch is required in the brain, the authors used RNAi-mediated Notch repression, restricted to the MBs by means of an MB-specific driver. MB Notch impairment led to an LTM defect (Presente et al., 2004). Finally, overexpression of a wild-type copy of Notch generates a protein synthesis-dependent memory resembling LTM after only one or two spaced training cycles, a protocol that normally induces only short-lasting memories (Ge et al., 2004). The identity of the Notch ligand(s), and the downstream gene(s) regulated by Notch signaling during LTM, remain to be discovered.

4.07.3.2.2.(ii) Translational regulation

4.07.3.2.2.(ii).a Staufen/pumilio pathways

Dubnau and colleagues (Dubnau et al., 2003a) tested a behavioral screen for LTM mutants, parallel to microarray experiments aimed to select genes with altered expression after LTM training. This work led to the identification of several proteins involved in mRNA processing as well as in translation (Dubnau et al., 2003a): (1) Pumilio is a protein known to act as a transcript-specific translational repressor, regulating localized mRNA translation in oocytes; (2) Staufen and Oskar mediate the translocation of several proteins to posterior poles of oocytes; and (3) eIF-5C is a translation initiation factor. In the adult brain, these genes are preferentially expressed in the MB. Disruption of either of these four genes impairs LTM. Thus LTM requires local mRNA translation regulation, possibly postsynaptically in the MB calyces or presynaptically in the vertical lobes.

4.07.3.2.2.(ii).b RNA-induced silencing complex

In an elegant study, Ashraf et al. (2006) showed that protein synthesis at the synapse is required for LTM, and that LTM formation depends on calcium/calmodulin-dependent kinase II (CaMKII) signaling, a pathway also implicated in synaptic plasticity in mammals (Kelleher et al., 2004). By driving the expression of a tagged CaMKII in projection neurons that link olfactory sensory neurons to MBs, they showed that the recruitment of CaMKII to postsynaptic sites in the antennal lobe glomeruli is required to induce LTM (Ashraf et al., 2006). By performing this expression in different mutant backgrounds, they further showed that synaptic protein synthesis is regulated by the RNA interference silencing complex (RISC).

4.07.3.2.2.(iii) Posttranslational regulation of long-term memory formation

4.07.3.2.2.(iii).a Crammer

We have described *crammer* (*cer*), a gene involved specifically in the formation of LTM (Comas et al., 2004). The *cer* mutant has reduced LTM but normal STM, MTM, and ARM. Interestingly, in the wild-type strain, *cer* expression is transiently reduced 3 hours after LTM training. As the Cer peptide is an inhibitor of cysteine proteinases, the decrease in its expression shortly after intensive training must lead to a transient activation of its cysteine proteinase(s) target(s) (Comas et al., 2004). The overexpression of *cer* in glial cells but not in MB neurons induces an LTM decrease, indicating that glial cells expressing *cer* might be involved in LTM formation. However, in this experiment Cer is overexpressed during development, and further experiments should be carried out to disrupt or overexpress Cer specifically during the adult stage in glia cells. Whether Cer is secreted to act on MB neurons or whether Cer is involved only within glial cells also remains to be resolved. In conclusion, this work suggests that regulation of cysteine proteases is required to perform LTM, possibly in glia cells surrounding the MBs (Comas et al., 2004).

4.07.3.2.2.(iii).b Tequila

Mutations in the human neurotrypsin gene are associated with nonsyndromic mental retardation (MR) (Molinari et al., 2002). An important question is whether the MR generated by this mutation is a consequence of a brain development defect and/or a consequence of a physiological plasticity defect. To address this question, we took advantage of the high degree of homology between the human and the fly genomes, and the genetic tools offered by the fly model. Tequila (teq), a serine protease, is the *Drosophila* ortholog of the human neurotrypsin. First, we showed that a constitutive *teq* mutant has normal STM and ARM, but presents an LTM defect. To study whether Teq regulation for LTM processing is required in the MBs, RNAi-mediated Teq inhibition was induced in these structures. LTM in these flies was specifically impaired. To further study

whether Teq is required only in the adult stage or during development, the inducible Gene-Switch system was used to disrupt Teq specifically in adult stage. LTM was disrupted, but not STM or ARM, which conclusively demonstrates that Teq is required for the plasticity process and not for development. Is Teq transcription finely regulated during LTM formation? Levels of Teq mRNA in adult heads were measured at different times after spaced conditioning and showed an approximately 30-fold increase 4 h after training. This increase took place in MBs, especially in the MB peduncle (a dense structure where axons project before giving rise to lobes), as shown by immunostaining 5 h after training. Interestingly, transient Teq silencing in the adult stage, before but not during training, had no impact on LTM, showing that LTM impairment is reversible if Teq is naturally expressed *de novo* after artificial disruption. We conclude that this serine protease is required for information processing and functional plasticity in *Drosophila* and could have a preponderant role in postnatal cognition processes in children (Didelot et al., 2006).

4.07.3.3 Dynamics of Memory Phases

The short protocol induces two labile phases: STM, which is disrupted in mutants affected for cAMP metabolism and lasts about 30 min, and MTM, which is disrupted in *amn* flies and lasts for a few hours. STM and MTM are both anesthesia-sensitive, as they are erased if flies are cooled down to 4 °C after conditioning. This property suggests that STM and MTM are sustained by brain electrical activity.

LTM is induced by the long protocol and can be measured for at least 1 week. What are the dynamics of memory phase interaction in *Drosophila*? In a previous model, information acquired during learning is processed into consolidated memories (ARM and LTM) by passing sequentially through two earlier memory phases (STM and MTM) (Tully et al., 1994). In contrast, we recently proposed a model that involves two parallel memory pathways, one with cAMP-dependent STM/MTM, and the other with ARM (**Figure 4**). Indeed, *dnc* and *rut* retain a significant level of early memory (Tully and Quinn, 1985), suggesting that an adenyl cyclase-Rut-independent learning might exist. Moreover, ARM levels in *rut*, *amn* are close to normal (Folkers et al., 1993; Tamura et al., 2003; Isabel et al., 2004), while their labile memories are strongly affected. Thus ARM does not seem to depend on STM/MTM as

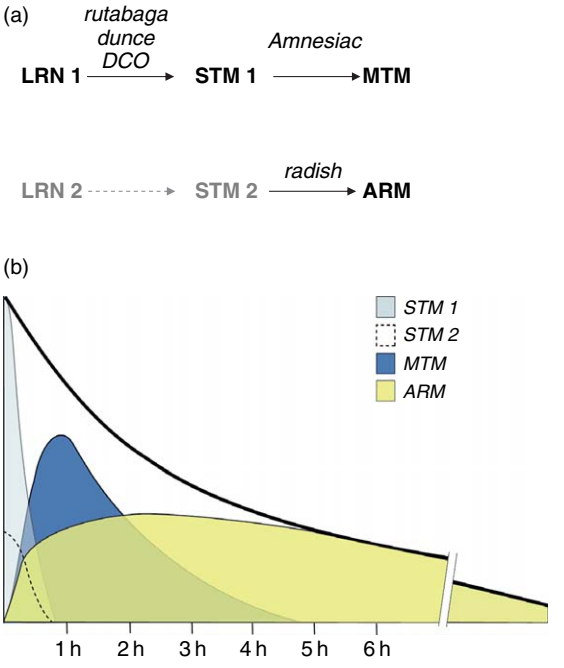

Figure 4 Model of associative memory phases (a) and temporal dynamics of memory phases (b) generated by a single cycle of conditioning (short protocol). LRN, learning; STM, short-term memory; MTM, middle-term memory; ARM, anesthesia-resistant memory; DCO, catalytic subunit gene.

previously suggested (Tully et al., 1994). Instead a second learning process could give rise to an STM 2 phase and later to ARM (**Figure 4**).

What are the relationships between ARM and LTM? To answer this question, the *ala* mutant was trained with the long protocol, and the memory of flies lacking vertical α/α' lobes was measured at 30 min and 5 h after the training. The 30-min memory was normal, but, surprisingly, the 5-h memory was close to zero. Memory performance was normal at 5 h when flies without vertical lobes were trained with the short protocol (Isabel et al., 2004) (**Figure 5**). Why does a longer training cycle give rise to weaker memory? The *ala* flies display no LTM because they lack the vertical lobes, the center for LTM. These flies show a normal ARM 5 h after the short protocol, but no ARM after the long protocol. This result suggests that ARM is erased after LTM conditioning. Thus the consolidated memory phases generated by olfactory conditioning are exclusive (**Figure 6**) (Isabel et al., 2004). Why is ARM erased after LTM conditioning? We propose that ARM could act as a gating mechanism for LTM formation, avoiding a heavy cascade of gene expression in absence of intensive spaced conditioning.

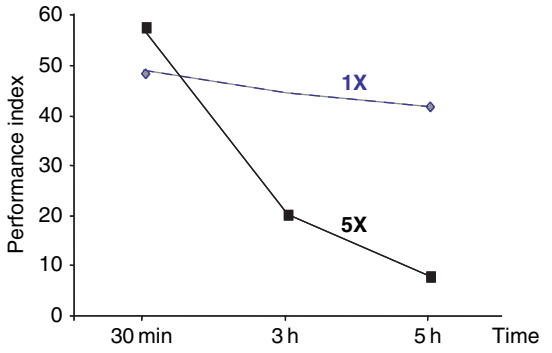

Figure 5 In flies without MB alpha lobes, which normally sustain long-term memory, the long protocol decreases memory performance at 5 h in comparison with the short protocol. Grey line, short protocol; black line, long protocol.

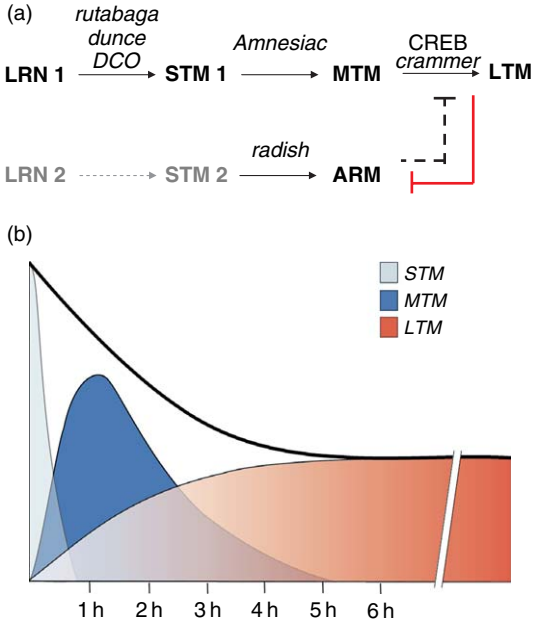

Figure 6 Model of associative memory phases (a) and temporal dynamics of memory phases (b) generated by five cycles of conditioning (long protocol). LRN, learning; STM, short-term memory; MTM, middle-term memory; ARM, anesthesia-resistant memory; LTM, long-term memory.

Despite the relative simplicity of the *Drosophila* brain, this model suggests a cognitive complexity more frequently associated with mammalian models. It supports the idea that *Drosophila* is a valid model to study some of the molecular and cellular mechanisms involved in normal or pathological human memory (Shulman et al., 2003).

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4.08 Molecular Mechanisms of Associative Learning in *Hermissenda*

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4.08.1 Introduction

A central focus in the history of studies of mentation is on how basic associations are formed and retained in memory. This characteristic of memory has been addressed from a descriptive perspective by the laws of association: similarity, contrast, and contiguity proposed by Aristotle, and further expanded by the philosophical school of British Associationism led by Locke, Berkeley, and Hartley (Watson, 1968). These early formulations led to a further elaboration of the role of contiguity in memory to include both simultaneous and successive associations. Within this historical context it is not surprising that the law of association by contiguity became the mainstay of several theories of learning proposed by behaviorists (Hilgard and Bower, 1966). Associations formed by contiguity are an essential feature of Pavlovian conditioning. Within the Pavlovian tradition, the response to the conditioned stimulus (CS) was conditional upon its pairing with the unconditioned stimulus (US), and the transfer of the response-evoking properties of the US to the CS as a result of pairing (contiguity) provided the critical evidence in support of the formation of an association in memory. In part, due to the detailed knowledge of the anatomy of sensory systems and the experimental control over the timing of stimulation of specific sensory pathways, Pavlovian conditioning paradigms have provided the opportunity to study the mechanisms underlying the formation of basic associations in memory at a cellular, synaptic, and molecular level. There is now a rich data base on Pavlovian conditioning generated from diverse species that represents sufficient complexity characteristic of different forms of memory.

The primary focus of cellular and molecular studies of Pavlovian conditioning has been on nondeclarative or procedural memory that is expressed by performance not dependent upon conscious recall. Recent studies of eyeblink conditioning employing trace procedures have explored the role of Pavlovian conditioning in declarative or conscious memory systems (Clark et al., 2002). The analysis of Pavlovian conditioning in the less complex nervous systems of higher invertebrates has been useful in elucidating the sites of memory storage, leading to an analysis of mechanisms of memory that underlie well-documented examples of associative learning.

The nudibranch mollusk *Hermissenda crassicornis* is one preparation that has contributed to an understanding of Pavlovian conditioning at the cellular, molecular, and systems levels. Pavlovian conditioning

in *Hermissenda* involves changes in intrinsic excitability and synaptic efficacy at multiple sites within the neural circuit supporting the generation of the conditioned response (CR). The modifications produced by Pavlovian conditioning involve the engagement of multiple cellular mechanisms within identified sensory neurons (photoreceptors) and interneurons that are expressed by alterations in the properties of channels in excitable membranes. Initial acquisition and long-term retention involve both presynaptic and postsynaptic mechanisms. The actions of several second messenger systems contribute to both acquisition and retention of associative memory. Short-term memory involves posttranslational modifications of proteins by several signaling pathways and is expressed by changes in both synaptic connections and intrinsic excitability. Intermediate-term memory requires translation and posttranslational modifications, but not transcription. Long-term memory requires posttranslational modifications, new mRNA and protein synthesis, and structural modifications and is expressed by long-term changes in intrinsic cellular excitability and synaptic efficacy.

4.08.2 Pavlovian Conditioning

Classical conditioning of *Hermissenda* follows the Pavlovian tradition where the CS and US elicit different responses prior to training. Stimulation of the CS and US pathways conveys different sensory information to the central nervous system. Before conditioning, light, the CS, does not elicit either of the unconditioned responses (UCRs) that have been studied in *Hermissenda:* foot-shortening and inhibition of forward ciliary locomotion. Stimulation of statocyst hair cells of the graviceptive system using rotation or orbital shaking, the US, elicits foot-shortening and a reduced rate of forward ciliary locomotion (Alkon, 1974; Crow and Alkon, 1978; Farley and Alkon, 1982; Lederhendler et al., 1986; Matzel et al., 1990b). Pavlovian conditioning produces both light-elicited inhibition of ciliary locomotion, which results in a suppression of *Hermissenda's* normal positive phototaxis (Crow and Alkon, 1978, 1980; Crow and Offenbach, 1983; Crow, 1985a), and CS-elicited foot-shortening (Lederhendler et al., 1986) (see **Figure 1**).

Both conditioned foot contraction and inhibition of ciliary locomotion involve the development or emergence of a new response to the CS, not the potentiation, through US presentations, of an already existing response to the CS referred to as alpha conditioning or reflex potentiation (e.g., Schreurs, 1989; Sahley and Crow, 1998). In both of the CRs there is a transfer of functional aspects of the response-evoking properties of the US to the CS (Crow and Alkon, 1978; Lederhendler et al., 1986;

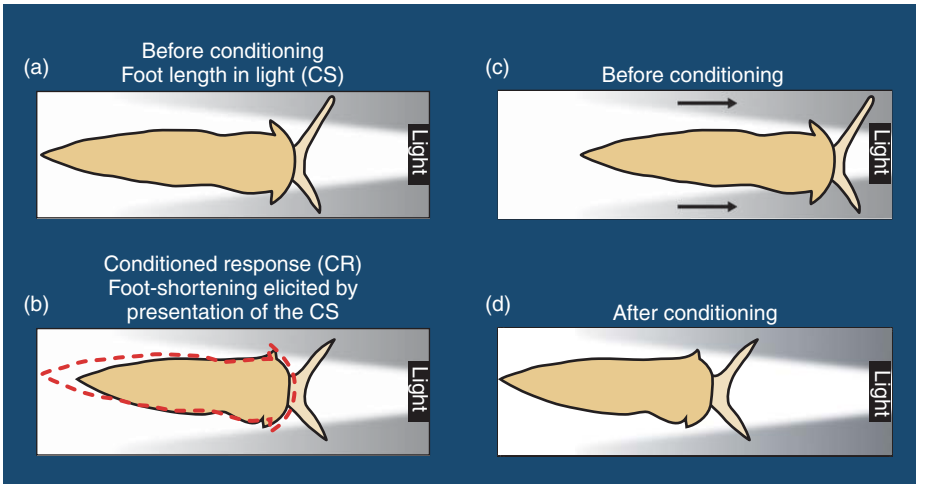

Figure 1 Pavlovian conditioning of foot-shortening and phototaxic inhibition in *Hermissenda.* (a) Foot length in light (CS) before conditioning. (b) CR foot-shortening elicited by the CS. Red outline indicates foot length in light before conditioning. (c) Light-elicited ciliary locomotion toward a light source (phototaxis) assessed before conditioning. (d) Inhibition of light-elicited ciliary locomotion detected after Pavlovian conditioning. Random or pseudorandom presentations of the CS and US do not produce either inhibition of ciliary locomotion or CS-elicited foot-shortening. Figure adapted from Crow T (2004) Pavlovian conditioning of *Hermissenda*: Current cellular, molecular, and circuit perspectives. *Learn. Mem.* 11: 229–238; used with permission from Cold Spring Harbor Laboratory Press.

Matzel et al., 1990b). The two CRs are proposed to develop independently (Matzel et al., 1990b), which is consistent with results showing that the neural circuitry supporting foot contraction and ciliary locomotion consists of different neuronal components (Crow and Tian, 2003a,b) (see the section titled 'Neural circuitry'). Retention of conditioned behavior persists for several days to weeks, depending upon the number of conditioning trials presented in initial acquisition (Crow and Alkon, 1978; Alkon, 1983; Harrigan and Alkon, 1985).

Pavlovian conditioning in *Hermissenda* has been shown to express many of the characteristics of Pavlovian conditioning in vertebrates, such as extinction (Richards et al., 1984), CS specificity (Crow and Offenbach, 1983), and conditioned inhibition (Britton and Farley, 1999). Conditioning is dependent upon the temporal association of the CS and US, involving both contiguity (Crow and Alkon, 1978) and contingency (Farley, 1987a,b). Extra CS and US presentations inserted into a sequence of CS-US pairings attenuate conditioning (Farley, 1987a). Conditioning in the two different behavioral response systems supporting the two CRs is sensitive to both CS-US contiguity and forward inter-stimulus-interval manipulations (Matzel et al., 1990c).

4.08.3 Neural Circuitry

The anatomy and synaptic organization of the two sensory structures (visual and graviceptive) mediating the CS and US have been described in detail (Alkon and Fuortes, 1972; Alkon, 1973a,b; Alkon and Bak, 1973; Detwiler and Alkon, 1973). In addition, many of the sites of convergence providing for synaptic interactions between the CS and US pathways have been identified (Alkon, 1973a,b; Alkon et al., 1978; Akaike and Alkon, 1980; Crow and Tian, 2000, 2002a,b, 2003a, 2004, 2006). Light produces a depolarizing generator potential and an increase in spike activity in the five photoreceptors in each eye (Dennis, 1967; Alkon and Fuortes, 1972). The primary sensory neurons of the pathway mediating the US consist of the 13 hair cells in each gravity-detecting statocyst. Rotation or gravity produces a depolarizing generator potential and an increase in the spike frequency of the stimulated hair cells (Alkon, 1975). There are multiple sites of convergence between the CS and US pathways.

1. The first site is between the primary sensory neurons, photoreceptors, and hair cells. Statocyst hair cells project monosynaptically to photoreceptors and receive monosynaptic input from photoreceptors (**Figure 2**). Stimulation of statocyst hair cells elicits a monosynaptic GABAergic (GABA: gamma-aminobutyric acid) inhibitory postsynaptic potential (IPSP) in type B photoreceptors (Alkon et al., 1993; Sakakibara et al., 1993; Blackwell, 2002a).

2. It has been proposed that hair cells also project polysynaptically to photoreceptors through a serotonergic modulatory pathway based upon behavioral, physiological, and immunohistochemical studies (Crow and Bridge, 1985; Land and Crow, 1985; Auerbach et al., 1989; Farley and Wu, 1989; Grover et al., 1989;

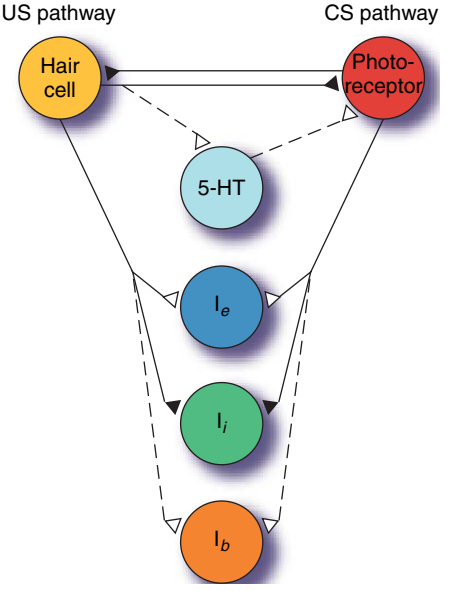

Figure 2 Sites of convergence between identified components of the CS and US pathways that result in intrinsic cellular plasticity in photoreceptors and type I_e interneurons, and proposed plasticity in type I_b interneurons. Statocyst hair cells project directly (monosynaptic) and indirectly (polysynaptically) through proposed serotonergic interneurons (5-HT) to identified photoreceptors. Caudal hair cells inhibit photoreceptors, and cephalic hair cells are inhibited by type B photoreceptors. Hair cells and photoreceptors form monosynaptic connections with type I_e and type I_i interneurons and form polysynaptic connections with type I_b interneurons. (▲) Inhibitory synaptic connections; (Δ) excitatory synaptic connections. Solid lines represent established monosynaptic connections, and dashed lines polysynaptic connections, with potential interneurons not yet identified. Figure adapted from Crow T (2004) Pavlovian conditioning of *Hermissenda*: Current cellular, molecular, and circuit perspectives. *Learn. Mem.* 11: 229–238; used with permission from Cold Spring Harbor Laboratory Press.

Crow and Forrester, 1991; Acosta-Urquidi and Crow, 1993; Rogers and Matzel, 1995; Yamoah and Crow, 1995, 1996; Tian et al., 2006). Serotonergic-immunoreactive varicosities encircle the optic nerve before entry into the cerebropleural ganglion (Land and Crow, 1985). However, the source of the serotonergic input to the photoreceptors has not yet been identified.

3. Statocyst hair cells and photoreceptors also form monosynaptic connections with type I_e and I_i interneurons (Akaike and Alkon, 1980; Crow and Tian, 2000, 2002a) and polysynaptic connections with type I_b interneurons (Crow and Tian, 2003a, 2004) (**Figure 2**).

The CS modulates ciliary locomotion through monosynaptic connections between photoreceptors and type I_e and I_i interneurons and polysynaptic connections between photoreceptors and type III_i inhibitory interneurons (see **Figure 3**). Type III_i inhibitory interneurons form monosynaptic connections with identified ciliary motor neurons located in the pedal ganglia. Activation of ciliary motor neurons is produced by a light-dependent reduction in the spike activity of type III_i inhibitory interneurons. Ciliary locomotion is reduced or inhibited by the US due to hair cell excitation of type I_e interneurons that in turn excite type III_i inhibitory interneurons, resulting in inhibition of ciliary motor neurons. An additional pathway that may modulate ciliary locomotion is the monosynaptic excitatory projections from type I_b interneurons to ciliary motor neurons (Crow and Tian, 2004). Interneurons projecting to motor neurons that innervate the foot have also been identified and have provided for the analysis of reflex movements of the foot modified by Pavlovian conditioning (Goh and Alkon, 1984, Goh et al., 1985; Crow and Tian, 2004).

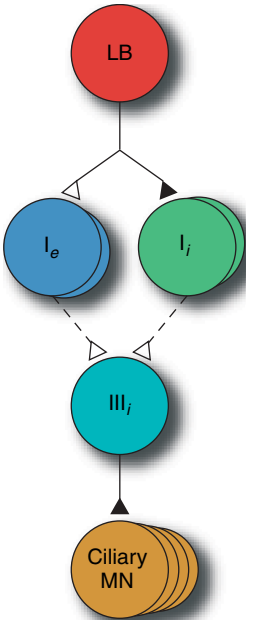

Figure 3 Components of the circuit involved in visually mediated ciliary locomotion in *Hermissenda.* Only synaptic connections with a single type B photoreceptor (LB) are shown. Monosynaptic connections are depicted by solid lines, and polysynaptic connections with dashed lines. Filled triangles denote inhibitory synapses, open triangles excitatory. As shown in the circuit diagram, identified photoreceptors project directly to aggregates of 'on' and 'off' neurons: type I_e and I_i interneurons. MN, motor neuron.

4.08.4 Cellular and Molecular Mechanisms Underlying Short-, Intermediate-, and Long-Term Memory Formation

Contemporary views of memory and its formation over time indicate that both declarative and nondeclarative forms of memory involve multiple stages with different underlying mechanistic requirements. A number of *in vivo* and *in vitro* procedures involving one or several training trials have been employed in Pavlovian conditioning studies of *Hermissenda* to examine the early events supporting the formation of short-, intermediate-, and long-term memory. The different protocols involving one or several conditioning trials produce behavioral changes and physiological modifications that can be detected within minutes following training (Crow, 1983; Farley and Alkon, 1987; Matzel et al., 1990a,b; Matzel and Rogers, 1993; Crow et al., 1998; Ramirez et al., 1998; Epstein et al., 2003; Kuzirian et al., 2006). Since the two sensory pathways mediating the CS and US are totally intact in the isolated nervous system, *in vitro* Pavlovian conditioning procedures can be applied to the isolated circumesophageal nervous system. Pairing the CS (light) with mechanical perturbations of the statocyst produced by piezoelectric stimulation (US) sufficient to depolarize hair cells, or rotation of the isolated nervous system (US), produces electrophysiological correlates in type B photoreceptors that are similar to correlates produced by multi-trial *in vivo* procedures (Matzel et al., 1990a; Matzel and Rogers, 1993; Gandhi and Matzel, 2000). A multi-trial *in vitro* procedure involving pairing the CS with extrinsic

current depolarization of identified statocyst hair cells (nominal US) also produces conditioning correlates in type B photoreceptors (Farley and Alkon, 1987). The results of these investigations depend upon the various conditioning protocols, the efficacy of the US, and the duration of CS-US stimulation.

4.08.4.1 One-Trial Conditioning

To more precisely control when the initial learning occurs and to not confound time after conditioning, when memory is tested, with varying numbers of conditioning trials, a one-trial *in vivo* conditioning procedure was developed that produces a pairing-specific long-term inhibition of normal light-elicited ciliary locomotion (Crow and Forrester, 1986). Pairing the CS (light) with the direct application of 5-hydroxytryptamine (5-HT, nominal US), one of the proposed transmitters of the US pathway to the exposed nervous system of otherwise intact *Hermissenda* produces inhibition of light-elicited ciliary locomotion when the animals are tested 24 h following the conditioning trial. An *in vitro* analog of the one-trial procedure involving pairing the CS with 5-HT application has been used with the isolated circumesophageal nervous system to examine mechanisms underlying the development of short-, intermediate-, and long-term memory. A one-trial *in vitro* procedure consisting of pairing the CS with mechanical perturbation of the statocyst produces a significant Ca^{2+}-dependent increase in input resistance of type B photoreceptors (a correlate of enhanced excitability) that is detected within minutes postconditioning (Matzel and Rogers, 1993). In addition, a one-trial *in vitro* procedure involving GABA application to the region of the photoreceptor terminal branches (nominal US) paired with a 10-s depolarization of the type B photoreceptors (nominal CS) produces an increase in the input resistance of the B photoreceptors that persists for at least 10 min (Matzel and Alkon, 1991). These studies indicate that the application of a neurotransmitter, when paired with depolarization resulting in a brief period of Ca^{2+} elevation, is sufficient to produce enhanced excitability, and the procedures may engage the essential components for the formation of associations underlying conditioning.

4.08.4.2 Long-Term Memory Following Multi-Trial Conditioning

The analysis of electrophysiological and biophysical modifications detected following multi-trial Pavlovian conditioning has focused upon two sites of convergence between the CS and US pathways. The first site is in the primary sensory neurons (photoreceptors) of the pathway mediating the CS (Crow and Alkon, 1980). Neural modifications in the primary sensory neurons of conditioned animals involve both enhanced excitability that is intrinsic to identified type A and type B photoreceptors (Crow and Alkon, 1980; Alkon et al., 1982, 1985; Farley and Alkon, 1982; West et al., 1982; Crow, 1985b; Frysztak and Crow, 1993, 1997) and facilitation of synaptic connections between identified photoreceptors (Frysztak and Crow, 1994, 1997; Gandhi and Matzel, 2000), and photoreceptors and interneurons (Crow and Tian, 2002b, 2003b). The intrinsic modifications in type B photoreceptors are expressed by enhancement of the amplitude of CS-elicited generator potentials and a concomitant increase in spike frequency, increased excitability to extrinsic current, decreased spike frequency accommodation, and a reduction in the peak amplitude of voltage-dependent (I_A, I_{Ca}) and Ca^{2+}-dependent ($I_{K(Ca)}$) currents (Crow and Alkon, 1980; Alkon et al., 1982, 1985, 1992, 1993; Farley and Alkon, 1982; Alkon, 1984; Crow, 1985b; Goh et al., 1985; Collin et al., 1988; Farley et al., 1990; Matzel et al., 1990a; Frysztak and Crow, 1993, 1994, 1997; Blackwell, 2000; 2002a,b; Muzzio et al., 2001).

The increase in the amplitude of CS-elicited generator potentials is in part the result of a reduction in I_A and $I_{K(Ca)}$. In type B photoreceptors of conditioned animals, the peak amplitude of I_A is significantly reduced and exhibits more rapid inactivation as compared to controls (Alkon et al., 1985). However, both the delayed rectifier (I_K) and inward rectifier (I_h) may play a role in conditioning-dependent enhanced excitability. The application of 5-HT to the isolated nervous system enhances the peak amplitude of I_h and decreases the peak amplitude of I_K and I_A in type B photoreceptors (Acosta-Urquidi and Crow, 1993). In addition, 5-HT reduces the amplitude of $I_{K(Ca)}$ and decreases I_{Ca} in type B photoreceptors (Yamoah and Crow, 1995). The reduction in $I_{K(Ca)}$ produced by 5-HT is a consequence of the decrease in I_{Ca} by 5-HT rather than a direct effect of 5-HT on $I_{K(Ca)}$. In conditioned animals, type A photoreceptors exhibit a decrease in the amplitude of light-elicited generator potentials, enhanced excitability to extrinsic current, increases in CS-elicited spike activity, and a significant increase in the magnitude of I_K (Farley et al., 1990; Frysztak and Crow, 1993, 1997; Farley and Han, 1997). Multi-trial conditioning does not result

in changes in either I_A or $I_{K(Ca)}$ in type A photoreceptors, in contrast to the modifications in type B photoreceptors (Farley and Han, 1997).

In addition to intrinsic enhanced excitability in sensory neurons produced by multi-trial conditioning, changes in synaptic strength between identified sensory neurons and interneurons occur following conditioning. The amplitude of the monosynaptic IPSP between the medial type B photoreceptor and medial type A photoreceptor is significantly enhanced in conditioned animals (Frysztak and Crow, 1994; Gandhi and Matzel, 2000). The second convergence site between the CS and US pathways is the monosynaptic connection between type B photoreceptors and type I interneurons. Multi-trial conditioning produces facilitation of monosynaptic and complex PSPs in identified type I_e and I_i interneurons (Crow and Tian, 2002b). In addition to conditioning-dependent synaptic facilitation, type I interneurons also express intrinsic enhanced excitability with conditioning. Extrinsic current pulses elicit significantly more spikes in type I_e interneurons of conditioned animals as compared to pseudorandom controls.

Therefore multi-trial conditioning in *Hermissenda* results in both presynaptic and postsynaptic modifications. The enhanced excitability of type B photoreceptors, expressed by an increase in both the amplitude of CS-elicited generator potentials and the number of action potentials elicited by the CS, may be a major contributor to changes in the duration and amplitude of CS-elicited complex PSPs and increased CS-elicited spike activity in type I interneurons of conditioned animals (Crow and Tian, 2002b). However, facilitation of the amplitude of the monosynaptic IPSP between type B photoreceptors and type I_i interneurons and the monosynaptic excitatory postsynaptic potential (EPSP) between type B photoreceptors and type I_e interneurons of conditioned animals may involve both pre- and postsynaptic mechanisms.

4.08.5 Second Messenger Systems

4.08.5.1 Protein Kinase C

Protein kinase C (PKC) activation contributes to enhanced excitability and synaptic facilitation underlying the formation of short- and long-term memory in *Hermissenda* (Farley and Auerbach, 1986; Neary et al., 1986; Matzel et al., 1990a; Crow et al., 1991; Farley and Schuman, 1991). PKC is translocated from the cytosol to membrane in the nervous system of *Hermissenda* by treatment with a phorbol ester (12-O-tetradecanoylphorbol-13-acetate (TPA)). The bath application of a phorbol ester (phorbol 12,13-dibutyrate (PDB)) and injection of PKC into type B photoreceptors results in a reduction in the peak amplitude of two K^+ currents, I_A and $I_{K,(Ca)}$, that resemble changes in conductances detected following multi-trial Pavlovian conditioning (Farley and Auerbach, 1986).

Nine conditioning trials produce a foot-shortening CR elicited by the CS that is detected within minutes after the last conditioning trial (Matzel et al., 1990a). An *in vitro* conditioning procedure consisting of nine training trials of the CS paired with rotation of the isolated circumesophageal nervous system (US) enhances type B photoreceptor excitability and increases the amplitude of the plateau phase of the CS-elicited generator potential. The conditioning-dependent change in excitability of type B photoreceptors is blocked by the broad-spectrum kinase inhibitor 1-(5-isoquinolinesulfonyl)-2-methylpiperizine (H-7) applied in artificial seawater during *in vitro* conditioning. A second *in vitro* procedure involving pairing the CS with extrinsic current depolarization of the B photoreceptors (nominal US) produced enhanced excitability of the B photoreceptors which is also blocked by preconditioning application of H-7 or sphingosine (Matzel et al., 1990a).

One-trial *in vivo* conditioning consisting of pairing the CS with the application of 5-HT to the exposed but otherwise intact circumesophageal nervous system produces short-term enhanced excitability, intermediate-term enhanced excitability, and long-term enhanced excitability of type B photoreceptors (Crow and Forrester, 1991, 1993a; Crow et al., 1991). The induction of short-term enhanced excitability following one-trial conditioning is blocked by the protein kinase inhibitors H-7 and sphingosine and by downregulation of PKC produced by pretreatment with TPA (Crow et al., 1991; Crow and Forrester, 1993b). However, while H-7, sphingosine, or downregulation of PKC by TPA blocks short-term enhanced excitability, the same treatments do not block long-term enhanced excitability produced by one-trial conditioning (Crow and Forrester, 1993b). Therefore short- and long-term enhanced excitability produced by one-trial *in vivo* conditioning involve independent or parallel processes and differential contributions of second messengers. *Thus, the expression of long-term memory produced by one-trial conditioning does not depend upon the induction of short-term memory.*

Consistent with previous studies, the induction of enhanced excitability in type B photoreceptors produced by five *in vitro* conditioning trials involving the CS paired with depolarizing current stimulation of an identified statocyst hair cell is blocked by pretreatment with PKC inhibitors (Farley and Schuman, 1991). However, the contribution of PKC to the expression of long-term enhanced excitability depends upon the conditioning protocol and the number of conditioning trials. Previously established long-term enhancement produced by one-trial *in vivo* conditioning is not reversed by the broad-spectrum kinase inhibitor H-7 or the PKC inhibitors sphingosine or staurosporine (Crow and Forrester, 1993a). In contrast, long-term enhanced excitability in type B photoreceptors produced by multi-trial Pavlovian conditioning is attenuated by H-7 or sphingosine, suggesting that long-term enhanced excitability is dependent upon persistent kinase activity (Farley and Schuman, 1991).

Lateral type A photoreceptors exhibit an increase in the number of spikes elicited by the CS and extrinsic current following multi-trial Pavlovian conditioning (enhanced excitability) (Frysztak and Crow, 1993). Injection of the PKC inhibitor peptide PKC(19-36) into lateral type A photoreceptors 24–48 h following multi-trial conditioning reverses enhanced excitability within 16 min postinjection, suggesting that either a long-lived activator or a constitutively active kinase contributes to the expression of enhanced excitability in lateral A photoreceptors (Frysztak and Crow, 1997). Injection of the control noninhibitory peptide [glu^{27}] PKC(19-36) does not reverse enhanced excitability in lateral A photoreceptors of conditioned animals. PKC activation also contributes to the induction of 5-HT-dependent synaptic facilitation, but persistent PKC activity is not required for long-term synaptic facilitation. Short-term synaptic facilitation of the connection between type B and type A photoreceptors is produced by bath application of 5-HT (Schuman and Clark, 1994; Frysztak and Crow, 1997). Injection of the PKC inhibitor peptide PKC(19-36) into medial type B photoreceptors blocks 5-HT-induced synaptic facilitation of the IPSP recorded in the medial type A photoreceptor (Frysztak and Crow, 1997). However, injection of PKC(19-36) into medial type B photoreceptors following multi-trial Pavlovian conditioning does not reduce or reverse established synaptic facilitation of the IPSP recorded in medial type A photoreceptors. Thus PKC contributes to the induction of short-term synaptic facilitation of the monosynaptic connection between types B and A photoreceptors, but not to the expression of long-term synaptic facilitation of the same monosynaptic connection between type B and A photoreceptors.

4.08.5.2 Extracellular Signal-Regulated Protein Kinase

One-trial *in vitro* conditioning of the isolated nervous system involving the CS paired with 5-HT results in the increased $^{32}PO_4$ labeling of a protein with an apparent molecular weight consistent with extracellular signal-related kinase (ERK). The increased phosphorylation of the protein following one-trial conditioning is blocked by pretreatment with the MEK1 (MAPK/ERK kinase) inhibitor PD098059 (Crow et al., 1998). Assays of ERK activity with brain myelin basic protein as a substrate shows greater ERK activity for nervous systems from one-trial *in vitro* conditioned animals as compared to controls that received the CS and 5-HT unpaired. In addition, Western blot analysis of phosphorylated ERK with a phospho ERK antibody shows a significant increase in ERK phosphorylation after one-trial conditioning as compared with unpaired controls. The increased phosphorylation is blocked by pretreatment with a MEK1 inhibitor (PD098059). Following a multi-trial conditioning procedure consisting of 10–15 trials, circumesophageal nervous systems from conditioned animals exhibit significantly greater ERK phosphorylation as compared with pseudorandom controls (Crow et al., 1998).

PKC contributes to the 5-HT-dependent activation of the ERK pathway. The phorbol ester TPA increases ERK phosphorylation that is blocked by pretreatment with PKC inhibitors. TPA-dependent ERK phosphorylation is also blocked by the MEK1 inhibitors PD0988059 or U0126. The increased phosphorylation of ERK by 5-HT is attenuated, but not blocked, by pretreatment with the Ca^{2+} chelator BAPTA-AM or pretreatment with PKC inhibitors Gö6976 or GF109203X (Crow et al., 2001). This suggests that Ca^{2+}-dependent PKC activation contributes to ERK phosphorylation, although a PKC-independent pathway also contributes to 5-HT-dependent ERK phosphorylation and activation.

4.08.5.3 Memory Formation Is Ca^{2+}-Dependent

The photoreceptors in the eyes of *Hermissenda* exhibit a spatial segregation of function. Phototransduction takes place in the apical region where the rhabdomere

abuts the lens, and spike generation occurs near the distal end of the axon close to the location of synapses on the terminal processes. Therefore light and rotation have spatially separated physiological consequences in type B photoreceptors. Both light and depolarization increase cytosolic Ca^{2+} levels in photoreceptors (Connor and Alkon, 1989; Sakakibara et al., 1993; Blackwell, 2000, 2002a,b; Muzzio et al., 2001). Light activates phospholipase C (PLC) to produce an increase in inositol trisphosphate (IP_3) and diacylglycerol (DAG) (Sakakibara et al., 1986, 1994). IP_3 opens rhabdomeric Na^+ and Ca^{2+} channels, which result in a depolarizing generator potential and Ca^{2+} influx (Blackwell, 2000). IP_3 also binds to its receptor (IP_3R), which triggers Ca^{2+} release from the endoplasmic reticulum (Blackwell and Alkon, 1999). The Ca^{2+} influx from the rhabdomere and the IP_3R-gated storage compartment can cause Ca^{2+} release from the ryanodine receptor-gated (RyR) compartment (Blackwell and Alkon, 1999).

Rotation (US) produces a depolarizing generator potential in identified statocyst hair cells and elicits a monosynaptic GABAergic IPSP in the photoreceptors (Alkon et al., 1993; Sakakibara et al., 1993; Rogers et al., 1994; Blackwell, 2002a). The US is also proposed to activate a polysynaptic serotonergic pathway that projects to type B photoreceptors (Land and Crow, 1985; Crow and Forrester, 1986, 1991). Both 5-HT (Rogers and Matzel, 1995; Yamoah and Crow, 1996) and GABA (Yamoah and Crow, 1996) are linked to a pertussis toxin–sensitive G-protein. These proteins can activate multiple second messenger systems (see **Figure 4**), several of which have been implicated in one-trial and multi-trial classical conditioning. The primary focus of 5-HT effects has been on the modulation of membrane conductances in type B photoreceptors (e.g., Farley and Wu, 1989; Acosta-Urquidi and Crow, 1993; Yamoah and Crow, 1996). In addition, the induction of 5-HT-dependent enhanced excitability in type B photoreceptors is Ca^{2+} dependent, since BAPTA loading of photoreceptors before 5-HT application blocks the induction of enhanced excitability (Falk-Vairant and Crow, 1992). However, the precise role of 5-HT in the induction and expression of long-term intrinsic enhanced excitability and synaptic facilitation is poorly understood. In contrast, it is proposed that GABA binding to G-protein-coupled receptors on photoreceptors activates phospholipase A_2 (PLA_2) to liberate arachidonic acid (AA) that interacts with Ca^{2+} to synergistically stimulate PKC (Muzzio et al., 2001) and create a back-propagating wave of Ca^{2+}

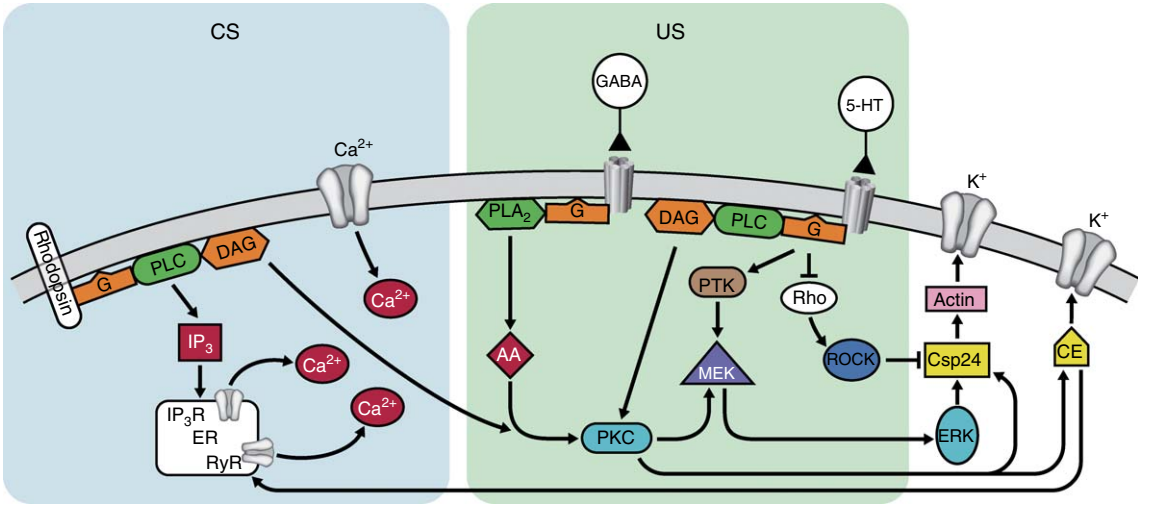

Figure 4 Mechanisms of memory formation produced by Pavlovian conditioning in *Hermissenda.* Acquisition involves the interaction of Ca^{2+} with the second messenger pathways regulated by neurotransmitter release in the unconditioned stimulus (US) pathway. Light (CS) activates phospholipase C (PLC) to produce an increase in inositol trisphosphate (IP_3) and diacylglycerol (DAG). The depolarizing generator potential and IP_3 effects on endoplasmic reticulum (ER) result in an increase in intracellular Ca^{2+}. Transmitters in the US pathway bind to G-protein-coupled receptors (G) to activate phospholipase A_2 (PLA_2), increase arachidonic acid (AA), and activate protein kinase C (PKC), nonreceptor protein tyrosine kinase (PTK), extracellular signal-regulated kinase (ERK), and the Rho GTPase/Rho-associated protein kinase (Rho/ROCK) pathway. Enhanced excitability is a consequence of short-term and long-term modification of K^+ channels by calexcitin (CE) and conditioned stimulus pathway protein 24 (Csp24). MEK, MAPK/ERK kinase; 5-HT, serotonin; GABA, gamma-aminobutyric acid.

released from intracellular stores (Ito et al., 1994; Blackwell, 2002a). When the CS and US are repeatedly paired, the Ca^{2+} influx due to light, IP_3R stores, RyR stores, and voltage-gated Ca^{2+} channels sums together (Blackwell and Alkon, 1999). The large increase in cytosolic Ca^{2+} combined with DAG and AA acts to synergistically activate PKC by translocation of PKC to the membrane (Lester et al., 1991). Each pairing of the CS and US has been proposed to incrementally increase the proportion of PKC translocated to the membrane that would contribute to the phosphorylation of K^+ channels (Muzzio et al., 1997, 2001; Alkon et al., 1998).

4.08.5.4 Long-Term Memory Depends Upon Translation and Transcription

The existence of mechanistic differences between short- and long-term enhanced excitability are illustrated by studies showing that inhibition of protein synthesis during one-trial *in vivo* conditioning blocks long-term enhanced excitability without affecting the induction or expression of short-term enhanced excitability (Crow and Forrester, 1990). Moreover, long-term enhanced excitability produced by one-trial conditioning is blocked by inhibition of mRNA synthesis, which does not affect the induction of short-term enhanced excitability (Crow et al., 1997). This result indicates that long-term memory following one-trial *in vivo* conditioning is dependent upon both translation and transcription.

The time-dependent development of enhanced excitability following one-trial *in vivo* conditioning is biphasic; enhancement reaches a peak at 3 h, decreases toward baseline control levels at 5–6 h, and increases to a plateau at 16 to 24 h postconditioning (Crow and Siddiqi, 1997). Enhanced excitability following one-trial conditioning involves an intermediate phase of memory consolidation that requires protein synthesis but not mRNA synthesis (Crow et al., 1999). The phosphorylation of a cytoskeletal-related protein, Csp24 (see the section titled 'Proteins regulated by Pavlovian conditioning') is associated with the intermediate phase, but not the short-term phase. Reducing the concentrations of 5-HT used in one-trial conditioning produces a short-term (< 1 h) associative enhancement of excitability that does not involve the posttranslational modification of Csp24 (Crow and Xue-Bian, 2000).

The conditioned foot contraction CR is expressed at a retention interval of 5 min following two or nine conditioning trials (Ramirez et al., 1998). However, nine conditioning trials are required for 90-min retention. *In vivo* incubation of animals with the protein synthesis inhibitor anisomycin during conditioning does not affect the expression of the CR at the 5-min retention interval, but attenuates conditioning at the 90-min interval for the group that received nine conditioning trials. A protocol involving *in vitro* conditioning of the isolated nervous system produces similar results to the effects of anisomycin on conditioned behavior. Two conditioning trials produce a short-term protein synthesis–independent increase in excitability that decreases within 45 min, and nine conditioning trials produce a persistent protein synthesis–dependent increase in type B photoreceptor excitability detected at 90 min (Ramirez et al., 1998). Applying anisomycin 5 min after the ninth conditioning trial does not affect the retention of enhanced excitability.

However, a recent study has challenged the view that protein synthesis occurring after the learning event is necessary and sufficient for the formation of long-term memory. PKC activation produced by bryostatin application on days before conditioning leads to the expression of proteins that can support long-term memory produced by later Pavlovian conditioning. Two conditioning trials typically result in a short-term (~7 min) foot-shortening CR. A 4-h exposure to bryostatin on two days preceding conditioning results in a long-term (>1 week) CR produced by two conditioning trials that is not blocked by anisomycin (Alkon et al., 2005).

4.08.6 Morphological Modifications in the Sensory Neurons of Conditioned Stimulus Pathway

Ultrastructural and electrophysiological analyses indicate that synaptic interactions between photoreceptors, other sensory neurons, and interneurons is in the neuropil of the cerebropleural ganglion (Crow et al., 1979). Changes in the morphology of secondary and terminal photoreceptor processes within the neuropil are produced by conditioning. Structural changes characterized by a contraction or reduction of dendritic boundary volumes enclosing labeled medial-type-B photoreceptor arborizations occur in conditioned animals as compared to unpaired controls (Alkon et al., 1990). This suggests that selective synaptic pruning may be a correlate of Pavlovian conditioning. Three-dimensional reconstructions of the volume of the terminal arborizations at the

synaptic connections between all pairs of identified photoreceptors double-labeled with different fluorescent dyes revealed that most project to the intermediate and lateral segments in the ventral region of the lateral B photoreceptor, a region of the lateral type B photoreceptor that is not contracted by conditioning (Kawai and Crow, 2004). The structural changes in type B photoreceptor dendritic volume observed with multi-trial conditioning have also been observed following an *in vitro* conditioning procedure. As compared to unpaired controls, five *in vitro* conditioning trials produce a contraction of the terminal branches along a central lateral axis of fluorescently labeled type B photoreceptors imaged with confocal microscopy (Kawai et al., 2002). The change in terminal branch morphology occurs within an hour after *in vitro* conditioning and is not observed at the synaptic connections between hair cells and photoreceptors (Kawai et al., 2002). The structural remodeling of the type B photoreceptor terminal branches following *in vitro* conditioning is blocked with anisomycin pretreatment (Kawai et al., 2003). In addition to changes in dendritic volume, changes in the volume of photoreceptor somas occur following activation of PKC with a phorbol ester (Lederhendler et al., 1990). Phorbol-induced changes involve outgrowths from the cell surface, similar to blebs, that alter the soma volume.

4.08.7 Proteins Regulated by Pavlovian Conditioning

Different *in vivo* and *in vitro* conditioning protocols have been used to study proteins regulated by conditioning. Multi-trial conditioning produces posttranslational modifications in a number of proteins; however, only a few of the full-length cDNAs have been cloned and the phosphoproteins fully characterized. Calexcitin (CE) is a guanosine triphosphate (GTP) and Ca^{2+}-binding protein found in *Hermissenda* photoreceptors (Neary et al., 1981; Alkon et al., 1998; Kuzirian et al., 2001). CE is activated by elevated Ca^{2+} and binds to the RyR to increase cytosolic Ca^{2+} concentrations (Ascoli et al., 1997; Nelson et al., 1996, 1999). CE is phosphorylated by PKC, which results in translocation of CE to membrane compartments where it decreases K^+ currents. Phosphorylation of CE also results in binding to the Ca^{2+}-ATPase transporter to increase the rate of Ca^{2+} removal from the cytosol (Alkon et al., 1998). Multi-trial conditioning increases the phosphorylation of CE (Neary et al., 1981) and increases CE in B photoreceptors, specifically in Ca^{2+} sequestering organelles such as endoplasmic reticulum (ER) and within mitochondria and photopigments (Kuzirian et al., 2001). The increased CE levels in B photoreceptors of conditioned animals results in increased excitability via K^+-channel inactivation and internal Ca^{2+} release from ER due to increased CE binding to ryanodine receptors.

In addition to CE, one-trial and multi-trial conditioning regulates other proteins found in the CS pathway and circumesophageal nervous system (Crow et al., 1996, 1997, 1999; Crow and Siddiqi, 1997; Crow, 2004). The phosphorylation of conditioned stimulus pathway protein 24 (Csp24) is regulated by Pavlovian conditioning and is involved in both intermediate-term and long-term memory. Csp24 is a cytoskeleton-related protein that is homologous to members of the family of multi-domain β-thymosin repeat proteins (Crow and Xue-Bian, 2000, 2002; Crow et al., 2003). Actin co-precipitates with Csp24 and is colocalized with Csp24 in the cytosol of B photoreceptor cell bodies (Crow and Xue-Bian, 2002). In addition, recombinant Csp24 binds to and sequesters G-actin *in vitro,* and phosphorylation of Csp24 by one-trial *in vitro* conditioning increases the co-precipitation of actin with anti-Csp24 (Redell et al., 2007).

Csp24 is phosphorylated by procedures that produce intermediate-term and long-term enhanced excitability, but not after *in vitro* procedures that result in only short-term enhanced excitability of photoreceptors (Crow and Xue-Bian, 2000). Several signaling pathways regulate Csp24 phosphorylation; thus it can integrate a number of signals that result in cytoskeletal remodeling. Inhibitors of PKC and MEK1 reduce Csp24 phosphorylation produced by *in vitro* conditioning. In addition to PKC and ERK regulation of Csp24, Rho GTPase activity and its downstream target Rho-associated protein kinase (ROCK) contribute to the posttranslational regulation of Csp24 through an inhibitory pathway (Crow et al., 2004). The ROCK inhibitor Y-27632 significantly increases Csp24 phosphorylation, and the Rho activator lysophosphatidic acid decreases Csp24 phosphorylation (Crow et al., 2004). In addition, the application of 5-HT to the isolated nervous system decreases Rho activity and increases the phosphorylation of Csp24. Inhibition of cyclin-dependent kinase 5 by butyrolactone also reduces Csp24 phosphorylation. Incubation of isolated *Hermissenda* nervous systems with *Csp* antisense oligonucleotides decreases Csp24 expression, and treatment with antisense oligonucleotides before

one-trial *in vitro* conditioning blocks intermediate-term enhanced excitability without affecting the induction of short-term immediate enhanced excitability (Crow et al., 2003).

Since Csp24 is associated with the actin cytoskeleton, its regulation by conditioning may influence K^+ channel activity by the spatial and temporal control of actin dynamics. One-trial *in vitro* conditioning of isolated type B photoreceptors produces a significant reduction in the amplitude of I_A and a depolarized shift in the steady-state activation curve of I_A without altering the inactivation curve (Yamoah et al., 2005). The conditioning-dependent changes in I_A are blocked by incubation of the isolated photoreceptors with *Csp* antisense oligonucleotide. Therefore Csp24 contributes to the regulation of voltage-gated channels associated with intrinsic enhanced excitability underlying Pavlovian conditioning.

Interestingly, the distribution of Csp24-like immunoreactivity in lateral type B photoreceptors is changed by one-trial *in vitro* conditioning. Conditioning results in a significant decrease in immunoreactivity in the soma and a significant increase in immunoreactivity in the terminal arborizations of identified lateral type B photoreceptors (Kawai and Crow, 2005).

4.08.8 Overview

Pavlovian conditioning in *Hermissenda* results in both intrinsic enhanced cellular excitability and modifications in synaptic efficacy at multiple loci within the neural circuit responsible for the generation of the CR. The first site of storage for the memory of the associated experience is in the primary sensory neurons of the CS pathway. The modifications in the sensory neurons are spatially segregated. There are alterations in the properties of K^+ channels in the soma that result in an enhancement of the amplitude of the CS-elicited generator potential and a concomitant change in channels in the spike-generating zone that results in a decrease in spike frequency accommodation. In addition, changes in synaptic efficacy result in facilitation of the monosynaptic connections between identified type B and type A photoreceptors and between identified photoreceptors and interneurons. Since the second site of memory storage is in the type I interneurons, the memory for Pavlovian conditioning involves both presynaptic and postsynaptic mechanisms.

Acquisition of short-term, intermediate-term, and long-term memory for Pavlovian conditioning involves the activation of several second messenger cascades, posttranslational modification of proteins, and the synthesis of mRNA and proteins. Acquisition engages the interaction of elevated intracellular Ca^{2+} and arachidonic acid to activate PKC and ERK that is dependent upon CS-US pairings. The mechanism for intrinsic enhanced excitability is different from the mechanisms supporting modifications in synaptic efficacy since long-term synaptic facilitation detected following multi-trial conditioning is not PKC-dependent.

Two proteins that have been fully characterized and are regulated by Pavlovian conditioning are CE and Csp24. The binding of CE to the plasma membrane decreases K^+ conductances and releases Ca^{2+} from internal stores. Csp24 phosphorylation is regulated by one-trial and multi-trial conditioning, is associated with actin, and contributes to long-term intrinsic enhanced excitability produced by the depolarized shift in the steady-state activation of I_A and the concomitant reduction in peak I_A. Therefore, the expression of Csp24 is important in both intermediate-term and long-term memory involving intrinsic enhanced excitability.

The analysis of mechanisms of memory in *Hermissenda* raises a number of questions that are important to an understanding of memory produced by Pavlovian conditioning. How are posttranslational modifications in proteins supporting short-term memory transformed into long-term memory involving both intrinsic enhanced excitability and changes in synaptic efficacy? What are the contributions of presynaptic and postsynaptic modifications to short-term, intermediate-term, and long-term memory? How does the regulation of CE and Csp24 by conditioning result in an alteration in the properties of K^+ channels in excitable membranes? Finally, how are modifications in intrinsic excitability and synaptic strength at several loci integrated within a neural circuit to reconfigure the circuit to support the generation of the conditioned response?

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4.09 Molecular Mechanism of Associative Learning in *Lymnaea*

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4.09.1 Introduction

4.09.1.1 Reductionist and Top-Down Approaches to Studying Molecular Mechanisms of Learning and Memory in Mollusks

Historically, there have been two different approaches to the analysis of the molecular mechanisms of learning and memory (*See* Chapters 4.06, 4.07, 4.08, 4.10, 4.38). One, often referred to as a reductionist or simple systems approach, is based on the investigation of molecular pathways involved in long-term neuronal plasticity (heterosynaptic faciliation, long-term potentiation, long-term depression) in neuronal cell cultures, invertebrate ganglia, or mammalian brain slices (Kandel, 2001). This approach rests on the assumption that these forms of synaptic plasticity underlie some forms of learning, such as sensitization or classical conditioning. The other type of approach, commonly known as a top-down approach, starts from the investigation of behavioral aspects of learning and memory and aims to establish causal links between learning-induced molecular changes and the learned behavior itself (Benjamin et al., 2000; Sadile, 1993; McGuire et al., 2005).

Gastropod mollusks (slugs and snails), such as *Aplysia californica, Hermissenda crassicornis, Helix pomatia, Lymnaea stagnalis, Pleurobranchaea californica,* and *Tritonia diomedia,* have been providing extremely useful experimental models for both types of approach since the 1970s (early reviews: Abraham et al., 1972; Willows, 1973; Kandel, 1979; Kandel and Schwartz, 1982; Carew and Sahley, 1986; Byrne, 1987). The main reason for the continuing success of gastropod preparations in learning and memory research is that snails and slugs have easily accessible central nervous systems with unusually large (up to 500 μm) neurons and a variety of well-defined reflex behaviors that can undergo plastic changes during both nonassociative (habituation, sensitization) and associative learning (classical and operant conditioning). These are known as implicit or nondeclarative forms of learning (Milner et al., 1998), during which the memory trace is formed and stored in the same network of neurons, making cellular-level analyses of both memory acquisition and storage feasible in the same system. There is

also an increasing amount of genomic and proteomic information available on mollusks, which has facilitated more in-depth molecular analyses of learning and memory in molluscan models, including the pond snail *Lymnaea stagnalis*.

4.09.1.2 The Roots of Top-Down Analyses of Associative Memory in *Lymnaea*

The predominantly top-down approach to investigations into the cellular and molecular mechanisms of associative learning in *Lymnaea* is rooted in two seemingly disparate fields of research, one almost exclusively physiological, the other purely behavioral (**Figure 1**). In the 1970s *Lymnaea* became a major experimental model system used for the analysis of the organization of central pattern generator (CPG) networks underlying the generation of rhythmic behaviors, such as feeding and respiration (**Figure 2**). Pioneering work in laboratories at Sussex University, Leeds University, and the University of Calgary led to a detailed understanding of how the feeding and respiratory CPGs worked in *Lymnaea* (Benjamin and Rose, 1979; Rose and Benjamin, 1979; McCrohan and Benjamin, 1980a,b; Elliott and Benjamin, 1985a,b; Kyriakides and McCrohan, 1989; Syed et al., 1990, 1992; Syed and Winlow, 1991; Elliott, 1992; Elliott and Kemenes, 1992; Elliott et al., 1992; Kemenes and Elliott, 1994). These studies, together with later work identifying serotonergic (mediated by the transmitter serotonin) and nitrergic (mediated by the gaseous transmitter nitric oxide, NO) modulatory mechanisms in the feeding system (Yeoman et al., 1994a,b, 1996; Elphick et al., 1995; Park et al., 1998; Kobayashi, 2000; Straub and Benjamin, 2001; Korneev et al., 2002), provided a firm physiological foundation for subsequent work aimed at understanding how learning can affect these CPG-driven behaviors (**Figure 1**).

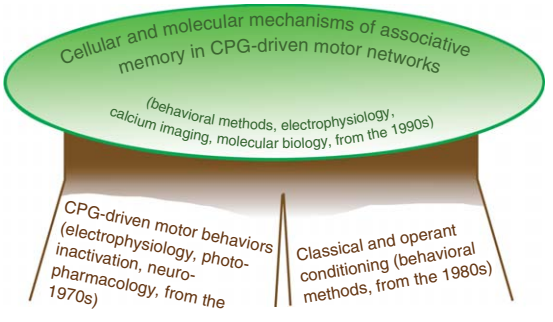

Figure 1 The two roots of current cellular and molecular studies of associative memory in *Lymnaea*.

Shortly after the publication of the first papers describing CPG interneurons, motor neurons, and modulatory neurons of the feeding network (Benjamin and Rose, 1979; Rose and Benjamin, 1979; McCrohan and Benjamin, 1980a,b), seminal behavioral work established the ability of *Lymnaea* to form long-term associative memory after a few pairings or even just a single pairing of a nonfood chemical stimulus and a food stimulus (Alexander et al., 1982, 1984; Audesirk et al., 1982). It was subsequently shown that *Lymnaea* is also capable of forming a positive association between tactile stimuli and food, but only after multiple trials (Kemenes and Benjamin, 1989a). Both the chemical and tactile conditioning paradigms revealed a number of characteristics of associative learning in *Lymnaea* that were shared by learning in vertebrates, such as dependence on age and motivational states, stimulus generalization, discriminative learning, and classical-operant interactions (Audesirk et al., 1982; Kemenes and Benjamin, 1989a,b, 1994). Later on it was also demonstrated that *Lymnaea* can form negative associations between a food conditioned stimulus (CS) and an aversive chemical unconditioned stimulus (US) (classical conditioning) (Kojima et al., 1996) or between a behavior and an aversive tactile stimulus (operant conditioning) (Lukowiak et al., 1996). The wealth of knowledge about the behavioral features of both operant and classical, aversive and reward conditioning, together with a detailed understanding of the neuronal networks underlying the unconditioned behaviors used in these paradigms (feeding and respiration, **Figure 2**), has made *Lymnaea* a very attractive experimental model for top-down analyses of learning and memory.

The two fields of research, physiological analysis of CPGs and behavioral analysis of associative conditioning, started to converge in the mid- to late 1990s when neuronal correlates of both classical and operant conditioning were first described (Whelan and McCrohan, 1996; Kemenes et al., 1997; Kojima et al., 1997; Staras et al., 1998, 1999; Spencer et al., 1999). The most important neuronal aspects of associative learning in *Lymnaea* are discussed in another chapter (*See* Chapter 1.30) as well as several review articles (Kemenes, 1999; Lukowiak and Syed, 1999; Benjamin et al., 2000; Lukowiak et al., 2003b). Detailed analyses of molecular mechanisms of associative memory started in the late 1990s after sufficient information had been obtained on both the behavioral and neuronal aspects of associative learning (**Figures 1** and **2**).

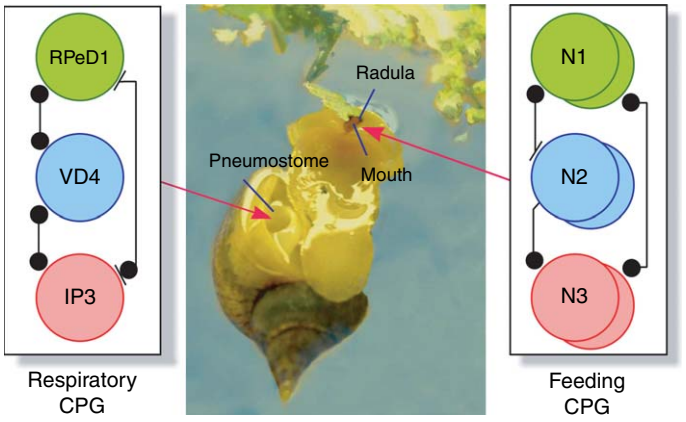

Figure 2 The respiratory and feeding behavior and the underlying CPG networks in *Lymnaea.* The photograph (courtesy of Dr. I. Kemenes) shows a snail hanging upside down on the water surface in an aquarium, simultaneously performing aerial respiration through the open pneumostome and feeding on a piece of lettuce. The cartoon to the left of the photograph shows the three-neuron respiratory CPG responsible for movements of the pneumostome. The cartoon on the right shows the feeding CPG responsible for the synchronized rhythmic movements of the snail's mouth parts, including the radula, which performs rasping movements during feeding. The feeding CPG consists of three main types of CPG neurons, each type having two different subtypes. The respiratory CPG produces a two-phase motor pattern (closure and opening of the pneumostome), whereas the feeding CPG produces a three-phase pattern (radula protraction, rasping, and swallowing). The arrows pointing from the CPG diagrams to the pneumostome and mouth parts, respectively, indicate causal relationships between CPG activity and motor functions performed by these organs. For more details on the two *Lymnaea* CPG networks see the references quoted in the text. Data sources: Benjamin PR and Elliott CJ (1989) Snail feeding oscillator: The central pattern generator and its control by modulatory interneurons. In: Jacklet J (ed.) *Neuronal and Cellular Oscillators*, p. 173. New York: Dekker; Lukowiak K (1991) Central pattern generators: Some principles learned from invertebrate model systems. *J. Physiol. (Paris)* 85: 63–70; Lukowiak K (2001) The *Lymnaea* respiratory system: Where are we going with learning? *Adv. Esp. Med. Biol.* 499: 321–326; Lukowiak K and Syed N (1999) Learning, memory and a respiratory central pattern generator. *Comp. Biochem. Physiol. A Mol. Integr. Physiol.* 124: 265–274; Benjamin PR, Staras K, and Kemenes G (2000) A systems approach to the cellular analysis of associative learning in the pond snail, *Lymnaea. Learn. Mem.* 7: 124–131; Benjamin PR, Kemenes G, and Staras K (2005) Molluscan nervous systems. In: *Encyclopedia of Life Sciences*. London: John Wiley; Elliott CJ and Susswein AJ (2002) Comparative neuroethology of feeding control in molluscs. *J. Exp. Biol.* 205: 877–896; Lukowiak K, Sangha S, Scheibenstock A, et al. (2003b) A molluscan model system in the search for the engram. *J. Physiol. Paris* 97: 69–76; Lukowiak K, Martens K, Orr M, Parvez K, Rosenegger D, and Sangha S (2006b) Modulation of aerial respiratory behaviour in a pond snail. *Respir. Physiol. Neurobiol.* 154: 61–72.

4.09.2 Molecular Analyses of Associative Memory in *Lymnaea*

4.09.2.1 Reward Classical Conditioning of Feeding

Classical (or Pavlovian) reward conditioning in *Lymnaea* is based on forming an association between a nonfood tactile or chemical CS and an unconditioned food stimulus. Associations are formed in the feeding network and lead to a learned change in the behavior, with trained animals producing stronger feeding responses to the CS compared to control animals. **Figure 2** shows a *Lymnaea* feeding on a piece of lettuce (an unconditioned food stimulus) and the CPG network underlying the generation of the three-phase rhythmic snail feeding behavior called rasping. A more detailed description of the *Lymnaea* feeding behavior and underlying neuronal circuitry is presented in another chapter (*See* Chapter 1.30) and previous review articles (Benjamin and Elliott, 1989; Elliott and Susswein, 2002; Benjamin et al., 2005).

4.09.2.1.1 Single-trial reward conditioning

To date, the most detailed information on the molecular mechanisms of associative long-term memory (LTM) in *Lymnaea* has been gained from experiments using single-trial food-reward (also known as appetitive) classical conditioning. In this paradigm, snails are subjected to a conditioning protocol using a single pairing of amyl acetate (pear drops) as a neutral CS with sucrose as a rewarding or appetitive US. In subsequent memory tests with the CS alone, the explicitly paired (CS/US) experimental group shows significantly greater feeding responses to amyl acetate over their own naive responses and all the standard control groups (random, explicitly unpaired, CS alone, US alone) (Alexander et al., 1984; Kemenes et al., 2002; also see **Figure 4**).

The most important finding from the original behavioral studies was that a single pairing of the amyl acetate CS and sucrose US resulted in LTM, which lasted for several weeks (Alexander et al., 1984). This is a remarkable example of robust single-trial learning, which is now very effectively used for analyses of the time course of the molecular mechanisms underlying memory processes. The use of this single-trial paradigm for the analysis of the molecular mechanisms of memory consolidation and reconsolidation has two main advantages. First, after single-trial conditioning, translation- and transcription-dependent memory emerges in a matter of hours (Fulton et al., 2005), allowing this type of memory to be studied on a timescale of a few hours to several weeks. This early emergence of LTM was utilized in recent *in vitro* conditioning experiments investigating the cellular and molecular mechanisms of memory formation in semi-intact preparations, which are only viable for up to 6 h (Ireland, 2006). Second, unlike multi-trial paradigms, single-trial conditioning allows the analyses of the amnestic effects of sharply timed manipulations of key molecular pathways during both memory consolidation (Kemenes et al., 2002; Fulton et al., 2005; Ribeiro et al., 2005) and reconsolidation after the retrieval of memory (Kemenes G et al., 2006).

In semi-intact preparations made from conditioned animals, electrophysiological correlates of the rhythmic conditioned feeding response to amyl acetate were recorded in nerves, motor neurons, and command-like neurons of the feeding system (Kemenes et al., 2002; Straub et al., 2004, 2006; Kemenes I et al., 2006). Moreover, in attempts to localize sites of plasticity, electrical changes, such as maintained depolarization of the modulatory neuron type cerebral giant cells (CGCs) after conditioning, have also been recorded (Kemenes I et al., 2006).

At the molecular level, the most important question to be addressed was whether or not LTM formation after single-trial conditioning (similar to 'flash-bulb' memory known in other systems, including humans, cf. Carew, 1996) is based on the same conserved pathways that were originally described using multi-trial paradigms in other systems. An important clue to suggest that this might be the case is that the broad-spectrum protein synthesis inhibitor anisomycin (ANI), injected in an early time window (10 min to 1 h) after conditioning, blocks the 24-h memory trace (conventionally regarded as LTM) (Fulton et al., 2005). An earlier (5 h) memory trace is blocked by both ANI and actinomycin-D (Act-D), an RNA synthesis blocker, confirming that LTM, defined as memory dependent on early post-training translation and transcription, is present as early as 5 h after single-trial conditioning (Fulton et al., 2005). This observation indicates that single-trial reward conditioning triggers molecular cascades that are involved in the rapid consolidation of long-lasting memory traces. Interestingly, there is only a single early time window of sensitivity to transcription and translation blockers (Fulton et al., 2005) (**Figure 3**), unlike in a number of other studies, which described a temporally distinct second window of protein synthesis-dependent LTM in both vertebrates and invertebrates (Grecksch and Matthies, 1980; Freeman et al., 1995;

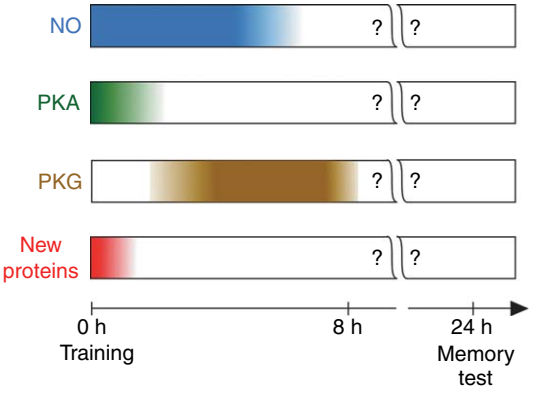

Figure 3 Time windows of requirement for NO, protein kinase A (PKA), protein kinase G (PKG), and new proteins for the consolidation of LTM after single-trial reward classical conditioning. Time windows were only investigated for up to 8 h post-training, so at present it cannot be ruled out (question marks) that there are further time windows between 8 h and 24 h. Data sources: Kemenes I, Kemenes G, Andrew RJ, Benjamin PR, and O'Shea M (2002) Critical time-window for NO-cGMP-dependent long-term memory formation after one-trial appetitive conditioning. *J. Neurosci.* 22: 1414–1425; Kemenes G, Kemenes I, Michel M, Papp A, and Müller U (2005) Early memory retrieval interferes with late memory expression in a PKA-dependent manner. In: *Abstracts of the 18th National Meeting of the British Neuroscience Association:* 39:10. Brighton, UK: British Neuroscience Association; Kemenes G, Kemenes I, Michel M, Papp A, and Müller U (2006) Phase-dependent molecular requirement for memory reconsolidation: Differential roles for protein synthesis and protein kinase A activity. *J. Neurosci.* 26:6298–6302; Fulton D, Kemenes I, Andrew RJ, and Benjamin PR (2005) A single time-window for protein synthesis-dependent long-term memory formation after one-trial appetitive conditioning. *Eur. J. Neurosci.* 21: 1347–1358; Korneev SA, Straub V, Kemenes I, et al. (2005) Timed and targeted differential regulation of nitric oxide synthase (NOS) and anti-NOS genes by reward conditioning leading to long-term memory formation. *J. Neurosci.* 25: 1188–1192; Ribeiro MJ, Schofield MG, Kemenes I, O'Shea M, Kemenes G, and Benjamin PR (2005) Activation of MAPK is necessary for long-term memory consolidation following food-reward conditioning. *Learn. Mem.* 12: 538–545.

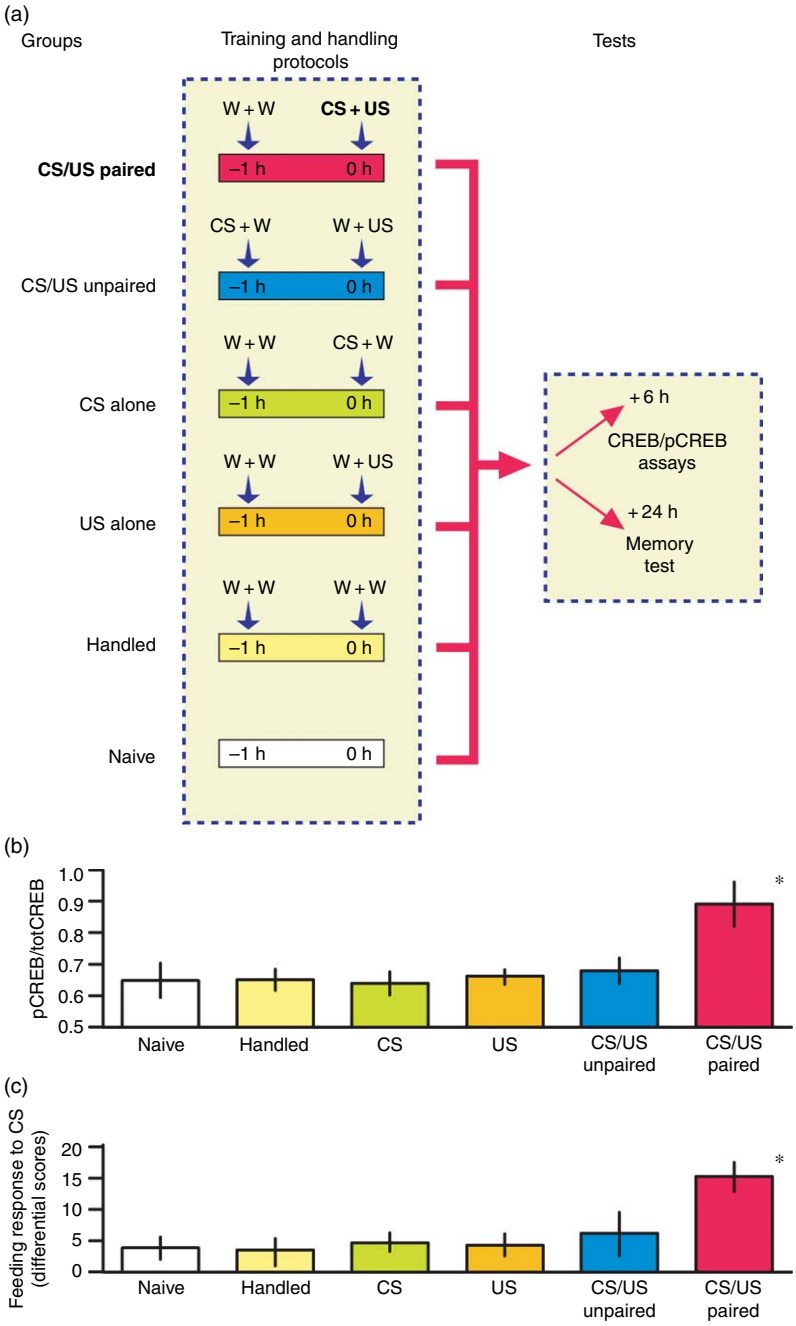

Figure 4 CREB is only phosphorylated after single-trial conditioning with contingent CS (amyl acetate) and US (sucrose) application. (a) The experimental design. An experimental (CS/US paired) and five different control groups were used in the same experiment. Water (W) stimuli were given to balance for disturbance in the experimental dish caused by applications of the CS and/or US solutions. After training or control treatment, each group was randomly divided into two subgroups. One of these groups was dissected for CREB/pCREB assays at 6 h posttraining; the other group was tested with the CS at 24 h posttraining to confirm that LTM had formed. (b) Significant (*, at least $p < .05$, analysis of variance (ANOVA) and post hoc tests) upregulation of pCREB levels in the experimental vs. all the control groups, indicating CS/US pairing-specific activation of CREB. (c) Significant (*, at least $p < .05$, ANOVA and post hoc tests) increase in the feeding response to the CS in the experimental vs. the control groups, indicating associative memory. The feeding response is shown as a difference score obtained by subtracting background feeding responses to water from the CS-induced feeding responses, both measured in 2-min observation periods. Data source: Michel M, Daniels M, Mueller U, Kemenes G (2007) Involvement of PKA and CREB in single-trial reward learning in *Lymnaea*. *Br. Neurosci. Assoc. Abstr.* 19: 57.

Bourtchouladze et al., 1998; Epstein et al., 2003). It has been suggested that 'strong' (i.e., effective) training protocols lead to rapid memory consolidation involving only a single wave of protein synthesis-dependent events, whereas 'weak' (i.e., less effective) training protocols are followed by a more prolonged consolidation phase, containing two or more windows of requirement for new protein synthesis (Bourtchouladze et al., 1998). According to this categorization, single-trial reward conditioning in *Lymnaea* certainly qualifies as a 'strong' training protocol, explaining both the rapid emergence and persistence of the associative memory trace, characteristic of 'flash-bulb' memory.

4.09.2.1.1.(i) CREB and LTM after single-trial reward conditioning Detailed molecular analyses identified highly conserved cyclic adenosine monophosphate (cAMP)-responsive element binding protein (CREB) genes (*Lym-CREB1* and *Lym-CREB2*) and CREB-like proteins in *Lymnaea* (Ribeiro et al., 2003; Sadamoto et al., 2004). A highly conserved CREB-binding protein (CBP) gene (*Lym-CBP*) also has been cloned in *Lymnaea* (Hatakeyama and Kemenes, 2005), further indicating the existence of functional CREB-dependent transcriptional mechanisms. These were important findings because regulation of gene expression during memory consolidation is known to involve a variety of transcription factors, with CREB playing a particularly important role in the switch between short-term and long-term memory storage in a variety of different species and paradigms (Frank and Greenberg, 1994; Stevens, 1994; Goda, 1995; Carew, 1996; Yin and Tully, 1996; Fletcher, 1997; Pittenger and Kandel, 1998; Silva et al., 1998; Alberini, 1999; Lamprecht, 1999; Scott et al., 2002; Tully et al., 2003; Josselyn and Nguyen, 2005). Consistent with a role for CREB in *Lymnaea* LTM is the observation that levels of phosphorylated CREB1 are increased in neurons of the feeding network following reward conditioning (Ribeiro et al., 2003). More recent experiments (Michel et al., 2007) showed that pCREB1 levels only increased in the CS/US paired group, and they remained unaffected in all the standard control groups (**Figure 4**).

4.09.2.1.1.(ii) NO and LTM after single-trial reward conditioning Historically, the first important discovery of the detailed molecular studies of learning and memory in *Lymnaea* was that consolidation of LTM after single-trial reward conditioning is dependent on the NO-cyclic guanosine monophosphate signaling pathway. There is a critical period of sensitivity up to 5 h after conditioning (**Figure 3**) when blocking this pathway by drug injection prevents LTM formation, which was established at both the level of whole animals and the neuronal network responsible for generating the feeding behavior (Kemenes et al., 2002).

Further evidence for a role of NO in LTM came from experiments on single isolated CGCs (Korneev et al., 2005). These giant modulatory neurons express mRNA transcripts from two related nNO synthase (nNOS) genes (*Lym-nNOS1* and *Lym-nNOS2*). Six hours after single-trial conditioning, *Lym-nNOS1* is upregulated compared with controls. This upregulation of the NOS-encoding transcript may be due to an earlier downregulation of the *Lymnaea anti-nNOS* transcript at 4 h that is known to be inhibitory on NOS transcript production. This is a rare example of analysis where the role of a specific signaling molecule in memory formation could be traced from the behavioral to the network and single neuronal level.

4.09.2.1.1.(iii) PKA, MAPK, and LTM after single-trial reward conditioning Other highly conserved molecular pathways that have been implicated in LTM after single-trial reward conditioning in *Lymnaea* are the protein kinase A (PKA) and mitogen-activated protein kinase (MAPK)-dependent signaling cascades. Inhibition of PKA catalytic subunit activity or MAPK phosphorylation blocked 24-h LTM without blocking sensory or motor pathways (Kemenes et al., 2005; Ribeiro et al., 2005). When measured 30 min after conditioning, increased levels of both PKA activity and MAPK phosphorylation were found (Ribeiro et al., 2005), with increased PKA activation also detected when measured in an earlier (5 min) and a later (1 h) time window (Kemenes et al., 2005), indicating a more prolonged dependence of LTM on PKA compared to protein synthesis (**Figure 3**).

A summary of the observations concerning the role of PKA, MAPK, CREB, and NOS/NO in 24-h LTM forming after reward conditioning is presented in **Table 1**. This direct comparison shows an important difference between MAPK and the other signaling molecules investigated in reward conditioning. Unlike the other factors, MAPK is activated not only in response to contingent CS/US application, but also when the CS or US were applied alone or in an unpaired manner (Ribeiro et al., 2005). This observation, together with the fact that preventing MAPK phosphorylation after training blocks LTM, shows that MAPK is necessary but definitely not sufficient for the consolidation of associative LTM

Table 1 A summary of the role of various molecular pathways in the consolidation of associative memory after single-trial reward classical conditioning in *Lymnaea*[a]

Molecule/pathway	*CS alone*	*US alone*	*CS/US unpaired*	*CS/US paired*	*Inhibition blocks associative LTM*
PKA	–	–	–	↑	Yes
MAPK	↑	↑	↑	↑	Yes
CREB	–	–	–	↑	?
NOS/NO	–	–	–	↑	Yes

[a]Abbreviations: – , no change after training; ↑, activation/upregulation after training; ?, no data.
Data source: Kemenes I, Kemenes G, Andrew RJ, Benjamin PR, and O'Shea M (2002) Critical time-window for NO-cGMP-dependent long-term memory formation after one-trial appetitive conditioning. *J. Neurosci.* 22: 1414–1425; Ribeiro MJ, Serfozo Z, Papp A, et al. (2003) Cyclic AMP response element-binding (CREB)-like proteins in a molluscan brain: Cellular localization and learning-induced phosphorylation. *Eur. J. Neurosci.* 18: 1223–1234; Ribeiro MJ, Schofield MG, Kemenes I, O'Shea M, Kemenes G, and Benjamin PR (2005) Activation of MAPK is necessary for long-term memory consolidation following food-reward conditioning. *Learn. Mem.* 12: 538–545; Kemenes G, Kemenes I, Michel M, Papp A, and Müller U (2005) Early memory retrieval interferes with late memory expression in a PKA-dependent manner. In: *Abstracts of the 18th National Meeting of the British Neuroscience Association:* 39:10. Brighton, UK: British Neuroscience Association; Kemenes G, Kemenes I, Michel M, Papp A, and Müller U (2006) Phase-dependent molecular requirement for memory reconsolidation: Differential roles for protein synthesis and protein kinase A activity. *J. Neurosci.* 26:6298–6302; Korneev SA, Straub V, Kemenes I, et al. (2005) Timed and targeted differential regulation of nitric oxide synthase (NOS) and anti-NOS genes by reward conditioning leading to long-term memory formation. *J. Neurosci.* 25: 1188–1192.

after single-trial reward conditioning. PKA, CREB, and NOS/NO are selectively activated/upregulated by the associative training protocol, so potentially each of these factors could be sufficient for memory consolidation. However, it is more likely to be the case that these and other signaling molecules make a synergistic contribution to the memory consolidation process, with each molecule and pathway playing an important role in LTM but none of them alone being sufficient for its consolidation.

Interestingly, unlike early inhibition of protein synthesis, inhibition of PKA catalytic subunit activity did not block the expression of memory at 6 h post-training (Kemenes et al., 2005), indicating the involvement of a different signaling molecule (possibly Ca^{2+}/calmodulin-dependent protein kinase II (CaMKII), Wan et al., 2007) in the protein synthesis-dependent consolidation of this earlier memory trace (**Figure 5** and **Table 2**).

Treatment of isolated cerebral ganglia with the adenylate cyclase activator forskolin resulted in massively increased CREB phosphorylation in neuronal nuclei (Ribeiro et al., 2003), indicating a potential link between training-induced PKA activation in the cerebral ganglia (Kemenes et al., 2005) and CREB phosphorylation in conditioned animals. Whether there is any linkage between the NO, PKA, and MAPK signaling pathways has yet to be determined.

4.09.2.1.1.(iv) Time windows of sensitivity of LTM to amnestic treatments after single-trial reward conditioning A summary of the known time windows of sensitivity of the 24-h LTM to inhibition of NO, PKA, protein kinase G (PKG), and protein synthesis is shown in **Figure 3**. Time windows of sensitivity were only investigated in the first 8 h after training, so we cannot rule out that there are also later time windows when these molecular processes are required for the consolidation of the 24-h memory trace. The observation that *Lym-nNOS1* mRNA levels increase at 6 h posttraining (Korneev et al., 2005) strongly suggests an additional later role for NO, perhaps in memory maintenance. The level of phosphorylated CREB1 was also found to be high at 6 h posttraining (Ribeiro et al., 2003), indicating that new transcription and translation still take place after this period. The time windows of requirement for NO and PKA are both wider (Kemenes et al., 2002, 2005) than the time window for requirement for early protein synthesis (Fulton et al., 2005), indicating that the functions of NO and PKA go beyond being upstream components of molecular pathways leading to new protein synthesis shortly after training.

Recently, we have found that, unlike PKA, NO, and new proteins, PKG is not involved in the early processes of memory consolidation (**Figure 3**). This kinase enzyme is, however, required for later memory consolidation processes (between 2 and 8 h posttraining), hardly overlapping with the temporal requirement for PKA (up to 2 h posttraining, Kemenes et al., 2005) but linked to the requirement for NO/soluble guanylyl cyclase (10 min to 6 h posttraining (Kemenes et al., 2002) in a delayed manner.

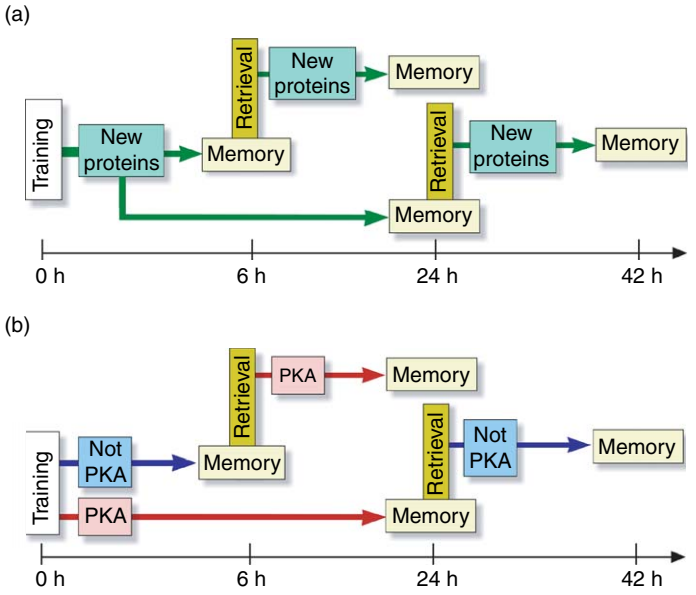

Figure 5 Time-dependent differential requirements for protein synthesis and PKA after training and memory retrieval. (a) New proteins are required for both 6-h and 24-h memory as well as memory reconsolidation after memory retrieval at both time points. (b) By contrast, PKA is only required for 24-h but not 6-h memory, whereas reconsolidation of the retrieved memory is PKA-dependent at 6 h but not at 24 h posttraining. These observations indicate that not PKA, but other signaling molecules are important for memory consolidation at 6 h and for memory reconsolidation at 24 h posttraining. Data sources: Kemenes G, Kemenes I, Michel M, Papp A, and Müller U (2005) Early memory retrieval interferes with late memory expression in a PKA-dependent manner. In: *Abstracts of the 18th National Meeting of the British Neuroscience Association:* 39:10. Brighton, UK: British Neuroscience Association; Kemenes G, Kemenes I, Michel M, Papp A, and Müller U (2006) Phase-dependent molecular requirement for memory reconsolidation: Differential roles for protein synthesis and protein kinase A activity. *J. Neurosci.* 26: 6298–6302.

Table 2 Differential time-dependent requirements for PKA activity and translation for memory consolidation after single-trial classical reward conditioning and reconsolidation after the retrieval of associative memory in *Lymnaea*

		Time of memory test or retrieval	
Requirement for	***Process required for***	***6 h***	***24 h***
Translation	Memory consolidation after training	Yes	Yes
	Memory reconsolidation after retrieval	Yes	Yes
PKA	Memory consolidation after training	No	Yes
	Memory reconsolidation after retrieval	Yes	No

Data sources: Kemenes G, Kemenes I, Michel M, Papp A, and Müller U (2005) Early memory retrieval interferes with late memory expression in a PKA-dependent manner. In: *Abstracts of the 18th National Meeting of the British Neuroscience Association:* 39:10. Brighton, UK: British Neuroscience Association; Kemenes G, Kemenes I, Michel M, Papp A, and Müller U (2006) Phase-dependent molecular requirement for memory reconsolidation: Differential roles for protein synthesis and protein kinase A activity. *J. Neurosci.* 26: 6298–6302.

4.09.2.1.1.(v) cAMP-dependent molecular cascades and neuronal plasticity contributing to LTM after single-trial reward conditioning

An important link between cAMP-dependent molecular cascades and neuronal plasticity contributing to LTM was found in voltage-clamp experiments, which demonstrated a long-lasting cAMP-induced increase in a low-threshold tetrodotoxin-resistant persistent sodium current of the CGCs (Nikitin et al., 2006). This current makes an important contribution to the CGC somal membrane potential (Staras et al., 2002; Nikitin et al., 2006), which in turn becomes

persistently depolarized after single-trial reward conditioning (Kemenes I et al., 2006). The CGC's synaptic output also became similarly enhanced by somal injection of cAMP (Nikitin et al., 2006) and artificial depolarization of the soma membrane (Kemenes I et al., 2006), lending further support to the notion that cAMP-dependent cascades might support long-lasting plastic changes in ion channel number and/or function, contributing to learning-induced maintained depolarization. The mechanism by which persistent somal depolarization affects synaptic output is likely to be based on a maintained increase in background calcium levels in axon terminals of the CGCs presynaptic to their target neurons (Kemenes I et al., 2006), similar to what was demonstrated previously in mammalian neurons (Awatramani et al., 2005).

4.09.2.1.1.(vi) PKA, protein synthesis, and memory reconsolidation after single-trial reward conditioning An interesting observation is that memory reconsolidation after retrieval at 6 h posttraining is both PKA- and protein synthesis-dependent, whereas reconsolidation after retrieval at 24 h depends on protein synthesis but not on PKA activity (Kemenes G et al., 2006) (**Figure 5** and **Table 2**). This finding indicates that, depending on how recent or remote consolidated memory is relative to the time of training, different molecular pathways are activated by memory retrieval and contribute differentially to memory reconsolidation (*See* Chapters 1.24, 4.14). At a more general level, this phase-dependent differential molecular requirement for reconsolidation supports the notion that even seemingly fully consolidated memories undergo further selective molecular maturation processes (the 'lingering consolidation' hypothesis, Dudai and Eisenberg, 2004), which however may only be revealed by analyzing the role of a variety of different specific pathways in memory reconsolidation after retrieval.

A key question concerning the molecular mechanisms of memory reconsolidation is whether or not they are a recapitulation of the processes active during memory consolidation (Nader et al., 2000; Sara, 2000; Dudai, 2002; Nader, 2003; Dudai and Eisenberg, 2004; Morris et al., 2006). If they are, memory retrieval should reactivate the same molecular cascades that were activated by training, and therefore the same amnestic treatments should impede both processes. Direct comparisons of the PKA and protein synthesis dependence of the consolidation of the 6-h and 24-h memory trace and the reconsolidation of memory after retrieval at these two time points (**Figure 5** and **Table 2**) support the notion that reconsolidation is a recapitulation of consolidation, only at a more general molecular level (protein synthesis), but not at the level of a specific signaling molecule (PKA). In fact, it seems that with regard to PKA, the molecular requirements for consolidation and reconsolidation are completely different: consolidation of the 6-h memory trace does not require PKA, but reconsolidation after memory retrieval at 6 h does, whereas reconsolidation after retrieval at 24 h takes place even when PKA activity is inhibited, despite the dependence of the consolidation of the 24-h memory on early posttraining PKA activity (Kemenes G et al., 2005, 2006). It remains to be elucidated if other specific signaling molecules already known to contribute to the consolidation of the 24-h memory trace (MAPK, NO) are also important for the 6-h memory trace and whether or not they are activated by retrieval at these two different time points.

4.09.2.2 Aversive Classical Conditioning of Feeding

Aversive classical conditioning of feeding in *Lymnaea* is based on pairing sucrose as a CS with an aversive chemical US such as KCl, which inhibits feeding and evokes a withdrawal response (conditioned taste aversion, CTA). After eight or more trials, trained animals showed a significantly weaker feeding response to sucrose than did controls, and this associative memory lasted for over 1 month (Kojima et al., 1996; Ito et al., 1999).

A neural analysis of CTA was carried out on isolated brains dissected from conditioned and control animals (Kojima et al., 1997, 2001). Like reward chemical conditioning, aversive chemical conditioning leads to specific changes in the feeding network, described in another chapter (*See* Chapter 1.30) and previous reviews (Ito et al., 1999; Benjamin et al., 2000; Lukowiak et al., 2003a).

4.09.2.2.1 PKA, CREB, and LTM after aversive conditioning

The transcription and translation dependence of aversive behavioral LTM or the necessity of the activation of specific molecular cascades for its consolidation have not been tested in intact *Lymnaea,* but *in vitro* experiments were performed to

investigate the role of the transcription factor CREB and its upstream activators in synaptic plasticity that may be linked to CTA. The injection of cAMP or PKA into the soma of the CGCs in isolated nervous system preparations led to a long-term enhancement of the synapse between the CGC and a follower motor neuron, B1 (Nakamura et al., 1999). The injection of a CRE oligonucleotide into the CGCs prevented this cAMP-induced long-lasting synaptic plasticity (Sadamoto et al., 2004). These *in vitro* experiments showed that one of the mechanisms of the previously described enhancement in the CGC's synaptic output induced by CTA learning could be the activation of the cAMP/PKA/CREB cascade. However, synaptic plasticity induced by CTA learning in the CGCs could depend on molecular mechanisms other than cAMP-dependent cascades (e.g., CaMKII), and cAMP/PKA and CREB-mediated synaptic plasticity in the CGCs could be involved in the formation of a variety of different memory traces arising from different training paradigms using food stimuli (e.g., visual conditioning (Andrew and Savage, 2000)). Thus, unlike LTM forming after reward conditioning, CTA has not yet been conclusively linked to PKA or the transcription factor CREB in *Lymnaea*.

4.09.2.2.2 C/EBP and LTM after aversive conditioning

Unlike in the case of PKA and CREB, a link has been found between CTA in intact *Lymnaea* and another conserved transcription factor, CCAAT/enhancer binding protein (C/EBP), and this was shown in the buccal ganglia and, specifically, the B2 gut motor neuron (Hatakeyama et al., 2006). Both the phosphorylated and total levels of the *Lym-C/EBP* protein increased in the buccal ganglia and neuron B2 during CTA consolidation, although *Lym-C/EBP* mRNA levels were reduced in the same time window (1 h posttraining) when the increases in protein levels were measured. One explanation for this paradoxical observation could be that the existing pool of *Lym-C/EBP* mRNA is rapidly translated and degraded early after CTA learning, resulting in fast turnover of newly transcribed mRNA, which may be required for a prolonged *de novo* synthesis of large amounts of *Lym-C/EBP* necessary for long-term memory after CTA learning. A more detailed analysis of the time course of changes in *Lym-C/EBP* mRNA and protein levels will be necessary to elucidate the relationship between *Lym-C/EBP* gene transcription, mRNA turnover, and protein synthesis in this system.

Although the CRE element upstream of *Lym-C/EBP* has not been investigated, based on data from *Aplysia, Helix,* and mammals (Niehof et al., 1997; Alberini, 1999; Grinkevich, 2002), the expression of *Lym-C/EBP* is likely to be regulated by *Lymnaea* CREB. It is not known which genes are targeted by C/EBP in *Lymnaea*. However, likely candidate downstream targets of C/EBP are the *Lym-nNOS* genes (Korneev et al., 2005). *Lym-C/EBP* and *Lym-nNOS* are colocalized in the B2 motor neurons (Hatakeyama et al., 2006), and in addition, *Lym-nNOS* genes have three putative *Lym-C/EBP* binding sites (Korneev et al., 2005), providing the necessary structural conditions for the interaction of C/EBP with NOS genes in the *Lymnaea* feeding network.

An interesting but so far unanswered question concerns the differences in the molecular mechanisms underlying reward and aversive conditioning of feeding. Plastic changes after both types of conditioning were found in the CGCs (Kojima et al., 1997; Kemenes I et al., 2006), which are therefore regarded as key neurons for both reward and aversive conditioning. In the case of reward conditioning, the plastic change (a persistent somal depolarization) is nonsynaptic in nature and increases the probability of a feeding response to the CS by recruiting feeding command-like neurons into the network of cells activated by the CS (Kemenes I et al., 2006). In the case of aversive conditioning, the plastic change increases the amplitude of a CGC-driven inhibitory synaptic input to the N1M-type CPG neurons (Kojima et al., 1997), whose inhibition suppresses feeding (Kemenes et al., 2001). It will be important to determine what molecular differences underlie the different cellular changes induced in the same neuron by reward versus appetitive conditioning and contributing either to the activation or suppression of the same behavior after classical conditioning.

4.09.2.3 Operant Conditioning of the Suppression of Aerial Respiration

The aerial respiratory behavior of *Lymnaea stagnalis* has been used in a series of experiments investigating the behavioral, neuronal, and molecular mechanisms of operant conditioning. The respiratory behavior and underlying neuronal circuitry are extremely well characterized and are reviewed in another chapter (*See* Chapter 1.30) as well as several previous reviews (Lukowiak, 1991, 2001; Lukowiak and Syed, 1999; Benjamin et al., 2000; Lukowiak et al., 2003b, 2006b). While single-trial classical conditioning has

provided detailed information on specific molecular mechanisms of LTM, work based on the operant conditioning of the suppression of aerial respiration has provided comprehensive information on the general molecular requirements for different memory phases (intermediate-term memory (ITM) and LTM) and active processes related to associative memory, such as forgetting, extinction, and reconsolidation.

The biological basis for operant conditioning in *Lymnaea* lies in the fact that these pond snails are bimodal breathers; they can breathe both through the skin (cutaneous respiration) and via a simple lung (aerial respiration), which is supplied with air through a respiratory orifice called the pneumostome (**Figure 2**). Hypoxia triggers pneumostome opening, the CPG-driven motor mechanism of aerial respiration (**Figure 2**). Tactile stimulation of the pneumostome area evokes reflexive pneumostome closure and thus suppresses aerial respiratory behavior. In an operantly trained group, a tactile stimulus was applied to the pneumostome area each time aerial respiration was attempted by the animal or semi-intact preparation in an artificially created hypoxic environment (Lukowiak et al., 1996). Suitable yoked and hypoxic control groups were also used. The number of openings, latency to first opening, and total breath durations were recorded in pre- and posttraining periods. Only the operantly conditioned group showed significant changes between the pre- and posttraining behaviors, with significant reductions in openings and total breathing time and significant increases in the latency to first breath. A memory for the operantly conditioned suppression of aerial respiration could persist for at least 4 weeks (Lukowiak et al., 1998), indicating the formation of LTM.

4.09.2.4 Intermediate and Long-Term Memory, Reconsolidation, and Extinction after Operant Conditioning

At the behavioral level, both ITM and LTM have been described based partly on the length of time the memory persists (Lukowiak et al., 2000) but also on the sensitivity to the protein and mRNA synthesis blockers ANI and Act-D, respectively (Sangha et al., 2003c). ANI prevents the formation of both ITM and LTM, whereas Act-D only prevents LTM. Both reconsolidation and extinction have been studied following operant conditioning, and both have been shown to be dependent on new RNA and protein synthesis (Sangha et al., 2003b,d). Extinction is viewed as a new type of associative memory that temporarily masks but does not replace the original memory. Thus following extinction trials, the loss of memory at 2 h is followed by full spontaneous recovery at 24 h.

4.09.2.4.1 Inhibition of macromolecular synthesis and dynamics of memory consolidation and forgetting after operant conditioning

In a series of intriguing experiments, Ken Lukowiak and colleagues showed that cooling, which inhibits macromolecular synthesis, can be used as a tool to prevent not only memory consolidation, but also forgetting, which is thought to be an active process itself, requiring both new learning and memory. When snails were kept at room temperature after operant conditioning, LTM was absent 7 days after training (i.e., forgetting occurred) (Sangha et al., 2005). However, when snails were placed into 4 °C water 1 h after the end of training and kept there for 7 days, LTM was preserved. Thus, unlike cooling for 1 h immediately after training, which impedes LTM (Sangha et al., 2003a), delayed and prolonged post-training cooling impeded forgetting instead (Sangha et al., 2005). Another interesting observation was that ITM can be boosted into LTM by cooling snails immediately after the first bout of a training protocol (two 30-min operant conditioning training sessions in hypoxic water separated by a 30-min rest interval in water with normal oxygen levels) that normally only results in ITM, but not LTM (Parvez et al., 2005).

4.09.2.4.2 Single-neuronal contribution to LTM, forgetting, extinction, and reconsolidation after operant conditioning

Neural changes associated with associative memory have been identified in the isolated central nervous system derived from operantly conditioned animals (Spencer et al., 1999; Spencer et al., 2002). For example, a higher percentage of right pedal dorsal 1 (RPeD1) CPG interneurons, which are important in the onset of the respiratory cycle (**Figure 2**), were silent in conditioned versus control preparations. This neuron became the main focus of work aimed at understanding the molecular processes involved in LTM, forgetting, extinction, and reconsolidation at the level of single identified neurons.

Evidence for the role of molecular processes active in the nucleus and somal cytoplasm of RPeD1 during memory formation came from both somal ablation experiments in intact animals

(Scheibenstock et al., 2002) and spike activity perturbation experiments in semi-intact preparations, where LTM formation was shown *in vitro* (Lowe and Spencer, 2006). Removal of the soma 2 h prior to conditioning of intact snails prevented LTM but had no effect on ITM, suggesting that the RPeD1 soma was necessary for LTM formation (Scheibenstock et al., 2002). Removal of the RPeD1 soma 1 h after conditioning had no effect on LTM, indicating that the effects of soma ablation were not related to memory access or retrieval. In semi-intact preparations, preventing RPeD1 spike activity between training sessions can directly augment memory formation (Lowe and Spencer, 2006), again emphasizing the important role this respiratory CPG neuron plays in memory consolidation.

Interestingly, forgetting, extinction, and reconsolidation also require the presence of the soma of RPeD1 (Sangha et al., 2003b,d, 2005), indicating both that these are all active processes and that RPeD1 is involved in all of them. The observations regarding the role of macromolecular synthesis and RPeD1 are summarized in **Table 3**.

Recent work has shown that snails with axotomized and subsequently regenerated RPeD1 axons can both form new LTM and retain already existing LTM (Lukowiak et al., 2006a). An important implication of this observation is that, despite the fact that similar second messenger cascades are utilized to elicit gene activity in both memory consolidation and regeneration (Feng et al., 1997; Zhang and Ambron, 2000; Carew and Sutton, 2001; cited in Lukowiak et al., 2006a), the effect of the complex molecular cascades activated during regeneration does not abolish the molecular processes necessary for memory consolidation or maintenance.

Table 3 Both macromolecular synthesis and the presence of the soma of RPeD1 (a CPG neuron of the respiratory network) are required for a variety of LTM-related processes after operant conditioning in *Lymnaea*

Memory-related process	*Protein/RNA synthesis required*	*RPeD1 soma required*
Consolidation of LTM	Yes	Yes
Reconsolidation of LTM	Yes	Yes
Forgetting of LTM	Yes	Yes
Extinction of LTM	Yes	Yes
Boosting ITM into LTM	Yes	Yes

Data sources: Scheibenstock A, Krygier D, Haque Z, Syed N, and Lukowiak K (2002) The soma of RPeD1 must be present for long-term memory formation of associative learning in *Lymnaea*. *J. Neurophysiol.* 88: 1584–1591; Sangha S, Morrow R, Smyth K, Cooke R, and Lukowiak K (2003a) Cooling blocks ITM and LTM formation and preserves memory. *Neurobiol. Learn. Mem.* 80: 130–139; Sangha S, Scheibenstock A, and Lukowiak K (2003b) Reconsolidation of a long-term memory in *Lymnaea* requires new protein and RNA synthesis and the soma of Right Pedal Dorsal 1. *J. Neurosci.* 23:8034–8040; Sangha S, Schiebenstock A, Mccomb C, and Lukowiak K (2003c) Intermediate and long-term memories of associative learning are differentially affected by transcription versus translation blockers in *Lymnaea*. *J. Exp. Biol.* 206: 1605–1613; Sangha S, Schiebenstock A, Morrow R, and Lukowiak K (2003d) Extinction requires new RNA and protein synthesis and the soma of the cell right pedal dorsal 1 in *Lymnaea stagnalis*. *J. Neurosci.* 23: 9842–9851; Sangha S, Schiebenstock A, Martens K, Varshney N, Cooke R, and Lukowiak K (2005) Impairing forgetting by preventing new learning and memory. *Behav. Neurosci.* 119: 787–796; Parvez K, Stewart O, Sangha S, and Lukowiak K (2005) Boosting intermediate-term into long-term memory. *J. Exp. Biol.* 208: 1525–1536; Lowe MR and Spencer GE (2006) Perturbation of the activity of a single identified neuron affects long-term memory formation in a molluscan semi-intact preparation. *J. Exp. Biol* 209: 711–721.

4.09.3 Conclusions

Studies based on the associative conditioning paradigms described in this article have yielded valuable information on a variety of general and specific molecular mechanisms contributing to memory consolidation, reconsolidation, forgetting, and extinction. Reconsolidation and extinction in operant conditioning have been shown to have similar general molecular requirements to memory consolidation. Memory consolidation after classical conditioning and reconsolidation after retrieval also share the same general molecular requirement (synthesis of new proteins), but the requirement for specific signaling molecules (such as PKA) for reconsolidation depends on how recent or remote the consolidated memory is relative to the time of training.

Studies of LTM in classical conditioning have emphasized the importance of regulation of gene expression by transcription factors such as CREB and C/EBP and the role of the PKA and MAPK signaling pathways, with the most detailed information gained from experiments using the single-trial classical conditioning paradigm. The importance of these molecular pathways in *Lymnaea* provides further evidence for the generality of these highly conserved mechanisms in learning, both across phylogenetic groups and across learning paradigms (nonassociative or associative, single- or multi-trial,

aversive or reward, operant or classical). NO has been shown to be important for memory consolidation in classical reward conditioning, and this appears to involve regulation of nNOS gene expression in a specific modulatory cell type, the CGCs. Work using *Lymnaea* as an experimental model has thus both provided important insights into the molecular mechanisms of associative learning and memory at the behavioral level and linked these mechanisms to learning-induced cellular and molecular changes in single identified neurons, hallmarks of a successful top-down approach to the study of the molecular mechanisms of associative memory.

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Dedicated to the memory of my father.

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4.10 Cellular Mechanisms of Associative Learning in *Aplysia*

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4.10.1 *Aplysia* Classical Conditioning and Operant Conditioning

The simple nervous system and the relatively large identifiable neurons of the marine mollusk *Aplysia* provide a useful model system to examine the cellular and molecular mechanisms of the two major forms of associative learning, classical conditioning and operant (instrumental) conditioning. The ability to associate a predictive stimulus with a subsequent salient event (i.e., classical conditioning) and the ability to associate an expressed behavior with the consequences (i.e., operant conditioning) allow for a predictive understanding of a changing environment. Although operationally distinct, there has been considerable debate whether at some fundamental level classical and operant conditioning are mechanistically distinct or similar (e.g., Rescorla and Solomon, 1967; Gormezano and Tait, 1976; Dayan and Balleine, 2002). Studies utilizing the defensive withdrawal reflexes of *Aplysia* have provided much information on the mechanisms underlying classical conditioning. Recent studies utilizing the feeding behavior of *Aplysia* are providing for a comparative analysis of the mechanisms underlying classical and operant conditioning, using the same behavior and studying the same neuron. This comparative analysis can help to resolve the issue of whether similar or different mechanisms underlie these two forms of associative learning.

4.10.2 Classical Conditioning

4.10.2.1 Behavioral Studies

The initial studies of classical conditioning in *Aplysia* focused on defensive reflex behaviors and used aversive conditioning procedures. A tactile or electrical stimulus delivered to the siphon of the animal resulted in a reflex withdrawal of the gill and siphon, a reaction which presumably protects sensitive structures from harmful stimuli. Aversive classical conditioning in this species can be demonstrated by presenting a conditioned stimulus (CS), a brief, weak tactile stimulus to the siphon which produced a small siphon withdrawal, and an unconditioned stimulus (US), a short-duration noxious electric shock to the tail which produced a large withdrawal of the siphon (the unconditioned response, UR). After repeated pairings of the CS and US, the CS alone produced a large siphon withdrawal (the conditioned response, CR). This withdrawal was enhanced beyond that produced by the US alone (sensitization control) or unpaired or random presentations of the CS or the US (Carew et al., 1981), and this conditioning persisted for as long as 4 days. Carew et al. (1983) also found that this reflex exhibited differential classical conditioning. CS (tactile stimulation) were delivered to either the siphon or to the mantle region. One CS (the CS^+) is paired with the US (electric shock to the tail) and the other is explicitly unpaired (the CS^-). After conditioning, the CS^+ produced a greater withdrawal than the CS^-.

In addition to the classical conditioning of defensive reflexes, classical conditioning can also be applied to feeding behavior. *Aplysia* feed by protracting a toothed structure called the radula into contact with seaweed. The radula grasps seaweed by closing and retracting, which results in the ingestion of the seaweed. Inedible objects can be rejected if the radula protracts while closed (grasping the object) and then opens as it retracts to release the object. Thus, the timing of radula closure determines which behavior will occur. Feeding behavior can be classically conditioned with an appetitive protocol (Colwill et al., 1997; Lechner et al., 2000a). This appetitive protocol (Lechner et al., 2000a) consisted of tactile stimulation of the lips with a fine-tipped paint brush (CS), and the US was a small piece of seaweed, which the animals were allowed to eat. The animals were trained by repeatedly pairing the CS and the US. After training, presentation of the CS elicited an increase in ingestive behavior (CR).

4.10.2.2 Neural Mechanisms of Aversive Classical Conditioning in *Aplysia*

A cellular mechanism called activity-dependent neuromodulation contributes to associative learning in *Aplysia* (Hawkins et al., 1983; Walters and Byrne, 1983; Antonov et al., 2001). A general cellular scheme of activity-dependent neuromodulation is illustrated in **Figure 1**. Two sensory neurons (SN1 and SN2) constitute the pathways for the conditioned stimuli (CS^+ and CS^-) and make weak subthreshold connections to a motor neuron. Delivering a reinforcing stimulus or US alone has two effects. First, the US activates the motor neuron and produces the UR. Second, the US activates a diffuse modulatory system that nonspecifically enhances transmitter release from all the sensory neurons. This nonspecific enhancement contributes to sensitization. Temporal specificity, which is characteristic of associative learning, occurs when there is pairing of the CS (spike activity in SN1) with the US, which causes a selective amplification of the modulatory effects in SN1. Unpaired activity does not amplify the effects of the US in SN2. The amplification of the modulatory effects in SN1 leads to an enhancement of the ability of SN1 to activate the motor neuron and produce the CR.

A reduced preparation for the siphon-withdrawal reflex was developed that consists of the isolated tail, siphon, and central nervous system (CNS) of the animal (Antonov et al., 2001). A classical conditioning

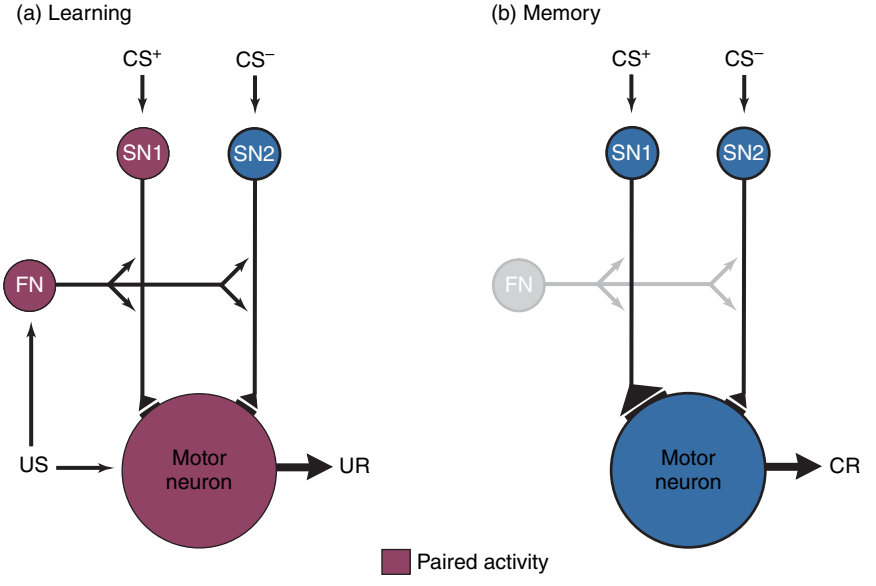

Figure 1 General model of activity-dependent neuromodulation. (a) Learning. A motivationally potent reinforcing stimulus (US) activates a motor neuron to produce the unconditioned response (UR) and a facilitatory neuron (FN) or modulatory system that regulates the strength of the connection between sensory neurons (SN1 and SN2) and the motor neuron. Increased spike activity in one sensory neuron (SN1) immediately before the modulatory signal amplifies the degree and duration of the modulatory effects, perhaps through the Ca^{+2} sensitivity of the modulatory evoked second messenger, with contributions from the postsynaptic neuron. The unpaired sensory neuron (SN2) does not show an amplification of the modulatory effects. (b) Memory: The amplified modulatory effects cause increases in transmitter release and/or excitability of the paired neuron, which in turn strengthens the functional connection between the paired sensory neuron (SN1) and the motor neuron. The associative enhancement of synaptic strength represents the conditioned response (CR).

training protocol was performed with tactile stimulation of the siphon as the CS and an electric shock to the tail as the US. Paired training significantly increased the amplitude of the siphon withdrawal, indicating successful conditioning. The classical conditioning protocol also produced a pairing-specific increase in the strength of the sensorimotor neuron synapse.

Experimental analyses of sensitization of defensive reflexes in *Aplysia* have shown that the neuromodulator released by the reinforcing stimulus, which is believed to be serotonin, activates the enzyme adenylyl cyclase in the sensory neuron. The activation of adenylyl cyclase increases the synthesis of the second messenger cyclic adenosine monophosphate (cAMP), which activates the cAMP-dependent protein kinase, and the subsequent protein phosphorylation leads to a modulation in several properties of the sensory neurons. These changes include modulation of membrane conductances and other processes which facilitate synaptic transmission. This facilitation results in the increased activation of the motor neuron and the sensitization of the reflex. The pairing specificity of the associative conditioning is at least partly due to an increase in the level of cAMP beyond that produced by serotonin alone (Ocorr et al., 1985; Abrams and Kandel, 1988). The influx of Ca^{+2} associated with the CS (spike activity) amplifies the US-mediated modulatory effect by interacting with a Ca^{+2}-sensitive component of the adenylyl cyclase (Abrams and Kandel, 1988). A critical role for Ca^{+2}-stimulated cyclase is also suggested by studies of *Drosophila* showing that the adenylyl cyclase of a mutant deficient in associative learning exhibits a loss of Ca^{+2}/calmodulin sensitivity.

The postsynaptic cell (i.e., motor neuron) also contributes to the plasticity of the synapse (Murphy and Glanzman, 1997; Bao et al., 1998). The postsynaptic membrane of the motor neuron contains *N*-methyl-D-aspartate (NMDA)-like receptors. If these receptors are blocked, then the associative modification of the synapse is disrupted. NMDA receptors require concurrent delivery of glutamate and depolarization in order to allow the entry of calcium. Activity in the sensory neuron (CS) provides the glutamate, and the US depolarizes the cell. The subsequent increase in intracellular Ca^{+2} may release a retrograde signal from the postsynaptic cell to the presynaptic terminal. This retrograde signal would then act to further enhance the cAMP cascade in the sensory neuron. Presynaptically blocking protein kinase A (PKA) by injecting a peptide inhibitor into the sensory neuron, or postsynaptically blocking Ca^{+2} by injecting BAPTA (1,2-bis-(o-aminophenoxy)-ethane-N,N,N′,N′-tetraacetic acid) into the motor neuron, also blocked the pairing-specific strengthening of the sensorimotor neuron synapse (Antonov et al., 2003) using the simplified preparation for classical conditioning mentioned earlier. The plasticity in the sensorimotor neuron synapse can be blocked by an injection to the postsynaptic motor neuron alone, suggesting that a retrograde signal is an integral part of the process.

This mechanism for associative learning appears to be an elaboration of a process already in place that mediates sensitization, which is a simpler form of learning (*See* Chapters 4.02, 4.03 for a review of the mechanisms of sensitization). This finding raises the interesting possibility that even more complex forms of learning may use simpler forms as building blocks, an idea that has been suggested by psychologists for many years but has only recently become testable at the cellular level.

4.10.2.3 Neural Mechanisms of Appetitive Classical Conditioning in *Aplysia*

The feeding system of *Aplysia* has many advantages. For example, much of the cellular circuitry controlling feeding behavior has been identified, so it is possible to study neurons with known behavioral significance. The *in vivo* training protocol for classical conditioning (Lechner et al., 2000a) has been used to examine correlates of classical conditioning in several neurons that are important for the expression of feeding behavior. Classical conditioning led to the pairing-specific strengthening of the CS-evoked excitatory synaptic input to pattern-initiating neuron B31/32, although no changes were observed in the membrane properties, such as input resistance or the threshold for bursting (Lechner et al., 2000b). If B31/32 receives a greater excitatory input from the CS, then this neuron would be more likely to initiate a feeding motor pattern, and the feeding motor patterns evoked by the CS would be mostly ingestive (Lechner et al., 2000b). However, B31/32 promotes the initiation of feeding motor patterns without defining which type of pattern (i.e., ingestive or egestive) is expressed (Hurwitz et al., 1996). Thus, the pairing-specific increase in excitatory input to B31/32 alone cannot account for the specific increase in the ingestive behavior that is observed following *in vivo* classical conditioning (Lechner et al., 2000a).

Correlates of classical conditioning were measured in neuron B51 following the *in vivo* classical

conditioning protocol (Lorenzetti et al., 2006). Neuron B51 is pivotal for the expression of ingestive motor patterns, and B51 exhibits a characteristic all-or-nothing sustained level of activity (i.e., plateau potential; Plummer and Kirk, 1990) during ingestive motor patterns (Nargeot et al., 1999a). Classical conditioning induced a significant pairing-specific increase in the CS-evoked excitatory synaptic input to B51, as well as an increase in the number of CS-elicited plateau potentials. The pairing-specific strengthening of the excitatory synaptic input can increase the likelihood that B51 is recruited into a motor pattern, which can contribute to the pairing-specific increase in the number of CS-evoked plateau potentials. An increase in the recruitment of B51 should bias the feeding central pattern generator (CPG) toward the expression of ingestive motor patterns (Nargeot et al., 1999b). Thus, the effects produced by classical conditioning appear to be distributed among elements of the feeding CPG such as B31/32 and B51, with the pairing-specific plasticity in B31/32 contributing to the increased number of motor patterns, while the pairing-specific plasticity in B51 is biasing the nature of the motor patterns toward ingestion.

In addition to the pairing-specific enhancement of the CS-evoked excitatory synaptic input to B51, classical conditioning can alter the intrinsic biophysical properties of B51. Classical conditioning raised the threshold for eliciting a burst (i.e., plateau potential) in B51 without affecting either the resting membrane potential or input resistance. This result adds to an increasing body of evidence that, in addition to changes in synaptic efficacy, changes in the intrinsic neuronal excitability also contribute to the storage of memory (for reviews see Daoudal and Debanne, 2003; Zhang and Linden, 2003; *See also* Chapter 4.40). The pairing-specific decrease in the excitability would make B51 less likely to be active, whereas the increase in the excitatory synaptic input would facilitate the recruitment of the neuron. However, the training produced more CS-evoked plateau potentials in B51 in the paired group as compared to the unpaired group. Thus, the factors that enhance the recruitment of B51 overpower the diminished excitability and bias B51 toward producing more plateau potentials, resulting in a greater number of ingestive motor patterns. This pairing-specific decrease in the excitability of B51 could be an adaptive mechanism to help shape the CS specificity produced by classical conditioning.

An *in vitro* analog of classical conditioning has been developed for the feeding system of *Aplysia* (Mozzachiodi et al., 2003). This preparation used isolated ganglia from naive animals. Stimulation of the anterior tentacular nerve was chosen as the analog of the CS. Specifically, the fourth branch of the anterior tentacular nerve was used because this branch innervates the lip region, which is the site of stimulation for the *in vivo* CS. Stimulation of the esophageal nerve was used as the analog of the US. The ganglia were trained by repeatedly pairing the CS with the US. After training, the ganglia produced more motor patterns (analogs of the CR) after CS delivery, indicating that conditioning was successful in this reduced analog. The *in vitro* analog of classical conditioning was then performed while monitoring the membrane properties and CS-elicited synaptic input to B31/32, CBI-2 (Mozzachiodi et al., 2003), and B51 (Lorenzetti et al, 2006). The *in vitro* training protocol produced an increase in the CS-elicited synaptic input to both B31/32 and to B51, an increase in the burst threshold of B51, and an increased number of CS-elicited plateau potentials in B51, similar to what was observed following *in vivo* training. In addition, *in vitro* classical conditioning led to a pairing-specific enhancement of the CS-elicited synaptic input to CBI-2 (Mozzachiodi et al., 2003), which is one of the command-like interneurons controlling the activity of the feeding CPG (Rosen et al., 1991). Synergism among these effects can help produce the pairing-specific increase in the number of CS-evoked bites observed following *in vivo* classical conditioning.

These results provide further support that memory can be distributed among multiple sites of plasticity, similar to what has been observed with other animal model systems. In *Lymnaea*, appetitive classical conditioning strengthened the CS-evoked excitatory synaptic drive to feeding motor neurons (Staras et al., 1999) and induced a persistent depolarization in the modulatory neuron CV1a (Jones et al., 2003). In *Aplysia*, empirical studies (e.g., Trudeau and Castellucci, 1993; for review see also Cleary et al., 1995) and theoretical work (White et al., 1993; Lieb and Frost, 1997) on the neural circuits controlling defensive withdrawal reflexes emphasize the role of both sensory neurons and interneurons as sites of learning-related plasticity underlying behavioral sensitization. In vertebrates, the plasticity produced by delay classical conditioning of the eyelid response is distributed between the cerebellar cortex and the deep cerebellar nuclei (for reviews see Raymond et al., 1996; Kim and Thompson, 1997). Trace

classical conditioning of the same reflex can also involve the hippocampus (for review see Christian and Thompson, 2003). Therefore, studies in both invertebrate and vertebrate neural circuits support the concept that multiple sites of plasticity contribute to the storage of information for associative and nonassociative forms of memory.

4.10.3 Operant Conditioning

4.10.3.1 Behavioral Studies

Feeding behavior in *Aplysia* can be modified by pairing feeding with an aversive stimulus. If food is wrapped in a tough plastic net, *Aplysia* bite and attempt to swallow the food. However, netted food cannot be swallowed, and so it is rejected. The inability to consume the food appeared to be an aversive stimulus that modified the feeding behavior, because the trained animals no longer attempted to bite the netted food (Susswein et al., 1986).

Feeding behavior can also be operantly conditioned with an appetitive stimulus (Brembs et al., 2002). The reinforcement signal for the *in vivo* training protocol was a brief shock to the esophageal nerve. The esophageal nerve is believed to be part of the pathway mediating food reward because bursts of activity in this nerve occur when the animal successfully ingests food (Brembs et al., 2002). In addition, lesions to this nerve blocked *in vivo* appetitive classical conditioning (Lechner et al., 2000a). Also, the *in vitro* analog of classical conditioning discussed earlier successfully increased the number of CS-elicited motor patterns when esophageal nerve shock was used as the US (Mozzachiodi et al., 2003). In the operant conditioning paradigm, the contingent reinforcement of biting behavior by a shock to the esophageal nerve produced an increase in the frequency of biting, when measured both immediately after training and 24 h after training, as compared to animals trained with a yoke-control procedure (Brembs et al., 2002).

4.10.3.2 Neural Mechanisms of Appetitive Operant Conditioning in *Aplysia*

We have previously discussed B51 (Plummer and Kirk, 1990) as being implicated in the expression of ingestive behavior and as a correlate of classical conditioning. B51 is active predominantly during the retraction phase (Nargeot et al., 1997), and when B51 is recruited into a pattern, it recruits radula closure motor neurons (see **Figure 2**).

The *in vivo* training protocol for operant conditioning was used to examine correlates in neuron B51 (Brembs et al., 2002). Operant conditioning led to changes in the membrane properties of B51. The input resistance was increased and the threshold for bursting was decreased. These changes increase the likelihood of B51 activation and thereby contribute to the conditioned increase in the ingestive response.

An *in vitro* analog of operant conditioning was developed using only the isolated buccal ganglia, which is responsible for generating the motor patterns involved in feeding (Nargeot et al., 1999a). These motor patterns can either be ingestive or egestive. In this analog of operant conditioning, motor patterns corresponding to ingestion were used as the analog of the behavior. The ingestive motor pattern was selectively reinforced by contingently shocking the esophageal nerve, which was the analog of the reinforcement. The conditioning procedure resulted in an increase in the likelihood of ingestive patterns being produced (Nargeot et al., 1999a). The contingent reinforcement also resulted in the modulation of the membrane properties of neuron B51 (Nargeot et al., 1999a). The input resistance increased and the threshold for eliciting a burst decreased in a manner similar to the *in vivo* operant conditioning protocol. These changes in the membrane properties of B51 make the cell more excitable and more likely to be recruited into a motor pattern, thus helping to explain the increase in the frequency of expression of the ingestive motor patterns following the *in vitro* analog of operant conditioning. Furthermore, these results for the membrane properties of B51 can be replicated when induced electrical activity in B51 was substituted for the analog of the behavior, instead of an ingestive motor pattern, which was then contingently reinforced with a shock to the esophageal nerve (Nargeot et al., 1999b).

The esophageal nerve, which is used to send both a reinforcement signal with operant conditioning and a US signal with classical conditioning, contains dopaminergic processes (Kabotyanski et al., 1998). Esophageal nerve stimulation produced a postsynaptic potential (PSP) in B51 and this PSP was blocked by the dopamine antagonist ergonovine (Nargeot et al., 1999c). This dopamine antagonist also blocked the acquisition of the associative changes induced by *in vitro* analogs of both operant conditioning (Nargeot et al., 1999c) and classical conditioning (Reyes et al., 2005).

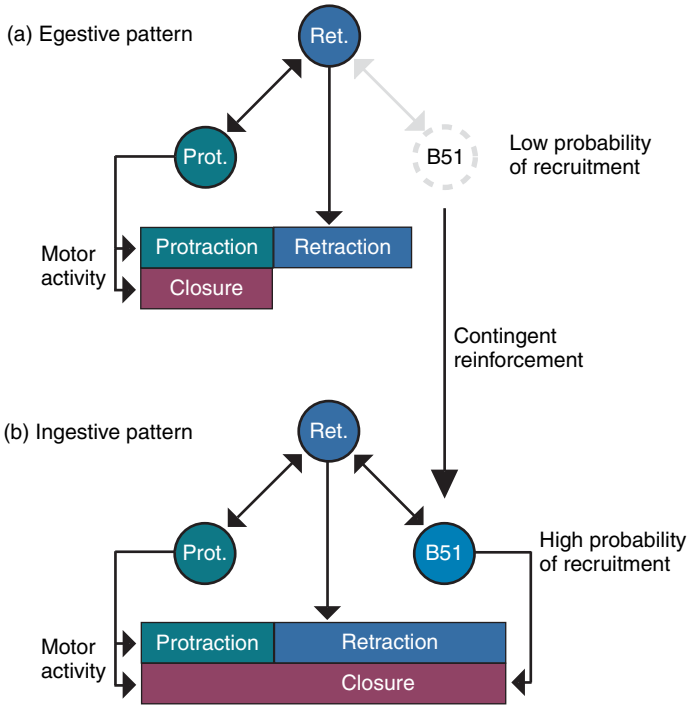

Figure 2 Model of operant conditioning of feeding in *Aplysia*. The cellular network that mediates feeding behavior is represented by the elements in circles. Motor activity comprising two basic feeding patterns is depicted below. (a) At first, the radula protraction-generating element (Prot.) is active, followed by the radula retraction element (Ret.). In the naive state, neuron B51 has a low probability for recruitment and thus does not take part in the feeding motor program. Radula closure occurs during the protraction phase. Consequently, the pattern elicited is egestive. (b) Neuron B51 now has a higher probability for recruitment following contingent reinforcement. B51 is now active during the motor program, leading to radula closure occurring primarily during the retraction phase. Thus, the pattern elicited will now be ingestive.

The analog was further reduced by removing neuron B51 from the ganglia and placing it in culture (Lorenzetti et al., 2000; Brembs et al., 2002). This single, isolated neuron was conditioned by contingently reinforcing induced electrical activity (the analog of behavior) with a direct and temporally discrete application of dopamine (the analog of reinforcement). After conditioning, the input resistance of B51 increased and the threshold for bursting decreased, similar to the *in vivo* and *in vitro* analogs of operant conditioning described above. The membrane properties of B51 were modulated such that the cell was more likely to be active in the future. Such a highly reduced preparation is a promising candidate to study the mechanisms of dopamine-mediated reward and the conditioned expression of behavior at the level of the intracellular signaling cascades.

The operant conditioning of the feeding behavior of *Aplysia* increased the expression of the ingestive responses. The excitability of B51 was also increased by the operant protocol, accounting for the bias in the output of the CPG toward ingestion. However, increasing the excitability of B51 is not likely to increase the total number of patterns expressed. Thus, another site of plasticity is probably induced by the operant protocol. A likely candidate for this additional site of plasticity is in a cell or synapse that is responsible for pattern initiation (e.g., B31). Though these possible sites of plasticity have not yet been explored, it seems likely that both operant and classical conditioning lead to the distribution of memory at multiple sites within the neural circuit.

4.10.4 Conclusions

The feeding system of *Aplysia,* with its relatively simple circuitry, provides a model system for a systematic comparison of the mechanisms underlying classical and operant conditioning. Some interesting similarities as well as differences are beginning to emerge (**Table 1**). One similarity is the nature of the reinforcement pathway and its neurotransmitter. The esophageal nerve mediates the reinforcement signal

Table 1 Comparative analysis between appetitive classical and operant conditioning of feeding in *Aplysia*

	Classical conditioning	*Operant conditioning*
Change in the number of bites	Increase	Increase
Pathway mediating US/reinforcement	Esophageal nerve	Esophageal nerve
Transmitter mediating US/reinforcement	Dopamine	Dopamine
B51 plateau potentials	Increase	Increase
B51 resting membrane potential	No change	No change
B51 input resistance	No change	Increase
B51 burst threshold	Increase	Decrease

for appetitive operant conditioning (Nargeot et al., 1997) and the US pathway for appetitive classical conditioning (Lechner et al., 2000a; Mozzachiodi et al., 2003). Also, this pathway appears to use dopamine as a transmitter, which is consistent with the long-held view that dopamine can mediate the US/reinforcement for appetitive forms of both classical and operant conditioning in both vertebrates and invertebrates (for review see Schultz, 2002).

Appetitive classical conditioning of feeding behavior in *Aplysia* produced two major changes in neuron B51. The synaptic input along the CS pathway into B51 was increased. This increase in the CS pathway suggests that the conditioned sensory pathway receives a preferential boost, while the other sensory pathways could remain unchanged. The second change seen with B51 was an increase in the burst threshold. This change acts on the level of the pattern generation machinery and makes the expression of ingestive feeding responses less likely. Thus, the animal would be less likely to feed unless the CS was present. Identical changes in the properties of B51 were expressed in intact animals trained with a classical conditioning protocol and in an *in vitro* analog of classical conditioning using isolated ganglia (Lorenzetti et al., 2006).

Appetitive operant conditioning of feeding behavior in *Aplysia* also produced two major changes in neuron B51. Both changes were made to the intrinsic membrane properties of the cell. First, the input resistance was increased. Second, the burst threshold was decreased. Both of these changes act in the same direction and would make B51 more likely to be active, thus accounting for the increased expression of the behavior following reinforcement. Identical changes in the membrane properties of B51 were expressed in intact animals trained with an operant conditioning protocol (Brembs et al., 2002), in an *in vitro* analog of operant conditioning using isolated ganglia (Nargeot et al., 1999a,b), and in a single-cell analog consisting of neuron B51 in culture (Brembs et al., 2002).

B51 is a cellular locus for the changes induced by both operant and classical conditioning. No pairing-specific changes in the input resistance were observed following classical conditioning, which was in contrast to the contingent-dependent increase in this parameter measured in B51 following both *in vivo* and *in vitro* operant conditioning. Both operant and classical conditioning modified the threshold level for activation of neuron B51, but in opposite directions, revealing key differences in the cellular mechanisms underlying these two forms of associative learning and suggesting a difference at the molecular level. B51 appears to be a coincidence detector for both the CS–US association (classical conditioning) and the contingency between ingestive behavior and reinforcement (operant conditioning). Because dopamine likely mediates both the US and the reinforcement, a key problem is the elucidation of the mechanisms that lead to the induction of the opposite effects on the burst threshold. One possibility is that the coincidence detector for classical conditioning involves an association between a transmitter released by the CS and dopamine, whereas for operant conditioning it involves an association between the cellular effects of B51 burst activity and dopamine.

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4.11 Neural and Molecular Mechanisms of Fear Memory

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4.11.1 An Overview of Pavlovian Fear Conditioning

Classical or Pavlovian fear conditioning has long been a tool of behavioral psychology to study simple forms of associative learning in the mammal. In this paradigm, an animal (or human) learns to fear an initially emotionally neutral stimulus (the *conditioned stimulus,* CS) that acquires aversive properties after being paired with a noxious stimulus (the *unconditioned stimulus,* US). First used by J. B. Watson and his colleague Rosalie Rayner in the now infamous studies of "Little Albert" (Watson and Rayner, 2000), fear conditioning is now most widely studied in rodents, where a discrete cue (such as a tone, light, or odor; CS) is paired with a brief electric shock to the feet (US). Before conditioning, the CS does not elicit fearful behavior. After as little as one CS-US pairing, however, the animal begins to exhibit a range of *conditioned responses* (CRs), both to the tone CS and to the context in which conditioning occurs (e.g., the conditioning chamber). In rats, these CRs include 'freezing' or immobility (the rat's species-typical behavioral response to a threatening stimulus), autonomic and endocrine alterations (such as changes in heart rate and blood pressure, defecation, and increased levels of circulating stress hormones), and potentiation of reflexes like the acoustic startle response (Blanchard and Blanchard, 1969; Kapp et al., 1979; LeDoux et al., 1988; Roozendaal et al., 1991; Davis, 1997).

4.11.2 The Amygdala and Fear Conditioning

4.11.2.1 The Neuroanatomy of Fear

There are few associative learning paradigms that have been better characterized at the neuroanatomical level than Pavlovian fear conditioning (see **Figure 1**). This is particularly true for the 'auditory fear conditioning' paradigm, where an animal learns to fear a tone (CS) that is paired with foot shock (US). In this review, we will therefore emphasize the findings from the auditory fear conditioning literature, although similar mechanisms have also been proposed for conditioning to visual stimuli (Davis, 1992, 1997).

Auditory fear conditioning involves transmission of auditory CS and somatosensory US information to the lateral nucleus of the amygdala (LA), an area that lesion and functional inactivation studies have shown to be critical for learning (LeDoux et al., 1990; Helmstetter and Bellgowan, 1994; Campeau and Davis, 1995; Muller et al., 1997; Wilensky et al., 2000). Anatomical tract tracing studies have shown that cells in the LA receive direct glutamatergic projections from areas of the auditory thalamus and cortex, specifically from the medial division of the medial geniculate body and the posterior intralaminar nucleus (MGm/PIN) and cortical area TE3, respectively (LeDoux et al., 1985; LeDoux and

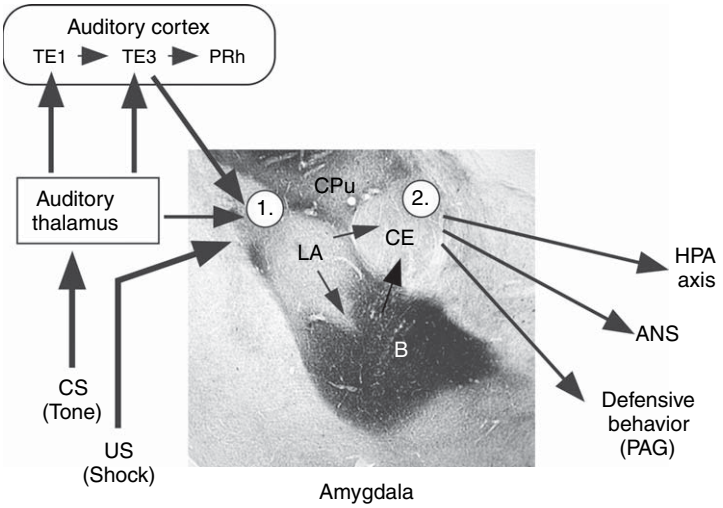

Figure 1 Anatomy of the fear system. (1) Auditory fear conditioning involves the transmission of CS sensory information from areas of the auditory thalamus and cortex to the lateral amygdala (LA), where it can converge with incoming somatosensory information from the foot shock US. It is in the LA that alterations in synaptic transmission are thought to encode key aspects of the learning. (2) During fear expression, the LA engages the central nucleus of the amygdala (CE), which projects widely to many areas of the forebrain and brainstem that control the expression of fear CRs, including freezing, hypothalamic-pituitary-adrenal (HPA) axis activation, and alterations in cardiovascular activity. CPu, caudate/putamen; B, basal nucleus of amygdala; ANS, autonomic nervous system; PRh, perirhinal cortex; PAG, periaqueductal gray.

Farb, 1991; Bordi and LeDoux, 1992; Romanski and LeDoux, 1993; McDonald, 1998; Doron and LeDoux, 1999). Neurophysiological evidence has indicated that inputs from each of these auditory areas synapse onto single neurons in the LA (Li et al., 1996), where they converge with inputs from the somatosensory US (Romanski et al., 1993). Individual cells in the LA are thus well suited to integrate CS and US information during fear conditioning, and it is here, as we will see, that alterations in synaptic transmission are thought to encode key aspects of the memory.

Thalamic and cortical inputs to the LA, while both capable of mediating fear learning (Romanski and LeDoux, 1992a), are believed to carry different types of information to the LA. The thalamic route (often called the 'low road') is believed to be critical for rapidly transmitting crude aspects of the CS to the LA, while the cortical route (known as the 'high road') is believed to carry highly refined information to the amygdala (LeDoux, 2000). Interestingly, while lesions of the MGm/PIN impair auditory fear conditioning (LeDoux et al., 1984, 1986), lesions of the auditory cortex do not (LeDoux et al., 1984; Romanski and LeDoux, 1992b). Thus, the thalamic pathway between the MGm/PIN and the LA appears to be particularly important for auditory fear conditioning. This is not to say, however, that the cortical input to the LA is not involved. Indeed, when conditioning depends on the ability of the animal to make fine discriminations between different auditory CSs, or when the CS is a complex auditory cue such as an ultrasonic vocalization, then cortical regions appear to be required (Jarrell et al., 1987; Lindquist et al., 2004).

During retrieval or expression of a fear memory, the LA, both directly and by way of the adjacent basal nucleus of the amygdala, engages the central nucleus of the amygdala (CE). The CE has traditionally been thought of as the principal output nucleus of the fear learning system, projecting to areas of the forebrain, hypothalamus, and brainstem that control behavioral, endocrine, and autonomic CRs associated with fear learning (Blanchard and Blanchard, 1969; Kapp et al., 1979; LeDoux et al., 1988; Roozendaal et al., 1991; Davis, 1997). Projections from the CE to the midbrain periaqueductal gray, for example, have been shown to be particularly important for mediating behavioral and endocrine responses such as freezing and hypoalgesia (LeDoux et al., 1988; Helmstetter and Landeira-Fernandez, 1990; Helmstetter and Tershner, 1994; De Oca et al., 1998), while projections to the lateral hypothalamus have been implicated in the control of conditioned cardiovascular responses (Iwata et al., 1986; LeDoux et al., 1988). Importantly, while lesions of these individual areas can selectively impair expression of individual CRs, damage to the CE interferes with the expression of all fear CRs (LeDoux, 2000). Thus, the CE acts to coordinate the collection of hard-wired, and typically species-specific, responses that underlie defensive behavior.

4.11.2.2 Synaptic Plasticity in the Amygdala and Fear Conditioning

In addition to being the recipient of CS and US information, the LA is also thought to be a critical site of synaptic plasticity underlying fear learning (LeDoux, 2000; Blair et al., 2001; Maren, 2001). In support of this view, numerous studies have shown that individual cells in the dorsal regions of the LA (LAd) alter their neurophysiological response properties when CS and US are paired during fear conditioning (**Figures 2(a)** and **2(b)**). For example, LAd neurons that are initially weakly responsive to auditory input respond vigorously to the same input after fear conditioning (Quirk et al., 1995, 1997; Rogan et al., 1997; Maren, 2000; Repa et al., 2001; Blair et al., 2003). This change in the responsiveness of LAd cells that occurs as the result of training has contributed to the view that neural plasticity in the LA encodes key aspects of fear learning and memory storage (Fanselow and LeDoux, 1999; Blair et al., 2001; Maren, 2001; Schafe et al., 2001), a topic to which we will return in a later section.

In the next several sections, we will discuss the biochemical and molecular mechanisms that likely underlie plasticity and memory formation at LA synapses. We begin with a discussion of long-term potentiation (LTP), as it has been proposed that this type of synaptic plasticity is the most likely type of mechanism that underlies memory formation in the mammalian brain (Bliss and Collingridge, 1993; Malenka and Nicoll, 1999), including in the LA (Maren, 1999; Blair et al., 2001; Schafe et al., 2001).

4.11.3 LTP as a Mechanism of Fear Learning

4.11.3.1 Why is LTP Important?

The change in the responsiveness of LA cells during fear conditioning suggests that alterations in excitatory synaptic transmission in the LA might be critical for fear conditioning. Accordingly, many of the recent studies that have examined the biochemical

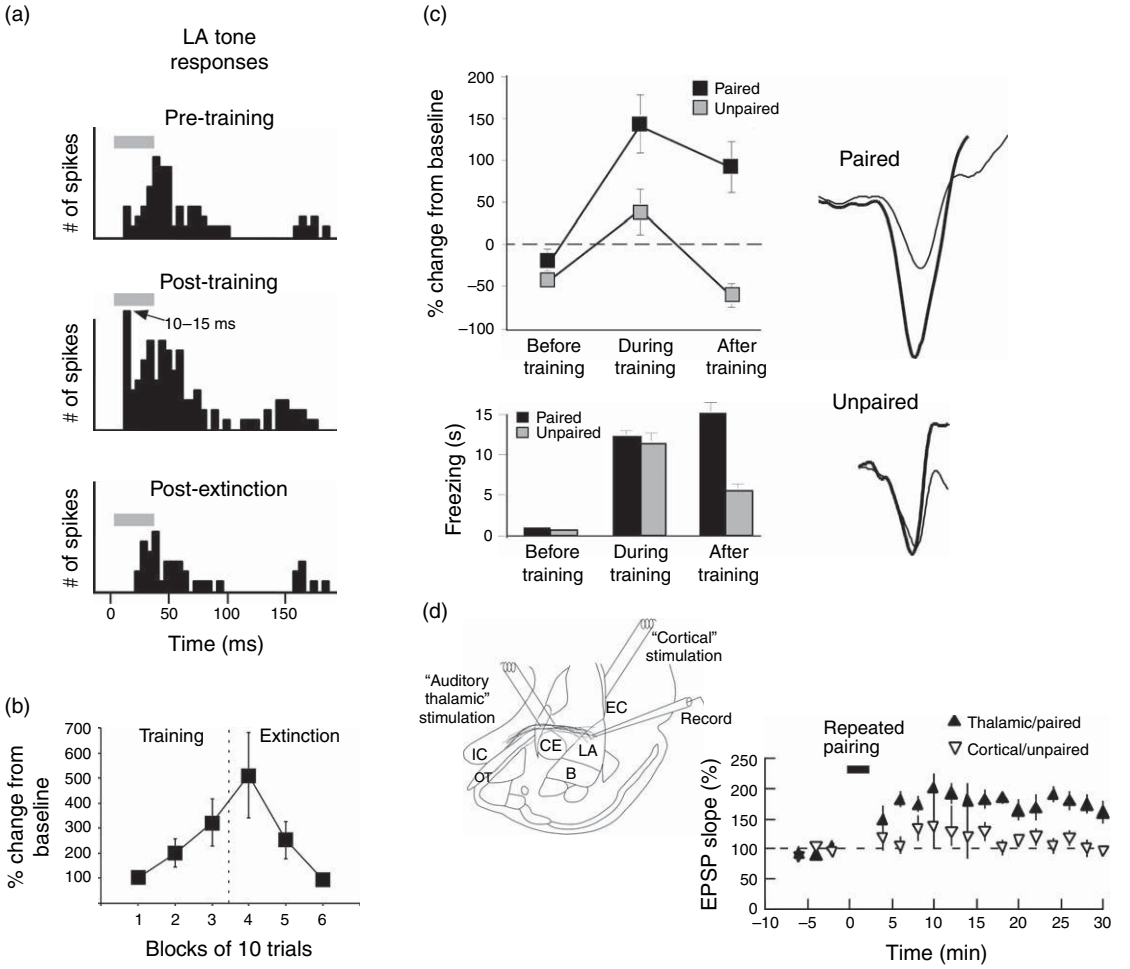

Figure 2 Synaptic plasticity in the lateral amygdala (LA) and fear conditioning. Pairing of CS and US during fear conditioning leads to changes in the responsiveness of LA cells to auditory stimuli, as measured by electrophysiological recording of single cells in the LA. (a) TOP: Prior to auditory fear conditioning, LA cells are weakly responsive to auditory stimuli. MIDDLE: Immediately after conditioning, the same cells respond vigorously, especially in the first few milliseconds of the tone (arrow). BOTTOM: Tone conditioning of LA cells decreases with extinction. In each figure, the onset of the tone stimulus is depicted by a gray bar. (b) Change in firing rate of LA cells over the course of training and extinction. The values represent averaged responses of 16 cells within the first few milliseconds of the tone stimulus and are expressed as percent change from preconditioning firing rates. (c) TOP: Fear conditioning leads to electrophysiological changes in the LA in a manner similar to long-term potentiation (LTP). The figure represents percent change in the slope of the auditory-evoked field potential in the LA before, during, and after conditioning in both paired and unpaired rats. BOTTOM: Freezing behavior across training and testing periods. Note that both paired and unpaired groups show equivalent freezing behavior during training, but only the paired group shows an enhanced neural response. Representative traces can be seen in the inset. (d) LEFT: Associative LTP is induced in the amygdala slice by pairing trains of presynaptic stimulation of fibers coming from the auditory thalamus with depolarization of LA cells. Stimulation of fibers coming from cortical areas serves as a control for input specificity. RIGHT: LTP induced by pairing as measured by the change in the slope of the excitatory postsynaptic potential (EPSP) over time. In this case, the thalamic pathway received paired stimulation, whereas the cortical pathway received unpaired stimulation (i.e., trains and depolarizations, but in a noncontingent manner). The black bar represents the duration of the pairing. IC, internal capsule; OT, optic tract; CE, central nucleus of amygdala; B, basal nucleus of amygdala; EC, external capsule.

basis of fear conditioning have drawn upon a larger literature that has focused on the biochemical events that underlie LTP, an activity-dependent form of synaptic plasticity that was initially discovered in the hippocampus (Bliss and Lømo, 1973). There are several good reasons behind this strategy, including the fact that LTP has been demonstrated in thalamic and cortical auditory input pathways to the

LA (Chapman et al., 1990; Clugnet and LeDoux, 1990; Rogan and LeDoux, 1995; Huang and Kandel, 1998; Weisskopf et al., 1999; Weisskopf and LeDoux, 1999), and that auditory fear conditioning itself has been shown to lead to neurophysiological changes in the LA that resemble artificial LTP induction (McKernan and Shinnick-Gallagher, 1997a; Rogan et al., 1997). Collectively, these findings provide strong support for the hypothesis that an LTP-like process in the LA may underlie fear conditioning (**Figures 2(c) and 2(d)**). This, in turn, suggests that fear memory acquisition and consolidation may share a common biochemical and molecular substrate with LTP.

4.11.3.2 The 'Consolidation' of LTP – E-LTP Versus L-LTP

There are several pharmacologically distinct forms of LTP, most of which have been identified in the hippocampus. One form critically involves the *N*-methyl-D-aspartate receptor (NMDAR), which is normally blocked by Mg^{2+}, but which can be opened following sufficient depolarization of the postsynaptic cell during LTP induction (Malenka and Nicoll, 1993). The other, less widely studied form involves the L-type voltage-gated calcium channel (L-VGCC). Other forms require a combination of both NMDARs and L-VGCCs (Grover and Teyler, 1990; Cavus and Teyler, 1996). Importantly, both NMDAR and L-VGCC-mediated forms of LTP have been discovered in the LA (Weisskopf et al., 1999; Bauer et al., 2002).

Regardless of how it is induced, the hallmark of each form of LTP is the entry of Ca^{2+} into the postsynaptic spine, which initiates a biochemical cascade of events that leads to strengthening of the synapse. Some of these biochemical cascades lead to a transient change in synaptic strength known as early LTP (E-LTP) that is independent of *de novo* RNA and protein synthesis. This type of LTP is thought to involve the activation of protein kinase signaling pathways near the postsynaptic density and the alteration in the conductance of number of a number of key synaptic proteins involved in glutamatergic signaling, including the NMDAR and the closely related alpha-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA) receptor (Soderling and Derkach, 2000). Other intracellular cascades lead to a more permanent alteration in cell excitability known as late LTP (L-LTP). Unlike E-LTP, L-LTP requires *de novo* RNA and protein synthesis and different classes of protein kinase signaling cascades that are thought to promote long-term synaptic plasticity by engaging activators of transcription in the nucleus (Nguyen et al., 1994; Nguyen and Kandel, 1996). Thus, the two forms of LTP have features in common with the traditional phases of memory consolidation. L-LTP is conceptually similar to long-term memory (LTM) formation, which is known to be dependent on *de novo* mRNA transcription and protein synthesis, while E-LTP is conceptually similar to short-term memory (STM), which is known to be short-lasting and independent of transcription and translation (Davis and Squire, 1984; Milner et al., 1998; Schafe et al., 2000). This pattern of findings suggests, in turn, that the consolidation process can be represented at the cellular level and understood through studies of LTP (Milner et al., 1998).

An exhaustive review of the biochemical mechanisms underlying LTP is beyond the scope of this chapter (for a more exhaustive review of this topic, see Milner et al., 1998). In the next several sections, however, we will review some of the key membrane-bound receptors and intracellular signaling pathways that have been most widely implicated in LTP and in memory formation of fear conditioning.

4.11.4 Biochemical Mechanisms of Fear Memory Formation and Consolidation

The fact that LTP is characterized by phases that differ as a function of the requirement for transcription and translation suggests that it is an excellent cellular model by which to study the biochemical mechanisms of memory consolidation. Accordingly, inspired by the success of studies that have defined the contribution of different cellular and molecular signaling cascades underlying both E-LTP and L-LTP, a number of recent studies have asked whether these same mechanisms might underlie short- and long-term formation of fear memories in the amygdala. In this section, we will summarize these findings, beginning with recent studies that have focused on glutamatergic signaling pathways and their contribution to fear acquisition and STM formation.

4.11.4.1 Short-Term Fear Memory Formation – Glutamatergic Signaling, CaMKII Activation, and AMPAR Trafficking in the Amygdala

Like E-LTP, STM is a short-lasting form of memory that does not require new protein or RNA synthesis (Milner et al., 1998). While no consistent time frame of STM has been defined in the literature, it is generally tested shortly after training, usually within 1 h. Further, deficits in STM formation are typically assumed to reflect deficits in memory acquisition, although it should be emphasized that acquisition and STM formation are likely subserved by distinct molecular mechanisms (Rodrigues et al., 2004b). In this section, we will examine how glutamatergic transmission, αCaMKII, and AMPA receptor (AMPAR) regulation and trafficking might contribute to fear memory acquisition and STM formation in the LA.

4.11.4.1.1 NMDA receptors

The NMDAR has a long history in the fear conditioning literature. Early pharmacological studies showed that blockade of NMDARs in the LA using the NMDAR antagonist D-2-amino-5-phosphonovaleric acid (APV) reliably impaired fear conditioning (Miserendino et al., 1990; Kim et al., 1991; Campeau et al., 1992), suggesting that an NMDAR-dependent form of synaptic plasticity was critical for fear learning. Later reports, however, indicated that infusion of APV into the LA also impaired the expression of previously acquired fear responses (Maren et al., 1996). These findings are consistent with neurophysiological evidence showing that NMDARs are involved, at least in part, in routine synaptic transmission in the LA (Weisskopf and LeDoux, 1999; Bauer et al., 2002). As such, it has been difficult to conclude unambiguously that NMDARs are required for fear acquisition independently of a role in routine synaptic transmission.

Several years ago, the role of NMDARs in fear conditioning was revisited by examining the effects of selective blockade of the NR2B subunit of the NMDA receptor in the LA. NMDARs are heteromeric complexes composed of several subunits, including the NR1 subunit, which is essential for channel function, as well as a range of NR2 subunits which regulate channel function (Monyer et al., 1992; Nakanishi, 1992). *In vitro* studies have shown that the NR1-NR2B complex exhibits longer excitatory postsynaptic potentials (EPSPs) than the NR1-NR2A complex (Monyer et al., 1992). This characteristic of NR2B-containing NMDARs is thought to provide a longer time window for coincidence detection, which is thought to be especially important during synaptic plasticity (Tsien, 2000). Indeed, recent molecular genetic studies have implicated the NR2B subunit in both synaptic plasticity and memory formation; overexpression of NR2B in the forebrain of mice results in enhanced LTP and memory formation for a variety of tasks, including fear conditioning (Tang et al., 1999).

In the amygdala, blockade of the NR2B by ifenprodil, a selective antagonist of the NR2B subunit of the NMDAR, dose-dependently impairs formation of both STM and LTM of fear conditioning (Rodrigues et al., 2001); that is, memory is impaired both at 1 h and 24 h after infusion and training (**Figures 3(a) and 3(b)**). In contrast, infusions of ifenprodil prior to testing at either time point have no effect on fear expression. These results suggest that ifenprodil lacks the nonspecific effects on routine transmission that are characteristic of the more global NMDAR antagonist APV. In support of this hypothesis, bath application of ifenprodil to amygdala slices also impairs LTP at thalamic inputs to LA neurons but has no effect on routine synaptic transmission (Bauer et al., 2002; **Figure 3(c)**). These results are also consistent with those of a recent study that examined the effects of APV on acquisition of fear-potentiated startle (Walker and Davis, 2000), showing that APV can, under certain circumstances, have selective effects on plasticity. Collectively, findings suggest that the NMDA receptor in the amygdala plays an essential role in both the acquisition and STM of conditioned fear.

4.11.4.1.2 Ca^{2+}/calmodulin-dependent protein kinase

One of the immediate downstream consequences of NMDAR-mediated activity-dependent increases in Ca^{2+} at the time of LTP induction is the activation of Ca^{2+}/calmodulin (CaM)-dependent protein kinase II (CaMKII). The alpha isoform of CaMKII has been widely implicated in synaptic plasticity and memory formation (Fukunaga and Miyamoto, 1999; Soderling and Derkach, 2000; Fink and Meyer, 2002; Lisman et al., 2002), in part for its ability to undergo a rapid 'autophosphorylation,' a state in which this enzyme can remain active in the absence of further Ca^{2+} entry (Soderling and Derkach, 2000). In this state, αCaMKII can phosphorylate and transiently enhance the conductance of a variety of membrane proteins, including AMPARs (Barria et al., 1997;

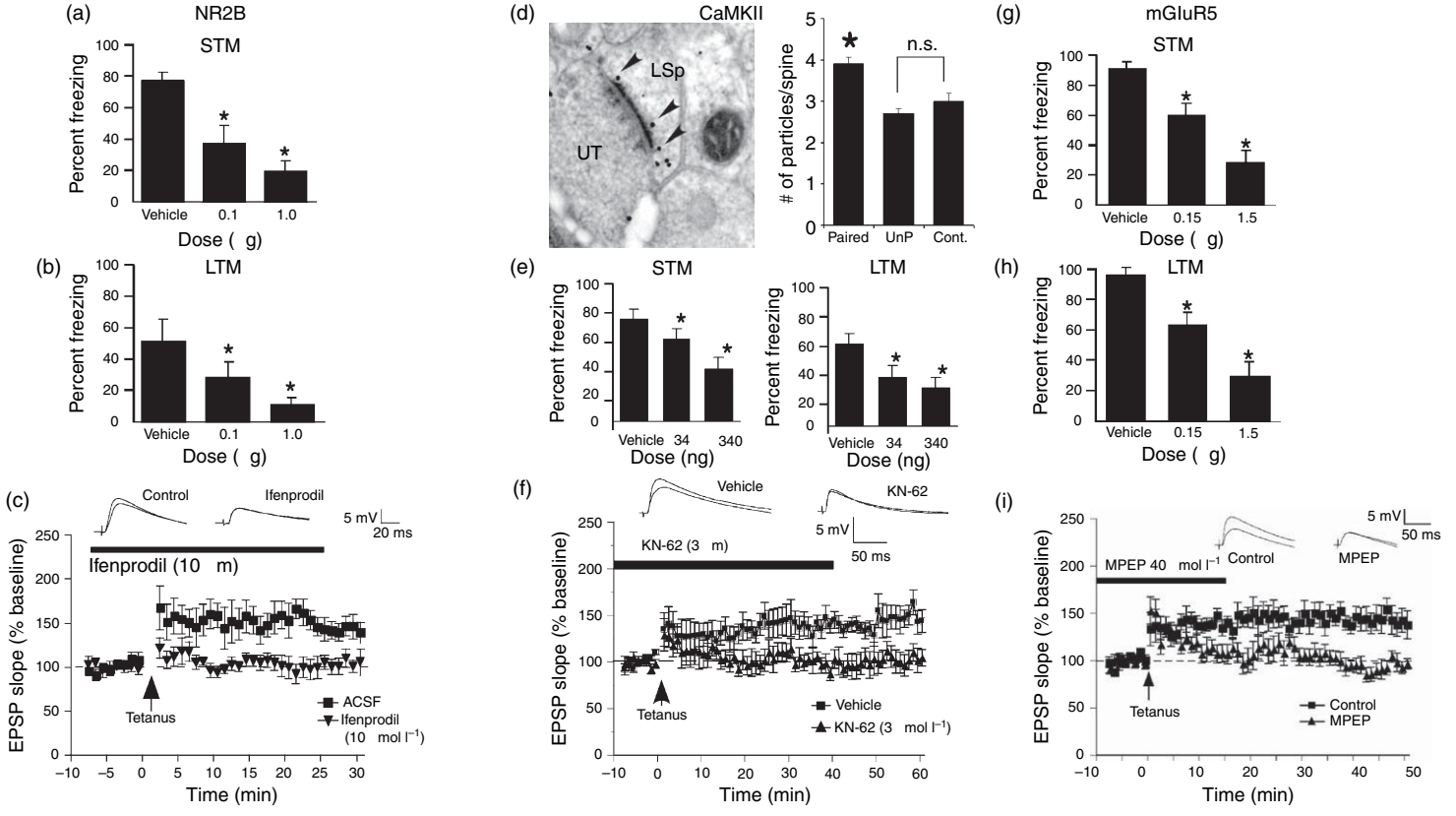

Figure 3 Glutamatergic mechanisms of fear acquisition and STM formation in the LA. (a–b) Both STM and LTM of auditory fear conditioning are dose-dependently impaired by intra-LA infusions of ifenprodil, a selective NR2B antagonist. Adapted from Rodrigues SM, Schafe GE, and LeDoux JE (2001) Intraamygdala blockade of the NR2B subunit of the NMDA receptor disrupts the acquisition but not the expression of fear conditioning. *J. Neurosci.* 21(17): 6889–6896, with permission from the Society for Neuroscience. (c) LTP at thalamic inputs to the LA is also impaired by ifenprodil. Adapted from Bauer EP, Schafe GE, and LeDoux JE (2002) NMDA receptors and L-type voltage-gated calcium channels contribute to long-term potentiation and different components of fear memory formation in the lateral amygdala. *J. Neurosci.* 22: 5239–5249, with permission from the Society for Neuroscience. (d) Fear conditioning results in an increase in autophosphorylated alpha Ca^{2+}/calmodulin-dependent protein kinase II (αCaMKII) in LA spines. Here, rats were conditioned, and activated αCaMKII was detected in LA spines using electron microscopy and an antibody against autophosphorylated αCaMKII at Thr^{286}. The image on the left shows a labeled spine (LSp) in the LA that contains numerous αCaMKII-immunogold labeled particles (arrowheads). The graph on the right shows that paired, but not unpaired (UnP), training leads to significant elevations in αCaMKII-labeled particles in LA spines. UT, unlabeled terminal. $p < .05$. (e) Both STM and LTM of auditory fear conditioning are impaired after intra-LA infusion of KN-62, a CaMKII antagonist. (f) LTP at thalamic inputs to the LA is also impaired by KN-62. (d–f) Adapted from Rodrigues SM, Farb CR, Bauer EP, LeDoux JE, and Schafe GE (2004a) Pavlovian fear conditioning regulates Thr286 autophosphorylation of Ca^{2+}/calmodulin-dependent protein kinase II at lateral amygdala synapses. *J. Neurosci.* 24: 3281–3288, with permission from the Society for Neuroscience. (g–h) Both STM and LTM of auditory fear conditioning are dose-dependently impaired by intra-LA infusions of 2-methyl-6-(phenylethynyl)-pyridine (MPEP), a selective mGluR5 antagonist. (i) LTP at thalamic inputs to the LA is also impaired by MPEP. (g–i) Adapted from Rodrigues SM, Bauer EP, Farb CR, Schafe GE, and LeDoux JE (2002) The group I metabotropic glutamate receptor mGluR5 is required for fear memory formation and long-term potentiation in the lateral amygdala. *J. Neurosci.* 22: 5219–5229, with permission from the Society for Neuroscience.

Mammen et al., 1997; Soderling and Derkach, 2000). Autophosphorylation of αCaMKII on Thr^{286}, for example, promotes the translocation of the kinase to synaptic sites (Shen and Meyer, 1999) and results in phosphorylation of the AMPAR subunit GluR1 on Ser^{831} (Barria et al., 1997; Mammen et al., 1997), an event which increases excitatory current influx into the postsynaptic cell (Derkach et al., 1999) and which is critical for LTP induction (Lee et al., 2000, 2003). Transgenic mice with a deletion of the αCaMKII gene display deficits in hippocampal LTP and hippocampal-dependent spatial memory (Silva et al., 1992a,b). Similarly, pharmacological inhibition of CaMKII blocks the induction of LTP in hippocampal area CA1 (Ito et al., 1991; Stanton and Gage, 1996) and impairs hippocampal dependent learning and memory (Tan and Liang, 1996).

Recent studies have also implicated αCaMKII in fear conditioning. Anatomical studies have shown that αCaMKII is robustly expressed in LA pyramidal neurons (McDonald et al., 2002), where it coexists with NR2B in LA spines postsynaptic to terminals that originate in the auditory thalamus (Rodrigues et al., 2004a). Fear conditioning leads to increases in the autophosphorylated form of αCaMKII at Thr^{286} in spines of LA neurons (**Figure 3(d)**). Further, intra-amygdala infusion or bath application of an inhibitor of CaMKII (KN-62) impairs acquisition and STM formation of fear conditioning and LTP at thalamic inputs to LA neurons, respectively (**Figures 3(e)** and **3(f)**). This latter finding is consistent with molecular genetic experiments indicating that induced overexpression of active αCaMKII by a transgene that replaces Thr^{286} with an aspartate residue in the amygdala and striatum results in a reversible deficit in fear conditioning (Mayford et al., 1996).

4.11.4.1.3 Metabotropic glutamate receptors and protein kinase C

While activation of αCaMKII and resultant GluR1 phosphorylation and receptor trafficking appears to be NMDAR dependent (Hayashi et al., 2000; Fu et al., 2004), it appears that Group I metabotropic glutamate receptors (mGluRs), including mGluR1 and mGluR5, are critical for the potentiation of NMDAR function via the Ca^{2+}/phospholipid-dependent protein kinase (PKC) (Ben-Ari et al., 1992; Kelso et al., 1992). Both mGluR1 and mGluR5, for example, are positively coupled to phospholipase C, activation of which leads to the hydrolysis of phosphatidylinositol 4,5-biphosphate into inositol 1,4,5-trisphosphate (IP3) and diacylglycerol (DAG), two substances that are directly upstream of PKC. In mGluR5 knockout mice, LTP of NMDAR currents in CA1 is absent, but can be rescued by activators of PKC (Jia et al., 1998). Further, an mGluR5 antagonist (CHPG) has been reported to induce a slowly developing, long-lasting potentiation of NMDAR currents via PKC (Doherty et al., 1997). Studies have suggested that two serine residues on the C-terminal domain of the NR2B subunit of the NMDAR, Ser^{1303} and Ser^{1323}, are the critical structural domain for PKC-mediated current potentiation (Liao et al., 2001). However, removal of all the PKC phosphorylation sites on NR1 and NR2 does not alter the PKC-induced potentiation of NMDAR currents (Zheng et al., 1999). Thus, it has been hypothesized that there is an intermediate step between PKC activation and NR2 subunit activation. One hypothesis is that this involves Src kinases (Ali and Salter, 2001; MacDonald et al., 2001). Src is the lead member of a family of protein tyrosine kinases, which also includes Fyn, Lyn, Lck, and Yes. It is thought that these kinases regulate the activity of NMDARs during LTP induction by phosphorylating tyrosine residues that, in turn, are responsible for increased channel conductance (Ali and Salter, 2001; MacDonald et al., 2001). The phosphorylation of NR2B on Tyr^{1472} is increased after tetanic stimulation in area CA1 (Nakazawa et al., 2001), and this appears to be Fyn mediated (Nakazawa et al., 2001, 2002). Further, mice lacking Fyn have impaired LTP in hippocampal area CA1 (Grant et al., 1992).

Several recent studies have examined the role of mGluRs in fear conditioning. Transgenic mice lacking mGluR5 are impaired in fear conditioning tasks (Lu et al., 1997), as are rats injected systemically with the selective mGluR5 antagonist 2-methyl-6-(phenylethynyl)-pyridine (MPEP) prior to fear conditioning (Fendt and Schmid, 2002). In a recent study, Rodrigues et al. showed that mGluR5 was localized in LA spines postsynaptic to auditory thalamic inputs and required for synaptic plasticity at thalamic inputs to LA neurons (Rodrigues et al., 2002). Further, in behavioral experiments, intra-amygdala infusion of MPEP prior to fear conditioning impaired formation of both STM and LTM of fear conditioning (Rodrigues et al., 2002; **Figures 3(g)** amd **3(h)**), and also impaired LTP at thalamic inputs to the LA (**Figure 3(i)**). Similar to the results with the NR2B antagonist ifenprodil, infusion of MPEP prior to training blocked both STM and

LTM, while infusion immediately prior to testing at either time point had no effect.

These findings suggest that mGluRs, and in particular mGluR5, are required for fear conditioning and STM formation in the amygdala. Future experiments, however, will be required to understand the exact mechanisms by which mGluRs contribute to fear conditioning. One attractive hypothesis, suggested by the LTP literature, is that activation of mGluR5 in the amygdala recruits the PKC signaling pathway and leads to modulation and trafficking of NMDARs via tyrosine phosphorylation of NR2B (Doherty et al., 1997; Anwyl, 1999; Liao et al., 2001). The role of PKC or tyrosine kinases in fear acquisition and STM formation has not been explicitly tested, although mice with a specific deletion of the β isoform of PKC have impaired fear conditioning when tested 24 h after training (Weeber et al., 2000). Additional experiments will be necessary to examine the role of mGluR5-mediated signaling in the LA in fear conditioning.

4.11.4.1.4 AMPA receptor regulation and trafficking

Alterations in the conductance properties of glutamatergic receptors are thought to be only one mechanism underlying LTP induction and E-LTP. Ample evidence, for example, has accumulated indicating that new AMPARs and NMDARs are trafficked and inserted into synapses during and after LTP (Grosshans et al., 2002; Malenka, 2003; Malinow, 2003). The insertion of GluR1 into synapses, for example, appears to be αCaMKII dependent, and blockade of αCaMKII-mediated synaptic delivery of GluR1 prevents LTP (Hayashi et al., 2000). Further, activation of PKC has been shown to drive NMDAR subunits into synapses, an effect which is blocked by tyrosine kinase inhibitors (Grosshans et al., 2002). Together with alterations in receptor conductance, these rapid physical alterations in the distribution of AMPARs and NMDARs represent one mechanism by which LTP might persist in the short term.

A recent study by Malinow and colleagues elegantly showed that intra-amygdala expression of a viral vector that prevents GluR1 from being inserted into synaptic sites impairs fear acquisition and synaptic plasticity in the LA (Rumpel et al., 2005). Thus, while additional studies are needed, these findings collectively suggest that activation of αCaMKII during fear acquisition may regulate the insertion of AMPARs at LA synapses and thereby contribute to the formation and maintenance of STM. It is currently unknown how long-lasting this effect is; e.g., whether it persists only over the course of hours or is also evident days after fear learning. Further, no studies have to date examined how fear conditioning might similarly regulate the trafficking of NMDARs. Additional experiments will be critical to examine each of these questions.

4.11.4.2 Long-Term Fear Memory Formation – Protein Kinase Signaling and Transcriptional Regulation in the Amygdala

As its name implies, LTM is a long-lasting phenomenon that can last many hours, days, weeks, or even years (Milner et al., 1998). Accordingly, LTM is typically tested at longer intervals after training, usually starting at 24 h. In this section, we discuss what is known about the mechanisms of LTM formation of fear conditioning in the amygdala. We begin with a discussion of L-VGCCs, as recent work has suggested that these channels play an essential role in promoting LTM formation in the LA.

4.11.4.2.1 L-VGCCs

Recent experiments have shown that LTP at thalamic input synapses to the LA is, under certain conditions, L-VGCC dependent and NMDAR independent (Weisskopf et al., 1999). These experiments used a pairing protocol in which subthreshold presynaptic stimulation of auditory afferents was paired with brief postsynaptic depolarizations (Magee and Johnston, 1997; Markram et al., 1997; Johnston et al., 1999). In this protocol, back-propagating action potentials (BPAPs) originating in the soma are thought to invade the dendrites and interact with EPSPs leading to Ca^{2+} influx through VGCCs (Magee and Johnston, 1997; Johnston et al., 1999; Stuart and Hausser, 2001). Accordingly, LTP induced by pairing in the thalamic pathway is blocked by application of the L-VGCC blockers nifedipine or verapamil (Weisskopf et al., 1999; Bauer et al., 2002).

Until recently, the contribution of L-VGCCs to fear conditioning had not been established. Bauer et al., however, examined the effect of intra-amygdala infusion of the L-VGCC blocker verapamil on the acquisition and consolidation of auditory fear conditioning (Bauer et al., 2002). The findings revealed that blockade of L-VGCCs prior to conditioning selectively impaired LTM formation of fear conditioning at 24 h after training; acquisition and STM, assessed at 1 h, were left intact. These findings,

together with those of studies that examined the role of NMDAR function in fear conditioning discussed earlier, suggest that there are two sources of Ca^{2+} in the LA that are critical for fear memory formation. One, mediated by NMDARs, appears to be selectively involved in fear acquisition and STM formation of fear conditioning (Walker and Davis, 2000; Rodrigues et al., 2001). The second, mediated by L-VGCCs, is selectively involved in LTM formation. While the effects of L-VGCC blockade are not apparent in fear conditioning for many hours after training, it is important to note that this is likely due to interference with a process that is set in motion at the time of CS-US pairing and fear acquisition. Consistent with that notion, recent reports have demonstrated that L-VGCCs play a selective role in signaling to the nucleus and initiating cyclic adenosine monophosphate (cAMP) response element (CRE)-mediated transcription, which is known to be required for long-term synaptic plasticity and memory formation (Dolmetsch et al., 2001). Additional experiments will be necessary to determine the contribution of L-VGCCs to activation of protein kinases and CRE-driven gene expression in the LA following fear conditioning.

4.11.4.2.2 Protein kinase A and mitogen-activated protein kinase

Activity-dependent increases in intracellular Ca^{2+} in LA neurons during fear acquisition is thought to lead, either directly or indirectly, to the activation of both protein kinase A (PKA) and the extracellular signal-regulated kinase/mitogen-activated protein kinase (ERK/MAPK). There has been a great deal of recent interest in each of these kinases, in part because they have been shown to be essential for the late phase of multiple forms of synaptic plasticity and memory (Milner et al., 1998; Sweatt, 2004). Once activated by stimulation that promotes L-LTP, each of these kinases is thought to engage activators of transcription. While PKA is directly capable of regulating transcription, recent evidence suggests that PKA may play a permissive role in transcriptional regulation by promoting the activation and nuclear translocation of ERK/MAPK (Roberson et al., 1999). As a result, it has been suggested that ERK/MAPK may represent a final common pathway through which different upstream kinases regulate transcription, long-term plasticity, and memory formation (Adams and Sweatt, 2002).

Both PKA and ERK/MAPK have also been implicated in fear conditioning. Mice that overexpress an inhibitory form of PKA, R(AB), exhibit impaired L-LTP in hippocampal area CA1 and selective deficits in LTM, but not STM, of contextual fear conditioning (Abel et al., 1997). Similarly, mice that lack *Ras-GRF,* an upstream regulator of ERK/MAPK, have impaired memory consolidation of auditory and contextual fear conditioning, as well as impaired amygdala LTP (Brambilla et al., 1997).

Recent pharmacological experiments have examined the role of PKA and ERK/MAPK in amygdala LTP and in fear conditioning. Huang et al. showed that bath application of inhibitors of PKA or ERK/MAPK to amygdala slices impairs LTP at thalamic and cortical inputs to the LA but has no effect on E-LTP (Huang et al., 2000). Consistent with those findings, infusion of a PKA inhibitor or of a peptide that blocks the association of PKA with the A-kinase anchoring protein (AKAP) in the LA impairs LTM, but not STM of fear conditioning (Schafe and LeDoux, 2000; Moita et al., 2002; **Figure 4(e)**). Further, fear conditioning results in a transient activation of ERK/MAPK in the LA (**Figures 4(a)–4(c)**), and infusion of an inhibitor of MEK, an upstream regulator of ERK/MAPK, into the LA prior to fear conditioning impairs memory consolidation; that is, rats have intact STM and impaired LTM (Schafe et al., 2000; **Figure 4(d)**). Collectively, these findings support the hypothesis that both PKA and ERK/MAPK contribute to fear memory formation by engaging cellular processes, possibly those in the nucleus, that are necessary for long-term synaptic plasticity and memory formation.

4.11.4.2.3 Neurotrophin signaling

In addition to Ca^{2+}-mediated signaling, neurotrophins have been widely implicated in driving protein kinase signaling pathways necessary for long-term synaptic plasticity and memory formation, including fear conditioning. In hippocampal neurons, direct application of brain-derived neurotrophic factor (BDNF) produces a long-lasting, transcription-dependent form of LTP (Kang and Schuman, 1995; Figurov et al., 1996). Further, blockade or genetic deletion of BDNF or its membrane-bound receptor tyrosine kinase, TrkB, impairs L-LTP in the hippocampus (Figurov et al., 1996; Patterson et al., 1996; Korte et al., 1998; Fanselow and LeDoux, 1999), and L-LTP is impaired in hippocampal slices in mice that lack BDNF (Patterson et al., 1996; Korte et al., 1998). Consistent with the importance of ERK/MAPK signaling in long-term synaptic plasticity and memory formation, recent studies have suggested that

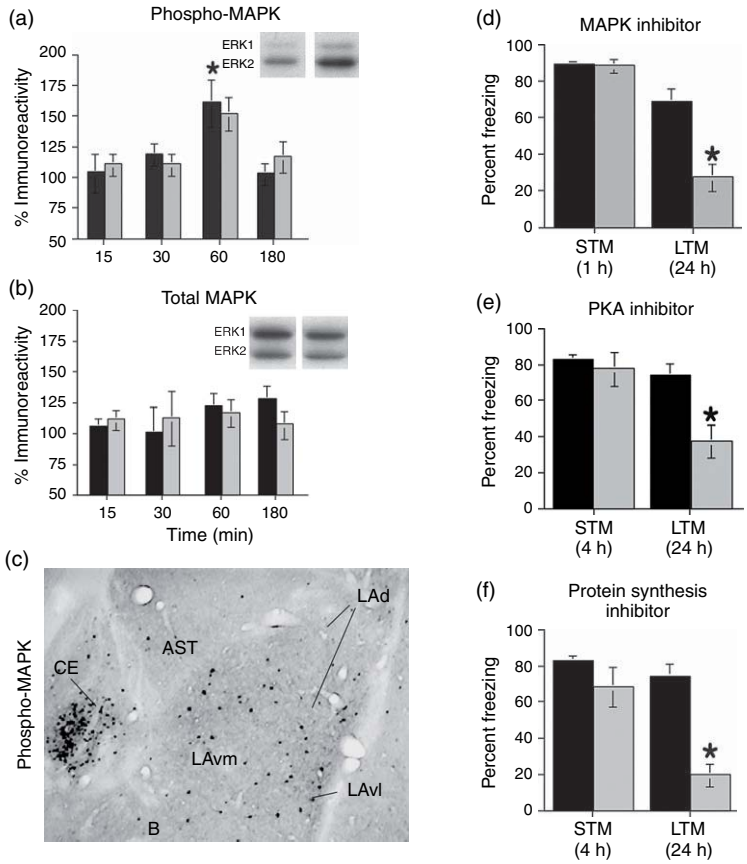

Figure 4 Protein kinase signaling pathways involved in long-term memory (LTM) formation in the lateral amygdala (LA). (a) Fear conditioning leads to an increase in phosphorylated extracellular signal-related kinase 1 (ERK1) and ERK2 at t = 60 min after training. In these experiments, rats were trained and sacrificed at different time points after conditioning, and LA homogenates were probed with antibodies that recognize phosphorylated ERK/mitogen-activated protein kinase (MAPK). ERK1 (black bars) and ERK2 (gray bars) are the two isoforms of ERK/MAPK recognized by the anti-phospho-ERK antibody. $p < .05$. (b) The increase in activated ERK/MAPK is not accounted for by a change in the amount of total (unphosphorylated) ERK/MAPK. (c) Immunocytochemical localization of phosphorylated ERK/MAPK in the LA after fear conditioning. The image shows ERK-labeled cells in three different regions of the LA (dorsal, LAd; ventromedial, LAvm; and ventrolateral, LAvl), with most of the label concentrated in the ventral portions of the LAd and throughout the LAvm and LAvl. Activated ERK/MAPK is also highly expressed in the nearby central nucleus (CE) and the amygdala-striatal transition zone (AST). B, basal nucleus of the amygdala. (a–c) Adapted from Schafe GE, Atkins CM, Swank MW, Bauer EP, Sweatt JD, and LeDoux JE (2000) Activation of ERK/MAP kinase in the amygdala is required for memory consolidation of Pavlovian fear conditioning. *J. Neurosci.* 20: 8177–8187, with permission from the Society for Neuroscience. (d–f) LTM, but not short-term memory (STM), in the LA requires MAPK, protein kinase A (PKA), and protein synthesis. In these studies, rats received intra-amygdala infusions of (d) U0126 (a MEK inhibitor, which is an upstream regulator of ERK/MAPK activation), (e) Rp-cAMPS (a PKA inhibitor), or (f) anisomycin (a protein synthesis inhibitor) at or around the time of training and were assayed for both STM (1–4 h later) and LTM (24 h later) of auditory fear conditioning. In each figure, vehicle-treated rats are represented by the gray bars, while drug-treated animals are represented by the black bars. $^{*}p < .05$ relative to vehicle controls. (d–f) Adapted from Schafe GE and LeDoux JE (2000) Memory consolidation of auditory Pavlovian fear conditioning requires protein synthesis and protein kinase A in the amygdala. *J. Neurosci.* 20: RC96, with permission from the Society for Neuroscience.

BDNF-TrkB-mediated signaling promotes long-term synaptic plasticity by engaging the ERK/MAPK signaling pathway (Patterson et al., 2001). Application of BDNF potently activates ERK/MAPK in hippocampal neurons (Ying et al., 2002), and treatment with an inhibitor of ERK/MAPK activation impairs BDNF-induced LTP (Ying et al., 2002). Collectively, these findings suggest that BDNF-induced ERK signaling plays an essential role in long-term synaptic plasticity.

A recent study has shown that BDNF-mediated signaling in the amygdala is critical to fear learning

(Rattiner et al., 2004, 2005). In that study, fear conditioning led to increases in both TrkB receptor phosphorylation and decreases in TrkB receptor immunoreactivity in the LA during the consolidation period, which is typically indicative of bound BDNF. Further, disruption of TrkB receptor signaling in the amygdala using either a Trk receptor antagonist or lentiviral overexpression of a dominant negative TrkB isoform impaired fear memory formation (Rattiner et al., 2004). While this study did not distinguish between acquisition and consolidation phases of fear learning, the assumption is that BDNF signaling in the LA plays a critical role in the establishment of long-term fear memories, possibly by promoting the activation and nuclear translocation of protein kinases such as ERK (Patterson et al., 2001; Ying et al., 2002). Additional experiments will be necessary to define the signaling pathways through which BDNF acts during fear learning.

4.11.4.2.4 Transcriptional regulation and macromolecular synthesis

Both L-LTP in the LA (Huang and Kandel, 1998; Huang et al., 2000) and LTM of fear conditioning (Bailey et al., 1999; Schafe and LeDoux, 2000) are known to require new RNA and protein synthesis in the LA (**Figure 4(f)**). The requirement for *de novo* RNA synthesis is particularly important, because it suggests that a nuclear event is required for the transition between short- and long-term memory formation.

As previously discussed, signaling via ERK/MAPK plays a critical role in memory formation by engaging activators of transcription in the nucleus. ERK/MAPK is thought to promote transcription by binding to and activating transcription factors, including, by way of the Rsk and MSK1 signaling pathways (Xing et al., 1996; Adams and Sweatt, 2002), the cAMP-response-element binding protein (CREB) (Impey et al., 1996, 1998a). It is the activation of CREB and CRE-mediated genes that ultimately leads to the protein and RNA synthesis-dependent functional and/or structural changes that are thought to underlie L-LTP (Frank and Greenberg, 1994; Yin and Tully, 1996; Silva et al., 1998; Stevens, 1998; Holt and Maren, 1999). While many of these genes and their functional roles remain to be elucidated, it has been suggested that the regulation of a number of CRE- and serum response element (SRE)-mediated immediate early genes (IEGs) plays a critical intermediate role in regulating the expression of late-response genes. These have included *Zif-268,* and its protein product EGR-1, and the activity-regulated cytoskeletal-associated protein (Arc). Importantly, each of these IEGs is known to be regulated by hippocampal LTP (Richardson et al., 1992; Abraham et al., 1993; Worley et al., 1993; Link et al., 1995) and required for hippocampal-dependent LTM formation (Guzowski et al., 2000; Jones et al., 2001).

Several transcription factors have been implicated in long-term synaptic plasticity and in memory formation, but CREB is perhaps the best studied. CREB is a family of transcription factors consisting of several functionally distinct isoforms. Some, known as activator isoforms, bind to DNA at CRE promoter regions and promote transcription. Others, known as repressor isoforms, compete with the binding of activator isoforms to DNA (Bartsch et al., 1995; Abel et al., 1998; Silva et al., 1998). CREB is an attractive candidate molecule for memory consolidation because it has direct interaction with the transcriptional machinery and also contains phosphorylation sites for the major protein kinase signaling pathways that are known to be involved in memory formation, including PKA, ERK/MAPK, and CaMKII (Silva et al., 1998).

The first evidence that suggested CREB might be involved in memory consolidation came from a study employing a Pavlovian conditioning task in *Drosophila.* Overexpression of a dominant negative (repressor) isoform of CREB in flies impaired LTM formation in a conditioned odor aversion task (Yin et al., 1994). Conversely, overexpression of an activator isoform of CREB facilitated LTM; that is, behavioral training that would normally produce only STM was effective at producing LTM (Yin et al., 1995).

CREB has also been implicated in fear conditioning. Mice lacking two critical isoforms of CREB, the α and δ, have impaired hippocampal L-LTP and memory consolidation for auditory and contextual fear conditioning; that is, LTM is impaired, while STM is intact (Bourtchuladze et al., 1994). Further, induced overexpression of a dominant negative isoform of CREB in the forebrain impairs LTM formation of fear conditioning (Kida et al., 2002). Conversely, overexpression of the transcription factor CREB in the LA facilitates fear memory formation (Josselyn et al., 2001). In the latter study, CREB was overexpressed locally in the LA, using viral transfection methods. Consistent with the role of CREB in long-term synaptic plasticity and memory formation, overexpression of CREB in the LA

facilitated LTM of fear conditioning, but had no effect on STM.

While CRE-mediated transcription clearly supports the development of long-term plasticity and memory, the downstream targets of CREB have remained largely unknown. However, a number of studies have shown that fear conditioning induces the expression of both IEGs (Beck and Fibiger, 1995; Rosen et al., 1998; Malkani and Rosen, 2000; Scicli et al., 2004) and downstream genes (Stork et al., 2001; Ressler et al., 2002; Rattiner et al., 2004) in the LA. While the specific contributions of many of these genes to fear conditioning is still unclear, it is widely believed that learning-induced gene expression ultimately contributes to changes in cell (especially synaptic) structure that stabilizes memory (Bailey and Kandel, 1993; Woolf, 1998; Rampon et al., 2000; Sweatt, 2004), presumably by altering the actin cytoskeleton underlying synaptic organization (van Rossum and Hanisch, 1999; Matus, 2000; Kasai et al., 2003). Such changes in synaptic structure have been well documented in invertebrates, where stimulation that promotes long-term synaptic plasticity has been shown to lead to an increase in new synaptic contacts (Bailey et al., 1992, 1994; Bailey and Kandel, 1993). Further, both learning and LTP result in a number of structural changes in the hippocampus and cortex, including an increase in spine head volume and widening and shortening of the spine neck (Van Harreveld and Fifkova, 1975; Fifkova and Van Harreveld, 1977; Fifkova and Anderson, 1981), spine perforation (Toni et al., 1999), and an increase in the total number of spines (Engert and Bonhoeffer, 1999; Leuner et al., 2003).

Recent studies have suggested that fear conditioning leads to alterations in cytoskeletal proteins and to new spine formation in the LA. Fear conditioning, for example, leads to the transcription of genes involved in cytoskeletal remodeling, including the CRE-mediated gene neurofilament-light chain (NF-l) (Ressler et al., 2002). Further, interference with molecular pathways known to be involved in structural plasticity during early development, such as the Rho GTPase (GTP: guanosine triphosphate) activating protein (Rho-GAP) signaling pathway, disrupts memory formation (Lamprecht et al., 2002), and fear conditioning drives actin cytoskeleton-regulatory proteins, such as profilin, into amygdala spines shortly after training (Lamprecht et al., 2006). Finally, a recent morphological study has suggested that fear conditioning leads to an increase in spinophilin-immunoreactive dendritic spines in the LA (Radley et al., 2006).

4.11.4.3 A Presynaptic Component to Fear Learning?

As outlined, most recent studies have focused on postsynaptic mechanisms and their role in amygdala LTP and memory formation (for review, see Schafe et al., 2001). There is growing evidence, however, that suggests that synaptic plasticity and memory formation in the LA involves a presynaptic process. McKernan and Shinnick-Gallagher (1997), for example, showed that auditory fear conditioning occludes paired-pulse facilitation (PPF) at cortical inputs to the LA, a type of short-term plasticity that is largely believed to be presynaptic. Similarly, Huang and Kandel (1998) observed that LTP at cortical inputs to the LA occludes PPF in this pathway. Further, bath application, but not postsynaptic injection, of a PKA inhibitor impairs LTP in LA neurons (Huang and Kandel, 1998). Conversely, bath application of forskolin, a PKA activator, in the presence of antagonists of postsynaptic NMDAR and AMPAR receptors, induces LTP and occludes PPF at cortical inputs (Huang and Kandel, 1998), suggesting that the presynaptic component of LTP in this pathway is PKA dependent. More recently, Tsvetkov et al. showed that auditory fear conditioning itself, in addition to LTP, occludes PPF at cortical inputs to LA (Tsvetkov et al., 2002). It is thus clear from the available evidence that a complete understanding of memory formation and synaptic plasticity in the LA will require attention to both sides of the synapse.

4.11.4.3.1 Nitric oxide signaling and fear learning

Recent evidence has suggested that nitric oxide (NO) signaling in the LA is critical to fear memory formation (Schafe et al., 2005) and may represent a mechanism whereby postsynaptic induction of plasticity induced by fear conditioning in LA neurons may engage accompanying presynaptic changes. NO is a highly soluble gas generated by the conversion of L-arginine to L-citrulline by the Ca^{2+}-regulated enzyme nitric oxide synthase (NOS) (Bredt and Snyder, 1992). In other memory systems, NO is thought to serve as a 'retrograde messenger' that engages aspects of presynaptic plasticity (Schuman and Madison, 1991; Zhuo et al., 1994; Arancio et al.,

1996; Doyle et al., 1996; Son et al., 1998; Ko and Kelly, 1999; Lu et al., 1999) and memory formation (Chapman et al., 1992; Bohme et al., 1993; Bernabeu et al., 1995; Holscher et al., 1996; Suzuki et al., 1996; Zou et al., 1998). One immediate downstream effector of NO, for example, is soluble guanylyl cyclase (Bredt and Snyder, 1992; Son et al., 1998; Denninger and Marletta, 1999; Arancio et al., 2001). This enzyme directly leads to the formation of cyclic guanosine monophosphate (cGMP) and in turn to the activation of the cGMP-dependent protein kinase (PKG). PKG, in turn, can have a number of effects, including targeting and mobilization of synaptic vesicles in the presynaptic cell, leading to enhanced transmitter release (Hawkins et al., 1993, 1998). In the hippocampus, pharmacological inhibition of NOS activation, guanylyl cyclase, or PKG impairs LTP in CA1 (Zhuo et al., 1994; Doyle et al., 1996; Son et al., 1998; Lu et al., 1999; Monfort et al., 2002). Conversely, bath application of exogenous NO or pharmacological activators of cGMP or PKG combined with weak tetanic stimulation, which would not produce LTP alone, induces long-lasting LTP (Zhuo et al., 1994; Son et al., 1998; Lu et al., 1999; Lu and Hawkins, 2002). Inhibition of NOS activity is equally effective at impairing LTP, whether the NOS inhibitor is injected directly into the postsynaptic cell or perfused over the entire slice, suggesting that the critical activation of NOS occurs postsynaptically (Schuman and Madison, 1991; Arancio et al., 1996; Ko and Kelly, 1999). However, NO is thought to act presynaptically, at least in part, because bath application of membrane-impermeable scavengers of NO also impairs LTP in CA1 (Schuman and Madison, 1991; Ko and Kelly, 1999). Collectively, this pattern of findings supports the notion that LTP in area CA1 is induced postsynaptically, but maintained or expressed presynaptically, at least in part, by an NO-dependent mechanism.

In our recent experiments, we showed that neuronal nitric oxide synthase (nNOS) is localized in LA spines (**Figure 5(a)**). Further, PPF was occluded by LTP at thalamic inputs to the LA, and bath application of either a NOS inhibitor or a membrane impermeable scavenger of NO impaired LTP at thalamo–LA synapses (**Figures 5(b)–5(d)**). Finally, intra-amygdala infusion of both compounds impaired fear memory consolidation; that is, LTM was impaired, while STM was intact (Schafe et al., 2005; **Figures 5(e)** and **5(f)**). While additional studies will be necessary, these are among the first to define a role for NO signaling in fear memory formation in the LA.

4.11.5 Is the Lateral Amygdala an Essential Locus of Fear Memory Storage?

In the previous sections, we have discussed the findings of lesion, neurophysiological, pharmacological, and biochemical studies. Collectively, these findings suggest that fear memory formation and consolidation involve alterations in synaptic transmission at LA synapses via an LTP-like mechanism. But is the LA really a site of fear memory storage? This question has proven extremely difficult to address experimentally. Recent findings, however, have provided a fresh look at this question and provide a new strategy for revealing the location of the fear engram.

4.11.5.1 An Alternative View of the Amygdala and Fear Conditioning

While an ever-increasing number of studies using lesion, neurophysiological, and most recently, pharmacological/biochemical techniques have suggested that the LA is an essential site of fear memory formation and storage, an alternative view has offered alternative interpretations of each of the aforementioned findings (Cahill et al., 1999). One obvious way to ask whether a brain structure might be involved in permanent storage of a memory is to lesion that structure at different time points after training (e.g., 1 day later, 1 week later, 1 year later). If memory is impaired as the result of the lesion at each of these time points it suggests that some type of permanent storage has occurred there, *provided that* the area of interest is not involved in some way in the expression of that type of learning. In support of the memory storage hypothesis, lesions of the amygdala impair fear learning even if given years after the initial training event (Gale et al., 2004). The conclusions drawn from these studies, however, have long been called into question due to the fact that the LA and its connections with the CE are also critical for fear expression (Cahill et al., 1999, 2001). Accordingly, lesion studies alone cannot unambiguously distinguish a role for the LA in fear acquisition from that of fear expression.

Neurophysiological and pharmacological studies supporting a role for the amygdala in memory storage have also been called into question. It has been pointed out, for example, that the LA is not unique but rather one of many regions of the wider fear network to exhibit training-related neurophysiological changes during and after fear conditioning.

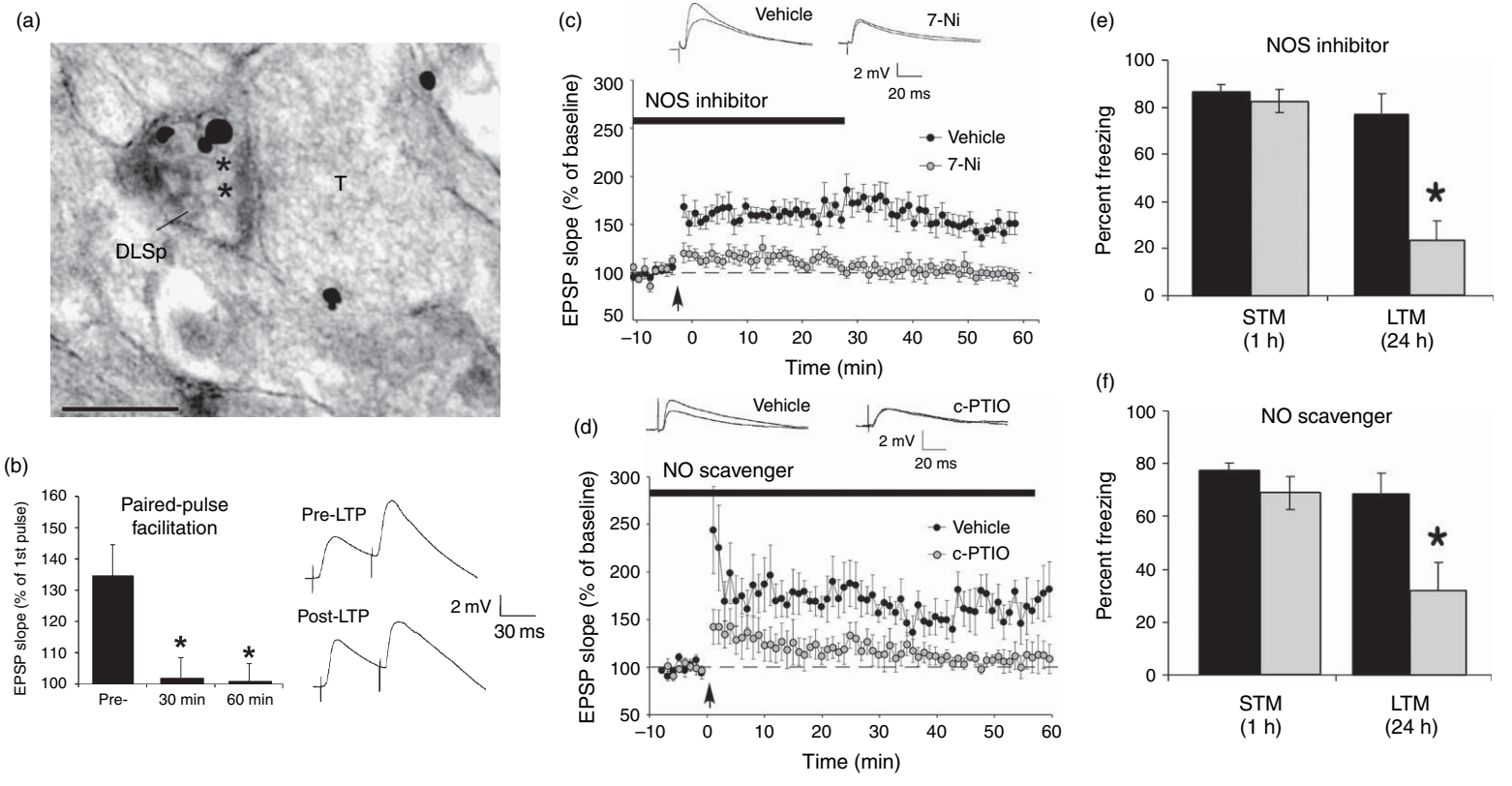

Figure 5 Nitric oxide (NO) signaling and fear memory formation. (a) Localization of neuronal NOS (nNOS) in LA spines. A terminal (T) forms an asymmetric synapse (asterisks) onto a nNOS- and αCaMKII-immunoreactivity dually labeled spine (DLSp) in the LA. The immunogold particles represent labeling of αCaMKII, while the peroxidase represents labeling of nNOS. (b) Paired-pulse facilitation at thalamic inputs to the LA before (Pre-) and 30 and 60 min after LTP induction. Each cell was given two stimulations that were spaced 50 ms apart, and the second pulse was expressed as a percentage of the first pulse. Representative traces can be seen at the right. (c–d) LTP at thalamic inputs to the LA is impaired by bath application of either (c) an inhibitor of nNOS (7-nitroindazole, 7-Ni) or (d) a membrane impermeable scavenger of NO (carboxy-PTIO, c-PTIO). (e–f) Fear memory consolidation is also impaired by 7-Ni and carboxy-PTIO. In both cases, STM is intact, while LTM is impaired. All figures adapted, with permission, from Schafe GE, Bauer EP, Rosis S, Farb CR, Rodrigues SM, and LeDoux JE (2005) Memory consolidation of Pavlovian fear conditioning requires nitric oxide signaling in the lateral amygdala. *Eur. J. Neurosci.* 22: 201–211. Copyright by Blackwell Publishing.

Auditory fear conditioning, for example, induces associative alterations in the activity of neurons not only in the LA, but also in the auditory cortex (Bakin and Weinberger, 1990; Edeline and Weinberger, 1993) and the auditory thalamus (Gabriel et al., 1975; Weinberger, 1993). Consequently, it has been difficult to argue with certainty that training-induced changes in the LA are of local origin rather than the result of a passive reflection of plasticity in these upstream regions (Cahill et al., 1999). This has been especially true of training-induced changes in the auditory thalamus (MGm/PIN), which are of short enough latency (e.g., 5–7 ms) to account for the shortest observed changes in the LA (e.g., 12–15 ms). Finally, rather than indicating that the LA is a site of storage of fear memories, it has been suggested that memory deficits observed after pharmacological manipulations may instead indicate that the LA is essential for triggering or modulating the strength of plasticity and memory storage in other regions of the wider fear network (Cahill et al., 1999). In support of this notion, recent studies have suggested that the acquisition of training-induced plasticity in the auditory thalamus is dependent on the amygdala (Maren et al., 2001; Poremba and Gabriel, 2001). Accordingly, one may argue that pharmacological manipulations of the LA that are aimed at disrupting synaptic plasticity may be doing so by modulating the strength of plasticity in regions of the wider fear network, such as the MGm/PIN, which is in turn reflected back to the LA.

4.11.5.2 A New Strategy for Tracking the Fear Engram

Recognizing that neurophysiological or pharmacological methods alone are unlikely to be able to answer the question of where fear memories are stored, a recent study from our lab has taken a new approach. In our studies, we combined simultaneous neurophysiological recordings from both LA and MGm/PIN with intra-LA infusion of the MAP kinase kinase (MEK) inhibitor U0126 (**Figure 6**). We reasoned that if local synaptic plasticity in the LA was necessary for fear memory formation and storage via an ERK/MAPK dependent mechanism, then local inhibition of MEK in the LA should selectively impair training-induced plasticity in the LA rather than the MGm/PIN. The findings showed that MEK inhibition in the LA impaired both memory consolidation of auditory fear conditioning (**Figures 6(a) and 6(b)**), and also the consolidation of training-induced synaptic plasticity in the LA (**Figures 6(c)–6(e)**). That is, acquisition and short-term retention of fear learning and cellular changes were intact, whereas long-term retention was impaired. Intra-LA infusion of the MEK inhibitor had no effect, however, on training-induced neurophysiological changes in the MGm/PIN (**Figures 6(f)–6(h)**).

Together, these findings strongly indicate that ERK/MAPK-mediated signaling in the LA is required for memory consolidation of fear conditioning as well as for consolidation of conditioning-induced synaptic plasticity in the LA. Further, our findings rule out the possibility that MEK inhibition in the LA may be impairing to fear memory formation by influencing synaptic plasticity (either short- or long-term) in the MGm/PIN. Further, these findings suggest that conditioned enhancement of CS responses in the auditory thalamus is not sufficient to support memory storage of fear conditioning, whereas ERK-dependent conditioned enhancement of CS responses in LA is necessary, at least in part, for memory storage. Importantly, it should be emphasized that these recent findings do not diminish the potential importance of the auditory thalamus and other structures in the encoding of different components of the whole fear memory trace, nor do they suggest, as we will see later, that the amygdala plays no role in modulating certain types of memory storage. However, these recent findings provide strong support to the notion that long-term storage of an emotional memory trace relies, in part, on local synaptic plasticity in the LA.

4.11.6 Distributed Versus Local Plasticity in the Amygdala

While the LA clearly appears to be a critical locus of synaptic plasticity, fear memory acquisition, and storage, it should not be assumed that LA synapses are the only critical synapses in the amygdala which undergo changes that are essential to fear memory formation and/or consolidation. Several recent studies, for example, have suggested that a distributed, rather than localized, network of plasticity in the amygdala underlies fear learning (Medina et al., 2002; Paré et al., 2004).

4.11.6.1 Distributed Plasticity within the LA

The distributed view of plasticity underlying fear learning begins in the LA itself, where plasticity at two sets of synapses has been linked to fear learning,

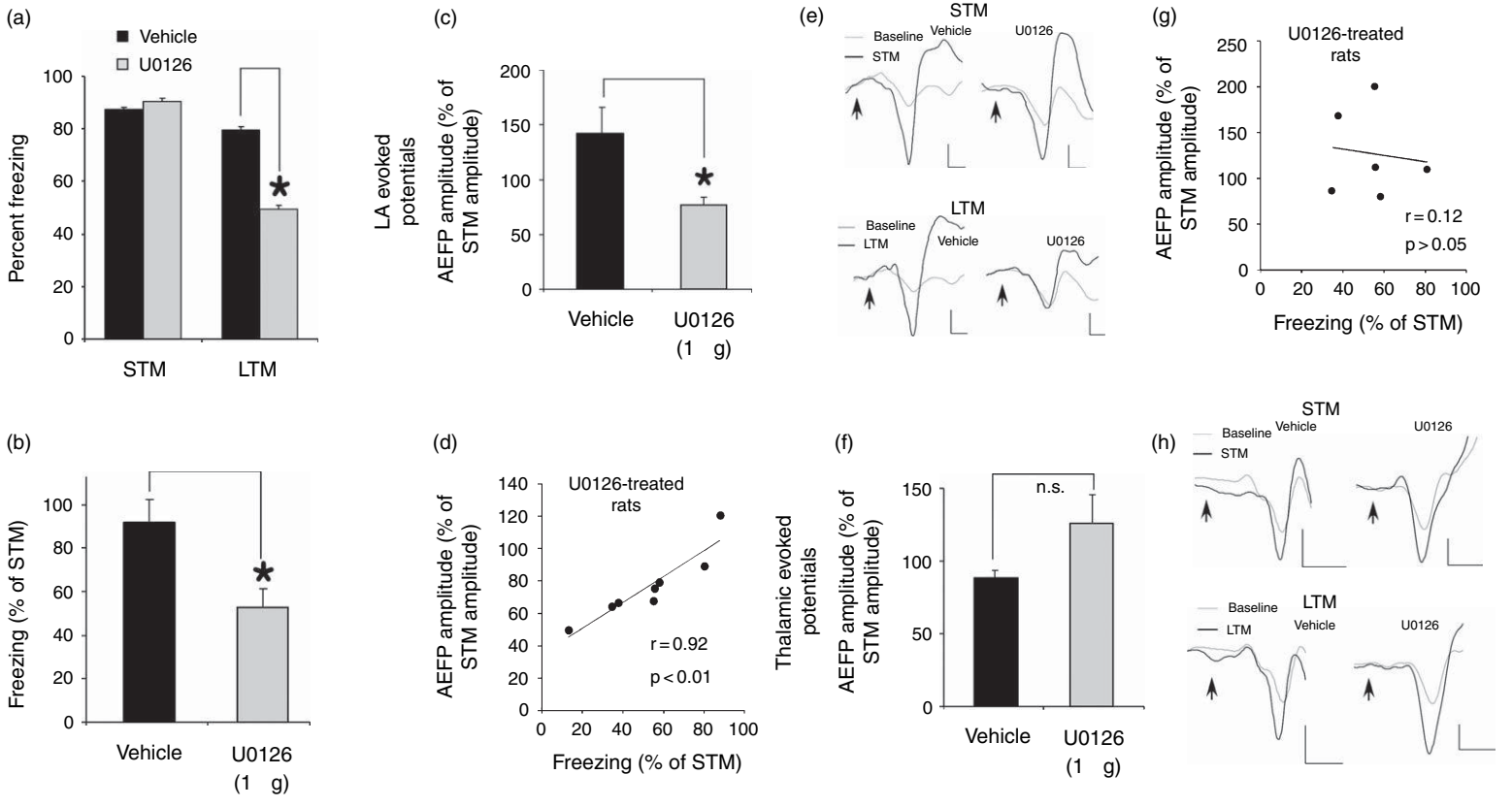

Figure 6 Local synaptic plasticity is required for fear memory consolidation in the lateral amygdala (LA). (a) Impaired fear memory in rats receiving intra-LA infusions of U0126 while neurophysiological recordings were made from both LA and the medial geniculate body and the posterior intralaminal nucleus (MGm/PIN). Mean (± SEM) percent freezing expressed during STM and LTM tests in rats treated with 50% dimethyl sulfoxide vehicle (black bars) or 1 μg U0126 (ray bars). (b) Mean (± SEM) retention of freezing in both groups; freezing during LTM is expressed as a percentage of that observed during the STM test. (c) Mean (± SEM) changes in the amplitude of LA auditory-evoked field potentials (AEFPs) during the LTM test, expressed as a percentage of that obtained during the STM test. (d) Correlation between freezing scores and LA AEFP amplitudes in U0126-treated rats (each expressed as a percentage of STM). (e) Representative AEFPs in the LA for each group (vehicle, U0126) during baseline, STM, and LTM tests. Scale = 20 μs, 5 ms. (f) Mean (± SEM) changes in the amplitude of MGm/PIN AEFPs during the LTM test, expressed as a percentage of that obtained during the STM test. (g) Correlation between freezing scores and LA AEFP amplitudes in U0126-treated rats (each expressed as a percentage of STM). (h) Representative AEFPs in the MGm/PIN for each group (vehicle, U0126) during baseline, STM, and LTM tests. Scale = 20 μs, 5 ms. Adapted from Schafe GE, Doyère V, LeDoux JE (2005b) Tracking the fear engram: The lateral amygdala is an essential locus of fear memory storage. *J. Neurosci.* 25: 10010–10015, with permission from the Society for Neuroscience.

and in unique ways. While most studies that have documented training-induced alterations in synaptic plasticity have focused on cells in the LAd (Quirk et al., 1995, 1997; Rogan et al., 1997; Maren, 2000; Blair et al., 2003), a relatively recent study has documented plastic changes in two populations of cells in the LA (Repa et al., 2001; **Figure 7**). The first is the traditionally studied dorsal population in the LAd that shows enhanced firing to the CS in the initial stages of training and testing and is sensitive to fear extinction. These so-called 'transiently plastic cells' exhibit short-latency changes (within 10–15 ms after tone onset) that are consistent with the involvement of rapid, monosynaptic thalamic input (**Figure 7(b)**). The second population of cells occupies a more ventral position in the LA. In contrast to the transiently plastic cells, these more ventral cells exhibit enhanced firing to the CS throughout training and testing and do not appear to be sensitive to extinction (**Figure 7(c)**). Further, these 'long-term plastic cells' exhibit longer latencies (within 30–40 ms after tone onset), indicative of a polysynaptic pathway. Thus, it has been hypothesized that a network of neurons within the LA is responsible for triggering and storing fear memories (Repa et al., 2001; Medina et al., 2002).

Interestingly, the cells that express activated ERK/MAPK after fear conditioning occupy a more ventral position in the LA, in the same anatomical location of cells that exhibit long-term plasticity during and after fear conditioning (Schafe et al., 2000; Repa et al., 2001; **Figure 7(d)**). In fact, very little activated ERK is observed in the dorsal region of the LA, the site of the majority of CS-US convergence and of cells that exhibit rapid, and transient, plastic changes during fear conditioning (Romanski et al., 1993; Repa et al., 2001). This pattern of findings is consistent with the hypothesis that fear conditioning induces long-term plastic change and memory formation in a ventral population of cells in the LA via the ERK/MAPK signaling cascade. It remains unknown whether this involves a rapid 'transfer' of plasticity between dorsal and ventral cells in the LA during fear conditioning, or an independent, parallel process.

4.11.6.2 Distributed Plasticity within Amygdala Nuclei

Recently, interest has also grown in the idea that distributed plasticity *between* amygdala nuclei may be critical for fear learning. This has been sparked, in part, by a recent study showing that the central nucleus of the amygdala may also be an important locus of fear memory acquisition and consolidation (Wilensky et al., 2006). In that study, functional inactivation restricted to *either* the LA or the CE impaired acquisition of auditory fear conditioning. Further, infusion of the protein synthesis inhibitor anisomycin into the CE impaired fear memory consolidation; that is, rats had intact STM but impaired LTM (Wilensky et al., 2006). These findings suggest that the CE plays an important role not only in fear expression, as has been previously thought, but also in the acquisition and consolidation of fear learning.

How might the CE participate in fear memory acquisition and consolidation? Since the CE, and particularly the medial division of the CE (CEm), also appears to be a recipient of somatosensory (Bernard and Besson, 1990; Jasmin et al., 1997) and possibly also auditory (LeDoux et al., 1987; Turner and Herkenham, 1991; Frankland et al., 1998; Linke et al., 2000) information, one possibility is that the CE encodes in parallel the same type of association that is encoded in the LA. In support of this possibility, a recent study showed that high-frequency stimulation of the auditory thalamus induces an NMDAR-dependent LTP in CEm neurons (Samson and Paré, 2005). If the CE were encoding memory in parallel to the LA, however, this would suggest that the CE should readily be capable of mediating fear learning when the LA is compromised, a finding which is not supported by the literature. Another possibility is that plasticity in the LA and the CE proceeds in a serial manner, such that plasticity and memory formation in the CE depends on plasticity in the LA. This view has been advocated in a recent model that proposes that plasticity in the LA enables CEm neurons to encode plasticity that is essential for fear conditioning, resulting in distributed plasticity and memory formation throughout the amygdala (Paré et al., 2004). The mechanism by which this distributed plasticity between the LA and the CE occurs is at present unknown, but likely involves projections from the LA to CEm neurons via the nearby intercalated cell masses which lie between the LA and the CE (Paré and Smith, 1993; Royer et al., 1999). Additional experiments employing single-unit recording techniques in both the LA and the CEm will be required to determine how these two regions influence one another during fear conditioning.

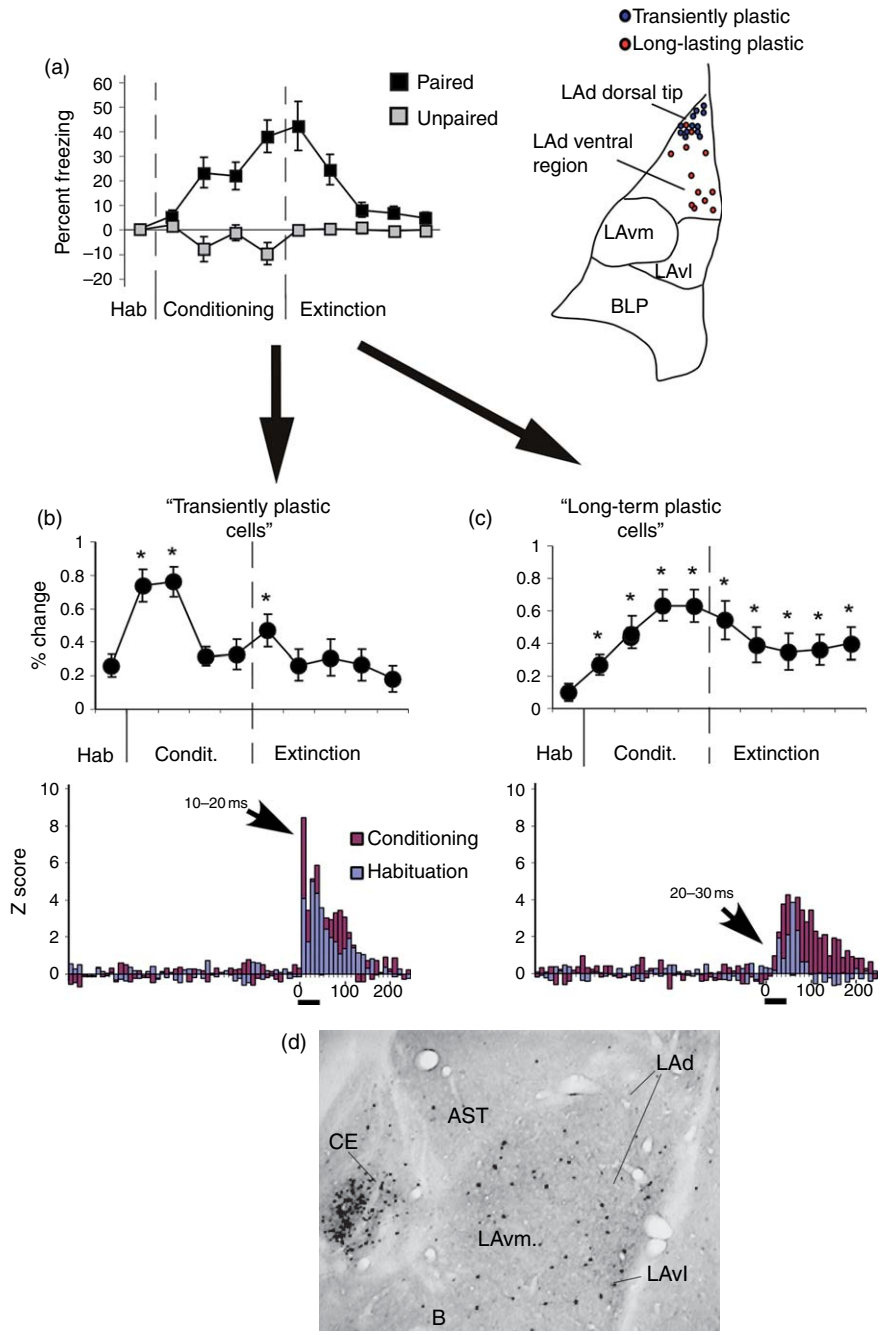

Figure 7 Distributed plasticity in the LA during fear conditioning. Pairing of CS and US during fear conditioning leads to changes in fear behavior (a) and also to changes in the responsiveness of single LA cells to auditory stimuli. During fear conditioning, there are two populations of cells that undergo plastic change. (b) 'Transiently plastic cells' are generally short latency and show enhanced firing shortly after training and during the initial phases of extinction, but not at other times. (c) 'Long-term plastic cells' are generally longer latency and show enhanced firing throughout training and extinction. INSET: 'Transiently plastic cells' are generally found in the dorsal tip of the LAd, where they may serve to trigger the initial stages of memory formation. 'Long-term plastic cells,' on the other hand, are found in the ventral regions of the LAd and may be important for long-term, extinction-resistant memory storage. (d) Location of cells expressing phospho-ERK following fear conditioning. Note the location of ERK-positive cells relative to 'long-term plastic cells.' CE, central nucleus of the amygdala; regions of the LA: LAd, dorsal; LAvm, ventromedial; LAvl, ventrolateral; AST, amygdala-striatal transition zone; B, basal nucleus of amygdala. (a–c) Adapted from Repa JC, Muller J, Apergis J, Desrochers TM, Zhou Y, and LeDoux JE (2001) Two different lateral amygdala cell populations contribute to the initiation and storage of memory. *Nat. Neurosci.* 4: 724–731, with permission from Nature Publishing Group. (d) Adapted from Schafe GE, Atkins CM, Swank MW, Bauer EP, Sweatt JD, and LeDoux JE (2000) Activation of ERK/MAP kinase in the amygdala is required for memory consolidation of Pavlovian fear conditioning. *J. Neurosci.* 20: 8177–8187, with permission from the Society for Neuroscience.

4.11.7 Summary: A Model of Fear Memory Acquisition and Consolidation in the Amygdala

In summary, the converging evidence from a number of recent studies supports a model of fear conditioning in which CS and US inputs converge onto individual LA neurons and initiate changes in synaptic function and/or structure (Blair et al., 2001; **Figure 8**). The convergence of CS and US inputs onto LA principal cells during training leads to Ca^{2+} influx through both NMDARs (Miserendino et al., 1990; Kim et al., 1991; Campeau et al., 1992; Walker and Davis, 2000; Rodrigues et al., 2001) and also L-VGCCs (Bauer et al., 2002). The NMDAR-mediated increase in intracellular Ca^{2+}, together with mGluR5 (Rodrigues et al., 2002), leads to the activation of a variety of local protein kinases at the postsynaptic density (PSD), including αCaMKII (Rodrigues et al., 2004a) and likely PKC, that promote STM formation by targeting and modulating the conductance and trafficking of glutamate receptors at LA synapses (Barria et al., 1997; Benke et al., 1998; Rumpel et al., 2005). The combined entry of Ca^{2+} through both NMDARs and L-VGCCs, together with signaling via the BDNF-TrkB pathway, however, may promote the activation of PKA and ERK/MAPK (Schafe et al., 2000; Schafe and LeDoux, 2000). These kinases, and particularly ERK/MAPK, appear to be exclusively involved in the formation of LTM, possibly via translocation to the cell nucleus and activation of transcription factors such as CREB (Josselyn et al., 2001). The activation of CREB by ERK/MAPK promotes CRE-mediated gene transcription (Bailey et al., 1999; Ressler et al., 2002) and the synthesis of new proteins (Schafe and LeDoux, 2000), which likely promotes LTM formation by leading to alterations in the structure of LA synapses (Lamprecht et al., 2002; Ressler et al., 2002; Radley et al., 2006). Intracellular signaling in the postsynaptic neuron, alone, however, does not appear to be sufficient for fear memory formation (McKernan and Shinnick-Gallagher, 1997b; Huang and Kandel, 1998; Tsvetkov et al., 2002). Modifications in presynaptic signaling, possibly engaged by retrograde signaling in the LA via NO and its downstream targets, also appears to be critical

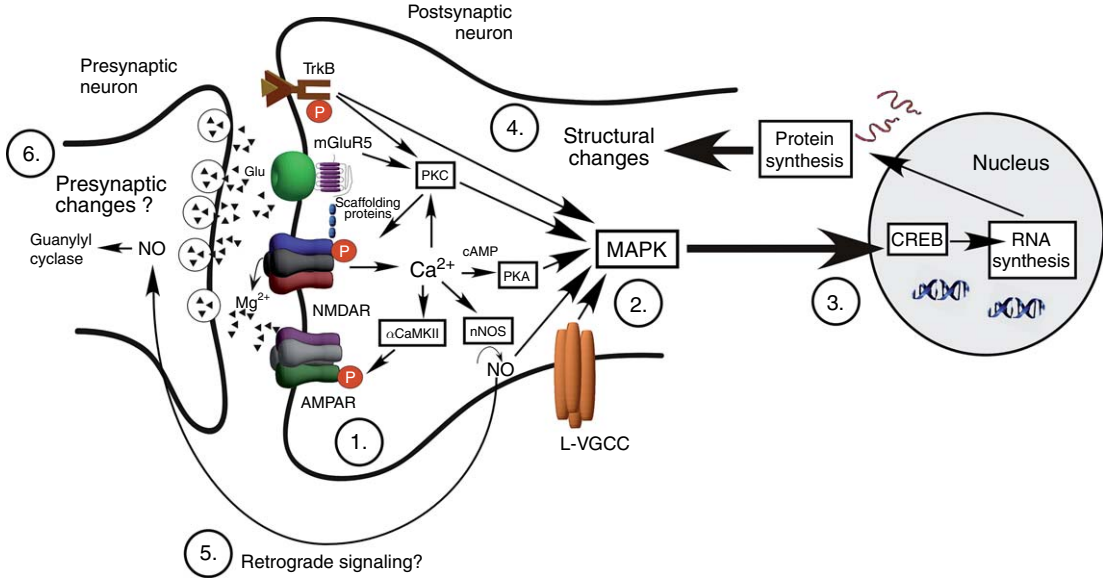

Figure 8 A model of fear memory consolidation in the amygdala. See text for details. (1) Acquisition and STM formation of fear conditioning requires events at the postsynaptic density, including activation of NMDARs, mGluR5, αCaMKII, and possibly PKC. Both αCaMKII and PKC may contribute to STM by influencing the conductance of NMDARs and AMPARs. (2) LTM formation of fear conditioning requires the activation of TrkB receptors, L-type VGCCs, and the cAMP-PKA signaling pathway. These pathways are thought to converge on ERK, which is thought to promote LTM and synaptic plasticity by translocating to the nucleus to influence gene expression. (3) CREB and CRE-mediated transcription are both required for LTM of fear conditioning. (4) The translation of CRE-mediated genes into proteins may lead to structural changes at LA spines that contribute to the permanence of LTM formation. (5) The activation of nNOS in LA neurons may promote retrograde signaling by NO and structural and/or functional changes on the presynaptic side of the synapse (6).

(Schafe et al., 2005). Finally, recent findings emphasizing a distributed network of plasticity in the amygdala have suggested that attention to synaptic plasticity at one synapse, or even one amygdala nucleus, will not be sufficient for a full understanding of how fear memories are acquired or consolidated (Repa et al., 2001; Paré et al., 2004). Accordingly, future experiments will need to consider not only how intracellular signaling mechanisms contribute to fear learning, but also how plasticity across amygdala synapses might be involved in fear memory formation.

4.11.8 Beyond 'Simple' Fear Conditioning

While great strides have been made in identifying the neural and molecular mechanisms that underlie auditory fear conditioning, we have also begun to learn a great deal about more complex aspects of fear learning. In this section, we will explore what is known regarding contextual fear learning, fear extinction, 'reconsolidation' of fear, instrumental fear learning, and memory modulation by the brain's fear system.

4.11.8.1 Contextual Fear Conditioning

In a typical auditory fear conditioning experiment, the animal not only learns to fear the tone that is paired with the foot shock, but also the context in which conditioning occurs. Contextual fear may also be induced by the presentation of foot shocks alone within a novel environment. In the laboratory, fear to the context is measured by returning the rat to the conditioning chamber on the test day and measuring freezing behavior (Blanchard et al., 1969; Fanselow, 1980).

In comparison to auditory fear conditioning, much less is known about the neural system underlying contextual fear. Much of the work examining the neuroanatomical substrates of contextual fear has relied exclusively on lesion methods, and, as in auditory fear conditioning the amygdala appears to play an essential role. For example, lesions of the amygdala, including the LA and basal nucleus, have been shown to disrupt both acquisition and expression of contextual fear conditioning (Phillips and LeDoux, 1992; Kim et al., 1993; Maren, 1998), as has reversible functional inactivation targeted to the LA (Muller et al., 1997). Contextual fear conditioning is also impaired by infusion of antagonists to NMDARs, mGluR5, and CaMKII into the LA, as well as inhibitors of PKA/PKC, RNA, and protein synthesis (Kim et al., 1991; Bailey et al., 1999; Goosens et al., 2000; Rodrigues et al., 2001, 2002, 2004a). Further, a recent study showed that memory consolidation for contextual fear is impaired by infusion of antisense oligonucleotides directed against EGR-1 (Malkani et al., 2004). Collectively, these findings suggest that essential aspects of the memory are encoded and stored in the amygdala via alterations in some of the same intracellular signaling mechanisms that underlie acquisition and consolidation of auditory fear conditioning. At this time, however, there are few data that allow us to distinguish between the involvement of different amygdala subnuclei in contextual fear, although recent lesion evidence suggests that the LA and anterior basal nuclei are critical, but not the posterior basal nucleus (Goosens and Maren, 2001). The CE is, of course, essential for the expression of contextual fear, as it is for auditory fear conditioning (Goosens and Maren, 2001). It remains unknown, however, whether the CE is also required for the acquisition and/or consolidation of contextual fear, or whether distributed plasticity in the LA underlies contextual fear learning.

The hippocampus has also been implicated in contextual fear conditioning, although its exact role has been difficult to define. A number of studies have shown that electrolytic and neurotoxic lesions of the hippocampus disrupt contextual, but not auditory, fear conditioning (Kim and Fanselow, 1992; Phillips and LeDoux, 1992; Kim et al., 1993; Maren et al., 1997). Posttraining lesions appear to be the most effective; pretraining lesions of the hippocampus have occasionally been shown to be without effect (Maren et al., 1997). This is presumably because the animal uses a nonhippocampal strategy to acquire fear to the contextual cues of the environment in the absence of an intact hippocampus during training. Posttraining hippocampal lesions, however, are only effective at impairing contextual fear if given shortly after training. If rats are given hippocampal lesions 28 days after training, there is no memory impairment (Kim and Fanselow, 1992). This 'retrograde gradient' of recall suggests that hippocampal-dependent memories are gradually transferred, over time, to other regions of the brain for permanent storage, an idea that is consistent with the findings of hippocampal-dependent episodic memory research in humans (Milner et al., 1998). The exact mechanism whereby these 'remote' contextual fear memories are consolidated remains unknown, but is thought to involve LTP-like changes in signaling between the

hippocampus and regions of the cortex that make up the individual elements of the contextual representation (Frankland et al., 2001).

What role does the hippocampus play in contextual fear? One prominent view is that it is necessary for forming a representation of the context in which conditioning occurs and for providing the amygdala with that information during training and CS-US integration (Phillips and LeDoux, 1992; Young et al., 1994; Frankland et al., 1998). In support of this view, the hippocampal formation has been shown to project to the basal nucleus of the amygdala (Canteras and Swanson, 1992). This pathway has been shown to exhibit LTP (Maren and Fanselow, 1995), thus providing a potential neuroanatomical substrate through which contextual fear associations can be formed (Maren and Fanselow, 1995). Further, it has recently been shown that intrahippocampal infusions of the protein synthesis inhibitor anisomycin impair the ability of the hippocampus to form a contextual representation, but not the ability of the animal to form a context-shock association (Barrientos et al., 2002). In these experiments, the 'immediate shock deficit' paradigm was used to tease apart the contribution of the hippocampus to learning about a context and learning to fear one. Normally, immediate shock (i.e., that which is given soon after introduction to the conditioning chamber) is not sufficient to support contextual fear conditioning, presumably because it takes time for the hippocampus to form a representation of the context in which the animal finds itself. However, if the animal is *preexposed* to the conditioning chamber briefly on the day before training, it can subsequently acquire contextual fear following immediate shock, presumably because the animal now enters the training situation with a contextual representation already intact (Fanselow, 1980). In the Barrientos et al. study (2002), rats were given an infusion of anisomycin or vehicle into the dorsal hippocampus immediately after exposure to a novel context on the day before they received immediate shock, or immediately after receiving immediate shock on the day after they received preexposure. The findings showed that intrahippocampal anisomycin resulted in impaired contextual learning only in the first group (Barrientos et al., 2002). This important finding suggests that the protein synthesis in the hippocampus is necessary for learning about contexts, but not for contextual fear conditioning. A similar finding has recently been reported by Frankland and colleagues using manipulations of NMDARs, CaMKII, and CREB in the hippocampus (Frankland et al., 2004).

It is clear, however, that the hippocampus undergoes plastic changes during fear conditioning, some of which may be necessary for memory formation of contextual fear. For example, intrahippocampal infusion of the NMDAR antagonist APV impairs contextual fear conditioning (Stiedl et al., 2000; Young and Wang, 2004), and contextual, but not auditory, fear conditioning is impaired in mice that lack the NR1 subunit of the NMDA receptor exclusively in area CA1 of the hippocampus (Rampon and Tsien, 2000). Further, fear conditioning leads to increases in the activation of αCaMKII, PKC, ERK/MAPK, and CRE-mediated gene expression in the hippocampus (Atkins et al., 1998; Impey et al., 1998b; Hall et al., 2000). These findings add support to the notion that NMDAR-dependent plastic changes in the hippocampus, in addition to the amygdala, are required for contextual fear conditioning. However, it should be emphasized that the exact contribution of this NMDAR-mediated signaling to contextual fear conditioning remains unclear. For example, most of these studies cannot distinguish between a role for NMDAR-mediated plasticity in formation of contextual representations as opposed to a role in fear memory acquisition and storage. Further, regulation of intracellular signaling cascades in the hippocampus by fear conditioning, while potentially indicative of some type of memory storage, does not necessarily indicate that these changes are related to the acquisition *fear* memories. They may be related to declarative or explicit memories of the training experience that are acquired at the same time as fearful memories (LeDoux, 2000). Indeed, a number of studies have shown that hippocampal cells undergo plastic changes during and after fear conditioning (Doyère et al., 1995; Moita et al., 2003), including auditory fear conditioning which is spared following hippocampal lesions (Kim and Fanselow, 1992). Clearly, more research is needed before a convincing picture of the role of the hippocampus in contextual fear conditioning emerges.

4.11.8.2 Fear Extinction

Extinction is a process whereby repeated presentations of the CS in the absence of the US leads to a weakening of the expression of conditioning responding. While extinction of conditioned fear has been well documented in the behavioral literature, until recently we learned comparatively little about its neurobiological substrates. Work in a

number of laboratories has recently implicated a number of structures, including the prefrontal cortex, amygdala, and hippocampus.

The medial prefrontal cortex (mPFC), and in particular the ventral mPFC, appears to play an important role in fear extinction. Early studies, for example, showed that selective lesions of the ventral mPFC retard the extinction of fear to an auditory CS while having no effect on initial fear acquisition (Morgan et al., 1993; Morgan and LeDoux, 1995). Further, neurons in the mPFC alter their response properties as the result of extinction (Garcia et al., 1999; Herry et al., 1999). Interestingly, studies by Quirk and colleagues suggest that the mPFC may not be necessary for fear extinction *per se,* but rather for the long-term recall of extinguished fear. For example, rats with mPFC lesions are able to extinguish within a session, but show impaired extinction between sessions (Quirk et al., 2000). Further, neurons in the mPFC fire strongly to a tone CS after behavioral extinction has occurred, and artificial stimulation of the mPFC that resembles responding in an extinguished rat is sufficient to inhibit behavioral expression of fear in nonextinguished rats (Milad and Quirk, 2002). Thus, it appears clear that the mPFC plays an essential role in long-term retention and/or expression of fear extinction. The question of whether the mPFC is a 'site of storage' of extinction or rather simply a region that is necessary for the long-term expression of extinguished memories has only begun to be explored. Recent studies, however, have shown that extinction training regulates the expression of the IEG cFos in regions of the mPFC (Santini et al., 2004). Further, intra-mPFC infusion of inhibitors to MEK or protein synthesis impairs long-term recall of fear extinction (Santini et al., 2004; Hugues et al., 2006), suggesting that essential aspects of the plasticity underlying extinction memory are localized in the mPFC.

The amygdala has also been shown to be an essential site of plasticity underlying fear extinction. Infusions of NMDAR antagonists or inhibitors of ERK/MAPK into the amygdala have been shown to impair fear extinction (Falls et al., 1992; Lu et al., 2001; Davis, 2002; Herry et al., 2006). Conversely, both systemic and intra-amygdala infusions of partial agonists of the NMDA receptor facilitate fear extinction (Walker et al., 2002). More recently, Ressler and colleagues showed that BDNF signaling in the amygdala was critical to the consolidation of fear extinction (Chhatwal et al., 2006). They showed, for example, that fear conditioning leads to an increase in BDNF expression in the LA and basal amygdala. Further, infusion of a viral vector encoding a dominant negative TrkB receptor into the amygdala impaired between-session, but not within-session, retention of fear extinction. These experiments suggest that some type of activity-dependent synaptic plasticity must take place in the amygdala during extinction learning, as it does during initial learning. After the memory of extinction is formed, the amygdala may then signal the mPFC to inhibit ongoing fear responses. Indeed, McDonald and colleagues have shown that the mPFC projects to GABAergic (GABA: gamma-aminobutyric acid) intercalated cells that are situated between the lateral and basal amygdala and the CE (McDonald et al., 1996), which may be important for regulating fear responses (Paré and Smith, 1993; Quirk and Gehlert, 2003; Quirk et al., 2003; Paré et al., 2004). In agreement with this hypothesis, a recent study has confirmed that stimulation of the mPFC neurons blunts the activity of CE neurons that are critical for the expression of fear responses (Quirk et al., 2003). Additional experiments will be necessary to define the exact contribution of connections between the mPFC and the amygdala in extinction processes, as well as the detailed biochemical mechanisms responsible for promoting fear extinction.

One of the more interesting facts about memories that have undergone extinction is that they are context specific. That is, an extinguished memory remains extinguished only in the context in which extinction has taken place, and responding returns or is subject to 'renewal' in a different context (Bouton and Bolles, 1979; Bouton and Ricker, 1994). This fact, along with the finding that fully extinguished memories are capable of 'reinstating' upon presentation of the US (Rescorla and Heth, 1975), has led to the long-held view that extinction does not result in the erasure of the original memory trace but is rather a new kind of learning that serves to inhibit expression of the old memory (Pavlov, 1927). Not surprisingly, recent studies have indicated that the hippocampus plays an important role in the contextual modulation of fear extinction. Maren and colleagues, for example, have shown that training-induced neurophysiological responses in the LA readily extinguish within a fear extinction session, but that this neural representation of extinction, like the behavior itself, is specific to the context in which extinction has taken place (Hobin et al., 2003). Further, functional inactivation of the hippocampus using the $GABA_A$ agonist muscimol can

impair the context-specific expression of fear extinction (Corcoran and Maren, 2001). While it remains unclear how the hippocampus might inhibit the expression of LA spike firing and fear behavior in a context-specific manner, it has been proposed that projections from the hippocampus to the mPFC may be critical (Hobin et al., 2003).

4.11.8.3 Retrieval and 'Reconsolidation' of Fear Memories

Fear extinction is not the only way to turn a fear memory off. Another, perhaps more clinically efficacious way, is to interfere with that fear memory's *reconsolidation*. The idea that memory undergoes a second phase of consolidation, or 'reconsolidation,' upon retrieval has been the subject of speculation for decades (Sara, 2000). Early studies showed that amnesic manipulations at or around the time of memory retrieval, rather than at the time of initial learning, resulted in loss of the memory on subsequent recall tests (Misanin et al., 1968; Lewis et al., 1972). These early findings suggested that the retrieval process could render a memory susceptible to disruption in a manner very similar to a newly formed memory.

Interest in the reconsolidation process has been rekindled in recent years, due in part to the progress that has been made in identifying the cellular and molecular mechanisms underlying long-term synaptic plasticity and the initial phases of memory consolidation (Milner et al., 1998). Accordingly, this has provided researchers with a set of tools and learning paradigms with which to study the reconsolidation process. Several years ago, for example, Nader and colleagues showed that infusion of the protein synthesis inhibitor anisomycin into the amygdala immediately after retrieval of auditory fear conditioning impaired memory recall on subsequent tests (Nader et al., 2000). This effect was clearly dependent on retrieval of the memory; that is, no memory deficit was observed if exposure to the CS was omitted. Further, the effect was observed not only when the initial recall test and drug infusion were given shortly after training (i.e., 1 day), but also if given 14 days later, suggesting that the effect could not be attributable to disruption of the late phases of protein synthesis necessary for the initial training episode. Thus, following active recall of a fear memory, that memory appears to undergo a second wave of consolidation that requires protein synthesis in the amygdala. More recent work has shown that this process does not appear to be attributable to rapid extinction of fear during the recall test, since fear memories that have failed to reconsolidate after intra-amygdala infusion of anisomycin fail to renew in a different context (Duvarci and Nader, 2004). Further, memories that fail to reconsolidate do not appear to be subject to reinstatement (Duvarci and Nader, 2004), a finding which suggests that manipulations of a fear memory at or around the time of retrieval may result in permanent impairment of the memory.

Reconsolidation does not appear to be unique to the amygdala; hippocampal-dependent contextual memories also appear to be sensitive to manipulation at the time of retrieval. In a recent study, Debiec et al. (2002) gave rats intrahippocampal infusions of anisomycin following recall of contextual fear conditioning and found that memory retrieval was impaired on subsequent tests. Interestingly, reconsolidation of contextual fear was impaired even when memory reactivation and intrahippocampal anisomycin treatment were given 45 days after the initial training session, a time when lesion studies have shown that contextual memories should no longer depend on the hippocampus (Kim and Fanselow, 1992). The initial experiments by Kim and Fanselow, however, used only a single recall test after training and hippocampal lesions; the ability of the animal to recall contextual fear on subsequent tests was not examined. Surprisingly, when Debiec et al. *reactivated* the contextual memory prior to making a lesion of the hippocampus, even as long as 45 days after training, subsequent recall was impaired (Debiec et al., 2002). Thus, hippocampal-dependent contextual memories appear to undergo both a cellular and a systems-level reconsolidation following memory retrieval. That is, recall of an older, hippocampal-independent contextual memory must return to the hippocampus during retrieval and undergo a protein synthesis–dependent process of reconsolidation to be maintained. As in most hippocampal studies, however, it remains unclear what information is being reconsolidated – the memory of the context or the contextual fear memory.

How might the reconsolidation process be accomplished at the cellular and molecular levels? Recent studies have shown that fear reconsolidation, like the initial phases of consolidation, requires both PKA and ERK/MAPK in the amygdala (Duvarci et al., 2005; Tronson et al., 2006). Further, transient overexpression of a dominant negative isoform of CREB in the forebrain at the time of memory retrieval impairs

reconsolidation of auditory and contextual fear conditioning (Kida et al., 2002). However, the reconsolidation process does not appear to be a mere recapitulation of the initial consolidation process; there have also been numerous reports of biochemical dissociations between consolidation and reconsolidation. These have included studies that have failed to find impairments in fear reconsolidation following inhibition of RNA synthesis (Parsons et al., 2006) or NO signaling (Schafe et al., 2005) in the amygdala. Further, reactivation of a contextual fear memory induces only a subset of genes in the hippocampus that are activated during the initial phases of memory consolidation (von Hertzen and Giese, 2005), and hippocampal-dependent reconsolidation of a contextual fear memory appears to be characterized by different classes of immediate early genes (Lee et al., 2004). Finally, a recent study has shown that blockade of β-adrenergic receptors in the LA impairs reconsolidation, but not consolidation, of fear conditioning (Debiec and Ledoux, 2004). Clearly, additional studies will be required for a full appreciation of how reconsolidation is accomplished at the cellular level.

4.11.8.4 Instrumental Fear Learning

In addition to its role in the rapid, reflexive learning that characterizes Pavlovian fear conditioning, the amygdala contributes to other fear-related aspects of behavior. Pavlovian fear conditioning, for example, is useful for learning to detect a dangerous object or situation, but the animal must also be able to use this information to guide ongoing behavior that is instrumental in avoiding that danger. In some experimental situations, the animal must learn to make a response (i.e., move away, press a bar, turn a wheel, etc.) that will allow it to avoid presentation of a shock or danger signal, a form of learning known as *active avoidance*. In other situations, the animal must learn *not* to respond, also known as *passive avoidance*. Both of these are examples of instrumental conditioning, and the amygdala plays a vital role in each.

Previously, we mentioned that only the LA and CE were critical for Pavlovian fear conditioning. However, we have recently begun to appreciate the significance of projections from the LA to the basal nucleus of the amygdala from studies that employ fear learning tasks that involve both classical and instrumental components (Killcross et al., 1997; Amorapanth et al., 2000). Amorapanth et al. (2000), for example, first trained rats to associate a tone with foot shock (the Pavlovian component). Next, rats learned to move from one side of a two-compartment box to the other to avoid presentation of the tone (the instrumental component), a so-called 'escape-from-fear' task. Findings showed that, while lesions of the LA impaired both types of learning, lesions of the CE impair only the Pavlovian component (i.e., the tone-shock association; **Figure 9(a)**). Conversely, lesions of the basal nucleus impaired only the instrumental component (learning to move to the second compartment; **Figure 9(b)**). Thus, different outputs of the LA appear to mediate Pavlovian and instrumental behaviors elicited by a fear-arousing stimulus (Amorapanth et al., 2000; **Figure 9(c)**). It is important to note, however, that these findings do not indicate that the basal nucleus is a site of motor control or a locus of memory storage for instrumental learning. Rather, the basal amygdala likely guides fear-related behavior and reinforcement learning via its projections to nearby striatal regions that are known to be necessary for instrumental learning and reward processes (Everitt et al., 1989, 1999; Robbins et al., 1989).

4.11.8.5 Memory Modulation by the Amygdala

Pavlovian fear conditioning is an implicit form of learning and memory. However, during most emotional experiences, including fear conditioning, explicit or declarative memories are also formed (LeDoux, 2000). These occur through the operation of the medial temporal lobe memory system involving the hippocampus and related cortical areas (Milner et al., 1998; Eichenbaum, 2000). The role of the hippocampus in the explicit memory of an emotional experience is much the same as its role in other kinds of experiences, with one important exception. During fearful or emotionally arousing experiences, the amygdala activates neuromodulatory systems in the brain and hormonal systems in the body via its projections to the hypothalamus, which can drive the hypothalamic-pituitary-adrenal (HPA) axis. Neurohormones released by these systems can, in turn, feed back to modulate the function of forebrain structures such as the hippocampus and serve to enhance the storage of the memory in these regions (McGaugh, 2000). The primary support for this model in animals comes from studies of *inhibitory avoidance learning*, a type of passive avoidance learning where the animal must learn not to enter a chamber in which it has previously received shock. In this paradigm, various pharmacological manipulations of the amygdala that affect neurotransmitter or

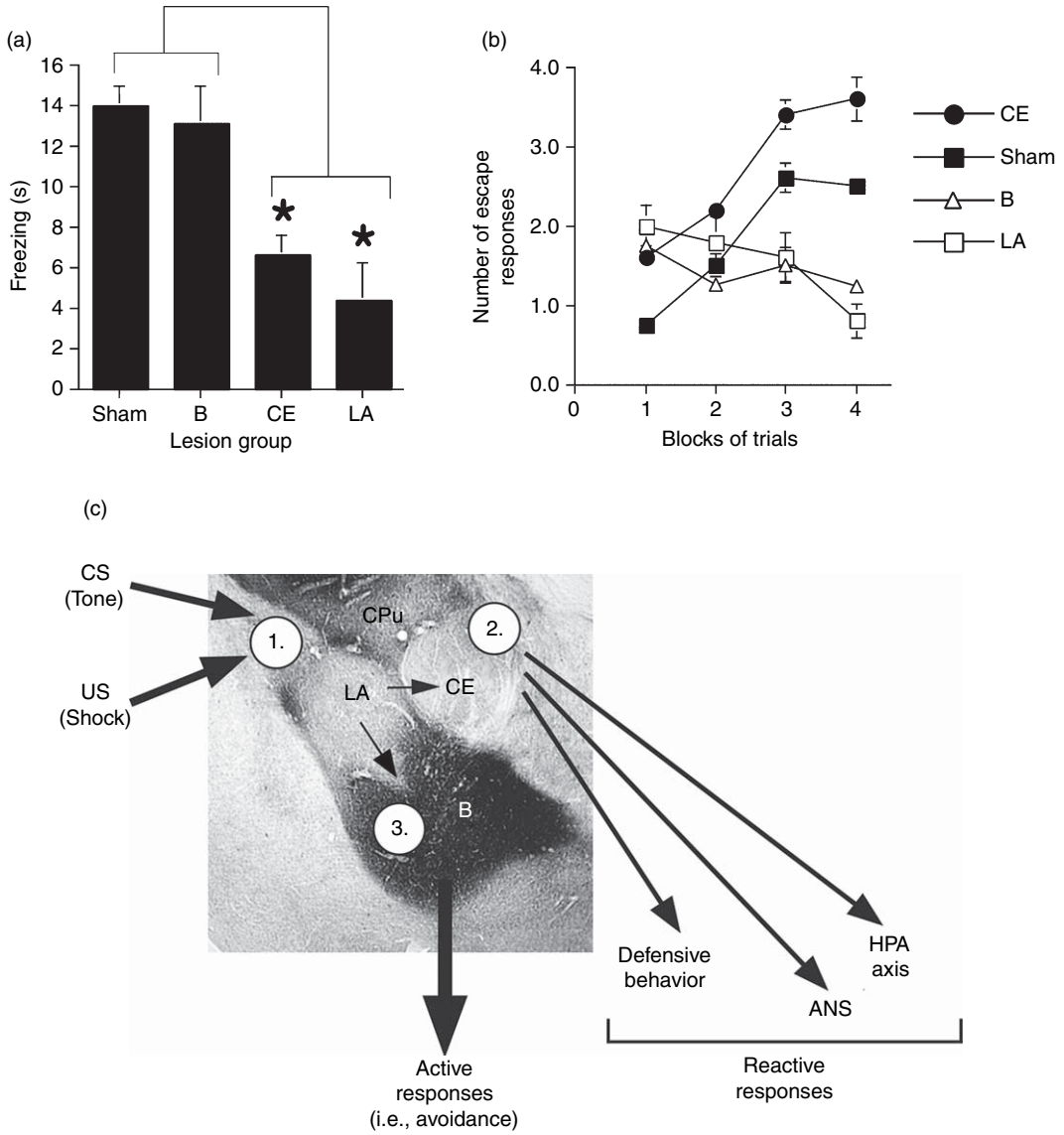

Figure 9 Active versus reactive fear. (a) Percent freezing in rats given auditory fear conditioning after receiving selective amygdala lesions. Auditory fear conditioning is impaired by lesions of the CE and LA, but spared by basal nucleus of the amygdala (B) lesions. (b) Number of escape responses across blocks of five trials during training in a one-way active avoidance task. Lesions of both LA and B impair this task, while lesions of CE do not. (c) The data are consistent with a model in which projections between LA and CE are sufficient for Pavlovian fear conditioning (reactive responses), while projections between LA and B are necessary for instrumental avoidance learning (active responses). HPA, hypothalamic-pituitary-adrenal; ANS, autonomic nervous system. Adapted from Amorapanth P, LeDoux JE, Nader K (2000) Different lateral amygdala outputs mediate reactions and actions elicited by a fear-arousing stimulus. *Nat Neurosci* 3: 74–79, with permission from Nature Publishing Group.

neurohormonal systems modulate the strength of the memory. For example, immediate posttraining blockade of adrenergic or glucocorticoid receptors in the amygdala impairs memory retention of inhibitory avoidance, while facilitation of these systems in the amygdala enhances acquisition and memory storage (McGaugh et al., 1993; McGaugh, 2000). The exact subnuclei in the amygdala that are critical for memory modulation remain unknown, as are the areas of the brain where these amygdala projections influence memory storage. Candidate areas include the hippocampus and entorhinal and parietal cortices (Izquierdo et al., 1997). Indeed, it would be interesting to know whether the changes in unit

activity or the activation of intracellular signaling cascades in the hippocampus during and after fear conditioning, as discussed earlier, might be related to formation of such explicit memories, and how regulation of these signals depends on the integrity of the amygdala and its neuromodulators. Interestingly, a recent study has shown that stimulation of the basal nucleus of the amygdala can modulate the persistence of LTP in the hippocampus (Frey et al., 2001), which provides a potential mechanism whereby the amygdala can modulate hippocampal-dependent memories.

4.11.9 Fear Learning in Humans

Within the last 10 years, considerable progress has been made in understanding how the human fear learning system is organized and what features it shares with the fear learning system of lower vertebrates. In this final section, we will briefly summarize these findings. For a more comprehensive look at this topic, see Phelps and LeDoux (2005).

4.11.9.1 The Human Fear Learning System – Lesion and fMRI Studies

It has long been known that amygdala damage in humans confers deficits in fear conditioning (Bechara et al., 1995; LaBar et al., 1995). In these studies, fear conditioning is typically accomplished by pairing the presentation of visual stimuli with either mild electric shock to the skin or an aversive high-amplitude (i.e., 100 dB or more) tone. Conditioned fear is then measured by changes in skin conductance upon presentation of the CS. Damage to the amygdala in humans produces deficits in conditioned emotional responding to a CS even though the knowledge of the CS-US contingency remains intact (Bechara et al., 1995). That is, a patient with amygdala damage will not respond fearfully to the CS after it has been paired with an aversive US, but is capable of stating that the CS was previously presented and followed by the US. Interestingly, patients with selective hippocampal damage exhibit the converse effect; they will respond fearfully to the CS but cannot tell you why (Bechara et al., 1995).

Fear conditioning in humans also leads to increases in amygdala activity, as measured by functional magnetic resonance imaging (fMRI) (Buchel et al., 1998; LaBar et al., 1998). These changes largely mirror what has been seen in neurophysiological studies of amygdala activity in rodents, namely, increases in CS-elicited amygdala activity during and after fear conditioning, a corresponding attenuation of CS-elicited amygdala activity, and an increase in CS-elicited activity in the mPFC with extinction of the behavioral response (LaBar et al., 1998; Phelps et al., 2004). Further, as suggested by the animal work, the human fear learning system appears preferentially suited to use subcortical 'low-road' information during fear learning. In a study by Morris and colleagues, CS-elicited increases in amygdala activity were observed even if the CS was presented too fast to be perceived consciously, a so-called 'unseen CS' (Morris et al., 1999). When the activity of the amygdala during fear conditioning is cross-correlated with the activity in other regions of the brain, the strongest correlations are seen with subcortical (thalamic and collicular) rather than cortical areas, further emphasizing the importance of the direct thalamo-amygdala pathway in the human brain (Morris et al., 1999).

4.11.9.2 Instructed Fear – Using the High Road

In humans, direct experience with an aversive US does not appear necessary for fear learning to occur. In a series of experiments, Phelps and colleagues have demonstrated that simply telling a human subject that presentation of a CS *might* lead to an aversive outcome is sufficient to induce a learned fear state, a phenomenon known as 'instructed fear' (Phelps et al., 2001).

Like fears that are learned from direct experience, instructed fears require the amygdala (Funayama et al., 2001). Interestingly, however, it is the left amygdala that appears to be the most critical in this type of fear learning. In fMRI studies, the left amygdala is preferentially active in a paradigm utilizing instructed fear (Phelps et al., 2001), and amygdala lesions confined to the left hemisphere are most effective at impairing this type of fear learning (Phelps et al., 2001). In general, this stands in contrast to studies that have examined amygdala activation to fears that have been acquired through experience, especially those involving an 'unseen' CS. In those studies, amygdala activity is typically observed to be lateralized to the right amygdala (Morris et al., 1999). It has been hypothesized that this left lateralization in the instructed fear paradigm is the result of a linguistic/cognitive fear representation acquired through

language, which, like other verbally mediated tasks, is mediated in the majority of individuals in the left hemisphere (Funayama et al., 2001).

4.11.9.3 Declarative Memory Formation and the Amygdala

It has long been recognized that memories formed during emotionally arousing situations are more vividly remembered than those formed under neutral circumstances. Earlier in this chapter, we reviewed evidence from the animal literature which provides a potential neural mechanism for this phenomenon, namely, that the amygdala and its various neurotransmitter systems modulate the strength of explicit or declarative memory formation by influencing the longevity of cellular processes such as LTP in the hippocampus (Frey et al., 2001). Does the human amygdala play a similar role in declarative memory formation? Evidence suggests that it does. For example, administration of the β-adrenergic antagonist propranolol to human subjects impairs long-term recall of an emotionally arousing short story (Cahill et al., 1994), while administration of the $\alpha 2$-adrenergic antagonist yohimbine, which is known to be anxiogenic, enhances recall (O'Carroll et al., 1999). A similar picture emerges in patients with bilateral amygdala damage; they cannot recall the details of an emotionally charged story to the extent that intact controls can (Cahill et al., 1995). Further, amygdala activity appears to correlate with the extent to which an emotionally arousing story is remembered. In one study, subjects in a positron emission tomography scanner were shown either emotionally arousing or emotionally neutral stories and tested for recall at a later time. The findings revealed that right amygdala blood flow during the emotionally arousing, but not neutral, stories correlated highly with the extent to which details of that story could be recalled at later test (Cahill et al., 1996). More recently, Dolan and colleagues studied amygdala-hippocampal activations and recall of emotionally arousing and neutral words in patients with varying degrees of hippocampal and amygdala damage. The findings revealed that left amygdala damage was inversely correlated with memory for the emotional words and also activity in the left hippocampus. Memory for neutral words, in contrast, was only related to the degree of hippocampal damage (Richardson et al., 2004). These findings parallel those found in the animal literature and suggest that interactions between the amygdala and hippocampal formation influence the strength of declarative memory in the human brain.

4.11.10 Conclusions

In this chapter, we have provided a comprehensive view of the neural system underlying fear learning, including the key synaptic events and downstream cellular cascades that are responsible for the acquisition and consolidation of fear memories in the amygdala. These findings provide a foundation for the continued study of the neural basis of emotional learning and memory at the cellular level, and also for bridging the gap between studies of memory formation and synaptic plasticity in the mammalian brain. These studies also provide us with a set of tools to continue our analysis of more complex and clinically relevant aspects of fear learning, including contextual control of learned fear, fear extinction, and reconsolidation. Finally, recent studies translating and extending what we have learned from laboratory rats to the human brain suggest that similar mechanisms and neural pathways are conserved across species.

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4.12 The Molecular Mechanisms of Reward

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4.12.1 Introduction

This article considers the molecular mechanisms which have been implicated in aspects of reward processing and reward-related learning. A brief description of the nature of reward and the animal learning theory associated with its assessment is provided. The neural circuitry involved in implicating these psychological processes is then described, with emphasis placed on the nucleus accumbens (NAc), amygdala, and frontal cortex. Dopaminergic regulation of these structures has been shown to play a pivotal role in mediating reward-related behavior, and the intracellular signaling cascades affected by dopamine release have provided us with novel insight into the molecular basis of reward processing. In particular, data are considered from research into both drug addiction and depression, with a focus on the transcription factors cyclic adenosine monophosphate response element binding protein (CREB) and ΔFosB as well as some of their downstream targets. Dissociable roles are identified for different molecules in the regulation of reward. Furthermore, in parallel to data from neurochemical investigations, the behavioral effects of manipulating molecular pathways depend on both the region targeted and the time course of action. Greater understanding of reward processing at the molecular level is being achieved through combining expertise developed within the fields of psychology and molecular biology. Such an approach can further our knowledge of the

detrimental changes in brain function which are inherent within addiction and affective disorders and which are associated with maladaptive assessment of reward and motivation.

4.12.2 Researching Reward Processes: What Do We Mean by Reward and How Do We Measure It?

Before we consider its molecular basis, we need to establish the nature of the psychological processes covered by the term 'reward.' This topic is dealt with in more detail elsewhere in this reference work (*See* Chapter 1.36); therefore, only a brief summary will be included here to enable discussion of subsequent experimental work. In its most simple terms, a reward is a positive stimulus, i.e., something which the individual values and enjoys. Rewards carry emotional significance, and individuals are motivated to expend effort to attain them. The study of how reward and reward-related stimuli inform behavior contributes a significant amount to our knowledge of learning and memory processes.

In terms of animal learning theory, an innately rewarding stimulus, such as food, is known as an unconditioned stimulus (US). This elicits an unconditioned, automatic response (UR), for example, salivation. If the US is repeatedly paired with a previously neutral stimulus, such as a light, an association will be learned between presentation of the light and food reward, such that illumination of the light alone will cause the animal to salivate. The light is now regarded as a conditioned stimulus (CS), and the response it elicits is termed a conditioned response (CR). This process is known as Pavlovian conditioning and forms the basis of associative learning. Although conceptually quite simple, the effects that a CS can exert over behavior are far-reaching and can influence goal-directed behavior. For example, a rat can learn to press a lever to earn a food pellet through instrumental conditioning processes (see **Figure 1**). If delivery of that food pellet is repeatedly paired with presentation of a light CS, the CS will acquire some of the appetitive value of the food and become rewarding in its own right such that the rat will press the lever to turn the light on. The CS is then called a conditioned reinforcer (CRf).

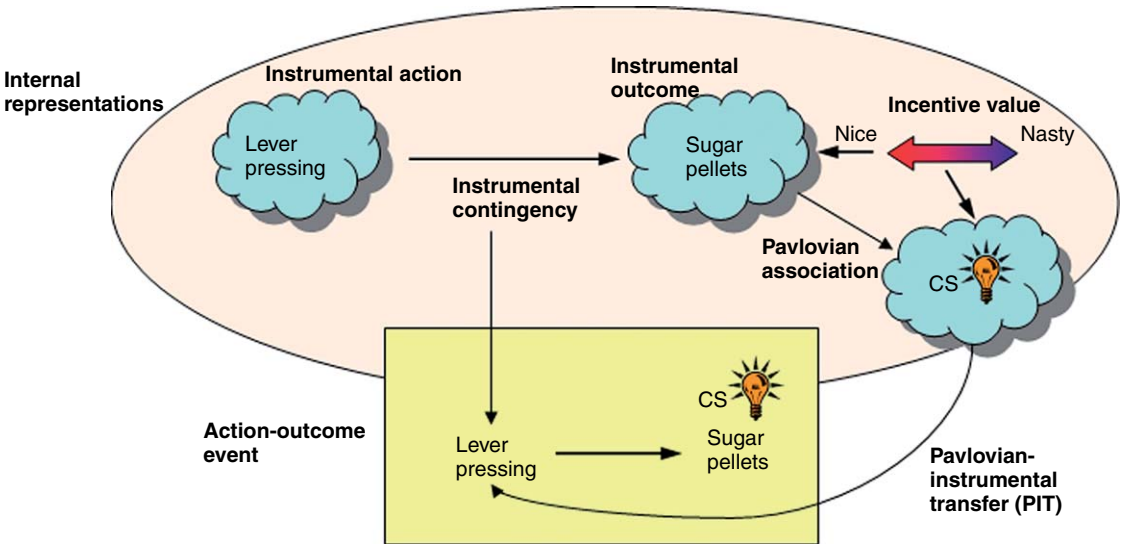

Figure 1 A schematic of some factors which affect instrumental learning. An action such as pressing a lever leads to the delivery of sugar pellets, accompanied by the onset of a stimulus light (the conditioned stimulus: CS). This action-outcome event (contained within the tan box) is detected and represented internally. Degrading the encoding of this contingency will have a direct effect on instrumental performance. The value of the outcome is determined by incentive learning (i.e., how nice the sugar pellets are). This can be affected by the motivational state of the animal. The attribution of incentive value affects the representation of the instrumental outcome, such that changing this value has an impact upon truly goal-directed instrumental behavior (see the section titled 'The prelimbic cortex'). The CS is also associated with the incentive value of the instrumental outcome through Pavlovian conditioning. Presentation of the CS can invigorate responding for the outcome through Pavlovian to instrumental transfer (PIT). Based on Cardinal RN, Parkinson JA, Hall J, and Everitt BJ (2002) Emotion and motivation: The role of the amygdala, ventral striatum, and prefrontal cortex. *Neurosci. Biobehav. Rev.* 26: 321–352; figure 2, p. 327; used with permission from Elsevier Science.

The noncontingent presentation of a CS can also influence ongoing instrumental responding in a process known as Pavlovian to instrumental transfer (PIT). For example, presentation of a tone associated with sucrose delivery will increase lever-pressing for sucrose (conditioned motivation). Conversely, if a light has been paired with a painful stimulus such as footshock, presentation of the light CS will decrease lever-pressing for reward (conditioned suppression).

These concepts inform more than just animal learning theory: experiments designed using these psychological constructs provide valuable insight into the processing of reward in psychopathology. The inability to find stimuli rewarding, as well as an excessive desire for certain rewards, are symptoms of several mental disorders, including depression, attention-deficit/hyperactivity disorder and drug addiction, among others. Research into the mechanisms of reward-related learning has therefore contributed to our understanding of these conditions. Just as a CS paired with food stimulates lever-pressing for reward in rats, stimuli paired with drug reward can lead to craving for drug and relapse to drug-seeking in both rats and human addicts (de Wit and Stewart, 1981; Childress et al., 1988). Failure to alter behavior when the incentive value of a reward changes also has obvious implications for substance abuse disorders, where addicts continue to use drugs despite increasingly negative consequences and reductions in the reward experienced. In rats, food reward can be devalued by pairing food delivery with an injection of lithium chloride, which induces nausea, or by feeding animals to satiation. Such devaluation procedures subsequently alter the way in which animals respond to food presentation or to a CS paired with that reward, making it possible to investigate the biological basis of this aspect of reward processing.

4.12.3 The Neural Circuitry of Reward

Considerable evidence has been amassed concerning the neural circuitry underpinning reward-related learning (see Cardinal, 2001, for review). The processing of reward occurs in a distributed network of structures comprising both cortical and subcortical areas, the majority of which are connected within the limbic or affective corticostriatal loop (**Figure 2**) (Alexander et al., 1986). Within this framework, structures involved in higher-order cognitive function such as the prefrontal cortex (PFC) interact with areas of the limbic system heavily implicated in emotional processing and memory, such as the amygdala and hippocampal formation. These structures are interconnected with the NAc, often described as the reward center of the brain. This circuit influences motor output and motivation via the ventral pallidum and mediodorsal thalamus, which also project back to the PFC. We will focus on three of the most studied areas: the NAc, the amygdala, and regions of the PFC. Given that the focus of this chapter is to discuss the molecular basis of reward, the majority of research of which has been undertaken in rodents, we will focus on data supporting a role for these regions in the reward system of the rat, although these areas and their homologues have been heavily implicated in reward processing in both monkeys and humans.

4.12.3.1 The Nucleus Accumbens

The NAc is probably the most widely studied region in terms of regulating reward-related learning. This region has been labeled the 'limbic-motor interface' due to its extensive connections with limbic structures, such as the amygdala, hippocampus, and PFC, in addition to its projections to motor output areas. The NAc is therefore thought to be a key node in the limbic corticostriatal loop, wherein diverse types of information from both cortical and subcortical structures are integrated and key signals generated to enable the implementation of behavioral change relevant to goal-seeking. The NAc can be divided into the core (NAc-C) and shell (NAc-Sh) subregions, which differ in both structure and function (Groenewegen et al., 1987; Voorn et al., 1989; Berendse et al., 1992). Whereas the NAc-C projects predominantly to the ventral pallidum, the shell also projects to subcortical structures, including the lateral hypothalamus and periaqueductal grey. Damage to the NAc does not prevent animals from making a response to earn food reward, or from adjusting their responding when the value of that reward changes, i.e., animals are still capable of goal-directed behavior (see the section titled 'The prefrontal cortex'). However, the ability of a CS to regulate behavior is profoundly affected by NAc lesions. Damage to the NAc-C disrupts PIT and the acquisition of autoshaping, a Pavlovian conditioning paradigm where presentation of a CS with food delivery leads animals to approach the CS (Parkinson et al., 1999; Hall et al., 2001). Damage to the NAc also impairs conditioned place preference, where animals learn to associate a specific context or place with reward delivery and therefore spend more time in this location. Neither

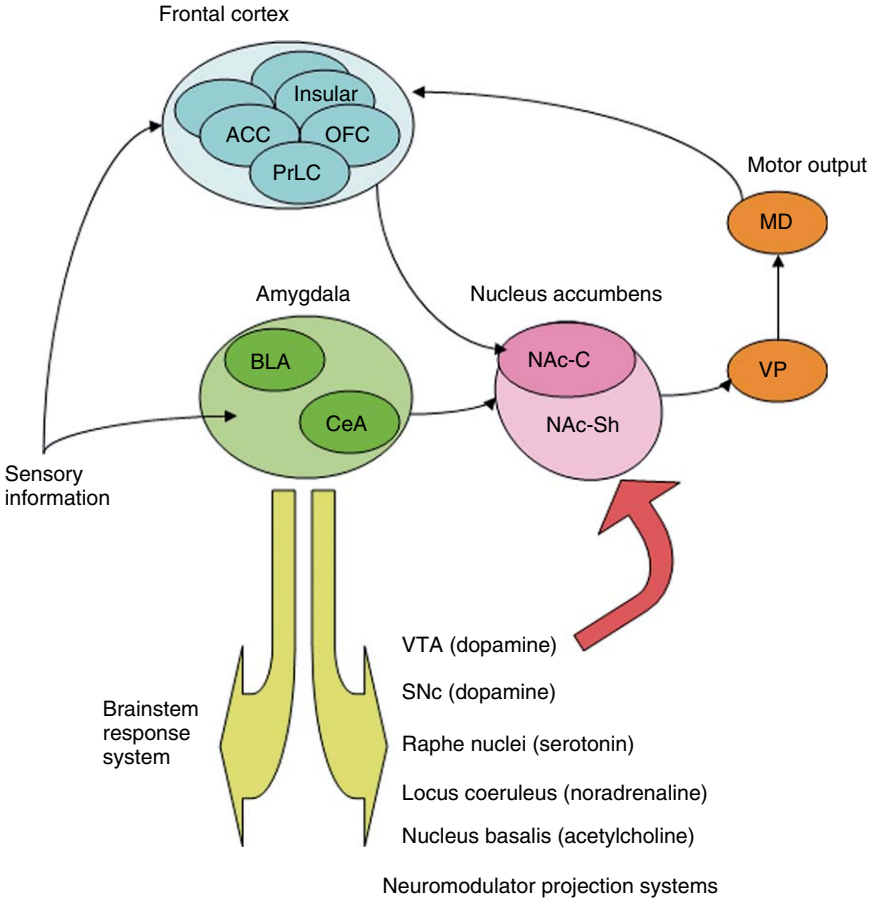

Figure 2 Simplified diagram of the limbic corticostriatal loop. (Abbreviations: ACC, anterior cingulate cortex, OFC, orbitofrontal cortex; PrLC, prelimbic cortex; BLA, basolateral amygdala; CeA, central amygdala; VTA, ventral tegmental area; SNc, substantia nigra pars compacta; NAc-C, nucleus accumbens core; NAc-Sh, nucleus accumbens shell; VP, ventral pallidum; MD, mediodorsal thalamus). The frontal cortex is functionally heterogeneous, and several frontal regions are involved in different aspects of instrumental responding. As discussed in the text, the PrLC, part of the medial prefrontal cortex, is involved in detecting the instrumental action-outcome contingency and is essential for the maintenance of goal-directed behavior. The functions of the ACC are complex and are not described in detail here, but involve resolving response conflict and error detection, whereas the insular cortex, containing the primary gustatory cortex, encodes the primary sensory qualities of specific foods. The OFC plays a role in integrating changes in the incentive value of a reward with representations of the expected outcome, a function which is thought to depend on its connections with the BLA. The BLA is one of the primary structures involved in encoding CS-US associations and is necessary for the presentation of a CS to trigger retrieval of the motivational value of its associated US. It can work in concert with the CeA to influence brainstem function, arousal, and neurotransmitter release. As the 'limbic-motor interface,' the NAc combines information from both frontal and amygdalar systems, as well as from other inputs, to generate motivational drive. The NAc-Sh signals the motivational properties of unconditioned (primary) reinforcers, whereas the NAc-C has a more pronounced role in mediating the motivational impact which Pavlovian conditioned stimuli have on behavior. Adapted from Cardinal RN, Parkinson JA, Hall J, and Everitt BJ (2002) Emotion and motivation: The role of the amygdala, ventral striatum, and prefrontal cortex. *Neurosci. Biobehav. Rev.* 26: 321–352; figure 3, p. 329; used with permission from Elsevier Science.

damage to the core or shell abolishes CRf *per se*, but manipulations of the dopaminergic innervation of the NAc can alter responding for CRf (see the section titled 'Dopamine and reward'). As well, damage to the NAc results in decreased tolerance to the delay of reward, such that animals will choose a small immediate reward over a larger but more delayed one, a concept which has direct relevance to models of impulsive behavior (Cardinal et al., 2001). In contrast, selective lesions of the NAc-Sh do not appear to have such a pronounced effect on the conditioned responses to rewards, i.e., reward-related learning, but this region plays an important role in the unconditioned response to primary reinforcers. In particular, the NAc-Sh appears to alter

the motivational impact of rewards, such that inhibition of NAc-Sh activity induces overeating, an effect attributable to its connections with the lateral hypothalamus (Stratford and Kelley, 1999). Inhibition of neuronal firing is also observed when animals engage in a sequence of reward-seeking and consumption (Taha and Fields, 2006), which results in disinhibition of activity in target brain regions such as the hypothalamus. Activity within the NAc may therefore act to gate appetitive behavior through its influence over reward-related brain circuitry.

4.12.3.2 The Amygdala

The first indication that the amygdala was one of the most important brain regions for the processing of affective stimuli was the discovery that damage localized to this area produced marked deficits in emotional display and apparent fearlessness in monkeys (Kluver and Bucy, 1939). Humans with damage to the amygdala show a variety of impairments in emotional perception and expression, and can unwittingly endanger themselves through failing to process danger or risk. The amygdala can be divided into multiple nuclei based on cytoarchitectonic distinctions (Pitkanen, 2000). Functional dissociations have also been observed between the different units. In particular, the central nucleus (CeA) and basolateral nucleus (BLA) of the amygdala have been implicated in divergent forms of affective processing (Everitt et al., 2000). Both the BLA and CeA receive sensory input, yet the BLA has more prominent connections with the frontal cortices and ventral striatum, while the CeA shares more numerous connections with areas within the hypothalamus and brainstem.

It is generally thought that the BLA plays an integral role in Pavlovian conditioning involving both appetitive and aversive stimuli (*See* Chapter 4.11) (Davis, 1998; LeDoux, 2000). One of the most commonly used paradigms to measure emotional learning is fear conditioning, in which animals form an association between a painful footshock and a particular stimulus such as a light or tone. Animals rapidly learn this association and freeze during subsequent presentations of the CS, indicative of a state of fear, yet this freezing is much less evident following BLA or CeA lesions. More sophisticated analysis of Pavlovian conditioning procedures suggests that the BLA is necessary in order for presentation of the CS to trigger retrieval of the value of the US with which it was paired. Although BLA-lesioned animals show evidence of learning simple CS-US associations, changing the value of a reward does not alter the way in which the animal responds to the associated CS. For example, BLA-lesioned rats show aversion to a devalued food, but still approach the food magazine when the CS paired with the devalued food is presented (Hatfield et al., 1996). Likewise, although BLA-lesioned monkeys show preference for different foods (i.e., can still make value judgments), they are unable to alter their choice preference when the value of a particular food has been changed through devaluation. This idea that the BLA is involved in processing the incentive value of rewards and reward-related stimuli associated with them has been very influential and is thought to depend on its connections with the orbitofrontal cortex (OFC) (Baxter et al., 2000; see the section titled 'The orbitofrontal cortex'). Given its proximity to the hippocampus and other limbic structures heavily implicated in memory processing and storage, the BLA is ideally positioned to mediate the effect of emotional arousal on memory.

4.12.3.3 The Prefrontal Cortex

The PFC is involved in numerous higher-order cognitive functions, such as decision-making, attention, problem solving, strategy development, and working memory (*See* Chapter 3.14). Such processes exert powerful control over goal-directed behavior. The PFC is both structurally and functionally heterogeneous, and a discussion of the role of each subregion is beyond the scope of this article. We will focus on two areas which appear to be particularly important in reward processing: the medial PFC (mPFC) as exemplified by the prelimbic and anterior cingulate regions, and the ventral PFC encompassing the orbitofrontal and agranular insular cortices.

4.12.3.3.1 The prelimbic cortex

This region of the rat mPFC is involved in the acquisition of goal-directed behavior. In order for action to be considered goal-directed, it must fulfill two criteria: (1) the animal must be aware of the *causal* link between the instrumental action and its outcome (i.e., a rat pressing a lever for food knows that pressing the lever results in delivery of food reward, and (2) the outcome for which the animal is responding must be considered a goal by the animal (i.e., the rat *wants* the food). Instrumental responding ceases to be goal-directed once it becomes habitual, that is, the animal is insensitive to changes in the incentive value of the reward or to the presence of an instrumental contingency. Behavior is instead controlled by simple stimulus-response (S-R)

associations in which stimuli or outcomes become directly associated with a motor response, so that the rat responds on the lever regardless of its motivational state. Damage to the prelimbic cortex (PrLC) not only retards acquisition of instrumental responding, but also disrupts the detection of instrumental contingencies (Balleine and Dickinson, 1998; Corbit and Balleine, 2003). These data indicate that rats with PrLC damage may acquire instrumental responses based on S-R associations and are no longer truly capable of goal-directed learning. The transition from goal-directed to habitual S-R responding can also happen naturally over time with repeated training and has some advantage in that it is thought to use fewer cognitive resources. However, habits are less flexible than goal-directed actions and can lead to maladaptive behavior, such as that commonly associated with addiction, where environmental stimuli trigger engagement in drug-taking even though drug intake itself is no longer rewarding.

In keeping with these data indicating that the mPFC is involved in maintaining cognitive flexibility, the PrLC is also thought to play an important role in extinction processes. Extinction refers to the decline in responding when that response no longer leads to the associated outcome. The role of the PrLC has been extensively studied in the extinction of conditioned fear (see Sotres-Bayon et al., 2004, for review). Repeated presentation of the CS in the absence of the associated shock reduces the ability of the CS to elicit fear-related responses such as freezing as the animal learns that the CS is no longer a reliable predictor of the US. This ability to update knowledge about what is, and what is not, an accurate predictor of a dangerous event is clearly important for adaptation and survival. Lesions to the mPFC encompassing the PrLC impair extinction of conditioned fear. The deficits in extinction observed in the absence of the PrLC may relate to the well-documented role of the frontal cortex in mediating behavioral inhibition and perseveration. Disconnection of the mPFC and the BLA also attenuates extinction of conditioned fear, suggesting that activity within the mPFC may act to inhibit the representations of the emotional value of the CS generated by the amygdala, highlighting the importance of prefrontal regulation of amygdala function in reward-related learning.

4.12.3.3.2 The orbitofrontal cortex

Perhaps more than any other region of the frontal cortex, the OFC has been heavily associated with the processing of rewarding or emotional stimuli and events. In humans, damage to the OFC produces a pattern of aberrant social behavior and maladaptive decision-making which is often described as impulsive. This behavior can be exemplified by performance of these patients on laboratory-based gambling tasks where subjects choose between different options to earn points. The optimal strategy is to choose options associated with small immediate gains but also low and infrequent losses, an approach which healthy volunteers learn. Persistent selection of options leading to large immediate gain but heavy losses in the long term is thought to reflect risky decision-making and is observed both in pathological gamblers (Cavedini et al., 2002) and substance abusers (Bechara et al., 2001) and in patients with damage to the OFC or BLA (Bechara et al., 1999). In the monkey, neurons within the OFC have been shown to fire preferentially to different food rewards and to decrease their firing rate specifically to a devalued reward (Critchley and Rolls, 1996). Similar to the BLA, the OFC therefore appears to be involved in creating representations of the incentive value of reward. The reciprocal connections between these two regions are well documented, and disconnection of the OFC and BLA prevents devaluation of reward from altering choice behavior in monkeys (Baxter et al., 2000). However, electrophysiology recordings in the rat suggest that the OFC may have a more sophisticated role to play in using this information. In a series of elegant studies, Schoenbaum and colleagues have developed the hypothesis that the OFC supports representations of outcome expectancy, that is, how rewarding the outcome of a certain action is anticipated as being (Schoenbaum et al., 2006). The BLA generates important information about the incentive value of reward-associated stimuli, which the OFC then uses to generate representations of the anticipated outcome predicted by those CS. Such outcome expectancy is then used to inform choice behavior.

Lesions to the OFC also affect aspects of impulsive and compulsive behavior in animals. As in humans, damage to the rodent or monkey OFC increases perseverative behavior and decreases cognitive flexibility. For example, in reversal learning paradigms, OFC-lesioned rats perseverate in responding to the previously rewarded stimulus (Schoenbaum et al., 2002; Chudasama and Robbins, 2003). In delay-to-reinforcement paradigms where rats choose between a small immediate versus a larger increasingly delayed reward, OFC-lesioned rats do not show such a strong aversion to the delay compared to their sham controls (Winstanley et al.,

2003). This deficit may arise from both perseverative tendencies as well as an inability to integrate the consequences of making a response with the incentive value of the reward, i.e., the delay does not sufficiently devalue the reward.

4.12.4 Dopamine and Reward

Converging evidence from numerous studies has implicated dopamine as the single most important neurotransmitter involved in the signaling of reward. Using intracranial self-stimulation techniques, where animals respond for electrical stimulation into a particular region of the brain, it has been shown repeatedly that animals will work for stimulation of their dopamine system (Wise and Rompre, 1989). Likewise, the addictive properties of drugs of abuse can be attributed in part to their ability to potentiate dopaminergic transmission. In particular, dopaminergic regulation of the NAc is critically involved in this process. The ventral tegmental area (VTA) sends dopaminergic projections to numerous regions within the brain including the NAc, PFC, and other parts of the limbic system. Both natural and drug rewards increase dopamine efflux in the NAc-Sh, whereas CS associated with such reward increase dopamine efflux in the NAc-C. Although animals are still capable of finding things rewarding or pleasurable in the absence of dopamine, they are no longer motivated to earn reward (Salamone et al., 2003), i.e., they are no longer capable of goal-directed behavior. Dopaminergic depletion of the NAc significantly decreases the amount of effort rats are willing to expend to earn reward, whereas manipulations which increase NAc dopamine function enhance goal-seeking.

Several groups have recorded from dopaminergic cells within the VTA in monkeys during Pavlovian conditioning paradigms. Using this methodology, it has been found that the firing of dopamine neurons may signal error prediction, i.e., they are particularly active when an unexpected reward is delivered, and firing is suppressed when an expected reward does not appear (Montague et al., 2004; Schultz, 2006). The potentiation of dopamine function caused by drugs of abuse may therefore generate a powerful signal that the reward was larger or better than expected regardless of the actual experience created by the drug (Hyman et al., 2006). Not only are psychostimulant drugs like amphetamine rewarding in their own right, they also enhance the effects of conditioned reinforcers, an effect which can be induced by direct application of amphetamine or dopamine agonists into the NAc, and which is blocked by dopaminergic lesions of the NAc and by ablation of the NAc-Sh (see Cardinal et al., 2002). Similarly, PIT can be enhanced by intra-NAc amphetamine and is abolished by dopamine receptor antagonists. This general potentiation of reward-related learning and reward-seeking likely plays an important role in the generation and maintenance of addiction.

Dopamine also regulates reward-related processing within the PFC. Data from both *in vivo* observations and computational modeling has led to the suggestion that phasic dopamine release acts as a gating mechanism, signaling when internal representations of reward and related stimuli need to be updated (Cohen et al., 2002). Damage to dopaminergic innervation of the PFC alters reward-related learning in a manner consistent with this theory. For example, lesions to dopaminergic inputs to the mPFC cause a deficit in fear extinction (Morrow et al., 1999), and ablation of dopaminergic terminals within the OFC leads to persistent choice of a larger but delayed reward, similar to excitotoxic lesions of these structures. Pharmacological manipulations also suggest that there is an optimum level of dopamine function within the PFC, and that too much as well as too little can have a negative impact on a range of cognitive behaviors (Arnsten, 1997). Long-term drug use leads to cognitive deficits that have been largely attributed to dysfunction of the frontal cortex (Rogers and Robbins, 2001), and hypofunction of the OFC has been observed in recently abstinent cocaine abusers (Volkow and Fowler, 2000). In rats, repeated exposure to addictive drugs has been shown to alter reward-related learning in tasks known to be dependent on the integrity of the OFC (Schoenbaum et al., 2004). Given the importance of the dopamine system in facilitating reward-related learning and the ability of addictive drugs to modulate this system, it seems likely that dysregulation of the dopaminergic input to frontal regions is responsible for these cognitive impairments.

4.12.5 Cellular and Molecular Targets of the Dopamine-Reward System: Insights from Drug Addiction

Given that the dopaminergic system has been heavily implicated in mediating the highly rewarding nature of addictive drugs, and that addictive behavior appears to arise from the hijacking of normal reward systems, a significant proportion of the data concerning the

molecular basis of reward have been obtained through studying the effects of drugs of abuse. The intracellular changes that occur following acute administration of an appetitive substance like cocaine can provide valuable information about the signaling cascades activated by such rewarding substances. However, the changes seen after repeated administration are of more relevance in determining the molecular basis of the alterations in reward-related learning underpinning the addicted state. Drug addiction is a chronic and often relapsing disorder, with human addicts remaining at risk of relapse even after years of abstinence. The fact that chronic drug intake produces such durable changes in brain function and behavior has led to the suggestion that long-lasting changes in gene transcription may play a prominent role (Nestler et al., 1993). This has led to a search for relatively stable markers of altered transcriptional regulation whose persistence matches the time course of aspects of addictive behavior. The contrasting effects of acute versus chronic administration of addictive drugs on intracellular signaling pathways can therefore provide information about different aspects of reward processing. The role played by these molecular mechanisms in the processing of natural rewards has also been studied in learning and memory paradigms, and also in animal models of depression.

The binding of dopamine, or any neurotransmitter or signaling molecule, by its membrane-bound receptors triggers the initiation of several intracellular signaling cascades which often culminate in the regulation of transcription factors (TFs), including those encoded by immediate early genes (**Figure 3**). In terms of our understanding of reward processing and addiction, a considerable amount of data is now available concerning the particular TF families activated by dopaminergic agents. In this section, we will focus on some specific examples of this aspect of gene regulation and consider the role of these different TFs in the development of addiction as well as in the response to natural rewards. In terms of reward-related learning, we will largely restrict our discussion to key areas within the affective corticostriatal loop highlighted in the previous section. However, it should be noted that many of these intracellular signaling pathways have been implicated in the emotional memory processes

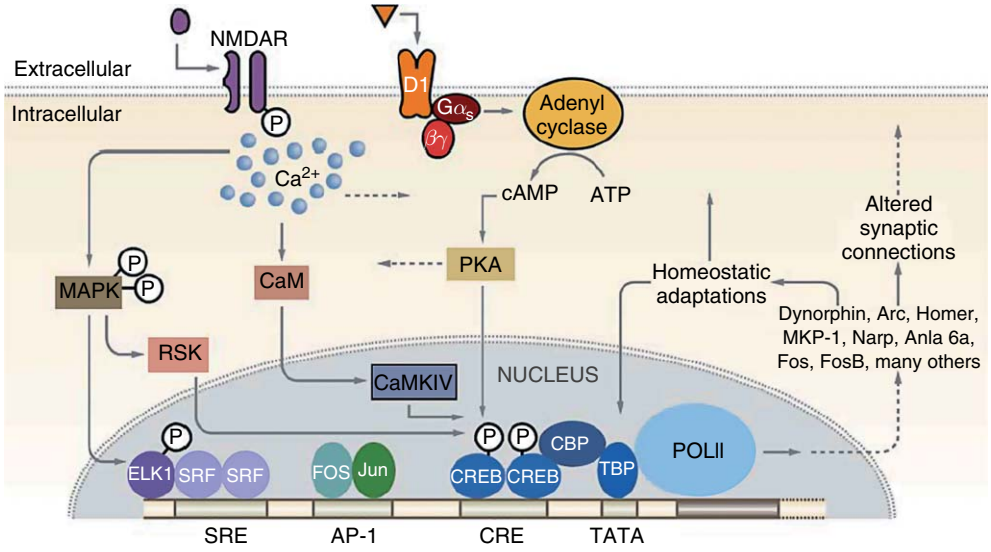

Figure 3 Regulation of gene expression within the striatum by dopamine and glutamate. Stimulation of dopaminergic D_1 receptors and glutamate receptors activates intracellular second messenger signaling cascades which result in changes in gene expression within the cell nucleus. Shown here are examples of DNA binding sites within the cFos promoter, including a serum response element (SRE), an activator protein-1 element (AP-1), and a cyclic adenosine monophosphate (cAMP) response element (CRE). Numerous other genes are also activated, including Homer, Arc, FosB, etc. Abbreviations: CBP, CREB binding protein; CREB, cAMP response element binding protein; MAPK, mitogen-activated protein kinase; NMDAR, *N*-methyl-D-aspartate glutamate receptor; PKA, protein kinase A; TBP, TATA binding protein; RSK, ribosomal S6 kinase; CaMKIV, Ca^{2+}/calmodulin-dependent kinase IV; ELK1, Ets-like transcription factor; SRF, serum response factor; POLII, RNA polymerase II; TATA describes a short sequence of base pairs that is rich in adenine (A) and thymidine (T) residues. Reprinted from Hyman SE, Malenka RC, and Nestler EJ (2006) Neural mechanisms of addiction: The role of reward-related learning and memory. *Annu. Rev. Neurosci.* 29: 565–598; used with permission from the Annual Reviews Permission Department.

involved in fear conditioning through their actions in the hippocampus (HPC).

Several techniques have been combined to determine whether these changes in TF regulation alter addiction-related behavior. Mice lacking these TFs have been developed, as have transgenic mice which either overexpress certain TFs or express mutant or dominant negative proteins which inhibit their effects. Reporter lines have also been developed, where the promoters regulated by a particular TF (e.g., cAMP response elements (CREs)) drive expression of a reporter gene expressing an easily visualized marker, such as β-galactosidase or green fluorescent protein (GFP). Such mice enable the consequences of different environmental manipulations on that TF to be determined. As well, viral vectors designed to express these proteins can be infused into specific regions using standard stereotaxic surgical techniques, which localizes the effects of changes in gene transcription to particular areas of interest. A small number of studies have infused antisense oligonucleotides into a certain brain area, although concerns remain about the toxicity and specificity of this approach. In addition to monitoring changes in cellular excitability and synaptic plasticity caused by these manipulations, development of these tools has made it possible to investigate the role these TFs play in reward-related learning. The majority of these studies have used conditioned place preference to assess the hedonic impact of substances of abuse given the relatively high throughput of this method. Instrumental responding for drug reward can also be assessed using self-administration paradigms, where animals learn to press a lever to obtain a drug infusion delivered into an indwelling intravenous catheter.

As mentioned before, one of the factors central to understanding addiction is the changes in behavior and brain function which occur following repeated rather than acute administration. In terms of behavioral output, one of the most widely studied phenomena is that of locomotor sensitization, whereby repeated administration of virtually any abused drug leads to a potentiation of the hyperlocomotor response seen after an acute drug injection in rodents. This increased sensitivity to the motor stimulating properties of addictive drugs is long-lasting, indicating that it could be mediated by some of the long-term changes in gene transcription and brain function which characterize the persistent nature of addiction. However, human addicts do not show sensitization to the arousing effects of drugs like cocaine following repeated use, with most users reporting tolerance of the drugs' stimulant effects. Nevertheless, it is hoped that understanding the changes in neuronal activity that accompany the development of locomotor sensitization may provide valuable insight into the changes in brain function caused by long-term drug use. The behavioral phenotype is very robust and easy to study, thereby facilitating the investigation of its underlying neurobiology. Furthermore, there is evidence to suggest that sensitization to psychostimulants enhances their ability to potentiate the impact of CS on behavior (Taylor and Horger, 1999). Some of the mechanisms underlying behavioral sensitization could also be involved in mediating the powerful ability of addictive drugs to influence goal-directed behavior and stimulate drug-seeking.

Given that addictive drugs heavily stimulate the dopamine system to cause long-term behavioral changes, it is likely that the regulatory mechanisms controlling neuronal plasticity within reward circuitry are targets of drugs of abuse. Repeated activation of neurotransmitter receptors leads to a change in the physiological state of neurons, rendering them more or less sensitive to subsequent stimulation. This could be mediated via changes in the effective strength of synaptic connections (referred to as synaptic plasticity), or via changes in the overall excitability of the affected neurons (referred to as whole-cell plasticity). The former in particular have been implicated in learning and memory processes. In keeping with this hypothesis, chronic administration of several, but not all, addictive drugs increases dendritic spine formation within the NAc (Robinson and Kolb, 2004). This increase in synaptic plasticity is proposed to underlie the locomotor sensitization discussed earlier. Considering the importance of the NAc in mediating the rewarding properties of addictive drugs, the majority of molecular studies have focused on manipulating gene transcription in this region.

4.12.5.1 The CREB and Fos Families of TFs

Ligand-binding at the dopamine D_1 receptor activates the cAMP second messenger signaling cascade, leading to the phosphorylation of protein kinase A (PKA) which can in turn phosphorylate downstream protein targets. Among the prominent targets of this signaling cascade is CREB. This transcription factor is constitutively expressed at fairly high levels throughout the brain, but needs to be phosphorylated for full transcriptional activity. In addition to PKA, CaM kinases such as Ca^{2+}/calmodulin kinase type IV (CaMKIV) and growth factor-associated kinases are all capable of

performing this phosphorylation event, indicating that CREB activation is a point of functional convergence for several signaling pathways. Dimers of CREB bind to CRE sites located within the promoter regions of certain genes and alter the rate at which they are transcribed. More information about this pathway can be found in other chapters in this reference work (*See* Chapters 4.21, 4.27).

Acute administration of psychostimulant drugs stimulates phosphorylation of CREB through D_1 receptor-dependent mechanisms at several nodes within the reward circuitry, including the VTA, amygdala, PFC, and NAc. Increased expression of cFos, a product of an immediate early gene, is observed in similar locations, and its induction may be partly dependent on CREB activation, at least for amphetamine (Konradi et al., 1994). This occurs through CRE sites present in the promoter region of the cFos gene. cFos expression is also induced by several other intracellular signaling cascades, in particular, serum response factor (SRF) acting on serum response elements (SREs) within the cFos promoter. cFos is a member of the Fos family of transcription factors, which includes FosB, Fra1, and Fra2. These proteins heterodimerize with members of the Jun protein family to form the activator protein 1 (AP-1) transcription factor complex, which then binds to AP-1 sites within gene promoter regions. Increases in the activation of both cFos and CREB are rapid and transient, returning to basal levels within hours of the acute stimulus. In fact, repeated administration of drug causes the induction of cFos to desensitize, so that subsequent drug exposure no longer induces the robust elevation seen following first administration (Nestler et al., 2001). A similar pattern is observed in the expression of FosB. In contrast, the activation of CREB appears to become greater and more persistent with repeated drug exposures, an effect most firmly established within the NAc (Shaw-Lutchman et al., 2003). This pattern of expression suggests a more pronounced role for CREB-mediated gene transcription in aspects of addiction.

However, in contrast to all the TFs mentioned so far, isoforms of a truncated splice variant of FosB, known as ΔFosB, is only induced at high levels within the same reward-related areas following chronic, *but not acute*, administration of addictive drugs (**Figure 4**) (Nestler et al., 2001). The 35–37 kDa isoforms of ΔFosB dimerize predominantly with JunD to form an active AP-1 complex. These isoforms of ΔFosB also have an unusually long half-life due to their resistance to degradation by the proteosome, a property conferred at least in part by its truncated C-terminus and by a casein kinase 2–mediated phosphorylation (McClung et al., 2004). Once induced, levels of ΔFosB have been detected up to 2 months after cessation of drug treatment. Such accumulation has been observed following treatment with virtually any addictive substance, including cocaine, *d*-amphetamine, morphine, nicotine, alcohol, and phencyclidine (PCP). This relatively unique pattern of induction and stability has lead to the suggestion that ΔFosB may be a particularly important mediator of long-term changes in gene regulation associated with addiction.

Activation of PKA within the NAc reduces the rewarding effects of cocaine in self-administration and relapse assays, whereas inhibition of PKA has the opposite effect (Self et al., 1998). Likewise, overexpression of CREB in the NAc, through viral mediated gene transfer, decreases place conditioning

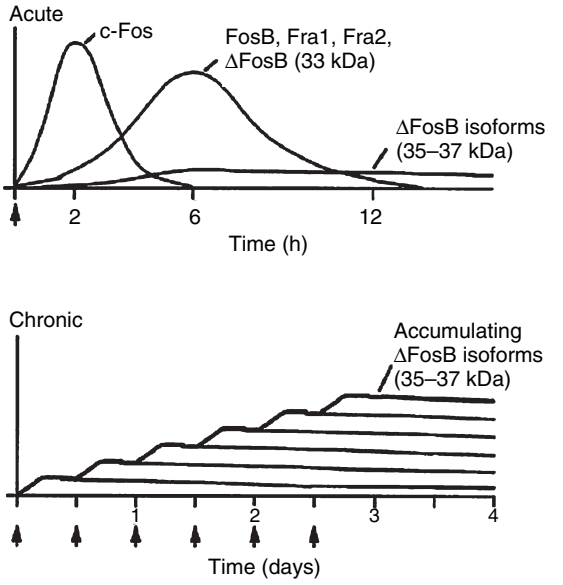

Figure 4 Diagrammatic representation of the induction of the Fos family of transcription factors (TFs) by acute and chronic drugs of abuse. The top panel charts the response to acute stimulation. cFos is rapidly induced, followed by FosB, Fra-1, and Fra-2. Levels of these TFs return to baseline between 6 and 12 hours poststimulation. Only a small increase is observed in levels of stabilized isoforms of ΔFosB. As shown in the bottom panel, this induction persists in the brain for a much longer time course and accumulates with chronic drug administration. Taken from Nestler EJ, Barrott M, and Self DW (2001) ΔFosB: A molecular switch for addiction. *Proc. Natl. Acad. Sci. USA* 98: 11042–11046; used with permission from National Academy of Sciences, USA.

to cocaine and to morphine, whereas overexpression of a dominant negative mutant form of CREB (mCREB), which cannot be phosphorylated due to a point mutation (Ser133 to Ala), potentiates the rewarding effects of both drugs (Carlezon et al., 1998; Barrot et al., 2002). Similar results are seen in transgenic mice that inducibly overexpress CREB or mCREB in the NAc and dorsal striatum. These data suggest that inhibition of CREB in the NAc enhances the hedonic value of cocaine and morphine; thereby animals 'like' the drug more, or alternatively could be facilitating the formation of the Pavlovian CS-US association between context and drug exposure. The viral infusions targeted the NAc-Sh, which is associated with encoding the rewarding properties of primary reinforcers rather than Pavlovian conditioning processes linking those rewards to environmental stimuli (see earlier). Therefore, it is likely that the behavioral changes observed arise from enhancing the rewarding effects of the drug. This interpretation is consistent with preliminary findings that mCREB decreases brain stimulation reward thresholds, while CREB has the opposite effect (Carlezon et al., 2005). Nevertheless, analysis of tissue from animals killed shortly after completion of the place conditioning test demonstrated increases in the phosphorylated form of CREB in the NAc core but not shell. Blocking this induction disrupted both the retrieval and consolidation of the CS-US association, indicating that CREB in the NAc-C is critically involved in this aspect of reward-related learning (Miller and Marshall, 2005). Thus, activation of CREB may play distinct roles in these two subregions of the NAc. CREB has been heavily implicated in multiple memory processes, particularly those underpinning long-term memory, which may reflect the known role for CREB in mediating certain changes in synaptic plasticity.

Transgenic mice have been developed which selectively overexpress ΔFosB within the NAc and dorsal striatum (Kelz et al., 1999). Furthermore, this overexpression is inducible (it occurs in adult animals) and is cell-type specific in that it is only observed in medium spiny neurons containing dynorphin/substance P (as opposed to those which contain enkephalin). The behavioral phenotype of these mice resembles animals treated chronically with drugs in several ways. The mice are more responsive to cocaine-induced hyperactivity, both acutely and after repeated administration, suggesting that ΔFosB expression may be involved in the development of locomotor sensitization. They also show enhanced place conditioning for cocaine and morphine, indicative of increased sensitivity to the rewarding properties of drugs or (as discussed earlier for CREB) of potential enhancement of the ability to form CS-US associations (Kelz et al., 1999; Zachariou et al., 2006b). Mice overexpressing ΔFosB self-administer lower doses of cocaine than wild-type controls and are more motivated to work for cocaine reward, as indicated by their elevated breakpoints in progressive ratio schedules (Colby et al., 2003). In contrast, mice overexpressing ΔcJun, a truncated form of cJun which acts as a dominant negative antagonist of all AP-1 mediated transcription, show reduced place conditioning to cocaine and morphine (Peakman et al., 2003). Together, these data may reflect more generalized increases in incentive motivation for reward.

One important molecular target of the dopamine system is dopamine and adenosine 3′5′-monophosphate-regulated phosphoprotein (32 kDa), or DARPP-32 as it is commonly known (Fienberg et al., 1998). DARPP-32 has been shown to be a potent modulator of both CREB and ΔFosB as well as many other facets of dopaminergic transmission. As with CREB, D_1-receptor-mediated activation of PKA induces the phosphorylation of DARPP-32 at threonine 34 (Thr34), which then acts as a potent inhibitor of protein phosphatase-1 (PP-1). In contrast, dopaminergic activation through D_2 receptors inhibits PKA signaling, through G-protein-coupled inhibition of adenylyl cyclase, leading to a decrease in phosphorylation of DARPP-32. Through its inhibition of PP-1, DARPP-32 regulates the phosphorylation of numerous proteins. With respect to TFs, mice lacking DARPP-32 show reduced phosphorylation of CREB in response to stimulant drugs of abuse, as well as reduced induction of cFos, in striatal regions (Fienberg et al., 1998). The mice also show reduced induction of ΔFosB after chronic stimulant administration. Consistent with these deficits in biochemical responses to drugs of abuse, DARPP-32 mutant mice show reduced responses to acute drug administration, including reduced locomotor activation and place conditioning (Zachariou et al., 2002). However, paradoxically, the mice show enhanced locomotor sensitization to chronic cocaine. The molecular basis of this latter abnormality is hard to explain on the basis of available data and requires more investigation (Hiroi et al., 1999). Mice in which Thr34 of DARPP-32 is mutated to Ala exhibit virtually the same biochemical and behavioral phenotype as DARPP-32 knockout mice, which demonstrates the importance of DARPP-32's phosphorylation by PKA in regulating its function (Zachariou et al., 2006a).

4.12.5.2 Clock

TFs traditionally associated with other roles of the dopamine system have also been recently implicated in reward-processing and the response to addictive drugs. For example, the dopaminergic system exerts an important influence over the entrainment of circadian rhythms during fetal development and in response to food and other stimuli, and disruption of the sleep cycle is observed in patients treated with dopaminergic drugs, such as *d*-amphetamine and L-DOPA. Abnormal circadian rhythms are also commonly found in substance abuse disorders. The most studied focus of circadian rhythms in brain is the suprachiasmatic nucleus (SCN) of the hypothalamus. This nucleus is particularly important for entraining the body's circadian rhythms to environmental lighting. The molecular basis of the circadian clock is now well established. The transcription factor Clock dimerizes with Bmal1 to form a transcription factor complex essential for accurate circadian rhythmicity (Vitaterna et al., 1994). The complex activates expression of Period and other proteins, which feed back and suppress their own expression in addition to regulating many other cellular targets. Increasing evidence indicates that this molecular clock operates in all tissues, which raises the interesting notion that many circadian rhythms are driven outside the SCN. For example, recent findings have shown that Clock is highly expressed within dopaminergic neurons of the VTA (McClung et al., 2005). Mice lacking functional Clock protein are hyperactive under baseline conditions, an effect which is most pronounced during the transition from the light to dark phases of their diurnal light cycle. Despite this hyperactivity, these mice show still greater activity following administration of cocaine as compared to littermate controls, and also show increased place conditioning to cocaine as well as decreased brain stimulation reward thresholds. These findings suggest that Clock, at the level of the VTA, may serve to dampen dopaminergic function and suppress reward, and that this may contribute to circadian rhythms in reward and motivation that have been well documented over the years.

4.12.6 The Role of CREB and ΔFosB in Response to Natural Rewards and Stress

Understanding the response to natural rewards has implications for research into depression due to the obvious relationship between value judgments and anhedonia. Pharmacologically, most currently used antidepressant treatments inhibit the reuptake of the monoamines serotonin and noradrenaline, or inhibit monoamine oxidase (a major catabolic enzyme for monoamine neurotransmitters). Although the dopamine system is critically associated with reward judgments, it is less well studied in the context of depressive etiology. However, changes in some of the same intracellular signaling mechanisms identified in drug addiction research are also affected in animal models of depression and within areas associated with reward-related learning such as the NAc, PFC, and BLA, as well as in areas more strongly associated with memory storage and retrieval such as the HPC (Nestler and Carlezon, 2006). Animal models of depression have generally focused on the response to stress, such as the forced swim test (FST), where antidepressants have been shown to increase the latency to immobility and decrease the total time rodents spend immobile when confined to a water-filled container. Similarly, antidepressants increase the time an animal struggles when suspended by its tail. A lack of struggling in these models is regarded as indicative of a state of behavioral despair. Likewise, animals exposed repeatedly to inescapable stressors, such as shocks, show an increased latency to escape when subsequently given the opportunity.

CREB activity in the NAc appears to play an important role in gating an individual's response to both rewarding and aversive stimuli (Barrot et al., 2002). Increased CRE-mediated transcription in the NAc has been observed following several stressors, including inescapable foot shocks, restraint stress, and the more natural stress of introducing an animal into a novel social group. Increased CREB expression reduces both the nociceptive reaction to painful stimuli and conditioned place aversion to naloxone withdrawal in morphine-dependent rats, whereas mCREB potentiates the response to these aversive stimuli. A similar pattern is observed in anxiety tests, where intra-NAc infusion of herpes simplex virus (HSV)-CREB appears to be anxiolytic and HSV-mCREB anxiogenic. In addition to modulating place conditioning for drug rewards, CREB in the NAc also alters preference for sucrose as assessed by a simple two-bottle choice test. Overexpression of CREB decreases sucrose preference, whereas mCREB increases sucrose preference. Conversely, levels of CRE-mediated transcription in the NAc decrease following protracted social isolation, a manipulation which increases anxiety and impairs initiation of sexual behavior. This

phenotype can be rescued by overexpressing CREB within the NAc using viral-mediated gene transfer. Likewise, overexpressing mCREB in this region mimics these effects of isolation in nonisolated rats, an effect that can be reversed with the anxiolytic diazepam (Barrot et al., 2005).

In summary, CREB appears to reduce the impact of emotionally significant stimuli, whereas inhibition of CRE-mediated transcription enhances emotional responsivity. Although mCREB is not a naturally occurring protein, the endogenous inhibitor of CREB function, inducible cAMP early repressor (ICER), is capable of mediating the same functions as mCREB *in vivo* and is induced by both stress and amphetamine (Green et al., 2006). In keeping with this hypothesis that CREB in the NAc numbs the emotional response to stimuli, overexpression of CREB in the NAc induces depressive-like behavior in the FST and learned helplessness test, whereas inhibition of CREB function in this region, through overexpression of either mCREB and ICER, induces antidepressant-like behavior in these tests (Pliakas et al., 2001; Green et al., 2006).

The proposal that CREB gates the response to emotional stimuli within the NAc is consistent with recent elecrophysiological findings, where CREB was shown to increase the electrical excitability of NAc neurons and mCREB to cause the opposite effect (Dong et al., 2006). Moreover, direct inhibition of NAc neurons, via viral-mediated overexpression of a K^+ channel, which would mimic the mCREB effect, increased an animal's behavioral response to cocaine. These findings are interesting in light of work, cited earlier, where inhibition of NAc neurons has been linked with increases in goal-directed behavior.

The effect of CREB in the NAc contrasts with its established role in the HPC where, as noted earlier, CREB is thought to mediate long-term memory formation. Virally mediated overexpression of CREB in the HPC also produces antidepressant-like behavior in rats, an effect potentially mediated in part by CREB-induced elevations of brain-derived neurotrophic factor (BDNF; see section 'Brain-derived neurotrophic factor'). Changing CRE-mediated gene transcription within different brain areas, therefore, has very different effects (Carlezon et al., 2005). Such functional dissociations are not uncommon when considering the effects of neurotransmitters such as dopamine or serotonin. Therefore, it is not surprising that the same intracellular signaling mechanisms activated by these neurotransmitters likewise produce region-specific changes in behavior.

In comparison to CREB, less is known regarding the role of ΔFosB in regulating the response to natural rewards or stressors. ΔFosB upregulation is seen after chronic wheel-running behavior, an activity which rodents are thought to find pleasurable but potentially compulsive or 'addictive,' and mice overexpressing ΔFosB in striatal regions exhibit greater compulsive wheel-running than their wild-type littermates (Werme et al., 2002). Moreover, overexpression of ΔFosB in striatal regions, either by viral vectors or in inducible transgenic mice, increases motivation for food in progressive ratio and instrumental learning tests (Olausson et al., 2006). These findings support the hypothesis, mentioned earlier, that ΔFosB in this neural pathway promotes reward.

4.12.7 Target Genes of CREB and ΔFosB

This section will primarily focus on examples of the downstream targets of CREB and ΔFosB associated with reward processing, addiction, and depression-like behavior at the level of the brain's reward pathways. A broad survey of CREB and ΔFosB targets in the NAc has been published recently (McClung and Nestler, 2003). However, changes in targets upstream of the TFs considered here have also been associated with the response to rewarding stimuli, including the enzymes responsible for the synthesis and degradation of cAMP, namely adenylyl cyclase and cyclic nucleotide phosphodiesterases, respectively. Kinases such as PKA and extracellular signaling kinases (ERKs) have been implicated in the effects of addictive drugs as well as in numerous facets of learning and memory. These aspects of the intracellular response to reward are considered in other chapters (*See* Chapters 4.21, 4.22, 4.25).

4.12.7.1 Dynorphin in the VTA-NAc Pathway

One of the primary mechanisms by which CREB is thought to affect reward-related learning and addiction is through induction of dynorphin within the NAc (**Figure 5**). Dopaminergic neurons in the VTA innervate GABAergic neurons in the NAc, which express dynorphin and in which activation of CREB has been observed after chronic treatment with addictive drugs. Dynorphin acts on κ opioid receptors expressed on the terminals of these

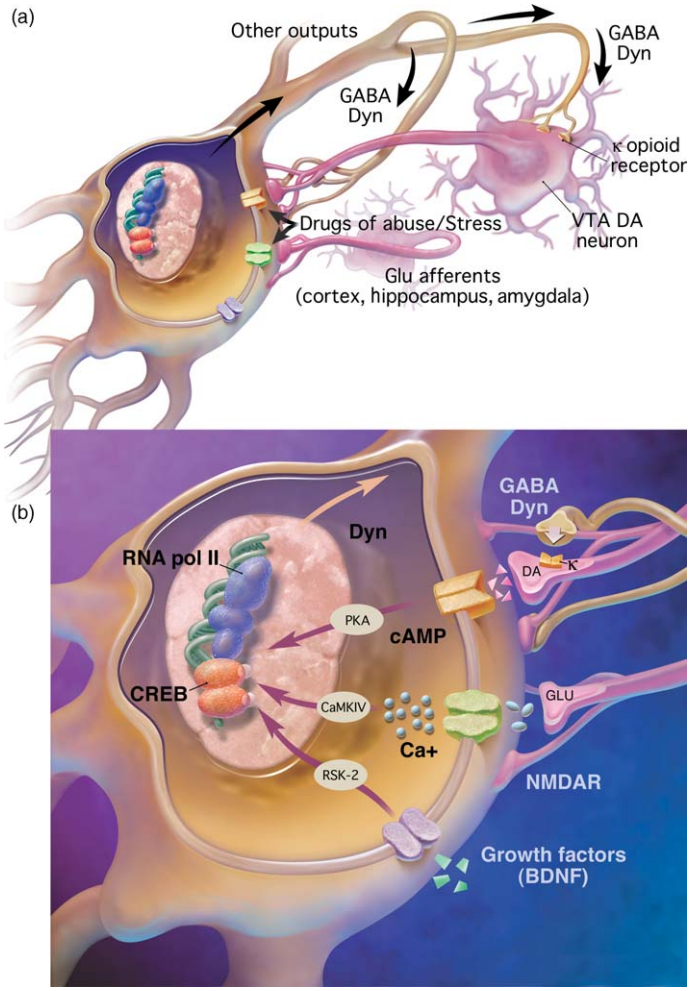

Figure 5 Regulation of NAc function by CREB and dynorphin (Dyn). The figure shows a dopaminergic neuron from the VTA innervating a medium spiny neuron within the NAc which expresses dynorphin. Glutamatergic input from other areas such as the PFC and amygdala, as well as BDNF (released from glutamatergic or dopaminergic projections) are also shown. Dynorphin acts as a negative feedback signal: when released, it binds to κ opioid receptors on dopaminergic neurons and inhibits their function. Drugs of abuse and stress increase CREB activity and induce dynorphin expression, upregulating this feedback loop. Activation of CREB could be caused by some of the mechanisms shown in the figure, all of which lead to its phosphorylation at Ser 133. Abbreviations: GABA, gamma-aminobutyric acid; NMDAR, *N*-methyl-D-aspartate receptor; PKA, protein kinase A; CaMKIV, Ca^{2+}/calmodulin-dependent protein kinase type IV; RSK-2, ribosomal S6 kinase-type 2; RNA pol II, RNA polymerase II complex. Taken from Nestler EJ, Barrot M, DiLeone RJ, Eisch AJ, Gold SJ, and Monteggia LM (2002) Neurobiology of depression. *Neuron* 34: 13–25; used with permission from Cell Press.

dopaminergic projections and inhibits their function, thereby forming a negative feedback loop to minimize the effects of dopaminergic stimulation. This dampening of the dopamine signal could contribute to the depressant-like effects of overexpressing CREB within the NAc and the reduction in place conditioning to addictive drugs (Carlezon et al., 2005). Increased ΔFosB is also primarily observed in dynorphin-containing cells within the NAc, but acts to decrease expression of dynorphin (Zachariou et al., 2006b) and to thereby potentiate dopaminergic signaling. Such reciprocal regulation of dynorphin by CREB and ΔFosB could explain some of the reciprocal behavioral changes observed following upregulation of these TFs. It could also account for the changes in reward processing that occur during different timepoints of withdrawal from addictive drugs. Drug-induced activation of CREB is relatively short-lived, yet increasing drug use potentiates CREB expression. The anhedonia and negative emotional symptoms which predominate during acute withdrawal could therefore arise partly from the ability of

CREB to downregulate dopaminergic signaling. In contrast, the sensitization to the rewarding effects of addictive drugs and the incubation of craving which predominate at later timepoints could be mediated in part by the prolonged expression of ΔFosB.

4.12.7.2 Cyclin-Dependent Kinase 5

Cyclin-dependent kinase 5 (Cdk5) was identified as a downstream target of ΔFosB within the NAc through microarray analysis (Bibb et al., 2001). Activation of Cdk5 alters dopaminergic signaling through phosphorylation of DARPP-32 at a different site from PKA, namely threonine 75 (Thr75) (see Benavides and Bibb, 2004). This converts DARPP-32 from an inhibitor of PP-1 to an inhibitor of PKA. The fact that DARPP-32 can function as either a protein phosphatase inhibitor or a protein kinase inhibitor, depending on the site at which it is phosphorylated, may be unique, and this high level of phosphorylation-site-specific regulation further highlights the importance of this molecule in intracellular signaling cascades. Furthermore, PKA activation can decrease phospho-Thr75 DARPP-32 through activation of protein phosphatase 2A (PP-2A). Acute administration of cocaine can increase phosphorylation of DARPP-32 at Thr34 and reduce it at Thr75 via activation of PKA and inhibition of the PP-2A pathway, respectively. However, chronic cocaine administration has the opposite effect, increasing phosphorylation of Thr75, and reducing the ability of D_1 receptor stimulation to activate PKA. Cdk5 is upregulated by chronic cocaine administration, and this effect appears to be mediated by ΔFosB: overexpression of ΔFosB induces Cdk5 expression, while expression of the dominant negative ΔcJun prevents the ability of cocaine to induce the enzyme (Bibb et al., 2001; Peakman et al., 2003).

The behavioral contribution of Cdk5 induction is complex. Intra-NAc infusion of the Cdk5 inhibitor roscovitine has been shown to potentiate the hyperlocomotor response to cocaine seen following chronic drug administration (Bibb et al., 2001). These behavioral data suggest that cocaine-induced upregulation of Cdk5 activity may be an attempt to compensate for overstimulation of the dopaminergic system. However, intra-NAc infusions of roscovitine also block the increase in dendritic spine proliferation seen in this region with chronic cocaine administration, which is correlated with the development of locomotor sensitization (Norrholm et al., 2003). As discussed earlier, these neuroplastic changes are one potential mechanism by which repeated drug administration perpetuates changes in learning and memory processes integral to the sensitized and addicted state. Chronic cocaine exposure, via ΔFosB, may therefore trigger an adaptive homeostatic response involving increased Cdk5 activity that ultimately commits the affected neurons to a maladaptive process of cytoarchitectural changes.

4.12.7.3 Nuclear Factor Kappa B

Nuclear factor kappa B (NFκB) is a transcription factor induced in many tissues by inflammation and immune responsiveness (see Chen and Greene, 2004). It is composed of two subunits, most commonly p50 and p65. Under basal conditions, it remains sequestered in the cytoplasm by inhibitory kappa B (IKB) protein. Upon phosphorylation by I kappa kinase (IKK), IKB releases an inactive dimer of p50 and p65, which can then be phosphorylated and transported to the nucleus where it can initiate gene transcription (*See* Chapter 4.28). This TF is more commonly associated with the field of immunology than neuroscience. However, in parallel to Cdk5, both ΔFosB overexpression and chronic cocaine treatment upregulate NFκB-related proteins such as p65, the precursor of p50 (p103), and IKB within the NAc (Ang et al., 2001). NFκB has been implicated in regulating cell survival and neuroplasticity and has been associated with long-term potentiation (LTP) and long-term depression (LTD), responses implicated in learning and memory processes. In terms of reward processing, intra-amygdala infusions of κB decoy DNA impaired fear-potentiated startle responses, suggesting that this molecule may play a role in the intracellular signaling pathway underpinning emotional CS-US learning (Yeh et al., 2002). Preliminary data also indicate that potentiating NFκB signaling within the NAc through overexpression of a constitutively active form of IKK increases place conditioning to cocaine and also increases local dendritic spine formation.

4.12.7.4 Brain-Derived Neurotrophic Factor

Neurotrophic factors facilitate neural growth and differentiation during development and also have a critical role to play in mediating neuronal survival and plasticity in adulthood. BDNF has been identified as an important downstream target of CREB and is implicated in numerous processes related to learning and memory, particularly within the HPC.

BDNF also modulates emotional learning within the amygdala, where increases in BDNF mRNA have been reported following fear conditioning. In addition, overexpression of a mutated dominant negative form of the tyrosine kinase B (TrkB) receptor within the amygdala blocks the acquisition of fear conditioning (Rattiner et al., 2004), indicating that the ability of BDNF to mediate changes in synaptic plasticity could be of particular import in the encoding of emotionally significant events in this region.

4.12.7.4.1 The neurotrophic hypothesis of depression

The neurotrophic hypothesis of depression suggests that a deficiency in neurotrophic support may contribute to the observed hippocampal pathology associated with depression (e.g., reduced hippocampal volume in depressed patients, decreases in dendritic arborization, decreased adult hippocampal neurogenesis), and that antidepressants relieve the symptoms of depression through increasing neurotrophic action. BDNF has been widely studied within the context of this hypothesis. Chronic administration of numerous antidepressant drugs increases expression of BDNF within the HPC despite their diverse pharmacological actions (Nibuya et al., 1995). Both acute and chronic stress decreases BDNF expression in hippocampal regions, effects which may contribute to the etiology of depression and which can be blocked by antidepressant treatment. Direct infusion of BDNF into the hippocampus also produces antidepressant-like effects on the FST and learned helplessness paradigms (Shirayama et al., 2002), while mice lacking BDNF do not show antidepressant behavioral responses (Monteggia et al., 2004, 2007), further indicating that BDNF may be important in mediating depressive symptoms.

Observations that both intra-cerebral infusions of BDNF (Pencea et al., 2001) and chronic administration of antidepressants (Malberg et al., 2000) increase adult neurogenesis has led to the suggestion that this may be one mechanism underlying the therapeutic action of antidepressants. However, a direct, causal relationship between neurogenesis, BDNF, and antidepressant action has proved difficult to demonstrate conclusively. Although X-ray irradiation of the brain blocks cell proliferation and also prevented the chronic effects of antidepressants in a novelty-suppressed feeding assay (Santarelli et al., 2003), irradiation also disrupts numerous intracellular signaling cascades, which may confound interpretation of these findings (Silasi et al., 2004).

Although it is clear that antidepressants can increase CREB and that CREB activity can increase BDNF expression, it is currently unclear as to whether CREB-mediated activation of BDNF is the critical pathway for the antidepressant actions of BDNF. Thus, the increase in BDNF caused by antidepressant administration is blocked in CREB-deficient mice (Conti et al., 2002), yet these mice still respond to antidepressant drugs in tests such as the FST. Although CREB phosphorylation is thought to have pro-survival properties in newly formed hippocampal neurons, the atypical antidepressant tianeptine increases hippocampal neurogenesis but does not activate the cAMP signaling cascade (Czeh et al., 2001). The role of CREB in the antidepressant effects of BDNF clearly merits further study (Malberg and Blendy, 2005).

4.12.7.4.2 BDNF within the VTA-NAc: Reward processing and addiction

In addition to its roles in neuroplastic responses, BDNF is critically involved in the regulation of dopaminergic neurotransmission. Through binding at TrkB receptors on dopaminergic terminals within the NAc, BDNF is capable of potentiating dopamine release in this region. In addition, BDNF acts directly on TrkB receptors expressed by NAc neurons. Hence, it is not surprising that this molecule has been implicated in addiction and reward-related learning. Direct administration of BDNF into the NAc or VTA increases cocaine-induced hyperactivity, whereas BDNF heterozygous knockout mice show reduced locomotor activity and reduced place conditioning to cocaine (Hall et al., 2003). Intra-NAc BDNF also increases responding for CRf (Horger et al., 1999), suggesting that induction of BDNF may contribute to the increases in incentive motivation for drugs that are associated with addiction. In support of this hypothesis, increases in BDNF have been observed in the NAc, BLA, and VTA following withdrawal from cocaine, and these increases appear to track the incubation (potentiation over time) of craving for cocaine as measured by drug-seeking behavior following presentation of a drug-paired CS (Grimm et al., 2003). A direct infusion of BDNF into the VTA can also potentiate such drug-seeking behavior (Lu et al., 2004).

However, despite these increases in incentive motivation for drugs and the potentiation of local dopaminergic transmission, increases in BDNF within the NAc and VTA appear to induce a pro-depressant phenotype. Intra-VTA infusions of BDNF decreased

the latency to immobility in the FST, whereas overexpression of a mutant TrkB receptor within the NAc (which inhibits BDNF signaling) produces an antidepressant-like effect in the same task (Eisch et al., 2003). Recent data using the social defeat model of stress in mice provide further insight into the role of BDNF in affective processing. In this paradigm, an animal is defeated by a larger, dominant, and aggressive mouse and is then housed in close confinement with the aggressor (although the animals can no longer fight). Animals defeated chronically in this way develop a behavioral syndrome characterized by numerous indices of anhedonia which may reflect aspects of human depression, such as decreased preference for natural rewards such as sucrose, sex, and social interaction, as well as a general decrease in locomotor activity. Some of these changes are particularly long-lasting and are reversed by chronic antidepressant treatment (Berton et al., 2006). Knocking out BDNF within the VTA selectively, by use of a viral vector expressing the Cre recombinase, prevents the development of this depressive-like syndrome following social defeat. These data suggest that BDNF within the VTA-NAc pathway plays an important role in reward-related learning, and that overstimulation of this signaling pathway by repeated drug intake or chronic stress could lead to potentiated and maladaptive learning, both to rewarding and aversive stimuli. As with CREB, the effects of BDNF in the HPC versus the VTA-NAc appear to be diametrically opposed. Indeed, the observation that stress decreases BDNF in the HPC, yet increases it in the VTA-NAc, indicates that these areas mediate very different aspects of an animal's behavioral repertoire in response to stress.

4.12.7.5 Glutamate Receptors

In addition to the changes observed in dopaminergic signaling, repeated administration of addictive drugs increases the expression of both alpha-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA) and *N*-methyl-D-aspartate (NMDA) glutamate receptor (GluR) subunits within the VTA. The GluR1 subunit of the AMPA receptor has received particular attention (Carlezon et al., 2002). Increases in the number of GluR1 subunits present in an AMPA receptor increase its overall conductance as well as its permeability to calcium (Ca^{2+}) ions. Ca^{2+} is involved in numerous intracellular signaling pathways, and changes in levels of intracellular Ca^{2+} can alter the regulation of gene expression. Given that sensitizing regimes of drug administration increase the electrophysiological responsiveness of dopaminergic cells within the VTA to AMPA receptor agonists (Thomas and Malenka, 2003), and that the increase in GluR1 subunits is most prominent in animals showing behavioral signs of sensitization (Churchill et al., 1999), it has been suggested that upregulation of the GluR1 subunit may be one molecular mechanism underlying the potentiated response to chronic drug treatment. In support of this hypothesis, increasing GluR1 expression within the VTA increases both conditioned place preference and hyperlocomotion caused by morphine (Carlezon et al., 1997). Recent evidence indicates that drug-induced upregulation of GluR1 in the VTA may be mediated via drug induction of CREB in this brain region (Olson et al., 2005).

In contrast, increases in GluR1 subunit expression within the NAc-Sh can facilitate the extinction of cocaine-seeking (Sutton et al., 2003), suggesting that increased glutamatergic action within this region can reverse some of the detrimental adaptations caused by chronic drug intake. Chronic cocaine treatment has been shown to reduce the electrophysiological sensitivity of NAc neurons to AMPA agonists (Thomas and Malenka, 2003). This effect may be accounted for by increased levels of GluR2 subunits within the NAc, which decrease the conductance of AMPA receptors and reduce their permeability to Ca^{2+}. Overexpression of ΔFosB increases GluR2 levels within the NAc, while ΔcJun prevents the ability of cocaine to induce the protein (Kelz et al., 1999; Peakman et al., 2003). Moreover, viral-mediated overexpression of GluR2 within the NAc mimics the effects of increased ΔFosB, in that both manipulations enhance place conditioning to cocaine (Kelz et al., 1999). Drug-induced adaptations in several postsynaptic density proteins, which modulate GluR function, have also been observed in the NAc (Yao et al., 2004). It would, therefore, appear that drug-induced changes in the expression of different GluR subunits within different reward-related regions may have opposing actions on cellular excitability, yet may both contribute to the addicted phenotype.

4.12.8 Molecular Changes within the PFC

The majority of work to date has focused on changes in transcriptional regulation within the subcortical regions of reward-related circuitry, with an understandable emphasis on the NAc and VTA. However,

changes in gene expression within the PFC have also been observed in models of addiction and depression, although less is known about their functional consequences (Kalivas, 2004). For example, a recent study examined patterns of cFos expression within the PrLC, NAc, and BLA following reexposure to a cocaine-associated context, as assessed by place conditioning. The authors observed a selective increase in GABAergic cells expressing cFos within the PrLC (Miller and Marshall, 2004). Similarly, a decrease in protein kinase C (PKC) has been observed in this region during retrieval of a discrete CS paired with cocaine during self-administration training (Thomas and Everitt, 2001). These data suggest that output from this region is reduced in response to cue exposure, a finding which may be of relevance to cue-elicited drug craving.

Alterations in G-protein-coupled receptor signaling pathways within the mPFC have recently been identified which may underlie potentiated responding to drug versus natural rewards and relapse to drug-seeking (Kalivas et al., 2005). The activator of G-protein signaling 3 (AGS3) is increased in the mPFC following withdrawal from cocaine self-administration, and reinstatement of cocaine seeking can be blocked by decreasing levels of AGS3. AGS3 sequesters the alpha subunit of inhibitory G proteins ($Gi\alpha$) and reduces signaling through $Gi\alpha$-coupled receptors such as D_2 dopamine receptors. It is thought that this reduction in D_2 receptor signaling leads to increased inhibition of PFC output to the NAc which can only be overcome by relatively strong inputs, such as drug reward. This hypothesis needs further investigation, but potentially provides a molecular mechanism to explain the increased control over goal-directed behavior exerted by drugs of abuse.

Increases in ΔFosB have also been reported in regions of the frontal cortex following chronic exposure to both addictive drugs and stressful manipulations (Perrotti et al., 2004). Recent evidence suggests that increased expression of ΔFosB within this region increases preference for sucrose, potentially indicative of an increased sensitivity to rewarding stimuli. Increased ΔFosB in this region also appears to sensitize animals to the locomotor stimulant actions of cocaine, yet produces tolerance to the disruptive effects of the psychostimulant on operant behavioral measures of motivation and impulsivity. These changes closely parallel those observed after chronic cocaine treatment. Further work aimed at understanding the changes in cognition caused by long-term drug use, and their underlying molecular basis, is clearly warranted.

4.12.9 Beyond Corticolimbic Circuitry: A Role for Hypothalamic Feeding Peptides in Reward-Related Learning?

The hypothalamus is one of the most important regions of the brain in terms of regulating more physiological aspects of reward such as the homeostatic control of hunger and thirst. Animals will preferentially self-stimulate the lateral hypothalamus (LH), a finding partially explained by the fact that dopaminergic fibers from the VTA to the NAc pass through this structure. Intriguingly, the threshold for LH self-stimulation in the perifornical region increases with weight loss, suggesting a relationship between the physiological homeostatic drive for natural rewards and the sensitivity of the brain to rewarding stimuli in general (see Shizgal et al., 2001). Although this region has not been associated with the more cognitive process of mediating goal-directed behavior, modulation of hypothalamic activity forms a critical part of the output pathway of the corticolimbic circuitry discussed earlier. The maintenance of energy balance depends on the allocation of behavior between feeding and competing activities; therefore, the signals of hunger or satiety generated by the hypothalamus have significant impact on the motivation for food reward and, therefore, potentially on numerous models of goal-seeking discussed previously. Whether modulation of this signal is also involved in assessment of the rewarding properties of addictive drugs is currently under investigation. The hypothalamic–pituitary adrenal axis is also one of the most prominent mechanisms by which the brain reacts to stress. Neurons in the paraventricular nucleus of the hypothalamus secrete corticotropin-releasing factor, which stimulates the release of adrenocorticotropin from the anterior pituitary. This in turn leads to production of glucocorticoids (cortisol in humans and corticosterone in rodents) within the adrenal cortex, which can have profound effects on behavior and brain function in numerous regions, as well as affecting general metabolism. Given that stress contributes to the development of affective psychiatric disorders such as depression and can trigger relapse to drug-seeking, a growing number of studies are addressing the role of signaling peptides within this region in reward-related learning and emotional processing. Some examples are considered in the following, though this is by no means an exhaustive list.

Melanin-concentrating hormone (MCH) is an orexigenic (pro-appetite) protein expressed within

the lateral hypothalamus. The MCH_1 receptor is highly expressed within the NAc, and intra-NAc infusions of MCH increase food intake, whereas antagonists of the MCH_1 receptor have the opposite effect. MCH_1 receptor antagonists acting within the NAc also exert antidepressant effects within the FST, an effect which is also observed in MCH knockout mice (Georgescu et al., 2005) and with systemic administration of MCH antagonists (Borowsky et al., 2002). These data suggest that molecules primarily thought to control the regulation of food intake can also have an effect on mood through their influence on NAc function.

Orexin (hypocretin) may have a similar role to play. Expressed within the lateral hypothalamus, orexin increases food intake by promoting a state of wakefulness and arousal, and deficits in orexin are known to cause the sleep disorder narcolepsy (Mignot, 2001). This debilitating condition, characterized by daytime sleepiness, cataplexy, and other sleep abnormalities, is frequently associated with depression, and some of the sleep-related symptoms are treated with antidepressants (Daniels et al., 2001). Narcolepsy and depression are both associated with alterations in circadian rhythms, and a dampening in the naturally occurring diurnal variation in orexin levels has been observed in depressed patients (e.g., Salomon et al., 2003). One mechanism by which hypothalamic peptides may influence reward processing may be via their modulation of the dopaminergic system. For example, orexin neurons project prominently to the dopaminergic cells of the VTA, where orexin binds to orexin 1 (OX_1) receptors to stimulate the neurons. Administration of an OX_1 receptor antagonist blocks the development of locomotor sensitization to cocaine (Borgland et al., 2006), whereas orexin precipitates relapse to drug-seeking in animals withdrawn from cocaine self-administration through induction of a stress-like state (Boutrel et al., 2005). Orexin knockout mice also show reduced physical dependence on morphine as indicated by a reduction in the physical signs of naloxone-precipitated withdrawal symptoms (Georgescu et al., 2003).

Functional interactions between the dopaminergic system and another hypothalamic peptide family, the melanocortins, have been reported and have likewise been implicated in mediating drug reward. Mice lacking the melanocortin-4 (MC_4) receptor, which is highly expressed within the NAc, fail to develop locomotor sensitization, and direct intra-NAc infusions of an MC_4 antagonist peptide, SHU-9119, reduces cocaine self-administration and place conditioning. As with BDNF, this peptide also prevents cocaine from potentiating the response to CRf (Hsu et al., 2005).

Neuropeptide Y (NPY) is perhaps best known for its ability to antagonize the behavioral consequences of stress within the central nervous system (CNS) (see Heilig, 2004). Administration of NPY is anxiolytic in numerous animal models, which is thought to result in part from its actions at Y_1 receptors within the amygdala. Acute stress decreases NPY expression, whereas chronic stress exposure, which leads to behavioral habituation, reverses this effect so that NPY is upregulated. The hypothesis that increased NPY expression could mediate coping responses is supported by the observation that NPY transgenic rats are less sensitive to stressful manipulations (Thorsell et al., 2000). In keeping with the view that stress promotes depression, at least in vulnerable individuals, antidepressant treatments also increase NPY within the frontal cortex, providing another mechanism by which antidepressant drugs may confer their therapeutic benefit. Dysregulation of NPY regulation has also been implicated in drug addiction, particularly in relation to alcoholism, where it is thought to mediate the anxiolytic properties of alcohol, thereby increasing motivation to consume the drug (see Valdez and Koob, 2004).

Whether induction of these hypothalamic feeding peptides is regulated by the same TFs as other proteins implicated in reward and addiction has yet to be determined. However, it is known that NPY is a downstream target of CREB, and whether the behavioral effects of NPY expression likewise vary depending on its locus of action remains a possibility.

4.12.10 Overview

Within this article, we have briefly considered the psychological processes involved in signaling rewarding events, and the roles played by different regions within the corticostriatal loop associated with reward-related learning. Through analysis of the intracellular signaling cascades affected by the dopaminergic system, specific molecules involved in mediating aspects of reward processing have been highlighted, and data pertaining to their influence over reward-related behavior have been discussed. In parallel to much of what is known regarding the neurochemical basis of reward signaling, it is clear that different molecules can have very different effects on behavior depending on their

locus of action. Likewise, the time course of molecular changes, whether transient or long-term, can have a profound influence on their behavioral consequences. The molecular tools have now been developed to directly manipulate intracellular signaling pathways and gene transcription at the level of different transcription factors and their downstream targets within highly circumscribed brain nuclei. As these advances in molecular biological techniques become more accessible, a wider array of behavioral and genetic studies will become possible. Although significant progress has been made in determining the role of such molecular events in reward-related learning, further integration between the fields of psychology and molecular biology will enable greater understanding of the biological basis of goal-directed behavior.

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4.13 Conditioned Taste Aversion and Taste Learning: Molecular Mechanisms

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4.13.1 Introduction

The sense of taste, together with that of odor, belongs to the family of chemical senses, and it is an important defensive sense, evolved to guide food intake and to aid in avoiding poisons. Moreover, the very existence of an organism is dependent on its ability to maintain intrinsic homeostasis in a continuously changing world. The guarding gate, both for the intake of energy and other metabolites necessary for the organism's survival and for the avoidance of poisonous substrates, is the sense of taste. In order to maintain homeostasis, the organism must recognize its bodily needs, identify the relevant substances that contain the means to satisfy these needs, and ensure that the process of ingestion maintains the balance between the needs and the intake. In addition and in parallel, the organism must avoid substances that will make it sick.

In order to consume the necessary and beneficial substances and to avoid the damaging ones that may cause malaise, the organism mainly uses taste and tags the substances as pleasant, indifferent, or unpleasant. However, the reaction of the organism to a specific substance is determined by a combination of automatic responses that developed during evolution and learning mechanisms that are plastic and can modify the perceived food value according to individual experience. An ethological view may account for the predisposition for different tastes according to the theory that the animals are well adapted to conditions that may occur with high probability and/or have critical survival value.

Learning the value of new tastes may be in line with or in contrast to this evolution-dependent genetic programming. There are just five different taste categories: Sweet, salt, bitter, sour, and the less well known umami (manifested in monosodium glutamate). However, combinations of different concentrations within these five categories and the additional information related to texture and temperature enable the characterization and identification of thousands of different tastes. Animals, including humans, can react to the various tastes by using two main strategies: Genetic programming (like sweet, dislike bitter), and complex learning mechanisms that involve the participation of several forebrain structures.

Taste learning has been found and studied in vertebrates and invertebrates and seems to be universal throughout the animal kingdom. However, most of the research into the biological mechanisms underlying taste learning has involved mice and rats, and the present chapter deals mainly with these studies. A simple sense stimulus such as taste can be very well defined in terms of a number of molecules (e.g., known molarities in a known volume). However,

even simple unimodal taste input includes not only the chemical properties of a substance, but also other physical dimensions, such as temperature and texture, and association with other cues and modalities.

This chapter does not address taste recognition on the receptor level, nor taste reactivity as it is defined genetically. The aim of this chapter is first to describe taste behavior in the context of laboratory attempts to identify molecular and cellular mechanisms of learning and memory, to present the various learning paradigms used in the laboratory, and the relevant neuroanatomy. Later, I will review and discuss in detail the molecular and cellular mechanisms of taste learning in the gustatory cortex, which reside in the insular cortex. I focus this chapter on recent publications, because the earlier development of the subject is covered in a seminal book by Jan Bures, Federico Bermudez-Rattoni, and Takashi Yamamoto (Bures et al., 1998). Finally, I present the current working model of taste memory formation, consolidation, and retention and suggest future research directions.

4.13.2 Measuring Taste Learning, Memory, and Consolidation: The Behavioral Paradigms

Learning is conventionally classified from the behavioral point of view into nonassociative (habituation and sensitization), associative (relationships between amounts and events), and incidental learning (learning in the absence of an explicit external reinforcer). In addition, memory can be classified according to the temporal phases of short- and long-term memories. The use of several different behavioral paradigms enables the different classification and temporal phases to be analyzed. Taste learning and conditioned taste aversion are considered to be implicit learning paradigms and they can result in short-term memory (hours) or lifelong memory (Bures et al., 1998; Houpt and Berlin, 1999). In animals, as in humans, a subject can prefer one taste over another without recognizing either (Adolphs et al., 2005).

The most familiar taste-learning paradigm is an association between taste and malaise: The process of conditioned taste aversion (CTA). In CTA learning, an animal learns to avoid a novel food associated with delayed poisoning (Garcia et al., 1955; Bures et al., 1988). CTA can be explained as an associative learning paradigm: The novel taste is the conditioned stimulus (CS), the malaise-inducing agent is the unconditioned stimulus (UCS), and the learned avoidance of the taste is the conditioned response (CR). However, it was clear from the first time that CTA was reported scientifically (Garcia et al., 1955) that CTA has very special and unique features.

The most prominent characteristic of CTA learning is the long delay between the novel food that serves as the CS and the toxic substance that serves as the UCS. This time frame of association is measured in hours (1–12 h) (Bures et al., 1998), which is in strong contrast to other forms of association, which tolerate time frames of a few seconds. The long delay between the CS and the UCS can be explained on the sensory level or in terms of the slow release of a substance from the stomach. Moreover, short time frames or backward conditioning do not yield good association and learning (Schafe et al., 1995). Thus, the long delay between the CS and the UCS in CTA should be explained in terms of the neuronal system subserving the CTA learning. Theoretically, the internal representation of the novel taste is kept in an on-hold position for many hours, ready for the UCS to produce the association. It is not clear how the internal representation of taste is stored, but it is hypothesized that it underlies ongoing activity that is dependent in part on the gustatory cortex (Katz et al., 2002; Bahar et al., 2003; Berman et al., 2003). CTA has other special features such as one-trial learning that produces strong and stable long-term memory; it can produce aversion to odor by odor potentiation taste aversion (Schneider and Pinnow, 1994), and, in contrast to the strong association between taste and malaise, there is no or very weak association between other sensory stimuli (e.g., sound, light) and malaise or discomfort. Similarly, there is hardly any association between taste and noninternal stresses, such as pain.

CTA can be affected if the CS is experienced either before the CTA, i.e., latent inhibition of CTA (LI-CTA), or after it, i.e., CTA extinction, as can other learning paradigms, but with specific characteristics for taste learning. In LI-CTA, if a taste stimulus was learned with no negative consequence there will be decreased aversion for the same taste following CTA (Rosenblum et al., 1993). This modulation in behavior can be attributed either to reduced strength of the association at the time of the CTA or to competition during the retrieval phase (Lubow, 1989). LI-CTA can be used successfully in a method

to study incidental taste learning, i.e., learning with no external reinforcer or association. One exposure to the novel taste has a significant effect on reducing the aversion elicited by CTA. However, a critical parameter is the amount of the novel taste consumed at the preexposure (Belelovsky et al., 2005), e.g., consuming 5 ml or less of a novel taste will produce no significant latent inhibition (LI) in rats (Belelovsky et al., 2005).

Experiencing a given taste after its association with malaise will reduce the aversion responses, i.e., the experience will cause an extinction of the learned CTA. The extinction of the association is dependent on the strength of the association, the number of extinction trials, and the amount of novel taste consumed during the session (Dudai, 2006). Extinction can be viewed as imparting an evolutionary advantage when the food supply is restricted and there is a constant need to test the negative or positive effects of a given food.

Two other behavioral phenomena that are important with respect to taste learning are attenuation of neophobia and taste interference. Neophobia to food, as to other stimuli, is manifested in the careful consumption of a novel food/taste. If a few exposures to the novel taste lead to no gastric consequences, consumption of the given food will increase and thus will reduce the primary neophobic response. In a similar way to extinction, attenuation of neophobia will mark a given taste as safe and will increase its consumption in response to other needs of the animal.

An interaction between different familiar and unfamiliar tastes can lead to overshadowing or blocking. However, in interference one taste can interfere with the learning of another taste (Merhav et al., 2006). Specifically, it was shown that when two novel tastes are given before an associated malaise, only the second taste will acquire the association (Best and Meachum, 1986), Moreover, by using the latent inhibition paradigm, it was shown that consumption of a novel taste after another taste will eliminate completely the effect of the first taste (Merhav et al., 2006). This interference was inversely correlated with the time between the two tastes, and could be established only if the second taste was novel but not if it was familiar.

The above behavioral paradigms were established mainly with rats and mice. In all methods, mild water restriction is needed to stimulate the animals to consume the taste in a specific time. However, harsh water deprivation can modulate the behavior, and the behavioral method itself determines the biological mechanisms underlying taste learning (Berkowitz et al., 1988; Bernstein et al., 1996a,b; El-Gabalawy et al., 1997; Koh et al., 2003; Wilkins and Bernstein, 2006). The following are the main methods used in these experiments.

1. Single pipette: Animals are presented with single pipette with a given taste. The amount of intake can be compared with the amount of water consumed on the previous day, following one of the behavioral manipulations described.
2. Multiple pipettes: A main problem with the single bottle setup is conflict between the animal's urges to drink, because it is water-deprived, and not to drink, because it underwent CTA. The most common behavioral test is to allow the animals to choose, during the retention phase, from a series of pipettes containing water or the taste that is under investigation. Usually, an aversion index is calculated (water/water + studied taste). The more aversive the animal is to the conditioned taste, the higher the aversion index will be.
3. Taste reactivity test: The amount of drinking measured in session with one or multiple pipettes does not necessarily mirror the attractiveness of a given taste. This can be measured directly according to several characteristic responses to palatable or unpalatable tastes, in a taste reactivity test (Grill and Norgren, 1978), or via the licking behavior (Halpern and Tapper, 1971).
4. Learning without ingestion: In some behavioral studies, it is possible or necessary, sometimes because of limitations of the experimental setup, e.g., when studying electrophysiology in the anesthetized animal, to test taste learning without ingestion or consumption of the novel food. In such cases, the test is performed by intraoral infusion of the food/taste onto the tongue to elicit the response of the taste buds. CTA and other taste learning can be acquired through this passive experience. However, it seems that learning with or without ingestion could involve different learning mechanisms (Bernstein et al., 1996a).

4.13.3 Neuroanatomy of Taste and Conditioned Taste Aversion Learning

The sensation of taste involves, similarly in principle to the other senses, chemical recognition but, in addition, the physical features are always associated with hedonic aspects of the sensory input. Indeed, functional analysis of the taste neuroanatomic

pathway reveals a strong association with the reward and feeding centers in the brain, including the ventral tegmental area (VTA), the nucleus accumbens (NAcb), the ventral palladium (VP), and the lateral hypothalamus (LH). The central gustatory pathway has been studied extensively in humans, monkeys, and rodents. **Figure 1** shows a schematic depiction of the rat's main taste pathway. Following activation of the taste buds, three cranial nerves (VII, IX, X) convey the taste input to the rostral part of the nucleus of the solitary tract (NTS), the first relay nucleus. In addition, the NTS receives input both from the area postrema (AP), which is sensitive to blood-transported toxins, and from the vestibular system, which is sensitive to nausea caused by motion. Lesioning this part of the NTS induces severe impairment of taste preference, but CTA can be still learned (Shimura et al., 1997). Taste information is that transduced from the NTS to the parabrachial nucleus (PBN) in the pons. The main taste-responsive neurons in the NTS project to medial subnuclei of the PBN, and the PBN projects both to the parvocellular part of the ventralis postmedial thalamic nucleus (VPMpc) and to other forebrain structures, including the amygdala, the lateral hypothalamus, the substati innominata, and the bed nucleus of the stria terminalis.

Yamamoto et al. (1995) studied the effects of lesions to various forebrain structures including the PBN, the hippocampus, the VPMpc, the gustatory

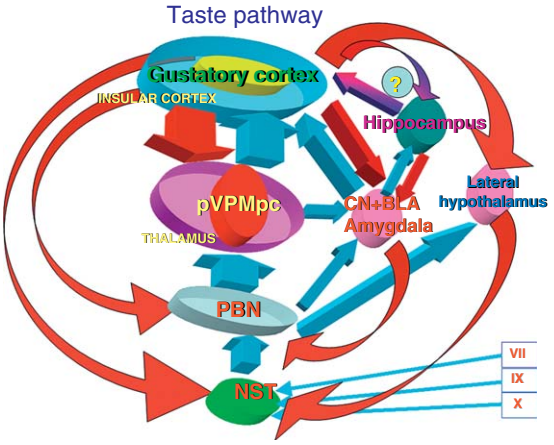

Figure 1 The neuroanatomy of the taste system. The processing of gustatory information begins with transduction of chemical stimuli which reach the oral cavity. Taste can be divided among five primary sensations: salty, sour, sweet, bitter, and umami. Typically, taste cells are broadly tuned and respond to several taste stimuli. The sensitivity to taste quality is not uniformly distributed throughout the oral cavity, and the same chemotopic arrangement is preserved to some degree at the gustatory relay. CN, central nucleus; BLA, basolateral amygdala.

Taste cells are innervated by cranial nerves VII, IX, X, which project to the primary gustatory nucleus in the brainstem (nucleus of solitary tract, NST). The NST sends information to three different systems:

1. The reflex system. This comprises medullary and reticular formation neurons which innervate the cranial motor nuclei (trigeminal, facial, hypoglossal).
2. The lemniscal system. The gustatory portion of the NST projects to the secondary nucleus situated in the dorsal pons (parabrachial nucleus, PBN). The PBN sends axons to the parvocellular part of the ventralis postmedial thalamic nucleus of the thalamus (VPMpc), which, in turn, relays gustatory information to the anterior part of the insular cortex (gustatory cortex, GC). The transition from the somatosensory lingual representation to the gustatory representation corresponds to the transition from the granular to the agranular insular cortex. The GC is thus situated dorsally to the intersection of middle cerebral artery and the rhinal sulcus and can be identified easily using these two markers. Although rodents have only a primary taste cortex, humans also have a second one.
3. The visceral-limbic system. The central gustatory pathway involves a collateral network of connections to the hypothalamus and limbic areas in the forebrain. The PBN is connected to the amygdala, the hypothalamus, and the bed-nucleus of the stria terminalis. All the limbic gustatory targets are interconnected with each other as well as with the PBN and the gustatory cortex. The GC and the thalamocortical system are required for acquisition and retention of taste information. The amygdala is required for learning the negative and possibly positive values of a taste. The prefrontal cortex is involved in CTA extinction. It is not clear what is the specific role of the hippocampus in taste learning, though it is hypothesized that it takes part in novel taste learning.

and enthorinal cortices, the amygdala, and the lateral and ventromedial hypothalamic nuclei, and reported that lesions to the PBN impaired both acquisition and retention of CTA. Other studies suggested that basic integration between taste and visceral inputs indeed took place at the level of the PBN.

The PBN projects to the VPMpc (Hamilton and Norgren, 1984), and from the relay station in the thalamus the taste information is transuded to the gustatory cortex (GC), which resides within the anterior portion of the insular cortex. Small lesions to the VPMpc did not affect CTA learning nor retrieval (Reilly and Pritchard, 1996), but a combination of lesions to the VPMpc and the GC eliminated CTA learning (Yamamoto, 1995).

Humans and monkeys have an additional, secondary area of taste, and it has been suggested that subdivisions within the insular cortex might serve as a secondary taste area in the rat brain. Similarly to the way the subcortical areas convert taste information, as described, the insular cortex also processes both taste information (in its anterior part) and visceral information (caudodorsally to the GC).

The first indication of the role of the GC in processing taste information was provided by Braun et al. (1972). Later, many experimental techniques, based on lesions, electrophysiology, imaging, correlative biochemistry, pharmacology, and, recently, direct imaging studies, were proven to be useful in analyzing the role of the GC in taste learning. It is clear that the GC plays a pivotal role in CTA acquisition and retention. Reversible inactivation of the amygdala and the insular cortex by microinjection of tetrodotoxin (TTX) to these two brain structures at different intervals before taste learning suggested that the insular cortex is pivotal for taste learning, whereas the amygdala is crucial for CTA formation (Gallo et al., 1992). The insular cortex and its gustatory portion can be anatomically divided into granular (normal neocortex), dysgranular, and agranular cortices (i.e., the gradual disappearance of the fourth layer). In rodents, most of the neurons that are responsive to taste stimuli reside within the dysgranular insular cortex. However, the input from the VPMpc terminates in both the granular and the dysgranular insular cortices. A topographical spatial organization of the GC in relation to the various taste stimuli was suggested recently by means of direct imaging of the GC *in vivo* (Accolla et al., 2007).

The experience of taste has other dimensions than the chemical input itself, including temperature and structure. These dimensions were hypothesized to be processed by the adjacent cortex, but also by the granular insular cortex itself (Simon et al., 2006).

The hippocampus, a forebrain structure known to be involved in many forms of learning, has been investigated also in relation to its role in taste learning. The role of the hippocampus in CTA is controversial; however, its involvement in neophobic responses to taste has been reported in several experiments. A temporal correlative response was found in the hippocampus and the GC. However, different molecular pathways were activated in the hippocampus and in the insular cortex (Yefet et al., 2006).

4.13.4 Long-Term Potentiation in the Insular Cortex

Long-term potentiation (LTP) is an attractive model for learning and memory: Activity-dependent, sustained increases in synaptic efficacy have been suggested to be the cellular manifestation of the learning process (Bliss and Collingridge, 1993; *See* Chapter 4.16). LTP was first described in the hippocampus but has been investigated in other brain structures, including the cortex; recently it was studied in the pathway from the basolateral amygdala to the insular cortex (Escobar et al., 1998) and in correlation with taste learning. High-frequency stimulation to the basolateral amygdala induced *N*-methyl-D-aspartate (NMDA)-dependent but metabotropic glutamate receptor (mGluR)-independent LTP in the insular cortex (Escobar et al., 1998, 2002). A pharmacological administration of the neurotrophin brain-derived neurotrophic factor (BDNF) locally to the insular cortex induced LTP that inclined slowly in a similar way to BDNF-induced LTP in the hippocampus (Escobar et al., 2003). Analysis of the molecular mechanisms of basolateral amygdala-insular cortex (IC) LTP identified correlative induction of extracellular signal-regulated protein kinase (ERK) activation and muscarine-dependent induction of several immediate early genes, including Zif268, Fos, Arc, and Homer (Jones et al., 1999). In a similar way to novel taste learning and to LTP in other brain structures, ERK was both correlative and necessary for LTP expression in the IC (Jones et al., 1999). It is clear from other studies that LTP and taste learning share molecular mechanisms in the IC. However, very little is known about the hypothesized possibility that LTP-like mechanisms in the IC subserve taste learning. A study that examined the possible interaction

between the two found that LTP in the insular cortex enhanced CTA retention (Escobar and Bermudez Rattoni, 2000). However, much more investigation is needed to achieve better identification of the relevant circuit within the IC, and to prove that LTP-like processes underlie taste learning in the GC.

4.13.5 Processing of Taste in the Gustatory Cortex

Neuronal responses in the GC are driven by somatosensory and chemosensory inputs received from the oral cavity (Yamamoto et al., 1989; Ogawa et al., 1992a,b). Neurons in the GC are responsive to both the quality of a given taste, i.e., chemical identification, and its hedonic value, i.e., attractive, palatable, or repulsive (Yamamoto et al., 1989), and it is difficult to dissociate the two from one another. This may represent a unique feature of the taste sensory information, whereby a given stimulus is always tagged as pleasant, indifferent, or repulsive, i.e., this value represents one of the dimensions of gustatory processing, including the chemical and physical dimensions. Relatively very few cells (<10%) are responsive to a specific taste (Yasoshima and Yamamoto, 1998; Bahar et al., 2003), and most of the others are broadly tuned. Recently, however, direct imaging of the insular cortex was used to reveal some spatial distribution of the various taste stimuli within the GC (Accolla et al., 2007). An interesting correlation between neuronal activity in the GC and novel taste input is that the increased response to a novel taste was not detected until 1 day following learning (Bahar et al., 2003). Currently, there are no good models of taste coding in the taste system. The two main models of taste coding in the neuronal system are labeled line and cross-fiber patterns. Both models are static and noninteractive and are insufficient to describe taste learning (Katz et al., 2002). Another hypothesis is that the anatomical divisions of the insular cortex, i.e., granular, dysgranular, and agranular, serve as primary, secondary, and tertiary sensory cortices, respectively.

A recent review on the subject suggested that the gustatory pathways, including the GC, use distributed, ensemble codes of the various dimension of taste stimuli, i.e., chemical, thermal, and tactile, to produce an internal representation of a given taste (Simon et al., 2006). Other reviews summarized a vast amount of seminal research on taste, olfactory, food texture, and control of food intake (Rolls, 2004, 2006), but that research is not within the scope of the current chapter and mainly addressed monkeys.

4.13.6 Molecular Mechanisms of Taste Learning in the Taste Cortex

The previous sections introduced the behavioral, neuroanatomical, and cellular levels of analysis. The sections below discuss aspects of molecular mechanisms that subserve taste learning in the taste cortex, within the frameworks of the behavior and the neuroanatomy, in more detail. The working hypothesis underlying the research in the field is that during learning, physical or chemical information about the world is depicted in part by the organism's sensory system, i.e., in the present case, the taste system. This information is transformed into neuronal activity that uses neurotransmitters to create an internal representation of the received sensory information in the central nervous system (CNS). This neuronal creation or modification of an internal representation, i.e., sensory learning, comprises several different temporal phases: Acquisition, which immediately follows experiencing the sensory input, the imprinting of the internal representation in the CNS, a consolidation phase that is divided into two major processes: molecular consolidation, which is dependent on functional protein synthesis in the relevant brain area and is limited to several hours following learning; and system consolidation, which involves the transfer of information between brain areas and which has a temporal domain ranging from many days to months. The consolidation phase is defined in negative terms; it is the time window during which the memory trace is still fragile and can be disrupted by various interventions: behavioral (e.g., Merahv et al., 2006), pharmacological (Rosenblum et al., 1993), or others (Bures et al., 1998). In addition, it is highly likely that the consolidation phase contains subdivisions. For a given internal representation, the last phase is retrieval. However, any retrieval may have the dimension of relearning, as discussed in detail elsewhere (*See* Chapters 1.05, 2.16).

Taste learning and CTA are highly suitable learning paradigms for studying the molecular mechanisms of learning and memory. Their relevant features include one-trial learning; strong incidental learning; clear and short learning time; minimal behavioral manipulation, since the animals can learn in their home cage with very little interference from

other modalities; clear definition of the sensory input, which can be quantified in molecular terms; and clearly defined cortical area(s) subserving the learning. Within the framework of the present chapter, we will specifically discuss molecular mechanisms taking place in the GC which underlie the various phases of taste learning. It is important to note that two basic means of acquiring information are studies of the correlations between taste behavior and molecular modifications/processes and causality experiments, which show that a given biochemical pathway is necessary for a given phase in taste learning. In addition, it is highly likely that one signal transduction cascade can operate in one brain area and not another. For example, protein kinase B (PKB)/Akt phosphorylation is correlated with taste learning in the hippocampus but not in the GC (Yefet et al., 2006); also, ERK is activated in the GC but not in the hippocampus after the same length of time after learning (Yefet et al., 2006). These examples suggest that from the neuroanatomical point of view, positive correlation between molecular changes and learning is informative, whereas negative correlation can teach us very little. A summary of recent molecular observation related to taste learning and mainly to the taste cortex is presented in the following sections.

4.13.7 The Neurotransmitters in the Gustatory Cortex Involved in Taste Learning

Information on the physical and chemical properties of a given taste and also on its prominence reaches the GC via different neurotransmitters. Several different neurotransmitter systems are released in the GC, and the relevant receptors for these neurotransmitters are expressed in the GC. These include acetylcholine (ACh), dopamine, noradrenaline, gamma-aminobutyric acid (GABA), glutamate, and various neuropeptides. However, only the muscarinic and NMDA receptors have been studied extensively for their role in taste memory acquisition, consolidation, and retention. I will, therefore, focus mainly on these two neurotransmitter systems and their possible role in taste memory formation. The physical and chemical taste information is transferred from the oral cavity to the cortex via fast neurotransmission mediated by the neurotransmitter glutamate. This is consistent with other known modalities (*See* Chapter 4.30). Glutamate is the main excitatory neurotransmitter in the mammalian CNS, and it acts via both ionotropic and metabotropic effects. However, the prominence of a given taste is hypothesized to be mediated via activation of the neuromodulatory system (e.g., Kaphzan et al., 2006). It is thus conceivable that the interaction between the two systems produces a long-term taste memory trace and that it coincides on specific neurons and probably molecules that can serve as coincidence detectors of the sensory input and its meaning (Kaphzan et al., 2006). Glutamate can affect four types of receptors – alpha-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA), NMDA, kainate, and mGluRs – that can produce complex fast (ion influx) and slow (second messenger) changes in the neuron. These receptors are structurally and functionally multifaceted molecules (*See* Chapter 4.30). Microdialysis studies of the amygdala and the IC demonstrated enhanced glutamate release in both the amygdala and the IC, following induction of malaise (Miranda et al., 2002).

Application of the AMPA/kainate antagonist, NBQX, specifically to the insular cortex impaired both acquisition and retrieval of taste memory, which suggests that, in agreement with the general understanding, the AMPA receptor plays a major role in mediating the physical properties of the taste (Berman et al., 2000) and that normal activity of the AMPA receptor is needed to mediate taste properties in either learning or recognition. In contrast to the effects on the AMPA receptor antagonist, microinjection of the NMDA antagonist aminophosphonovaleric (APV) into the GC induced severe impairment of CTA and taste memory acquisition, but not of retrieval (Rosenblum et al., 1997; Berman et al., 2000). Thus, taste learning and CTA are dependent on the NMDA receptor in the insular cortex similarly to many other learning paradigms. Moreover, LTP in the insular cortex is NMDA-dependent, which suggests that similar molecular mechanisms subserve learning and LTP (Jones et al., 1999).

Application of the NMDA antagonist specifically to the insular cortex blocked taste memory formation and reduced the correlative activation of ERK (Rosenblum et al., 1997; Berman et al., 2000). It did not, however, reduce the basal level of ERK phosphorylation (Berman et al., 2000) as it did in hippocampal slices (Kaphzan et al., 2006). In addition, the NMDA antagonist, APV, did not affect the correlative increase in tyrosine phosphorylation of the NMDA receptor, suggesting that this increased phosphorylation is

dependent on neuromodulatory neurotransmitters, most probably ACh (Rosenblum et al., 1997). Interestingly, APV microinjection to the insular cortex impaired long-term memory but did not affect short-term memory (Ferreira et al., 2002). These results are in contrast to the dogma that the NMDA receptor is crucial for memory formation but not for its consolidation, and may hint at the existence of unique molecular mechanisms that subserve the CS in the CTA case and that remain active for many hours.

Posttranslation modification and, especially, phosphorylation of the glutamate receptors, was hypothesized to play a major role in learning and LTP induction (*See* Chapter 4.30). The dogma suggests that changes in the steady state of the phosphorylation site of a specific receptor, e.g., the AMPA receptor, caused by modulation of the activity of kinases or phosphatases, alter the function of the receptor so as to increase or decrease synapse efficacy and thus to imprint a new cellular structure in the brain, which is manifested in the formation of an internal representation. However, on the assumption that the kinase/phosphatase modulation of activity is reversible, the memory of such a change is limited to the time span of the receptor that underwent phosphorylation. Different proteins are known to have different half-lives but, in any case, this cannot account for structural stability lasting more than a few days. In addition, the very rapid turnover of the receptors between different subcellular compartments shortens the time during which the receptor is expressed in the brain. Phosphorylation occurs on serine, threonine and, to a much smaller extent, tyrosine residues. However, tyrosine phosphorylation events have been found to be crucial in many cellular functions (Huang and Reichardt, 2003).

In the forebrain of mature animals, the NMDA receptor is composed of the NR1 subunits and a combination of NR2A and B subunits (*See* Chapter 4.20). The main tyrosine phosphorylated protein in the synapse is the 2B subunit of the NMDA receptor (Moon et al., 1994). However, it is clear that the 2A subunit is phosphorylated on tyrosine also, and in response to physiological inputs (Thornton et al., 2003). Tyrosine phosphorylation of both subunits induces modulation in the receptor function (Kalia et al., 2004), and it can be measured for a specific residue of a given protein, e.g., 1472 of the NR2B subunit, or as total tyrosine phosphorylation of a given protein after immunoprecipitation, or of a population of proteins. This measurement can be taken using antiphosphotyrosine antibodies for a population of proteins. Measuring tyrosine phosphorylation using general antiphosphotyrosine revealed that, compared with other tissues, the brain is highly phosphorylated on tyrosine residues in the absence of stimulation. In addition, following CTA or novel taste learning, tyrosine phosphorylation of a set of proteins was increased, but a familiar taste did not induce this correlative effect (Rosenblum et al., 1995). Following taste learning, the main molecular weights to be modulated in the insular cortex were 100, 115, and 180 kDa. At the same time, other proteins were unaffected by learning (Rosenblum et al., 1995). With regard to the findings of many other studies that identified correlations between posttranslation modifications and learning, the following questions can be raised in attempting to understand mechanistically the role of tyrosine phosphorylation in the GC during the formation of the internal representation of a taste:

- What is the identity of the tyrosine-phosphorylated proteins?
- What is the identity of the specific residue that is phosphorylated in a given protein?
- Is this phosphorylation event a mere correlation or a necessary step in memory formation?
- Can similar modifications be found in other learning paradigms or learning models?
- What is the time window and what is the physiological function of such phosphorylation?
- What are the upstream events that induce this phosphorylation in correlation with learning, and which neurotransmitters induce the modifications?
- What, and in which cortical layer, are the cells that are involved in these modifications? What is the localization, i.e., subcellular compartment, of such modification within the cells?

There are answers to some of these questions, whereas others are still subjects of ongoing research. It is clear that the NR2B is tyrosine phosphorylated in correlation with taste learning in the taste cortex (Rosenblum et al., 1997); a similar increase in NR2B tyrosine phosphorylation was detected in the hippocampus following LTP (Rosenblum et al., 1996; Rostas et al., 1996). As for the specific residue, the main tyrosine to be phosphorylated in the sequence of the NR2B is residue 1472. Indeed, a clear increase in pY1472 was correlated with taste learning in the GC. A recent study of genetic replacement of pY1472 in the mouse forebrain identified malfunction in amygdala-dependent learning, and suggested

that the localization of the NR was defective in these transgenic mice (Nakazawa et al., 2006). In any case, mapping of the phosphorylation sites specifically on the NR and generally in synaptic proteins, which might explain synaptic plasticity, is currently the subject of proteomic investigation (Schrattenholz and Soskic, 2006).

In order to test the hypothesis that induced tyrosine phosphorylation is necessary for taste learning, the general tyrosine kinase inhibitor, genistein, was injected locally into the GC during taste learning; it inhibited the induced tyrosine phosphorylation of the NR2B and attenuated taste learning, which suggests that tyrosine phosphorylation during learning is crucial for memory formation.

What might be the identity of the neurotransmitter that induces tyrosine phosphorylation of the NR2B? Surprisingly, local blocking of the NMDA receptor itself by microinjection of APV into the GC blocked taste learning but did not affect the induced level of its tyrosine phosphorylation correlated with learning (Rosenblum et al., 1997). Thus, it seems that the process is not Ca^{2+} dependent and is involved in cross-talking with other receptors. Indeed, pharmacological activation of the muscarinic ACh receptor (AChR) in the GC induced tyrosine phosphorylation of the NR2B (Rosenlum et al., 1996). This cross talk between the two receptors may partly account for the important role played by muscarinic AChRs in novel taste learning. An interesting point is that the time window of increased tyrosine phosphorylation correlated with taste learning. The induction was still clear several hours following learning, in clear contrast to the behavior of other phosphorylation events that can be monitored only within minutes following learning. It is therefore suggested that the synaptic tyrosine phosphorylation represents an intermediate phase of molecular mechanisms and that it is not involved in the acquisition phase but rather in the consolidation phase. Another possibility is that this prolonged increase in tyrosine phosphorylation following learning is unique to taste learning and plays a role in the ability of the taste to be on hold, ready for association with malaise.

The involvement of muscarinic AChRs in taste and CTA learning was studied mainly through the administration of antagonists during the various phases of taste learning, followed by microdialysis measurements of ACh in the GC. Indeed, local application of atropine or scopolamine to the GC disrupted both taste learning and CTA (Naor and Dudai, 1996; Gutierrez et al., 2003; Berman et al., 2000). It was suggested that microinjection of scopolamine but not APV to the insular cortex attenuates the acquisition of familiarity of a novel taste (Gutierrez et al., 2003), though APV clearly affected latent inhibition of CTA (Rosenblum et al., 1997). Another study suggested that APV treatment impaired only long-term but not short-term taste memory (Ferreria et al., 2002). In a similar way to the prolonged duration of NR2B tyrosine phosphorylation, this result implies that the NR is more involved in the consolidation than in the acquisition processes. Microdialysis experiments have shown that ACh is released in the GC following consumption of novel but not of familiar tastes (Shimura et al., 1995; Miranda et al., 2000) in a similar way to other modalities. It is yet to be determined whether the prolonged endogenous release of ACh is the physiological reason for tyrosine phosphorylation of the NR; what is the exact function of such interaction? Other biochemical interactions between these two receptors may occur and await further analysis.

The glutamatergic and cholinergic systems were studied in detailed in the GC following novel taste aversion. Fascinatingly, the correlated expression of different immediate early genes in the GC that followed LTP induction was dependent both on NMDA and muscarinic AChR activation. However, the involvement of other neurotransmitters is well documented. Local microinjection of antagonists for the $GABA_AR$, dopamine (D1, 5), mGluR, and β-adrenergic neurotransmitters into the GC impaired both novel taste learning and CTA acquisition. However, only application of the AMPA/kainate antagonist and the $GABA_AR$ impaired CTA retrieval (Berman et al., 2000). A recent report suggests that metabotropic GABA receptors are differentially involved in CTA learning. The $GABA_{B(1a)}$ receptor was found necessary for CTA learning, whereas the $GABA_{B(1b)}$ receptor was involved in extinction of CTA (Jacobson et al., 2006), but the brain locus involved in these manipulations could not be identified. Various pharmacological tools have been used to assay the correlative induction of ERK with taste learning. It was found that antagonists for AMPA/kainate, dopamine (D1, 5), mGluR, and agonist of the $GABA_AR$ reduced the basal level of ERK activation, whereas $GABA_AR$ antagonist induced the levels of ERK activation in the GC (Berman et al., 2000). However, antagonists of the muscarinic AChR, NMDAR, AMPA/kainate, dopamine (D1, 5), and mGluR inhibited the induced expression of ERK with learning (Berman et al., 2000). It is clear from these experiments

that cortical excitation is positively correlated with ERK activation and that antagonizing the muscarinic and the NMDA receptors is correlative with both taste and CTA learning, and with the correlative induction of ERK.

4.13.8 The Role of the MAPK/ERK Pathway in the Gustatory Cortex

ERK1 and ERK2 belong to the mitogen-activated protein kinase (MAPK) family of signal cascades. ERK is activated in and is necessary for the development of several forms of memory, such as fear conditioning, CTA memory, spatial memory, step-down inhibitory avoidance, and object recognition memory (*See* Chapter 4.25). Inhibition of MAP/ERK (MEK), the upstream kinase of ERK, affected both early and late phases of LTP in the hippocampus (Rosenblum et al., 2002). The role of ERK activation in the GC was first studied by means of radioactive kinase assays and, indeed, increased ERK activity was found in the GC, in correlation with novel taste learning. Later, most of the experiments in the field used phospho-specific antibodies that recognized the phosphorylated state of ERK as well as the activated state of these proteins in this unique case (*See* Chapter 4.25).

ERK activation was correlated with novel taste learning, whereas the actual amount of ERK protein was unchanged (Berman et al., 1998; Belelovsky et al., 2005). The time scale of the induced activation was a few minutes following consumption of a novel taste, and it could not be detected after more than 1 h. Microinjection of the MEK inhibitor into the GC prior to learning attenuated CTA memory; examination of other members of the MAPK family revealed that Jun N-terminal kinase 1/2 (JuNK1/2) was activated 1 h following novel taste learning, whereas p38 was not modified at any of the examined time points (Berman et al., 1998). A similar study in mice identified different temporal activation of ERK following novel taste learning (Swank and Sweatt, 2000). Further examination of the phosphorylation of the ERK substrate ELK-1 found a similar time scale for the relation between ERK activation and ELK-1 phosphorylation following novel taste learning as well as similarity in the neurotransmitters that induce ELK-1 phosphorylation in the GC in response to taste learning (Berman et al., 2003). Interestingly, the expression of LTP in the GC was found to be ERK dependent, and ERK was activated in correlation with LTP induction in the GC (Jones et al., 1999).

Similarly to other brain areas, ERK activation is correlated with learning, and possibly mainly with the consolidation phase of learning. Moreover, as discussed, the upstream mechanisms for ERK activation have been studied extensively in the GC and elsewhere. However, we know very little about the downstream targets of ERK or about the mechanisms in neurons that affect learning and synaptic plasticity. Much more proteomic research is needed to identify ERK substrates and to understand the effects of ERK on various neuronal processes, including trafficking, membrane properties, and nuclear targeting. One major role attributed to the ERK pathway is regulation of gene expression and, recently, translation regulation (Govindarajan et al., 2006).

4.13.9 The Role of Translation Regulation in Taste Memory Consolidation

Memory consolidation is defined biochemically by its dependence on functional protein synthesis within the relevant brain structures (Davis and Squire, 1984). Indeed, local application of the protein synthesis inhibitor anisomycin to the GC attenuated CTA and taste learning, as measured in the latent inhibition paradigm (Rosenblum et al., 1993). However, another study found that the same treatment had no effect on short-term memory, which suggests that short-term taste memory is independent of protein synthesis (Houpt and Berlin, 1999). Local application of anisomycin had a dose-dependent effect on CTA learning: Whereas 50- and 75-μg doses had no effect, 100- and 150-μg doses abolished CTA. This study (Rosenblum et al., 1993) is one of very few that measured the effect of anisomycin on protein synthesis *in vivo*. Local application of anisomycin inhibited more then 90% of protein synthesis in the GC for hours, but not in the hippocampus. Application of the same amount of anisomycin to the lateral ventricles did not affect either taste learning or CTA, and had a weaker and much faster effect on protein synthesis in the GC (Rosenblum et al., 1993; Meiri and Rosenblum, 1998). Usually, in order to achieve an effect on memory and LTP consolidation, one should apply the protein synthesis inhibitor(s) just before or immediately after learning, though some studies have suggested the existence of two or more sensitive periods for application of protein synthesis inhibitors. A recent study determined the temporal phase of sensitivity for

protein synthesis inhibitors, and found that up to 100 min following novel taste learning, local application of anisomycin attenuated taste learning, which suggests that the short period after learning, i.e., the beginning of consolidation, is not the only sensitive period, but so, also, is a more advanced phase of consolidation.

As discussed, sensitivity for protein synthesis is a negative definition of memory consolidation. One may assume that following learning there is increased expression of proteins in the relevant brain areas (*See* Chapters 4.22, 4.33), but it was only recently that scientists began to explore directly the possibility that the translation machinery is regulated during memory consolidation (Govindarajan et al., 2006). Translation comprises initiation, elongation, and termination phases. The initiation phase is the most highly regulated step and it is modulated via phosphorylation of initiation factors and ribosomal proteins (Proud, 2000). Analysis of taste learning in mice that lacked the translation repressor eukaryotic initiation factor 4E-binding protein (4E-BP2) revealed no difference in taste recognition but enhanced CTA learning (Banko et al., 2007). Similarly, mice with reduced phosphorylation of eukaryotic initiation factor 2 (eIF2α) exhibited enhanced taste learning, with no effect on taste recognition (Costa-Matiolli et al., 2006). In addition, it is clear that in these mice other forms of learning and plasticity are enhanced during the consolidation phase. Both the 4E-BP2 and eIF2α mice represent genetic modifications that enhanced translation initiation and thus induced better taste learning and memory. In contrast, knockout mice for both S6K1 and S6K2, which are characterized by reduced initiation rates, exhibited impaired taste learning.

Analysis of the initiation phase suggests a simple image: More initiation, better taste learning. However, the picture is more complex when the elongation phase of translation is analyzed.

The elongation phase requires activity of eukaryotic elongation factors (eEFs). Eukaryotic elongation factor 2 (eEF2) mediates ribosomal translocation (Ryazanov and Davydova, 1989) and is phosphorylated on Thr56 by a specific Ca^{+2}/calmodulin-dependent kinase. Phosphorylation of the kinase inhibits its activity and leads to general inhibition of protein synthesis (Nairn and Palfrey, 1987). Analysis of eEF2 phosphorylation in the GC, following novel taste learning, revealed that, in contrast to the simple hypothesis, the phosphorylation of eEF2 was increased and not decreased, which indicates an attenuation in translation elongation (Belelovsky et al., 2005). At the same time and among the same samples, the phosphorylation levels of S6K1 and ERK were increased, which suggests that the initiation levels were indeed increased (Belelovsky et al., 2005). On the assumption that the increased initiation and decreased elongation were taking place in the same neurons in the GC, one may suggest that this situation might lead to increased expression of mRNAs that are poorly initiated (see **Figure 2** for the proposed model). The suggested mechanisms could serve as a switch-like mechanism to express a specific set of mRNAs for a restricted time in a cellular microdomain such as the synapse.

It is clear that in the near future much more research will be aimed at understanding how regulation of the various phases of translation subserves consolidation processes. Other important proteins such as mammalian target of rapamycin (mTOR) and ERK are thought to be molecules that integrate the information delivered by the various neurotransmitters, in time and space, and translate it into a cellular decision that enables the consolidation of memories. In the near future, these very same molecules could serve as targets for possible cognitive enhancers in the consolidation phase.

4.13.10 Modulation of Specific Protein/mRNA Expression During Taste Learning and Consolidation

In many studies, a positive correlation was identified between enhanced expression of protein and/or mRNA, on the one hand, and learning in the relevant brain area(s), on the other hand (e.g., Guzowski, 2002). This correlation was assumed to be the positive aspect of the dependence of memory and synaptic plasticity on protein and mRNA inhibitors. As shown in **Figure 3** and discussed in detail earlier, many phosphorylation events are correlated with both novel taste learning and CTA learning. However, two conceptual observations can be derived from these findings. First, the posttranslation modulation that is correlated with novel taste learning is attributed to the meaning/prominence/novelty dimension and not to the physical/chemical dimension of the taste information (**Figure 3**); a familiar taste does not induce these correlations. One may suggest that the synaptic and neuronal activities underlying the formation of the taste memory *per se* are so small compared with the ongoing activity in the cortex that the biochemical methods used in these studies are not sensitive enough

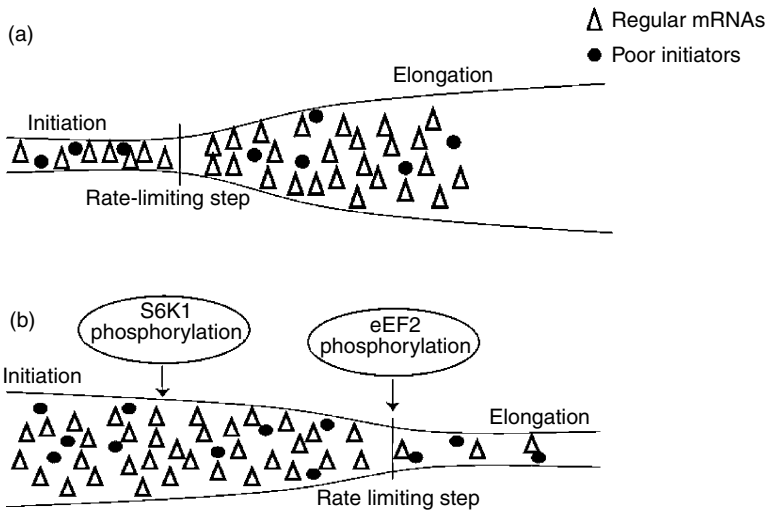

Figure 2 A proposal for the role of translation regulation underlying memory consolidation. (a) In a normal situation the rate-limiting step of protein synthesis is the initiation phase. The amount of protein synthesis is dependent on mRNA availability and the initiation rate as determined by ribosomal proteins and initiation factors. For a given amount of mRNA, regular mRNAs (triangles) are translated more than poor initiators (circles). A component of the intracellular signaling can alter the initiation rate, e.g., as in MAPK activation. (b) Taste memory consolidation is correlated with an increased initiation rate, which can be measured as increased ERK2 activation and decreased eIF2α phosphorylation and S6K1 phosphorylation. Within the same time frame, and possibly within the same cellular compartment, there is also a decrease in translation elongation, which can be detected in the increase in eEF2 phosphorylation. The end result of increasing initiation and decreasing elongation may be a shift in the rate-limiting step to the elongation phase. Together with the decrease in total protein synthesis, the synthesis of poorly initiated proteins such as α-Ca^{2+}/calmodulin-dependent protein kinase II (α-CaMKII) is probably increased. The mechanism described here can perform a switch-like function for various biological processes that shift protein expression patterns within a restricted time scale. In the cortex the mechanisms described can serve as molecular mechanisms that consolidate changes in synaptic strength over time. Adapted from Belelovsky K, Elkobi A, Kaphzan H, Nairn AC, and Rosenblum K (2005) A molecular switch for translational control in taste memory consolidation. *Eur. J. Neurosci.* 22: 2560–2568 with permission.

to detect the biochemical alterations subserving the physical properties of the taste. However, it is clear that some of the observed correlations (Rosenblum et al., 1997; Belelovsky et al., 2005) can be detected in the synaptosomal fraction. Another possibility lies in the strong link between taste and its perceived quality that is less inherent in other modalities. Taste is always perceived as good, bad, or indifferent, and thus the qualitative value of the physical input is a major building block in the memory of any taste.

The second observation is the difficulty of distinguishing between the biochemical correlations with the CTA or with the UCS itself, the malaise. In many experiments, the correlation between CTA and biochemical alterations can be detected following the UCS itself. In addition, the feeling of sickness is a strong input that can modulate several different parts of the brain.

What about mRNA or protein expression in the insular cortex that is correlated with taste learning or CTA?

The most common immediate early gene to be studied in correlation with learning is c-fos. Indeed, acute suppression, but not chronic genetic deficiency, of c-fos attenuated CTA learning (Yasoshima et al., 2006). Correlative studies for c-fos expression were carried out in Ilen Bernstein's laboratory (Wilkins and Bernstein, 2006; Koh et al., 2003; Koh and Bernstein, 2005) and elsewhere (Houpt et al., 1996; Ferriera et al., 2006). Exposure to a novel but not to a familiar taste induced c-fos expression in the central amygdala and the GC. The injection of the malaise-inducing agent LiCl induces c-fos in several brain structures, including the central amygdala and the IC. It is important to note that the posterior part of the IC processes visceral information and, therefore, it is possible that c-fos expression is not located in the same brain loci. The peak in c-fos expression can be detected about 1 h following novel taste learning and the malaise induced by the LiCl injection, and is degraded thereafter similarly to the processes in other brain areas subserving other learning paradigms (Wilkins and Bernstein, 2006).

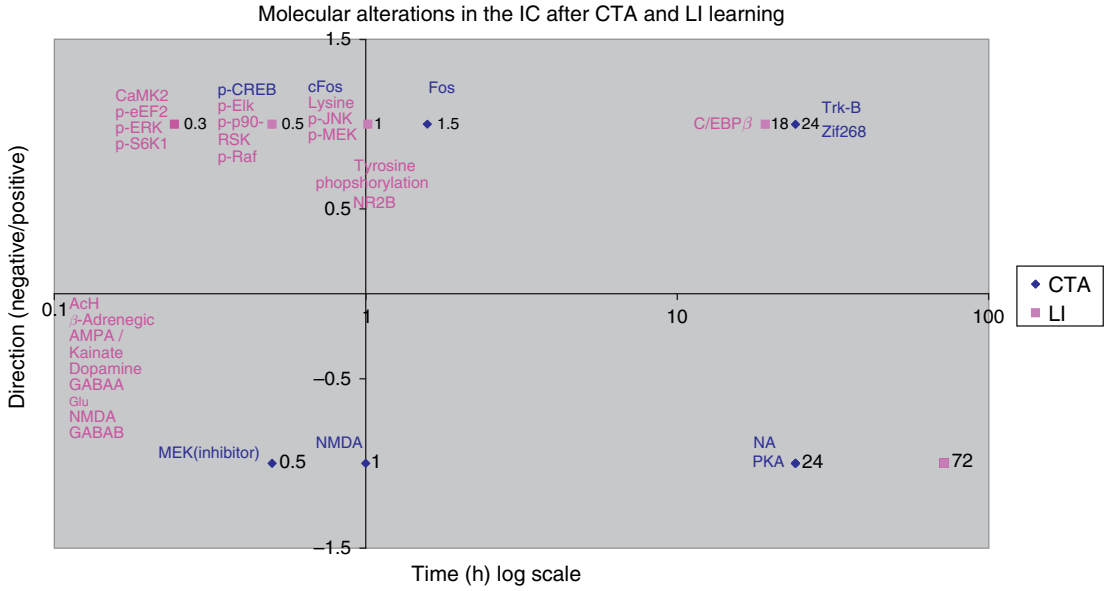

Figure 3 Summary of recent molecular analysis of taste learning in the rodent brain. The figure summarizes recent publications that examine the molecular mechanisms of taste learning; it shows whether the changes are correlative or are necessary for memory formation, and the direction of the changes. In addition, the figure indicates the method that was used and the temporal window of the changes. As can be seen, the research by Swank and Sweatt (2001) that used solid food with high metabolic value revealed a different time scale from that revealed by the other studies, which used liquid. In addition, the data in the figure can be divided into those related to CTA or novel taste learning; gene expression modulation, e.g., c-fos, or posttranslation regulation, e.g., MAPK activation; and manipulation affecting acquiring, retention, or both.

Another protein to be induced in correlation with taste learning in the GC is CCAAT enhancer binding protein β (C/EBPβ). However, the striking difference lies in the temporal phase of its induced expression. C/EBPβ is induced in both the hippocampus and the GC 18 h after taste learning (Yefet et al., 2006). Such late expression can be found following other learning paradigms (Taubenfeld et al., 2001). The correlative temporal increase in C/EBPβ 18 h following novel taste learning suggests that in a simple implicit learning task such as taste learning, both the hippocampus and the cortex are engaged in memory consolidation many hours after the acquisition phase. Moreover, another study demonstrated that this correlative induction of C/EBPβ can be deleted if another novel taste is consumed after the studied taste. A second novel taste induces memory interference that can be measured in behavior. However, at the same time, the second taste interferes with the correlative expression of C/EBPβ. Both the biochemical and behavioral interference suggest that a very long process of consolidation of taste information takes place, at least in part, in the GC (Merhav et al., 2006).

Careful analysis of the literature reveals very few correlations between mRNA-induced expression and taste learning. There are a few reports that identified induced expression of various mRNAs following CTA or LiCl injection (Lamprecht and Dudai, 1995, 1996). These investigations did not observe any modulation in mRNA expression in the insular cortex, following novel taste learning *per se*. Interestingly, LTP induced strong elevation in the expression of various mRNAs in the GC. These induced expressions were dependent on NMDA and muscarinic AChR (Jones et al., 1999). It is yet to be determined whether the major process that induces protein in the GC, in correlation with taste learning, is indeed translation and not transcription. Another possibility is that the temporal window for mRNA induction is prolonged and different from those in other learning paradigms, which would be consistent with the ability of the CS (novel taste) to maintain an on-hold position and to be associated with malaise hours later.

4.13.11 Temporal Phases in Taste Learning

The main feature of CTA learning is the long delay between the taste-related CS and the aversive internal symptoms (UCS). As a matter of fact, when first

published in the scientific community, this long delay put the behavioral results into question (e.g., Garcia et al., 1955). The delayed CS–UCS association cannot be explained as a phenomenon related to transfer of the taste input but as one related to the processing machinery itself within the CNS. In a way that is not clear, a better association is created with a time interval in the range of many minutes than with shorter time intervals (Sachfe et al., 1995). Thus, the system is well suited for prolonged delay between the CS and the UCS. It may be assumed that taste sampling creates a taste memory that remains ready for association, for many minutes. This taste memory trace is both short-lived for an association (usually a few hours) and also long-lived (weeks or more), as can be measured in the latent inhibition paradigm. The creation of taste memory is dependent on the functional taste cortex. One way to study the stability of the taste memory trace, which is dependent on GC activity, is to examine the interactions between taste inputs. Indeed, it was shown recently that taste memory is fragile and can be disrupted for hours by learning another novel taste input (Merhav et al., 2006). This intrinsic limitation of the taste system may be related to its capacity for prolonged association. Interestingly, the interference interaction is only for a second taste over the first one. The inverse interaction of the first novel taste on the second one induces a facilitation effect for the second taste. This facilitation effect has a time window of a few hours; it is shorter than the interference effect and it may be attributed to molecular interactions within the same neuron, i.e., a similar phenomenon to that in the tagging hypothesis model in LTP or to interactions among neuromodulators released within vast GC areas over a long period.

In any case, the unique ability of the taste system to form an association in such a delayed manner presents the researcher with the possibility of studying associative learning in a time frame that allows biochemical/molecular experiments, and not only electrophysiological manipulations and measurements.

4.13.12 Summary and New Directions

It was clear from the first scientific report of CTA that some of its features do not suit the mainstream learning models (Garcia et al., 1955). The two main differences, which may in fact be linked to one another, are the long delay between the CS (taste) and the malaise, and the built-in meaning of a given taste input. Thus, the chemical internal representation of a given taste always has a value: good, bad, or indifferent. It is indeed possible that any taste input is remembered by its association with its value. Definitely, the biochemical correlates of taste learning in the taste cortex – those that have been identified so far – are always correlated with the novelty of the taste input, which is a major factor in the saliency of any input. This means that, according to the current synaptic-Hebbian view of memory formation, the biochemical correlates cannot be attributed to encoding of the physical properties of the taste input. It is possible that these biochemical and cellular modifications are too small to be detected with the currently available technology.

One may argue that any long-term memory trace is associated with a value; however, it is clear that incidental taste learning (i.e., with no known external associations) is genetically strongly associated with the implication of the input for the animal, and it is clear that food consumption is a major factor in animal survival. Whereas the various senses can report to the brain regarding the potential of a given food for the animal (e.g., sound, color, smell, texture, and shape), the taste conveys the essence of the food for the animal. It is interesting that in Hebrew the word 'taste' is synonymous with meaning or reasoning, while 'vision' (I see) is analogous to understanding in English.

In any case, the perceived value of a given taste can be changed rapidly. The association with malaise is strong (CTA), but past experience (the behavioral paradigm of LI-CTA) and future experience (the behavioral paradigm of CTA extinction) can modify the taste value, and all three learning modes are dependent, at least in part, on the GC.

Taste learning offers unique opportunities to study the biological mechanisms underlying learning and memory. In the last decade, a number of laboratories have contributed to vast progress in this field. The development of new research tools (e.g., multielectrode arrays, genetic and molecular tools, and imaging techniques) enables us to ask new questions and to propose new hypotheses. Here are some of the major questions that will be addressed in the near future.

1. A major question in the neurobiology of learning and memory is whether the diverse experiences that create the internal representations are

modifications of a major internal representation or represent competition between different internal representations. It seems that the current knowledge of the taste system favors competition between different internal representations. However, further research is needed to obtain better evidence for one or the other of the options.

2. CTA learning is a hybrid between conscious learning, i.e., learning the taste information, and unconscious learning, i.e., visceral information. A major question concerns where this association takes place within the brain and neuronal circuit. The data so far indicate that the mainly subcortical structures, most probably the PBN, subserve the association. New tools and further analysis may enable us to answer this question.

3. Taste information is encoded, at least in part, by the GC, which resides within the insular cortex. However, very little is known about the circuit within the GC, and there are no good models that encompass the correlation between the electrophysiological and molecular information, on the one hand, and taste learning, on the other hand. A major line of research involves dissecting out the functional organization of the insular cortex on the circuit level, in order to better understand the encoding, consolidation, and retrieval processes, and it includes a number of questions. What types of cells produce the plasticity? Are they inhibitory interneurons, or excitatory cells? In which layer within the cortex do the correlative biochemical alterations take place? Does a given neuron contribute to the encoding of different tastes? Where within a given neuron do the modifications take place? The current experimental tools, which provide good spatial and temporal resolution, should provide answers to these basic questions in the near future.

4. Taste information can be kept on hold for many hours, waiting for an association, in contrast to other associations that have time windows measured in seconds. It is not clear if this unique ability represents distinct biological hardware, or if it is a prolongation of similar biological processes that underlie conventional association processes. A major question is how this associative potential is retained for many hours. If the mechanisms are similar to those in other learning paradigms, this unique ability of CS, which derives from processing mechanisms within the CNS, could be exploited to study association mechanisms by means of research tools with resolution times of minutes to hours, which could not be used in other learning paradigms, i.e., most molecular/biochemical tools.

5. The time frame of molecular modifications in the GC that are correlated with taste learning (see **Figure 2**) opens with posttranslation modifications within minutes after learning (e.g., ERK, eEF2; see **Figure 2** for more details), proceeds with other posttranslation modifications to synaptic proteins on a time scale of hours (e.g., tyrosine phosphorylation of the NR2B), and continues with protein expression on a time scale of hours to days following learning (e.g., the inductions of postsynaptic density 95 and C/EBPβ, which occur 3 and 18 h following learning, respectively). A major task will be to organize a temporal and spatial chart of these correlations in order to understand the stream of molecular and cellular events underlying taste memory formation.

In addition, the known identities of molecular events, and their time windows, are in accord with other brain structures that subserve other learning paradigms, for example, the expression of C/EBPβ in the relevant brain structure 18 h after learning. However, some of the events are different. For example, there are several indications that functional NMDA receptor in the GC is necessary for the consolidation phase, in contrast to other learning and LTP protocols, whereas the NMDA receptor is necessary mainly for the acquisition/induction phase. One possibility is that this is a unique feature of taste learning and that it is related to the ability of the taste memory trace to be associated after such a long delay. However, further analysis is needed to understand the role of NMDA receptor activity, localization, and posttranslation modification within the GC during taste memory formation.

6. In the last few years, the role of translation regulation in taste learning consolidation has been studied extensively. The results demonstrate intrinsic regulation of both the initiation and the elongation phases during taste memory formation. Moreover, it is clear that those genetic and pharmacological modifications that enhance the initiation phase also enhance performance. Much more research is needed to achieve understanding of translation regulation in memory consolidation to answer such questions as: What are the upstream neurotransmitters and signal transduction cascades? What are the targets and what are the mRNA populations and their localizations? However, the taste system can serve as a platform from which to study new targets for drugs, to be used as cognitive enhancers.

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4.14 Memory Reconsolidation

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4.14.1 Definitions and History of Memory Reconsolidation

Memory is an organism's ability to store, retain, and subsequently recall information.

The formation of memory takes place through complex and very dynamic processes. Following learning, the newly acquired information is in a fragile state and stabilizes over time to be finally stored as a long-lasting representation of the learned experience. This process of progressive stabilization of the memory is known as memory consolidation, and the internal representation of the memory is referred to as the memory trace or engram.

The concept of memory consolidation evolved from many studies over the last century in both humans and animal models. Toward the end of the nineteenth century, clinical observations showed that head injury or other traumatic insults to the brain lead to forgetting of more recent memories, while gradually sparing older ones. This gradient of retrograde amnesia (RA) was later reproduced using human verbal learning by the pioneering studies of Muller and Pilzecker around 1900. These authors reported that memory for a newly learned list of words is disrupted by the learning of a second list of words if it is presented shortly after the original one, a phenomenon known as retroactive interference. The authors concluded that neural processes activated by recently acquired information require time in order to become permanently 'fixated' or consolidated (Muller and Pilzecker, 1900).

The consolidation hypothesis has greatly influenced the neurobiological studies of memory throughout the last century. Starting from 1940, many laboratories investigated RA and experimentally reproduced the human data using animal models, particularly mice and rats. These animal studies confirmed that, if within a limited time window after acquiring the information the subject encounters some type of interfering stimuli, memory does not stabilize and the experience is forgotten. Stimuli able to disrupt memory consolidation can be different in nature and include, among others, brain trauma, new learning, seizure, electroconvulsive shock (ECS), brain cooling, and pharmacological treatments that inhibit basic molecular functions such as transcription and translation (Dudai, 2004). During the same years, in parallel with the multitude of studies on RA, an interesting complementary cellular view of how neuronal mechanisms store long-lasting changes was proposed. Inspired by Freud's earlier theories, in 1949 Hebb proposed that memories strengthen and stabilize through reverberating neural circuits and suggested a possible neuronal mechanism for the temporal gradient of RA: the disruption of reverberating circuits prevents the synaptic changes necessary for memory consolidation, and this effect gradually decreases as the changes progress over time. This concept of reverberating circuits, together with the conclusions from the findings on RA, led to the hypothesis that memory consolidation builds on biological modifications occurring over time, which become integrated and preserved as a lasting engram or impression (trace) in the brain.

One important issue that emerged from these original consolidation studies and still remains to be understood is the duration of the consolidation process. This has not yet been clarified, possibly because interferences of different nature likely affect different phases of the consolidation process itself. Various types of interfering stimuli have revealed at least

two levels, or phases, of consolidation. An early, rapid phase occurs over minutes or hours and is sensitive to molecular intervention, such as inhibition of RNA or protein synthesis or disruption of the expression of specific genes. In addition, at a later time, a second, slower phase of consolidation that can last for weeks, months, or even years in humans is believed to reorganize the circuits that bear the memory trace. This late consolidation phase can be disrupted by events that produce lesions or inactivation of neuronal circuits (e.g., hippocampal inactivation) (Squire et al., 2001). Despite that, this important issue remains unsettled: Until very recently, memory consolidation has been considered to be a one-time process that from its initial fragile state progressively strengthens memory over time (McGaugh, 2000). This chapter, unless otherwise specified, refers to the use of the term 'consolidation' that indicates the early phase of memory fragility, which depends upon new synthesis of RNA or proteins or the expression of specific molecules.

Discoveries reported in the 1960s and 1970s showed that, in contrast to the idea of a unitary process of memory consolidation, a memory that has become sufficiently stable and insensitive to various interfering stimuli, including the administration of ECS or RNA and protein synthesis inhibitors, could return to a fragile state for a limited time if the memory was retrieved or in some way 'reactivated.' During this fragile state, memory could be disrupted by the same interfering stimuli that were known to affect the initial consolidation. Consequently, a different hypothesis of memory stabilization was proposed: it is not the age of the memory that makes a memory more consolidated and resilient to disruption but, rather, its 'active' state (Lewis, 1979). Thus, a memory in its 'active' state (e.g., after training or retrieval) is fragile, whereas an 'inactive' memory is stable. It was also found that a memory could be reactivated by several means, the most common of which is the recall or retrieval of the learned experience (e.g., a conditioned stimulus (CS) exposure for Pavlovian conditioning memories). Memory reactivations, however, could also occur effectively by reexperiencing the motivational aspect of the learned event (e.g., drive states such as hunger) or by reliving the entire learning experience (e.g., another training trial). Thus, it was concluded that, after becoming again active and therefore fragile, memory needs to undergo again a stabilization process that, as revealed by RA, shares similarities to posttraining consolidation. For this reason, this process was later named reconsolidation.

After this series of experiments, in the 1970s, controversies set in, in part because of some failure to reproduce the findings of RA after memory reactivation and in part because, in several cases, spontaneous recovery from amnesia was reported. Therefore, alternative views that could explain postreactivation RA were put forward, and the lack of memory retention was considered a retrieval deficit rather than a failure of consolidation or reconsolidation (Riccio et al., 2006), raising a question that, as discussed below, still remains to be answered.

Subsequently, the issue of postreactivation memoryfragility quieted down for several decades, with the exception of a few sporadic studies, but then strongly reemerged in 2000, when an important study extended the notion that an established fear-conditioningmemory is disrupted if, after its retrieval, protein synthesis is inhibited in the amygdala (Nader et al., 2000a). The authors, in agreement with previous hypotheses, proposed that an established memory, once reactivated, becomes transiently labile and undergoes another consolidation phase in order to be maintained. This process was therefore named *reconsolidation* (Nader et al., 2000a,b; Sara, 2000a). In the last 6 years, a large number of manuscripts have been published on this subject, reflecting the exponential increase of the acquired knowledge about memory reconsolidation. All these studies have proven that numerous types of memories elicited with different protocols in different species ranging from snails to humans undergo reconsolidation (**Table 1**). In the following subchapters, the major recent findings about the mechanisms, features, circuits, and functions of memory reconsolidation are summarized.

4.14.2 Mechanisms and Circuits of Memory Reconsolidation

An important question that has been the focus of attention during the last 6 years and partially still remains to be fully understood is whether and to what extent the posttraining consolidation phase and reconsolidation are mechanistically similar at the molecular and cellular levels.

A premise that needs to be made here is that the identification of which molecular mechanisms underlie both memory consolidation and reconsolidation is one of the biggest technical challenges in neurobiology. Understanding the complexity of the

Table 1 Species, learning and memory tasks, brain regions, and molecules involved in memory reconsolidation

Species	*Tasks*	*Brain regions*	*Molecules*
Caenorhabditis elegans	Contextual fear conditioning	Hippocampus	C/EBPβ
Helix lucorum	Inhibitory avoidance	Amygdala	Zif268
Lymnaea stagnalis	Classical conditioning	Insular cortex	CREB
Chasmagnathus granulata	Implicit conditioning (mobile conjugate	Nucleus accumbens	NFκB
Honeybee	reinforcement)	Ventromedial prefrontal	PKA
Medaka fish	Radial arm-maze	cortex	MEK
Chick	Nonassociative learning (tap response)		SGK3
Mouse	Aerial respiratory operant conditioning		ERK
Rat	Appetitive odor discrimination		ERK2
Human	Object recognition		Choline
	Cue-induced drug seeking		AMPAR
	Conditioned withdrawal		CB-1R
	Conditioned place preference		NMDAR
	Motor skill finger tapping		mGluR5
			β-AR
			Glucocorticoid R
			$GABA_AR$

molecular changes accompanying behavioral responses and their functional role requires both the identification and functional testing of multiple molecules and mechanisms ongoing in different brain regions over time, an arduous task with the technology currently available. Currently, the most comprehensive approaches available can only identify correlative changes to behavioral responses occurring within specific brain regions or cell populations and are based on differential screenings using DNA microarrays or proteomics. These techniques offer the advantage of simultaneously evaluating the expression levels of thousands of genes or proteins, respectively, in a given experimental condition (e.g., time, space, treatment). The comparison among different experimental conditions (e.g., trained vs. controls) leads to the identification of specific genes or proteins. Although these techniques are incredibly powerful, presently they still possess several limitations: they are very laborious and expensive and are still not sufficiently sensitive for detecting small changes in heterogeneous tissues such as brain regions. Moreover, they can target only one time point at a time, and therefore each experiment only reveals a single snapshot of the ongoing molecular changes. Despite these limitations, several studies in the last few years have investigated comprehensive changes during posttraining consolidation using DNA microarrays or proteomics. However, perhaps due to its more recent reemergence, very little progress has been made investigating the reconsolidation process. The approaches thus far employed to uncover the nature of the molecular mechanisms underlying memory reconsolidation have been based on testing the potential roles of individual molecules which had been previously found to play a critical function during the consolidation phase. This approach, which has limited the potential for identifying new molecules and mechanisms, has been mainly used for another purpose: addressing the question of whether the reconsolidation process is a recapitulation of consolidation (Dudai and Eisenberg, 2004; Alberini, 2005; Nader et al., 2005). This question has been the focus of many debates. For example, it has been argued that it is expected that reconsolidation is not an exact copy of the posttraining consolidation, as new learning is distinct from a reactivation process, which often occurs through only a partial reexperience (i.e., reactivation by recall, in which, for example, for Pavlovian conditioning only a CS presentation occurs). However, for the precise reason that reactivation is generally different than training, other authors have felt that it is important to determine how similar or different their underlying mechanisms of stabilization actually are. With this in mind, several studies have examined whether the disruption of protein or RNA synthesis or of the expression of specific molecules that were known to block consolidation affected the reconsolidation of the same memories. Disruption of specific molecules was achieved by stereotactic injection of molecular inhibitors such as specific antagonists or antisense

sequences, or by region-restricted gene expression interference in transgenic animals. The conclusions from these studies revealed that a number of mechanisms are commonly used by both consolidation and reconsolidation, while other mechanisms are regulated and required for the consolidation of a particular memory but not during its reconsolidation. In summary, reconsolidation appears to be only a partial recapitulation of the initial posttraining consolidation process (von Hertzen and Giese, 2005). One study also reported that molecular mechanisms required in the hippocampus during either consolidation or reconsolidation are distinct or show distinct temporal dynamics. The genes examined thus far belong to different functional classes including kinases such as mitogen-activated protein kinase (MAPK), serum and glucocorticoid-induced kinase 3 (SGK3), and cAMP-dependent protein kinase A (PKA); transcription factors such as cAMP-response element binding protein (CREB), CCAAT enhancer binding protein β (C/EBPβ), zif 268, and nuclear factor kappa B (NFκB); neurotrophic factors such as brain-derived growth factor (BDNF); and membrane receptors, such as glutamate, noradrenergic, cannabinoid, and glucocorticoid receptors (**Table 1**).

In addition to specific mechanisms, it was also investigated whether similar or distinct brain areas (circuits) are recruited during consolidation or reconsolidation. These questions have been addressed by disrupting neuronal activity (inactivation) or specific molecules within restricted brain areas or even distinct cell populations within brain regions. The results indicate that not all the same brain regions engaged during memory consolidation are also involved during the reconsolidation of the same memory. Hence, in a variety of memories, similar to what has been observed for the molecular mechanisms, only a partial overlapping of circuitry between the two processes was revealed.

In summary, the two processes of consolidation and reconsolidation appear to only partially share molecular mechanisms and brain areas. Hence, the term 'reconsolidation' refers to the concept of memory restabilization and does not indicate the reiteration of underlying mechanisms.

Our understanding of how memory reconsolidation, but also consolidation, occurs mechanistically at the molecular and cellular levels is still very primitive. While, as mentioned above, several molecules required for memory reconsolidation have been pinpointed, the sequence of the molecular events that are activated during the onset and development of the process is unknown. Some recent studies have found intriguing correlations between the expression level of glutamate receptors and memory reconsolidation, suggesting that, like memory consolidation, a critical role might be carried out by the regulation of alpha-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA) receptors (Rose and Rankin, 2006). Further studies will be important for unraveling the sequence of molecular events necessary to restabilize a memory after it has been reactivated.

4.14.3 Temporal Constraints

Two types of temporal constraints have been found to characterize memory reconsolidation: (1) the persistence and duration of the amnesia and (2) the age of the memory.

4.14.3.1 Persistence and Duration of Amnesia Induced by Postreactivation Interference

Some researchers have found that, whereas the amnesia caused by protein synthesis inhibition after training is long lasting, the amnesia resulting from inhibition of protein synthesis after memory reactivation spontaneously recovers over time (Anokhin et al., 2002; Lattal and Abel, 2004; Salinksa et al., 2004) or recovers after exposure to a reminder event (Fischer et al., 2004). Thus, several authors believe that the amnesia caused by postreactivation interference results from a retrieval deficit. On the other hand, these results have not always been reproduced, and in several cases memory disruption after reactivation lasted for a long time (weeks or months in animals), and memory retention never returned even following a strong reminder. Several reasons may explain these opposing results. First, the question of the persistence of the amnesia induced by protein synthesis inhibitors after reconsolidation may be related to the degree and duration of protein synthesis inhibition. Unfortunately, very few studies have determined the rate of protein synthesis inhibition with the conditions and protocols used in reconsolidation experiments. The same issue had been under debate for the consolidation process a few decades ago, and a possible explanation offered for the temporary effect was that protein synthesis was only partially inhibited or inhibited for an insufficient length of time. Indeed, it has been reported

that stronger memories require longer (stronger) inhibition of protein synthesis to be permanently disrupted (Flood et al., 1973, 1975; Barraco and Stettner, 1976; Davis et al., 1978). In addition, in agreement with these results, it has been recently demonstrated that a partial inhibition of protein synthesis during the consolidation or reconsolidation phase precisely results in temporary impairment that recovers at later times (Milekic et al., 2006; Miller and Sweatt, 2006). A second possibility that can explain why amnesia results more frequently after postretrieval rather than posttraining interference is that disrupting a memory that had been already stabilized is more difficult than disrupting a newly acquired memory. Logically, a memory trace that has been previously established would be more resistant to disruption than a newly developing trace. Therefore, the same amnesic treatments may result in a total amnesia for new memories, but only a partial amnesia for a reactivated memory. In the latter case, a reminder may increase the strength of the memory trace and result in a memory recovery, which would be misinterpreted in favor of a potential retrieval failure rather than a loss of stored information. Third, by definition, it is always impossible to exclude that an amnesia results from a retrieval deficit, because even though no recovery might be observed under any circumstances, currently there is no direct method to prove that the memory trace has been completely erased.

4.14.3.2 Age of the Memory

The hypothesis of memory reconsolidation predicts that when a memory is active (following training or reactivation) it is in a labile state, and when inactive it is stable. This implies that *every time* a memory is reactivated it becomes transiently labile. A number of recent studies have tested whether this hypothesis is correct or whether the memory changes over time. The results have clearly shown that, in most cases, the passage of time influences the stability of a memory and that the older a memory becomes the less susceptible it is to disruption following its reactivation. These results have been found using several fear conditioning types of tasks in many different species including rat, mouse, chick, and Medaka fish. In general, an increasing resilience to disruption following memory reactivation has been reported over several weeks. For example, in rat inhibitory avoidance 2- and 7-day-old memories became fragile and are disrupted by protein synthesis inhibitors administered after recall, while older, 14- and 28-day-old memories are resistant to the same treatment (Milekic and Alberini, 2002) (**Figure 1**). Similarly, in Medaka fish, the administration of a Na^+ channel blocker upon reactivation disrupts a 4-day-old memory but has no effect on a 15-day-old memory and an intermediate effect on a 9-day-old memory (Eisenberg and Dudai, 2004). Likewise, numerous other studies in different species have confirmed the existence of a temporal gradient of progressive resilience to memory disruption after reactivation (Litvin and Anokhin, 2000; Suzuki et al., 2004; Boccia et al., 2006; Frankland et al., 2006). An important extension of these findings revealed that, in addition to the age of the memory, the strength of the memory and the reactivation intensity interact to influence the degree of vulnerability of a reactivated memory (Suzuki et al., 2004). In fact, whereas protein synthesis inhibitors can disrupt a 24-h- to 3-week-old contextual fear memory after reactivation in mice, an 8-week-old memory remains insensitive to disruption by a similar reactivation session. However, if the reactivation session is prolonged, even the older memory can be disrupted. Finally, weak (one shock) versus strong (three shocks) training protocols correlate with more or less susceptibility to disruption after reactivation, respectively. Interestingly, using associative learning in *Lymnaea*, it was found that reconsolidation at both 6 and 24 h, which requires protein synthesis, undergoes progressive molecular changes, because only reconsolidation at 6 h after training, but not at 24 h, requires protein kinase A activity. This phase-dependent differential molecular requirement for reconsolidation supports the idea that even

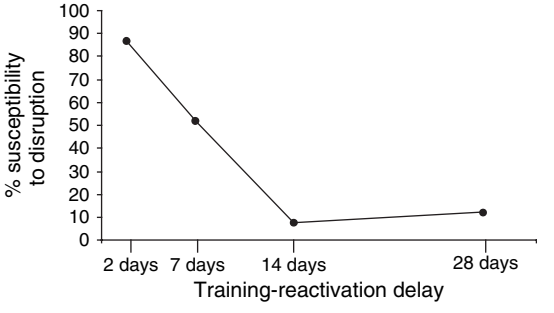

Figure 1 Temporally graded decrease of susceptibility to disruption of a reactivated memory. Susceptibility to disruption by anisomycin of reactivated inhibitory avoidance memories of different ages. Taken from Milekic MH and Alberini CM (2002) Temporally graded requirement for protein synthesis following memory reactivation. *Neuron* 36: 521–525, with permission from Elsevier.

seemingly consolidated memories undergo further selective molecular maturation processes (Kemenes et al., 2006).

In conclusion, an important temporal constraint characterizes memory reconsolidation: as the time interval since training lengthens, there is increasing resistance to postreactivation disruption. This indicates that the reconsolidation process is a manifestation of a lingering consolidation (Dudai and Eisenberg, 2004; Alberini, 2005).

Some authors disagree with this interpretation. Some studies show that 2-day- and 2-week-old memories of cued fear conditioning as well as a 45-day-old contextual fear conditioning memory in rats are disrupted by postretrieval bilateral injections of anisomycin into the amygdala or hippocampus, respectively (Nader et al., 2000a; Debiec et al., 2002). In contrast, other groups have reported opposite results using contextual fear conditioning in mice (Suzuki et al., 2004; Frankland et al., 2006). Furthermore, memories that are based on multiple training trials appear to show a long-lasting temporal window of postreactivation fragility: a 21-day-old appetitive instrumental response (sucrose self-administration) is disrupted by systemic injection of the beta-adrenergic antagonist propranolol, and a 27-day-old cocaine-induced conditioned place preference is disrupted by postreactivation amygdala injections of zif 268 antisense (Diergaarde et al., 2006; Lee et al., 2006). These opposing data can be explained by the findings discussed above showing that the nature of the learning, the intensity of training, and reactivation protocols produce gradients of protein synthesis requirements after reactivation that show different temporal evolutions. Hence, under certain experimental conditions (e.g., the reactivation intensity and time points chosen), the memory may still lie within the labile period. Following the same logic, it will be consistent with a lingering consolidation and the results of RA affecting remote memories that the stabilization process may take a long time, and therefore, some memories may remain sensitive to postretrieval interference for an extended period of time.

Importantly, the existence of a gradient does not exclude the possibility that reactivation of fully consolidated memories is accompanied by a phase of *de novo* protein synthesis. However, new molecular changes induced by the reactivation of a fully consolidated memory may occur without manifested fragility of the consolidated information. This view is in agreement with a hypothesis that had been proposed to explain memory stabilization over time, which suggested that memory stability correlates with the distributed nature of the memory trace (Nadel and Moscovitch, 1997) and is also in line with the hypothesis mentioned above of reverberating circuits proposed by Hebb (1949).

The mechanisms underlying the progressively decreasing gradient of memory fragility are not yet clear. However, future investigations should be able to address this question.

4.14.4 Functions of Memory Reconsolidation

The reason why memories become labile after reactivation is still unknown. Two main, non–mutually exclusive hypotheses have been proposed. First, as suggested above by the hypothesis of a lingering consolidation, a function of reconsolidation might be to strengthen memory retention (Sara, 2000b). A second possible role of reconsolidation may be to allow the integration of new information into the background of the past. In other words, reconsolidation might be required "to associate new learning with already established and reactivated memories" (Lewis, 1979; Sara, 2000a; Dudai, 2004). Although no direct proof has yet been provided, the results of several studies support the idea that memory reactivation leads to increased memory strength and prevents forgetting. For example, electrical stimulation of the mesencephalic reticular formation (MRF) improves the retention of memories, including fear conditioning and linear maze, when the stimulus is applied after memory reactivation (DeVietti et al., 1973; Sara, 2000b), indicating that memory retention can be enhanced during a postreactivation phase. In addition, recent studies in *Lymnea stagnalis* showed that the reexperience of a conditioned stimulus in the same context where the subject previously underwent a training that elicits an intermediate-term memory converts a residual memory trace into long-term memory (Parvez et al., 2006). Finally, several modulatory compounds, including angiotensin II and agonists of protein kinase A, have been found to increase memory retention if present during the reconsolidation phase (Frenkel et al., 2005; Tronson et al., 2006), suggesting once again that after reactivation, memory can be strengthened.

The second proposed function, that the reconsolidation process mediates the formation of associations between new and reactivated information, is now

beginning to be understood. Toward this end, one study utilized a second-order conditioning paradigm (Tronel et al., 2005) in which two associations are established in series. First, a conditioned stimulus (CS1) is paired with an unconditioned stimulus (US), such as when a given context (context A) is paired with a footshock. Then the established properties of CS1 are paired with a new stimulus, CS2, producing a CS1-CS2 association. For example, the subject that has learned the context A-US pairing is then exposed to a different context (context B) in which one cue of context A is presented. This experience reevokes the result of the CS1-US association, which now becomes linked with context B. Therefore, the subject learns to fear context B even though this context has never been associated with a shock.

Using inhibitory avoidance, it was found that systemic inhibition of protein synthesis after the exposure to context B (which also contains a cue from context A) disrupts both the new association (CS2-CS1) and the recalled memory (CS1-US). Region-restricted inhibition of the transcription factor C/EBPβ allows the functional dissociation of the consolidation and reconsolidation of inhibitory avoidance. Inhibition of C/EBPβ in the hippocampus disrupts inhibitory avoidance consolidation but not reconsolidation, and conversely, inhibition of C/EBPβ in the amygdala disrupts reconsolidation but not consolidation. If memory reconsolidation mediates the incorporation of new information into a reactivated memory, then it is predicted that after the exposure to context B the inhibition of C/EBPβ in the amygdala should disrupt both memories. Instead, it was found that such a C/EBPβ inhibition only and selectively disrupted the old memory for context A but spared the new memory for context B. Thus, the reconsolidation of the original memory takes place independently from the formation of the new association. Conversely, the memory for context B was selectively disrupted by hippocampal C/EBPβ inhibition, suggesting that the formation of the new association is mediated by molecular mechanisms similar to those underlying the initial consolidation of a new memory. In conclusion, although the old memory needs to be retrieved in order to associate new information, its reconsolidation process is not engaged in establishing the link between the new information and the reactivated memory.

Another study investigated memory 'updating' by approaching this question from a different angle. Repeated learning presentations of the same event using attenuation of neophobia (AN) in rats were used to test (1) how the acquisition of a new learning trial becomes linked to the consolidated memory established by the previous trials and (2) the fragility of both the updated and previously established memories (Rodriguez-Ortiz et al., 2005).

In AN, the animal gradually increases the intake of a tastant after repeated presentations of the tastant itself. Each additional learning could be considered as a memory updating event. Using multiple training trials, each separated by 24 h, it was found that, while after the first and second trials an infusion of anisomycin into the insular cortex completely disrupted memory retention, after the third trial a partial disruption was observed. Moreover, after six trials, a similar treatment had no significant effect. Thus, it was concluded that, while additional learning is acquired, part of the older, consolidated memory in the insular cortex becomes independent of protein synthesis. Furthermore, rats that after seven trials of AN were trained to conditioned taste aversion (CTA) for the same taste in the presence of anisomycin developed amnesia for CTA the following day. In light of these results, it was concluded that "protein synthesis is required to update previously consolidated memory trace regardless of the valence of the tastant." This study, in agreement with the first one, indicates that the formation of a new association linked to a previously established memory requires protein synthesis. Because it was not determined whether the protein synthesis required for the reconsolidation or persistence of the old memory is recruited to mediate the incorporation of new information, it is possible that AN updating, like inhibitory avoidance (IA) second-order conditioning, utilizes consolidation-like mechanisms and not the reconsolidation process of the original memory. In agreement with this hypothesis, the AN study shows that the old and new memories are dissociable processes because, while the old memory can become resistant (consolidated), the updating is sensitive to disruption. In conclusion, it is important to experimentally dissect what is being disrupted – the process that mediates an association between a new experience and a retrieved information, or the restabilization process of a reactivated, previously established memory.

4.14.5 Clinical Applications

The reemergence of research focusing on memory reconsolidation has stimulated interest in the medical community, particularly among mental health professionals treating disorders that are based on

pathogenic memories. Two examples of these directives are posttraumatic stress disorder (PTSD) and drug addiction, both of which can be viewed as the result of dysfunctional memory processes.

PTSD can develop after an exposure to an emotionally or physically traumatic event, and the illness is characterized by repeated, intrusive memories of the experienced trauma. Patients suffering from PTSD have difficulty sleeping and feel detached or estranged, and these symptoms can be so severe and persistent as to significantly impair the person's daily life. A behavioral model of PTSD onset has been proposed: the traumatic event (US) triggers a strong hormonal stress response, which mediates the formation of a robust and enduring memory for the trauma. Subsequent recall of the event in response to cues and reminders (CS) releases more stress hormones (conditioned response) and even further consolidates the memory leading to PTSD symptoms, such as flashbacks, nightmares, and anxiety (Pitman and Delahanty, 2005). The persistence of PTSD can be explained in terms of a trauma-induced strengthening of the memory trace. More specifically, it is hypothesized that noradrenergic hyperactivity and stress hormones facilitate the encoding and consolidation of the memory (Pitman, 1989; O'Donnell et al., 2004; Yehuda, 2006).

Although it is not possible to precisely reproduce PTSD in an animal model, fear conditioning in rodents can be used to mimic and elucidate some aspects of PTSD, including the processing of fearful stimuli and the encoding of emotional memory. As described above, fear conditioning is readily induced in laboratory animals by pairing a CS, such as contextual cues, with a fear-inducing US, such as a foot shock. Presentation of the neutral CS at some later time evokes a stereotypic fear response in the animal, including the stress response and activation of the autonomic nervous system. This is similar to the scenario in which contextual cues associated with a traumatic event can precipitate onset of PTSD symptoms in patients. Thus, it is possible to closely approximate some of the PTSD symptoms in an animal model that can be used for preclinical research. In principle, all pharmacological compounds thus far shown to have the ability to disrupt fear memory reconsolidation could potentially be useful for designing new drugs that can be used in clinical trials. Some of these compounds, including antagonists of beta-adrenergic or glucocorticoid receptors, which target the stress pathways, are already employed in clinical pharmacology for treating other diseases. Hence, they represent the most readily available potential therapies.

Targeting memory reconsolidation and the noradrenergic system in an attempt to treat PTSD has already been explored at the preclinical level and is currently being used in clinical trials. One method involves administration of the beta-adrenergic blocker propranolol, commonly used to treat hypertension, following recall of the traumatic memory. Several years ago, researchers first tested the effect of propranolol on memory reconsolidation using inhibitory avoidance in rats (Przybyslawski et al., 1999). Systemic administration of propranolol given after the reactivation of the inhibitory avoidance memory disrupted the memory at subsequent tests. More recently, using another paradigm known as auditory fear conditioning, other researchers have shown that propranolol injected either systemically or into the lateral nucleus of the amygdala (LA) after reactivation of a 2-month-old memory weakened the fear response when tested 48 h later (Debiec and LeDoux, 2004, 2006).

Another pharmacologically targeted pathway for the potential treatment of PTSD is the glucocorticoid pathway. The endogenous stress hormone corticosterone bidirectionally modulates memory retention (McGaugh and Roozendaal, 2002; Roozendaal, 2002). Low doses increase memory retention, while high doses disrupt it. Recent studies have reported that glucocorticoids, when administered after the reactivation of a contextual fear memory, have an amnesic effect on the original memory and provide evidence that a possible mechanism for this effect is an enhancement of extinction of the expression of the original memory (Cai et al., 2006). Another study, however, revealed that intra-amygdala blockade of glucocorticoid receptors with the antagonist mifepristone after reactivation of inhibitory avoidance memory significantly disrupts the original memory, probably interfering with its reconsolidation process (Tronel and Alberini, 2007). In light of these results, the role of the glucocorticoid pathway following memory reactivation has clearly been established and promises to be an additional site of pharmacologic intervention for PTSD.

Substance abuse generally leads to a chronic condition believed to result from an addict's inability to permanently abstain from drug use. Drug addicts repeatedly relapse to drug seeking even after years of abstinence, and this pathologic behavior is frequently induced by the recall of memories and environmental

stimuli intimately connected to the rewarding effects of the drug. Therefore, disruption of memory reconsolidation provides an unprecedented potential strategy to disrupt memories that facilitate drug addiction. Promising results have recently been reported in animals dependent on morphine or cocaine by injecting, after memory reactivation, inhibitors of protein synthesis or ERK (extracellular signal-regulated kinase), or by disrupting the expression of the immediate-early gene *zif268* both peripherally and within specific brain regions, such as the amygdala, hippocampus, or nucleus accumbens (Lee et al., 2005; Miller and Marshall, 2005; Milekic et al., 2006; Valjent et al., 2006). In some of these studies, inhibitors were administered to animals that had previously acquired a place preference in response to the drug of abuse, a learning known as conditioned place preference. Animals learned to associate the euphoria of the drug with a specific location, choosing to spend more time there. Administration of several of these inhibitors after reactivation of the drug-related memory interfered with its reconsolidation and abolished the place preference. Other studies investigated a different type of task in which animals form a CS–drug association during drug self-administration training, a model of drug seeking (Lee et al., 2005, 2006). These studies showed that infusion of zif268 antisense oligodeoxynucleotides into the basolateral amygdala, prior to the reactivation of a CS–cocaine association, abolishes its impact on the learning of a new cocaine-seeking response or maintenance of cocaine seeking as well as relapse to a previously established drug-seeking behavior. Furthermore, the same group later demonstrated that conditioned withdrawal could be disrupted following the reactivation of a CS–withdrawal association (Hellemans et al., 2006).

How might a clinical strategy for disruption of reconsolidation of a pathogenic memory be designed? An example of methodology involves pairing pharmacotherapy with memory retrieval within a carefully controlled clinical setting. For example, a patient's memory could be evoked through reexposure to key sensory components of the memory, or reminder cues, immediately followed by treatment with an antagonist or inhibitor that interferes with the reconsolidation of that particular memory. Another strategy might involve patient self-administration during a spontaneous memory flashback, presumably when the memory becomes pliable. Better understanding the mechanism of memory reconsolidation and the consequences of its disruption on human behavior will ultimately help clinicians to target mental illnesses based on pathogenic memories.

Acknowledgements

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4.15 Molecular Aspects of Memory Dysfunction in Alzheimer's Disease

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4.15.1 Introduction

Alzheimer's disease (AD) is the foremost human disease of memory. Independent streams of research on the molecular mechanisms of AD and the molecular basis of memory, which historically were quite distinct, are now converging in exciting ways. The study of AD may provide novel insights into basic mechanisms of memory, and even more importantly for the over 24 million patients with AD worldwide (Ferri et al., 2005), discoveries about the molecular basis of memory are advancing our understanding of the disease and yielding new treatment targets.

AD typically begins with mild forgetfulness in the elderly. Early on, before memory problems interfere with day-to-day functioning, a diagnosis of mild cognitive impairment (MCI) is often made. As years go by, deficits become more severe and patients are forced to curtail their usual activities. Deficits in other cognitive domains become increasingly apparent, including executive dysfunction, anomia and other language problems, visuospatial dysfunction, and ultimately, global cognitive impairment. Median survival is about 12 years after first symptom onset (Roberson et al., 2005).

Although the diagnosis of probable AD is made during life, definite diagnosis is made only at autopsy. The hallmark pathological features of AD are extracellular amyloid plaques and intracellular neurofibrillary tangles (NFTs) (**Figure 1**), combined with neuronal and synaptic loss, vascular amyloidosis, astrocytosis, and microgliosis. Most of these changes occur in a stereotypical regional distribution, with medial temporal structures involved in memory affected earliest and most severely.

Biochemical and genetic studies of AD have identified several molecules that may play a causal role in its pathogenesis (**Figure 2**). For the most part, these molecules have been studied primarily in the context of AD. In the first half of this chapter, we consider the impact of these AD-related molecules on memory. In addition, many molecules that have been studied primarily in the context of synaptic plasticity and memory, including a variety of receptors, channels, second messengers, kinases, and transcription factors, are also involved in AD. In the second half of the chapter, we consider AD-related memory impairment in relation to these molecules.

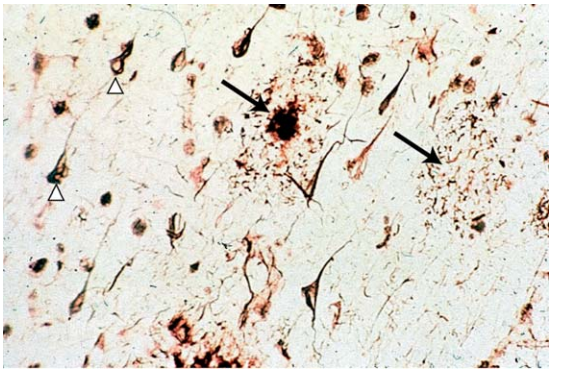

Figure 1 Plaques and tangles. This silver-stained section of cerebral cortex from a patient with Alzheimer's disease shows extracellular amyloid plaques (black arrows) and intracellular neurofibrillary tangles (white arrowheads). (Courtesy of Dr. Nitya R Ghatak, Virginia Commonwealth University Health System.)

4.15.2 Memory Impairment by AD-Related Molecules

In this section, we examine several molecules that likely contribute to the pathogenesis of AD, including the amyloid precursor protein (APP), amyloid-β peptide (Aβ), the β- and γ-secretases that release Aβ from APP by proteolytic cleavage, the microtubule-associated protein tau, the lipid carrier apolipoprotein E (apoE), and α-synuclein, which also plays an important role in Parkinson's disease. For each molecule, we briefly review its normal function and the evidence for its involvement in AD and then examine studies of its relationship to memory deficits in both humans and animal models.

4.15.2.1 APP and Aβ

APP is a ubiquitously expressed, multifunctional type I membrane protein with potential roles in signaling (Turner et al., 2003), axonal transport (Kamal et al., 2000; Satpute-Krishnan et al., 2006),

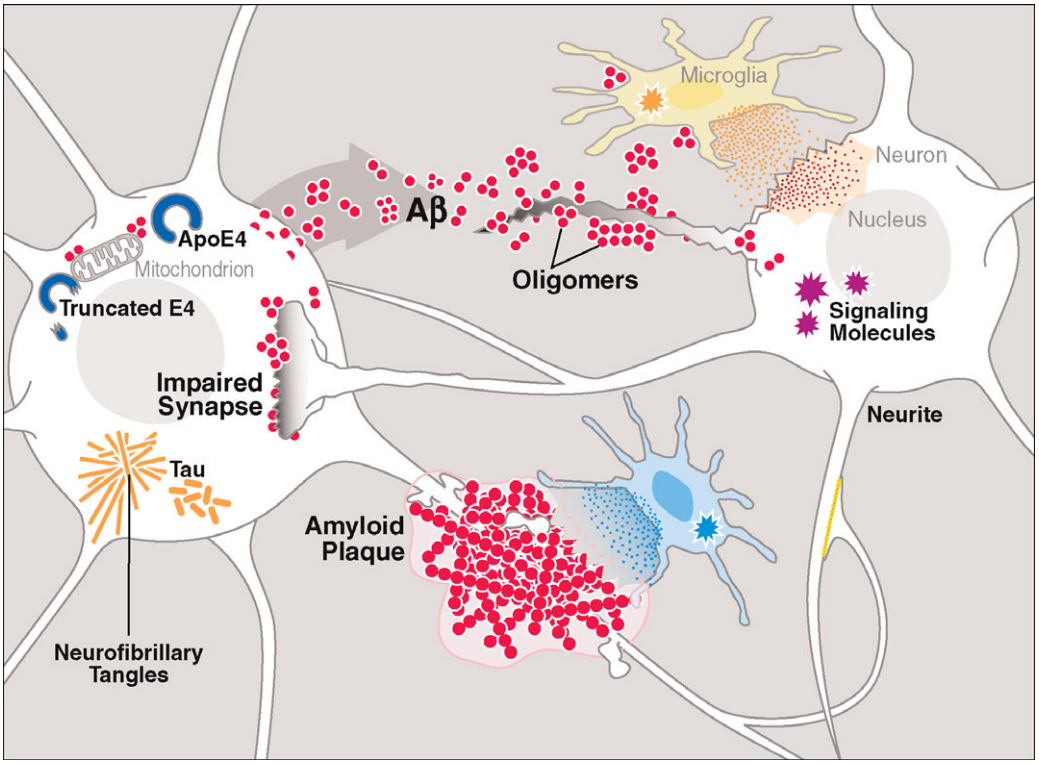

Figure 2 Key molecules involved in Alzheimer's disease pathogenesis. Aβ peptides produced by neurons and other brain cells aggregate into a variety of assemblies, including amyloid plaques containing Aβ fibrils and soluble nonfibrillar oligomers. Some of these Aβ assemblies impair synapses and neuronal dendrites, either directly or through the engagement of pathogenic glial loops. However, other glial activities protect neurons against dysfunction and degeneration. ApoE4 and tau promote Aβ-induced neuronal injury and also have independent adverse effects. (From Roberson ED and Mucke L [2006] 100 years and counting: Prospects for defeating Alzheimer's disease. *Science* 314: 781–784.)

transcriptional regulation (Cao and Südhof, 2001), neurite outgrowth (Leyssen et al., 2005), synaptogenesis (Mucke et al., 1994; Morimoto et al., 1998), and regulation of glutamate uptake (Masliah et al., 1998). APP-null mice have deficits in learning and memory, abnormal long-term potentiation (LTP), and loss of synapses (Dawson et al., 1999; Seabrook et al., 1999).

Proteolytic cleavage of APP produces several biologically active fragments. Among them are Aβ peptides, produced by the sequential action of two proteases, β- and γ-secretase, on APP (**Figure 3**). Aβ peptides of 40 (Aβ40) or 42 (Aβ42) amino acids in length are the primary species produced. Aβ42 is believed to be more pathogenic, since it is more prone to aggregation and more toxic when applied to cells than Aβ40 (Jarrett et al., 1993; Seilheimer et al., 1997). Aβ has been shown to decrease synaptic transmission (Hsia et al., 1999; Kamenetz et al., 2003; Priller et al., 2006) or LTP (Lambert et al., 1998; Walsh et al., 2002), which may represent physiological functions, pathogenic mechanisms, or both. Neuronal activity stimulates the production and release of Aβ, which in turn depresses excitatory synaptic transmission, at least at certain synapses (Kamenetz et al., 2003). Under normal circumstances, this feedback loop could help regulate synaptic activity. In AD, aberrant neuronal activity might turn this feedback loop into a vicious cycle that increases Aβ production and impairs neurotransmission (Palop et al., 2006, 2007).

A great deal of additional biochemical and genetic evidence suggests an involvement of Aβ in AD, which forms the basis of the 'amyloid hypothesis' of AD pathogenesis (Hardy and Higgins, 1992; Tanzi and Bertram, 2005). Aβ is the major constituent of amyloid plaques, one of the pathological hallmarks of AD (Glenner and Wong, 1984). Mutations in APP cause autosomal dominant AD (Goate et al., 1991); AD-causing APP mutations are concentrated around the Aβ domain, and most increase the Aβ42/Aβ40 ratio or total Aβ production (Theuns et al., 2006). Presenilin mutations, the other major known cause of autosomal dominant AD, also increase the relative

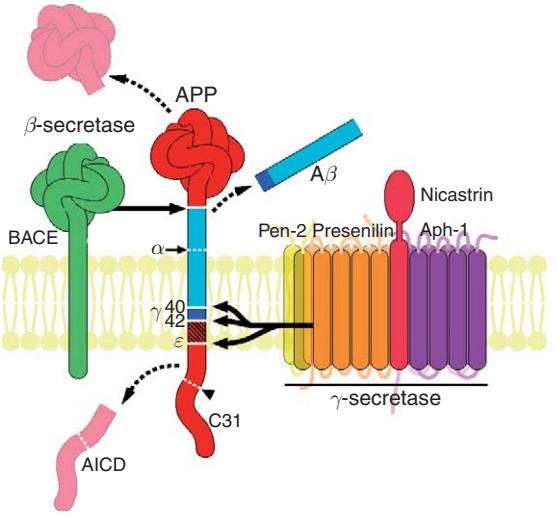

Figure 3 APP, Aβ, and the secretases. Aβ production depends on sequential proteolytic cleavage of APP by β-secretase, also known as β-site APP-cleaving enzyme 1 (BACE1), and the γ-secretase complex, composed of presenilin and other proteins. γ-Secretase can cleave APP at different locations to generate Aβ40, Aβ42, or the APP intracellular domain (AICD). Caspase cleavage at the carboxy-terminus yields the C31 fragment. (Modified from Roberson ED and Mucke L [2006] 100 years and counting: Prospects for defeating Alzheimer's disease. Science 314: 781–784.)

abundance of Aβ42 (see the section titled 'γ-Secretase') (Levy-Lahad et al., 1995; Rogaev et al., 1995; Sherrington et al., 1995). In addition, polymorphisms in the gene for insulin-degrading enzyme, which degrades Aβ, may contribute to AD risk (Ertekin-Taner et al., 2004).

The first animals with prominent AD-like pathology, and still the most widely studied, were transgenic mice with neuronal expression of human APP (hAPP) carrying familial AD mutations (Games et al., 1995; McGowan et al., 2006). These mice have high levels of Aβ and several, but not all, of the neuropathological hallmarks of AD. For example, age-dependent plaque deposition is a universal feature, whereas tangles do not develop. Because hAPP mice develop age-dependent learning and memory abnormalities (Kobayashi and Chen, 2005; McGowan et al., 2006), these models provide an opportunity to test hypotheses about how Aβ contributes to cognitive impairment in AD.

At a molecular level, there are a variety of hypotheses for how Aβ might interfere with normal neuronal function and survival. For example, Aβ can bind directly to lipid bilayers, altering membrane fluidity (Müller et al., 2001). It also forms pores or otherwise permeabilizes membranes, increasing their conductance to ion flow (Pollard et al., 1995; Kayed et al., 2004). In addition, Aβ binds to and influences the function of a variety of cellular proteins (Verdier et al., 2004), including the α7 nicotinic acetylcholine receptor (Oddo and LaFerla, 2006), integrins (Sabo et al., 1995), the receptor for advanced glycation end-products (RAGE; Yan et al., 1996), mitochondrial enzymes (Lustbader et al., 2004), and the APP holoprotein (Shaked et al., 2006). Several of these interactions are discussed in more detail in the section titled 'Memory-related molecules in AD.'

4.15.2.1.1 Aβ and plaques

The deposition of Aβ into amyloid plaques has been the focus of much AD research, given the prominence of plaques in AD patients and related animal models and their relative ease of detection. Plaque deposition in AD brains has historically been studied with neuropathological methods, including histochemical staining with amyloid-binding dyes and immunostaining with Aβ antibodies. It can now be imaged *in vivo* in living human subjects by positron emission tomography with a plaque-binding agent termed Pittsburgh compound B (PIB) (Klunk et al., 2004) (**Figure 4**).

The leading hypothesis for how plaques might disrupt neuronal function is by inducing changes in surrounding neurites. A subset of amyloid plaques in both AD patients and mouse models, termed neuritic plaques, are surrounded by swollen and tortuous neuronal processes (Knowles et al., 1999; Tsai et al., 2004). Plaque-associated neuritic dystrophy may alter the electrophysiological properties of the circuits involved (Knowles et al., 1999; Stern et al., 2004).

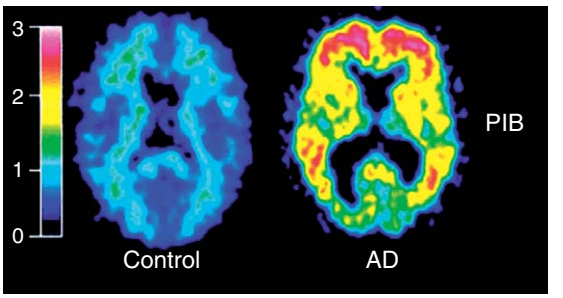

Figure 4 *In vivo* imaging of amyloid plaques with Pittsburgh compound B (PIB). PIB–positron emission tomography scan of a 79-year-old patient with AD shows a robust signal attributable to amyloid plaque binding, compared with a 67-year-old control subject. (From Klunk WE, Engler H, Nordberg A, et al. [2004] Imaging brain amyloid in Alzheimer's disease with Pittsburgh Compound-B. *Ann. Neurol.* 55: 306–319.)

However, plaque deposition does not correlate well with memory deficits in AD. Amyloid deposition tends to be an early event in AD pathogenesis that plateaus and does not worsen as cognition deteriorates throughout the disease course (Arriagada et al., 1992; Ingelsson et al., 2004; Giannakopoulos et al., 2007). Also, plaques do not have the preferential distribution in medial temporal structures involved in memory that is seen with NFTs (see the section titled 'NFTs, neuronal death, and memory loss'). Rather, plaques are seen first in the neocortex, particularly in prefrontal and posterior parietal regions (Price et al., 1991; Buckner et al., 2005; Kemppainen et al., 2006). Data from animal models also support a mechanistic dissociation between plaques and cognitive deficits (Holcomb et al., 1998; Westerman et al., 2002; Palop et al., 2003; Kobayashi and Chen, 2005; Lesné et al., 2006; Cheng et al., 2007). While neuritic dystrophy correlates with cognitive deficits better than plaque load (McKee et al., 1991), many dystrophic neurites in AD brains are not associated with plaques (Wang and Munoz, 1995). Recent findings in hAPP transgenic mice suggest that vascular amyloidosis and oxidative stress may contribute to the pathogenesis of plaque-independent neuritic dystrophy (Esposito et al., 2006).

4.15.2.1.2 Soluble Aβ oligomers

Because deposition of fibrillar Aβ in plaques does not correlate well with cognitive deficits, interest has grown in nonfibrillar forms of Aβ. Increasing evidence points to a pathogenic role for oligomeric Aβ assemblies that are soluble in aqueous solutions. Multiple types of Aβ oligomers have been described, including low-molecular-weight species (dimers and trimers) and larger globular species (including dodecamers and Aβ-derived diffusible ligands) (Podlisny et al., 1995; Lambert et al., 1998; Lesné et al., 2006). Putative Aβ oligomers have been detected in the cerebrospinal fluid (CSF) and brain tissue of AD patients (Podlisny et al., 1995; Kuo et al., 1996; Pitschke et al., 1998; Kayed et al., 2003; Lacor et al., 2004) and hAPP transgenic mice (Podlisny et al., 1995; Lambert et al., 1998; Lesné et al., 2006; Cheng et al., 2007). Extracellularly, oligomers seem to bind preferentially to dendritic and synaptic regions (Lacor et al., 2004; Barghorn et al., 2005). Aβ42 is more prone to oligomer formation than Aβ40 (Dahlgren et al., 2002; Bitan et al., 2003), which may relate to their relative pathogenicity (Aβ42 > Aβ40).

In mixed neuronal/glial cultures and brain slices, oligomeric Aβ is a potent neurotoxin, inducing neuronal death within 24 h (Roher et al., 1996; Lambert et al., 1998; Chen et al., 2005a). Direct experimental comparisons suggest that oligomeric Aβ is more toxic than fibrillar or monomeric Aβ (Klein et al., 2001; Dahlgren et al., 2002).

At physiological concentrations, Aβ oligomers interfere with synaptic plasticity, and memory. Both low-molecular-weight and larger globular oligomers interfered with LTP when acutely applied to hippocampal slices, whereas monomers did not (Lambert et al., 1998; Wang et al., 2004; Barghorn et al., 2005; Walsh et al., 2005). Injection of oligomeric Aβ into the cerebral ventricles blocked LTP *in vivo* and impaired learning and memory (Walsh et al., 2002; Cleary et al., 2005; Lesné et al., 2006). The effects of Aβ on LTP are acute and independent of neuronal death (Chen et al., 2000). Although oligomer-induced blockade of LTP in hippocampal slices could not be reversed by washing out Aβ (Townsend et al., 2006), *in vivo* impairment of LTP by oligomers could be blocked when Aβ antibodies were injected after the Aβ (Klyubin et al., 2005).

4.15.2.1.3 Neuronal dysfunction versus neuronal death

The ability of Aβ to induce both neuronal dysfunction and neuronal death raises the question of which effect is primarily responsible for the memory deficits in AD. Evidence from animal models points toward an important role for neuronal dysfunction. First, although hAPP mice have memory deficits, most lines do not have prominent neuron loss in the areas most affected in AD, including entorhinal cortex and hippocampal area CA1 (Irizarry et al., 1997a,b; Takeuchi et al., 2000). Also, memory impairment in hAPP mice can be rapidly reversed by treatments that lower Aβ levels but are not likely to change neuron numbers (Dodart et al., 2002; Kotilinek et al., 2002). Rather than frank neuronal loss, hAPP mice have synaptodendritic changes, including reduction of presynaptic terminals, loss of postsynaptic spines, and simplification of dendritic arborization, that may contribute to cognitive impairment (Mucke et al., 2000; Buttini et al., 2002; Lanz et al., 2003; Chin et al., 2004; Moolman et al., 2004; Wu et al., 2004; Spires et al., 2005). Whether these alterations would ultimately lead to neuronal loss if mice had a longer lifespan is unknown. It is also unknown how long it takes neurons to die in the brains of humans with AD.

Differentiating the relative contributions of neuronal dysfunction and neuronal loss to cognitive deficits in AD patients has been difficult, because

neuron loss is commonly found early in the disease (Price et al., 2001). However, several observations suggest that dysfunction may at least add to the impairments that are likely to result from progressive neuronal loss. Synaptodendritic alterations similar to those observed in hAPP mice are also observed in AD (Terry et al., 1991; Masliah et al., 2001a; Shim and Lubec, 2002; Moolman et al., 2004). Synaptic loss is an early event that correlates with cognitive impairment in AD (Terry et al., 1991; Masliah et al., 2001a). In addition, synaptic failure may precede and contribute to neuronal death (Selkoe, 2002). Finally, the frequent fluctuations in cognition seen in AD patients cannot be explained by sudden changes in neuronal numbers and are likely a manifestation of neuronal dysfunction (Palop et al., 2006).

4.15.2.1.4 Other APP fragments

Aβ is not the only biologically active APP fragment. γ-Secretase cleavage liberates a carboxy-terminal fragment known as the APP intracellular domain (AICD). The AICD activates the adapter protein Fe65, which translocates to the nucleus and regulates gene expression (Cao and Südhof, 2001, 2004). The AICD/Fe65 pathway stimulates expression of many genes, including neprilysin, a peptidase that degrades Aβ (Pardossi-Piquard et al., 2005), the P53 tumor suppressor, which controls programmed cell death (Alves da Costa et al., 2006), and components of the cytoskeleton (Müller et al., 2006). The AICD also modulates calcium stores in the endoplasmic reticulum (Leissring et al., 2002). While AICD-dependent changes in gene expression may be a factor in patients with APP mutations (Wiley et al., 2005), their importance in sporadic AD remains unclear. AICD levels and some cytoskeletal target genes are unchanged, while others are upregulated, and neprilysin expression is decreased (Wang et al., 2005; Müller et al., 2006).

The ectodomain of APP can be shed by α- or β-secretase, generating secreted APP fragments known as sAPPα and sAPPβ, respectively. Ectodomain shedding is a prerequisite for γ-secretase cleavage and, thus, Aβ and AICD production. sAPP may have independent effects, particularly neuroprotective and neurotrophic activities (Mattson, 1997; Kerr and Small, 2005; Zheng and Koo, 2006).

Finally, APP is cleaved by caspase(s) at a site 31 amino acids from the carboxy terminus; the resulting C-terminal fragment is termed C31 (Gervais et al., 1999; Lu et al., 2000). Transgenic mice expressing hAPP with a mutation that prevents this cleavage have less pronounced cognitive impairment than mice expressing caspase-sensitive hAPP (Galvan et al., 2006). The favorable effect of this mutation may be a result of reduced generation of C31, which is neurotoxic (Lu et al., 2000; Galvan et al., 2002). It may also be caused by changes in protein–protein interactions involving the APP carboxy terminus, including APP multimerization (Lu et al., 2003) and interactions with motor proteins (Satpute-Krishnan et al., 2006).

4.15.2.2 BACE

The first step in production of Aβ from APP is shedding the large APP ectodomain by β-secretase (**Figure 3**). The primary β-secretase enzyme is an aspartyl protease termed β-site APP cleaving enzyme (BACE1, also known as Asp2, memapsin 2) (Hussain et al., 1999; Sinha et al., 1999; Vassar et al., 1999; Yan et al., 1999). Genetic deletion of BACE1 dramatically reduces Aβ levels (Roberds et al., 2001) and prevents Aβ-dependent cognitive deficits in hAPP mice (Ohno et al., 2006). Thus, BACE1 inhibition is an attractive potential AD therapy.

Two aspects of BACE physiology are of particular interest in relation to synaptic transmission and neuronal function. First, BACE activity, at least its cleavage of APP, is dynamically regulated and increases with neuronal activity (Kamenetz et al., 2003). This effect contributes to a putative feedback loop whereby increased neuronal activity stimulates Aβ production, which in turn can suppress synaptic transmission (Kamenetz et al., 2003). Alterations in BACE regulation may be important in AD pathogenesis, as BACE activity is increased in AD (Holsinger et al., 2002) and after other types of neuronal injury (Blasko et al., 2004). Second, BACE1 has other substrates beyond APP, many of which affect neuronal function (**Table 1**). Among these are neuregulin 1, a

Table 1 Selected BACE1 substrates

Aβ
APP
APP-like proteins
Low density lipoprotein receptor-related protein
Neuregulin-1
P-selectin glycoprotein ligand-1
ST6Gal I sialyltransferase
Voltage-gated sodium channel β subunit

Adapted from Willem M, Garratt AN, Novak B, et al. (2006) Control of peripheral nerve myelination by the β-secretase BACE1. *Science* 314: 664–666.

ligand in the ErbB signaling pathway (Willem et al., 2006). In the absence of BACE cleavage, lack of neuregulin/ErbB signaling results in peripheral nerve hypomyelination (Willem et al., 2006). Cleavage of substrates besides APP may also contribute to AD. Mice overexpressing human BACE1 have neurodegeneration and memory deficits (Rockenstein et al., 2005). However, these effects are not a result of increases in Aβ levels, which are actually lower in BACE-overexpressing mice than in mice with normal BACE levels (Lee et al., 2005; Rockenstein et al., 2005), presumably because BACE overexpression promotes APP processing in an earlier component of the secretory pathway (Lee et al., 2005).

4.15.2.3 Presenilins

Shortly after the discovery that mutations in the APP gene cause autosomal dominant AD, two other genes were found in other families with early-onset AD: presenilin 1 (PS1) on chromosome 14 and presenilin 2 (PS2) on chromosome 1 (Levy-Lahad et al., 1995; Rogaev et al., 1995; Sherrington et al., 1995). PS1 mutations have since proven to be the most common cause of dominantly inherited AD, responsible for more than 50% of cases. Over 150 different mutations spanning the protein have been identified. PS2 mutations are much less common and tend to produce a somewhat less severe phenotype (Bertram and Tanzi, 2004). PS1 and PS2 are 67% identical and are ubiquitously expressed in the brain and other tissues.

The normal functions of the presenilins were unknown at the time of their discovery and are still being elucidated today. Most attention has focused on presenilin's role in Aβ production as a key component of γ-secretase. However, diverse γ-secretase-independent roles of presenilins continue to emerge, and controversy remains about the degree to which these roles contribute to AD and whether the AD-linked mutations cause primarily a gain or loss of function.

4.15.2.3.1 γ-Secretase

The presenilins are membrane-embedded aspartyl proteases that form the catalytically active center of the γ-secretase complex, along with nicastrin, Aph-1, and Pen-2 (De Strooper et al., 1998; Edbauer et al., 2003). γ-Secretase cleaves type I membrane proteins within their transmembrane domains (reviewed in Wolfe, 2006). Its substrate selectivity is rather broad, with the main requirement being a short extracellular domain (Struhl and Adachi, 2000). Thus, γ-secretase has many substrates (**Table 2**).

Table 2 Selected γ-secretase substrates

γ-protocadherin
APLP1
APLP2
APP
CD43
CD44
DCC
Delta
E-Cadherin
ErbB-4
Jagged
LRP
Voltage-gated sodium channel β2 subunit
N-Cadherin
Nectin-1α
Notch
NRADD
P75
Syndecan-1
Tyrosinase
Tyrosinase-related proteins 1 and 2

From Vetrivel KS, Zhang YW, Xu H, et al. (2006) Pathological and physiological functions of presenilins. *Mol. Neurodegener.* 1:4.

One of these is, of course, APP. Shedding of the large extracellular domain of APP by α- or β-secretase generates carboxy-terminal fragments (CTFs) that make suitable substrates for γ-secretase (**Figure 3**). The γ-secretase complex can cleave β-CTF at different sites, generating either Aβ40 or Aβ42. PS mutations favor production of Aβ42 over Aβ40 (Borchelt et al., 1996; Scheuner et al., 1996). Given the considerable evidence that Aβ42 is more pathogenic (see the section titled 'APP and Aβ'), this effect is believed to be an important mechanism by which PS mutations lead to AD. γ-Secretase is also responsible for generation of the AICD (see the section titled 'Other APP fragments').

4.15.2.3.2 γ-Secretase-independent roles of presenilins

In addition to their role in Aβ production, presenilins have several other functions, many of which are independent of γ-secretase, as they are not blocked by γ-secretase inhibitors or by point mutations that abolish secretase activity.

Several of these functions relate to calcium regulation. Presenilin mutations increase calcium release from the endoplasmic reticulum induced by inositol-1,4,5-trisphosphate (IP_3) (reviewed in LaFerla, 2002). One mechanism for this effect seems to be overfilling endoplasmic reticulum (ER) Ca^{2+} stores (Leissring et al., 2000). Presenilins form calcium leak channels

in the ER membrane; this function is lost in AD-associated mutants, leading to overfilling of the ER with Ca^{2+} (Tu et al., 2006). Others have pointed to an upregulation of IP_3 receptors in presenilin-deficient cells as another possible cause for increased calcium release (Kasri et al., 2006). Whatever the underlying mechanism, the resulting increases in intracellular calcium release induced by presenilin mutations are likely to contribute to neuronal dysfunction.

Presenilin also regulates intracellular signaling pathways that control tau phosphorylation. Presenilin stabilizes cadherin–cadherin complexes that interact with and activate phosphatidylinositol-3 kinase, stimulating Akt activity, which, in turn, suppresses glycogen synthase kinase (GSK) activity and tau phosphorylation (Baki et al., 2004). This γ-secretase-independent effect of presenilin in preventing tau phosphorylation is lost in AD-associated mutants and, thus, may enhance tau-mediated neurotoxicity (Baki et al., 2004).

Conditional PS1/PS2 double-knockout mice have learning and memory impairments, LTP deficits, aberrant tau phosphorylation, and neurodegeneration, although their Aβ levels are not increased (Saura et al., 2004), suggesting that mutations impairing APP-independent PS functions could also contribute to AD-related deficits.

4.15.2.4 Tau

Tau is a small microtubule-associated protein (MAP) and a member of the MAP2 superfamily (Weingarten et al., 1975; Cleveland et al., 1977; Dehmelt and Halpain, 2005). It has a variety of functions, including stabilizing microtubules, enabling neurite outgrowth, regulating axonal transport and controlling neuronal susceptibility to overexcitation (Shahani and Brandt, 2002; Avila et al., 2004; Roberson et al., 2007). Tau knockout mice are surprisingly normal, with no abnormalities in general health, fertility, longevity, gross brain cytoarchitecture, or learning and memory (Harada et al., 1994; Ikegami et al., 2000; Dawson et al., 2001; Tucker et al., 2001; Roberson et al., 2007). This may be, at least in part, the result of compensation by other MAPs, since double knockouts lacking both tau and MAP1B have 80% mortality in the first few weeks postnatally (Takei et al., 2000). Microdeletions on chromosome 17q21 including the tau gene are associated with mental retardation in humans (Lupski, 2006), but these deletions also involve several other genes. The fact that tau knockout mice have a very mild phenotype suggests that the loss of other genes might underlie the deficits associated with these deletions.

Tau was first implicated in AD by the discovery that NFTs are composed of heavily phosphorylated tau forming paired helical filaments (Grundke-Iqbal et al., 1986; Kosik et al., 1986; Wood et al., 1986; Lee et al., 1991). Interestingly, mutations in tau cause frontotemporal dementia, but not AD (Rademakers et al., 2004). However, the tau gene contains several polymorphisms that are in linkage disequilibrium, creating several unique haplotypes, one of which is associated with AD (Myers et al., 2005). The high-risk haplotype, known as H1c, is associated with roughly 10% more tau expression than other haplotypes (Kwok et al., 2004). This is consistent with the observation that reducing tau expression is protective in mouse models of AD (Roberson et al., 2007). The H1c haplotype also affects splicing of tau. Transcripts of the single tau gene are alternatively spliced to generate six different isoforms in adults (**Figure 5**). The most important distinction is between those isoforms with three copies of the microtubule-binding domain (termed 3R tau) and those with four (4R). The H1c haplotype is associated with slightly higher production of 4R than 3R tau (Myers et al., 2007). The mechanism by which these differences in tau expression raise AD risk is unclear.

4.15.2.4.1 NFTs, neuronal death, and memory loss

The study of tau's contribution to memory dysfunction in AD has concentrated largely on NFTs. Interest in NFTs dates all the way back to Alzheimer's original report (1907), which focused more on tangles than on plaques. Two observations form the basis of the hypothesis that tangles produce memory deficits in AD. First, the regional distribution of NFTs, which evolves in a stereotypical manner over the course of the illness, begins in medial temporal structures involved in memory (Braak and Braak, 1991). Tangles first appear in the transentorhinal region (stage I), then the entorhinal cortex (stage II), hippocampus (stage III), temporal neocortex (stage IV), and eventually other neocortical areas (stages V–VI) (**Figure 6**). Second, NFT counts correlate with clinical dementia severity, unlike amyloid plaque deposition, which does not (Giannakopoulos et al., 2007; but see Näslund et al., 2000).

Neuron loss may be a key mechanism underlying the connection between tau aggregation into NFTs and memory deficits. NFT burden correlates with the severity of neuronal loss (Giannakopoulos et al., 2007). NFT counts also correlate with levels of CSF tau, which increase in AD, possibly as a result of tau release

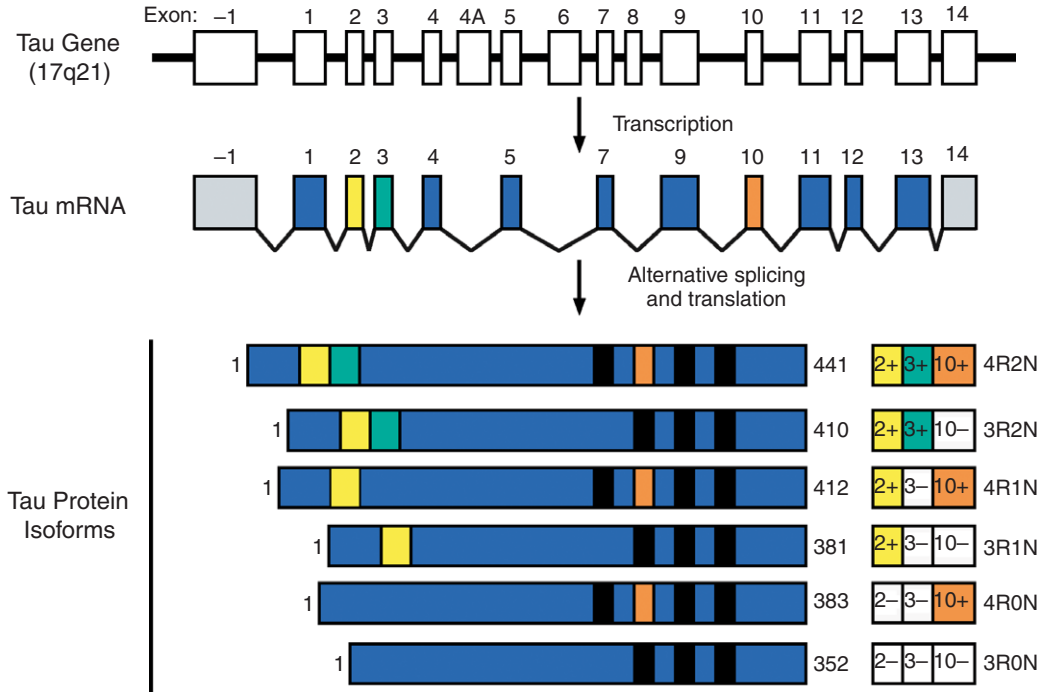

Figure 5 Alternative splicing of tau isoforms. The tau RNA can be alternatively spliced to include zero, one, or two amino-terminal inserts encoded by exons 2 and 3. Isoforms contain either three or four microtubule-binding domains, depending on whether exon 10 is included. (Modified from Buée L, Bussière T, Buée-Scherrer V, et al. [2000] Tau protein isoforms, phosphorylation and role in neurodegenerative disorders. *Brain Res. Rev.* 33: 95–130.)

from dying neurons (Arai et al., 1995; Tapiola et al., 1997; Giannakopoulos et al., 2007). Increased CSF tau is associated with poorer cognitive performance (Wallin et al., 2006) and a higher risk of progressing from MCI to AD (Blennow and Hampel, 2003).

Animal and cellular models also support a link between tau aggregation and neuron death (McGowan et al., 2006). Many transgenic mouse lines expressing human tau with mutations that favor tau aggregation display memory deficits and neuron loss (Lewis et al., 2000; Tatebayashi et al., 2002; Pennanen et al., 2004; McGowan et al., 2006). Overexpression of tau in large neurons of the lamprey leads to fibrillar tau aggregates and neuronal degeneration, an effect blocked by compounds that inhibit tau aggregation (Hall et al., 2001, 2002). Expression of aggregation-prone tau mutants in cultured neuroblastoma cells also causes toxicity that can be reversed by point mutations or small molecules that block aggregation (Khlistunova et al., 2006).

4.15.2.4.2 Tangle-independent roles for tau

There are limitations to the data suggesting a connection between NFTs and cognitive decline. Because much of the human data are correlational, they cannot establish causal relationships. Notably, animal model data indicate that aggregation is not the only means by which tau can impair neuronal and cognitive functions. Tau overexpression induced neurodegeneration in *Drosophila* in the absence of NFTs (Wittmann et al., 2001). Even in tau transgenic mice that have NFTs, neurodegeneration affects cells that do not have tau aggregates (Andorfer et al., 2005; Spires et al., 2006). Nonfibrillar tau might induce neuronal death by stimulating cell cycle reentry in normally postmitotic neurons (Andorfer et al., 2005; Khurana et al., 2006), although other mechanisms are possible also. The rTg4510 model has an inducible mutant tau transgene that, when turned on, causes NFT formation, severe neuronal loss, and spatial memory impairment (Ramsden et al., 2005; SantaCruz et al., 2005). However, suppressing transgene expression reverses the memory deficits, even though NFT formation continues, dissociating these processes (SantaCruz et al., 2005).

The mechanisms underlying these effects of tau on neuronal function have not yet been determined, although some leads exist. Abnormal tau can interfere with axonal transport (Ebneth et al., 1998; Ishihara et al., 1999), which seems consistent with its role as a

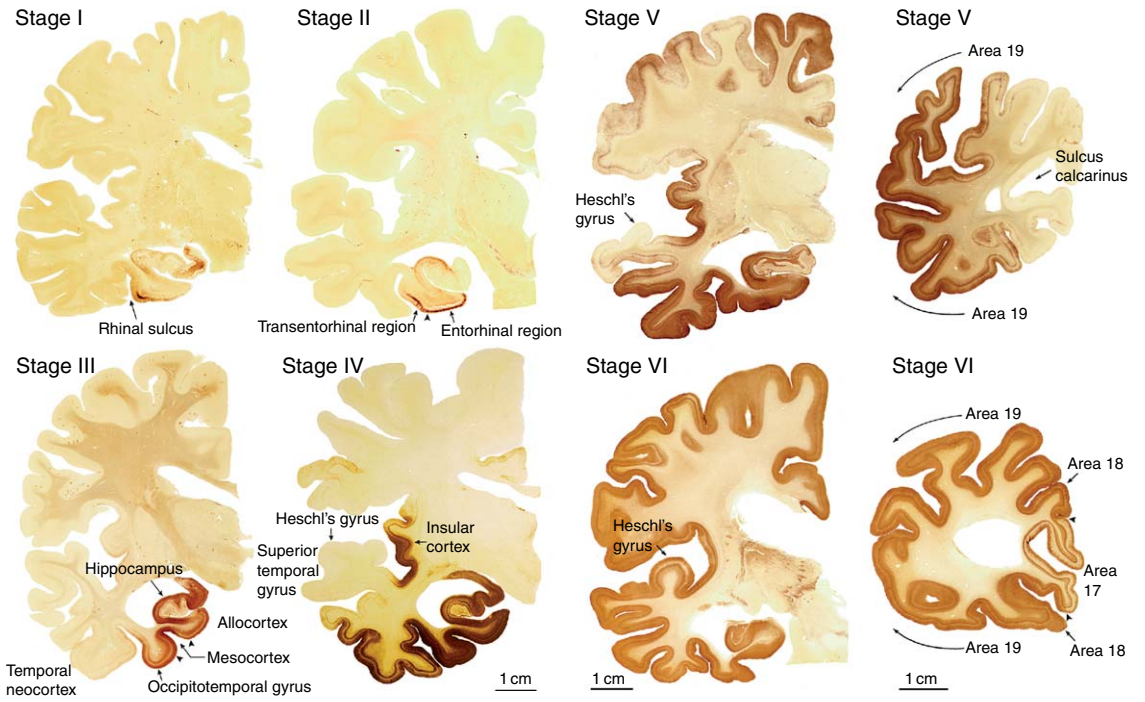

Figure 6 Stages of neurofibrillary pathology in Alzheimer's disease. Whole-brain sections were immunostained with antibody to phosphorylated tau. In stage I, involvement is limited to the transentorhinal region. Neurofibrillary pathology spreads to the entorhinal cortex in stage II. Stage III involves the hippocampus. Stage IV involves spread to the insula and inferior temporal neocortex. Finally, in stages V–VI even more neocortical areas are affected. (From Braak H, Rüb U, Schultz C, et al. [2006]. Vulnerability of cortical neurons to Alzheimer's and Parkinson's diseases. *J Alzheimers Dis* 9:35–44, with permission from IOS Press.)

microtubule-binding protein. Tau's influence on microtubule stability could also affect plasticity-related structural rearrangements, and some of these effects may relate to normal tau functions. Reversible synaptic regression during hibernation in certain rodents is associated with changes in tau, especially changes in its phosphorylation (Arendt et al., 2003). At young ages, before formation of aggregates or NFTs, tau transgenic mice have better-than-normal LTP in the dentate gyrus and longer-lasting memory than nontransgenic littermates (Boekhoorn et al., 2006), suggesting a role of soluble tau in cognitive function.

4.15.2.4.3 Tau phosphorylation and other posttranslational modifications

Aberrant tau phosphorylation is a hallmark of AD and seems to be carried out by many of the kinases involved in learning and memory (see the section titled 'Kinases'). Fully 19% of the amino acids in tau are potential phosphorylation sites (Ser, Thr, and Tyr), and many are in fact phosphorylated in AD brains (Stoothoff and Johnson, 2005). The vast majority of the phosphorylation sites are highly conserved across species and surround tau's microtubule-binding domains (**Figure 7**). Much effort has been devoted to

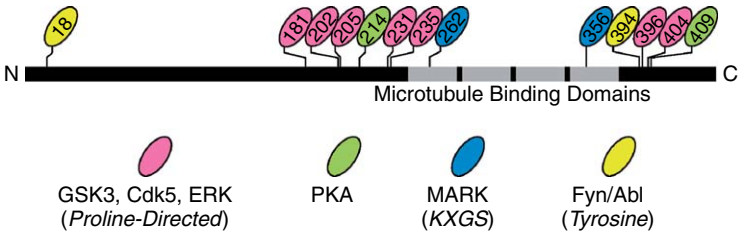

Figure 7 Tau phosphorylation sites. The four microtubule-binding domains are indicated by shading in a schematic line drawing of tau protein. Most tau phosphorylation sites surround the microtubule-binding regions.

sorting out which sites and kinases are most important in the pathogenesis of AD.

- Proline-directed kinase sites. Many tau phosphorylation sites are substrates for proline-directed kinases, which target Ser/Thr residues directly adjacent to a proline. These sites are substrates for glycogen synthase kinase 3 (GSK3), cyclin-dependent kinase 5 (Cdk5), and extracellular-signal regulated kinase (ERK), among others. Phosphorylation at these sites seems to be involved in the tau aggregation/cell death pathways mentioned above (Lucas et al., 2001; Augustinack et al., 2002; Cruz et al., 2003; Noble et al., 2003). For example, GSK3 stimulates tau aggregation (Sato et al., 2002), and GSK3 inhibition reduces tau aggregation and neurodegeneration (Noble et al., 2005).
- PKA sites. Tau is phosphorylated by cyclic AMP-dependent protein kinase (PKA), preferentially at Ser214 (Scott et al., 1993). Phosphorylation of Ser214 inhibits tau's microtubule binding and stabilizing activity (Illenberger et al., 1998). The PKA sites are near the proline-directed sites, and tau phosphorylation by PKA facilitates phosphorylation by proline-directed kinases (Singh et al., 1996; Liu et al., 2004) but may inhibit aggregation (Schneider et al., 1999).
- KXGS sites. The PKA and proline-directed sites are concentrated at both ends of the four microtubule-binding domains of tau. Within these domains are sites with a Lys-Xxx-Gly-Ser (KXGS) consensus sequence, most notably Ser262 and Ser356. These sites are phosphorylated by microtubule-affinity regulating kinase (MARK), which dramatically reduces tau's ability to stabilize microtubules (Drewes et al., 1997) and facilitates neurite outgrowth (Biernat et al., 2002). This phosphorylation may also be a prerequisite for phosphorylation at proline-directed kinase sites (Nishimura et al., 2004; but see Biernat and Mandelkow, 1999).
- Tyrosine phosphorylation. In AD brains, tau is also phosphorylated on tyrosine residues, primarily Tyr18 by Fyn and Tyr394 by Abl (Williamson et al., 2002; Lee et al., 2004; Derkinderen et al., 2005). There is considerable evidence for a role of Fyn in AD (Lambert et al., 1998; Chin et al., 2004, 2005; Lee et al., 2004), as reviewed further in the section titled 'Fyn.' Interestingly, interactions between tau and Fyn may contribute to the subcellular localization of Fyn and, thus, affect its substrate availability (Lee et al., 1998). In addition, disease-associated tau phosphorylation and mutations strongly increase Fyn binding (Bhaskar et al., 2005).
- Tau proteolysis. The carboxy terminus of tau can be cleaved by activated caspases, producing a truncated tau that aggregates more easily than full-length tau (Cotman et al., 2005). This may be an important step leading to development of tangles in AD, as tau cleavage at this site is a relatively early event in NFT formation (Rissman et al., 2004; Guillozet-Bongaarts et al., 2005). Tau is also cleaved by calpain, which produces a 17-kD tau fragment that is toxic to cultured neurons (Park and Ferreira, 2005).

4.15.2.4.4 *Tau and Aβ*

Given the prominence of Aβ and tau in AD pathology, their relationship is of considerable interest. Although expression of human Aβ does not induce NFTs in hAPP transgenic mice, it increases tangle formation in hAPP mice coexpressing mutant human tau; in contrast, expression of mutant human tau does not seem to worsen Aβ-dependent pathologies in hAPP mice (Lewis et al., 2001; Götz et al., 2001). In such multiple transgenic models, Aβ immunotherapy decreases tau pathology, further suggesting that Aβ acts upstream of tau (Oddo et al., 2004). Plausible mechanisms include Aβ-induced kinase activation and resulting tau phosphorylation (see the section titled 'Kinases'). In addition, caspase activation by Aβ may stimulate tau proteolysis that favors aggregation (Cotman et al., 2005).

Calpain-mediated tau proteolysis seems to play an important role in Aβ toxicity *in vitro*. Tau-deficient primary neurons are resistant to the rapid neurodegeneration induced by Aβ application, apparently because they lack calpain-induced tau fragments that are toxic to neurons in culture (Rapoport et al., 2002; Park and Ferreira, 2005). Reducing tau even by just 50% ameliorated Aβ-induced memory deficits in a mouse model of AD (Roberson et al., 2007). Interestingly, this effect did not seem to involve removal of a tau species with Aβ-induced posttranslational modifications. Rather, tau reduction prevented Aβ-induced epileptiform activity and compensatory inhibitory remodeling of hippocampal circuits (Palop et al., 2007; Roberson et al., 2007).

4.15.2.5 ApoE

ApoE is a multifunctional lipoprotein originally discovered for its role in intercellular transport and distribution of cholesterol throughout the body (Mahley, 1988). The brain is second only to the liver

as a producer of apoE. In addition to mediating lipid transport within the central nervous system, apoE is involved in the response to neural injury and regulation of neurite outgrowth (Mahley and Rall, 2000).

Three main apoE isoforms are produced from different alleles ($\varepsilon 2$, $\varepsilon 3$, and $\varepsilon 4$) of a single *APOE* gene on chromosome 19; apoE2 and apoE4 differ from each other and from the more frequent apoE3 by single amino acid substitutions, which have major effects on apoE structure and function (**Figure 8**) (Hatters et al., 2006; Mahley et al., 2006).

ApoE was implicated in AD by the near-simultaneous discoveries that it binds Aβ and colocalizes to amyloid plaques (Namba et al., 1991; Strittmatter et al., 1993) and that *APOE* genotype has dramatic effects on AD risk and onset (Corder et al., 1993). Individuals with one, and particularly those with two, $\varepsilon 4$ alleles are much more likely to develop AD and have an earlier age of onset compared with $\varepsilon 3$ carriers, while $\varepsilon 2$ carriers are most resistant to the disease (**Figure 9**) (Corder et al., 1993). *APOE* $\varepsilon 4$ appears to be the most important genetic risk factor for sporadic AD (Farrer et al., 1997; Raber et al., 2004; Bertram et al., 2007); 40–60% of all sporadic AD patients have at least one $\varepsilon 4$ allele (Saunders et al., 1993).

These genetic studies established that apoE4 decreases the age at which AD becomes manifest. Interestingly, even in the absence of frank AD, individuals with apoE4 have abnormalities in cognitive performance and functional neuroimaging. In apoE4 carriers, the normal age-related decline in performance on episodic memory tasks occurs at an earlier age and progresses at a faster rate than in noncarriers, while other cognitive domains do not appear to be affected (Caselli et al., 1999, 2004). ApoE4 carriers are also more likely to develop cognitive deficits following open heart surgery or head injury (Tardiff et al., 1997; Teasdale et al., 1997). Even before cognitive impairment is detectable, apoE4 carriers have hypometabolism in the same regions affected in AD, including posterior parietal, posterior cingulate, and frontal cortex (Reiman et al.,

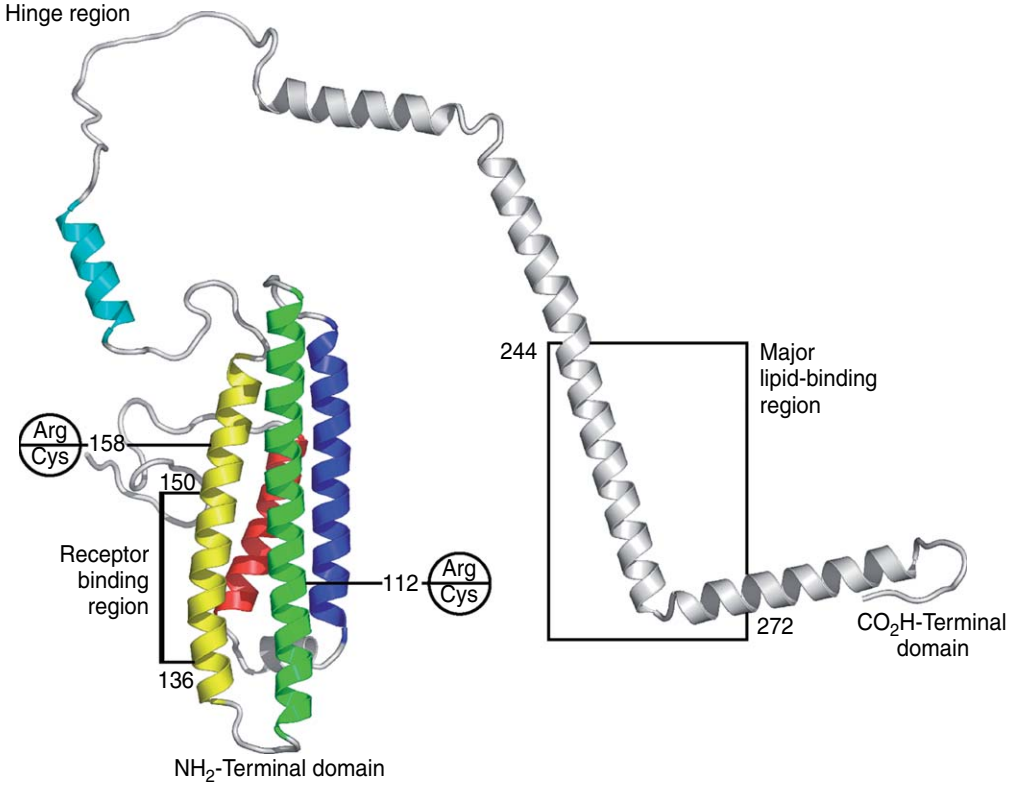

Figure 8 ApoE structure and isoforms. The apoE molecule consists of a globular amino-terminal domain that mediates receptor binding, while the lipid-binding region is in the carboxy-terminal domain. ApoE2 and apoE4 isoforms differ from the most common apoE3 by single amino acid substitutions. At amino acid positions 112 and 158, apoE3 contains Cys and Arg, whereas apoE2 contains Cys and Cys and apoE4 contains Arg and Arg. (From Hatters DM, Peters-Libeu CA, and Weisgraber KH [2006] Apolipoprotein E structure: Insights into function. *Trends Biochem. Sci.* 31: 445–454.)

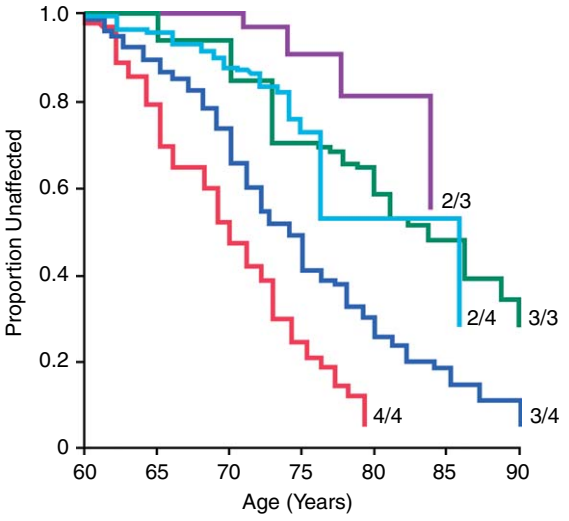

Figure 9 Effect of apoE genotype on AD risk. ApoE4 increases AD risk in a gene dose–dependent manner, while apoE2 lowers risk. (From Strittmatter WJ and Roses AD [1996] Apolipoprotein E and Alzheimer's disease. *Annu. Rev. Neurosci.* 19: 53–77.)

2004, 2005). These abnormalities are seen in apoE4 carriers as young as 20–39 years (Reiman et al., 2004).

4.15.2.5.1 Interactions between Aβ and apoE

ApoE4-related cognitive impairment is also seen in animal models, including mice expressing human apoE4 with or without hAPP/Aβ in neurons. ApoE3, but not apoE4, protects against hAPP/Aβ-induced cognitive deficits (Raber et al., 2000) and synaptic loss (Buttini et al., 2002). Remarkably, this effect is seen well before such mice form amyloid plaques. Consistent with these results, oligomeric Aβ impairs LTP more in slices from apoE4 knockin mice than apoE3 knockin mice (Trommer et al., 2005).

In addition, apoE has a prominent effect on Aβ aggregation and deposition. ApoE binds directly to Aβ and is a component of amyloid plaques (Namba et al., 1991; Strittmatter et al., 1993). ApoE4 is associated with increased deposition of amyloid plaques both in AD (Schmechel et al., 1993) and after head trauma (Nicoll et al., 1995). ApoE-deficient hAPP mice have almost no amyloid plaques (Bales et al., 1997). Compared with apoE3, apoE4 greatly increases amyloid plaque deposition in aged hAPP mice (Holtzman et al., 2000; Buttini et al., 2002). Given their effects on Aβ aggregation into fibrils and plaques, apoE isoforms may also have differential effects on Aβ aggregation into oligomers, although this has not yet been shown.

In addition to direct effects on Aβ aggregation, apoE4 increases Aβ production by stimulating endocytosis of APP-containing vesicles, which enter the endosomal pathway where much of Aβ is produced (Ye et al., 2005). ApoE also increases intracellular Aβ by enhancing its uptake from the extracellular space via the LDL receptor-related protein (LRP), although this effect is not isoform-dependent (Zerbinatti et al., 2006). Finally, once Aβ has been taken up into lysosomal vacuoles, apoE4 potentiates Aβ-induced lysosomal leakage and apoptosis (Ji et al., 2006).

4.15.2.5.2 Aβ-independent mechanisms for apoE4-induced neuronal impairments

Compared with apoE-deficient mice, female mice with neuronal expression of apoE4 develop age-dependent deficits in learning and memory even in the absence of Aβ (Raber et al., 1998, 2000), presumably because of apoE4-induced reductions in andogen receptor levels in the brain (see following). ApoE4 knockin mice also have LTP impairments not seen in apoE3 mice (Trommer et al., 2004). Neuronal expression of human apoE3, but not apoE4, protected mice lacking endogenous apoE against synaptodendritic damage elicited by excitotoxic drugs (Buttini et al., 1999). Notably, these excitoprotective effects of apoE3 were eliminated when apoE3 and apoE4 were coexpressed in the same mice, suggesting a dominant adverse effect of apoE4 (Buttini et al., 2000).

ApoE is also found in NFTs and has effects on tau (Namba et al., 1991). ApoE3 binds to tau, whereas apoE4 does not (Strittmatter et al., 1994). ε4 carriers have more NFTs than age-matched ε3 homozygotes (Ohm et al., 1999). ApoE4 also stimulates microtubule depolymerization (Nathan et al., 1995), possibly through effects on tau. Neuronal, but not astroglial, expression of apoE4 in transgenic mice increases tau phosphorylation (Tesseur et al., 2000). Neuronal expression of endogenous apoE occurs primarily after neuronal injury (Xu et al., 2006b). In neurons, apoE4 undergoes cleavage by a chymotrypsin-like protease activity, and E4 is more susceptible to cleavage than E3 (Huang et al., 2001). The resulting C-terminally truncated apoE stimulates tau phosphorylation and NFT formation (Huang et al., 2001; Brecht et al., 2004). Truncated apoE can escape the secretory pathway and enter the cytosol, where it binds to mitochondria and impairs their function (Mahley et al., 2006).

ApoE also has cerebrovascular effects that may contribute to memory dysfunction. ApoE4 carriers have higher plasma cholesterol levels (Hallman et al., 1991) and are at higher risk of carotid atherosclerosis (Terry

et al., 1996), coronary artery disease (Chen et al., 2003), and ischemic stroke (McCarron et al., 1999). The overlap between these cerebrovascular risks and AD is becoming increasingly clear (Martins et al., 2006).

There are interesting gender differences in the effects of apoE that point toward important interactions with sex hormones. Among ε4 carriers, women are more likely to develop AD than men (Payami et al., 1996), and female apoE4 mice are more susceptible to memory deficits than their male counterparts (Raber et al., 1998, 2000; Grootendorst et al., 2005). This difference may be the result of effects of apoE4 on androgen receptors. ApoE4 decreases androgen receptor levels in males and females, and females may be more susceptible to this effect because of their lower circulating androgen levels (Raber et al., 2002; Raber, 2004).

Last, apoE4 is intrinsically less stable than apoE3 (Morrow et al., 2002). As a result, brain apoE levels are lower in individuals with the ε4 allele, an isoform difference that has also been identified in apoE4 and apoE3 knockin mice (Gregg et al., 1986; Ramaswamy et al., 2005). Thus, in addition to the adverse gain-of-function effects described above, apoE4 may contribute to neurological impairments by a loss-of-function mechanism. Consistent with this notion, ApoE-deficient mice display age-dependent synaptic loss, deficient regenerative axonal sprouting after perforant pathway transection, and greater susceptibility to diverse neural injuries (Masliah et al., 1995a, b; Buttini et al., 1999, 2000; Krzywkowski et al., 1999).

4.15.2.6 α-Synuclein

α-Synuclein is a small, cytosolic protein that is enriched in presynaptic terminals; it regulates presynaptic function and neurotransmitter release (Chandra et al., 2004; Fortin et al., 2005). It is also the main component of Lewy bodies, neuronal inclusions associated with most forms of Parkinson's disease (PD) and other Lewy body diseases (Spillantini et al., 1997). Mutations in α-synuclein are linked to rare forms of autosomal dominant PD (Polymeropoulos et al., 1997). α-Synuclein also seems to play a role in the intriguing clinical and neuropathological overlap between PD and AD. Many AD patients have Lewy bodies, sometimes known as the Lewy body variant of AD, and many PD patients develop AD-like dementia (Perl et al., 1998), emphasizing the fact that AD is a polyproteinopathy combining the accumulation of abnormal assemblies or fragments of Aβ, tau, apoE, and α-synuclein.

The initial link between α-synuclein and dementia was established through the discovery of a so-called 'nonamyloid component' (NAC) of plaques in AD brains, which turned out to be a fragment of α-synuclein (Uéda et al., 1993; Iwai et al., 1995). α-Synuclein promotes Aβ aggregation *in vitro* (Yoshimoto et al., 1995), although it doesn't seem to increase amyloid plaque deposition *in vivo* (Masliah et al., 2001b). α-Synuclein does, however, worsen Aβ-induced neuronal deficits independently of plaques. Doubly transgenic mice expressing hAPP/Aβ and wild-type human α-synuclein in neurons displayed more synapse loss, greater reductions in choline acetyltransferase-positive neurons, and more severe cognitive impairments than the singly transgenic parental strains, even though α-synuclein had no effect on plaque formation *in vivo* (Masliah et al., 2001b). This study also revealed that Aβ strongly promotes α-synuclein aggregation, both *in vitro* and *in vivo*. α-Synuclein concentrations are elevated in synaptic boutons in AD brains, suggesting that α-synuclein also plays a role in synaptic pathology in the human condition (Masliah et al., 1996). Whether the underlying mechanisms relate to α-synuclein's normal functions or to its abnormal aggregation remains to be determined.

4.15.3 Memory-Related Molecules in AD

Although some of the major players in the pathobiochemistry of AD have been identified, it is just now beginning to be understood how these molecules affect neuronal function and impair learning and memory. Mouse models of AD recapitulate many aspects of the human disease, both in terms of pathology and in relation to behavioral/memory impairments. Indeed, transgenic mouse models have provided a unique opportunity for synergy between two historically separate fields of biomedical research – AD research and the basic scientific analysis of learning and memory. As reviewed below, research in the last 15 years has revealed that AD-relevant molecules such as Aβ, tau, and apoE affect several aspects of neuronal function and cellular mechanisms of plasticity that have been implicated in the formation of long-lasting memories.

4.15.3.1 Neurotransmitter Release

Reliable neurotransmission requires a steady supply of synaptic vesicles filled with neurotransmitter to be ready for release at the presynaptic terminal. Since trafficking of new synaptic vesicles from the cell body

can take several hours, nerve terminals are equipped with a special machinery that allows for local recycling and refilling of synaptic vesicles, a process that replenishes vesicle pools within seconds to minutes (Südhof, 2004; Fernandez-Alfonso and Ryan, 2006; Kavalali, 2006; Ryan, 2006). The number of recycling vesicles and the efficiency with which they fuse with (exocytosis) and are retrieved from (endocytosis) the presynaptic terminal plasma membrane set the boundaries on the duration and frequency of neurotransmission, particularly during repetitive stimulation. Thus, factors that affect vesicle cycling, particularly steps that are rate limiting, can have profound consequences on synaptic efficacy.

The fusion of synaptic vesicles with the presynaptic membrane and their recovery through endocytosis require a number of neuron-specific proteins. The levels of many proteins that coordinate the docking and fusion of synaptic vesicles are decreased in AD as well as in experimental models (Honer, 2003; Scheff and Price, 2003). Synaptophysin, one of the most abundant membrane proteins on synaptic vesicles, is integral to the vesicle fusion process for neurotransmitter release. Decreases in synaptophysin have been used extensively as a measure of synaptic impairments in both AD and transgenic mouse models of the disease (Masliah et al., 1993; Mucke et al., 2000; Honer, 2003; Scheff and Price, 2003). Decreased levels of proteins involved in vesicle release, such as synaptophysin, may represent decreased expression at intact synapses, synaptic degeneration, or both.

In addition, several proteins involved in the local endocytosis and recycling of vesicles, including synaptotagmin, AP2, AP180, and dynamin I, are altered in AD and in some mouse models of the disease, as illustrated in **Figure 10** (Honer, 2003; Yao, 2004; Nixon, 2005). Animal models in which any of these proteins are mutated or ablated exhibit abnormal synaptic vesicle size and number, synaptic transmission deficits, and even mortality in extreme cases (reviewed in Yao, 2004; Kavalali, 2006). For some factors, both protein and mRNA levels are decreased in AD, suggesting that the expression of the corresponding genes may be dysregulated. Other factors may be depleted by increased cleavage and degradation (Yao, 2004; Kelly et al., 2005; Kelly and Ferreira, 2006; but see Yao et al., 2005). Impaired vesicle recycling may also contribute to some of the

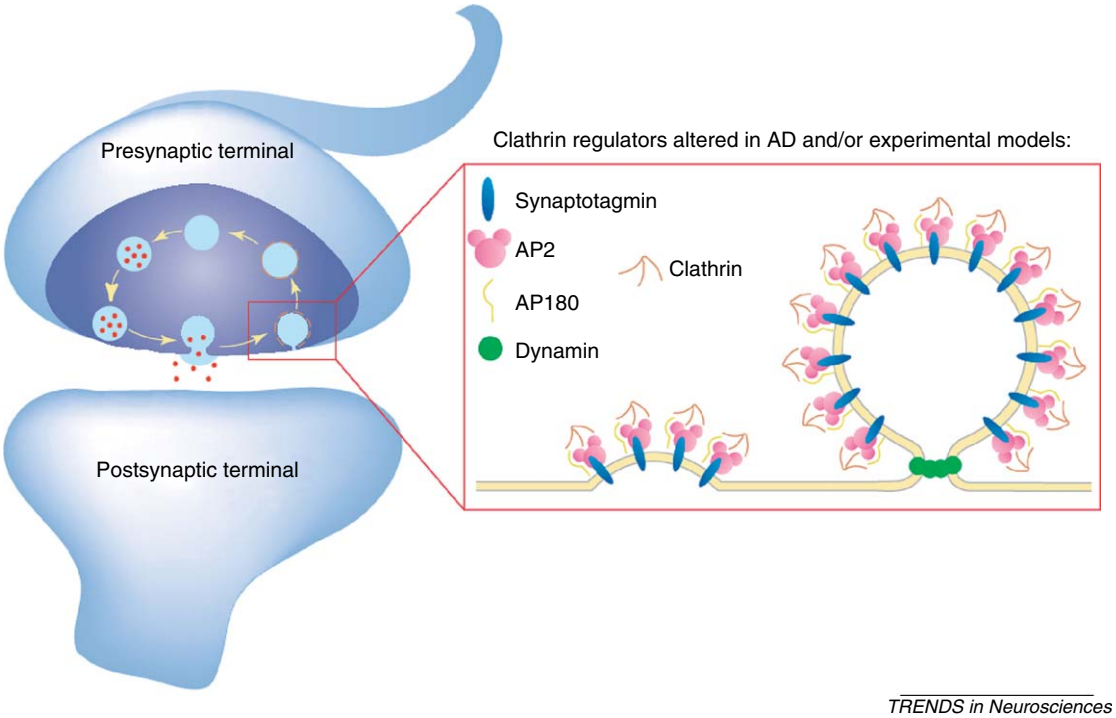

Figure 10 Alzheimer's disease (AD) affects clathrin-mediated synaptic vesicle recycling in synapses. Synaptic vesicles are recycled through clathrin-mediated endocytosis, which requires clathrin and regulatory proteins. The levels of several key regulators (see red box) of this process are decreased in AD as well as in experimental models of the disease. (From Yao P [2004] Synaptic frailty and clathrin-mediated synaptic vesicle trafficking in Alzheimer's disease. *Trends Neurosci.* 27: 24–29.)

ultrastructural changes found in AD brains and related mouse models. Decreases in synaptic density, attributed to synapse loss, are accompanied by an increase in size of the remaining synapses (reviewed in Scheff and Price, 2003). Although it has been hypothesized that such increases represent compensatory changes to maintain overall synaptic contact area, it has also been proposed that impaired endocytosis for local generation of vesicles leads to an overall accumulation of membrane and enlargement of nerve terminals (Yao, 2004).

In summary, alterations in levels of key components in synaptic vesicle trafficking may contribute to deficits in neurotransmitter release in AD.

4.15.3.2 Receptors and Channels

A large number of cell surface receptors and channels are located on the postsynaptic membrane, ready to receive and transduce input from presynaptic contacts. The strength of any given synapse can remain stable over time, increase, or decrease based on the number, localization, and activity of the neurotransmitter receptors and ion channels at the postsynaptic membrane. In this section, we review several receptors and channels that play important roles in synaptic plasticity and describe how alterations in their levels, localization, or function may contribute to AD-related cognitive dysfunction.

4.15.3.2.1 NMDA receptors

N-methyl-D-aspartate (NMDA) receptors play a critical role in the induction of LTP by acting as coincidence detectors of presynaptic glutamate release and postsynaptic depolarization (reviewed in Wang et al., 2006). Subsequent influx of calcium through the NMDA receptor triggers a series of intracellular signaling events that culminate in the induction of gene expression required for long-term changes in synaptic efficacy.

Currently approved therapies for the treatment of AD include an NMDA receptor antagonist, which is thought to protect neurons against increased calcium permeability of NMDA receptors and to increase the synaptic signal-to-noise ratio (Tariot and Federoff, 2003; Jacobsen et al., 2005). Studies in transgenic mouse models of AD and cell culture experiments are beginning to unravel the mechanisms by which AD-relevant molecules such as Aβ might alter NMDA receptor functions and impact synaptic plasticity.

As reviewed in the section titled 'Soluble Aβ oligomers,' many studies have reported that Aβ potently inhibits the induction of LTP both *in vitro* and *in vivo* (reviewed in Walsh and Selkoe, 2004). Although it is uncertain whether Aβ directly binds NMDA receptors, several lines of evidence demonstrate that at least part of Aβ's effect on LTP may be a result of alterations in the level, availability, or activity of NMDA receptors at the postsynaptic membrane.

In vitro, low concentrations of synthetic Aβ peptides acutely augment NMDA receptor–mediated calcium influx and synaptic transmission (Wu et al., 1995; Wu and Dun, 1995; Kelly and Ferreira, 2006). Furthermore, Aβ-dependent degradation of dynamin and the scaffolding protein PSD-95 can be blocked by NMDA receptor antagonists (Almeida et al., 2005; Roselli et al., 2005; Kelly and Ferreira, 2006). However, Aβ produces a delayed NMDA receptor–dependent reduction in synaptic transmission (Cullen et al., 1996). Because prolonged NMDA receptor stimulation leads to down-regulation of receptor activity through the recruitment of negative feedback loops (Oster and Schramm, 1993; Resink et al., 1995; Salter and Kalia, 2004; Braithwaite et al., 2006), it is possible that acute, Aβ facilitates glutamatergic transmission and sensitizes neurons to excitotoxic events (Mattson et al., 1993), whereas chronically, it leads to a down-regulation of NMDA receptor activity.

In vivo, hAPP transgenic mice that produce high Aβ levels have decreased levels of phosphorylation of Tyr-1472 of NR2B subunits of the NMDA receptor, particularly in the dentate gyrus (Palop et al., 2005). Phosphorylation at this residue affects the gating of the channel and is positively correlated with NMDA receptor currents (Lu et al., 1999; Alvestad et al., 2003; reviewed in Salter and Kalia, 2004), suggesting that the decreased phosphorylation found in hAPP mice may contribute to an attenuation of NMDA receptor-dependent signaling. Importantly, decreased levels of Tyr-1472 phosphorylation were associated with decreased activity of Fyn, a src-kinase family member that phosphorylates NR2B subunits at this residue, and increased expression of striatal-enriched phosphatase (STEP), a tyrosine phosphatase that negatively regulates Fyn activity (Chin et al., 2005). STEP can dampen NMDA receptor-dependent activity when engaged by high levels of stimulation (Pelkey et al., 2002; Braithwaite et al., 2006). The regulation of tyrosine kinases, such as Fyn, by Aβ is discussed in more detail in the section titled 'Kinases.'

Phosphorylation at Tyr-1472 also regulates the interaction of NMDA receptors with the scaffolding protein PSD-95 and with AP-2, an adaptor molecule that triggers clathrin-mediated endocytosis (Lavezzari

et al., 2003). *In vitro* experiments demonstrate that Aβ-dependent dephosphorylation of Tyr-1472 induces endocytosis of NMDA receptors, resulting in decreased surface expression of this key regulator of LTP induction (Snyder et al., 2005). The results of this study suggested a model in which extracellular Aβ binds α7 subunit-containing nicotinic acetylcholine receptors and thereby activates the phosphatases PP2B (calcineurin) and STEP, resulting in dephosphorylation of NR2B (**Figure 11**). Thus, Aβ may impair synaptic plasticity by inducing the dephosphorylation of Tyr-1472 and attenuating NMDA receptor activity through a variety of mechanisms.

The attenuation of glutamatergic transmission by Aβ may have different consequences depending on the brain region affected. If brain regions that control neuronal excitability on a global scale are particularly susceptible to Aβ-induced impairments of glutamatergic transmission, aberrant increases in overall brain activity may result. Such a notion is supported by findings that hAPP mice with high levels of Aβ exhibit nonconvulsive seizure activity in EEG recordings, which was associated with the induction of compensatory inhibitory mechanisms and impairments in synaptic plasticity (Palop et al., 2007).

4.15.3.2.2 AMPA receptors

α-amino-3-hydroxy-5-methyl-4-isoxazole-propionic acid (AMPA) receptors are glutamate-gated channels that mediate most of the fast excitatory synaptic transmission in the brain and provide the primary means of postsynaptic depolarization in glutamatergic neurotransmission. AMPA receptor localization is dynamically regulated by neuronal activity, and rapid insertion and removal of these receptors into/from the postsynaptic membrane are key mechanisms by which long-term changes in

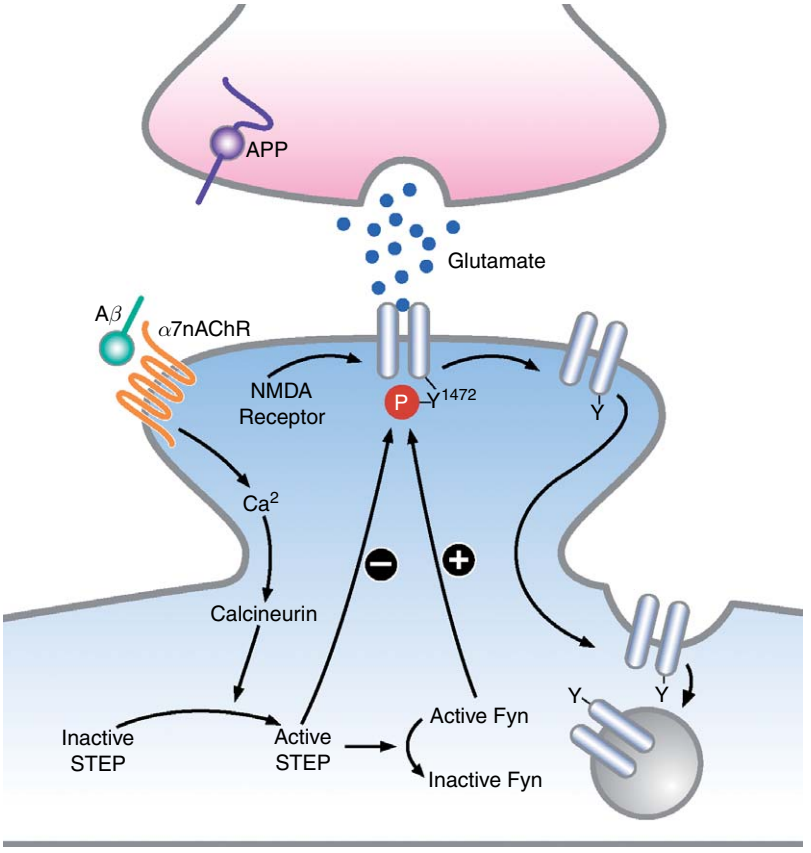

Figure 11 Aβ attenuates *N*-methyl-D-aspartate (NMDA) receptor signaling. Under normal circumstances, phosphorylation of the NMDA receptor at Tyr1472 is controlled by a balance between phosphorylation by Fyn and dephosphorylation by STEP. STEP also negatively regulates Fyn. In models of AD, Aβ activates α7 nAChRs and increases STEP activity, resulting in a net decrease in Fyn activity and Tyr1472 phosphorylation. In the absence of Fyn-mediated tyrosine phosphorylation, NMDA receptors are endocytosed.

synaptic strength (LTP and LTD) are expressed (Bredt and Nicoll, 2003; Esteban, 2003).

Such dynamic regulation of AMPA receptor density at the synapse requires that a pool of receptors be available for use at any given time. Indeed, recycling endosomes in dendritic compartments maintain a pool of AMPA receptors that can be rapidly mobilized and shuttled to the synaptic membrane in response to NMDA receptor activation to effect increases in synaptic strength (**Figure 12**) (Park et al., 2004; reviewed in Kennedy and Ehlers, 2006), whereas LTD-inducing stimuli result in endocytosis and removal of AMPA receptors from the synapse (Bredt and Nicoll, 2003).

In addition to their role in rapidly modifying synaptic strength, AMPA receptors play a critical role in another, slower form of plasticity called synaptic scaling (reviewed in Turrigiano and Nelson, 2004). In this type of homeostatic plasticity, the overall synaptic strength of a neuron is modulated to regulate its excitability depending on its history of activity. Periods of reduced activity result in increased levels of AMPA receptors at the synapse, whereas periods of increased activity lead to removal of AMPA receptors from the synapse. Moreover, regulation of AMPA receptor density at the postsynaptic membrane partly underlies distance-dependent scaling, in which synapses that lie farther from the soma are endowed with increased synaptic strength so they can transmit information with similar fidelity as synapses located closer to the soma (Andrasfalvy and Magee, 2001; Smith et al., 2003).

Several factors contribute to the ability of AMPA receptors to fulfill these critical roles in dictating synaptic strength and homeostatic control of neuronal activity, including expression levels, dendritic transport, and local synaptic trafficking within the endosomal pathway. Thus, impairments in any one of these processes may lead to deficits in synaptic plasticity and in the regulation of activity levels.

It is particularly interesting in this regard that reductions in several AMPA receptor subunits have been documented in the entorhinal cortex and hippocampus in AD and related transgenic mouse models (Yasuda et al., 1995; Chan et al., 1999; Wakabayashi et al., 1999; Carter et al., 2004; Chang et al., 2006; Palop et al., 2007). In AD, AMPA receptors also appear to be cleaved by caspases (Chan et al., 1999). In primary neurons or slices treated with Aβ or isolated from hAPP transgenic mice, surface expression of AMPA

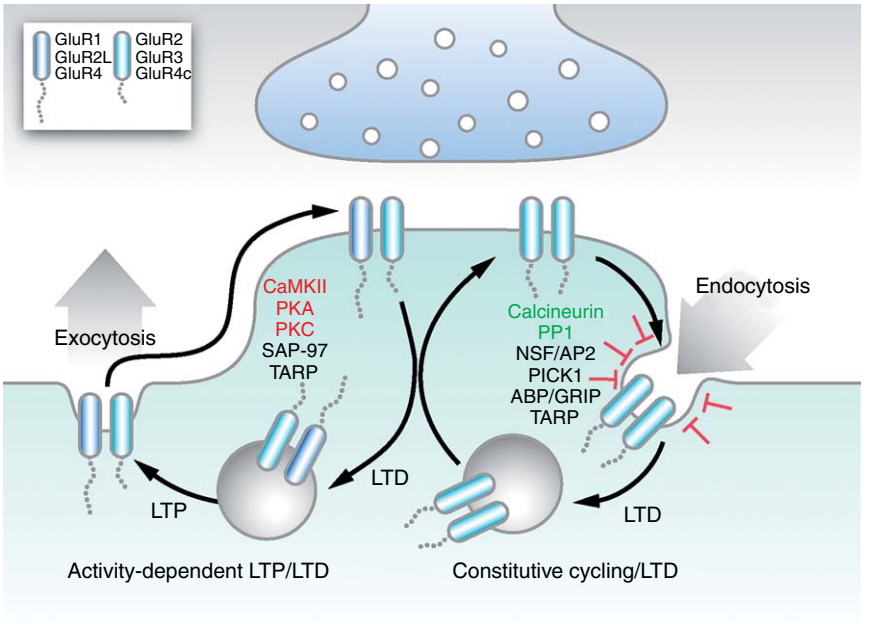

Figure 12 α-Amino-3-hydroxy-5-methyl-4-isoxazole-proprionic acid (AMPA) receptor trafficking. The trafficking of AMPA receptors between the synaptic membrane and recycling endosomes regulates synaptic strength. The insertion of AMPA receptors into the synapse (in long-term potentiation [LTP]) and the endocytosis of synaptic AMPA receptors (in long-term depression [LTD]) are governed by the activities of several kinases, phosphatases, and binding proteins. In Alzheimer's disease or related models, the activities of several kinases are decreased (red font), whereas the activities of several phosphatases are increased (green font), which may contribute to overall decreases in synaptic strength.

receptors is decreased (Almeida et al., 2005; Roselli et al., 2005; Hsieh et al., 2006). Such alterations may contribute to the decreased AMPA-mediated currents and increased NMDA/AMPA current ratios found in several *in vitro* and transgenic mouse models of AD (Hsia et al., 1999; Chang et al., 2006; Shemer et al., 2006).

Since AMPA receptors play such key roles in synaptic and homeostatic plasticity, alterations in their expression or trafficking may contribute to deficits in synaptic plasticity and learning and memory in AD and related models. As reviewed in the next section, it has been hypothesized that increased cholinergic activity in early stages of AD may be recruited to support synaptic scaling, perhaps in response to the loss of normal mechanisms underlying this homeostatic plasticity (Small, 2004).

4.15.3.2.3 Nicotinic acetylcholine receptors

Neuronal nicotinic acetylcholine receptors (nAChRs) are key modulators of neurotransmission. Presynaptically localized receptors enhance neurotransmitter release, postsynaptic receptors transduce fast excitatory transmission and calcium-regulated signaling, and perisynaptic or nonsynaptic receptors modulate neuronal excitability (Dani and Bertrand, 2006).

Although cholinergic activity is increased in early stages of AD, the loss of cholinergic neurons, particularly in the basal forebrain, is a characteristic neuropathological feature of AD that is thought to contribute to cognitive decline (Auld et al., 2002). The levels of acetylcholine receptors in AD brains, particularly the primarily presynaptically localized α7 subunit–containing nAChRs, are also decreased in postmortem tissues and by *in situ* imaging of receptor binding in live patients (Burghaus et al., 2000; Guan et al., 2000; reviewed in Auld et al., 2002; and Oddo and LaFerla, 2006). It is unclear whether this decrease simply reflects the loss of neurons expressing the receptors. Some studies report that α7nAChR mRNA levels are increased in the hippocampus of AD patients (Hellstrom-Lindahl et al., 1999; but see Mousavi et al., 2003), suggesting that decreases in protein levels and receptor binding may indeed reflect synaptic loss or neuronal degeneration. The use of acetylcholinesterase inhibitors to boost cholinergic signaling has long been a standard treatment for mild to moderate AD (reviewed in Jacobsen et al., 2005; Roberson and Mucke, 2006), but these regimens do not appear to provide long-lasting benefits.

A number of studies have shed light on how Aβ peptides may alter cholinergic signaling in AD. *In vitro* evidence demonstrated that Aβ binds α7nAChRs with high affinity, although experimental conditions and cell types can determine whether Aβ inhibits or stimulates the receptor (Wang et al., 2000; Liu et al., 2001a; Dineley et al., 2001, 2002; Pettit et al., 2001). Aβ-induced stimulation of α7nAChRs can appear to block receptor function but may simply occlude the effect of other ligands such as nicotine by potently stimulating the receptor and inducing calcium influx (Dougherty et al., 2003). Unlike the majority of membrane-bound receptors, nAChR expression is increased upon receptor stimulation (Fenster et al., 1999), which may account for several reports that Aβ increases α7nAChR levels *in vitro* and in hAPP transgenic mice (Dineley et al., 2001; Chin et al., 2005; Snyder et al., 2005).

Moreover, α7nAChRs can be targeted to somatodendritic compartments and have been found in perisynaptic regions on postsynaptic membranes, where they are in a strategic position to modulate intracellular signaling processes through their high calcium permeability (Fabian-Fine et al., 2001; Xu et al., 2006a). In primary cortical neurons, Aβ activates the calcium-dependent phosphatase PP2B (calcineurin) by engaging postsynaptic α7nAChRs (Snyder et al., 2005). One consequence of this activation is activation of the phosphatase STEP and subsequent dephosphorylation of the Tyr-1472 of the NR2B subunit of the NMDA receptor, either through direct dephosphorylation by STEP or through STEP-mediated desphosphorylation and inactivation of tyrosine kinases such as Fyn that are known to phosphorylate the NMDA receptor at this residue (see **Figure 11**).

These *in vitro* findings are in line with observations in hAPP mice, in which increased levels of α7nAChRs are concomitant with increased STEP levels, Fyn suppression, and decreased phosphorylation of Tyr-1472 of NR2B subunits (Chin et al., 2005; Palop et al., 2005). It remains to be determined whether the engagement of α7nAChRs by Aβ in AD and in experimental models of the disease represent primary pathogenic mechanisms or compensatory mechanisms to boost synaptic scaling (Small, 2004; Geerts and Grossberg, 2006) or combat excitotoxicity (Palop et al., 2007).

4.15.3.2.4 Potassium channels

The activities of potassium (K^+) channels, which efflux K^+ and hyperpolarize cells, play an important

role in neuronal survival, because they govern membrane excitability and because the intracellular potassium level is a determinant of apoptosis (Yu, 2003). New discoveries have highlighted how diverse K^+ channels in neuronal dendrites fine-tune excitability and affect neuronal information processing by regulating the induction of NMDA receptor–dependent synaptic plasticity in the hippocampus (reviewed in Yuan and Chen, 2006).

Phosphorylation-dependent modulation of K^+ channel activity, particularly of the Kv4.x family, is a major means by which synaptic plasticity is regulated and dendritic information processing is achieved (reviewed in Birnbaum et al., 2004; Yuan and Chen, 2006). Based on the kinetics of time-dependent inactivation, K^+ currents can be separated into different components. One such component, the fast-inactivating A-type K^+ current, plays a particularly important role in regulating membrane excitability, because it responds quickly to subthreshold depolarization and its activation delays the generation of action potentials (**Figure 13**). Inhibition of this current by pharmacological agents that block ion flux or stimulate phosphorylation of the channel markedly increases intracellular calcium (Hoffman et al., 1997; reviewed in Yuan and Chen, 2006).

Interestingly, Aβ inhibits A-type K^+ currents in primary hippocampal and neocortical neurons, increasing dendritic calcium influx and neuronal excitability (Good et al., 1996; Ye et al., 2003; Chen, 2005). This process may contribute to the observation that nonfibrillar Aβ assemblies increase neuronal excitability in various *in vitro* models (Hartley et al., 1999; Jhamandas et al., 2001; Turner et al., 2003; Ye et al., 2004; but see Yun et al., 2006). Aβ may inhibit K^+ currents through the aberrant engagement of tyrosine kinases, as tyrosine kinase inhibitors abrogated the ability of Aβ to increase excitability of cholinergic neurons (Jhamandas et al., 2001). Aβ treatment also induces K^+ channel abnormalities in cultured fibroblasts that are similar to K^+ channel abnormalities detected in fibroblasts isolated from AD patients (Etcheberrigaray et al., 1993, 1994). While regulated increases in intracellular calcium are important for synaptic plasticity, sustained increases in intracellular calcium through Aβ's effect on K^+ channels could impair synaptic plasticity and sensitize neurons to excitotoxic injuries (Xie, 2004).

Although A-type K^+ channel currents are generally inhibited by Aβ, the expression levels of K^+ channel subunits that contribute to the A-type current are increased in early stages of AD and in primary cerebellar neurons after treatment with Aβ (Angulo et al., 2004; Plant et al., 2006). Such increases in subunit expression may represent compensatory mechanisms aimed at restoring membrane excitability.

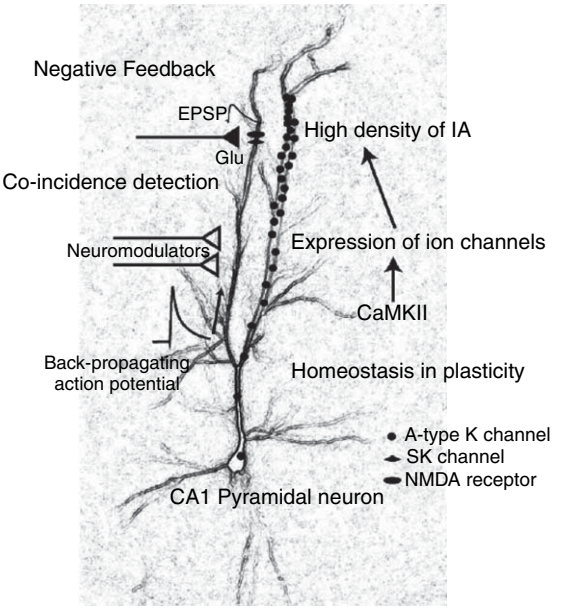

Figure 13 Dendritic K^+ channels influence neuronal information processing. SK and Kv4.2-encoded A-type K^+ channels are expressed at high levels in dendrites. Propagation of back-propagating action potentials and release of glutamate and neuromodulators within an appropriate time window ensures *N*-methyl-D-aspartate (NMDA) receptor activation through three coinciding events (as depicted in left dendrite above). Regulation of K^+ channel expression is also a homeostatic mechanism regulated by CaMKII. Activation of CaMKII promotes expression of both α-amino-3-hydroxy-5-methyl-4-isoxazole-propionic acid receptors and A-type K^+ channels, resulting in antagonizing effects on neuronal responsiveness. Alzheimer's disease–related decreases in the activity of A-type K^+ channels increase neuronal excitability and intracellular calcium and may contribute to excitotoxicity. (From Yuan L and Chen X [2006] Diversity of potassium channels in neuronal dendrites. *Prog. Neurobiol.* 78: 374–389.)

4.15.3.3 Calcium Signaling

Downstream of cell surface receptors and ion channels at the postsynaptic membrane, a myriad of intracellular signaling molecules await instruction. Depending on the dynamics of receptor/channel activity, local Ca^{2+} plumes of various magnitudes are generated to direct the kinase cascades and other signaling pathways that transduce signals to the nucleus or other subcellular compartments. In

this section, we discuss several aspects of calcium homeostasis that impact the dynamics of postsynaptic signaling and describe kinase pathways that play key roles in the induction and maintenance of long-term changes in synaptic efficacy. Special emphasis is placed on those aspects that are altered in AD and related models and on how their dysregulation contributes to plasticity deficits. Finally, we review the mechanisms by which extracellular, neuromodulatory factors such as brain-derived neurotrophic factor (BDNF) and Reelin influence synaptic plasticity and how AD-related alterations in these factors exacerbate impairments in synaptic function.

Calcium (Ca^{2+}) plays fundamental roles in synaptic plasticity and neuronal survival. Calcium signals, either from the extracellular milieu or from intracellular stores, must be precisely regulated both temporally and spatially to achieve tight control over intracellular signaling pathways that transduce signals from extracellular sources (Yuste et al., 2000; Berridge et al., 2003). Disruptions in neuronal Ca^{2+} homeostasis and calcium-regulated signaling likely play important roles in normal aging and AD, impairing synaptic plasticity and cognitive function, and contributing to neuronal loss in vulnerable regions (Mattson and Chan, 2003; Xie, 2004; Smith et al., 2005a; Kelly et al., 2006). Acute treatment of primary neurons and cell lines of neuronal origin with Aβ induces rapid, transient increases in intracellular calcium levels, whereas chronic exposure to Aβ leads to slower, more progressive increases in resting calcium levels (Xie, 2004; Kelly and Ferreira, 2006). Aβ peptides, particularly oligomeric assemblies, have been suggested to insert into neuronal cell membranes and form calcium-fluxing pores (reviewed in Pollard et al., 1995; Glabe and Kayed, 2006). Alternatively, such assemblies may increase intracellular calcium levels by increasing influx through calcium channels, releasing calcium from intracellular stores, or modulating intracellular calcium dynamics through alterations in endogenous calcium binding proteins (**Figure 14**).

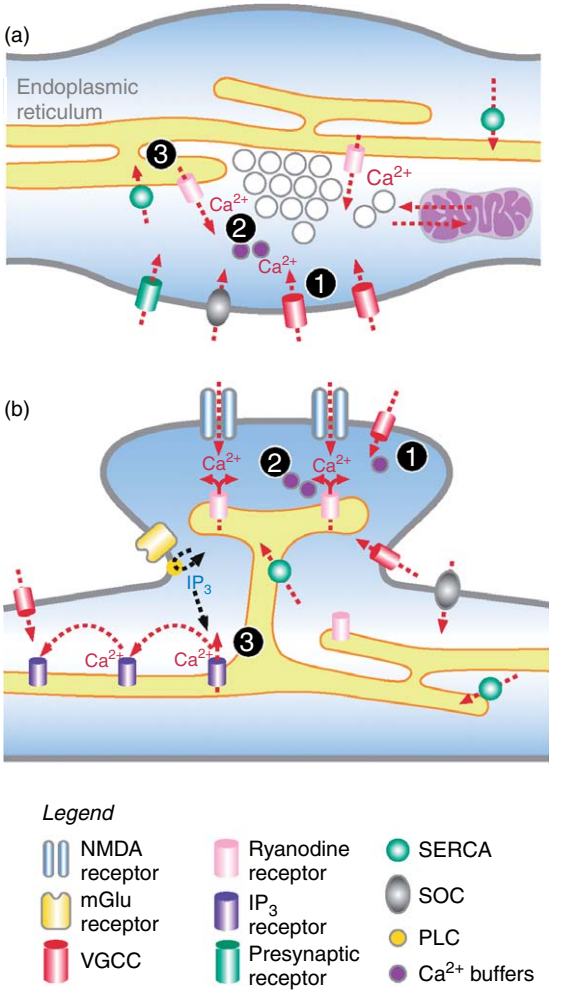

Figure 14 Ca^{2+} dysregulation in AD. Intracellular Ca^{2+} levels in the presynaptic terminal (top) and postsynaptic spine (bottom) are regulated by the influx of Ca^{2+} through Ca^{2+} channels (1), the buffering of free Ca^{2+} by Ca^{2+}-binding proteins (2), and the release of Ca^{2+} from intracellular Ca^{2+} stores (3). AD-related alterations in the levels and/or activities of Ca^{2+} channels, receptors, and Ca^{2+}-binding proteins perturb calcium homeostasis and may contribute to deficits in synaptic plasticity as well as increase susceptibility to excitotoxicity. (Modified from Bardo S, Cavazzini MG, and Emptage N [2006] The role of the endoplasmic reticulum Ca^{2+} store in the plasticity of central neurons. *Trends Pharmacol. Sci.* 27: 78–84).

4.15.3.3.1 Calcium channels

Ca^{2+} channels play diverse roles in synaptic transmission, on both the presynaptic and the postsynaptic side (Augustine et al., 2003). Presynaptic N- and P/Q-type voltage-sensitive Ca^{2+} channels are primarily involved in triggering synaptic vesicle exocytosis, whereas postsynaptic L-type voltage-sensitive Ca^{2+} channels contribute to the integration of synaptic activity and the transduction of signals that trigger a transcriptional response. Synaptic activity induces Ca^{2+} entry through NMDA receptors and L-type channels. The magnitude and temporal dynamics of the combined calcium influx determine which signaling pathways become engaged (Bradley and Finkbeiner, 2002; Deisseroth et al., 2003; Thiagarajan et al., 2006).

Aβ potentiates currents through L-type voltage-sensitive Ca^{2+} channels in primary neurons (Brorson

et al., 1995; Ueda et al., 1997; Ekinci et al., 1999; Fu et al., 2006). Although the underlying mechanisms have not been fully characterized, one possibility is Aβ-stimulated phosphorylation of L-type Ca^{2+} channels, which would increase conductance through the channel (Ekinci et al., 1999). In addition, because L-type Ca^{2+} channels are voltage sensitive, Aβ could indirectly modulate Ca^{2+} currents by blocking voltage-gated potassium channels (see earlier section), altering neuronal excitability and prolonging membrane depolarization (Good et al., 1996; Birnbaum et al., 2004). Finally, Aβ exposure leads to increased expression of particular L-type Ca^{2+} channel subunits on surface membranes in neuronal cell lines (Scragg et al., 2005; Chiou, 2006), which may contribute to long-term changes in Ca^{2+} homeostasis. Because Ca^{2+} influx through L-type channels could contribute to deranged Ca^{2+} homeostasis and signaling, L-type Ca^{2+} channel blockers are now in clinical trials for the treatment of AD (reviewed in Jacobsen et al., 2005; Roberson and Mucke, 2006).

4.15.3.3.2 Calcium-binding proteins

The level of intracellular free Ca^{2+} is governed by a balance between the entry of Ca^{2+} into the cytoplasm, either from extracellular sources or from intracellular stores, and the removal of Ca^{2+} by buffers, pumps, and exchangers (reviewed in Berridge et al., 2003). Many cell types contain particular calcium-binding proteins that act as buffers by rapidly binding and sequestering free Ca^{2+}. The primary cytosolic calcium-buffering proteins include calbindin-D_{28K}, calretinin, and parvalbumin, which are differentially expressed in various populations of neurons and play important regulatory roles in the maintenance of Ca^{2+} homeostasis (Hof et al., 1999). Parvalbumin is expressed in interneurons that modulate local circuitry in the neocortex and hippocampus, whereas calbindin and calretinin are expressed by both interneurons and pyramidal cells in the neocortex and hippocampus. Since disruption of neuronal Ca^{2+} homeostasis appears to contribute to AD pathogenesis, the levels and distribution of these types of calcium buffers in various areas of the AD brain and AD models have been the focus of a rapidly increasing number of studies.

Neuronal populations that express the calcium buffers calretinin and parvalbumin appear to be relatively preserved in AD (Hof et al., 1993; Fonseca and Soriano, 1995; Sampson et al., 1997). Losses of parvalbumin-expressing neurons have been documented in the entorhinal cortex and hippocampus of AD brains, but this loss appears to occur at late stages of the disease and may be secondary to degeneration of principal neurons in the same region (Solodkin et al., 1996; Brady and Mufson, 1997; Mikkonen et al., 1999). Such findings supported the hypothesis that neurons containing high levels of calcium-buffering proteins, and presumably a high calcium-buffering capacity, are relatively resistant to AD-related neurotoxicity (Hof et al., 1993). Late-stage loss of calcium-binding proteins was related to loss of neurons producing these proteins (Solodkin et al., 1996).

The calcium-binding protein calbindin is expressed in local circuit interneurons and pyramidal cells of the neocortex. In addition, it is very highly expressed in granule cells of the dentate gyrus and in Purkinje cells of the cerebellum (Celio, 1990). Calbindin regulates intracellular Ca^{2+} levels and is important for synaptic plasticity and learning and memory (Molinari et al., 1996). Calbindin levels in dentate granule cells are depleted in hAPP mice with high hippocampal levels of Aβ, and the magnitude of this depletion correlates tightly with cognitive deficits (Palop et al., 2003). Transgenic mice expressing the carboxy terminus of hAPP also exhibit depletions of calbindin in the dentate gyrus (Lee et al., 2006). Similar calbindin depletions occur in the dentate gyrus of AD patients, in whom the greatest depletions were seen in individuals with the most severe dementia (Palop et al., 2003). Moreover, calbindin mRNA levels in the hippocampus are also reduced in AD and in hAPP mice, supporting the hypothesis that calbindin depletions result from decreased expression of the calbindin gene rather than from loss of dentate granule cells (Iacopino and Christakos, 1990; Sutherland et al., 1993; Palop et al., 2003).

Similar calbindin depletions in the dentate gyrus have been observed after chronic neuronal overexcitation, for example, in human temporal lobe epilepsy, $GABA_B$ receptor-deficient mice, and models of kindling or kainate-induced chronic excitotoxicity (Tonder et al., 1994; Magloczky et al., 1997; Nägerl et al., 2000; Ruttimann et al., 2004; Palop et al., 2007). Calbindin reductions in the dentate gyrus of hAPP mice and in AD brains may also result from an imbalance between excitatory and inhibitory inputs (Palop et al., 2003, 2006, 2007). The ability of granule cells to downmodulate calbindin over a wide dynamic range may explain, at least in part, why these neurons are relatively resistant to degeneration in AD (West et al., 1994; Irizarry et al., 1997b; Palop et al., 2003), since calbindin reduction can lead to an

inactivation of voltage-gated calcium channels, limiting calcium entry and protecting against excitotoxicity (Nägerl et al., 2000).

4.15.3.3.3 Intracellular stores

In addition to influx from the extracellular compartment, the release of Ca^{2+} from intracellular stores in the ER is a major source of free Ca^{2+} that is available for signaling and synaptic plasticity (Rose and Konnerth, 2001; Berridge et al., 2003; Bardo et al., 2006). To maintain such a supply of Ca^{2+}, the ER faces the daunting task of sustaining an immense concentration gradient of Ca^{2+} across its membrane: The concentration of free Ca^{2+} in the ER lumen is about one thousand times greater than resting levels in the cytosol. Sarco-ER Ca^{2+} ATPases (SERCAs) actively transport Ca^{2+} into the ER to clear Ca^{2+} from the cytosol and fill ER stores. The liberation of Ca^{2+} from these internal stores is regulated by two types of channels in the ER membrane: the ryanodine receptor (RyR) and the inositol triphosphate receptor (IP_3R).

RyRs are activated by cytosolic Ca^{2+}, and their sensitivity is modulated by several factors: caffeine binding, oxidation, and high luminal Ca^{2+} levels increase sensitivity, whereas phosphorylation (by PKA) and calmodulin binding decrease activity (reviewed in Berridge et al., 2003; Bardo et al., 2006). IP_3Rs must be activated by the second messenger IP_3, which is generated upon stimulation of Gq-coupled receptors on the plasma membrane, such as metabotropic glutamate receptor types 1 and 5 ($mGluR_{1,5}$), serotonin receptors ($5\text{-}HT_2$), and muscarinic receptors (M1–3). The binding of IP_3 to IP_3Rs then sensitizes the receptors to Ca^{2+}, which increases receptor activity at low concentrations but inhibits it at high concentrations, such as those reached after release of Ca^{2+} from the ER (Berridge et al., 2003). IP_3Rs are often tethered to IP_3-producing cell surface receptors by scaffolding proteins such as Homer, linking the source of IP_3-production to its site of action (Ehrengruber et al., 2004). IP_3R activity can also be influenced by phosphorylation, which modulates the sensitivity of IP_3Rs in different directions, depending on the kinase involved. For example, phosphorylation by Ca^{2+}/calmodulin-dependent protein kinase II (CaMKII) decreases activity, whereas phosphorylation by Fyn kinase increases activity (Cui et al., 2004; Bare et al., 2005). Together, the activities of SERCAs, RyRs, and IP_3Rs monitor intracellular Ca^{2+} levels and regulate release of Ca^{2+} from the ER in a process called Ca^{2+}-induced Ca^{2+} release (CICR).

Several aspects of Ca^{2+} dysregulation in AD and AD mouse models have been linked to alterations in ER Ca^{2+} signaling. AD-related mutations in presenilin 1, presenilin 2, and APP increase cellular sensitivities to IP_3, caffeine activation of RyRs, and blockade of SERCA pumps, enhancing Ca^{2+} liberation from the ER (Smith et al., 2005a; Stutzmann, 2005). Overfilling of ER stores or excess phosphorylation of IP_3Rs or RyRs through aberrant activation of kinases by Aβ or other AD-related molecules may result in ER hypersensitivity and exaggerated Ca^{2+} release upon physiological stimulation of these receptors (discussed in the section titled 'Intracellular calcium stores'). As a result, even normal stimuli, such as synaptic activity and activation of mGluRs, could disrupt the intracellular Ca^{2+} homeostasis.

Mutations in presenilin 1 have been particularly linked to dysregulation of ER Ca^{2+} signaling by mechanisms that are unrelated to γ-secretase activity. As mentioned in Section 4.15.2.3.2, wild-type, but not AD-mutant, presenilin 1 and 2 can act as low-conductance Ca^{2+}-permeable ion channels, which may account for the majority of passive Ca^{2+} leaks from the ER (Tu et al., 2006). This Ca^{2+}-fluxing activity is important for maintaining normal steady-state intraluminal levels of Ca^{2+} and is exhibited by the unprocessed, holoprotein form of presenilin in the ER (Tu et al., 2006). In contrast, the secretase activity of presenilin emerges only in later compartments (trans-Golgi network, endosome) after assembly with other components of the γ-secretase complex (Tandon and Fraser, 2002). AD-related mutations in PS1 (PS1-M146V) or PS2 (PS2-N141I) abrogate the Ca^{2+} fluxing properties of the presenilins and abolish the passive efflux of Ca^{2+} out of the ER, overloading the ER with Ca^{2+}.

AD-related increases in levels of RyRs may also contribute to ER hypersensitivity and exaggerated Ca^{2+} release. RyR binding is increased in the entorhinal cortex and hippocampus in early stages of AD, suggesting increased levels of RyRs in these areas (Kelliher et al., 1999). Presenilin mutations are associated with increased levels of RyRs in transgenic mice and cell culture models (Chan et al., 2000; Smith et al., 2005b; Stutzmann et al., 2006). hAPP mice and primary cortical neurons treated with Aβ also show increases in RyRs (Supnet et al., 2006), suggesting that increased levels of Aβ are the unifying mechanism.

Together, these studies indicate that dysregulation of ER Ca^{2+} dynamics may contribute to impairments in synaptic plasticity and cognitive function associated with AD.

4.15.3.4 Kinases

Kinase activity is often coupled to the activity of receptors and channels at the plasma membrane and is crucial to the transduction of extracellular signals to cytosolic or nuclear targets. Signaling specificity is conferred by the type of receptor activated by synaptic activity, the dynamics and distribution of the ensuing Ca^{2+} influx, and the scaffolding of particular kinases to receptors/channels. Phosphorylation events triggered by active kinases can alter enzymatic activities or protein conformations of target molecules, setting in motion molecular cascades that culminate in cytoplasmic changes or nuclear events including gene transcription. Over the years, researchers have uncovered important roles for many kinases in synaptic plasticity and demonstrated how the orchestration of their activities leads to the induction, expression, and maintenance of long-term changes in synaptic efficacy. Notably, the levels, localization, or activities of many of these kinases are disrupted in AD, providing clues into the mechanisms by which AD impairs synaptic and cognitive function. In this section, we discuss several kinases whose roles in synaptic plasticity have been well characterized and how AD-related alterations in these kinases or associated signaling pathways may contribute to synaptic dysfunction.

4.15.3.4.1 MAPKs

The mitogen-activated protein kinase (MAPK) superfamily comprises three major subclasses of Ser/Thr kinases that are involved in the regulation of growth, differentiation, and cellular responses to stress and/or inflammatory cytokines. The extracellular signal-regulated kinases (ERKs) regulate growth, proliferation, and differentiation in many cell types and are essential for short-term increases in synaptic efficacy and for the expression and maintenance of LTP (Pearson et al., 2001; Thomas and Huganir, 2004; Davis and Laroche, 2006). The p38 branch of the MAPK family was originally characterized as key transducers of stress and inflammatory responses to cytokines but has recently been discovered to also mediate the induction and expression of LTD (Pearson et al., 2001; Thomas and Huganir, 2004). The activity or localization of ERK and p38 family members are altered in AD and related models, and their misregulation has been implicated in impairments of synaptic plasticity (Johnson and Bailey, 2003; Haddad, 2004). The third branch of the MAPK family is made up of the c-Jun N-terminal kinase/stress-activated protein kinases (JNK/SAPKs), which transduce stress signals, including oxidation and DNA damage, as well as growth and differentiation signals (Raivich and Behrens, 2006). Aβ-induced generation of reactive oxygen species activates JNK/SAPK, and such activation has been documented in AD and in AD models (reviewed in Zhu et al., 2004; Smith et al., 2006). Aβ-related engagement of JNK/SAPKs has been associated with overt cell death rather than more subtle effects on synaptic/neuronal functions. We will thus focus our discussion of MAPKs in AD-related synaptic impairments on ERK and p38.

ERK1/2 is activated rapidly after the induction of LTP. Although ERK1/2 activity is not necessary for the induction of LTP, it is critical for its maintenance and for learning and memory (English and Sweatt, 1997; reviewed in Thomas and Huganir, 2004; Davis and Laroche, 2006). In combination with other signaling pathways, LTP-induced ERK1/2 activation increases the expression of proteins necessary for long-term changes in synaptic efficacy, including the activity-regulated cytoskeletal protein (Arc/Arg3.1) (Roberson et al., 1999; Waltereit et al., 2001; Ying et al., 2002). Additional targets of ERK1/2 that are important for the expression or maintenance of LTP include cytoskeletal proteins such as MAP-2 and Tau, which modulate the structural organization of neurites; Kv4.2 potassium channels, which control dendritic depolarization and neuronal excitability; AMPA receptors, which are inserted into the membrane and increase synaptic strength; and mTOR, a component of the ribosomal machinery that controls synthesis of new proteins (reviewed in Haddad, 2004; Birnbaum et al., 2004; Kelleher et al., 2004; Sweatt, 2004). Clearly, tight regulation of ERK1/2 activity, dynamics, and localization is necessary to orchestrate its many effects on synaptic efficacy (**Figure 15**).

Increased levels of active ERK1/2 in AD brains are typically associated with neurofibrillary tangles and amyloid plaques (Trojanowski et al., 1993; Pei et al., 2002; Haddad, 2004; Webster et al., 2006). ERK1/2 phosphorylation of tau, described above in the section titled 'Tau phosphorylation and other posttranslational modifications,' has been well documented as a means by which hyperphosphorylated tau is generated in AD (reviewed in Haddad, 2004). In addition, the dysregulation of ERK1/2 activity may contribute in other ways to synaptic dysfunction, as indicated by studies in animal and *in vitro* models of AD. ERK1/2 activity has been shown to mediate the effects of Aβ on synaptic plasticity, including Aβ's effect on L-type calcium channels

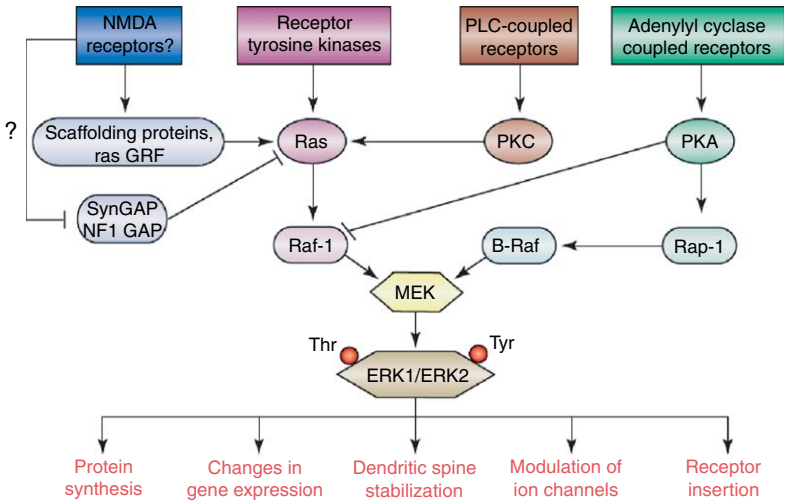

Figure 15 Regulation and targets of extracellular signal-regulated kinase (ERK)1/2 signaling in neurons. The ERK/mitogen-activated protein kinase cascade is activated by a number of receptors and pathways, and therefore plays a critical role in the integration of a wide variety of signals. The targets of ERK1/2 modulate processes that are crucial for synaptic plasticity (red font), all of which are impaired in Alzheimer's disease and experimental models. (From Sweatt JD [2004] Mitogen-activated protein kinases in synaptic plasticity and memory. *Curr. Opin. Neurobiol.* 14: 311–317.)

(reviewed in the section titled 'Calcium channels'). Interestingly, the kinetics of ERK1/2 activity depend on the duration of Aβ exposure and on Aβ's assembly state. Exposure of primary neurons or hippocampal slices to oligomeric Aβ acutely activated ERK1/2, but chronically decreased ERK1/2 activity, while exposure to fibrillar Aβ progressively increased ERK1/2 activity (Rapoport and Ferreira, 2000; Bell et al., 2004). Similarly, young hAPP transgenic mice exhibit increased ERK1/2 activity, whereas older hAPP mice exhibit decreased ERK1/2 activity, in particular in hippocampal subregions (Dineley et al., 2001; Chin et al., 2005; Palop et al., 2005). Certain brain regions may be able to downregulate ERK1/2 activity through compensatory mechanisms. For example, the phosphatase STEP dephosphorylates and inactivates ERK1/2 and is increased by Aβ *in vitro* and in the hippocampus of hAPP mice (Chin et al., 2005; Snyder et al., 2005; Braithwaite et al., 2006). Although the downregulation of aberrant ERK1/2 activity may be neuroprotective, it may also increase Aβ production (Kim et al., 2006) and decrease the expression of gene products required for the formation of long-term memories.

Although much emphasis has been placed on p38-mediated phosphorylation of tau, this process does not contribute greatly to the hyperphosphorylation of tau in AD (reviewed in Johnson and Stoothoff, 2004). p38 signaling also regulates synaptic plasticity by mediating long-term depression (LTD) of synaptic strength (reviewed in Thomas and Huganir, 2004). Inhibitors of p38 activity block LTD mediated by mGluRs or NMDARs (Bolshakov et al., 2000; Zhu et al., 2002), and inhibition of either p38 or mGluR activity prevents Aβ-induced LTP deficits in hippocampal slices (Wang et al., 2004). Furthermore, levels of phosphorylated, active p38 are increased in AD brains and related mouse models (Hensley et al., 1999; Zhu et al., 2000; Savage et al., 2002; reviewed in Johnson and Bailey, 2003; Hwang et al., 2005). Thus, Aβ-induced neuronal p38 activation may impair synaptic function in AD by promoting LTD.

4.15.3.4.2 CaMKII

Calcium/calmodulin-dependent protein kinase II is a major constituent of the postsynaptic density (PSD) that interacts with NMDA receptors and the cytoskeletal protein α-actinin (*See* Chapter 4.23). When activated by calcium influx during high-frequency stimulation, CaMKII translocates to the PSD and undergoes autophosphorylation at Thr286 of the αCaMKII subunit, resulting in prolonged calcium/calmodulin-independent CaMKII activity. This process is thought to underlie, at least in part, the conversion of a transient calcium signal to long-lasting enhancement of synaptic strength. Genetic or pharmacological manipulations that decrease levels of CamKII or prevent its autophosphorylation abolish LTP and

impair learning and memory (reviewed in Colbran and Brown, 2004).

In addition to its influence on gene transcription, CaMKII's cytoplasmic targets have also received great attention for their roles in the expression and maintenance of LTP. CaMKII promotes the insertion of alpha-amino-3-hydroxyl-5-methyl-4-isoxazolepropionate receptors (AMPARs) into the synapse, surface expression of Kv4.2 potassium channels, and activity of R-type calcium channels (reviewed in Colbran and Brown, 2004). Moreover, CaMKII-dependent modulation of cytoskeletal proteins regulates modifications of dendritic spine morphology associated with LTP (reviewed in Carlisle and Kennedy, 2005).

Although levels of CaMKII are relatively preserved in AD brains, levels of active, autophosphorylated CaMKII are significantly decreased (Mah et al., 1992; Simonian et al., 1994; Amada et al., 2005). These changes may be subregion specific, as decreases in autophorylated CaMKII were found in the hippocampus, but not in the amygdala (Amada et al., 2005). Consistent with these findings, Aβ acutely inhibits the ability of high-frequency stimuli to induce αCaMKII autophosphorylation and subsequent LTP in hippocampal slices (Zhao et al., 2004).

Autophosphorylation of αCaMKII, and thus CaMKII activity, are negatively regulated by the phosphatase PP1, which acts downstream of the calcium-dependent phosphatase calcineurin (Blitzer et al., 1998; Hedou and Mansuy, 2003). Particularly interesting in this regard are the findings by multiple groups that Aβ activates calcineurin *in vitro* and in transgenic mouse models of AD (Chen et al., 2002; reviewed in Xie, 2004; Cardoso and Oliveira, 2005; Snyder et al., 2005). Calcineurin levels and activity are also increased in AD (Hata et al., 2001; Liu et al., 2005; but see Lian et al., 2001). Together, these results suggest that enhanced negative regulation of CaMKII may diminish its activity in AD and impair synaptic plasticity.

4.15.3.4.3 PKC

Considerable evidence indicates that protein kinase C (PKC) is critical for long-term synaptic plasticity (Hvalby et al., 1994; Bortolotto and Collingridge, 2000). Indeed, ablation of PKC or inhibition of its activity impairs LTP as well as learning and memory (reviewed in Battaini and Pascale, 2005). Part of PKC's role in LTP may relate to its actions on AMPARs (Chung et al., 2000; Boehm et al., 2006). In addition, crosstalk between the PKC and PKA pathways can amplify ERK1/2 signaling (Roberson et al., 1999).

PKC is kept in a folded, inactive conformation by the binding of its pseudosubstrate domain to the substrate-binding site in the catalytic domain. Activation of the conventional, calcium-dependent isoforms of PKC, which are highly expressed in the brain, is regulated by binding to the second messengers calcium and diacylglycerol (DAG, reviewed in Battaini and Pascale, 2005). Upon binding and activation by second messengers, PKC translocates to the membrane via interactions with the scaffolding protein RACK1, which stands for receptor for activated C kinase (reviewed in Sklan et al., 2006). Interactions with RACK1 therefore aid in localizing PKC to its substrates.

Decreased activity of PKC has been implicated in the pathogenesis of AD. PKC levels are reduced in AD brains (Cole et al., 1988). In addition to deficits in PKC activation, which may result from decreased synaptic transmission and depletion of growth factors, PKC does not translocate effectively from the cytosolic to the membrane fraction in samples from AD brains, possibly because of decreased levels of RACK1 (Wang et al., 1994; Battaini et al., 1999).

Increased PKC immunoreactivity has been found in some AD cases and in hAPP transgenic mice at the beginning stages of amyloid deposition (Saitoh et al., 1993; Rossner et al., 2001), suggesting an early hyperactivation of PKC by Aβ, which may be followed by chronic suppression. Indeed, chronic activation is known to downregulate PKC activity (reviewed in Battaini and Pascale, 2005).

The consequences of reduced PKC activity are several-fold. In addition to decreasing the potential for synaptic plasticity, reduction of PKC activity may enhance the production of neurotoxic Aβ peptides. PKC increases the processing of APP by α-secretase in the nonamyloidogenic pathway, releasing the neurotrophic sAPPα fragment and precluding Aβ production (reviewed in Olariu et al., 2005). Treatment of APP/PS1 transgenic mice with small molecule activators of PKC significantly increased sAPPα, decreased Aβ levels, and reduced premature mortality (Etcheberrigaray et al., 2004). In addition, PKC regulates Aβ levels by increasing its clearance. Overexpression of the epsilon isoform of PKC activated endothelin-converting enzyme, an Aβ-degrading enzyme, and decreased Aβ levels, plaque deposition, neuritic dystrophy, and reactive astrocytosis in hAPP transgenic mice (Choi et al., 2006).

Together, these results suggest that enhancement of PKC activity may provide some benefit in AD.

4.15.3.4.4 PKA

Since the discovery in the 1980s that activation of cyclic AMP-dependent PKA increases synaptic efficacy in *Aplysia* neurons, numerous roles for PKA have been described in both short-term and long-term plasticity (reviewed in Nguyen and Woo, 2003; Waltereit and Weller, 2003). PKA is activated rapidly by calcium influx, which leads to increased synthesis of cyclic adenosine monophosphate (cAMP) by calcium/calmodulin-sensitive adenyl cyclases. Short-term actions of PKA include phosphorylation of potassium channels, to acutely increase excitability, and phosphorylation of synaptic vesicle proteins, to increase neurotransmitter release.

LTP-inducing stimuli lead to the degradation of the regulatory subunits of PKA, resulting in sustained activity of the catalytic subunits, which translocate to the nucleus and phosphorylate the transcription factor cAMP response element binding protein (CREB) to initiate gene transcription (reviewed in Kandel, 2001). Activity of PKA is necessary for long-lasting LTP in the hippocampus: it initiates gene transcription by direct phosphorylation of transcription factors and synergizes with other kinases, such as PKC, to activate ERK1/2 signaling (Roberson et al., 1999; Impey et al., 1998; reviewed in Waltereit and Weller, 2003). The combined actions of PKA and ERK1/2 are necessary for the transcription of immediate-early genes such as Arc/Arg3.1 that are critical to memory consolidation (Waltereit et al., 2001). PKA also increases current conductance and synaptic strength via phosphorylation of synaptic AMPA receptors (reviewed in Nguyen and Woo, 2003).

Alterations in PKA signaling have been implicated in several aspects of AD. PKA contributes to tau hyperphosphorylation by direct phosphorylation and by rendering tau susceptible to phosphorylation by GSK-3 (Liu et al., 2004; reviewed in Gong et al., 2005). Although PKA is responsible for a large proportion of tau hyperphosphorylation in AD, PKA activity is decreased in AD as well as in animal and cell culture models of the disease (Kim et al., 2001; Vitolo et al., 2002; Gong et al., 2006), possibly because Aβ inhibits the proteasomal degradation of PKA's regulatory subunits (**Figure 16**) (Vitolo et al., 2002). Increasing cAMP levels by inhibition of phosphodiesterases that break down cAMP ameliorates Aβ-induced deficits in synaptic plasticity and

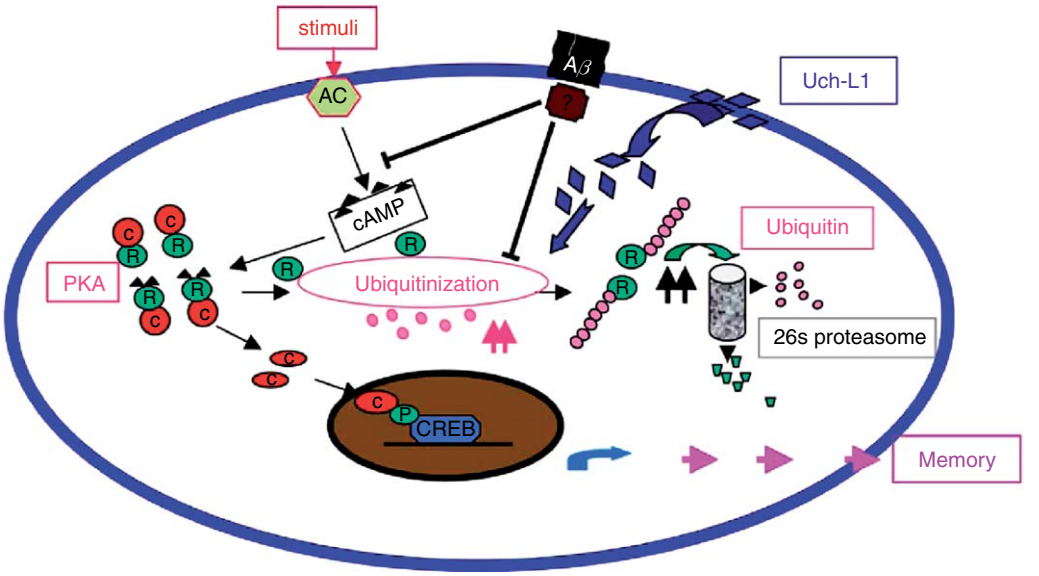

Figure 16 Aβ modulation of the ubiquitin-proteasome-protein kinase A (PKA)-cyclic adenosine monophosphate response element binding protein (CREB) pathway. Aβ inhibits adenylate cyclase activity and proteasomal degradation of the regulatory subunits of PKA, resulting in their accumulation and a shift in the PKA complex toward the inactive tetramer. Consequently, CREB phosphorylation and initiation of transcription is impaired. Transduction of Uch-L1 promotes proteasomal activity, normalizing levels of the PKA regulatory subunit and freeing the active catalytic subunit. (From Gong B, Cao Z, Zheng P, et al. [2006] Ubiquitin hydrolase Uch-L1 rescues β-amyloid-induced decreases in synaptic function and contextual memory. *Cell* 126: 775–788.)

learning and memory (Vitolo et al., 2002; Gong et al., 2004). Exogenous ubiquitin C-terminal hydrolase L1 (Uch-L1), which boosts proteasome activity, reversed Aβ-induced LTP deficits in hippocampal slices (Gong et al., 2006). Moreover, endogenous Uch-L1 activity was decreased in APP/PS1 transgenic mice, and treatment of these mice with exogenous Uch-L1 restored PKA activity and contextual memory (Gong et al., 2006).

4.15.3.4.5 Fyn

The tyrosine kinase Fyn can be activated through diverse receptors and participates in signaling pathways that control a broad spectrum of biological activities, including long-term changes in synaptic efficacy (Thomas and Brugge, 1997; Roskoski, 2004; Salter and Kalia, 2004). Ablation of Fyn abolishes LTP and impairs spatial learning and memory (Grant et al., 1992). Postnatal overexpression of Fyn can restore LTP, indicating that Fyn is a critical modulator of long-term synaptic efficacy (Kojima et al., 1997). Anchored to NMDA receptor complexes through interactions with PSD-95, Fyn phosphorylates the NR2B subunit at tyrosine residue 1472 (Tyr1472), which increases calcium conductance by altering channel gating properties and controls the internalization of the receptors by preventing AP-2 binding, a signal for endocytosis (reviewed in Salter and Kalia, 2004; Prybylowski et al., 2005). In addition, Fyn can modulate cytoskeletal dynamics by altering the phosphorylation and/or localization of cytoskeletal elements such as tau, adducin, and β-catenin (Williamson et al., 2002; Lilien and Balsamo, 2005; Gotoh et al., 2006). Moreover, phosphorylation by Fyn influences the integrity of synaptic AMPA receptors by rendering them less susceptible to proteolytic cleavage (Rong et al., 2001).

A number of findings suggest that misregulation of Fyn activity may play a role in AD. The distribution and levels of Fyn are altered in AD brains (Shirazi and Wood, 1993; Ho et al., 2005), and the toxic effects of Aβ oligomers on hippocampal slices can be blocked by the genetic ablation of Fyn (Lambert et al., 1998). Ablation of Fyn decreases – whereas overexpression of Fyn increases – Aβ-induced synaptotoxicity and premature mortality in hAPP transgenic mice (Chin et al., 2004). In addition, Fyn phosphorylates tau and binds it in a manner that is modulated both by AD-related hyperphosphorylation and by disease-related mutations in tau (Bhaskar et al., 2005; Lee, 2005). Together with *in vitro* studies demonstrating that acute application of Aβ leads to activation of Fyn signaling pathways and increased interactions with binding partners, these results suggested that Aβ may derange synaptic functions by aberrantly engaging Fyn-related pathways (Zhang et al., 1996; Williamson et al., 2002). Indeed, the overexpression of Fyn in hAPP mice with moderate levels of Aβ rendered the mice as severely impaired, with respect to biochemical and behavioral alterations, as hAPP mice with high levels of Aβ (Chin et al., 2005). These results suggest that Fyn activity sensitizes neurons to Aβ-induced neuronal impairments. Fyn also exacerbates Aβ-induced aberrant increases in neuronal activity (Palop et al., 2007).

Interestingly, aberrant engagement of Fyn activity also appears to trigger compensatory mechanisms that limit Fyn activity in the presence of elevated Aβ levels. hAPP transgenic mice exhibit significant increases in levels of the phosphatase STEP, which dephosphorylates and inactivates Fyn, and have corresponding decreases in levels of active Fyn (see **Figure 11**) (Chin et al., 2005). The increase in STEP and decrease in Fyn activity are most prominent in the dentate gyrus, a region particularly susceptible to Aβ-related synaptic dysfunction. The compensatory downregulation of Fyn activity in this region has consequences on NMDA receptor phosphorylation, calcium gating, and receptor internalization that likely further contribute to deficits in synaptic plasticity and learning and memory (Chin et al., 2005; Palop et al., 2005; Snyder et al., 2005).

4.15.3.4.6 Cdk5

Cyclin-dependent kinase 5 is an unusual Cdk that lacks a role in the cell cycle and is activated by two noncyclin activators, p35 and p39. With an extensive list of substrates, the primarily neuronal Cdk5 regulates cell death and survival, as well as a variety of specific cellular functions (reviewed in Cheung and Ip, 2004; Cruz and Tsai, 2004). Cdk5 activity is increased in AD brains and in neurons and cell lines treated with Aβ and mediates tau hyperphosphorylation (reviewed in Giese et al., 2005). It also modulates synaptic plasticity, with consequences on learning and memory (reviewed in Cruz and Tsai, 2004; Cheung et al., 2006; Angelo et al., 2006).

Presynaptic roles for Cdk5 that influence synaptic transmission include the regulation of synaptic vesicle exocytosis through phosphorylation of P/Q-type calcium channels and synapsin 1, which increases calcium influx and releases synapsin's tethering of synaptic vesicles in a reserve pool (reviewed in Angelo et al., 2006). Cdk5 also modulates endocytosis for the recycling of synaptic vesicles by phosphorylating dynamin

I and amphiphysin I, proteins necessary for clathrin-mediated endocytosis (reviewed in Angelo et al., 2006).

Postsynaptic roles for Cdk5 in synaptic plasticity include phosphorylation of PSD-95, which suppresses its multimerization and decreases PSD-95-dependent clustering of NMDA receptors and Kv1.4 potassium channels (Morabito et al., 2004). Cdk5 also phosphorylates NR2A subunits of NMDA receptors and increases calcium conductance (Li et al., 2001). Moreover, Cdk5 activity regulates dendritic spine remodeling through actions on proteins that modulate cytoskeletal dynamics, including Rho GTPases and PAK1 (Nikolic et al., 1998).

Direct evidence for a role of Cdk5 in learning and memory has come from studies of transgenic mice expressing p25, a truncated form of the Cdk5 activator p35 that results in constitutive activation of Cdk5. Transient expression of p25 led to improved synaptic plasticity and learning in hippocampus-dependent tasks (Fischer et al., 2005). These improvements were accompanied by increased spine density and synapse formation.

Thus, Cdk5 activity must be well regulated in order to maintain control over the numerous aspects of neuronal function that it modulates. p25 levels are higher in AD brains than in normal controls (Lee et al., 1999; Patrick et al., 1999; reviewed in Giese et al., 2005). Although transient expression of p25 increases synaptic plasticity, prolonged neuronal expression of p25 in transgenic mice leads to synaptic impairments, learning and memory deficits, neurofibrillary tangle formation, and neurodegeneration (Cruz and Tsai, 2004; Fischer et al., 2005). In addition, sustained Cdk5 activity increases Aβ production (Cruz et al., 2006). Thus, the generation of p25 may be initiated in early stages of AD as a compensatory mechanism to support waning plasticity and memory, but its continued presence and the resulting overactivation of Cdk5 may eventually contribute to synaptic impairments and other neuronal deficits.

4.15.3.5 Neurotrophic and Neuromodulatory Factors

During development of the nervous system, secreted neurotrophic and neuromodulatory factors regulate axonal outgrowth, dendritic maturation, synapse formation, and synaptic strength. These processes overlap widely with those necessary for synaptic plasticity and the maintenance of long-term changes in synaptic function. Therefore, it is not surprising that nature has recycled many of these same neurotrophic and neuromodulatory factors to effect synaptic plasticity in adult organisms. Two such neuromodulatory factors are brain-derived neurotrophic factor (BDNF) and Reelin. We discuss their roles in synaptic plasticity and how AD-related alterations in their levels may contribute to synaptic and cognitive dysfunction.

4.15.3.5.1 BDNF

BDNF belongs to the neurotrophin family of signaling proteins that also includes nerve growth factor (NGF), neurotrophin 3 (NT-3), and neurotrophins 4/5(NT-4/5), all of which participate in regulating the survival and differentiation of specific neuronal populations during development. However, BDNF is unique among its family members in its ability to modulate activity-dependent synaptic plasticity in the developing and the adult brain (reviewed in Lu, 2003).

The actions of BDNF have long been studied in the context of learning and memory in animal models, but the recent discovery that a Val→Met mutation in the prodomain of BDNF is linked to memory impairment and susceptibility to neuropsychiatric disorders in humans has fueled additional research (reviewed in Bath and Lee, 2006). This mutation affects the trafficking of BDNF to the secretory pathway, resulting in reduced secretion of BDNF in target regions. Moreover, BDNF mRNA and protein levels are decreased in brain regions that are vulnerable in AD, suggesting that alterations in BDNF may reflect or contribute to cognitive impairments in AD (reviewed in Murer et al., 2001; Allen and Dawbarn, 2006).

BDNF has multiple distinct functions in synaptic plasticity that can be divided into two broad categories: permissive and instructive (reviewed in Schinder and Poo, 2000; Bramham and Messaoudi, 2005). Permissive actions of BDNF prepare synapses to be LTP-competent but do not actually generate LTP. Such actions include the maintenance of the presynaptic release machinery (vesicle docking and vesicle pool dynamics), which allows neurons to follow high-frequency stimuli.

Instructive signals from BDNF are initiated in response to high-frequency stimuli that induce LTP and result in the activity-dependent expression and release of BDNF. Some of these signals modulate postsynaptic calcium influx through voltage-gated sodium channels, reducing the amount of stimulation necessary for LTP induction (gating) (reviewed in Blum and Konnerth, 2005). The majority of BDNF's

effects are mediated by TrKB receptors and subsequent signaling events that engage ERK1/2 and regulate the expression of genes, including the immediate-early gene Arc/Arg3.1 (reviewed in Blum and Konnerth, 2005; Bramham and Messaoudi, 2005). BDNF also regulates the translation of dendritically localized mRNAs associated with synapse-specific LTP (reviewed in Schuman et al., 2006). Together, BDNF's actions lead to long-lasting increases in synaptic strength.

Regulated BDNF expression also plays a central role in homeostatic synaptic scaling, through which the overall activity of a neuronal network is maintained over time (reviewed in Turrigiano and Nelson, 2004). As mentioned above, BDNF expression is activity dependent. Under situations of reduced activity, BDNF expression is reduced. GABA expression in inhibitory interneurons is then reduced, diminishing inhibition and promoting the firing rate of pyramidal neurons (Rutherford et al., 1997, 1998; reviewed in Turrigiano and Nelson, 2004).

The role of BDNF in scaling is particularly interesting in light of the decreased levels of BDNF observed in vulnerable brain regions in AD. Does the decrease in BDNF result from a primary insult and exacerbate synaptic deficits and plasticity in AD, or does it represent the attempts of an impaired network to increase neuronal activity and maintain synaptic connections? Answers to these questions are pending.

An additional complexity in considering the role of BDNF in AD is that the regulation and effects of BDNF have different outcomes depending on the state of the neuronal network on which it acts (Turrigiano and Nelson, 2004). The role of BDNF reductions in homeostatic scaling described above is evident in conditions in which normal activity has been abolished. However, in situations containing normal levels of background activity, BDNF's potentiating activity prevails. Transgenic mice overexpressing BDNF have increased seizure severity after kainic acid challenge and develop hyperexcitability in the entorhinal cortex and the CA regions of the hippocampus (Croll et al., 1999). Transgenic mice overexpressing BDNF's receptor TrKB also have a reduced threshold for kainate-induced seizures (Lahteinen et al., 2003). These studies suggest that a reduction of BDNF in AD may represent a compensatory mechanism against hyperexcitability. Consistent with this idea, removal of one BDNF allele in mice leads to increased synaptic inhibition (Olofsdotter et al., 2000).

4.15.3.5.2 Reelin

Reelin is a large glycoprotein of the extracellular matrix involved in neuronal migration and positioning during development (Tissir and Goffinet, 2003). In the mature brain, it modulates neuronal function and synaptic plasticity and regulates tau phosphorylation as well as axonal growth and dendritic spine morphology (Hiesberger et al., 1999; Liu et al., 2001b; Fatemi, 2005; Herz and Chen, 2006; Qiu et al., 2006b).

In most of the brain, Reelin is expressed by GABAergic interneurons that regulate the activity and function of neighboring glutamatergic neurons (Pesold et al., 1998; Ramos-Moreno et al., 2006). Interestingly, Reelin is also expressed highly by glutamatergic pyramidal neurons in layer II of the entorhinal cortex (Pesold et al., 1998; Perez-Garcia et al., 2001; Ramos-Moreno et al., 2006), a population of neurons that is affected early and severely by AD (Blennow et al., 2006). These neurons project primarily to the dentate gyrus and area CA1 of the hippocampus (Ramos-Moreno et al., 2006; van Groen et al., 2003), which are also vulnerable to AD (Blennow et al., 2006; Palop et al., 2003). Although Reelin does not appear to undergo calcium-dependent exocytosis (Lacor et al., 2000), its localization in secretory vesicles, axons, and dendritic spine-rich neuropils suggests that it may be released from both the cell soma and synaptic terminals (Pesold et al., 1998; Lacor et al., 2000; Pappas et al., 2001; Ramos-Moreno et al., 2006). Reelin immunoreactivity is also present in the axonal projections of glutamatergic pyramidal neurons in layer II of the entorhinal cortex (Ramos-Moreno et al., 2006), suggesting that Reelin produced by these cells is transported down axons and may impact neuronal function in target regions such as the dentate gyrus and CA1.

Two neuronal cell surface receptors that bind apoE and transport cholesterol into neurons, very low density lipoprotein receptor (VLDLR) and apolipoprotein E receptor 2 (ApoER2), bind Reelin and cooperate to transduce its signals (reviewed in Herz and Chen, 2006). The close proximity between Reelin receptors and NMDA receptors allows crosstalk between the two signaling pathways: Through a series of phosphorylation events, Reelin increases NMDA receptor function and thereby enhances the induction of LTP (**Figure 17**) (Weeber et al., 2002; Beffert et al., 2005; Chen et al., 2005b). Reelin also enhances

synaptic function by stimulating the translation of dendritically expressed mRNAs, such as Arc/Arg3.1 mRNA (Dong et al., 2003). Reelin-deficient mice have a diminished capacity for hippocampus-dependent memory, indicating that Reelin is necessary for normal plasticity and memory formation (Qiu et al., 2006a).

The localization of Reelin expression and its roles in regulating synaptic plasticity as well as tau phosphorylation suggest that decreases in Reelin might exacerbate synaptic impairments in AD. In addition to memory deficits, Reelin-deficient mice exhibit robust increases in levels of hyperphosphorylated tau (Hiesberger et al., 1999; reviewed in Herz and Chen, 2006), suggesting a role for Reelin in the generation of tau pathology in AD. Furthermore, apoE competes with Reelin for binding to VLDLR/ApoER2 receptors and decreases Reelin signaling (D'Arcangelo et al., 1999; Herz and Bock, 2002). It has been suggested that apoE4 competes more efficiently than apoE3 (reviewed in Herz and Bock, 2002), providing yet another mechanism by which apoE4 may increase the susceptibility to AD (**Figure 17**; see also the section titled 'Aβ-Independent mechanisms for apoE-induced neuronal impairments').

A few recent studies have begun to examine whether AD is associated with Reelin alterations hAPP transgenic mice with high levels of Aβ were found to have significantly fewer Reelin-expressing

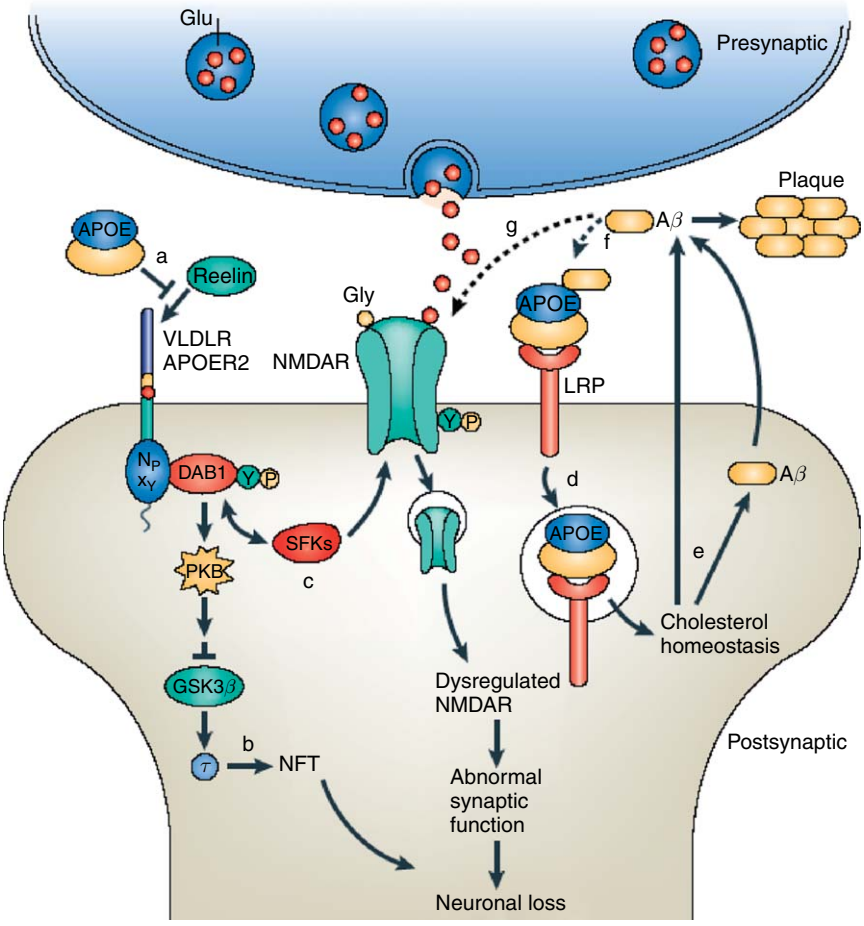

Figure 17 Reelin and ApoE signaling – implications for AD. Binding of Reelin to VLDLR and ApoER2 receptors initiates a cascade of events that leads to modulation of NMDA receptor function and enhanced long-term potentiation. ApoE can impede Reelin signaling by competing for receptor binding. Impaired Reelin signaling results in impaired synaptic plasticity as well as in elevated tau phosphorylation, which could contribute to neurofibrillary tangles associated with AD. In addition, binding of ApoE to the LRP lipoprotein receptor results in internalization of the ligand-bound receptor. Cholesterol homeostasis modulates the production and trafficking of Aβ. Secreted Aβ can bind apoE and be cleared through receptor-mediated endocytosis, promote the internalization of NMDA receptors, and deposit into plaques (From Herz J and Chen Y [2006] Reelin, lipoprotein receptors and synaptic plasticity. *Nat. Rev. Neurosci.* 7: 850–859.)

pyramidal cells in the entorhinal cortex and corresponding reductions in Reelin levels in the hippocampus relative to nontransgenic mice (Chin et al., 2007). In contrast, the number of Reelin-expressing GABAergic interneurons was not altered in either the entorhinal cortex or the hippocampus. Underscoring the relevance of these findings, qualitatively similar reductions of Reelin-expressing pyramidal neurons were found in the entorhinal cortex of AD brains (Chin et al., 2007). Increased fragments of Reelin were found in the CSF of AD patients, suggesting altered processing of Reelin (Saez-Valero et al., 2003). Increased levels of Reelin were found in the frontal cortex of AD brains (Botella-López et al., 2006). Conceivably, increases in Reelin in frontal brain regions reflect the kind of hyperactivation of frontal areas that is presumed to compensate for the failure of more vulnerable brain regions in AD (Buckner, 2004; Pariente et al., 2005; Palop et al., 2006).

4.15.3.6 Gene Expression

Short-term synaptic plasticity is effected by noncovalent modifications of existing proteins, such as ion channels, receptors, or components of the vesicle-release machinery that lead to acute modulation of synaptic strength. Long-lasting synaptic plasticity, however, requires protein synthesis and structural changes for the long-term maintenance of changes in synaptic strength (reviewed in Kandel, 2001; Carlisle and Kennedy, 2005). It is clear from the discussion in the section titled 'Kinases' that the derangement of kinase pathways in AD may disrupt the expression of pertinent genes. We consider here two proteins that play particularly important roles in gene transcription necessary for long-term plasticity (CREB) and in consolidation of long-term memories (Arc/Arg3.1), and how alterations in their expression or localization may contribute to AD-related synaptic and cognitive impairments.

4.15.3.6.1 CREB

The transcription factor CREB is essential for many forms of learning and memory (reviewed in Lonze and Ginty, 2002; and Tully et al., 2003) (*See* Chapter 4.27). CREB is a nuclear protein that modulates the transcription of genes containing cAMP-responsive elements (CREs). Phosphorylation of CREB at Ser133 leads to the recruitment of other components of the transcription machinery to CREs. Both PKA and Ca^{2+}/calmodulin kinase type IV (CaMKIV) can phosphorylate CREB at Ser133 and are responsible for rapid initial increases in CREB phosphorylation in response to neuronal activity, whereas ERK1/2-dependent phosphorylation of Ser 133 occurs with slower kinetics, involves an intermediate such as RSK or MSK family kinases, and leads to prolonged phosphorylation of CREB.

CREB target genes encompass diverse proteins, ranging from proteins involved in neurotransmission, the transcriptional machinery, or signal transduction to growth factors such as BDNF, structural proteins, channels, and transporters (reviewed in Lonze and Ginty, 2002). Consequently, disruptions in CREB activation impair numerous neurological functions.

Decreased activity of PKA and ERK1/2 in AD and related experimental models is accompanied by decreased levels of phosphorylated CREB (Dineley et al., 2001; Vitolo et al., 2002; Gong et al., 2004, 2006). Efforts are being made to determine whether promoting CREB activity is an effective means to enhance memory in normal aging and in AD (Tully et al., 2003). Indeed, treatment of transgenic mouse models of AD with agents that boost cAMP levels (and thus PKA activity) do restore PKA/CREB signaling and are associated with an amelioration of deficits in synaptic plasticity and hippocampus-dependent memory (Vitolo et al., 2002; Gong et al., 2004, 2006).

4.15.3.6.2 Arc/Arg3.1

The activity-regulated cytoskeletal protein/activity-regulated gene 3.1 is an immediate early gene (IEG) that is critical for LTP maintenance and for the consolidation of memories (Tzingounis and Nicoll, 2006). IEGs are rapidly and transiently activated at the transcriptional level after neuronal stimulation by neurotransmitters or growth factors. Some IEGs, for example, c-fos, encode transcription factors that modulate the expression of genes for proteins that affect synaptic strength, while others, such as Arc/Arg3.1, are effector IEGs whose products directly effect or maintain long-term changes in synaptic strength.

Arc/Arg3.1 was identified in 1995 by two independent groups searching for an IEG that might serve as an effector of long-term changes in synaptic strength (Link et al., 1995; Lyford et al., 1995). Both groups posited that the expression of such an effector should be (1) rapidly stimulated by neuronal activity, (2) blocked by NMDA receptor antagonists, and (3) localized to dendritic compartments. Using these criteria, both groups identified the same IEG, now known by the combined name of Arc/Arg3.1.

Arc/Arg3.1 is rapidly activated by patterned synaptic activity, including seizure activity, LTP, exploration of a novel environment, and memory-inducing behavioral paradigms (reviewed in Guzowski, 2002; Tzingounis and Nicoll, 2006). Newly synthesized Arc/Arg3.1 mRNA is rapidly transported to dendrites and accumulates in the particular synapses that were previously activated (Steward and Worley, 2001a,b). Because of these properties, the expression of Arc/Arg3.1 has been used to image behaviorally relevant activity in neuronal networks (Guzowski et al., 1999; Temple et al., 2003; Burke et al., 2005; Tagawa et al., 2005; Zou and Buck, 2006). For example, the sequential exposure of rats to two different environments results in the activation of distinct patterns of Arc/Arg3.1 expression in the hippocampus, particularly in the dentate gyrus, suggesting that Arc/Arg3.1 represents the activity of neuronal ensembles involved in the encoding of contextual information (Guzowski et al., 1999; reviewed in Guzowski et al., 2005).

Arc/Arg3.1 expression has also been used to examine the susceptibility of particular neuronal populations to Aβ-induced impairments in transgenic mouse models of AD (**Figure 18**). The induction of Arc/Arg3.1 after various stimuli is diminished in hAPP mice and hAPP/PS1 mice (Dickey et al., 2004; Chin et al., 2005; Palop et al., 2005). After exploration of a novel environment, Arc/Arg3.1 expression is reliably induced in the dentate gyrus of nontransgenic rodents (reviewed in Guzowski et al., 2005), but not in hAPP transgenic mice (Chin et al., 2005; Palop et al., 2005). Basal levels of Arc/Arg3.1 were also markedly reduced in the dentate gyrus. These alterations in Arc/Arg3.1 expression were accompanied by

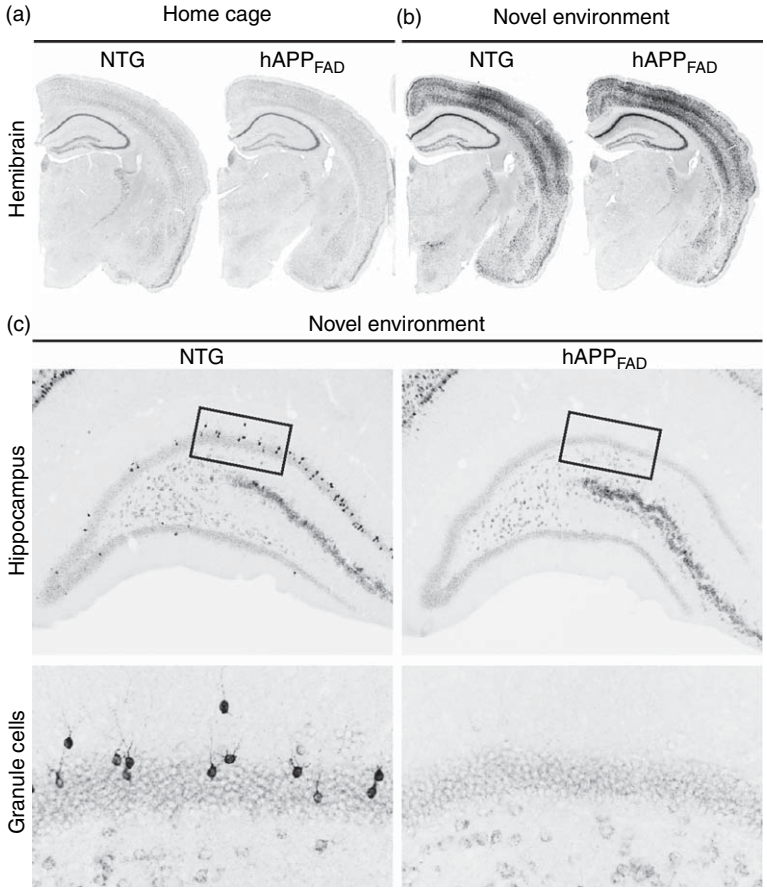

Figure 18 Imaging neuronal network activity using Arc/Arg3.1 expression. (a, b) After exploration of a novel environment, Arc/Arg3.1 expression is reliably induced in nontransgenic (NTG) mice in several brain regions including neocortex and hippocampus. hAPP transgenic mice show normal induction in the neocortex and CA1, but a striking lack of induction in the dentate granule cells of the hippocampus (a–c). (From Palop JJ, Chin J, Bien-Ly N, et al. [2005] Vulnerability of dentate granule cells to disruption of Arc/Arg3.1 expression in human amyloid precursor protein transgenic mice. *J. Neurosci.* 25: 9686–9693. Copyright 2005 by the Society for Neuroscience.)

decreased activities of NMDA receptors and ERK1/2, which regulate Arc/Arg3.1 expression. Other calcium-regulated proteins, for example, Fos and calbindin, are also depleted in the dentate gyrus of hAPP mice, and these depletions correlate well with deficits in learning and memory (Palop et al., 2003). Together, these studies indicate that the dentate gyrus is particularly vulnerable to Aβ-induced deficits in synaptic function, encoding of spatial information, and learning and memory.

Recent work by several groups has begun to shed light on Arc/Arg3.1 activities that may be particularly relevant to the synaptic and cognitive deficits observed in AD. Arc/Arg3.1 regulates AMPA receptor trafficking through interactions with endophilin and dynamin, proteins critically involved in the endocytotic recycling of synaptic vesicles (Chowdhury et al., 2006). Overexpression of Arc/Arg3.1 increases recycling rates and decreases surface expression of AMPA receptors, with corresponding decreases in AMPA receptor-mediated currents (Chowdhury et al., 2006; Rial Verde et al., 2006). In contrast, ablation of Arc/Arg3.1 increases surface expression of AMPA receptors and impairs long-term memory (Plath et al., 2006; Shepherd et al., 2006). Notably, the regulation of Arc/Arg3.1 and AMPA receptors is bidirectional, as AMPA receptor activity downregulates Arc/Arg3.1 expression (Rao et al., 2006).

The dynamic interactions between Arc/Arg3.1 and AMPA receptors may play a critical role in homeostatic scaling of synaptic strength (reviewed in Turrigiano and Nelson, 2004; Davis, 2006). Synaptic scaling is an important mode of plasticity by which neuronal networks maintain an optimal equilibrium of activity over time, and impairments of this plasticity may exacerbate synaptic and cognitive deficits in AD (Small, 2004; Palop et al., 2006).

4.15.4 Conclusions

We have surveyed here a multitude of molecules whose functions are perturbed in AD and animal models of the disease. The complexity of the pathways and interactions involved can be overwhelming, and it may be tempting to ask whether any molecule in the brain is left unaffected by the disease. Such a question has several potential answers.

Perhaps not coincidentally, this situation evokes similarities to LTP and the molecular basis of synaptic plasticity, where an equally long (and largely overlapping) list of molecules is involved (Roberson et al., 1996; Sanes and Lichtman, 1999; Malenka and Bear, 2004). This parallel highlights the fact that AD is a disease of memory not just in terms of neuropsychology but also at the molecular level, and that addressing AD may be one of the most critical applications of basic knowledge about the molecular basis of synaptic plasticity.

Second, the molecular changes in AD are not unlimited and are, in fact, bounded by multiple levels of specificity. Many are restricted to specific anatomic structures (e.g., hippocampus vs. neocortex) or even to specific subregions (e.g., CA1 vs. CA3). It is increasingly apparent that this specificity extends to the level of individual cells (e.g., principal cells vs. interneurons), an observation with important implications. Aβ-induced activation of a neurotransmitter receptor on an excitatory principal neuron might result in overexcitation of its circuit, whereas activating the same receptor on inhibitory interneurons could shut the network down, (Palop et al., 2006) (**Figure 19**). Even at the molecular level, AD-related changes can be quite specific; among the important neurotrophic factors, BDNF seems to play an important role in AD, whereas in NT3 does not appear to be involved (Hock et al., 2000).

Finally, there is at least one great boon to the long list of molecules involved in AD: a surfeit of potential targets for treating the disease. Indeed, treatments aimed at many of the molecules discussed here are now in trials for AD (Jacobsen et al., 2005; Roberson and Mucke, 2006). In terms of the AD-associated molecules in the section titled 'Memory impairment by AD-related molecules,' diverse approaches are under study, including (1) reducing the production or speeding the removal of potentially toxic proteins, for example, with β- or γ-secretase inhibitors or via immune-mediated clearance (Citron, 2004; Weiner and Frenkel, 2006); (2) preventing unwanted post-translational processing or aggregation of Aβ, tau, and apoE into particularly toxic forms (Harris et al., 2003; Khlistunova et al., 2006; McLaurin et al., 2006); (3) restoring normal functions lost by AD-related modifications, such as the microtubule-stabilizing effect of tau (Zhang et al., 2005); and (4) in the case of apoE, forcing apoE4 to adopt more apoE3-like structure and function (Mahley et al., 2006). The plasticity-related molecules highlighted in the section titled 'Memory-related molecules in AD' may provide good complementary targets. Here, the goal is to restore plasticity, boost related memory mechanisms, and protect neurons against aberrant network

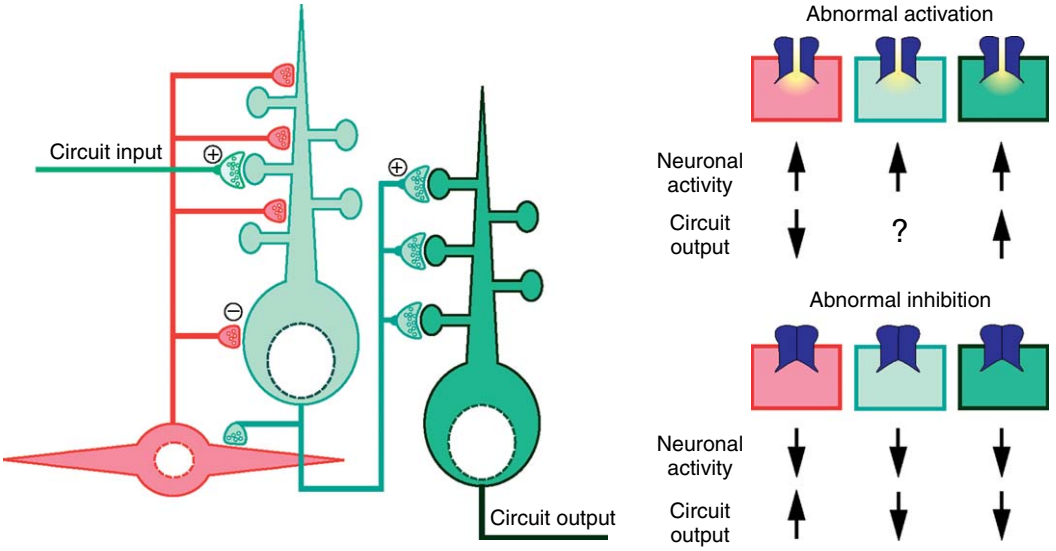

Figure 19 Consequences of the same molecular process on different cell types. The same molecular process can have a very different effect on circuit output depending on whether the cell it affects is an excitatory principal neuron (shades of green) or an inhibitory interneuron (red). Abnormal activation of an excitatory principal neuron will lead to overexcitation of the circuit, whereas abnormal activation of an inhibitory interneuron will shut the circuit down.

activities, even in the presence of pathogenic protein assemblies (Palop et al., 2006).

Thus, while complexity is certainly a feature of our current molecular understanding of AD, such issues pose exciting opportunities for neuroscientists working at the interface between AD and plasticity research, which is rapidly becoming one of the most active fronts in the battle against AD.

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4.16 Long-Term Potentiation: A Candidate Cellular Mechanism for Information Storage in the CNS

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In a very practical way, this chapter is a transition point for this volume. With this chapter, we transition from analyzing behavior to investigating cellular and molecular mechanisms for altering synaptic strength. We transition to attempting to understand mammalian memory by the reductionist approach of studying a simpler cellular phenomenon at the molecular level. Thus, this chapter will serve to place the many molecular details presented in other chapters into a broader context. In addition, wherever possible this chapter will be used as a launching point for further reading of other chapters in the volume by specifically citing other chapters at appropriate points along the way.

The particular circuits and neuronal connections that underlie most forms of mammalian learning and memory are mysterious at present, especially for hippocampus-dependent forms of learning. There is little understanding of the means by which complex memories are stored and recalled at the neural circuit level – this will be a very important avenue of future research. Thus in many ways the study of long-term potentiation (LTP) serves as a surrogate for studying hippocampus-dependent memory directly. LTP can only be viewed as a surrogate at present because very few studies are available directly implicating LTP (especially hippocampal LTP, which has been most widely studied) in defined memory behaviors. Even considering the vast number of published studies of LTP, we are left with a tentative causal link between LTP and memory *per se*.

Nevertheless, this chapter will focus on LTP. We will focus on it for three main reasons. First, it has been extensively studied and is the form of synaptic plasticity that is best understood at the molecular level. Second, it is a robust form of synaptic plasticity and worthy of investigation in its own right. Finally, it is a

specific candidate cellular mechanism for mediating certain forms of associative learning, spatial learning, and adaptive change in the central nervous system (CNS), in particular in the amygdala, hippocampus, and cerebral cortex, respectively.

4.16.1 Hebb's Postulate

Despite the various caveats concerning the specific role of LTP in hippocampus-dependent memory formation, there is a general hypothesis for memory storage that is available and broadly accepted. This hypothesis is the following:

> Memories are stored as alterations in the strength of synaptic connections between neurons in the CNS.

The significance of this general hypothesis should be emphasized – this is one of the few areas of contemporary cognitive research for which there is a unifying hypothesis.

This general hypothesis has a solid underlying rationale. Learning and memory manifest themselves as a change in an animal's behavior, and scientists capitalize on this to study these phenomena by observing and measuring changes in an animal's behavior in the wild or in experimental situations. However, all the behavior exhibited by an animal is a result of activity in the animal's nervous system. The nervous system comprises many kinds of cells, but the primary functional units of the nervous system are neurons. Because neurons are cells, all of an animal's behavioral repertoire is a manifestation of an underlying cellular phenomenon. By extension, changes in an animal's behavior such as occurs with learning must also be subserved by an underlying cellular change.

In general, the vast majority of the communication between neurons in the nervous system occurs at synapses. As synapses mediate the neuron–neuron communication that underlies an animal's behavior, changes in behavior are ultimately subserved by alterations in the nature, strength, or number of interneuronal synaptic contacts in the animal's nervous system. The capacity for alterations of synaptic connections between neurons is referred to as *synaptic plasticity*, and as described earlier, one of the great unifying theories to emerge from neuroscience research in the last century was that synaptic plasticity subserves learning and memory. LTP (of some sort at least) is the specific form of synaptic plasticity that is the leading candidate as a mechanism subserving behavior-modifying changes in synaptic strength that mediate higher-order learning and memory in mammals.

One of the pioneers in advancing the idea that changes in neuronal connectivity are a mechanism for memory was the Canadian psychologist Donald Hebb, who published his seminal formulation as what is now generally known as Hebb's postulate:

> When an axon of cell A . . . excites cell B and repeatedly or persistently takes part in firing it, some growth process or metabolic change takes place in one or both cells so that A's efficiency as one of the cells firing B is increased. (Hebb, 1949)

Note the important contrast between Hebb's postulate and its popular contemporary formulation – one (Hebb's) specifies cell firing, and the other (the modern formulation) specifies synaptic change. These two phenomena are clearly different, and the current, exclusively synaptic, variant is incomplete. Changes in synapses are certainly important in information storage in the CNS, but we need to consider that the postsynaptic receptors sit in a membrane whose biophysical properties are carefully controlled. Regulation of membrane sodium channels, chloride channels, and potassium channels also contributes significantly to the net effect in the cell that any neurotransmitter-operated process can achieve.

Thus, limitations arise from ignoring potential long-term regulation of membrane biophysical properties. We need to consider that local changes in dendritic membrane excitability may be involved in cellular information processing and also that global changes in cellular excitability that alter the likelihood of the cell firing an action potential may also be a mechanism for information storage. These topics are addressed elsewhere in this volume (*See* Chapters 4.39, 4.40). Another potential mechanism involved in memory that involves the entire cell and not specific synapses is adult neurogenesis, the growth and functional integration of new neurons in the adult CNS. Neurogenesis will be addressed in other chapters (*See* Chapter 4.42). Finally, one can think of inhibition (e.g., GABAergic (GABA: gamma-aminobutyric acid) modulation) as operating above the level of the single synapse because it can control the likelihood of the cell firing an action potential. The role of inhibition in plasticity, and the plasticity of inhibition *per se*, are discussed in another chapter (*See* Chapter 4.18).

The possibility that global or cellwide alterations might be involved in memory is also relevant when considering global genomic (transcriptional) and

epigenomic changes, which affect the nucleus and thereby potentially the entire cell as well. These mechanisms are discussed elsewhere (*See* Chapters 4.27, 4.28, 4.42). One solution to the problem of global changes due to altered transcription is specific trafficking of the products of global changes in transcription. Various aspects of this are dealt with in other chapters (*See* Chapters 4.25, 4.29, 4.30, 4.33).

The idea of the involvement of processes such as excitability in memory, processes that encompass the entire cell, has been criticized as too limiting because with global changes in excitability, one loses the computational power of selectively altering the response at a single synaptic input (i.e., synapse specificity). However, we don't know how the neuron or the CNS computes a memory output. The fundamental unit of information storage may not be the synapse but the neuron. Future experiments will be necessary to resolve this issue, but it is nevertheless worthwhile to keep in mind the possibility that regulation of excitability, and regulation of neuronal properties cellwide, as well as the more typically considered alterations in synaptic connections, may play roles in memory storage.

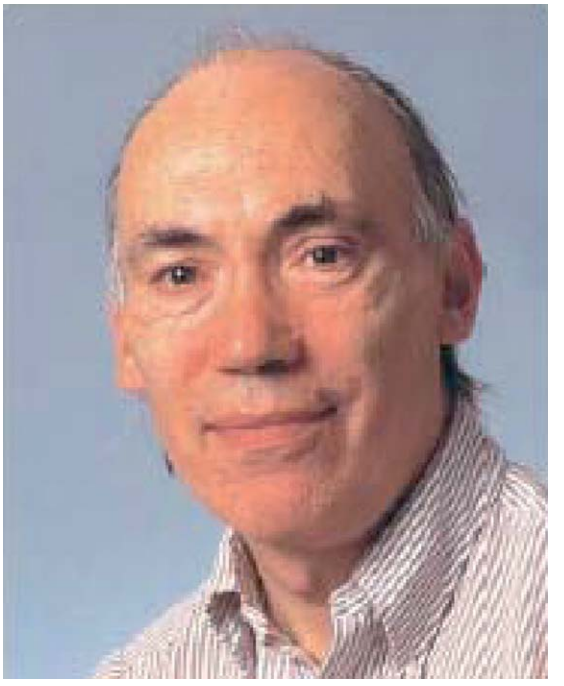


Figure 1 T. V. P. Bliss, *FRS*. Photo courtesy of Tim Bliss.

4.16.2 A Breakthrough Discovery – LTP in the Hippocampus

As a young postdoctoral researcher, Tim Bliss (**Figure 1**) set out to find a long-lasting form of synaptic plasticity in the hippocampus. By teaming up with Terje Lomo in Per Anderson's laboratory in Oslo, Bliss did just that. The seminal report by Bliss and Lomo in 1973, describing a phenomenon they termed long-term potentiation of synaptic transmission, set the stage for what is now over three decades of progress in understanding the basics of long-term synaptic alteration in the CNS.

In their experiments, Bliss and Lomo recorded synaptic responses in the dentate gyrus, stimulating the perforant path inputs from the entorhinal cortex (Bliss and Lomo, 1973). They used extracellular stimulating and recording electrodes implanted into the animal, and the basic experiment was begun by recording baseline synaptic transmission in this pathway. They discovered that a brief period of high-frequency (100-Hz 'tetanic') stimulation led to a robust increase in the strength of synaptic connections between the perforant path inputs from the entorhinal cortex onto the dentate granule neurons in the dentate gyrus (**Figure 2**). They also observed an increased likelihood of the cells firing action potentials in response to a constant synaptic input, a phenomenon they termed E-S (excitatory postsynaptic potential (EPSP)-to-spike) potentiation. These two phenomena together were termed LTP. LTP lasted many, many hours in this intact rabbit preparation. The appeal of LTP as an analog of memory was immediately apparent – it is a long-lasting change in neuronal function that is produced by a brief period of unique stimulus, exactly the sort of mechanism that had long been postulated to be involved in memory formation. This pioneering work of Bliss and Lomo set in motion a several-decades-long pursuit by numerous investigators geared toward understanding the attributes and mechanisms of LTP. Much of the progress in this area is described in the remaining chapters of this volume.

4.16.2.1 The Hippocampal Circuit and Measuring Synaptic Transmission in the Hippocampal Slice

Bliss and Lomo did their experiment using the intact rabbit, stimulating and recording in the anesthetized animal using implanted electrodes. In recent times, this preparation has been largely supplanted by the use of recordings from hippocampal slices maintained *in vitro* (**Figure 3**). Because most of the LTP

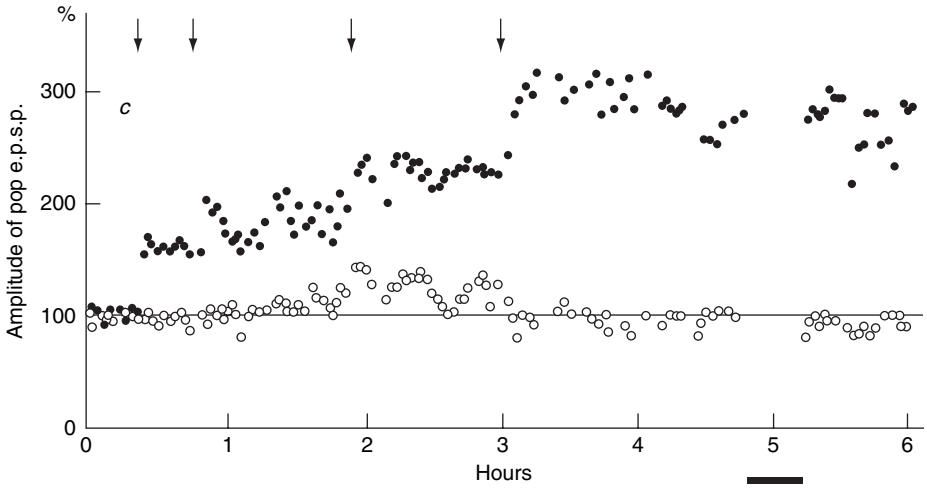


Figure 2 Bliss and Lomo's first published LTP experiment. As described in more detail in the text, in this pioneering work Tim Bliss and Terje Lomo demonstrated LTP of synaptic transmission. This specific experiment investigated synaptic transmission at perforant path inputs into the dentate gyrus (see **Figure 4**). Arrows indicate the delivery of high-frequency synaptic stimulation, resulting in LTP. Filled circles are responses from the tetanized pathways; open circles are a control pathway that did not receive tetanic stimulation. The bar, where no data points are available, indicates a period of time where Tim Bliss fell asleep. Data acquisition in this era involved the investigator directly measuring by hand synaptic responses from an oscilloscope screen. Moreover, it was not unusual for experiments to extend overnight due to the long amount of time involved in preparing the rabbit for the experiment, implanting the electrodes into the brain, and establishing a stable recording configuration. From Bliss TV and Lomo T (1973) Long-lasting potentiation of synaptic transmission in the dentate area of the anaesthetized rabbit following stimulation of the perforant path. *J. Physiol.* 232: 331–356. Used with permission.

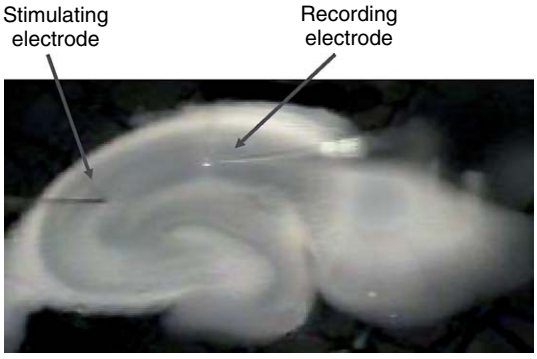


Figure 3 Electrodes in a living hippocampal slice. This photograph illustrates the appearance of a mouse hippocampal slice, maintained in a recording chamber. Responses in area CA1 are recorded using a saline-filled glass micropipette electrode (right) and a bipolar platinum stimulating electrode (left). See text and **Figure 4** for additional details.

experiments that will be described in the rest of the book come from this type of preparation, the next section will describe the hippocampal neuronal and synaptic circuit and give an overview of extracellular recording in a typical LTP experiment.

The main information processing circuit in the hippocampus is the relatively simple trisynaptic pathway, and much of this basic circuit is preserved in transverse slices across the long axis of the hippocampus (**Figure 4**). Various types of LTP can be induced at all three of these synaptic sites, and we will discuss later some mechanistic differences among the various types of LTP that can be induced. Most experiments on the basic attributes and mechanisms of LTP have been studies of the synaptic connections between axons from area CA3 pyramidal neurons that extend into area CA1. These are the synapses onto CA1 pyramidal neurons that are known as the Schaffer collateral inputs.

The main excitatory (i.e., glutamatergic) synaptic circuitry in the hippocampus, in overview, consists of three modules (see **Figure 4**) (van Groen and Wyss, 1990; Johnston and Amaral., 1998; Naber and Witter, 1998). Information enters the dentate gyrus of the hippocampal formation from cortical and subcortical structures via the perforant path inputs from the entorhinal cortex (**Figure 4**). These inputs make synaptic connections with the dentate granule cells of the dentate gyrus. After synapsing in the dentate gyrus, information is moved to area CA3 via the mossy fiber pathway, which consists of the axonal outputs of the dentate granule cells and their connections with pyramidal neurons in area CA3. After

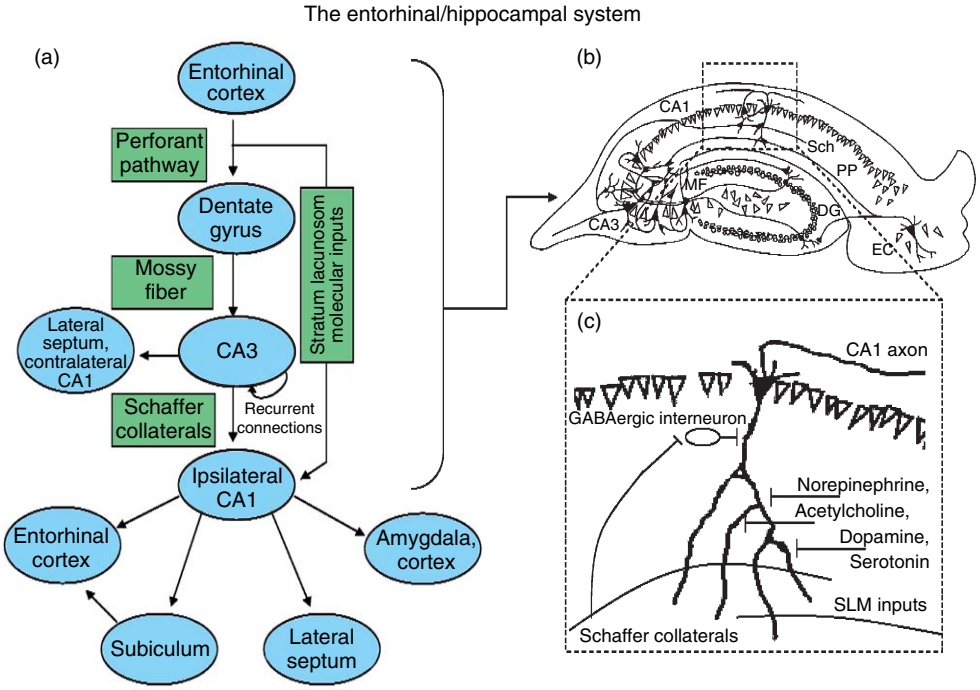


Figure 4 The entorhinal/hippocampal system. (a) This panel diagrams the principal inputs, outputs, and intrinsic connections. (b) In this panel, the central components of the circuit are delineated in a more anatomically correct fashion, illustrating the principal intrinsic connections of the dentate gyrus and hippocampus proper. (c) This is an expansion of area CA1 showing some of the synaptic inputs onto a single pyramidal neuron in area CA1. See text for additional details. Diagram by J. David Sweatt and Sarah E. Brown. Hippocampal diagram from Johnston D and Wu SM (1995) *Foundations of Cellular Neurophysiology*, p. 433. Cambridge, MA: MIT Press. Used with permission.

synapsing in area CA3, information is moved to area CA1 via the Schaffer collateral path, which consists largely of the axons of area CA3 pyramidal neurons along with other projections from area CA3 of the contralateral hippocampus as well. After synapsing in CA1, information exits the hippocampus via projections from CA1 pyramidal neurons and returns to subcortical and cortical structures.

The connections in this synaptic circuit are retained in a fairly impressive manner if one makes transverse slices of the hippocampus, as the inputs, 'trisynaptic circuit,' and outputs are laid out in a generally laminar fashion along the long axis of the hippocampal formation. This is a great advantage for *in vitro* electrophysiological experiments.

It is important to emphasize that the trisynaptic circuit outlined earlier is a great oversimplification, as there are a great many additional synaptic components of the hippocampus. For example, inhibitory GABAergic interneurons make synaptic connections with all of the principal excitatory neurons outlined earlier. These GABAergic inputs serve in both a feedforward and feedback fashion to control excitability. There are many recurrent and collateral excitatory connections between the excitatory pyramidal neurons as well, particularly in the area CA3 region. There is a direct projection from the entorhinal cortex to the distal regions of CA1 pyramidal neuron dendrites, a pathway known as the stratum lacunosum moleculare.

Finally, there are many modulatory projections into the hippocampus that make synaptic connections with the principal neurons (see **Figure 4**). These inputs are via long projection fibers from various anatomical nuclei in the brainstem region, and they are generally not directly excitatory or inhibitory, but rather serve to modulate synaptic connectivity in a fairly subtle way. There are four predominant extrinsic modulatory projections into the hippocampus. First, there are inputs of norepinephrine (NE)-containing fibers that project from the locus ceruleus. Second, there are dopamine (DA)-containing fibers that arise from the substantia nigra. There also are inputs using acetylcholine (ACh) from the medial septal nucleus and 5-hydroxytryptamine (5HT, serotonin) from the raphe nuclei.

4.16.2.2 LTP of Synaptic Responses

In a popular variation of the basic LTP experiment, extracellular field potential recordings in the dendritic regions of area CA1 are utilized to monitor synaptic transmission at Schaffer collateral synapses (see **Figure 5**). A bipolar stimulating electrode is placed in the *stratum radiatum* subfield of area CA1 and stimuli (typically constant current pulses ranging from 1 to 30 μA) are delivered. Stimuli delivered in this fashion stimulate the output axons of CA3 neurons that pass nearby, causing action potentials to propagate down these axons. Cellular responses to this stimulation are recorded using extracellular or intracellular electrophysiologic recording techniques.

The typical waveform in an extracellular recording consists of a fiber volley, which is an indication of the presynaptic action potential arriving at the recording site and the excitatory postsynaptic potential (EPSP) itself. The EPSP responses are a manifestation of synaptic activation (depolarization) in the CA1 pyramidal neurons. For measuring field (i.e., extracellularly recorded) EPSPs, the parameter typically measured is the initial slope of the EPSP waveform (see **Figure 5**). Absolute peak amplitude of EPSPs can also be measured, but the initial slope is the preferred index. This is because the initial slope is less subject to contamination from other sources of current flow in the slice. For example, currents are generated by feedforward inhibition due to GABAergic neuron activation. Also, if the cells fire action potentials, this also can contaminate later stages of the EPSP, even when one is recording from the dendritic region.

Extracellular field recordings measure responses from a population of neurons, so EPSPs recorded in this fashion are referred to as population EPSPs (pEPSPs). Note that pEPSPs are downward deflecting for stratum radiatum recordings (see **Figure 5**). If one is recording from the cell body layer (stratum pyramidale), the EPSP is an upward deflection, and if

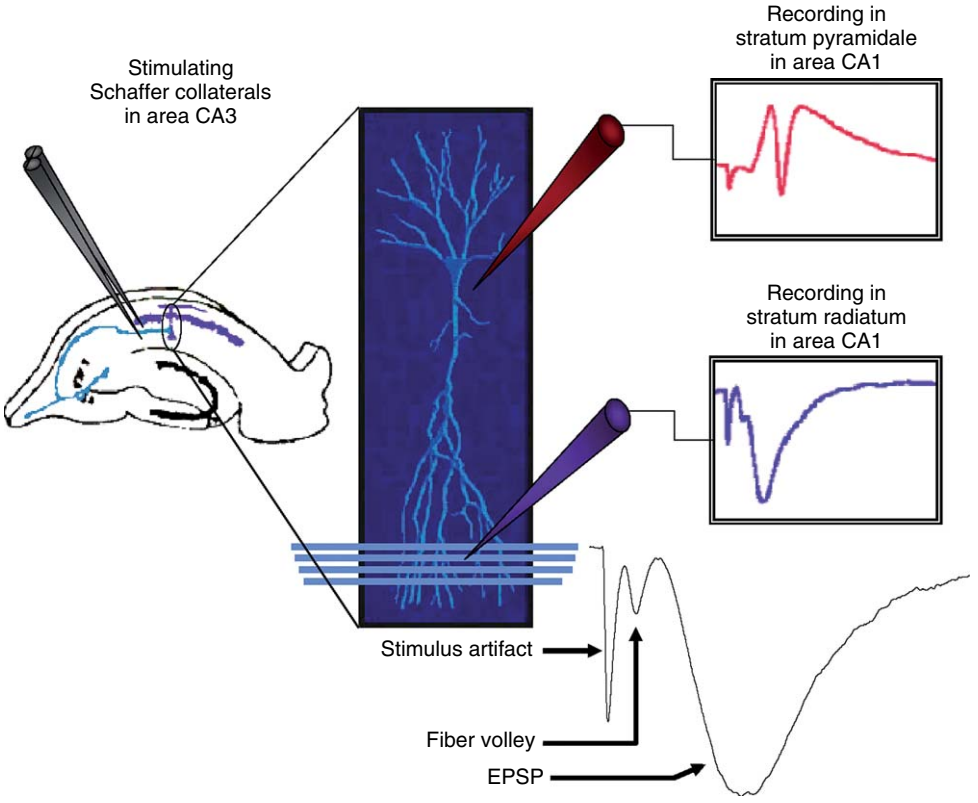


Figure 5 Recording configuration and typical physiologic responses in a hippocampal slice recording experiment. Electrode placements and responses from stratum pyramidale (cell body layer) and stratum radiatum (dendritic regions) are shown. In addition, the typical waveform of a population excitatory postsynaptic potential (EPSP) is illustrated, showing the stimulus artifact, fiber volley, and population EPSP. Figure and data by Joel Selcher.

the cells fire action potentials, the EPSP has superimposed on it a downward deflecting spike, the population spike. As mentioned earlier, for both stratum radiatum and stratum pyramidale recordings the EPSP slope measurements are taken as early as possible after the fiber volley to eliminate contamination by population spikes.

As a prelude to starting an LTP experiment, input-output (I/O) functions for stimulus intensity versus EPSP magnitude are recorded in response to increasing intensities of stimulation (see **Figure 6**). For the remainder of the experiment, the test stimulus intensity is set to elicit an EPSP that is approximately 35–50% of the maximum response

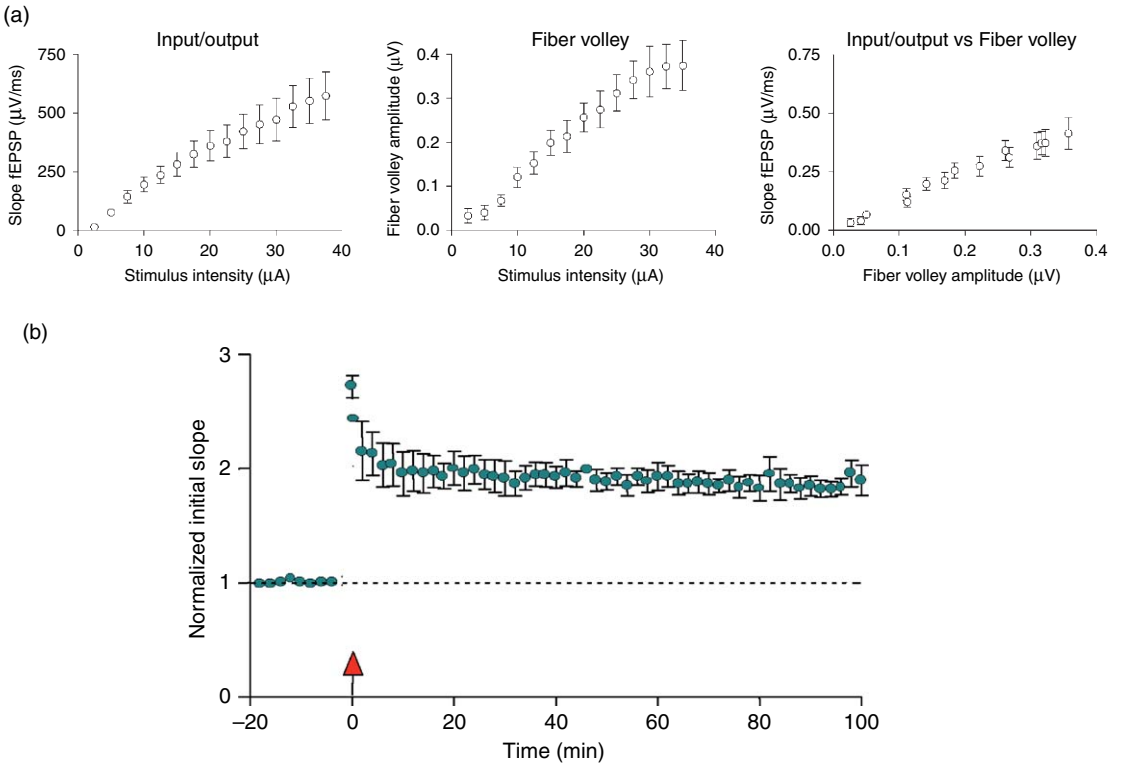


Figure 6 An input-output curve and typical LTP experiment. (a) The relationship of EPSP magnitude (field EPSP, EPSP) versus stimulus intensity (in microamperes), and the same data converted to an input-output relationship for EPSP versus fiber volley magnitude to allow an evaluation of postsynaptic response versus presynaptic response in the same hippocampal slice. (b) A typical high-frequency stimulation-induced potentiation of synaptic transmission in area CA1 of a rat hippocampal slice *in vitro*. The arrow indicates the delivery of 100-Hz (100 pulses/second) synaptic stimulation. Data courtesy of Ed Weeber and Coleen Atkins. In most pharmacologic experiments using physiologic recordings in hippocampal slice preparations, effects of drug application on baseline synaptic transmission can be evaluated by simply monitoring EPSPs before and after drug application, using a constant stimulus intensity. A more elaborate alternative is to produce input-output curves for EPSP initial slope (or magnitude) versus stimulus intensity for the presynaptic stimulus. These types of within-slice experiments are very straightforward, but in some experimental comparisons, this type of within-preparation design is not possible. For example, if one is comparing a wild type with a knockout animal, there of necessity must be a comparison across preparations. How does one evaluate if there is a difference in basal synaptic transmission in this situation? The principal confound is because although one has control over magnitude of the stimulus one delivers to the presynaptic fibers, differences in electrode placement, slice thickness, etc., from preparation to preparation cause variability in the magnitude of the synaptic response elicited by a constant stimulus amplitude. One commonly used approach to compare from one preparation (or animal strain) to the next is to quantitate the EPSP relative to the amplitude of the fiber volley in that same hippocampal slice. The rationale is that the fiber volley, which represents the action potentials firing in the presynaptic fibers, is a presynaptic physiologic response from within the same slice and that one can at least normalize the EPSP to a within-slice parameter. The underlying assumption is that the magnitude of the fiber volley is representative of the number of axons firing an action potential. Although not a perfect control, evaluating input-output relationships for fiber volley magnitude versus EPSP is a great improvement when making comparisons between different types of animals. If differences are observed, an increase in the fiber volley amplitude – EPSP slope relationship suggests an augmentation of synaptic transmission.

recorded during the I/O measurements. Baseline synaptic transmission at this constant test stimulus intensity is usually monitored for a period of 15–20 min to ensure a stable response.

Once the health of the hippocampal slice is confirmed as indicated by a stable baseline synaptic response, LTP can be induced using any one of a wide variety of different LTP induction protocols. Many popular variations include a single or repeated period of 1-s, 100-Hz stimulation (with delivery of the 100-Hz trains separated by 20 s or more) where stimulus intensity is at a level necessary for approximately half-maximal stimulation (see **Figure 6**). A variation is a strong induction protocol where LTP is induced with three pairs of 100-Hz, 1-s stimuli, where stimulus intensity is near that necessary for a maximal EPSP. This latter protocol gives robust LTP that lasts for essentially as long as one can keep the hippocampal slice alive. A final major variation is high-frequency stimulation patterned after the endogenous hippocampal theta rhythm; this will be described in more detail in a later section of this chapter.

4.16.2.3 Short-Term Plasticity: PTP and PPF

Two types of short-term plasticity are exhibited at hippocampal Schaffer collateral synapses and elsewhere that are activity dependent, just as is LTP. These are paired-pulse facilitation (PPF) and posttetanic potentiation (PTP). PPF is a form of short-term synaptic plasticity that is commonly held to be due to residual calcium augmenting neurotransmitter release presynaptically. When two single-stimulus pulses are applied with interpulse intervals ranging from 20 to 300 ms, the second EPSP produced is larger than the first (see **Figure 7**). This effect is referred to as PPF. The role of this type of synaptic plasticity in the behaving animal is unknown at this time; however, it clearly is a robust form of temporal integration of synaptic transmission and could be used in information processing behaviorally. The second form of short-term plasticity, PTP, is a large enhancement of synaptic efficacy observed after brief periods of high-frequency synaptic activity. For example, in experiments where LTP is induced with one or two 1-s, 100-Hz tetani, a large and transient increase in synaptic efficacy is produced immediately after high-frequency tetanus (see **Figure 7**). This is PTP. The mechanisms for PTP are unknown, but both PTP and PPF are *N*-methyl-D-aspartate (NMDA) receptor-independent phenomena. Both PPF and PTP, and some candidate molecular mechanisms that might underlie them, are discussed in another chapter (*See* Chapter 4.36) of this volume.

4.16.3 NMDA Receptor Dependence of LTP

In 1983, Graham Collingridge made the breakthrough discovery that induction of these tetanus-induced forms of LTP is blocked by a blockade of a specific subtype of glutamate receptor, the NMDA receptor (Collingridge et al., 1983). Collingridge's fascinating discovery was that the glutamate analog

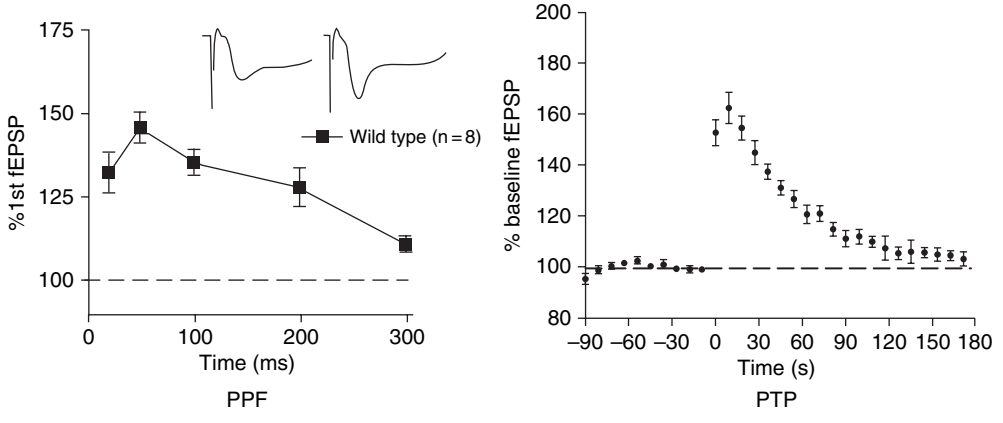


Figure 7 Paired-pulse facilitation (left) and posttetanic potentiation (right). See text for details. Data for both panels courtesy of Michal Levy. fEPSP, field EPSP.

aminophosphonovaleric acid (APV), an agent that selectively blocks the NMDA subtype of glutamate receptor, could block LTP induction while leaving baseline synaptic transmission entirely intact (Figure 8).

This was the first experiment to give a specific molecular insight into the mechanisms of LTP induction. The properties of the NMDA receptor that allow it to function in this unique role of triggering LTP are important, and we will return to a detailed analysis of regulation of the NMDA receptor later in this volume (*See* Chapter 4.20). For our purposes right now, pharmacologic blockers of NMDA receptor function have allowed the definition of different types of LTP that can be selectively induced with various physiologic stimulation protocols. For example, subsequent work has shown that an NMDA receptor-independent type of LTP can be induced in area CA1 and elsewhere in the hippocampus (mossy fibers to be precise), as well as in other parts of the CNS. We will return to a brief description of these types of LTP at the end of this chapter, but for now, we will continue to focus on NMDA receptor-dependent types of LTP.

Early studies of LTP used mostly high-frequency (100-Hz) stimulation in repeated 1-s-long trains as the LTP-inducing stimulation protocol. Although these protocols are still widely used to good effect, it is clear that such prolonged periods of high-frequency firing do not occur physiologically in the behaving animal. However, LTP can also be induced by stimulation protocols that are much more like naturally occurring neuronal firing patterns in the hippocampus. To date the forms of LTP induced by these types of stimulation have all been found to be NMDA receptor dependent in area CA1. Two popular variations of these protocols are based on the natural occurrence of an increased rate of hippocampal pyramidal neuron firing while a rat or mouse is exploring and learning about a new environment. Under these circumstances hippocampal pyramidal neurons fire bursts of action potentials at about 5 bursts/s (i.e., 5 Hz). This is the hippocampal 'theta' rhythm that has been described in the literature. One variation of LTP-inducing stimulation that mimics this pattern of firing is referred to as theta-frequency stimulation (TFS), which consists of 30 s of single stimuli delivered at 5 Hz. Another variation, theta-burst stimulation (TBS) consists of three trains of stimuli delivered at 20-s intervals, each train composed of ten stimulus bursts delivered at 5 Hz, with each burst consisting of four pulses at 100 Hz (see Figure 9). It is worth noting that these patterns of stimulation, which are based on naturally occurring firing patterns *in vivo*, lead to LTP in hippocampal slice preparations as well.

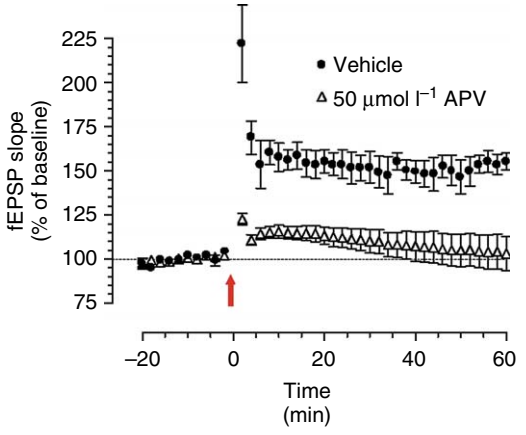


Figure 8 APV block of LTP. These data are from recordings *in vitro* from mouse hippocampal slices, demonstrating the NMDA receptor dependence of tetanus-induced LTP. Identical high-frequency synaptic stimulation was delivered in control (filled circles) and NMDA receptor antagonist (APV, open triangles) treated slices. fEPSP, field EPSP. Data courtesy of Joel Selcher.

4.16.3.1 Pairing LTP

Of course, much more sophisticated electrophysiologic techniques than extracellular recording can be used to monitor synaptic function. Intracellular recording and patch clamp techniques that measure electrophysiologic responses in single neurons have also been used widely in studies of LTP. These types of recording techniques perturb the cell that is being recorded from and lead to 'run-down' of the postsynaptic response in the cell impaled by the electrode. This limits the duration of the LTP experiment to however long the cell stays alive – somewhere in the range of 60 to 90 min for an accomplished physiologist. Regardless, in these recording configurations one can induce synaptic potentiation using tetanic stimulation or theta-pattern stimulation and measure LTP as an increase in postsynaptic currents through glutamate-gated ion channels or as an increase in postsynaptic depolarization when monitoring the membrane potential.

Control of the postsynaptic neuron's membrane potential with cellular recording techniques also allows for some sophisticated variations of the LTP induction paradigm. In one particularly important

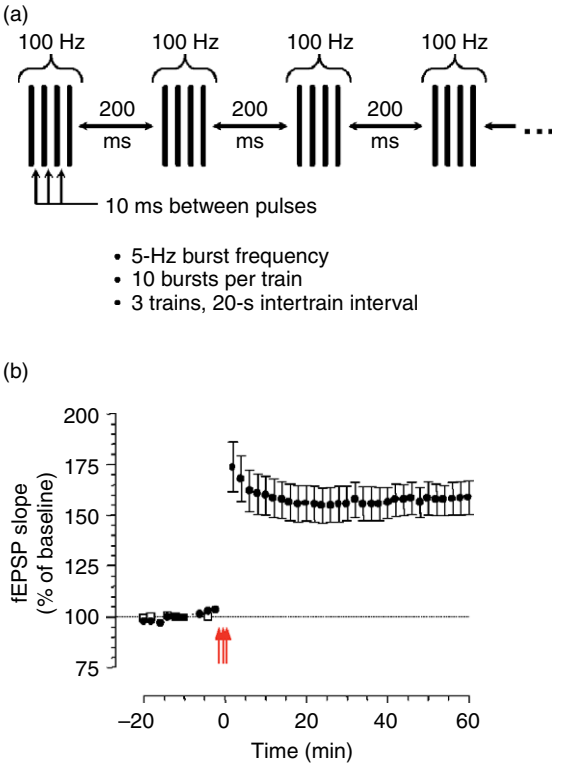


Figure 9 LTP triggered by theta-burst stimulation (TBS) in the mouse hippocampus. (a) Schematic depicting TBS. This LTP induction paradigm consists of three trains of 10 high-frequency bursts delivered at 5 Hz. (b) LTP induced with TBS (TBS-LTP) in hippocampal area CA1. The three red arrows represent the three TBS trains. fEPSP, field EPSP.

series of experiments, it was discovered that LTP can be induced by pairing repeated single presynaptic stimuli with postsynaptic membrane depolarization, so-called pairing LTP (Wigstrom and Gustafsson, 1986) (**Figure 10**).

The basis for pairing LTP comes from one of the fundamental properties of the NMDA receptor (**Figure 11**). The NMDA receptor is both a glutamate-gated channel and a voltage-dependent one. The simultaneous presence of glutamate and a depolarized membrane is necessary and sufficient (when the coagonist glycine is present) to gate the channel. Pairing synaptic stimulation with membrane depolarization provided via the recording electrode (plus the low levels of glycine always normally present) opens the NMDA receptor channel and leads to the induction of LTP.

How does the NMDA receptor trigger LTP? The NMDA receptor is a calcium channel, and its gating leads to elevated intracellular calcium in the postsynaptic neuron. This calcium influx triggers LTP, and indeed many other chapters in this volume deal with the various processes this calcium influx triggers. It is important to remember that it is not necessarily the case that every calcium molecule involved in LTP induction actually comes through the NMDA receptor. Calcium influx through membrane calcium channels and calcium released from intracellular stores may also be involved.

The gating of the NMDA receptor/channel involves a voltage-dependent Mg^{++} block of the channel pore. Depolarization of the membrane in which the NMDA receptor resides is necessary to drive the divalent Mg^{++} cation out of the pore, which then allows calcium ions to flow through. Thus, the simultaneous occurrence of both glutamate in the synapse and a depolarized postsynaptic membrane are necessary to open the channel and allow LTP-triggering calcium into the postsynaptic cell.

These properties, glutamate dependence *and* voltage dependence, of the NMDA receptor allow it to function as a coincidence detector. This is a critical aspect of NMDA receptor regulation, and this allows for a unique contribution of the NMDA receptor to information processing at the molecular level. Using the NMDA receptor, the neuron can trigger a unique event, calcium influx, specifically when a particular synapse is both active presynaptically (glutamate is present in the synapse) and postsynaptically (when the membrane is depolarized).

This confers a computational property of associativity on the synapse. This attribute is nicely illustrated by 'pairing' LTP, as described earlier, where low-frequency synaptic activity paired with postsynaptic depolarization can lead to LTP. The associative property of the NMDA receptor allows for many other types of sophisticated information processing as well, however. For example, activation of a weak input to a neuron can induce potentiation, provided a strong input to the same neuron is activated at the same time (Barrionuevo and Brown, 1983). These particular features of LTP induction have stimulated a great deal of interest, as they are reminiscent of classical conditioning, with depolarization and synaptic input roughly corresponding to unconditioned and conditioned stimuli, respectively.

The associative nature of NMDA receptor activation allows for synapse specificity of LTP induction as well, which has been shown to occur experimentally. If one pairs postsynaptic depolarization with activity at one set of synaptic inputs to a cell, while leaving a second input silent or active only during

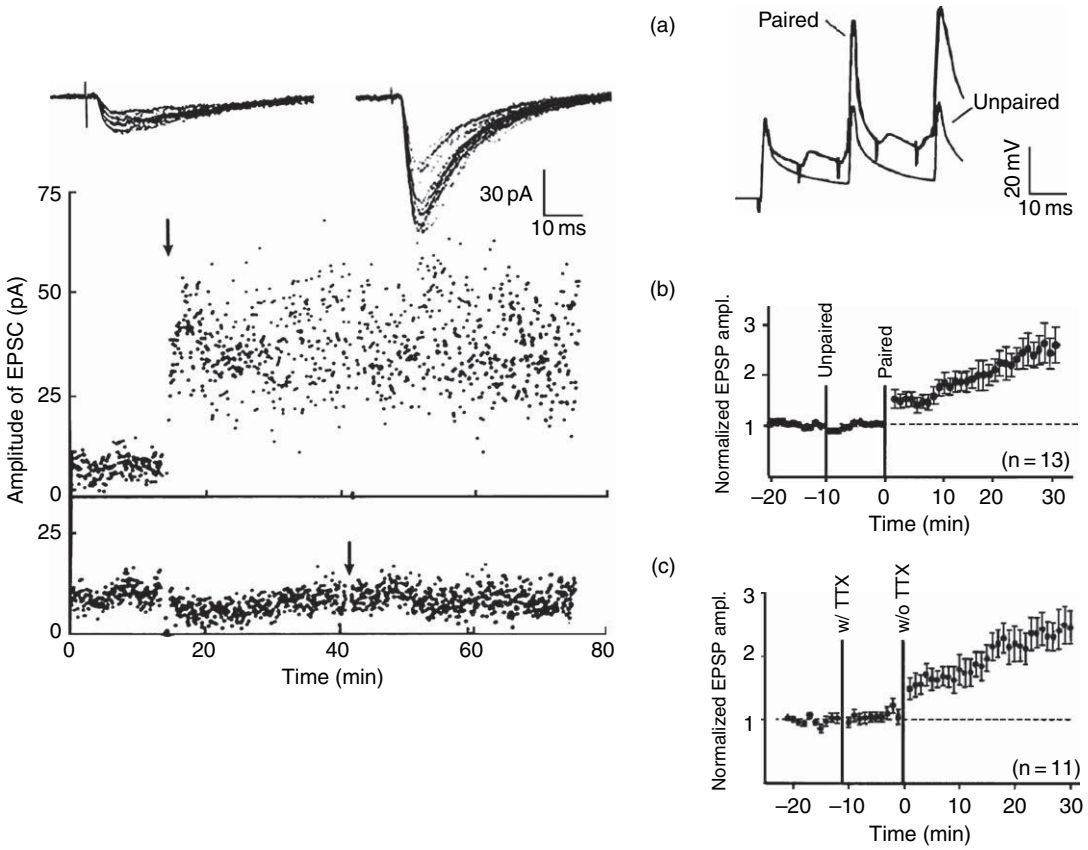


Figure 10 Pairing LTP. (Left panel) LTP of synaptic transmission induced by pairing postsynaptic depolarization with synaptic activity. The upper panels illustrate postsynaptic currents (EPSCs) recorded directly from the postsynaptic neuron using voltage-clamp techniques. The data shown are a pairing LTP experiment (upper) and control, nonpaired pathway (lower). In the pairing LTP experiment, hippocampal CA1 pyramidal neurons were depolarized from −70 mV to 0 mV while the paired pathway was stimulated at 2 Hz 40 times. Control received no stimulation during depolarization. From Malinow R and Tsien RW (1990) Presynaptic enhancement shown by whole-cell recordings of long-term potentiation in hippocampal slices. *Nature* 346: 177–180. (Right panels) Pairing small EPSPs with back-propagating dendritic action potentials induces LTP. Inset (a): Subthreshold EPSPs paired with back-propagating action potentials increase dendritic action potential amplitude. Voltage-clamp recording at approximately 240 μm from soma, that is, in the dendritic tree of the neuron (see **Figure 12**). Action potentials were evoked by 2-ms current injections through a somatic whole-cell electrode at 20-ms intervals. Alone, action potential amplitude was small (unpaired). Paired with EPSPs (5 stimuli at 100 Hz), the action potential amplitude increased greatly (paired). Inset (b): Grouped data showing normalized EPSP amplitude after unpaired and paired stimulation. The pairing protocol shown in (a) was repeated 5 times at 5 Hz at 15-s intervals for a total of 2 times. Inset (c): A similar pairing protocol was given with and without applying the sodium channel blocker tetrodotoxin (TTX, to block action potential propagation) to the proximal apical dendrites to prevent back-propagating action potentials from reaching the synaptic input sites. LTP was induced only when action potentials fully back-propagated into the dendrites. Reproduced with permission from Magee JC and Johnston D (1997) A synaptically controlled, associative signal for Hebbian plasticity in hippocampal neurons. *Science* 275: 209–213.

periods at which the postsynaptic membrane is near the resting potential, then selective potentiation of the paired input pathway occurs.

Similarly, in field stimulation experiments LTP is restricted to tetanized pathways – even inputs convergent on the same dendritic region of the postsynaptic neuron are not potentiated if they receive only baseline synaptic transmission in the absence of synaptic activity sufficient to adequately depolarize the postsynaptic neuron (Anderson et al., 1977). This last point illustrates the basis for LTP cooperativity. LTP induction in extracellular stimulation experiments requires cooperative interaction of afferent fibers, which in essence means there is an intensity threshold for triggering LTP induction. Sufficient total synaptic activation by the input fibers must be

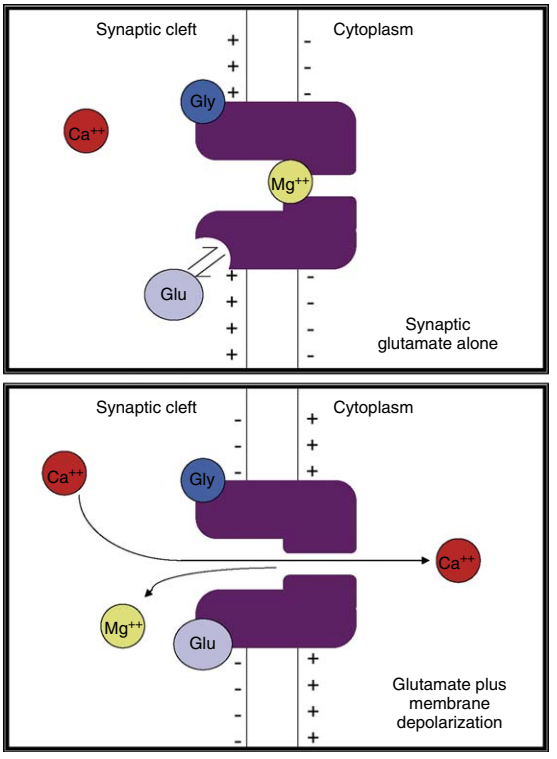


Figure 11 Coincidence detection by the NMDA receptor. The simultaneous presence of glutamate and membrane depolarization is necessary for relieving Mg^{++} blockade and allowing calcium influx. Figure by J. David Sweatt and Sarah E. Brown.

achieved such that the postsynaptic membrane is adequately depolarized to allow opening of the NMDA receptor (McNaughton et al., 1978).

4.16.3.2 Dendritic Action Potentials

In the context of the functioning hippocampal neuron *in vivo*, the associative nature of NMDA receptor activation means that a given neuron must reach a critical level of depolarization for LTP to occur at any of its synapses. Specifically, in the physiologic context the hippocampal pyramidal neuron generally must reach the threshold for firing an action potential, although there are some interesting alternatives to this that we will discuss later in this chapter and elsewhere in this volume (*See* Chapter 4.39). Although action potentials are, of course, triggered in the active zone of the cell body, hippocampal pyramidal neurons along with many other types of CNS neurons can actively propagate action potentials into the dendritic regions: the so-called back-propagating action potential (Magee and Johnston, 1997) (*See* **Figure 12** and Chapters 4.35 and 4.39). These dendritic action potentials are just like action potentials propagated down axons in that they are carried predominantly by voltage-dependent ion channels such as sodium channels. The penetration of the back-propagating action potential into the dendritic region provides a wave of membrane depolarization that allows for the opening of the voltage-dependent NMDA receptor/ion channels. *Active* propagation of the action potential is necessary because the biophysical properties of the dendritic membrane dampen the passive propagation of membrane depolarization, thus an active process such as action potential propagation is required. As a generalization, in many instances in the intact cell, back-propagating action potentials are what allow sufficient depolarization to reach hippocampal pyramidal neuron synapses to open NMDA receptors. In an ironic twist, this has brought us back to a more literal reading of Hebb's postulate, where, as we discussed at the beginning of this chapter, Hebb actually specified *firing* of the postsynaptic neuron as being necessary for the strengthening of its connections.

In fact, the timing of the arrival of a dendritic action potential with synaptic glutamate input appears to play an important part in precise, timing-dependent triggering of synaptic plasticity in the hippocampus (Magee and Johnston, 1997) (*See* **Figures 10** and **13**). It has been observed that a critical timing window is involved vis-à-vis back-propagating action potentials: glutamate arrival in the synaptic cleft must slightly precede the back-propagating action potential for the NMDA receptor to be effectively opened. This timing dependence arises in part due to the time required for glutamate to bind to and open the NMDA receptor. The duration of an action potential is, of course, quite short, so in essence the glutamate must be there first and already be bound to the receptor for full activation to occur. (Additional factors are also involved; *See* Bi and Poo, 1998; Kamondi et al., 1998; Linden, 1999; and Johnston et al., 2000 for a discussion.)

This order-of-paring specificity allows for a precision of information processing – not only must the membrane be depolarized but also as a practical matter, the cell must fire an action potential. Moreover, the timing of the back-propagating action potential arriving at a synapse must be appropriate. It is easy to imagine how the nervous system could capitalize on these properties to allow for forming precise timing-dependent associations between two events.

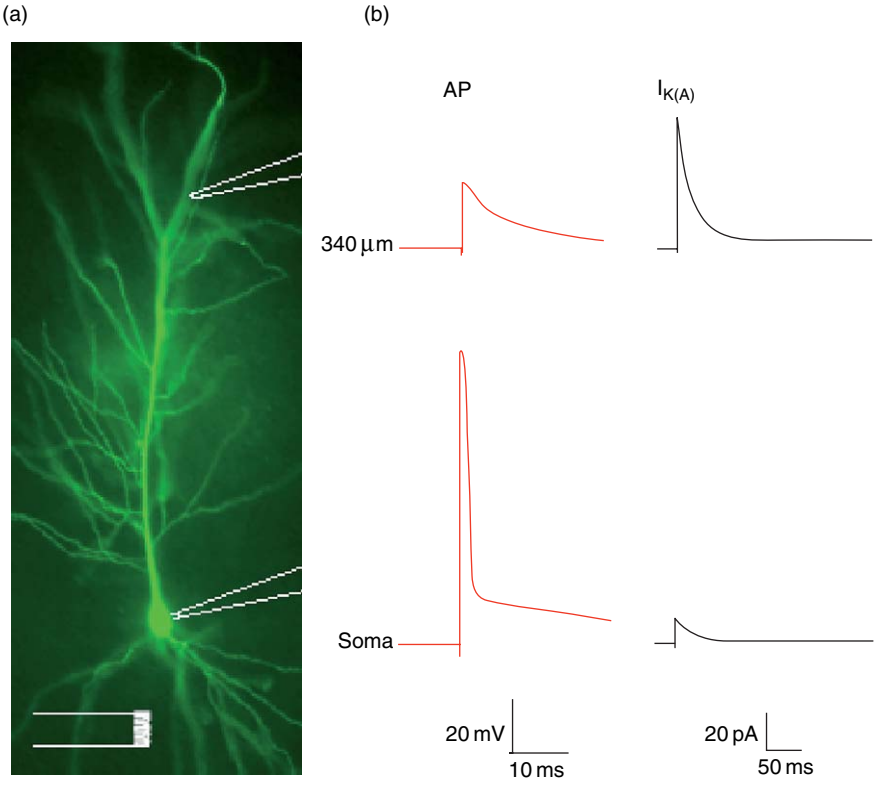


Figure 12 Back-propagating action potentials in dendrites of CA1 pyramidal neurons. (a) Indicates the recording setup, with a bipolar stimulating electrode used to trigger action potentials at the cell body region (lower left), a recording electrode in the cell soma to monitor firing of an action potential, and a recording electrode in the dendrites (upper right) to monitor propagation of the action potential into the distal dendritic region. (b) Traces in (b) indicate the data recorded from the soma (lower) and dendritic (upper) electrodes. The left-hand traces from (b) (labeled AP) indicate the membrane depolarization achieved at the soma and dendrite when an action potential is triggered and propagates into the dendritic region. Note that the dendritic action potential is of lower magnitude and broader due to the effects of dendritic membrane biophysical properties as the action potential propagates down the dendrite. The right half of (b) shows current flow through 'A-type' voltage-dependent potassium currents observed in the soma and dendrites. The density of A-type potassium currents increases dramatically as one progresses outward from the soma into the dendritic regions, as illustrated by the much larger potassium current observed in the distal dendritic electrode. These voltage-dependent potassium channels are key regulators of the likelihood of back-propagating action potentials reaching various parts of the dendritic tree. Data and figure reproduced from Yuan LL, Adams JP, Swank M, Sweatt JD, and Johnston D (2002) Protein kinase modulation of dendritic K+ channels in hippocampus involves a mitogen-activated protein kinase pathway. *J. Neurosci.* 22: 4860–4868, with permission.

One twist to the order-of-pairing specificity is that if the order is reversed and the action potential arrives before the EPSP, then synaptic depression is produced. The mechanisms for this attribute are under investigation at present. One hypothesis is that the backward pairing by various potential mechanisms leads to a lower level of calcium influx, which produces synaptic depression (*See* following discussion and Chapter 4.17).

In other chapters we will discuss in more detail the molecular mechanisms by which local effects regulating membrane depolarization within specific dendritic branches or dendritic subregions may be achieved. Moreover, we will discuss the signal transduction mechanisms by which modulatory neurotransmitter systems can regulate the likelihood of action potential back propagation through controlling dendritic potassium channels, and we will discuss how this might allowing for sophisticated information processing through an interplay of action potential propagation, glutamate release, and neuromodulation (*See* Chapter 4.39). All of these things become possible because the dendritic membrane in which the NMDA receptors reside is not passive, but contains voltage-dependent ion channels. Thus controlling the postsynaptic membrane biophysical properties can be a critical determinant for regulating the triggering of synaptic change.

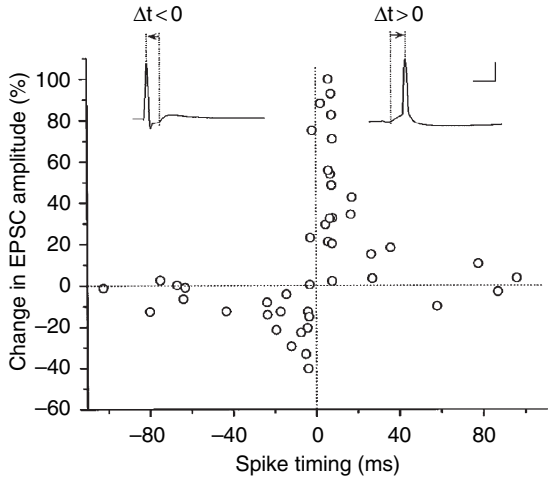


Figure 13 The timing of back-propagating action potentials with synaptic activity determines whether synaptic strength is altered, and in which direction. Precise timing of the arrival of a back-propagating action potential (a 'spike') with synaptic glutamate determines the effect of paired depolarization and synaptic activity. A narrow window when the arrival of the synaptic EPSP immediately precedes or follows the arrival of the back-propagating action potential determines whether synaptic strength is increased, is decreased, or remains the same. See text for additional discussion. EPSC, excitatory postsynaptic current. Figure adapted from Bi GQ and Poo MM (1998) Synaptic modifications in cultured hippocampal neurons: Dependence on spike timing, synaptic strength, and postsynaptic cell type. *J. Neurosci.* 18: 10464–10472, with permission.

4.16.4 NMDA Receptor-Independent LTP

Although the vast majority of studies of LTP and its molecular mechanisms have investigated NMDA receptor-dependent processes, as mentioned earlier there also are several types of NMDA receptor-independent LTP. The next section will briefly describe a few different types of NMDA receptor-independent LTP as background material and to highlight them as important areas of investigation.

4.16.4.1 200-Hz LTP

NMDA receptor-independent LTP can be induced at the Schaffer collateral synapses in area CA1, the same synapses discussed thus far. This allows for somewhat of a comparison and contrast of two different types of LTP at the same synapse. A protocol that elicits NMDA receptor-independent LTP in area CA1 is the use of four 0.5-s, 200-Hz stimuli separated by 5 s (Grover and Teyler, 1990). LTP induced with this stimulation protocol is insensitive to NMDA receptor-selective antagonists such as APV (*See* **Figure 14**). It is interesting that simply doubling the rate of tetanic stimulation from 100 Hz to 200 Hz appears to shift activity-dependent mechanisms for synaptic potentiation into NMDA receptor independence. At the simplest level of thinking, this indicates that there is some unique type of temporal integration going on at the higher frequency stimulation that allows for superseding the necessity for NMDA receptor activation. What might the 200-Hz stimulation be uniquely stimulating? One appealing hypothesis arises from the observation that 200-Hz LTP is blocked by blockers of voltage-sensitive calcium channels. Thus, the current working model is that 200-Hz stimulation elicits sufficiently large and sufficiently prolonged membrane depolarization, resulting in the opening of voltage-dependent calcium channels, to trigger elevation of postsynaptic calcium sufficient to trigger LTP synaptic potentiation. One observation consistent with this hypothesis is that injection of postsynaptic calcium chelators blocks 200-Hz stimulation-induced LTP.

4.16.4.2 TEA LTP

NMDA receptor-independent LTP in area CA1 can also be induced using tetraethylammonium (TEA^+) ion application, a form of LTP that is referred to as LTP_k (Aniksztejn and Ben-Ari, 1991; Powell et al., 1994). TEA^+ is a nonspecific potassium channel blocker, the application of which greatly increases membrane excitability. Like 200-Hz LTP, LTP_k is insensitive to NMDA receptor antagonists and is blocked by a blockade of voltage-sensitive calcium channels. Moreover, LTP_k is also blocked by postsynaptic calcium chelator injection as well. The induction of LTP_k is dependent on synaptic activity, as AMPA receptor antagonists block its induction. Similar to 200-Hz LTP, the current model for TEA LTP is that synaptic depolarization via alpha-amino-3-hydroxy-5-methyl-4 isoxazole propionic acid (AMPA) receptor activation, augmented by the hyperexcitable membrane due to K^+ channel blockade, leads to a relatively large and prolonged membrane depolarization. This leads to the triggering of LTP through postsynaptic calcium influx.

4.16.4.3 Mossy Fiber LTP in Area CA3

The predominant model system for studying NMDA receptor-independent LTP is not the Schaffer collateral synapses, but rather the mossy fiber inputs into area CA3 pyramidal neurons. Considerable excitement

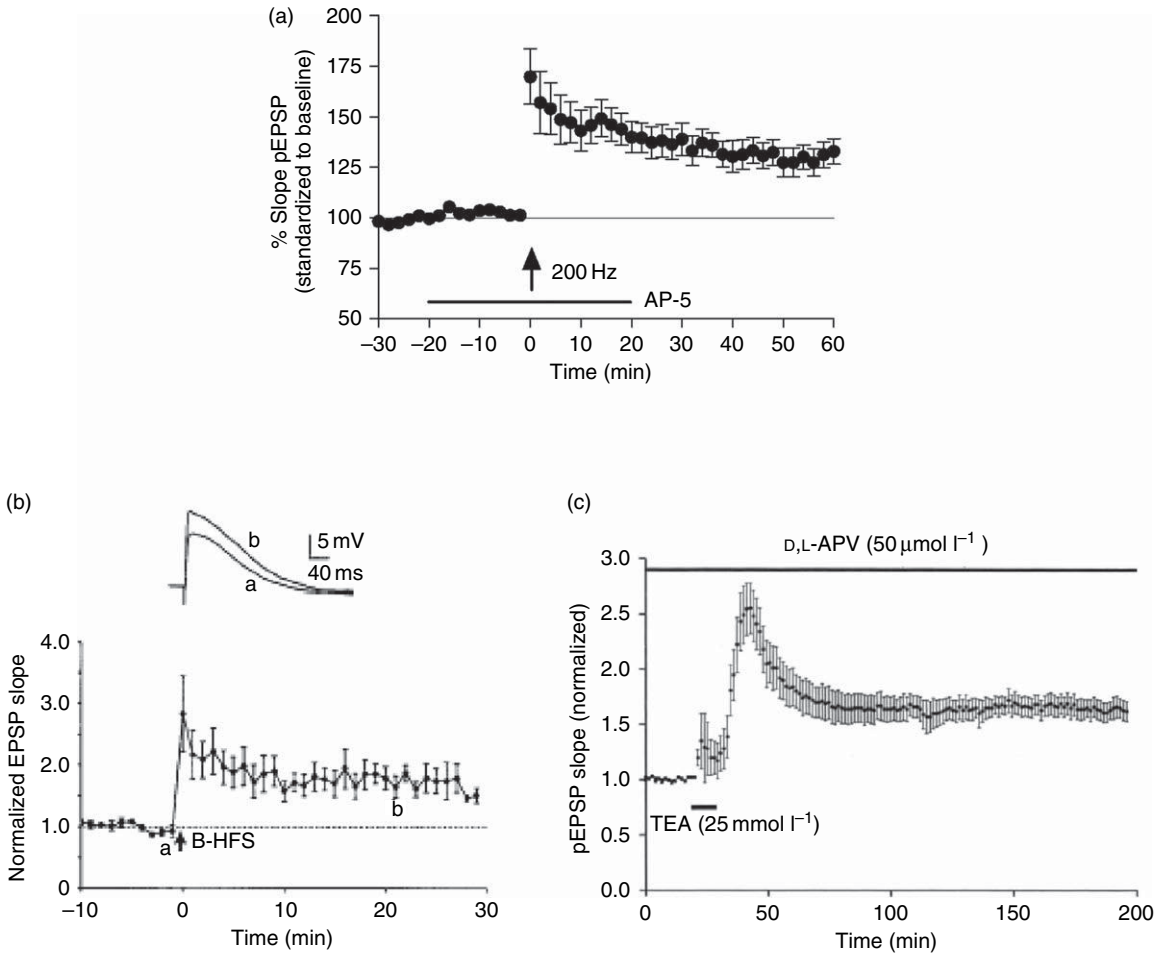


Figure 14 Examples of NMDA receptor-independent LTP. (a) 200-Hz stimulation in area CA1 elicits LTP even in the presence of the NMDA receptor antagonist APV. pEPSP, population EPSP. Data courtesy of Ed Weeber. (b) LTP at mossy fiber inputs into area CA3 is also NMDA receptor independent – the potentiation shown occurred in the presence of blockers of the NMDA receptor. Data courtesy of Rick Gray. From Kapur A, Yeckel MF, Gray R, and Johnston D (1998) L-Type calcium channels are required for one form of hippocampal mossy fiber LTP. *J. Neurophysiol.* 79: 2181–2190; used with permission. (c) Application of the K channel blocker tetra-ethyl ammonium (TEA) also elicits NMDA receptor-independent LTP in area CA1. Data courtesy of Craig Powell (Ph.D. thesis, Baylor College of Medicine, p. 50).

accompanied the discovery of NMDA receptor-independent LTP at these synapses by Harris and Cotman (1986). The mossy fiber synapses are unique, large synapses with unusual presynaptic specializations, and there has been much interest in comparing the attributes and mechanisms of induction of mossy fiber LTP (MF-LTP) with those of NMDA receptor-dependent LTP in area CA1.

However, subsequent progress in investigating the mechanistic differences between these two types of LTP has been relatively slow for several reasons. First, the experiments are technically difficult physiologically – typically area CA3 is the first part of the hippocampal slice preparation to die *in vitro*. The local circuitry in area CA3 is complex, with many recurrent excitatory connections between neurons there: synapses that also are plastic and exhibit NMDA receptor-dependent LTP. Most problematic has been that there has been an ongoing controversy about the necessity of postsynaptic events, especially elevations of postsynaptic calcium, for the induction of MF-LTP. There are basically two schools of thought on MF-LTP. One line of thinking is that MF-LTP is entirely presynaptic in its induction and expression (Zalutsky and Nicoll, 1990). A second line of thinking is that MF-LTP has a requirement for postsynaptic signal transduction events for its induction (*See*, e.g., Kapur et al., 1998; Yeckel et al., 1999). Based on the available

literature, it is difficult to come up with a definitive answer to the locus and mechanisms of induction of MF-LTP. For the purposes of this chapter, which focuses on NMDA receptor-dependent forms of LTP, I will simply note that this has been an area of controversy. Evidence supporting a presynaptic locus for MF-LTP is discussed in more detail in another chapter (*See* Chapter 4.36).

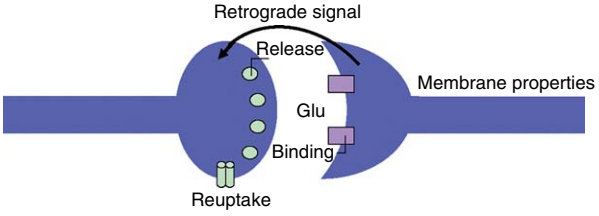
4.16.5 A Role for Calcium Influx in NMDA Receptor-Dependent LTP

In contrast to the story with MF-LTP, NMDA receptor-dependent LTP at Schaffer collateral synapses has achieved a broad consensus of a necessity for elevations of postsynaptic calcium for triggering LTP (Lynch et al., 1983). In fact, this is one of the few areas of LTP research in which there is almost universal agreement.

The case for a role for elevated postsynaptic calcium in triggering LTP is quite clear-cut and solid. It is well established and has been reviewed adequately a sufficient number of times (Johnston et al., 1992; Nicoll and Malenka, 1995; Chittajallu et al., 1998), so this material will only be presented in overview here. A principal line of evidence is that injection of calcium chelators postsynaptically blocks the induction of LTP. Also, inhibitors of a variety of calcium-activated enzymes also block LTP induction, including when they are specifically introduced into the postsynaptic neuron. Fluorescent imaging experiments using calcium-sensitive indicators have clearly demonstrated that postsynaptic calcium is elevated with LTP-inducing stimulation. Finally, elevating postsynaptic calcium is sufficient to cause synaptic potentiation (although there has been some controversy on this point). Thus the hypothesis of a role for postsynaptic calcium elevation in triggering LTP has met the three classic criteria (block, measure, mimic) necessary for 'proving' a hypothesis (Sweatt, 2003), and this idea is on a solid experimental footing.

4.16.6 Presynaptic versus Postsynaptic Mechanisms

One of the most intensely studied and least satisfactorily resolved aspects of LTP concerns the locus of LTP maintenance and expression. One component of LTP is an increase in the EPSP, which could arise from increasing glutamate concentrations in the synapse or by increasing the responsiveness to glutamate by the postsynaptic cell (**Figure 15**). The 'pre' versus 'post' debate is whether the relevant changes reside presynaptically, manifest as an increase in neurotransmitter release or similar phenomenon, or whether they reside postsynaptically as a change in glutamate receptor responsiveness. Over the last 15 years or so there have been numerous experiments performed to try to address this question, and as of yet there is no clear consensus answer. Popularity of the 'pre' hypothesis versus the 'post' hypothesis has waxed and waned, and this oscillation may continue for some time yet. The next few paragraphs will summarize a few representative findings to provide background on these issues. Reading the recent papers by Choi et al. (2000), Bolshakov et al. (1997), and Nicoll and Malenka (1999) will provide a feel for the nature of the ongoing debate.



Presynaptic = Altered
- Neurotransmitter amount in vesicles
- Number of vesicles released
- Kinetics of release
- Glutamate reuptake
- Probability of vesicle fusion

Postsynaptic = Altered
- Number of AMPA receptors
- Insertion of AMPA receptors
- Ion flow through AMPA channels
- Membrane electrical properties

Additional possibilities include changes in number of total synaptic connections between two cells

Figure 15 Potential sites of synaptic modification in LTP. Figure by J. David Sweatt and Sarah E. Brown.

In some of the earliest studies to begin to get at LTP mechanistically, it became clear that infusing compounds into the postsynaptic cell led to a block of LTP. A few of these studies involving calcium chelators were described in the last section, and some studies investigating protein kinases are described in other chapters of this volume (*See* Chapters 4.21, 4.22, and 4.23). If compounds that are limited in their distribution to the postsynaptic compartment block LTP, the most parsimonious hypothesis is that LTP resides postsynaptically.

However, shortly thereafter, evidence began to accumulate suggesting that presynaptic changes were involved in LTP expression as well. For example, various types of 'quantal' analysis that had been successfully applied at the neuromuscular junction to dissect presynaptic changes from postsynaptic changes suggested that LTP is associated with changes presynaptically. In a series of investigations, several laboratories used whole-cell recordings of synaptic transmission in hippocampal slices and found an increase in the probability of release, a strong indicator of presynaptic changes in classic quantal analysis (Dolphin et al., 1982; Bekkers and Stevens, 1990; Malinow and Tsien, 1990; Malinow, 1991; Malgaroli et al., 1995; Zakharenko et al., 2001). These findings fit nicely with earlier studies from Tim Bliss's laboratory suggesting an increase in glutamate release in LTP as well (Dolphin et al., 1982). Given findings supporting postsynaptic locus on the one hand and presynaptic locus on the other, why not just hypothesize that there are changes both presynaptically and postsynaptically? The rub came in that some of the quantal analysis results seemed to exclude postsynaptic changes as occurring.

These findings in the early 1990s ushered in an exciting phase of LTP research that was important independent of the pre versus post debate *per se.* If there are changes presynaptically but these changes are triggered by events originating in the postsynaptic cell, as the earlier inhibitor-perfusion experiments had indicated, then the existence of a *retrograde messenger* is implied. A retrograde messenger is a compound generated in the postsynaptic compartment that diffuses back to and signals changes in the presynaptic compartment – the opposite (retrograde) direction from normal synaptic transmission. Moreover, if the compound is generated intracellularly in the postsynaptic neuron then the compound must be able to traverse the postsynaptic membrane somehow. The data supporting presynaptic changes in LTP implied the existence of such a signaling system, and this hypothesis launched a number of interesting and important experiments to determine what types of molecules might serve such a role – some of these are highlighted in **Figure 16** and described in other chapters (*See* Chapters 4.21, 4.36, and 4.37).

However, in the mid-1990s the pre/post pendulum began to swing back in the opposite direction, toward the postsynaptic side. Several groups found evidence for postsynaptic changes that could account for the apparently presynaptic changes identified by quantal analysis studies. Specifically, evidence was generated for what are termed *silent synapses* (see **Figure 17**). These are synapses that contain NMDA receptors but no AMPA receptors – they are capable of synaptic plasticity mediated by NMDA receptor activation but are physiologically silent in terms of baseline synaptic transmission. Silent synapses are rendered active by NMDA receptor-triggered

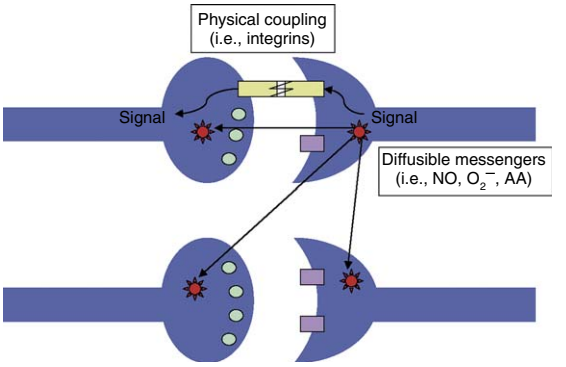


Figure 16 Potential mechanisms for retrograde signaling. Figure by J. David Sweatt and Sarah E. Brown.

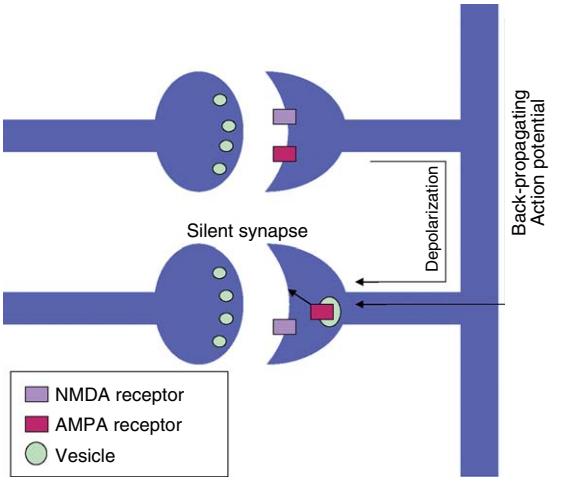


Figure 17 A simplified model of silent synapses. Figure by J. David Sweatt and Sarah E. Brown.

activation of latent AMPA receptors postsynaptically. Such uncovering of silent AMPA receptors could involve membrane insertion or posttranslational activation of already-inserted receptors. Activation of silent synapses is a postsynaptic mechanism that could explain the effects (decreased failure rate, for example) in quantal analysis experiments that implied presynaptic changes. Thus, there is now an argument that all of LTP physiology and biochemistry could be postsynaptic.

This model for conversion of silent synapses into active synapses by AMPA receptor insertion is an entirely postsynaptic phenomenon. However, there has been a variation of this idea proposed, which has been referred to as a whispering synapse. A whispering synapse has both AMPA and NMDA receptors in it, but because of a number of hypothetical factors such as glutamate affinity differences between NMDA and AMPA receptors, kinetics of glutamate elevation in the synapse, or spatial localization of the receptors, the AMPA receptors are silent. A whispering synapse is converted to being fully active by a presynaptic mechanism. An increase in glutamate release presynaptically, resulting in an elevation of glutamate levels in the synapse, then allows the effective activation of preexisting AMPA receptors with baseline synaptic transmission. By this mechanism, a synapse that was previously silent with respect to baseline synaptic transmission is rendered detectably active. However, this alternative mechanism requires no change in the postsynaptic compartment whatsoever.

Like the retrograde messenger hypothesis, the silent synapses hypothesis has also led to a number of important and interesting experiments that warrant attention aside from the pre versus post debate. Specifically, these experiments have focused new attention on the importance of considering the postsynaptic compartment in a cell-biological context. Mechanisms of receptor insertion, trafficking, and turnover that had been studied in nonneuronal cells are now beginning to get the attention they deserve in neurons as well. Other chapters address these issues in greater detail (*See* Chapters 4.30, 4.31, and 4.36). Like retrograde signaling, experiments arising from investigating mechanisms for activation of silent synapses have led to important 'spin-off' studies that are important independent of the precipitating issue of pre versus post.

Given the variety of evidence described so far, should one conclude that LTP resides presynaptically, postsynaptically, or both? Although there is not yet an unambiguous consensus in the pre versus post debate, overall the available literature indicates that changes are occurring in both the presynaptic and postsynaptic compartments. Two different types of approaches are briefly mentioned here, but these types of experiments are described in much more detail in other chapters in this volume (*See* Chapters 4.34, 4.35, 4.36, 4.38). First, a number of experiments using sophisticated imaging techniques have found LTP to be associated with presynaptic changes such as increased vesicle recycling and increased presynaptic membrane turnover (see, for example, Malgaroli et al., 1995; Zakharenko et al., 2001). Also, direct biochemical measurements of the phosphorylation of proteins selectively localized to the presynaptic compartment have shown LTP-associated changes. Conceptually similar experiments looking at phosphorylation of postsynaptic proteins have found the same thing. (These types of experiments are described in other chapters (*See* Chapters 4.30, 4.31, 4.32). Thus, imaging and biochemistry studies have fairly clearly illustrated that sustained biochemical changes are happening in both the presynaptic and postsynaptic cell.

This conclusion and indeed all of the pre versus post experiments have a very important caveat to keep in mind. In trying to reach a consensus conclusion, one is making a comparison across a wide spectrum of different types of experiments and different preparations. For example, one is comparing results with cultured cells versus hippocampal slices. One is trying to compare results for different types of LTP, LTP induced using pairing versus tetanic stimulation protocols. One likely is looking at different stages of LTP in comparing results from different experimental time points. Finally, in these experiments the various investigators are using material from different developmental stages in the animal, where the neurons under study are in different stages of their differentiation pathway. These considerations are a good reason to exercise caution in interpreting the experiments at this point, and indeed these issues may be contributing greatly to the apparent incompatibility of the results obtained in different labs. Another chapter of this volume presents a nice discussion of these various issues (*See* Chapter 4.30).

4.16.7 LTP Can Include an Increased Action Potential Firing Component

Another caveat to keep in mind is that the preceding discussion deals only with mechanisms contributing to increases in synaptic strength. The increased EPSP is typically measured in field recording

experiments as an increase in the initial slope of the EPSP (or EPSP magnitude), and as was discussed earlier, a second component of LTP is referred to as EPSP-spike (E-S) potentiation. As was already mentioned, E-S potentiation was identified by Bliss and Lomo in the first published report of LTP (1973) and is defined as an increase in population spike amplitude that cannot be attributed to an increase in synaptic transmission (i.e., initial EPSP slope in field recordings). Thus, E-S potentiation is a term used to refer to the postsynaptic cell having an increased probability of firing an action potential at a constant strength of synaptic input.

E-S potentiation at Schaffer collateral synapses can be observed using recordings in stratum pyramidale, as is illustrated in **Figure 18**. In this example, Eric Roberson generated I/O curves for the initial slope of the EPSP and the population spike amplitude, using various stimulus intensities, before and after LTP induction. E-S potentiation is manifest as an increase in population spike amplitude, even when responses are normalized to EPSP slope. Roberson's research also found that the probability of induction and magnitude of EPSP-spike (E-S) potentiation in area CA1 is more variable than LTP of synaptic transmission. A similar greater variability in E-S potentiation was observed by Bliss and Lomo in their original report as well.

What is the mechanism for this long-term increase in the likelihood of firing an action potential? One possibility is that there are changes in the intrinsic excitability of the postsynaptic neuron. Particularly appealing is the idea that long-term downregulation of dendritic potassium channel function could cause a persisting increase in cellular excitability and action potential firing. Although investigations of this hypothesis are still at an early stage, some recent work has suggested that E-S potentiation has a component due to intrinsic changes in the postsynaptic neuron. This idea is discussed further in another chapter (*See* Chapter 4.40).

Progress in testing this hypothesis has been slow due to the technically difficult nature of the experiments.

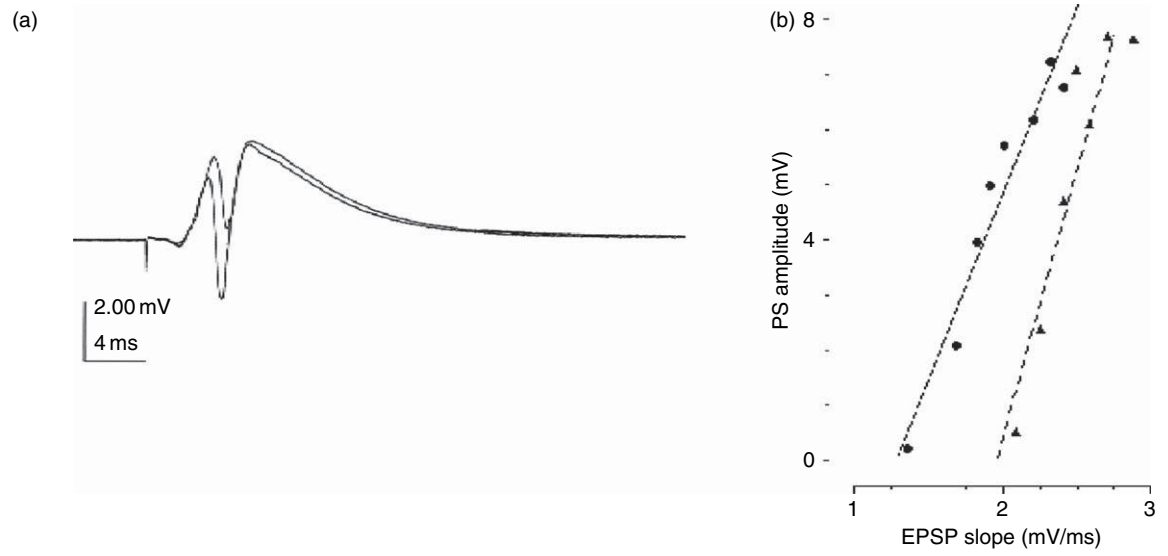


Figure 18 EPSP-spike (E-S) potentiation in area CA1. Extracellular recordings were made in the cell body layer of area CA1 (using stimulation of the Schaffer collateral inputs), and input-output (I/O) curves were performed using a range of 5 to 45 μA constant current stimulation. Initial slopes of the EPSP and population spike (PS) amplitude were then determined from the tracings and the data plotted as PS amplitude versus EPSP slope. (a) Superimposed representative tracings for before and 75 min after tetanic stimulation, showing the increased PS amplitude after tetanic stimulation. (b) Plots are shown for pretetanus (triangles) and 75 min posttetanus (circles). In this experiment five 100-Hz tetani were delivered. E-S coupling was assayed in hippocampal slices by taking a second set of I/O measurements after the induction of LTP. The baseline I/O curve and the poststimulation I/O curve were then compared to assess whether a change in excitability has occurred over the course of the experiment. Although in the illustration both EPSP slope and PS amplitude were measured from the same waveform, recording from the cell body layer, the preferred approach is to record EPSPs in the dendritic region and simultaneously record spikes independently from the cell body layer. This minimizes cross contamination of the PS changes in the EPSP measurements and vice versa. Data and figure courtesy of Erik Roberson.

Most patch-clamp physiologic studies of LTP have utilized recordings from the cell body, which are not capable of detecting changes in channels localized to the dendrites due to technical limitations. Thus, testing the idea of changes in dendritic excitability as a mechanism contributing to E-S potentiation requires dendritic patch-clamp recording, which at present only a few laboratories do routinely.

However, a more thoroughly investigated mechanism for E-S potentiation is based on alterations in feed-forward inhibitory connections onto pyramidal neurons in area CA1 (see **Figure 19**). These mechanisms for altered excitability and, indeed, plasticity of inhibitory synapses are discussed in detail in another chapter in this volume (*See* Chapter 4.18). However, the next few paragraphs present an overview of this area.

A number of different types of neurons in the hippocampus are called interneurons (or intrinsic neurons) because their inputs and outputs are restricted to local areas of the hippocampus itself. In other words, they only communicate with other neurons nearby in the hippocampus. Most of these neurons in area CA1 use the inhibitory neurotransmitter GABA, and their actions are to inhibit firing of CA1 pyramidal neurons. Different GABAergic interneurons make connections in all the dendritic regions of CA1 pyramidal neurons as well as the initial segment of the axon where the action potential originates. A single GABAergic interneuron may contact a thousand pyramidal neurons; thus, the effects of altered interneuron function are not generally limited to a single follower cell.

Interneurons in area CA1 receive glutamatergic Schaffer collateral projections just as the pyramidal neurons do – in fact, the inputs to the interneurons are branches of the same axons impinging the pyramidal neurons. Glutamate release at these interneuron synapses activates the interneurons and causes downstream release of GABA onto the pyramidal neurons. This inhibitory action is of course slightly delayed at the level of the single cell that receives input from the same Schaffer collateral axon that is activating the GABAergic interneuron, because there is an extra synaptic connection involved.

How does this local circuit contribute to E-S potentiation? Two different groups have shown that the same stimulation that produces LTP at the Schaffer collateral pyramidal neuron synapses simultaneously produces a decreased efficacy of coupling (long-term depression, LTD) of the Schaffer collateral interneuron synapses (McMahon and Kauer, 1997; Lu et al., 2000). Thus, although the excitatory input to the pyramidal neuron is being enhanced, the feed-forward inhibitory GABA input is diminished. This causes a net increase in excitability and increased likelihood of firing an action potential, added on top of the increased EPSP due to the normal LTP mechanisms. This is, of course, the definition of E-S potentiation.

There are a couple of interesting properties for this LTD at the Schaffer collateral interneuron synapse. First, it is NMDA receptor dependent just like LTP. This explains why one does not see E-S potentiation independent of synaptic potentiation in

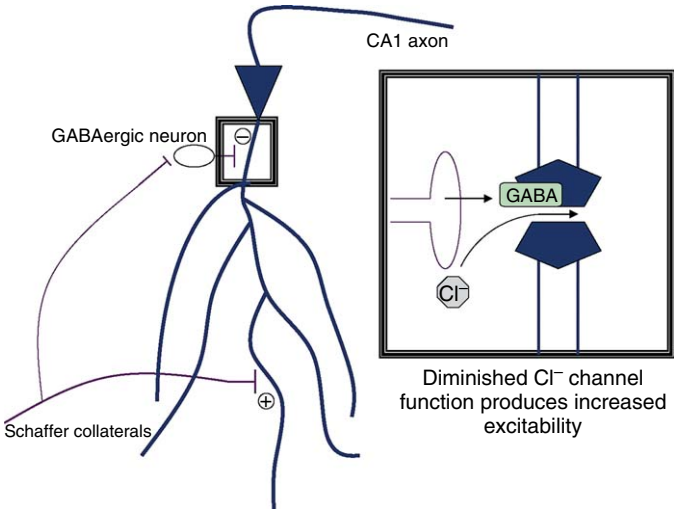


Figure 19 The GABAergic interneuron model of ES potentiation. One potential mechanism for E-S potentiation is diminution of inhibitory feed-forward inhibition through GABAergic interneurons in area CA1. Specific possible sites for this effect include LTD of the Schaffer collateral inputs onto GABAergic neurons, or synaptic depression of the interneuron-CA1 pyramidal neuron synapse. Figure by J. David Sweatt and Sarah E. Brown.

experiments where APV is infused onto the slice. Second, and more interesting, the LTD is not specific to the activated synapse – other Schaffer collateral inputs onto the same interneuron are also depressed (McMahon and Kauer, 1997). Therefore, there is decreased feed-forward inhibition across all the inputs (and outputs of course) for the whole interneuron. The interneuron has a diminished response to all its inputs and, therefore, decreased feedforward inhibition to all its outputs. Thus, the interneuron LTD appears to be serving to modulate the behavior of an entire small local circuit of neuronal connections. The precise role this interesting attribute plays in hippocampal information processing is unclear at present, but it is under study.

4.16.8 Temporal Integration Is a Key Factor in LTP Induction

At one level, it is a statement of the obvious to say that LTP induction depends on temporal integration. After all, the characteristic that distinguishes LTP induction protocols from baseline stimulation is that during an LTP induction protocol, stimulation is delivered at a higher rate. It obviously is the case that if the only attribute that is different is that the synapse is seeing activity at 100 pulses per second rather than once per 20 seconds, then unique timing-dependent processes are triggering LTP, which is simply a restatement of one definition of temporal integration. But what are the unique events that are happening physiologically with high-frequency stimulation? Stated briefly, the answer to this question is that temporal integration is occurring such that the cell is reaching a threshold of depolarization to fire an action potential (Scharfman and Sarvey, 1985; Johnston et al., 1999; Linden, 1999). This action potential firing then leads to membrane depolarization to allow opening of NMDA receptors. The following paragraphs will describe two different ways in which this can happen.

The first mechanism can be illustrated by considering what happens during the 1-s period of 100-Hz tetanus. Such closely spaced stimulation means that postsynaptic depolarization from the first EPSP carries over into the second stimulation, and so on, and so on, 96 more times. Stated more precisely, the postsynaptic membrane potential does not recover to the original resting potential before an additional depolarizing EPSP is triggered, and temporal summation of postsynaptic depolarization occurs. The summed depolarization eventually reaches threshold for the cell to fire an action potential. This is one of the classic examples of neuronal temporal integration, and of course, such a process is not limited to hippocampal pyramidal neurons. One unique aspect of this in hippocampal neurons, and probably other cortical neurons as well, is that triggering of the action potential is used to generate a back-propagating action potential into the dendrites, which is involved in depolarizing the NMDA receptor and triggering synaptic plasticity.

A second example comes from considering LTP induced by theta-pattern stimulation. With this type of LTP induction protocol, delivered at the slower 5 Hz (once/200 ms) rate, temporal integration is similarly involved but occurs via a different route. After all, 200 ms is long enough for the postsynaptic membrane potential to completely recover before the next wave of depolarization, so temporal integration of the sort described earlier is inadequate as an explanation. Joel Selcher investigated this question by examining the physiologic events occurring during the period of TFS. For illustrative purposes, his results with TFS stimulation will be discussed, although he and others observed similar effects with TBS as well.

These experiments used TFS consisting of 30 s of 5-Hz stimulation. This stimulation paradigm evokes stable LTP as described earlier and illustrated in **Figure 20**. Population spikes were assessed during the TFS period, utilizing a dual-recording electrode technique. The stimulating electrode remained in hippocampal area CA3 and activated Schaffer collateral fibers innervating area CA1. One recording electrode was positioned in stratum radiatum of area CA1 to record synaptic responses, field EPSPs (**Figure 20(b)**). Another electrode was placed in stratum pyramidale, the cell body layer, to record action potential firing in response to the same input. For each single stimulus, the initial slope of the EPSP recorded in stratum radiatum and the amplitude of the population spike recorded in stratum pyramidale were measured throughout the period of 5-Hz stimulation.

TFS resulted in a short-lived increase in action potential firing during the 30 seconds of 5-Hz stimulation (see **Figures 20(c)** and **20(d)**). For roughly the first 20 s of the stimulation, the amplitude of the population spike increased dramatically. Meanwhile, over this same time period, the EPSP slope recorded in stratum radiatum gradually declined. Therefore, the ratio of the population spike amplitude to the EPSP slope increased over time, indicating an increased likelihood of action potential firing over

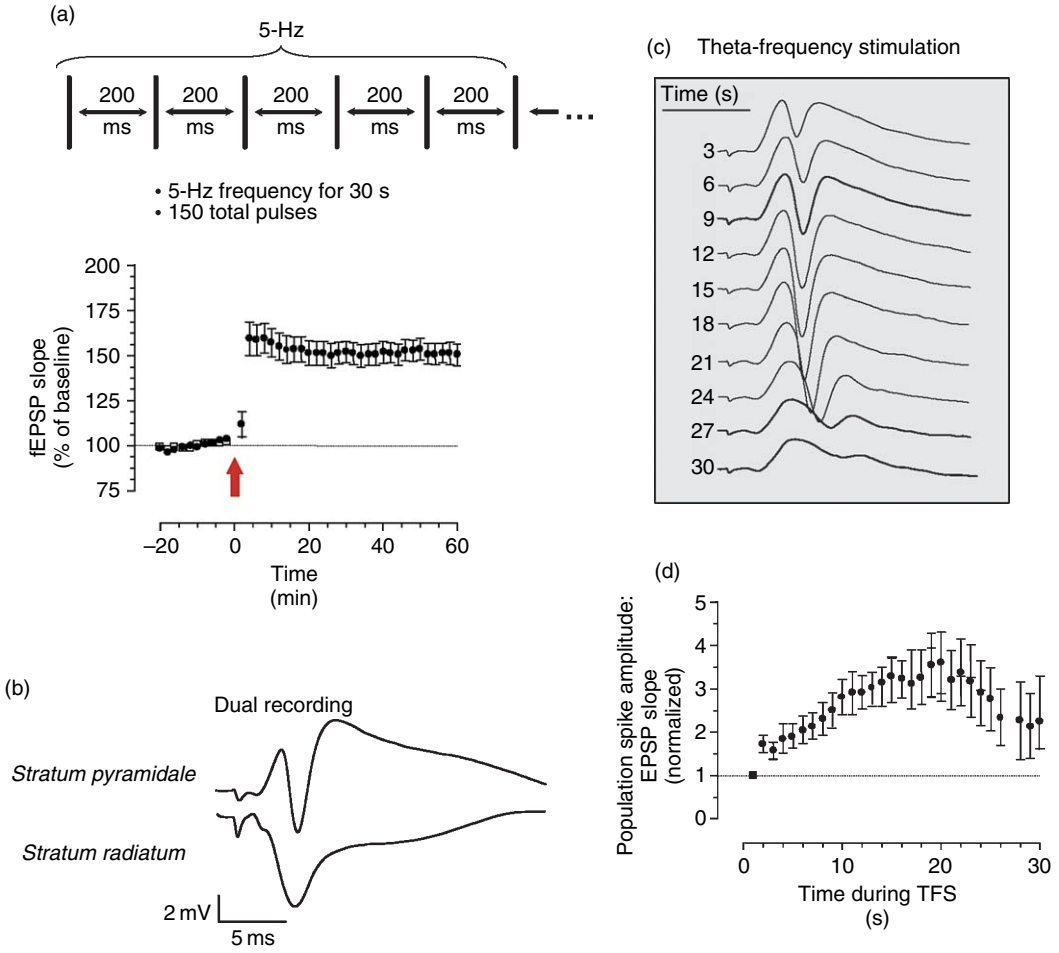


Figure 20 Increased action potential firing over the course of theta-frequency stimulation (TFS). (a) The TFS protocol and TFS-induced LTP in mouse hippocampal slices. (b) Electrode placement configuration for recording EPSP and population spikes simultaneously during TFS. (c) Representative traces in response to TFS from a hippocampal slice. Note the difference in the population spike between the first and 18th stimulation of the stimulation paradigm. (d) Quantitation of increased spike amplitude during TFS. Population spike counts recorded in stratum pyramidale of hippocampal area CA1 during theta-burst stimulation is plotted versus burst number during TFS. Slices showed a progressive increase in spike generation during the first two-thirds of TFS. Data and figures courtesy of Joel Selcher.

the short time course of the TFS (**Figure 20(d)**). Once again, for TFS as for 100-Hz tetanic stimulation, some temporal integration process is taking place to cause action potential firing during the period of LTP-inducing stimulation.

The mechanism for this temporal integration is not clear at present: clearly temporal summation of the sort operating in 100-Hz stimulation is not sufficient to explain it. However, a variety of previous studies have suggested that for LTP induced by TFS, there is an important role for attenuation of feed-forward GABAergic inhibition onto pyramidal neurons (Davies et al., 1991; Mott and Lewis, 1991; Chapman et al., 1998) (**Figure 21**). One current hypothesis is that short-term synaptic depression in the GABAergic local circuit during TFS, due to stimulation of presynaptic GABA-B autoreceptors, leads to a loss of GABA-mediated inhibition, increased excitability, and increased firing of action potentials during the period of TFS.

4.16.9 LTP Can Be Divided into Phases

Contemporary models divide very long-lasting LTP (i.e., LTP lasting in the range of 5 to 6 h) into at least three phases. LTP comprising all three phases can be

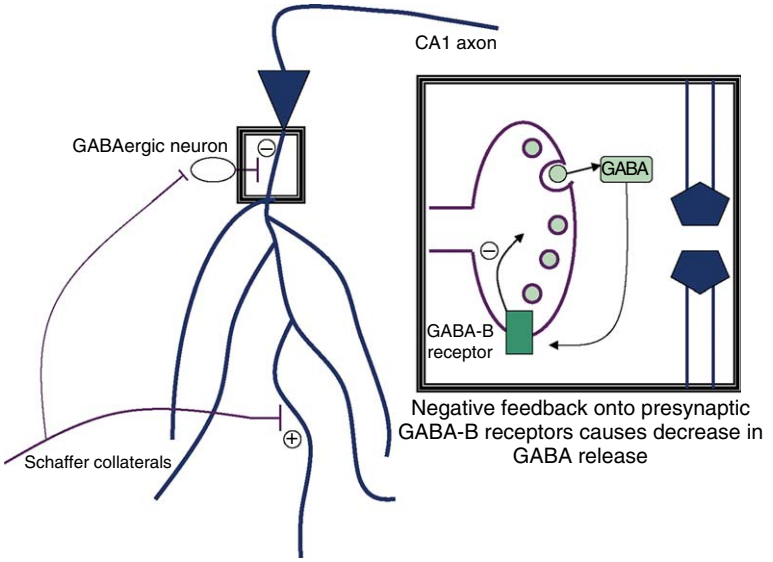


Figure 21 GABA-B receptors in temporal integration with TBS. This figure presents one model for the increased excitability that occurs during TBS, based on autoinhibition at GABAergic inputs onto CA1 pyramidal neurons during the period of stimulation. Figure by J. David Sweatt and Sarah E. Brown.

induced with repeated trains of high-frequency stimulation in area CA1 (see **Figure 22**), and the phases are expressed sequentially over time to constitute what we call 'LTP.' Late LTP (L-LTP) is hypothesized to be dependent for its induction on changes in gene expression, and this phase of LTP lasts many hours (see also Winder et al., 1998). Early LTP (E-LTP) is likely subserved by persistently activated protein kinases, as we will discuss in other chapters (*See* Chapters 4.22 and 4.23), and starts at around 30 min or less posttetanus and is over in about 2–3 h. The first stage of LTP, generally referred to as short-term potentiation (STP), is independent of protein kinase activity for its induction and lasts about 30 min. I prefer to refer to the first stage of LTP as initial LTP to emphasize that it is a persistent form of NMDA receptor-dependent synaptic plasticity that is induced by LTP-inducing tetanic stimulation and is a prelude to E-LTP and L-LTP (Roberson et al., 1996). The mechanisms for initial LTP (aka STP) are essentially a complete mystery at present.

Readers may note some degree of ambiguity in the times specified for each phase of LTP. This is in part because the phases are very descriptive, and different labs often use slightly different conditions for their LTP experiments. For example, for technical reasons most L-LTP experiments are performed at room temperature, or 27–28°C, because it is much easier to maintain a healthy slice for many hours at these lower

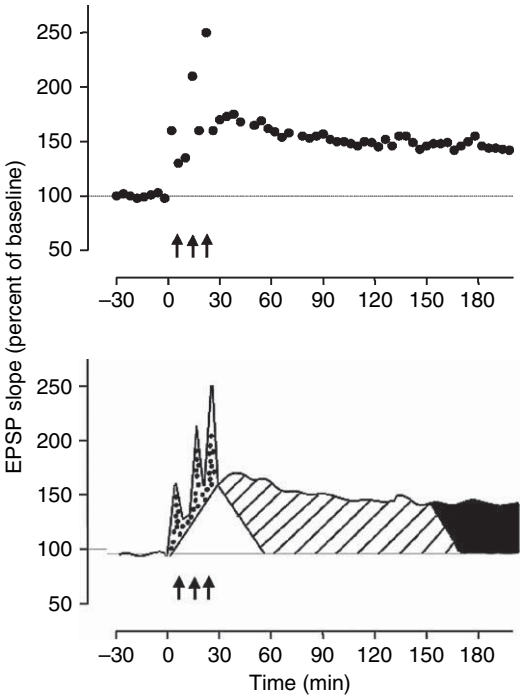


Figure 22 Immediate, early, and late LTP. The upper panel is real data from a late-phase LTP experiment, courtesy of Eric Roberson. The lower panel is a cartoon adaptation of the same data approximating the initial, early, and late stages of LTP. Adapted from Roberson ED, English JD, and Sweatt JD (1996) A biochemist's view of long-term potentiation. *Learn. Mem.* 3: 1–24, with permission.

temperatures. Many E-LTP experiments, especially those involving direct biochemical measurements, are performed at 32–35°C. Comparing studies done at different temperatures is complicated by the pronounced temperature dependence of essentially all chemical reactions. A doubling of reaction rate for a change from room temperature to 32°C is fairly common for biochemical reactions. For these many reasons, it is difficult to try to compare experiments done at one temperature to experiments done at another. 'Late' LTP may start at 3 h at room temperature, start at 1.5 h at 32°C, and start at 45 min *in vivo*.

4.16.9.1 E-LTP and L-LTP – Types versus Phases

E-LTP and L-LTP refer to different *temporal* phases of LTP. These phases are subserved by different *maintenance* mechanisms of different time courses and durations. These two phases of LTP, E-LTP and L-LTP, are not exclusive of each other. In fact, depending on the LTP induction protocol used, E-LTP can be ongoing while L-LTP is developing, and one supplants the other over time. This has certain theoretical implications that are discussed in more detail in Roberson et al. (1996). These definitions are important as we transition to molecular mechanisms in other chapters of this volume (*See* Chapters 4.20, 4.21, 4.22, 4.23, 4.25, 4.27, 4.28, 4.29, 4.30, 4.31, 4.32, 4.33, 4.34, and 4.36). These definitions contain an underlying assumption about the biochemistry of LTP that is an organizing principle for the rest of this volume, that is, that different phases of LTP are subserved by distinct molecular mechanisms.

However, the terms E-LTP and L-LTP have been used in a slightly different fashion as well, in particular as popularized by the Kandel laboratory (*See* Winder et al., 1998). The Kandel laboratory and others use a terminology that divides the NMDA receptor-dependent form of LTP in area CA1 into E-LTP and L-LTP as well. E-LTP and L-LTP in this terminology refer to what one can characterize as two subtypes of LTP – a transient form (typically lasting 1–2 h) and a long-lasting form (lasting at least 5 h or more). The latter form of LTP is characterized by its dependence on intact protein synthesis, and the induction of this form of LTP requires delivery of multiple tetanic stimuli. E-LTP in this alternative nomenclature is induced by fewer tetanic stimuli and is protein synthesis independent. In this usage, E-LTP and L-LTP are defined as different *types* of LTP, not as temporal phases of LTP. Thus, one must keep in mind that two slightly different variations in the use of the terms E-LTP and L-LTP exist in the literature.

Before turning to a discussion of some implications of LTP having phases, a final set of three terms must be introduced – three terms widely used in the LTP literature. These terms arose from pharmacological inhibitor studies of LTP, and these types of studies will be reviewed in a moment. However, first we will simply introduce the terms.

Induction refers to the transient events serving to trigger the formation of LTP. *Maintenance*, or more specifically a maintenance mechanism, refers to the persisting biochemical signal that lasts in the cell. This persisting biochemical signal acts on an effector, for example, a glutamate receptor or the presynaptic release machinery, resulting in the *expression* of LTP.

It is important to keep in mind that, depending on the design of the experiment, induction, maintenance, and expression could be differentially inhibited (see **Figure 23**). The simplest type of experiment does not do this – for example, imagine if one applies an enzyme inhibitor (or knocks out a gene) before, during, and after the period of LTP-inducing high-frequency stimulation, this manipulation may block LTP. However, this does not distinguish whether the missing activity is required for the induction, the expression, or the maintenance of LTP. To distinguish among these possibilities, imagine instead applying the inhibitor selectively at different time points during the experiment. If inhibitor is applied only during the tetanus and then washed out and it blocks the generation of LTP, one can conclude that the enzyme is necessary for LTP induction. If the inhibitor is applied after the tetanus and it reverses the potentiation, it may be blocking either the maintenance or expression of LTP, as was nicely illustrated in an early experiment by Malinow et al. (1988), where they applied a protein kinase inhibitor after LTP induction. In this experiment, transient application of a kinase inhibitor after tetanus blocked synaptic potentiation, but the potentiation recovered after removal of the inhibitor. This is a blockade of LTP *expression*. However, if the kinase inhibitor had caused the potentiation to be lost irreversibly, the inhibitor would then by definition have blocked the *maintenance* of LTP.

Finally, it is important to synthesize the concepts of induction, maintenance, and expression with the concept of phases. Simply stated, three phases of LTP (initial-, E-, and L-LTP) times three distinct

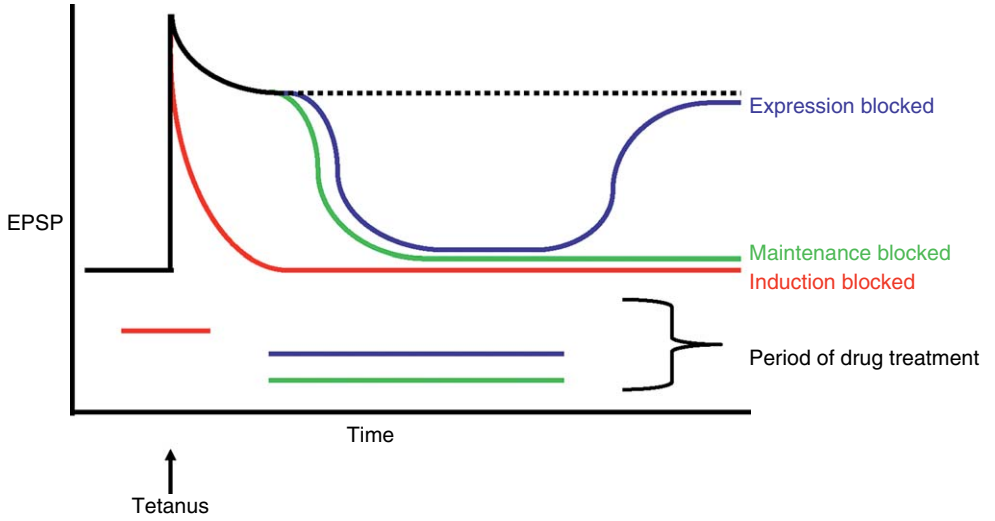


Figure 23 Induction, maintenance, and expression of LTP. This schematic illustrates the different experimental approaches to dissecting effects on the biochemical mechanisms subserving LTP induction, maintenance, or expression. See text for additional details.

underlying mechanisms for each phase (induction, maintenance, and expression) give nine separate categories into which any particular molecular mechanism contributing to LTP may fit (see Figure 24). Added to this is the complexity that one phase could be largely presynaptic and another largely postsynaptic. Interesting implications begin to arise from thinking about LTP this way: How is it that the different mechanisms for the different phases interact with each other? Is the maintenance mechanism for one phase the induction mechanism for the next, or do the mechanisms for the phases operate independently? If the maintenance and expression mechanisms for the phases are independent, how does the magnitude of LTP stay constant as the shorter-lasting phase decays? How does the mechanism for L-LTP know where to stop, so that the magnitude of L-LTP is the same as the magnitude that E-LTP had attained? Roberson et al. (1996) discusses some hypothetical answers to these questions. It also is important to keep in mind that in many ways the same considerations apply to memory itself. If memory is encoded as some complex set of molecular changes, how is it that fidelity of memory maintained as short-term memory fades into long-term memory, for example? Although we will not arrive at an answer to these many questions, it is instructive to begin to formulate a hypothetical framework for their discussion.

4.16.10 Spine Anatomy and Biochemical Compartmentalization

So far we have discussed the synapse in largely abstract terms related mostly to its function. However, the synapse is also a physical entity, and the structural attributes of this entity confer some interesting properties (reviewed in Chapter 4.34 of this volume). This brief section will describe certain physical aspects of the synapse that will be important to consider before moving on to other chapters of this volume, which discuss details about the molecular mechanisms for LTP. In brief, three points are highlighted in this

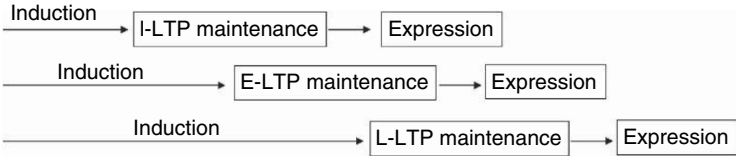


Figure 24 Mechanisms of induction, maintenance, and expression. This diagram highlights the importance of considering that each different phase of LTP may have separate and parallel induction, maintenance, and expression mechanisms. Figure by J. David Sweatt and Sarah E. Brown.

section. First, most synapses in the CNS and almost all excitatory synapses in the hippocampus are at specialized structures called *dendritic spines*. Second, spines are small, well-circumscribed biochemical compartments that localize proteins and signal molecules to a specific postsynaptic compartment. Third, spines are, of course, contiguous with dendrites and thus continuously sense the local dendritic membrane potential.

A picture of part of the dendritic region of an area CA1 pyramidal neuron is shown in **Figure 25**. The fuzzy appearance of the CA1 dendritic tree in this picture is due to the abundance of small dendritic spines protruding at right angles to the dendritic shaft. Almost all (about 95%) of the Schaffer collateral synapses we have been discussing in the abstract are actually physically present at spines. Most spines have a fairly simple, elongated, mushroomlike (i.e., chicken drumstick) shape, although there is clearly great diversity of their morphology. For example, a low percentage (about 2%) of CA1 pyramidal neuron spines are bifurcated and actually have two synapses on them. Spines have an actin-based cytoskeleton, and most have both smooth endoplasmic reticulums that can contribute to local calcium release and polyribosomes, where local protein synthesis occurs. In hippocampal pyramidal neurons, microtubules and mitochondria are limited to the dendritic shaft.

A distinguishing feature of the area of synaptic contact at the spine is the postsynaptic density, or PSD. This is a highly compact biochemical structure containing scaffolding proteins, receptors, and signal transduction components. The calcium/calmodulin-sensitive protein kinase CaMKII is particularly enriched at the PSD, as is a structural protein called PSD-95, a name that is based on its molecular weight. These molecules are discussed in much more detail elsewhere (*See* Chapters 4.20, 4.23, and 4.32).

The dendritic spine membrane surrounds the PSD and the area immediately below it and thus circumscribes a discrete biochemical compartment. The spine neck, however, is open to the dendritic shaft so there is still considerable diffusion of soluble spine contents (such as calcium and second messengers) into the local dendritic region. Nevertheless, on short time scales the spine compartment may serve to effectively localize signaling molecules to a specific synapse. Moreover, molecules tethered to the PSD by scaffolding proteins and the like probably have fairly limited diffusion because the spine compartment will make them tend to rebind at the same PSD as they unbind and rebind. Thus, this spine morphology is likely to be an important component for achieving synaptic specificity in LTP and other forms of synaptic plasticity.

The compartmentalization of molecules by the dendritic spine is not generally paralleled by an electrical compartmentalization. At one point, a popular line of thinking was that the shape and properties of the spine neck might regulate the capacity of electrical signals to get to and from the spine head compartment. This idea is no longer considered tenable, and as a first approximation, we can assume that the spine membrane potential reflects the local

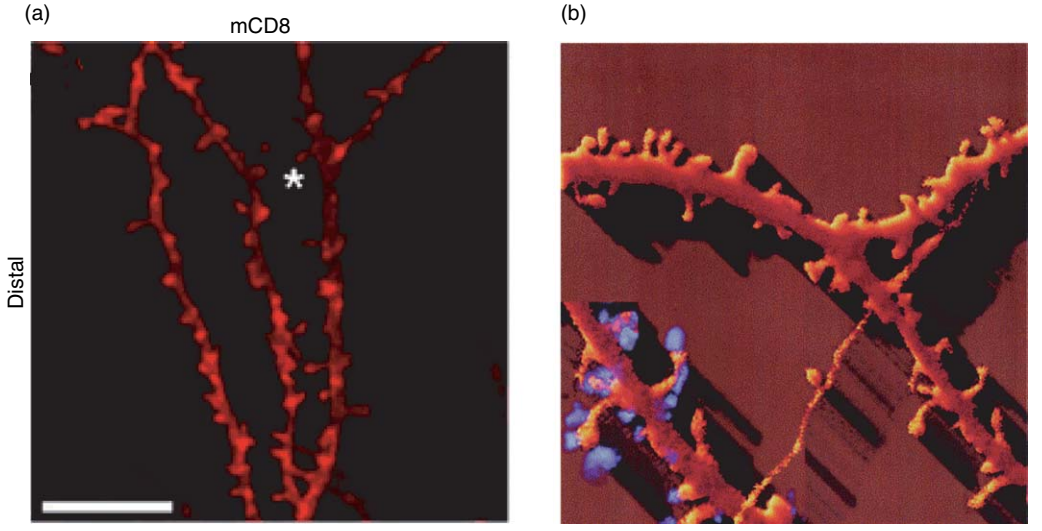


Figure 25 Dendrites with spines in a hippocampal pyramidal neuron. This figure illustrates the presence and shapes of dendritic spines on pyramidal neurons in the hippocampus. The spines are the small mushroom-shaped lateral projections containing synaptic contacts. (a) Courtesy of Liqun Lou, Stanford University. (b) Courtesy of E. Korkotian, The Weizmann Institute.

dendritic shaft membrane potential. However, it is likely that electrical compartmentalization does occur in dendrites, but this is at the level of the various dendritic branches as well as a component contributed by their overall distance from the soma (Johnston and Wu, 1995). This introduces the fascinating possibility that local generation and restricted propagation of action potentials within a specific dendritic subregion might be used as a mechanism for generating dendritic branch-specific plasticity.

4.16.11 LTP Outside the Hippocampus

The abundance of literature dedicated to studying LTP in the hippocampus might lead a newcomer to the field to suppose that LTP is somehow restricted to these synapses. However, plasticity of synaptic function, including phenomena such as LTP and LTD, is the rule rather than the exception for most forebrain synapses. LTP outside the hippocampus has been mostly studied in the cerebral cortex and the amygdala. The likely functional roles for LTP at these other sites are quite diverse, but two specific examples are worth highlighting. LTP-like processes in the cerebral cortex play a role in activity-dependent development of the visual system and other sensory systems. LTP in the amygdala has received prominent attention as a mechanism contributing to cued fear conditioning. The role of LTP in amygdala-dependent fear conditioning, in fact, is the area for which the strongest case can be made for a direct demonstration of a behavioral role for LTP. This will be discussed in more detail elsewhere (*See* Chapter 4.11). It is important to keep in mind through the rest of this volume that cortical LTP and amygdalar LTP probably exhibit some mechanistic differences from the NMDA receptor-dependent LTP that we will be focusing on. However, the molecular similarities are likely to greatly outweigh the differences (Schafe et al., 2000).

4.16.12 Modulation of LTP Induction

In one sense, the hippocampal slice is a denervated preparation. In the intact animal, the hippocampus receives numerous input fibers that provide modulatory inputs of the neurotransmitters DA, NE, 5HT, and ACh. Functionally these inputs are largely lost as a necessity of physically preparing the hippocampal slice for the experiment. However, these lost modulatory inputs can be partially reconstituted by directly applying the neurotransmitters (or more commonly pharmacologic substitutes) to the slice preparation *in vitro*. This approach has been used quite successfully to gain insights into the physiologic mechanisms and functional roles of these inputs in the intact brain.

NE, DA, and ACh-mimicking compounds can all modulate the induction of LTP at Schaffer collateral synapses. Specifically, agents acting at various subtypes of receptors for these compounds can increase the likelihood of LTP induction and the magnitude of LTP that is induced. Several examples of this type of modulation experiment are shown in **Figure 26**. In one example (panel A), 5-Hz stimulation of Schaffer collateral synapses, for 3 min, gives essentially no potentiation. Coapplication of isoproterenol, a beta-adrenergic receptor agonist that mimics endogenous NE, converts a nonpotentiating signal into a potentiating one (Thomas et al., 1996). Under other conditions beta-adrenergic agonists can augment the *magnitude* of LTP induced as well, if different physiologic stimulation protocols are used that evoke modest LTP. Similar types of effects can be observed for activation of various subtypes of receptors for ACh and DA (see Yuan et al., 2002).

One known site of action of neuromodulators is regulation of back-propagating action potentials in pyramidal neuron dendrites. All of these agents, which modulate LTP induction, can modulate the magnitude of back-propagating action potentials (see **Figure 26(b)**). The augmentation of back-propagating action potentials is a means by which these neurotransmitters can enhance membrane depolarization and thereby enhance NMDA receptor opening.

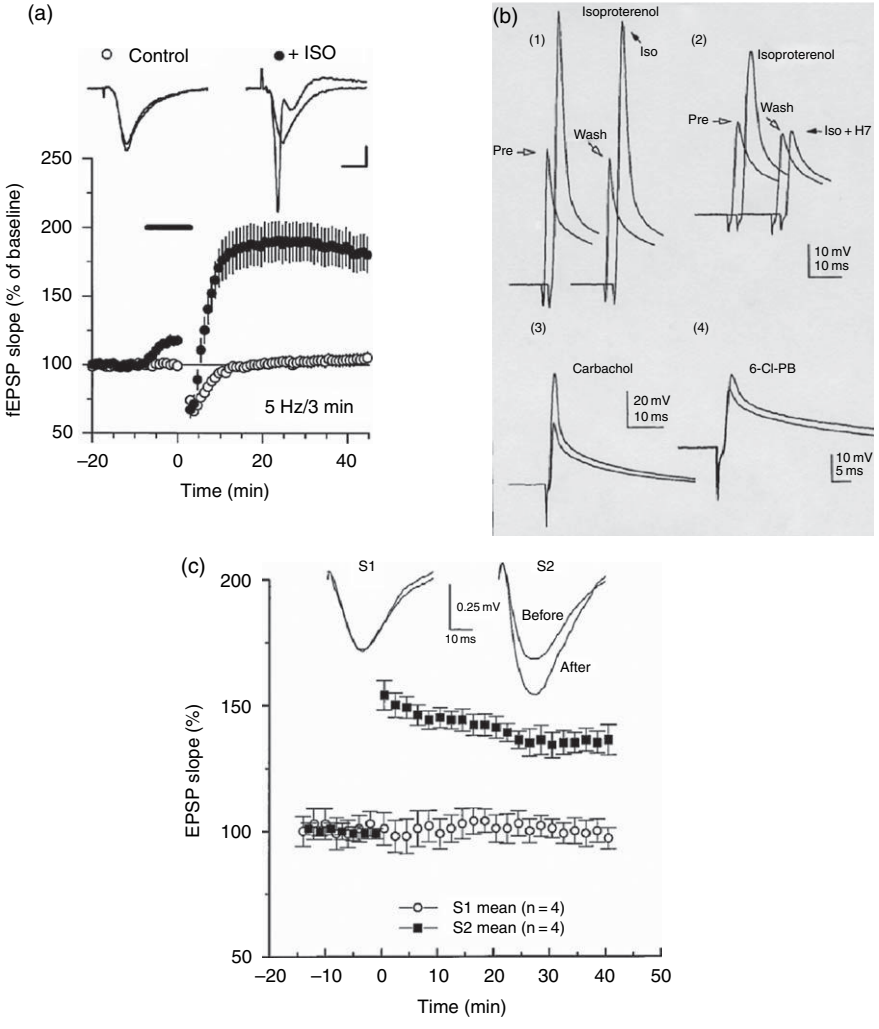
The growth factor BDNF (brain-derived neurotrophic factor) can also modulate the induction of LTP by a number of mechanisms, at least one of which is presynaptic (Gottschalk et al., 1998; Lu and Chow, 1999; Xu et al., 2000; **Figure 26(c)**). BDNF, acting through its cell-surface receptor TrkB, acts on presynaptic terminals to selectively facilitate neurotransmitter release during high-frequency stimulation. This is an interesting example of modulation of LTP induction that is activity dependent but localized to the presynaptic compartment. The mechanisms controlling the levels of BDNF in the adult hippocampus are not entirely clear at this point, but it is fairly well established that hippocampal BDNF levels can be regulated by a variety of neuronal activity-dependent processes and indeed in response to environmental signals impinging on the behaving animal.

4.16.13 Depotentiation and LTD

If synapses can be potentiated and this potentiation is very long-lasting, over time the synapses will be driven to their maximum synaptic strength. In this condition, there is no longer synaptic plasticity and no further capacity for that synapse to participate in synaptic-plasticity-dependent processes. Worse yet, over the lifetime of an animal, synapses will by random chance experience LTP-inducing conditions (presynaptic activity coincident with a postsynaptic action potential, for example) numerous times. If LTP is irreversible, ultimately every synapse will be maximally potentiated – obviously not a desirable condition vis-à-vis memory storage.

Consideration of this conundrum raises two implications. First, synapses that are involved in lifelong memory storage must be rendered essentially aplastic. In order to have good fidelity of memory storage over the lifetime of an animal, a synapse involved in permanent memory storage must be rendered immutable to a change in synaptic strength due to the random occurrence of what would normally be LTP-inducing stimulation.

But what about synapses like those in the hippocampus that are not sites of memory storage, but rather whose plasticity is part of the active processing of forming new long-term memories? To retain their plasticity and hence their capacity to contribute to memory formation, their potentiation must be reversible. Schaffer collateral synapses can undergo activity-dependent reversal of LTP, a phenomenon termed depotentiation (see **Figure 27**). Another activity-dependent way to decrease synaptic strength



is LTD, the mirror image of LTP. LTD is a long-lasting decrease of synaptic strength below baseline. Using a logic similar to that of the first paragraph of this section, the phenomenon of dedepression of synaptic transmission is implied, although this has not been widely studied at this point.

As a practical matter it is often difficult to separate depotentiation from LTD experimentally. For example, a 'baseline' response in hippocampal slices or *in vivo* likely is a mixture of basal synaptic activity and activity at previously potentiated synapses. Moreover, for the most part the stimulation protocols used to induce depotentiation are variations of the protocols used to induce LTD. Nevertheless, mechanistic investigations have made clear that depotentiation and LTD use different mechanisms (Lee et al., 1998; Lee et al., 2000) and thus must be considered as distinct processes. This is addressed elsewhere in this volume (*See* Chapter 4.31).

Physiologic LTD (and depotentiation) induction protocols generally involve variations of repetitive 1-Hz stimulation (Lee et al., 1998; Kemp et al., 2000). A common protocol is to deliver 900 stimuli at 1 Hz, but there also are LTD protocols that use random small variations in frequency in the 1-Hz region and variations that use paired-pulse stimuli delivered at 1 Hz. Synaptic depression appears to be fairly robust *in vivo*, but is quite difficult to induce in hippocampal slices from adult animals. LTD *in vitro* is almost always studied using slices from immature animals, or cultured immature neurons, and it is possible that LTD as it is currently studied *in vitro* is largely a manifestation of what is normally a developmental mechanism.

One ironic aspect of the LTP/LTD story is that both phenomena at Schaffer collateral synapses can be blocked by NMDA receptor antagonists. This suggests that calcium influx triggers both processes, and indeed current models of LTD induction hypothesize that

Figure 26 Neuromodulation of LTP induction. (a) Modulation of LTP induction by the beta-adrenergic agonist isoproterenol (ISO). Activity-dependent β-adrenergic modulation of low-frequency stimulation-induced LTP in the hippocampus CA1 region. In control experiments (no ISO), 3 min of 5-Hz stimulation (delivered at time = 0, open symbols, n = 26) had no lasting effect on synaptic transmission (45 min after 5-Hz stimulation, field EPSPs (fEPSPs) were not significantly different from pre–5-Hz baseline, t(25) = 1.01). However, 3 min of 5-Hz stimulation delivered at the end of a 10-min application of 1.0 mmol l^{-1} ISO (indicated by the bar) induced LTP (closed symbols, $p < 0.01$ compared with baseline). The traces are fEPSPs recorded during baseline and 45 min after 5-Hz stimulation in the presence and absence (control) of ISO. Calibration bars are 2.0 mV and 5.0 ms. Reproduced from Thomas MJ, Moody TD, Makhinson M, and O'Dell TJ (1996) Activity-dependent beta-adrenergic modulation of low frequency stimulation induced LTP in the hippocampal CA1 region. *Neuron* 17: 475–482, with permission. (b) One potential mechanism for neuromodulation is regulation of back-propagating action potentials in CA1 dendrites. The data shown illustrate amplification of dendritic action potentials by isoproterenol (1) and its susceptibility to inhibition by the protein kinase inhibitor H7 (2). The traces shown are from dendritic patch-clamp recordings from hippocampal pyramidal neurons. Muscarinic agonist (carbachol, (3)) and the dopamine receptor agonist 6-Cl-PB also can give various degrees of action-potential modulation as well. (1) Bath application of 1 μmol l^{-1} isoproterenol resulted in a 104% increase in amplitude, from 41 mV ('Pre') to 84 mV, of an antidromically initiated action potential recorded 220 μm from the soma. Wash-out of isoproterenol amplitude (38 mV; 'wash'). With a second application of isoproterenol (dark arrow labeled 'Iso'), the amplitude again increased twofold to 80 mV. (2) In a different recording 300 μmol l^{-1} H-7, a generic kinase inhibitor, was included in the control sine during the wash-out of isoproterenol. The subsequent second application of isoproterenol failed to lead to a second increase in amplitude (dark arrow labeled 'Iso + H7'). (3) In a distal recording (300 μM), 1 μmol l^{-1} carbachol increased the action potential amplitude by 81%, from 27–60 mV. In the carbachol experiments, cells were held hyperpolarized to –80 mV to remove Na+ channel inactivation. (4) One of the 6 out of 10 recordings where 6-Cl-PB led to an increase in amplitude. In a recording 220 μm from the soma, 10 μmol l^{-1} 6-Cl-PB increased dendritic action potential amplitude by 26%, from 21 to 26.5 mV. The cells were held at –70 mV in all 6-Cl-PB experiments. Adapted from Johnston D, Hoffman DA, Colbert CM, and Magee JC (1999) Regulation of back-propagating action potentials in hippocampal neurons. *Curr. Opin. Neurobiol.* 9: 288–292, with permission. (c) BDNF also modulates LTP induction in response to theta-frequency type stimulation. Two stimulating electrodes were positioned on either side of a single recording electrode to stimulate two different groups of afferents converging in the same dendritic field in CA1. Stimulation was applied to Schaffer collaterals alternately at low frequency (1 per min). After a period of baseline recording, LTP was induced with a theta-burst stimulation applied at time 0 only to one pathway (S1, filled squares). Simultaneous recording of an independent pathway (S2, open circles) showed no change in its synaptic strength after the theta burst was delivered to S1. BDNF (closed squares) selectively facilitates the induction of LTP in the tetanized pathway without affecting the synaptic efficacy of the untetanized pathway. EPSPs were recorded in the CA1 area of BDNF-treated slices. Synaptic efficacy (initial slope of field EPSPs) is expressed as a percentage of baseline value recorded during the 20 min before the tetanus. Representative traces of field EPSPs from S1 and S2 pathways were taken 10 min before and 40 min after the theta-burst stimulation. Adapted from Gottschalk W, Pozzo-Miller LD, Figurov A, and Lu B (1998) Presynaptic modulation of synaptic transmission and plasticity by brain-derived neurotrophic factor in the developing hippocampus. *J. Neurosci.* 18: 6830–6839, with permission.

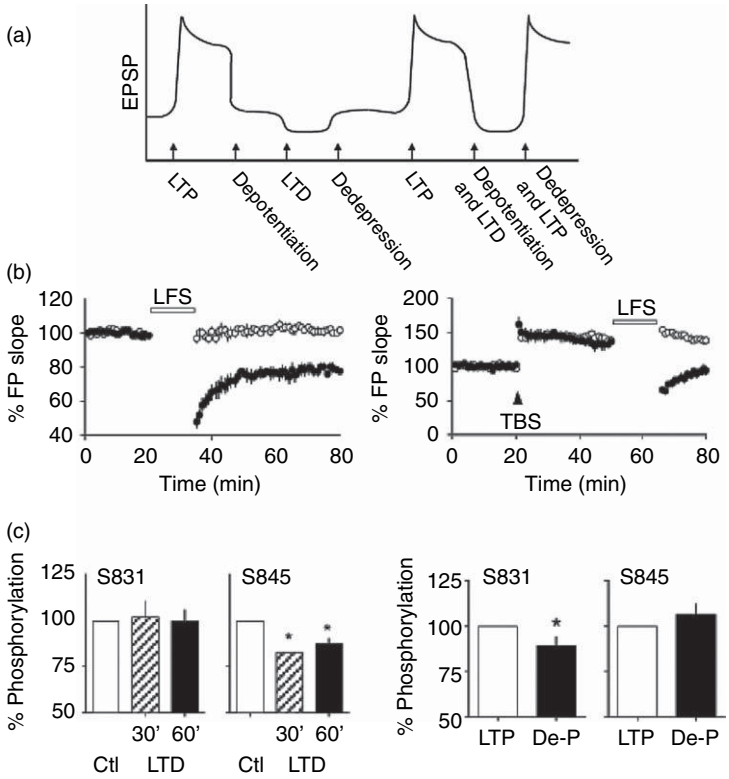


Figure 27 Depotentiation and LTD. (a) Schematic illustrating LTP, depotentiation, LTD, dedepression, and combinations of them. Figure by J. David Sweatt and Sarah E. Brown. (b) LTD and depotentiation in hippocampal neurons. Simultaneous recording of slices receiving baseline stimulation (control, open circles) and 1-Hz stimulation (closed circles). FP, Field potential. Regulation of distinct AMPA receptor phosphorylation sites during bidirectional synaptic plasticity. (c) Homosynaptic LTD in CA1 is associated with dephosphorylation of GluR1 at a PKA site (ser845). Depotentiation gives dephosphorylation at a CaMKII/PKC site (ser831). Adapted from Lee HK, Barbarosie M, Kameyama K, Bear MF, and Huganir RL (2000) Regulation of distinct AMPA receptor phosphorylation sites during bidirectional synaptic plasticity. *Nature* 405: 955–959, with permission from Elsevier.

LTD is caused by an influx of calcium that achieves a lower level than that needed for LTP induction. This lower level of calcium is hypothesized to selectively activate protein phosphatases and, by this mechanism, to lower synaptic efficacy.

Another very different type of LTD is cerebellar LTD. Cerebellar LTD occurs at synapses onto Purkinje neurons in the cerebellar cortex. Cerebellar LTD is a very interesting phenomenon because its behavioral role is much better understood than the hippocampal plasticity phenomena we are discussing throughout this book. Among other things, cerebellar LTD is involved in associative eye-blink conditioning, a cerebellum-dependent classical conditioning paradigm. Considerable progress has been made in investigating the roles and mechanisms of cerebellar LTD, as will be described in another chapter (*See* Chapter 4.17).

4.16.14 Summary

Like learning, LTP can be defined as a long-lasting change in output in response to a transient input. The persistence of this effect has been demonstrated to extend many hours *in vitro* and several weeks *in vivo*. We do not know how LTP relates to memory, and there is evidence for and against the hypothesis that hippocampal LTP is involved in memory. Regardless, it is the best-understood example of long-lasting synaptic plasticity in the mammalian CNS, and it is a model for how long-lasting memory-associated changes are likely to occur in the CNS. One premise of many chapters of this volume is that understanding LTP will yield valid insights into the mechanisms of plasticity that underlie learning and memory in the brain. The bona fide changes in neuronal connections that occur *in vivo* may or may not be identical to LTP as

it is presently studied in the laboratory, but this does not diminish its utility as a cellular model system for studying lasting neuronal change in the mammalian CNS.

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4.17 LTD – Synaptic Depression and Memory Storage

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4.17.1 Introduction

A widely held assumption among neuroscientists is that experience is capable of persistently modifying the properties of synapses, and that this use-dependent modification is central to both neuronal memory storage and the refinement of connections in brain development. This general idea was initially voiced by Sechenov and Cajal and was later formalized by Hebb (1949) in his famous synaptic modification postulate:

> When an axon of cell A is near enough to excite a cell B and repeatedly or persistently takes part in firing it, some growth process or metabolic change takes place in one or both cells such that A's efficiency, as one of the cells firing B, is increased. (Hebb, 1949)

However, it was not until many years later that an electrophysiological model system emerged that appeared to embody this idea of activity-dependent synaptic memories in the mammalian brain. Bliss and Lomo (1973) showed that brief, high-frequency

stimulation of a population of axons, the perforant path projection to the hippocampal dentate gyrus, produced an increase in the strength of these synapses which could last for hours. This phenomenon, called long-term potentiation (LTP), has since been seen to last for days to weeks in chronic preparations. The duration of LTP, together with its initial discovery in the hippocampus, a brain region known from behavioral studies to be important for the storage of declarative memory, produced a surge of interest in LTP as a putative cellular model system for memory. This interest was only increased when it became clear that under certain conditions LTP could display some of the formal properties of learning such as specificity (LTP is confined to activated synapses) and associativity (weak stimulation of an input to a postsynaptic cell will only induce LTP when paired with a neighboring strong input to that same cell).

While the first studies of LTP relied upon field potential recording in the intact hippocampus, this phenomenon has subsequently been observed in almost every type of glutamatergic synapse in the brain and has been extensively studied in reduced preparations such as brain slices and cultures of embryonic neurons. At the same time that LTP was gradually 'escaping' from the hippocampus, it was becoming clear that it was not the only form of use-dependent synaptic modification. The converse phenomenon, long-term depression (LTD) was also initially observed in the hippocampus before being found in other brain regions. At present, it appears likely that there are no synapses that express only LTP or LTD. In most synapses, LTP and LTD are typically evoked by brief, strong stimulation and sustained, weak stimulation, respectively. The direction of change in synaptic strength (LTP vs. LTD) is believed to be determined, at most types of synapses, by the amount of postsynaptic activity (as indexed by Ca influx) which occurs during induction: a small amount of postsynaptic Ca influx results in LTD, while a larger amount results in LTP (see Linden, 1999; Zucker, 1999, for review).

If LTP in the mature brain truly functions to underlie memory storage, then what is the function of LTD? One proposal has been that LTD is a "neuronal substrate of forgetting" (Tsumoto, 1993). While there is no definitive evidence to dispute this view, there is no definitive support for it either. A potentially more useful construct is to consider that information is likely to be stored in the brain, at least in part, as an array of synaptic weights. If these synapses are driven to their maximal or minimal strengths, then those elements of the array become limited in their ability to contribute to subsequent plasticity. Thus, neural circuits containing synapses that can actively both increase and decrease their strength are at a distinct computational advantage.

Experience-dependent refinement of connections during brain development can also potentially benefit from having both LTP and LTD mechanisms. Synapses which undergo strong, correlated activity can be strengthened and thereby retained, while synapses which have weak uncorrelated activity can be weakened and ultimately removed. Like memory storage, one could imagine that developmental refinement of connections could proceed using either LTD or LTP alone, but the presence of both allows for faster and more flexible change.

In this article we will not attempt to provide a comprehensive overview of LTD at the many synapses in the brain where it has been studied. Rather, we will focus on the two best-understood forms (LTD at the hippocampal Schaffer collateral/commissural-CA1 pyramidal cell synapse and LTD at the cerebellar parallel fiber–Purkinje cell synapse) as case studies to examine both the cellular processes which underlie LTD and its larger role in behavior and development.

4.17.2 LTD of the Hippocampal Schaffer Collateral-CA1 Synapse

The Schaffer collateral-CA1 synapse is widely used as a model synapse for the study of LTP and of synaptic plasticity in general. The hippocampus is not necessarily the brain area of choice when it comes to relating synaptic gain changes to their behavioral consequences, because it is many synapses from the sensory periphery. Nevertheless, the hippocampus does store certain types of information, and it is reasonable to expect that synaptic plasticity participates in this process (Riedel et al., 1999). Moreover, available evidence suggests that what has been learned about synaptic plasticity in the hippocampus is also widely applicable to synapses elsewhere in the brain. For example, it seems that synaptic plasticity at glutamatergic synapses onto some (but not all) neocortical pyramidal cells operates using similar rules for induction as its counterpart at CA1 hippocampal synapses.

Although the original discovery of LTP (in the dentate gyrus, by Terje Lømo) was accidental (the unexpected outcome of experiments designed to study synaptic responses during repetitive stimulation), it was quickly embraced as a potential synaptic

mechanism for memory (Bliss and Lomo, 1973). Excitement grew in the mid-1980s, when the properties of LTP in CA1 were shown to satisfy the requirements of Hebb's famous postulate that active synapses strengthen when their activity correlates specifically with a strong postsynaptic response (Wigstrom and Gustafsson, 1985; Kelso et al., 1986; Malinow and Miller, 1986; Sastry et al., 1986). However, theoreticians had concluded years before that 'Hebbian' synaptic modifications alone were not likely to be sufficient to account for memory storage; the efficient storage of information by synapses requires bidirectional synaptic modifications; i.e., LTD as well as LTP. Thus, the search for homosynaptic LTD in the hippocampus was theoretically motivated; it was not an accident (the reasons that it was not stumbled on accidentally will become clear in the discussion below). The theoretical suggestion was that synapses should depress when their activity *fails* to correlate with a strong postsynaptic response. To realize this situation experimentally, induction of LTD was attempted in CA1 using prolonged trains of presynaptic stimulation, delivered at frequencies (0.5–10 Hz) that fail to evoke a strong postsynaptic response (Dudek and Bear, 1992). Trains of low-frequency stimulation (LFS) are now the standard protocol for induction of homosynaptic LTD in CA1 and at synapses throughout the forebrain.

When discussing induction and expression mechanisms of LTD, it is important to note that LFS induces at least two, and possibly three (Berretta and Cherubini, 1998), mechanistically distinct forms of LTD in CA1, whose discovery solved previously existing contradictions (see Bear and Abraham, 1996). One form depends on the activation of *N*-methyl-D-aspartate receptors (NMDARs); another depends on the activation of group 1 metabotropic glutamate receptors (mGluRs), which are postsynaptic glutamate receptors coupled to phosphoinositide metabolism. These two forms will be described separately here.

4.17.3 Theoretical Framework

The bidirectional modification of excitatory synaptic transmission is not just an abstract theoretical construct. We need to understand mechanisms of bidirectional synaptic plasticity because direct experimental observations have shown, repeatedly, that synapses in the cerebral cortex are, in fact, bidirectionally modifiable. The value added by a theoretical structure is that it helps to make sense of what bidirectional synaptic plasticity accomplishes with respect to information storage and provides insight into how it might be implemented.

Neurons throughout the cerebral cortex, including area CA1 of the hippocampus, have stimulus-selective receptive fields. Chronic recording from cortical neurons has shown that as something new is learned, stimulus selectivity changes – some synaptic inputs potentiate and others depress. In CA1, for example, neurons show selectivity for positions in space, and this selectivity shifts rapidly as animals learn a new spatial environment (Breese et al., 1989; Wilson and McNaughton, 1993). What does a stable shift in selectivity tell us about memory? Neural network theory suggests that the selectivity shift reflects the creation of new neural representations. The memory is encoded by changing the pattern of synaptic weights across the network of neurons (Bear, 1996).

Now consider what happens when more new information is learned: stimulus selectivity (i.e., the pattern of synaptic weights) shifts further. An implication of this finding is that previously encoded memories can remain stable, even as the pattern of synaptic strengths is again modified to create new representations. According to this way of thinking, memory requires the episodic (if not continual) bidirectional modification of synaptic transmission to fine-tune the patterns of synaptic weights in the neural network. It is important to emphasize, of course, that in the absence of new learned information, synaptic weights must remain stable. Passive decay of synaptic weight (that is, back to an initial value that might be larger or smaller) leads to a loss of the stored representations.

The bidirectional modification of synaptic transmission obviously requires that individual synapses on neurons be capable of some form of LTP and some form of LTD. However, every theory of memory storage that assumes bidirectional synaptic modification places an important constraint on the mechanisms of LTP and LTD: reversibility. Consider the problem that would arise if the LTP and LTD mechanisms were distinct and irreversible. While it is true that synaptic weights could be fine-tuned initially by simple summation of the two independent processes, eventually saturation would occur as the synapses underwent rounds of bidirectional modification (see **Figure 6**). This problem does not occur if LTP and LTD are inverse processes mechanistically.

Now we come to the question of what distinguishes stimulation conditions that yield synaptic potentiation from those that yield synaptic depression. To specifically encode memory, synaptic modifications must depend on the presynaptic activation of the synapses bringing information into the network. In other words, the modifications must be 'homosynaptic.' The variables that determine the polarity or sign of the modification, in principle, could be the absolute amount of presynaptic activity, the concurrent level and timing of postsynaptic activity, or some combination of these variables. There are many abstract theoretical 'learning rules' based on these variables, but the most useful are those that attempt to account for what has actually been observed experimentally. One very influential proposal was made by Bienenstock, Cooper, and Munro (1982) in what is known as the BCM theory. In order to account for the development and plasticity of neuronal stimulus selectivity, they proposed that active synapses are potentiated when the total postsynaptic response exceeds a critical value, called the 'modification threshold' (qm), and that active synapses are depressed when the total postsynaptic response is greater than zero but less than qm. In addition, it was proposed that the value of qm varies as a function of the average integrated postsynaptic activity.

Once the requirements for LTP induction in CA1 had been elucidated, a specific physiological basis for the BCM theory became apparent. The proposal was made (1) that the term qm corresponds to the critical level of postsynaptic depolarization at which the Ca flux through the NMDAR exceeds the threshold for inducing LTP; (2) that LTD should be a consequence of presynaptic activity that consistently fails to evoke a postsynaptic Ca response large enough to induce LTP; and (3) that the postsynaptic threshold for LTP should vary depending on the stimulation history of the postsynaptic neuron (Bear et al., 1987). These hypotheses have now all been validated experimentally.

The BCM theory motivated the search for LTD using LFS (Dudek and Bear, 1992). The rationale was to provide a high level of presynaptic activity that did not evoke a large postsynaptic response. Critical variables for LTD induction in rat hippocampal slices proved to be the stimulation strength (it could not be so strong as to elicit orthodromic action potentials), healthy inhibition, stimulation frequency (<10 Hz), the number of stimuli (typically hundreds), and the age of the animal (greater magnitude before 35 days of age). LTD was found to be a very reliable phenomenon when the appropriate conditions were met. Even so, there was concern that LTD might be an artifact. First, there was heightened skepticism because several previous reports of LTD induction using different protocols had proven difficult to replicate in the hippocampus; second, the same LFS protocol that was found to induce LTD had previously been reported by others to be ineffective in altering baseline synaptic transmission; and third, synaptic depression could easily be dismissed as a pathological change rather than a form of synaptic plasticity. Most of these concerns faded when it was found that at least one form of LTD depended specifically upon activation of NMDARs and a rise in postsynaptic Ca, that the same synapses that showed LTD could subsequently be potentiated, and that LTD could be elicited *in vivo* (Dudek and Bear, 1992, 1993; Mulkey and Malenka, 1992; Thiels et al., 1994; Heynen et al., 1996; Debanne et al., 1997; Manahan-Vaughan, 1997).

It is now apparent that multiple forms of LTD exist in CA1, possibly at the same synapses. Remarkably, however, all forms of LTD can be elicited using variations of the LFS protocol, specifically under conditions that fail to evoke a large postsynaptic response. Thus, the hypothesis of homosynaptic LTD, inspired by the BCM theory, has been amply confirmed. As we will discuss further, it is also noteworthy that the NMDAR-dependent form of LTD appears to be the functional inverse of LTP, thus satisfying the theoretical requirement that synaptic modifications be both bidirectional and reversible. Thus, at least in theory, the mechanisms of LTD as well as LTP can contribute to the receptive field plasticity underlying memory storage in the hippocampus and elsewhere.

4.17.4 NMDAR-Dependent LTD

4.17.4.1 Induction by Calcium

Induction of homosynaptic LTD in CA1 using the standard 1-Hz LFS protocol *in vivo*, and under most experimental conditions *in vitro*, is blocked by NMDAR antagonists (Dudek and Bear, 1992; Mulkey and Malenka, 1992; Heynen et al., 1996; Manahan-Vaughan, 1997). Although this form of LTD shares with LTP a dependence upon NMDA receptor activation and a rise in postsynaptic Ca ion concentration, there is a systematic difference in the

type of stimulation that yields the two types of synaptic modification. This difference is easily demonstrated simply by varying the frequency of tetanic stimulation. In rat CA1, for example, 900 pulses at 0.5–3 Hz typically yields LTD, whereas the same amount of stimulation at frequencies greater than 10 Hz yields LTP (**Figure 1(a)**; Dudek and Bear, 1992). The different consequences of stimulation at different frequencies have been attributed to systematic differences in the postsynaptic Ca currents through the postsynaptic NMDA receptors. Indeed, it is now well established that the critical variables are postsynaptic depolarization and Ca entry, not stimulation frequency *per se*. For example, while 1-Hz stimulation normally produces LTD, postsynaptic hyperpolarization during conditioning prevents any change, and depolarization leads to induction of LTP (Mulkey and Malenka, 1992). Likewise, while high-frequency stimulation normally produces LTP, it produces LTD instead if delivered in the presence of subsaturating concentrations of an NMDA receptor antagonist (Cummings et al., 1996; **Figure 1(b), (c)**).

The appropriate activation of postsynaptic NMDARs appears to be sufficient to induce LTD; presynaptic activity is not necessary. This conclusion is supported by the observation that photolysis of caged extracellular glutamate (Kandler et al., 1998; Dodt et al., 1999) and brief bath application of NMDA (Lee et al., 1998; Kamal et al., 1999) induce LTD without concurrent presynaptic stimulation. In agreement with the idea that Ca passing through NMDARs is the trigger for LTD, photolysis of caged Ca in the postsynaptic neuron can also induce synaptic depression (Neveu and Zucker, 1996). This finding is important, as it indicates that LTD can be induced by Ca entry through the NMDAR without the need to invoke any other Ca-independent signaling or triggering process. Curiously, however, a modest, brief elevation in Ca concentration ([Ca]) was found to induce LTP and LTD with equal probability. Subsequent analysis suggests that LTD is most reliably induced (and LTP is never induced) by a modest ($\sim$0.7 µmol l^{-1}) but prolonged ($\sim$60 s) rise in [Ca]. LTP, in contrast, is most reliably induced by a large ($\sim$10 µmol l^{-1}) and brief ($\sim$3 s) increase in [Ca] (Yang et al., 1999). Although these findings are all consistent with the proposal that LTD and LTP are triggered by distinct Ca responses during NMDAR activation (Bear et al., 1987; Lisman, 1989; Artola and Singer, 1993; Bear and Malenka, 1994) and with imaging studies that confirmed

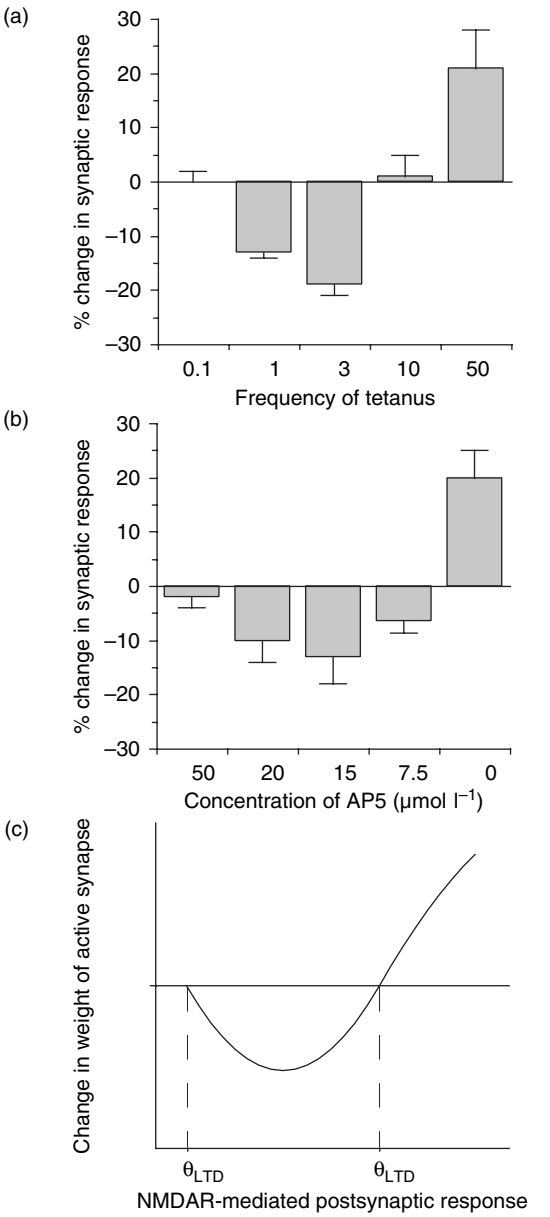

Figure 1 Induction of NMDAR-dependent bidirectional plasticity of the Schaffer collateral synapse in CA1. (a) Summary of the effects of a 900-pulse tetanus delivered at different frequencies (replotted from Dudek SM and Bear MF (1992) Homosynaptic long-term depression in area CA1 of hippocampus and effects of NMDA receptor blockade. *Proc. Natl. Acad. Sci. USA* 89: 4363–4367, with permission from the National Academy of Sciences, USA). (b) Summary of the effects of a 600-pulse, 20-Hz tetanus in different concentrations of the NMDA receptor antagonist AP5 (replotted from Cummings JA, Mulkey RM, Nicoll RA, and Malenka RC (1996) Ca signalling requirements for long-term depression in the hippocampus. *Neuron* 16: 825–833, with permission from Elsevier). (c) A synaptic 'learning rule' based on data such as those given in (a) and (b). This learning rule is formally similar to that proposed in the BCM theory.

that LTP-inducing stimuli elicit larger Ca transients than LTD-inducing stimuli (Hansel et al., 1997; Cormier et al., 2001), they argue against a simple relationship between synaptic modification and Ca level. The dynamics of the Ca response are also important determinants of the polarity of synaptic modification.

Perhaps not surprisingly, under certain circumstances voltage-gated Ca channels may also contribute to the Ca signal that triggers LTD (Christie et al., 1996). However, this role appears to be supplementary, since NMDAR activation is still required to observe homosynaptic LTD under these conditions, and Ca channel activation is not always necessary (Selig et al., 1995b). Nonetheless, the modulation of synaptic plasticity by active dendritic Ca conductances can be striking. Markram et al. (1997) made the remarkable observation that a back-propagating dendritic action potential, precisely timed to occur a few milliseconds before a synaptically evoked excitatory postsynaptic potential (EPSP), could promote induction of LTD in neocortical pyramidal neurons by stimuli that otherwise are ineffective. Similar findings have been reported in hippocampal cultures (Bi and Poo, 1998). The relative timing of coincident pre- and postsynaptic activity determines the amplitude of Ca transients in dendritic spines: Ca transients are larger when an EPSP precedes an action potential (AP) than when it follows it (Koester and Sakmann, 1998). As the former condition favors LTP induction (Markram et al., 1997), these results demonstrate that, under physiological conditions, larger Ca signals are indeed associated with LTP induction, whereas lower Ca signals more likely result in the induction of LTD.

An appealing hypothesis for this observation is that voltage-gated Ca influx inactivates NMDARs, thus causing a relatively lower Ca transient upon subsequent synaptic activation (Linden, 1999; Zucker, 1999). From a computational perspective, the observation that LTP results from EPSPs followed by APs, whereas LTD results from activation in the reverse order, provides a perfect correlate of Hebbian (and anti-Hebbian) concepts. APs following EPSPs suggest that the activated input repetitively and successfully contributed sufficient depolarization so that the spike threshold was reached, which is a Hebbian requirement for strengthening of that synapse. On the other hand, EPSPs following APs can be interpreted as random, uncorrelated presynaptic activity, leading to weakening of those synaptic inputs.

To summarize what has been learned to date, LTD is induced by an elevation of Ca that is constrained, apparently, by three variables: (1) proximity to the postsynaptic membrane, (2) peak concentration, and (3) duration. Synaptic stimulation causes LTD when it yields the appropriate response within the 'box' defined by these parameters. While LFS at 1 Hz is usually effective, under different experimental conditions different protocols may be required (e.g., Thiels et al., 1994; Debanne et al., 1994). Ca entry through the NMDA receptor is sufficient to induce LTD, but this can also be supplemented by other Ca sources (Christie et al., 1996; Reyes and Stanton, 1996).

4.17.5 The Role of Calcium-Dependent Enzymatic Reactions

A key component in the theory of synaptic memory formation is that synaptic efficacy is controlled by the phosphorylation state of alpha-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid receptors (AMPARs) which can mediate biophysical changes at the individual receptor level and/or can modify the insertion/internalization balance of AMPARs (for review see Song and Huganir, 2002). Induction of LTP requires activation of Ca-dependent serine-threonine protein kinases in the postsynaptic neuron. Key molecules in hippocampal LTP are Ca/calmodulin-dependent protein kinase II (CaMKII; Malenka et al., 1989; Malinow et al., 1989; Silva et al., 1992; Pettit et al., 1994; Lledo et al., 1995) and protein kinase C (PKC), whose ζ isoform is upregulated during LTP maintenance (Hrabetova and Sacktor, 1996). Lisman (1989) proposed that LTD might result from activation of a protein phosphatase cascade, leading to dephosphorylation of the same synaptic proteins that are involved in LTP. Subsequent experiments by Mulkey and coworkers confirmed an essential role for postsynaptic protein phosphatase 1 (PP1) and calcineurin (PP2B) in the induction of LTD with LFS (Mulkey et al., 1993, 1994).

The basic working hypothesis continues to be that LTD results from dephosphorylation of postsynaptic PP1 substrates and that CaMKII and PP1 act as a kinase/phosphatase switch, regulating the phosphorylation state of AMPARs and their auxiliary proteins (Lisman and Zhabotinsky, 2001; Malleret et al., 2001). As will be discussed, there is now direct evidence for dephosphorylation of synaptic proteins following

LTD induction protocols (Lee et al., 1998, 2000, 2003; Ramakers et al., 1999). Moreover, there is evidence that PP1 activity is persistently increased by LTD-inducing stimulation (Thiels et al., 1998) and that peptides that inhibit PP1 binding to target proteins block LTD induction (Morishita et al., 2001). PP1 is regulated by the protein inhibitor-1 (I-1). When I-1 is phosphorylated by protein kinase A (PKA), PP1 is inactive. Dephosphorylation of I-1 by PP2B releases PP1 from inhibition (Cohen, 1989; Nairn and Shenolikar, 1992). PP2B is activated by Ca/calmodulin and, therefore, is believed to be key for translating an increase in [Ca] into LTD (**Figure 2**).

Before moving on to consider expression mechanisms, it should be noted that there are also data suggesting that Ca/calmodulin triggers LTD by activating nitric oxide synthase (Izumi and Zorumski, 1993; Gage et al., 1997). The proposed mechanism of nitric oxide action is the retrograde activation of a second messenger cascade in the presynaptic terminal involving soluble guanylyl cyclase and cyclic guanosine monophosphate (cGMP)-dependent protein kinase (Reyes et al., 1999). This scenario is not universally agreed upon, however, since others report no effect of nitric oxide inhibitors on LTD (Cummings et al., 1994). At the present time, it appears that this mechanism lies in parallel with

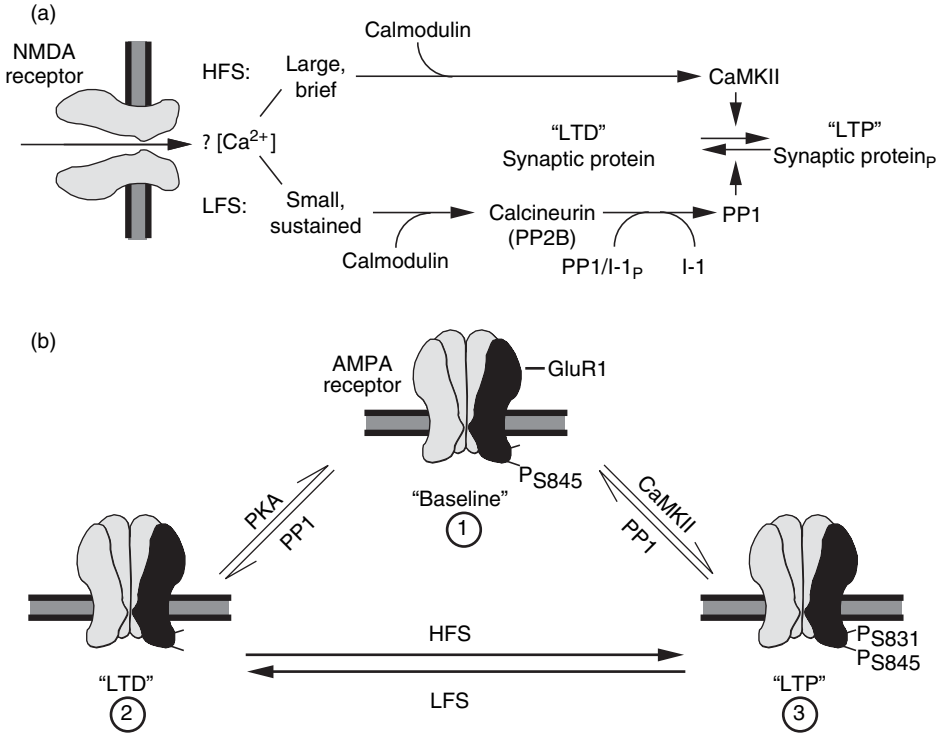

Figure 2 Molecular models for bidirectional plasticity of the Schaffer collateral synapse in CA1. (a) Induction. Strong NMDA receptor activation resulting from high-frequency stimulation (HFS) causes a large, brief rise in intracellular calcium. This calcium signal triggers activation of CaMKII and the induction of LTP by phosphorylation of synaptic proteins. Weak NMDA receptor activation resulting from low-frequency stimulation (LFS) produces a smaller but more sustained rise in intracellular calcium. This calcium signal selectively activates calcineurin which dephosphorylates inhibitor-1 (I-1). Dephosphorylation of I-1 relieves protein phosphatase 1 (PP1) from inhibition. LTD results from dephosphorylation of the same synaptic proteins involved in LTP. (b) Expression. (1) Under baseline conditions, the GluR1 subunit of AMPA receptors is highly phosphorylated at ser-845, a protein kinase A (PKA) substrate. (2) LFS causes dephosphorylation of ser-845 and LTD. (3) From the baseline state, HFS causes phosphorylation of ser-831, a CaMKII substrate, and LTP. According to this model, LTD and LTP result from bidirectional modifications of AMPA receptor phosphorylation, but at different sites. Thus, dedepression and LTP are not formally equivalent, nor are depotentiation and LTD. (4) The fourth possible state, in which ser-831 is phosphorylated and ser-845 is not, has not been observed experimentally. Adapted from Kameyama K, Lee HK, Bear MF, and Huganir RL (1998) Involvement of a postsynaptic protein kinase A substrate in the expression of homosynaptic long-term depression. *Neuron* 21: 1163–1175, with permission from Elsevier.

that involving postsynaptic phosphatase activation. It is plausible that nitric oxide signaling is involved in the mGluR-dependent form of LTD (discussed in the following section), but this remains to be examined explicitly.

4.17.5.1 Expression Mechanisms

It now seems very clear that a modification of postsynaptic glutamate sensitivity is a major expression mechanism for the NMDAR-dependent form of LTD. As mentioned earlier, liberating caged glutamate in CA1, under conditions where synaptic transmission is blocked, results in LTD of the glutatmate-evoked currents. This LTD is restricted spatially to the site of glutamate release and depends upon NMDAR activation and postsynaptic protein phosphatase activity (Kandler et al., 1998). The possibility remains that this form of LTD is actually expressed at extrasynaptic glutamate receptors and therefore could be mechanistically distinct from LFS-induced LTD. However, very similar findings have recently been obtained in rat neocortex where, in addition, it was shown that synaptically induced LTD results in a decrease in sensitivity to laser-stimulated photolysis of caged glutamate. Moreover, LFS-induced LTD occluded further synaptic depression by glutamate pulses (Dodt et al., 1999). The close similarities between CA1 and neocortical LTD (Kirkwood et al., 1993; Kirkwood and Bear, 1994) suggest that these findings may apply generally to NMDAR-dependent LTD in the cerebral cortex, at least in some layers.

A very interesting picture has emerged recently to account for decreased glutamate sensitivity following LTD. The model of bidirectional synaptic modification through reversible changes in the phosphorylation of postsynaptic substrates (Lisman, 1989; Bear and Malenka, 1994) begged the question of which synaptic phosphoproteins are involved. Now there is direct evidence that LTD is associated with dephosphorylation of AMPAR subunits, the consequence of which is known to be depression of glutamate-evoked currents. In addition, there are converging lines of evidence that LTD is associated with the removal of glutamate receptors from the postsynaptic membrane (for review see Song and Huganir, 2002; Collingridge et al., 2004). We shall discuss each of these mechanisms, in turn.

AMPARs are heteromeric complexes assembled from four homologous subunits (GluR1–4) in various combinations (Seeburg, 1993; Hollmann and Heinemann, 1994). The large majority of AMPARs in the hippocampus contain both GluR1 and GluR2 subunits (Wenthold et al., 1996). Hippocampal LTP largely rests on modifications of the GluR1 subunits, which determine trafficking behavior in GluR1/GluR2 heteromers (Song and Huganir, 2002). The GluR1 subunit is highly regulated by protein phosphorylation and contains identified phosphorylation sites on the intracellular carboxy-terminal domain. Serine-831 is phosphorylated by CaMKII and PKC, while serine-845 is phosphorylated by PKA (Roche et al., 1996; Barria et al., 1997a). Phosphorylation of either of these sites has been shown to potentiate AMPAR function through distinct biophysical mechanisms (Derkach et al., 1999). Phosphorylation site-specific antibodies have been used to measure the phosphorylation state of receptors *in situ* (Mammen et al., 1997). The PKA site shows higher basal phosphorylation than the CaMKII/PKC site (Lee et al., 1998).

To investigate the changes in AMPAR phosphorylation that occur following synaptic plasticity, Lee et al. (1998) devised a method to induce LTD chemically in hippocampus slices. The rationale behind this approach was to increase the probability of detecting biochemical changes by maximizing the number of affected synapses in the slice. They showed that brief bath application of NMDA induces synaptic depression (called chem-LTD) that shares a common expression mechanism with LFS-induced LTD. Biochemical analysis showed a selective dephosphorylation of serine-845 (the PKA site) following induction of chem-LTD. Phosphorylation of serine-831 (the CaMKII/PKC site), in contrast, was not altered by the treatment. This result was unexpected, since serine-831 is the site phosphorylated during LTP (Barria et al., 1997b). Thus, these findings contradict the simple notion that LTD and LTP reflect bidirectional changes in the phosphorylation of the same site. By refining the biochemical detection method, these findings have now been confirmed using synaptically induced LTD and LTP (Lee et al., 2000). LFS delivered to naive synapses causes dephosphorylation of the PKA site and LTD. Conversely, theta-burst stimulation (TBS) delivered to naive synapses causes phosphorylation of the CaMKII/PKC site and LTP. Both types of synaptic change are reversible, however. Thus, LFS delivered after prior induction of LTP causes dephosphorylation of the CaMKII/PKC site and depotentiation of the synaptic response. TBS delivered after prior induction of LTD causes phosphorylation of the

PKA site and de-depression of the synaptic response (**Figure 2**; Kameyama et al., 1998). This PKA-mediated recovery from depression is associated with AMPA receptor reinsertion (Ehlers, 2000).

Despite the progress that has been made in characterizing the phosphorylation events underlyling LTP and LTD induction, some important aspects still remain unsolved. While the CaMKII/PKC phosphorylation site serine-831 is phosphorylated during LTP (Lee et al., 2000) and this phosphorylation event leads to an increase in receptor conductance (Derkach et al., 1999), serine-831 phosphorylation does not affect receptor trafficking (Hayashi et al., 2000). A current hypothesis is that synaptic targeting of GluR1/GluR2 receptors ultimately requires CaMKII-mediated phosphorylation of a family of small transmembrane AMPAR regulatory proteins (TARPs), such as stargazin (for review see Nicoll et al., 2006). Phosphorylation at the PKA site serine-845 seems to act as a 'priming' step for GluR1 membrane insertion (Esteban et al., 2003). As described above, dephosphorylation at serine-845 occurs during LTD (Lee et al., 2000), and indeed promotes internalization of AMPARs (Lee et al., 2003). PKC also plays a role in the delivery of GluR1 subunits to synapses. In a recent study, it was demonstrated that a PKC (all isoforms)-mediated phosphorylation at serine-818 is required for GluR1 insertion and LTP (Boehm et al., 2006). Moreover, it has been shown that the constitutively active PKC isoform PKMζ promotes AMPAR membrane insertion and might be crucial for maintaining increased receptor numbers (Ling et al., 2006). Taken together, the available data suggest that all three GluR1 phosphorylation sites discussed here, namely serine-818, -831, and -845, are involved in the induction of LTP, which likely sets constraints for LTP-inducing stimuli, but also opens up routes to modify the probability for GluR1 insertion and LTP.

While the available evidence for the role of GluR1 insertion in LTP is compelling and the remaining questions mostly focus on the underlying phosphorylation steps, the events underlying hippocampal LTD induction are less clear. As pointed out earlier, it has been shown that dephosphorylation of the GluR1 subunit at serine-845 is involved in LTD and GluR1 endocytosis. In mutant mice, in which phosphorylation at serine-831 and -845 is prevented by mutations to alanine ('phospho-free mice') NMDAR-dependent LTD is blocked (Lee et al., 2003). This finding is consistent with earlier reports demonstrating that inhibition of PP2B blocks GluR1 internalization, which has been interpreted as showing that phosphatase activity is required for GluR1 endocytosis and LTD (Beattie et al., 2000). However, a recently promoted hypothesis gains weight, which states that LTP is mediated by membrane delivery of AMPA receptors with long cytoplasmic termini (i.e., GluR1-, GluR2L-, GluR4-containing AMPARs), whereas LTD is mediated by the endocytosis of AMPA receptors with short cytoplasmic termini (GluR2-containing AMPARs) (for review see Malinow, 2003). The refilling of receptor 'pools' required to maintain plasticity would then occur through a slow exchange of AMPARs (McCormack et al., 2006). Time will tell how these findings can be reconciled. However, there is indeed plenty of evidence showing that GluR2 subunits can cycle in and out of the membrane as well.

The carboxy terminus of GluR2 binds to N-ethylmaleimide-sensitive factor (NSF), a protein previously shown to play an essential role in membrane fusion events (Nishimune et al., 1998; Osten et al., 1998; Song et al., 1998; Lüscher et al., 1999; Noel et al., 1999). NSF-GluR2 binding promotes GluR2 insertion at hippocampal synapses (Lüscher et al., 1999). Under physiological conditions, NSF is activated by NO-mediated S-nitrosylation, which enables NSF to bind to GluR2, thus promoting GluR2 surface expression (Huang et al., 2005). Conversely, GluR2 phosphorylation at serine-880 promotes a clathrin-mediated GluR2 endocytosis (Man et al., 2000), which leads to LTD (Seidenman et al., 2003). GluR2 endocytosis involves unbinding of GluR2 from the glutamate receptor-interacting protein GRIP1 (Dong et al., 1997) and binding to protein interacting with C-kinase 1 (PICK1) (Xia et al., 1999; Chung et al., 2000).

The pool of NSF-regulated AMPARs appears to be required for expression of LTD, because LFS has no effect after these receptors are internalized (Lüscher et al., 1999; Luthi et al., 1999). Three lines of evidence suggest that AMPAR internalization is an expression mechanism for LTD. First, in hippocampal slices, prior saturation of LTD renders the AMPARs at the depressed synapses (but not at other synapses on the same neuron) insensitive to inhibitors of the NSF-GluR2 interaction (Luthi et al., 1999). Second, in hippocampal cell culture, field stimulation at 5 Hz causes an NMDAR-dependent depression of spontaneous miniature excitatory postsynaptic current (EPSC) amplitudes and the loss of surface-expressed GluR1 (Carroll et al., 1999). Third, in the adult hippocampus *in vivo*, there is an NMDAR-dependent loss

of GluR1 and GluR2 from the synaptoneurosomal biochemical fraction following induction of LTD (Heynen et al., 2000).

In keeping with the evidence cited, the magnitude of the postsynaptic response to quantal release of glutamate (the quantal size) is decreased after LTD (Oliet et al., 1996). However, in addition, a robust finding is that the number of quantal responses to synaptic stimulation (the quantal content) is also decreased (Stevens and Wang, 1994; Goda and Stevens, 1996; Oliet et al., 1996; Carroll et al., 1999). According to traditional assumptions, decreased quantal content reflects the failure of neurotransmitter release in response to a presynaptic action potential. It is interesting, therefore, that a decrease in quantal content is also a consequence of disrupting the NSF-GluR2 interaction (Luthi et al., 1999). Presumably this results from the total loss of AMPARs from some synapses. Thus, although presynaptic changes may also occur following LFS (Ramakers et al., 1999), there is apparently no need to invoke a presynaptic mechanism to account for the key properties of NMDAR-dependent LTD of AMPAR-mediated responses.

Another robust finding is that responses to activation of NMDARs are also depressed following LTD (Xiao et al., 1994, 1995; Selig et al., 1995a). While these observations are consistent with the parallel loss of AMPA receptors and NMDA receptors from synaptoneurosomes following LTD *in vivo* (Heynen et al., 2000), they could also reflect a component of LTD expression that is presynaptic. The GluR internalization in response to intracellular manipulations of the NSF-GluR2 interaction (Luthi et al., 1999), or of clathrin-mediated endocytosis (Lüscher et al., 1999; Man et al., 2000; Wang and Linden, 2000), has been restricted to AMPARs only. Thus, the mechanism for NMDAR regulation is apparently distinct from that for AMPAR regulation.

4.17.6 Modulation of LTD

As discussed, induction of LTD depends on postsynaptic phosphatase activation by Ca passing through NMDAR channels. Thus, it comes as no surprise that LTD is subject to modulation by factors that alter the Ca flux in response to synaptic stimulation and by factors that alter the intracellular enzymatic response to a change in Ca concentration.

An example of the first type of modulation is the effect of altered inhibition. Under some experimental conditions, reduced inhibition may be required to allow the NMDAR activation that is necessary to induce LTD (Wagner and Alger, 1995). However, under other conditions, reduced inhibition may suppress LTD in response to LFS at certain frequencies by facilitating induction of LTP instead (Steele and Mauk, 1999). Similarly, the conditions required for LTD induction depend on the properties of postsynaptic NMDARs. For example, overexpression of the NR2B subunit, which leads to a prolongation of NMDAR-mediated synaptic currents, changes the frequency-response function to promote LTP and suppress LTD across a range of stimulation frequencies (Tang et al., 1999). Modulation of inhibition and NMDAR subunit composition are physiologically relevant, as these parameters change during development and are regulated by activity.

The intracellular response to a change in Ca depends on the availability of Ca binding proteins, such as calmodulin, and on the location, concentration, and activity of the kinases and phosphatases that regulate synaptic strength. Mutations that alter these parameters have been shown to enhance (Mayford et al., 1995) or disrupt (Brandon et al., 1995; Qi et al., 1996; Migaud et al., 1998) LTD. PKA seems to play a pivotal role in the intracellular regulation of LTD. According to the current model for LTD induction, activation of PKA would be expected to inhibit LTD by preventing the activation of PP1 (via I-1 phosphorylation) and by maintaining AMPAR phosphorylation at a high level. Regulation of LTD via PKA is also physiologically relevant. For example, there is evidence that PKA activation in response to stimulation of noradrenergic b-receptors shifts the frequency response function to favor LTP over LTD (Blitzer et al., 1995, 1998; Thomas et al., 1996; Katsuki et al., 1997). Conversely, activation of muscarinic acetylcholine receptors facilitates LTD (Kirkwood et al., 1999), possibly by PKC-mediated inhibition of adenylyl cyclase (Stanton, 1995; Nouranifar et al., 1998).

Other variables that impact LTD are postnatal age and the behavioral state of the animal. Although the mechanism remains to be determined, it is well established that the magnitude and reliability of LTD decline with increasing age (Dudek and Bear, 1993; Errington et al., 1995; Wagner and Alger, 1995a; Kamal et al., 1998), supporting the idea that this mechanism plays an important role in the refinement of circuits during critical periods of development (Rittenhouse et al., 1999). However, the existence of LTD in the adult hippocampus has been the subject of some controversy, with some labs reporting

success (Thiels et al., 1994; Heynen et al., 1996; Manahan-Vaughan, 1997) and others reporting failure (Errington et al., 1995; Doyle et al., 1997; Staubli and Scafidi, 1997) to observe LTD *in vivo*. Resolution of this controversy may now be at hand. First, it has been established that there are rat strain differences in the expression of LTD (Manahan-Vaughan and Braunewell, 1999). Second, and most importantly, it has been shown that LTD is powerfully modulated by the behavioral state of the animal. When LTD-resistant animals are exposed to mild stress (Kim et al., 1994; Xu et al., 1997), or even simply to a novel environment (Manahan-Vaughan and Braunewell, 1999), there is a striking facilitation of LTD. The stress effect may be mediated by glucocorticoids (Coussens et al., 1997; Xu et al., 1998b); the mechanism for the novelty effect is unknown, although modulation by acetylcholine has been suggested (Bear, 1999). Whatever the mechanism, the results show that brain-state is a crucial variable that must be controlled during studies of LTD in adults.

The effect of novelty exposure on LTD is particularly interesting. When LFS is delivered as animals explore a novel environment, the resulting LTD lasts for weeks regardless of the strain of rat. However, the facilitation of synaptic plasticity is so marked that the usual 1-Hz tetanus is no longer required to induce LTD. The low-frequency electrical stimulation normally used to monitor synaptic transmission (5 pulses given at 0.1 Hz every 5 min for 15 min) is enough to significantly depress synaptic transmission for several hours if it is delivered during novelty exposure. An exciting possibility is that the pattern of electrical stimulation imposed on the brain during the novel experience is incorporated into the memory of that experience, and this memory is stored as LTD of the synapses that were active at that time. Indeed, recordings from neurons in the temporal lobes have consistently revealed that a cellular correlate of recognition memory is a diminished response to the learned stimulus (Xiang and Brown, 1998). Perhaps this reduced response, and the memory trace, is accounted for by the mechanisms of homosynaptic LTD.

A final type of LTD modulation was suggested by the BCM theory. The idea was that the value of qm (the LTD-LTP crossover point) should vary depending on the history of the integrated postsynaptic response (Bear et al., 1987; Bear, 1995). After periods of strong postsynaptic activity, the modification threshold slides to promote LTD over LTP; after periods of postsynaptic inactivity, the threshold adjusts to promote LTP over LTD. In this way, the properties of synaptic plasticity adjust to keep the network of modifiable synapses within a useful dynamic range. There is now compelling evidence from a number of systems that the stimulation requirements for induction of LTD are indeed altered by prior postsynaptic activity (Kirkwood et al., 1996; Holland and Wagner, 1998; Wang and Wagner, 1999). The mechanisms for this plasticity of synaptic plasticity, or 'metaplasticity' (Abraham and Bear, 1996), remain to be determined, but the obvious candidates are clear from this discussion of LTD modulation. Changes in inhibition (Huang et al., 1999b; Steele and Mauk, 1999), NMDAR properties (Quinlan et al., 1999), and the balance of postsynaptic kinases and phosphatases (Mayford et al., 1995; Migaud et al., 1998) have all been proposed as mechanisms for the sliding modification threshold of the BCM theory.

4.17.7 mGluR-Dependent LTD

4.17.7.1 Induction

In addition to activating ionotropic receptors, glutamate stimulates G-protein coupled mGluRs. There are three classes of mGluR, defined by their pharmacology and coupling to second messenger pathways (Pin and Bockaert, 1995). Group 1 mGluRs (designated mGluR1 and mGluR5) stimulate phosphoinositide (PI) turnover via activation of phospholipase C (PLC). It is of historical interest to note that, based on theoretical considerations, the proposal was made that PI-coupled mGluRs play a role in triggering synaptic depression in the cerebral cortex (Dudek and Bear, 1989), and that the protocol of using LFS to induce homosynaptic LTD was designed originally with the aim of testing this hypothesis (Dudek and Bear, 1992). Although this early work implicated NMDARs instead, it was not long before a role for mGluRs was suggested for LTD. In particular, Bolshakov and Siegelbaum (1994) found that the mGluR antagonist α-methyl-4-carboxyphenylglycine (MCPG) prevents homosynaptic LTD in response to LFS in slices from very young rats (postnatal day 3–7). LTD in this preparation also required a rise in intracellular [Ca] and activation of voltage-gated Ca channels during LFS, but not activation of NMDARs.

Confusion about mGluR involvement in LTD persisted for a number of years due to some failures to replicate (Selig et al., 1995b), exacerbated by the finding that MCPG is actually a very weak antagonist of the action of glutamate at mGluR5 (Brabet et al., 1995; Huber et al., 1998). Fortunately, the smoke has now

cleared. First, it is now clear that the activation of mGluRs necessary to induce LTD is often not achieved using the usual 1- to 5-Hz stimulus trains. Protocols that work reliably are those that enhance glutamate release during conditioning stimulation, such as delivering prolonged trains of paired pulses (Kemp and Bashir, 1997a), or by antagonizing the adenosine inhibition of glutamate release (de Mendonca et al., 1997; Kemp and Bashir, 1997b). These protocols produce LTD of large magnitude with a component that cannot be blocked with NMDAR antagonists. Second, the use of new, potent mGluR antagonists and genetically altered mice has established that the NMDAR-independent LTD requires activation of mGluR5, and, conversely, that induction of NMDAR-dependent LTD does not (Bortolotto et al., 1999; Huber et al., 2001; Sawtell et al., 1999).

It has now been established that activation of group 1 mGluRs induces LTD by a mechanism that is entirely distinct from that engaged by NMDAR activation (Oliet et al., 1997). This mGluR-dependent LTD (mGluR-LTD) can be induced by synaptic stimulation in the presence of NMDAR antagonists, or by simple pharmacological activation of mGluRs using the group 1 mGluR-selective agonist DHPG ((RS)-3,5-dihydroxyphenylglycine) (Fitzjohn et al., 1999; Huber et al., 2001). Remarkably, the specific group 1 mGluRs involved differ for chemically and synaptically induced LTD. Whereas DHPG-induced LTD involves mGluR1 and mGluR5 activation, synaptically induced LTD is only mGluR5-dependent (Volk et al., 2006). The requirement of voltage-gated Ca entry for induction of mGluR-dependent LTD (mGluR-LTD) by synaptic stimulation has been confirmed, although the type of channel (L- or T-type) apparently varies depending on the circumstances (Bolshakov and Siegelbaum, 1994; Oliet et al., 1997; Otani and Connor, 1998). In addition, synaptically induced mGluR-LTD requires activation of postsynaptic PLC (Reyes and Stanton, 1998) and PKC (Bolshakov and Siegelbaum, 1994; Oliet et al., 1997; Otani and Connor, 1998). Unlike the NMDAR-dependent LTD (NMDAR-LTD), mGluR-LTD is not affected by inhibition of postsynaptic PP1 (Oliet et al., 1997).

Biochemical experiments suggest that activation of group 1 mGluRs in synaptoneurosomes stimulates, in a PKC-dependent manner, the aggregation of ribosomes and mRNA, and the synthesis of the fragile X mental retardation protein (Weiler and Greenough, 1993; Weiler et al., 1997). Thus, it is of considerable interest that mGluR-LTD is prevented by manipulations that interfere with protein synthesis. Huber et al. (2001) have shown that induction of mGluR-LTD is prevented by the postsynaptic inhibition of mRNA translation during conditioning stimulation. Because the LTD is homosynaptic and occurs even when the dendrites are isolated from their cell bodies, a requirement for rapid, synapse-specific synthesis of proteins from preexisting mRNA is strongly suggested. These findings are consistent with a number of converging lines of evidence suggesting a major role for mRNA translation in the mechanisms of mGluR5 action (Merlin et al., 1998; Raymond et al., 2000).

The discovery of polyribosomes at the base of dendritic spines has long invited speculation that synaptic activity regulates the protein composition, and therefore function, of synapses in the brain (Steward et al., 1988). Available data now indicate that mGluR5 activation triggers synapse-specific mRNA translation, and that one functional consequence is LTD (**Figure 3**). The obvious questions to be examined next concern the mechanism of translation regulation, the identity of the essential transcripts, and the mechanism that couples new protein synthesis to a change in synaptic function. The mGluR-LTD model should prove extremely valuable for answering these questions.

4.17.7.2 Expression

At the present time, more is known about how mGluR-LTD is *not* expressed than about how it is expressed. Specifically, mGluR-LTD is not expressed via the same mechanism as NMDAR-LTD. This conclusion is supported by the finding that the two forms of LTD are additive and do not mutually occlude one another. Moreover, while NMDAR-LTD is reversed by induction of LTP (and vice versa), mGluR-LTD is not (Oliet et al., 1997; Fitzjohn et al., 1999; Huber et al., 2001). It has been reported that after mGluR-LTD the quantal content, but not the quantal size, is reduced (Bolshakov and Siegelbaum, 1994; Oliet et al., 1997), consistent with a presynaptic expression side. However, this observation could also be explained by an all-or-none loss of postsynaptic AMPARs at individual synapses (all-or-none because a graded decrease would be reflected in a decrease in quantal size). Studies on the phosphorylation state of AMPARs in mGluR-LTD could not provide a consistent view on the expression side of mGluR-LTD so far. Whereas one study reported that

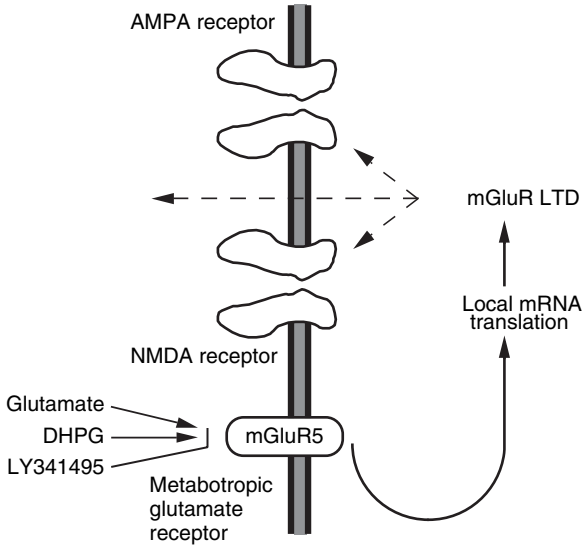

Figure 3 Model for metabotropic glutamate receptor-dependent LTD (mGluR-LTD) in CA1. Activating mGluR5 during synaptic stimulation, or by the selective agonist DHPG, triggers LTD that can be prevented by the selective mGluR antagonist LY341495. Synaptically evoked mGluR-LTD requires local translation of preexisting mRNA.

mGluR-LTD, unlike NMDAR-LTD, is not associated with a dephosphorylation of AMPA receptors (Huber et al., 2001), another found that in DHPG-induced LTD the activation of tyrosine phosphatases leads to a tyrosine dephosphorylation of AMPARs and their subsequent endocytosis (Moult et al., 2006). A possible explanation for this discrepancy might be that a developmental switch occurs between the second and the third postnatal week from a pre- to a postsynaptic expression side of mGluR-LTD (Nosyreva and Huber, 2005).

4.17.8 Depotentiation

The term 'depotentiation' refers to the reversal of previously established LTP, which can be elicited by variations of the LFS protocol. Confusion has arisen because the same term has been used to describe two phenomena. One type of depotentiation, of course, is homosynaptic LTD of synapses from a potentiated baseline, which can be induced at any time following LTP induction and utilizes the same mechanisms discussed above. The second type of depotentiation refers to the disruption of LTP that occurs when LFS is delivered within a relatively brief time window immediately following LTP induction. This time-sensitive depotentiation apparently is caused by interference with the transient intracellular biochemical reactions that are required to 'fix' LTP in the period that follows strong NMDAR activation.

4.17.8.1 Time-Sensitive Depotentiation

High-frequency synaptic stimulation (HFS) typically induces LTP in CA1. However, establishment of stable LTP is prevented if the HFS is followed by certain types of synaptic stimulation including, but not restricted to, LFS (Hesse and Teyler, 1976; Barrionuevo et al., 1980; Arai et al., 1990; Staubli and Lynch, 1990; Fujii et al., 1991; Barr et al., 1995; Holscher et al., 1997). This retrograde disruption of LTP is time dependent. LFS within 5 min of HFS can completely prevent LTP; however, the same stimulation may have no effect when delivered 1 h after HFS.

A clear picture of the mechanism for time-sensitive depotentiation (TS-DP) has finally emerged (**Figure 4**). Although the upstream regulation varies depending on the type of stimulation used, the critical downstream requirement for TS-DP is activation of postsynaptic PP1 during the sensitive period (O'Dell and Kandel, 1994; Staubli and Chun, 1996; Huang et al., 1999a). This period coincides with the time when LTP can be disrupted by inhibition of protein kinases (Huber et al., 1995). Thus, the data indicate that stable establishment of LTP requires the active serine-threonine phosphorylation of synaptic substrates for a defined time period. If this phosphorylation is prevented or reversed, so is LTP.

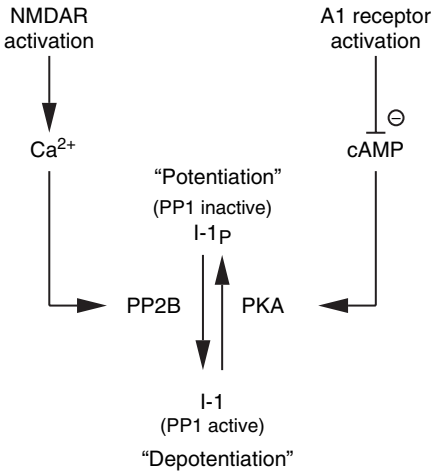

Figure 4 Converging pathways to depotentiation. Depotentiation occurs if synapses are given LFS in a narrow time window immediately following induction of LTP. Available evidence suggests that depotentiations results from dephosphorylation of postsynaptic proteins by protein phosphatase 1 (PP1). PP1 is activated when inhibitor 1 (I-1) is dephosphorylated by calcineurin (PP2B) at a PKA site. Stimuli that cause depotentiation include those that activate PP2B (e.g., NMDAR activation) and those that inhibit PKA (e.g., adenosine A1 receptor activation).

Upstream regulation of TS-DP can occur in different ways. One route for TS-DP induction appears to be the now-familiar pathway involving NMDAR stimulation (Fujii et al., 1991; O'Dell and Kandel, 1994; Barr et al., 1995; Xiao et al., 1996) followed by activation of PP2B and dephosphorylation of I-1 (O'Dell and Kandel, 1994; Zhuo et al., 1999). A second route to TS-DP induction, also leading to dephosphorylation of I-1, is the activation of A1 adenosine receptors (Larson et al., 1993; Staubli and Chun, 1996; Fujii et al., 1997; Huang et al., 1999a). A1 receptor activation inhibits adenylyl cyclase and, as a consequence, PKA (Dunwiddie and Fredholm, 1989). Injection of PKA activators into the postsynaptic neuron can prevent depotentiation caused by A1 receptor activation (Huang et al., 1999a).

Despite the apparent mechanistic similarities of TS-DP and NMDAR-LTD, the two phenomena differ. For example, TS-DP shows much less developmental regulation than LTD, and the patterns of activity that are optimal for induction are different (O'Dell and Kandel, 1994). In addition, TS-DP shows greater sensitivity to PP1 inhibitors and to deletion of the Aα isoform of PP2B than does LTD (O'Dell and Kandel, 1994; Zhuo et al., 1999). Interestingly, however, like LTD, TS-DP *in vivo* is dramatically facilitated by exposure of animals to a novel environment (Xu et al., 1998a).

A simple way to reconcile the findings is if we assume that LTP induction results in the transient exposure of postsynaptic phosphorylation sites to both protein kinases and phosphatases. Normally, the kinase activation that follows strong NMDAR stimulation leads to a net phosphorylation of these sites and LTP. However, the sites are also vulnerable to dephosphorylation if PP1 is activated. Because the phosphorylation sites are exposed, the threshold level of PP1 activation for TS-DP is much lower than that for NMDAR-LTD. Thus, while stimulation that induces LTD also can always produce TS-DP, the converse is not true. However, the common requirement for PP1 activation makes both forms of synaptic modification subject to very similar types of modulation.

4.17.8.2 Time-Insensitive Depotentiation

Under conditions in which *de novo* LTD is induced by LFS, the same stimulation can also reverse LTP that was induced hours before. The precise mechanism of LTD and LTP reversal may not be identical, however. For example, LTD *de novo* is associated with the dephosphorylation of GluR1 at a PKA site. However, the same induction protocol given 1 h after induction of LTP causes dephosphorylation of the CaMKII/PKC site instead (Lee et al., 2000; see **Figure 2(b)**). On the other hand, both LTD and LTP reversal *in vivo* are associated with a parallel decrease in AMPAR and NMDAR protein in the synaptoneurosomal biochemical fraction (Heynen et al., 2000).

4.17.9 LTD of the Cerebellar Parallel Fiber–Purkinje Cell Synapse

The second type of synapse that we want to use as an example to discuss LTD mechanisms is the parallel fiber–Purkinje cell synapse in the cerebellum. For comparison, we will also describe a more recently characterized form of LTD at the climbing fiber–Purkinje cell synapse. Why bother to study synaptic plasticity in an obscure and atypical part of the brain like the cerebellum? The answer is that it is one of only two locations where learning and memory can be understood at the level of circuits (the other being the amygdala; for comparison see Medina et al., 2002). In contrast, the hippocampus, for all of its experimental utility, receives information that is so

highly processed that its content cannot be easily characterized (what is the nature of the information conveyed by the perforant path?).

4.17.10 Cerebellar Anatomy and Some Useful Models

The cerebellum functions largely to integrate various forms of sensory information to smooth and fine-tune complex voluntary movements and reflexes (see Ito, 1984, for review). Therefore, cerebellar damage in humans is associated not with outright paralysis, but rather with dysmetric and ataxic syndromes, as well as impairments in motor learning. In addition, some recent work on human cerebellar lesions complemented by functional imaging studies has implicated the cerebellum in certain forms of non-motor procedural learning as well (see Schmahmann, 1997, for review).

The cerebellum comprises ~10% of the total weight of the human brain, but contains >50% of the total number of neurons, packed into the most infolded and convoluted structure in the brain. This degree of specialization suggests that, throughout evolution, fast, accurate, coordinated movements have been highly adaptive. To coordinate many joints and muscles, it is necessary that sensory and proprioceptive signals from any location in the body or sensory world be able to influence motor commands to any muscle in the body. Essentially, this requires a giant switchboard, which is implemented in the following way. The cerebellar circuitry is essentially composed of a relay station in the deep cerebellar nuclei (DCN) and a cortical 'side-loop' (see **Figure 5(a)**). The neurons of the DCN receive their main excitatory drive from glutamatergic mossy fibers which are the axons of a large number of precerebellar nuclei. The main outflow of information from this structure is carried by excitatory axons which originate from the large neurons of the DCN and project to premotor areas including the red nucleus and thalamus. In addition, there are small projection neurons in the DCN which are GABAergic (Kumoi et al., 1988; Batini et al., 1992) and send axons to the inferior olive (Fredette and Mugnaini, 1991).

The sole output of the cortical side-loop is the inhibitory, GABAergic (GABA: gamma-aminobutyric acid) projection from Purkinje cells to the neurons of the DCN (both large and small projection neurons are innervated; see De Zeeuw and Berrebi, 1995; Teune et al., 1998). Purkinje cells receive two major excitatory inputs, which are organized in very different ways. Each Purkinje cell is innervated by a single climbing fiber. This climbing fiber, which originates in the neurons of the inferior olive, will innervate ~10 Purkinje cells. This is potentially the most powerful synaptic contact in the brain, as each Purkinje cell receives ~1400 synapses from a single climbing fiber axon (Strata and Rossi, 1998). Climbing fibers also provide a very weak innervation of the DCN, consisting of a few synapses in the most distal dendrites, the function of which is poorly understood. In contrast, each Purkinje cell receives ~200 000 synapses from parallel fibers, which are the axons of granule cells. Because of the large number of granule cells (~50 billion) and the divergent output of their parallel fibers (each contacts ~1000 Purkinje cells), this synapse is the most abundant of any in the brain. Closing the loop, granule cells receive excitatory synapses from branches of the same mossy fibers which innervate the DCN directly. Because there are ~10 000-fold more granule cells than DCN cells, the innervation of granule cells by mossy fibers is highly convergent.

Putting this circuit together, it appears as if cerebellar output is driven by direct excitatory input from the mossy fibers and is modulated by the inhibitory input from the Purkinje cell axons, the latter of which will reflect computations and interactions in the Purkinje cell. These computations will be performed upon very subtle and informationally rich excitatory parallel fiber input and massive, synchronous excitation produced by the climbing fiber. This striking anatomical organization has inspired some notable models of motor learning. In particular, Marr (1969) proposed that the parallel fiber–Purkinje cell synapses could provide contextual information, that climbing fiber–Purkinje cell synapses could signal an 'error' in motor performance that required alteration of subsequent behavior, and that the conjunction of these two signals could strengthen the parallel fiber–Purkinje cell synapse to create a memory trace for motor learning. This model was modified by Albus (1971), who noted that a decrease in synaptic strength would be more appropriate, given the sign-reversing function of the Purkinje cell inhibitory output. Importantly, Albus also noted that this model is analogous to classical conditioning, with the parallel fibers conveying a conditioned stimulus (CS), the climbing fiber an unconditioned stimulus (US), and a depression of the parallel fiber–Purkinje cell synapse giving rise to a conditioned response (CR) via disinhibition of

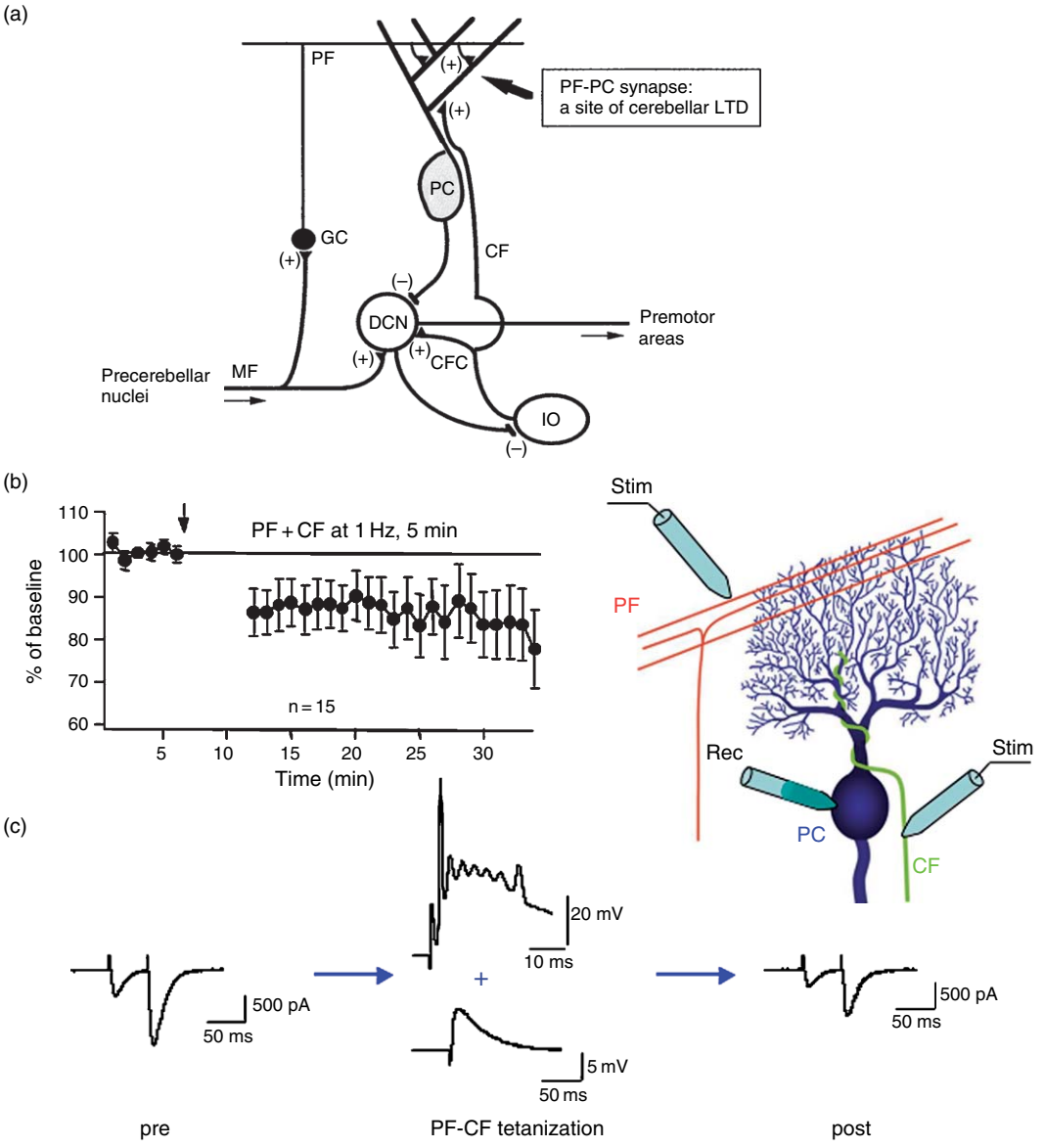

Figure 5 Basic cerebellar functional anatomy and LTD of the parallel fiber–Purkinje cell synapse. (a) A simplified diagram of cerebellar circuitry. Information flow through the main relay pathway consisting of precerebellar nuclei, their axons, the mossy fibers (MF), their targets in the deep cerebellar nuclei (DCN), and DCN excitatory axons projecting to premotor centers, is indicated with arrows. Excitatory synapses are denoted with a (+) and inhibitory synapses with a (−). IO, inferior olive; CF, climbing fiber; CFC, climbing fiber collateral; PC, Purkinje cell; GC, granule cell; PF, parallel fibers. (b) Left: parallel fiber LTD is obtained after paired parallel fiber and climbing fiber tetanization (1 Hz, 5 min; n = 15). Arrow indicates the time point of tetanization. Right: Diagram showing the electrode arrangement used for LTD induction *in vitro.* Whole-cell patch-clamp recording (rec) is used to monitor electrical responses to parallel fiber (PF) and/or climbing fiber (CF) stimulation. For extracellular stimulation, glass pipettes are used (stim) that are filled with ACSF. (c) Test responses to PF stimulation are recorded before and after tetanization in voltage-clamp mode. The paired-pulse facilitation ratio is monitored to screen for presynaptic changes. For tetanization, recordings are switched to current-clamp mode. CF activation results in a typical complex spike (top). The PF-EPSP is shown here in isolation (bottom), but in these recordings is masked by the complex spike. Following tetanization, the PF-EPSC amplitude is reduced.

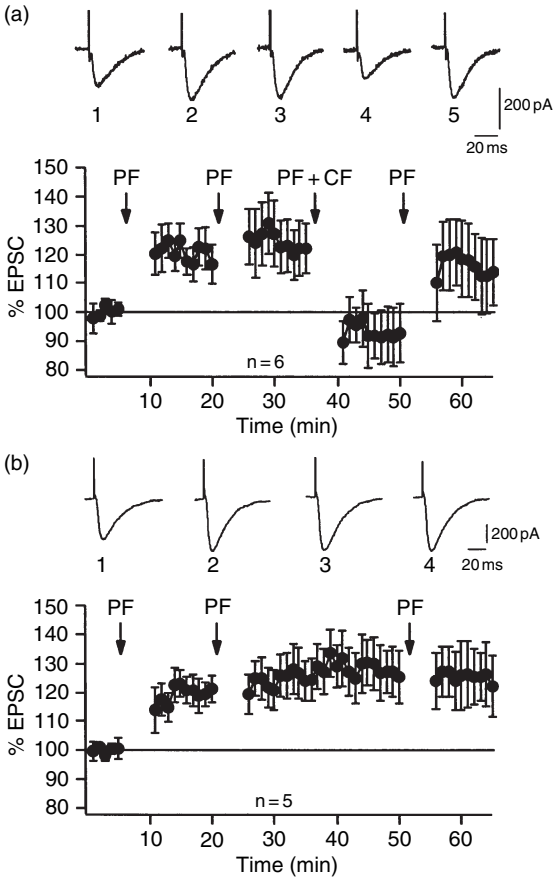

Figure 6 Reversal of parallel fiber LTP by LTD. (a) Saturated LTP is reversed by the application of the LTD protocol. Two LTP protocols were followed by an LTD protocol and, finally, by a third LTP protocol (n = 6). The LTD protocol consists of a paired parallel fiber and climbing fiber stimulation at 1 Hz for 5 min. The LTP protocol consists of the same parallel activation pattern in the absence of climbing fiber activation. Tetanization periods are indicated by the arrows. (b) Omission of the LTD protocol reveals LTP saturation after the application of two LTP protocols (n = 5). In (a) and (b), traces on top show EPSCs from the time points indicated. This figure is taken from Coesmans M, Weber JT, De Zeeuw CI, and Hansel C (2004) Bidirectional parallel fiber plasticity in the cerebellum under climbing fiber control. *Neuron* 44: 691–700, with permission from Elsevier.

the DCN. To place this model in a behavioral context, let us consider a well-characterized form of classical conditioning, associative eyeblink conditioning in the rabbit. Before training, an airpuff to the eye (US) gives rise to an immediate reflexive blink (the unconditioned response, UR). During training, a neutral stimulus such as a tone (CS) is paired with the airpuff stimulation so that the tone onset precedes the airpuff and the two stimuli coterminate. As the rabbit acquires the association, it performs a blink carefully timed to immediately precede the airpuff (CR). This associative learning can also be actively reversed. In well-trained animals which reliably perform CRs, this response can undergo rapid extinction if tone stimuli are repeatedly presented without airpuffs.

4.17.11 The Role of the Cerebellum in Associative Eyeblink Conditioning

There is extensive evidence to support the involvement of cerebellar circuits in associative eyeblink conditioning (see Kim and Thompson, 1997, for review). Similar evidence implicates the cerebellum in other forms of motor learning such as limb-load adjustment and adaptation of the vestibulo-ocular reflex (VOR; du Lac et al., 1995; De Zeeuw et al., 1998). Extracellular recording showed that populations of cells in the nucleus interpositus (a particular portion of the DCN) discharge during the UR before training and, in well-trained animals, begin to fire during the CS-US interval. This firing is predictive of and correlated with the performance of the CR, suggesting that the CR behavior is expressed in the firing rate and pattern of DCN neurons (McCormick and Thompson, 1984a,b; Berthier and Moore, 1986, 1990). This notion is further supported by the finding that microstimulation in the appropriate region of the nucleus interpositus elicited a strong eyelid response in either trained or untrained animals (McCormick and Thompson, 1984a). Moreover, during training, stimulation of mossy and climbing fibers can substitute for the CS and US, respectively (Mauk et al, 1986; Steinmetz et al., 1986, 1989).

The data obtained using lesions and reversible inactivation have been somewhat more complex (see Mauk, 1997, for review). A Marr/Albus model would predict that lesions of the cerebellar cortex would both delete the memory trace in previously trained animals and prevent further learning. Initially, it was observed that lesioning either the whole cerebellum (ipsilateral to the trained eye) or the anterior interpositus nucleus completely abolished the CR but *not* the UR (McCormick et al., 1982; McCormick and Thompson, 1984a,b; Yeo et al., 1985a; Steinmetz et al., 1992; but see Welsh and Harvey, 1989). These experiments suggested that cerebellar lesions abolished the memory trace for eyeblink conditioning, but their irreversibility made it difficult to dissociate this interpretation from a performance deficit. A more convincing case was made when experiments

showed that reversible inactivation of the DCN with muscimol (a $GABA_A$ receptor agonist) prevented the acquisition of the eyeblink CR, but not the performance of the UR (Krupa et al., 1993; Hardiman et al., 1996; Krupa and Thompson, 1997; but see Bracha et al., 1994). In contrast, inactivation of the superior cerebellar peduncle or red nucleus, sites through which excitatory DCN output is conveyed, prevented the expression of the CR during training, but not its acquisition, as evidenced by the fact that the CR was present after inactivation (Krupa et al., 1993; Krupa and Thompson, 1995). These studies suggest that the cerebellum and its associated projections are essential for acquisition and expression of the eyeblink CR. More specifically, the memory trace seems to be localized 'upstream' of the red nucleus, in the cerebellar cortex and/or the DCN.

While there is general agreement that lesions or inactivation of the DCN block the acquisition of the eyeblink CR, there has been considerable debate over the specific role of the cerebellar cortex in eyeblink conditioning. Reports using lesions and inactivation of the cerebellar cortex have ranged from those which have found a complete blockade of CR acquisition (Yeo et al., 1985b), to those which have slowed, but not prevented acquisition (Lavond and Steinmetz, 1989; Yeo and Hardiman, 1992), to those which have found no effect at all (McCormick and Thompson, 1984a,b). Some recent reports point to a potential resolution of this problem. Lesions which included the anterior cerebellar cortex (a region previously thought not to be important), or infusion of picrotoxin (a $GABA_A$ receptor antagonist, the opposite of muscimol) into the DCN to block Purkinje cell input, did not abolish the CR entirely but affected its timing (Perrett et al., 1993; Perrett and Mauk, 1995; Garcia and Mauk, 1998). Recently, a model has been proposed to explain these findings. In this model, the memory trace of the eyeblink CR is sequentially stored, initially as a depression of the parallel fiber–Purkinje cell synapse in the cerebellar cortex. This would result in an attenuation of Purkinje cell firing and hence Purkinje cell–DCN synaptic drive, thereby disinhibiting the DCN targets. This disinhibition, when coupled with activation of the mossy fiber–DCN synapse, could then potentiate the latter, resulting in storage of the CR at the mossy fiber–DCN synapse while the timing of the conditioned response is retained in the cerebellar cortex (Raymond et al., 1996; Mauk, 1997; Mauk and Donegan, 1997; Medina and Mauk, 1999).

4.17.12 Potential Cellular Substrates of Associative Eyeblink Conditioning

LTD of the parallel fiber–Purkinje cell synapse has been proposed as a cellular mechanism which could, at least in part, underlie the acquisition of associative eyeblink conditioning. This phenomenon, which was first described by Ito and colleagues (1982), results when the climbing fiber (corresponding to the US) and parallel fiber (corresponding to the CS) inputs are activated together at low frequencies (1–4 Hz; see **Figure 5(b)**). In addition, stimulation of parallel fibers alone can produce LTP of the parallel fiber–Purkinje cell synapse, thus providing a form of bidirectional control (Lev-Ram et al., 2002; Coesmans et al., 2004). LTD in the parallel fiber–Purkinje cell synapse requires association of parallel fiber (CS) and climbing fiber (US) activation and would result in decreased firing of the Purkinje cell, causing increased firing of DCN neurons and enhanced expression of the CR. Essentially, this is a cellular restatement of the Marr/Albus model. Conversely, repeated activation of the parallel fiber (CS) alone could, through enhanced inhibition resulting from parallel fiber LTP, decrease firing of the DCN and thereby reduce expression of the CR during extinction.

As indicated by the lesion and inactivation studies described above, it is likely that the parallel fiber–Purkinje cell synapse is not the only site of information storage during cerebellar motor learning. At a cellular level, extinction of the CR, as results from repeated application of a tone CS, could be mediated not only by LTP of the parallel fiber–Purkinje cell synapse, but also by LTD of the mossy fiber–DCN synapse. This idea is consistent with reports that both cortical lesions which include the anterior region (Perrett and Mauk, 1995) and reversible inactivation of the DCN with muscimol (Hardiman et al., 1996; Ramnani and Yeo, 1996) block CR extinction.

Recently, both LTD and LTP have been described at the mossy fiber–DCN synapse. Whereas LTD can be observed after high-frequency mossy fiber burst stimulation, either alone or paired with postsynaptic depolarization (Zhang and Linden, 2006), LTP can be elicited when high-frequency mossy fiber stimulation is paired with postsynaptic hyperpolarization followed by a rebound current (Pugh and Raman, 2006). The existence of mossy fiber LTP has been suggested by a model of Mauk and coworkers, in which disinhibition of the DCN

from reduced Purkinje cell input, when coupled with activation of the mossy fiber–DCN synapses, results in LTP of mossy fiber–DCN synapses, constituting a portion of the memory trace of the CR (Raymond et al., 1996; Mauk, 1997; Mauk and Donegan, 1997). A recent computational analysis has suggested that an LTP induction rule for the mossy fiber–DCN synapse that depends upon specific patterns of Purkinje cell input (plus ongoing mossy fiber activity) could constitute a memory trace that is unusually resistant to degradation by ongoing 'background' activity in the cerebellar circuit (Medina and Mauk, 1999). The dependence of mossy fiber LTP on paired hyperpolarization and subsequent rebound currents (Pugh and Raman, 2006), mimicking the response to Purkinje cell activity and the transient interruption of inhibition (e.g., related to a complex spike pause), fits this theoretical framework and underlines the importance of a specific timing of mossy fiber and Purkinje cell activity for LTP induction. A missing piece that remains in the puzzle, however, is whether previous parallel fiber–LTD induction (resulting in a reduction of the inhibitory tone imposed by Purkinje cells) not only leads to increased activity levels in DCN cells, but also facilitates the induction of mossy fiber LTP.

4.17.13 Parallel Fiber LTD Induction

4.17.13.1 Parametric Requirements

Cerebellar LTD was first described in the intact cerebellum (Ito et al., 1982) and, since that time, has been analyzed in acute slice preparations, primary cultures, acutely dissociated Purkinje cells, and macropatches of Purkinje cell dendrite. In slice or *in situ*, the standard induction protocol consists of stimulating the parallel and climbing fiber inputs together at low frequency (1–4 Hz) for a period of 2–6 min. This results in a selective attenuation of the parallel fiber–Purkinje cell synapse (typically a 20–50% reduction of baseline synaptic strength), which reaches its full extent in ~10 min and persists for the duration of the experiment, typically 1–2 h.

LTD is said to result from coactivation of parallel fibers and climbing fibers, but what are the precise timing constraints on this coactivation? This is an important point, because if parallel fiber LTD underlies associative eyeblink conditioning, then the temporal constraints on CS/US association should be reflected in the temporal constraints on LTD induction. One study, using intracellular recording in rabbit cerebellar slice, has indicated that LTD is optimally induced when climbing fiber stimulation precedes parallel fiber stimulation by 125–250 ms (Ekerot and Kano, 1989). Another study using a similar preparation has shown that LTD may be induced by climbing fiber–parallel fiber stimulation with an interval of 50 ms, but claims that LTD induced by climbing fiber–parallel fiber pairing will not occur unless disynaptic inhibition is blocked by addition of a $GABA_A$ antagonist (Schreurs and Alkon, 1993). Neither of these intervals (in which the US precedes CS) will support robust eyeblink conditioning. However, with slightly different stimulation protocols (small trains of parallel fiber stimulation instead of single pulses in one case) parallel fiber before climbing fiber pairing, at intervals which support eyeblink conditioning, may also be effective in inducing LTD in the absence of $GABA_A$ receptor blockade (Chen and Thompson, 1995; Schreurs et al., 1996). In a more recent study, which combined whole-cell patch-clamp recordings with two-photon Ca imaging, it was shown that LTD is optimally induced when parallel fiber activation precedes climbing fiber activation by 50–200 ms and that coincident parallel fiber and climbing fiber activation results in supralinear Ca signals (Wang et al., 2000). Thus, the timing requirements found in cerebellar motor learning paradigms can indeed be matched by timing requirements characterized in cerebellar slice preparations. Moreover, the optimal timing conditions for LTD induction also yielded the largest spine Ca signals, providing an explanation why this particular activation sequence was beneficial for LTD induction.

4.17.13.2 Climbing Fiber Signals

The climbing fiber contributes to LTD induction by causing sufficient postsynaptic depolarization (through activation of AMPARs) to strongly activate voltage-sensitive Ca channels in the dendrites, thereby causing a complex spike and a large Ca influx (see **Figure 7**; for review see Schmolesky et al., 2002). In fact, climbing fiber activation may be replaced in the LTD induction protocol by direct depolarization of the Purkinje cell (Crepel and Krupa, 1988; Hirano, 1990; Linden et al., 1991). Furthermore, LTD induction is blocked by postsynaptic application of a Ca chelator (Sakurai, 1990; Linden and Connor, 1991; Konnerth et al., 1992), electrical inhibition of Purkinje cells during parallel

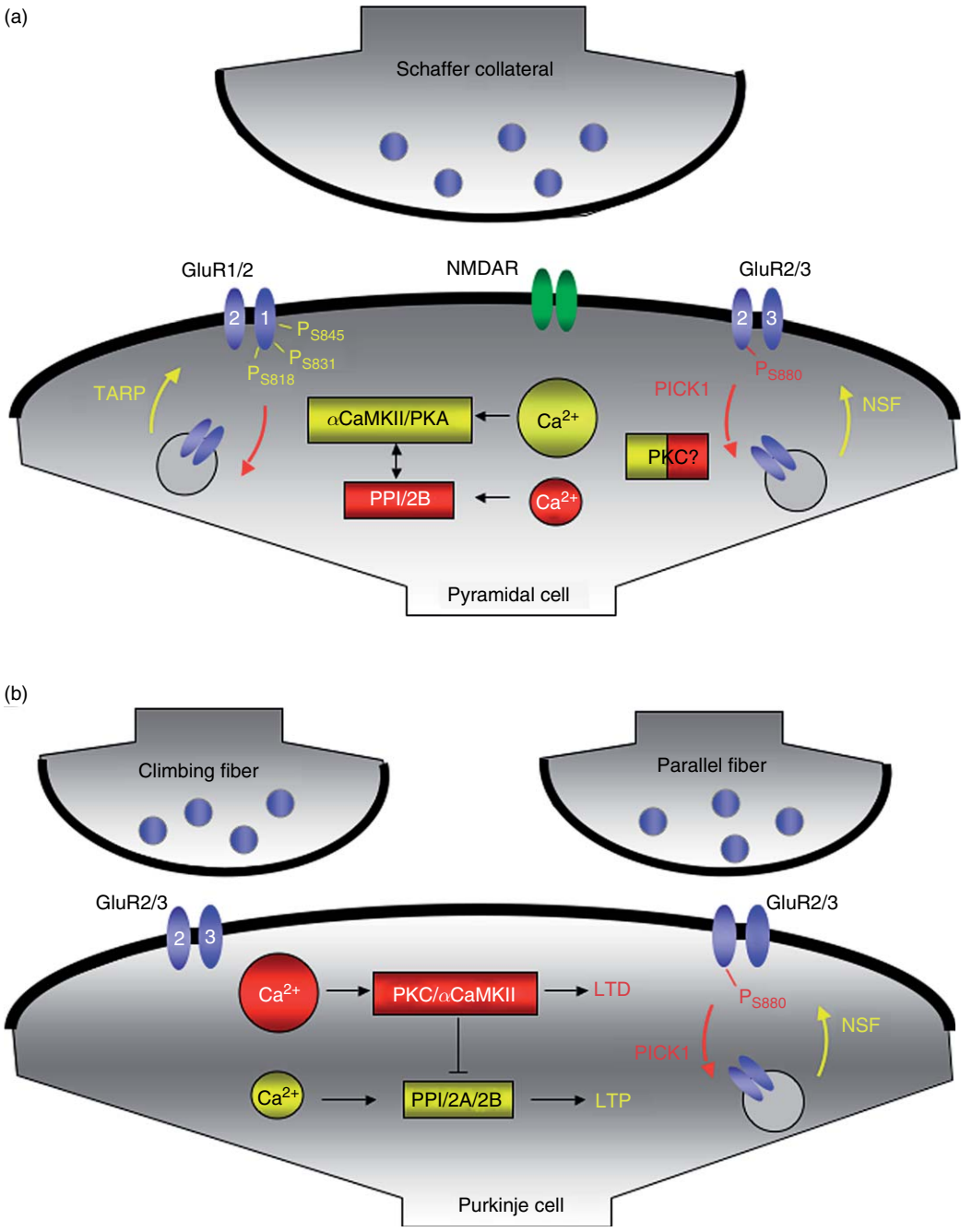

Figure 7 Comparison of LTP and LTD induction cascades at CA1 hippocampal (a) and cerebellar synapses (b). LTP induction cascades are shown in yellow, and LTD induction cascades in red. (a) LTP and LTD induction at Schaffer collateral synapses onto CA1 hippocampal pyramidal cells. Functional NMDARs are present in pyramidal cells, but not in Purkinje cells. Other calcium sources present in both types are neurons are not displayed. Note that PKA and protein phosphatase 1 (PP1) are not directly calcium activated. PKC has been implicated in hippocampal LTP, but also in the endocytosis of GluR2 subunits. Abbreviations are used as explained in the text. (b) LTP and LTD induction at cerebellar parallel fiber–Purkinje cell synapses. For simplicity, climbing fiber and parallel fiber terminals are shown to contact the same postsynaptic compartment; (b) is modified from Hansel C (2005) When the B-team runs plasticity: GluR2 receptor trafficking in cerebellar long-term potentiation. *Proc. Natl. Acad. Sci. USA* 102: 18245–18246, with permission from the National Academy of Sciences, USA.

fiber/climbing fiber conjunctive stimulation (Ekerot and Kano, 1985, 1989; Hirano, 1990; Crepel and Jaillard, 1991), or removal of external Ca (Linden and Connor, 1991). In addition, studies using optical indicators have shown large Ca accumulations in Purkinje cell dendrites following climbing fiber stimulation (Ross and Werman, 1987; Knöpfel et al., 1990; Konnerth et al., 1992). These Ca transients reach supralinear levels when the climbing fiber stimulation is paired with parallel fiber activation (see above; Wang et al., 2000). While these studies have suggested that Ca influx is the sole mediator of climbing fiber action, another view has come from studies which have examined a peptide released from climbing fiber terminals, corticotropin releasing factor. Miyata et al. (1999) have found that LTD induced by either parallel fiber/climbing fiber conjunction or parallel fiber/depolarization conjunction can be blocked by antagonists of the corticotropin releasing factor receptor in a slice preparation. Furthermore, parallel fiber/depolarization conjunction fails to induce LTD in slices prepared from rats in which climbing fibers were chemically prelesioned, but this may be restored with exogenous corticotropin releasing factor. These observations have led to the suggestion that corticotropin releasing factor plays a permissive role in LTD of parallel fiber synapses. A similar facilitatory role of corticotropin releasing factor can be found for LTD induction at climbing fiber–Purkinje cell synapses (see following; Schmolesky et al., 2007).

4.17.13.3 Parallel Fiber Signals

Parallel fiber activation results in glutamate release, which activates glutamate receptors in the Purkinje cell dendrite. While mature Purkinje cells do not express functional NMDARs, they are found on both cultured embryonic Purkinje cells and acutely dissociated Purkinje cells in early postnatal life (Linden and Connor, 1991; Rosenmund et al., 1992). Purkinje cells also express AMPARs of the GluR2-containing, Ca-impermeable variety (Linden et al., 1993; Tempia et al., 1996) as well as a particular metabotropic receptor, mGluR1, at high levels in the dendritic spines where parallel fiber synapses are received (Martin et al., 1992).

The first evidence indicating that activation of metabotropic receptors was required for parallel fiber LTD induction came from experiments using cerebellar cultures, which showed that agonists that activated both AMPA and metabotropic receptors (such as glutamate and quisqualate) could substitute for parallel fiber activation during LTD induction, but that agonists that failed to activate metabotropic receptors (such as AMPA or aspartate) could not (Kano and Kato, 1987; Linden et al, 1991). Complementary evidence was found in which metabotropic receptor antagonists blocked LTD induction (Linden et al., 1991; Hartell, 1994; Narasimhan and Linden, 1996; Lev-Ram et al., 1997a). These results, while they indicated that metabotropic receptor activation was required, did not specify which metabotropic receptor(s) were important for LTD induction. The first findings to address this issue were those of Shigemoto et al. (1994), who demonstrated that specific inactivating antibodies directed against mGluR1 could block LTD induction in cell culture. This result was confirmed and extended by two different groups using mGluR1 knockout mice (Aiba et al., 1994; Conquet et al., 1994).

Activation of mGluR1 results in the activation of phospholipase C and the consequent production of two initial products, inositol-1,4,5-trisphosphate (IP_3) and 1,2-diacylglycerol. The former binds to specific intracellular IP_3 receptors, resulting in the liberation of Ca from internal stores, while the latter results in activation of PKC. Are both of these products required for parallel fiber LTD induction? Purkinje cells express IP_3 receptors, particularly the type I isoform, at unusually high levels (Nakanishi et al., 1991), and it has been shown through photolysis of caged IP_3 that these receptors are functionally coupled to intracellular Ca release in situ (Khodakhah and Ogden, 1993; Wang and Augustine, 1995). Several lines of evidence have supported a role for IP_3 receptor activation in the induction of parallel fiber LTD. First, compounds which interfere with IP_3 receptor function have been shown to block LTD. Application of heparin, a nonspecific (Herbert and Maffrand, 1991; Bezprozvanny et al., 1993) inhibitor of the IP_3 receptor, blocked LTD induced by glutamate/depolarization conjunction in cultured Purkinje cells (Kasono and Hirano, 1995) or by parallel fiber/depolarization conjunction in Purkinje cells in a cerebellar slice (Khodakhah and Armstrong, 1997). A specific inactivating antibody directed against the IP_3 receptor was similarly effective (Inoue et al., 1998). Application of thapsigargin, a drug which depletes internal Ca stores through inhibition of the endoplasmic reticulum Ca-ATPase, also blocked LTD induction (Kohda et al., 1995). Thapsigargin would be expected to deplete Ca stores gated by both the IP_3 receptor and the ryanodine receptor, the latter of which mediates Ca-induced Ca

release. Second, photolysis of IP_3 in cultured Purkinje cells can induce LTD when combined with depolarization plus AMPAR activation (Kasano and Hirano, 1995). Similarly, IP_3 photolysis combined with depolarization can induce LTD in slices derived from either wild-type (Khodakhah and Armstrong, 1997) or mGluR1 knockout mice (Daniel et al., 1999). Finally, parallel fiber LTD is blocked in slices derived from a mutant mouse which lacks the type I IP_3 receptor (Inoue et al., 1998).

While these experiments would appear to provide a strong case for the involvement of IP_3 receptors in cerebellar LTD induction, it is worth noting that not all evidence has been consistent with this view. For example, thapsigargin application in slices was found to block LTD induced by bath application of the mGluR agonist trans-DL-1-amino-1,3-cyclopentanedicarboxylic acid (ACPD) together with depolarization, but not parallel fiber/depolarization conjunction (Hemart et al., 1995). Furthermore, Narasimhan et al. (1998) performed ratiometric imaging of free cytosolic Ca on both acutely dissociated and cultured Purkinje cells. It was determined that the threshold for glutamate pulses to contribute to LTD induction was below the threshold for producing a Ca transient. Furthermore, the Ca transients produced by depolarization alone and glutamate plus depolarization were not significantly different. In addition, the potent and selective IP_3 receptor channel blocker xestospongin C (an improvement over heparin) was not found to affect the induction of LTD in either acutely dissociated or cultured Purkinje cells at a concentration which was sufficient to block mGluR1-evoked Ca mobilization. Finally, replacement of mGluR1 activation by exogenous synthetic diacylglycerol in an LTD induction protocol was successful. At present it is not clear why an IP_3 signaling cascade is not required for induction of cerebellar LTD in these experiments using reduced preparations, while other experiments using both slice and culture preparations have suggested otherwise.

4.17.13.4 Second Messengers

Two major postsynaptic signals resulting from cerebellar LTD induction are 1,2-diacylglycerol and Ca. These signals are known to synergistically activate the enzyme PKC. The involvement of PKC in LTD induction was suggested by experiments in which PKC inhibitors blocked induction when applied during glutamate/depolarization conjunction in cultured Purkinje cells (Linden and Connor, 1991). Application of these compounds after LTD had been induced had no effect, suggesting that continued PKC activation is not required for LTD to persist. Blockade of LTD induction by PKC inhibitors has since been confirmed using several preparations including cerebellar slices (Hartell, 1994; Freeman et al., 1998), acutely dissociated Purkinje cells and Purkinje cell dendritic macropatches (Narasimhan and Linden, 1996), as well as cultured Purkinje cells derived from a transgenic mouse which expresses a PKC inhibitor peptide (De Zeeuw et al., 1998). These observations are complemented by the finding that bath application of PKC-activating phorbol esters induces an LTD-like attenuation of Purkinje cell responses to exogenous glutamate or AMPA (Crepel and Krupa, 1988; Linden and Connor, 1991) which occludes pairing-induced LTD. In a recent study using αCaMKII knockout mice, it was shown that αCaMKII is involved in cerebellar LTD induction as well (Hansel et al., 2006). Moreover, LTD was blocked when the CaMKII inhibitor KN-93 was bath applied, but not when its inactive analogue, KN-92, was used. These observations show that, similar to hippocampal LTP (but operating in an 'inverse' manner), multiple kinases are involved in the induction process.

In addition to PKC and αCaMKII activation, a number of studies have indicated that release of the gaseous second messenger, nitric oxide (NO) by the action of the Ca/calmodulin-sensitive enzyme NO synthase (NOS), is necessary for parallel fiber LTD induction. They have shown that an LTD-like phenomenon could be induced when climbing fiber stimulation was replaced by bath application of NO via donor molecules such as sodium nitroprusside (Crepel and Jaillard, 1990; Shibuki and Okada, 1991; Daniel et al., 1993; but see Glaum et al., 1992). Likewise, induction of LTD by more conventional means could be blocked by inhibitors of NOS (such as N^G-nitro-L-arginine), agents that bind NO in the extracellular fluid (such as hemoglobin), or genetic deletion of the neuronal isoform of NOS (Lev-Ram et al., 1997b). Application of NO donors, cGMP analogs, or cGMP phosphodiesterase inhibitors directly to the Purkinje cell (via a patch pipette) also resulted in depression of parallel fiber responses (Daniel et al., 1993; Hartell, 1994, 1996), while postsynaptic application of a NOS inhibitor did not block LTD induction (Daniel et al., 1993). In contrast, postsynaptic application of a specific guanylyl cyclase inhibitor was effective in blocking LTD induction (Boxall and Garthwaite, 1996;

Lev-Ram et al., 1997a). These findings suggested a model in which climbing fiber activation resulted in NO production, which then diffused to the Purkinje cell to activate soluble guanylyl cyclase. However, this model was complicated by the fact that both climbing fibers (Bredt et al., 1990; Vincent and Kimura, 1992; Ikeda et al., 1993) and Purkinje cells (Bredt et al., 1990; Vincent and Kimura, 1992; Crepel et al., 1994) lack NOS.

A proposal which addresses this complication has been that climbing fiber–evoked Ca influx into Purkinje cell dendrites causes K-efflux, which depolarizes adjacent parallel fiber terminals, resulting in Ca influx and the consequent activation of NOS in these compartments (Daniel et al., 1998). Another approach has been taken by Lev-Ram et al. (1995), who found that photolysis of caged NO loaded into Purkinje cells could substitute for parallel fiber activation in LTD induction. When NO photolysis was followed by direct Purkinje cell depolarization within a 50-ms window, LTD of parallel fiber EPSCs was produced. LTD induced in this manner could be blocked by a postsynaptic application of a Ca chelator or NO scavenger, but not external application of a NOS inhibitor or an NO scavenger. In contrast, LTD produced by parallel fiber/depolarization conjunction could be blocked by either an internally or externally applied NO scavenger, or an externally applied NOS inhibitor, but not an internally applied NOS inhibitor. This pattern of results suggests a model in which activation of parallel fibers causes an anterograde NO signal which acts inside the Purkinje cell. A subsequent investigation by this group showed that, when photolysis of caged Ca was used in place of Purkinje cell depolarization, the coincidence requirement for NO and Ca pairing was <10 ms, and the resultant LTD could be blocked by an inhibitor of soluble guanylyl cyclase (Lev-Ram et al., 1997a). However, when caged Ca and cGMP were used, the inhibition of guanylyl cyclase could be overcome, and the coincidence requirement was lengthened to ~200 ms.

The production of cGMP by this cascade is likely to be exerting its effect through activation of cGMP-dependent protein kinase (PKG). LTD induced by parallel fiber/depolarization conjunctive stimulation (Hartell, 1994) or photolytic NO/depolarization stimulation (Lev-Ram et al, 1997a) may be blocked with PKG inhibitors. While the mechanisms by which PKG might contribute to LTD induction are not known, one suggestion has been that phosphorylation of G-substrate by PKG could result in inhibition of protein phosphatases (Ito, 1990), which in turn promote LTP induction (Belmeguenai and Hansel, 2005).

In contrast to the extensive evidence indicating a requirement for a NO/cGMP/PKG cascade in slice preparations, LTD of glutamate currents produced without synaptic stimulation in cultured Purkinje cells is unaffected by reagents that stimulate (sodium nitroprusside) or inhibit (hemoglobin, N^G-nitro-L-arginine) NO signaling (Linden and Connor, 1992). Furthermore, in cerebellar cultures made from neuronal NOS knockout mice, LTD was indistinguishable from that in cultures from wild-type mice (Linden et al., 1995). In wild-type cultures, neither an activator of soluble guanylate cyclase, nor an inhibitor of type V cGMP-phosphodiesterase, nor inclusion of cGMP analogs in the patch pipette produced an LTD-like effect. Induction of LTD was not blocked by inclusion in the patch pipette of three different PKG inhibitors. These results suggest that a NO/cGMP/PKG cascade is not required for cerebellar LTD induction in culture.

A recent study suggests that in slices NO might indeed be required for LTD induction, but that NO is not released from parallel fiber terminals, but instead from interneurons (Shin and Linden, 2005). Using combined patch-clamp recordings and presynaptic confocal Ca imaging, the authors demonstrated that there are no NMDAR-mediated Ca transients in parallel fiber terminals, which had previously been suggested to promote NOS activation and subsequent NO production (Casado et al., 2002). Rather, functional NMDAR and NMDAR-mediated Ca signaling were found in the somata, dendrites, and presynaptic terminals of stellate cells (Shin and Linden, 2005). Thus, it is likely that under certain activation conditions NO diffuses from interneurons to Purkinje cells and facilitates LTD induction by promoting phosphatase inhibition.

4.17.14 Parallel Fiber LTD Expression

While considerable attention has been paid to the molecular mechanisms of cerebellar LTD induction, significant effort has only recently been focused upon its expression. A widely accepted notion has been that LTD is expressed, at least in part, as a down-regulation of the postsynaptic sensitivity to AMPA, as LTD may be detected using AMPA or glutamate test pulses in intact (Ito et al., 1982), slice (Crepel and Krupa, 1988), and culture (Linden et al., 1991)

preparations. These experiments have been extended with ultrareduced preparations that completely lack functional presynaptic terminals (Linden, 1994) including outside-out dendritic macropatches and acutely dissociated Purkinje cells (Narasimhan and Linden, 1996, Narasimhan et al., 1998) which provide definitive evidence for a postsynaptic locus of expression. This idea has received further support from a study showing that the coefficient of variation of parallel fiber EPSCs was altered by manipulations known to act presynaptically (such as transient synaptic attenuation produced by addition of adenosine), but was not altered by induction of LTD (Blond et al., 1997).

In CA1 pyramidal cells, synaptic plasticity is implemented by changes both in the properties of postsynaptic AMPA receptors and in the insertion/internalization balance of AMPA receptor subunits (see above). In contrast, parallel fiber LTD is not associated with changes in AMPA receptor properties, such as glutamate affinity, conductance, or kinetics (Linden, 2001; for review see Ito, 2001). Rather, synaptic plasticity at parallel fiber synapses seems to rely entirely on changes in AMPA receptor trafficking. In Purkinje cells, GluR1 expression is low (Baude et al., 1994), and GluR2/GluR3 heteromers constitute the majority of AMPA receptors. PF-LTD results from a clathrin-mediated endocytosis of GluR2 (Wang and Linden, 2000), which requires PKCα-dependent phosphorylation of GluR2 at serine-880 (Chung et al., 2003; Leitges et al., 2004). The internalization of GluR2 involves unbinding from GRIP (Dong et al., 1997) and binding to PICK1 (Xia et al., 1999, 2000; Chung et al., 2000; Steinberg et al., 2006), thus using the same molecular machinery as described for GluR2 endocytosis in CA1 pyramidal cells (**Figure 7**). The role of αCaMKII activation, which is needed for LTD induction (Hansel et al., 2006), in GluR2 subunit trafficking remains to be examined.

4.17.15 Another Type of Cerebellar LTD: Climbing Fiber LTD

In the classic Marr-Albus-Ito models of cerebellar function and learning, paired parallel fiber and climbing fiber stimulation leads to LTD at the parallel fiber synapses, but the climbing fiber input was considered invariant (Marr, 1969; Albus, 1971; Ito, 1984). This reputation is a result of early studies by Eccles and colleagues demonstrating a high degree of reliability of complex spike occurrence upon tetanization at frequencies exceeding 100 Hz (e.g., Eccles et al., 1966). However, a different perspective of the 'invariance' of the complex spike emerges when it comes to modifications of the complex spike waveform. The search for plasticity at climbing fiber synapses was actually started using recordings of climbing fiber–mediated EPSCs in voltage-clamp mode, enabling an electrophysiological isolation of synaptic events underlying the complex spike. Climbing fiber activation at 5 Hz for 30 s resulted in a lasting reduction of EPSC amplitudes (Hansel and Linden, 2000; Carta et al., 2006). This form of LTD at the climbing fiber input was not accompanied by changes in parallel fiber responses, but it turned out that climbing fiber LTD shares induction requirements with parallel fiber LTD, namely a dependence on postsynaptic Ca transients, mGluR1, and PKC activation (Hansel and Linden, 2000). Climbing fiber LTD is not accompanied by changes in the paired-pulse depression ratio (Hansel and Linden, 2000) and also does not alter the degree of AMPA receptor blockade by a low-affinity competitive antagonist, γ-D-glutamylglycine, which unbinds rapidly from AMPA receptors and can be used as a reporter for changes in glutamate release (Shen et al., 2002). These results suggest that climbing fiber LTD, just like parallel fiber LTD, is postsynaptically expressed. When discussing plasticity at the climbing fiber synapse, it has to be kept in mind that this is a very unusual type of synapse, as climbing fiber activity under physiological conditions results in an all-or-none complex spike. The waveform of complex spikes is composed of an initial Na action potential in the soma, followed by smaller spikelets on top of a plateau. These spikelets likely reflect calcium spike activity in the dendrite, although at the somatic level (where typically the recordings are performed) resurgent Na conductances might be involved as well (for review see Schmolesky et al., 2002). As complex spikes are the physiological responses to climbing fiber stimulation, climbing fiber plasticity was also characterized when complex spikes were recorded in current-clamp mode. Under these conditions, 5-Hz climbing fiber activation for 30 s resulted in a selective reduction of slow complex spike components (most reliably the first spikelet) (Hansel and Linden, 2000; see also Hansel et al., 2001). This effect could be mimicked by partial AMPA receptor blockade using NBQX (1,2,3,4-tetrahydro-6-nitro-2,3-dioxo-benzo-[f]quinoxaline-7-sulfonamide) (Weber et al., 2003), suggesting that

climbing fiber LTD is primarily caused by a reduction in AMPAR-mediated transmission, which subsequently affects dendritic Ca spike activity. This interpretation is supported by experiments in which whole-cell patch-clamp recordings and microfluorometric Ca measurements were simultaneously performed. These recordings showed that climbing fiber LTD is accompanied by a long-term depression of dendritic Ca transients (Weber et al., 2003). Finally, it was shown that climbing fiber LTD is associated with a reduction in the afterhyperpolarization (AHP) following complex spikes (Schmolesky et al., 2005), which might result from the depression of Ca transients. These observations show that LTD at the climbing fiber synapse affects all components of a climbing fiber response, namely the underlying synaptic transmission and subsequently the complex spike, the complex spike-evoked Ca transient, as well as the complex spike AHP. As will be discussed, these alterations (particularly the 'Ca-LTD') have a large effect on the function of Purkinje cells and on parallel fiber plasticity.

4.17.16 Interactions Between LTP and LTD at Parallel Fiber Synapses

Parallel fiber LTD is postsynaptically induced and expressed (for review see Hansel et al., 2001; Ito, 2001, 2002). A mechanism allowing for activity-dependent reversibility of LTD therefore needs to operate postsynaptically as well. Recently, a postsynaptic form of parallel fiber LTP has indeed been described (Lev-Ram et al., 2002; Coesmans et al., 2004). LTP had been reported in earlier studies under conditions when LTD was blocked (e.g., Sakurai, 1990; Shibuki and Okada, 1992), but in these studies the expression side of LTP was not specifically addressed. The first type of parallel fiber LTP that was actually examined in detail turned out to be a presynaptic phenomenon (Salin et al., 1996).

This presynaptic form of LTP is typically induced by brief (4–8 Hz) parallel fiber tetanization and requires presynaptic Ca influx (Salin et al., 1996; Linden, 1997) and activation of Ca-sensitive adenylyl cyclase I (Storm et al., 1998), an enzyme which is concentrated in granule cell presynaptic terminals. The resulting cyclic adenosine monophosphate elevation then activates PKA (Salin et al., 1996) in this same compartment (Linden and Ahn, 1999). A PKA substrate involved in the induction of presynaptic parallel fiber LTP is the active zone protein RIM1α (Lonart et al., 2003).

The postsynaptic form of parallel fiber LTP can be obtained with the same parallel fiber tetanization protocol used for the induction of parallel fiber LTD (1 Hz; 5 min), but in the absence of climbing fiber activity (Lev-Ram et al., 2002; Coesmans et al., 2004). Therefore, the polarity of synaptic gain changes at the parallel fiber input depends on the activity level of the heterosynaptic climbing fiber input. Climbing fiber activity can exert such function as a polarity switch, because LTD induction requires a larger Ca transient than LTP induction (Coesmans et al., 2004). This is a remarkable observation, as it indicates that bidirectional parallel fiber plasticity is governed by a Ca-threshold mechanism that operates inverse to its hippocampal counterpart (**Figure 7**). The additional Ca needed for parallel fiber LTD induction is contributed by climbing fiber–evoked complex spike activity (Konnerth et al., 1992; Wang et al., 2000). In fact, previous induction of climbing fiber LTD (and the associated reduction in dendritic Ca transients) reduces the probability for subsequent induction of parallel fiber LTD (Coesmans et al., 2004). Postsynaptic parallel fiber LTP depends on the activation of protein phosphatases 1, 2A, and 2B, but neither requires PKC activity (Belmeguenai and Hansel, 2005) or αCaMKII activity (Hansel et al., 2006). The postsynaptic expression side of this form of parallel fiber LTP suggests that it might provide a reversal mechanism for LTD. The postsynaptic expression of LTP was confirmed by the absence of changes in the paired-pulse facilitation ratio (Lev-Ram et al., 2002; Coesmans et al., 2004) and by the absence of changes in the degree of AMPA receptor blockade by the low-affinity competitive AMPA receptor antagonist γ-DGG (Coesmans et al., 2004). Postsynaptic LTD and LTP can indeed reverse each other: when LTP was saturated first by applying the LTP-inducing protocol twice, application of the LTD protocol resulted in a depotentiation (**Figure 6**). Subsequent application of the LTP protocol for the third time again potentiated the parallel fiber EPSCs, indicating that the previous LTD protocol had reversed LTP and that the LTP mechanism was not saturated any longer (Coesmans et al., 2004). The mutual reversibility of postsynaptic parallel fiber LTD and LTP is also suggested by the observation that LTP induction involves an NSF-dependent membrane delivery of GluR2 subunits (Kakegawa and Yuzaki, 2005), which are internalized during LTD (**Figure 7**).

4.17.17 Comparison of Bidirectional Plasticity at Hippocampal and Cerebellar Synapses

While this article largely focuses on the description of LTD mechanisms and the function of LTD, we have also discussed how at both hippocampal and cerebellar synapses LTD relates to LTP. This relation is crucial for an understanding of brain plasticity when it comes to the cellular machinery involved in synaptic memory storage, but also when it comes to the functions of LTP and LTD, respectively. A postsynaptic form of parallel fiber LTP was only recently discovered (Lev-Ram et al., 2002), but its arrival on the scene finally establishes bidirectional plasticity at parallel fiber synapses and allows us to directly compare the cellular mechanisms involved in LTP and LTD induction at hippocampal synapses to their cerebellar counterparts. To start with, at both types of synapses different 'tools' are available for plasticity: in contrast to pyramidal neurons, mature Purkinje cells lack functional NMDARs (Crepel et al., 1982) and only weakly express the AMPAR subunit GluR1 (Baude et al., 1994). Purkinje cells, in turn, express the orphan glutamate receptor $\delta 2$ (GluR$\delta 2$), which plays a not well-understood role in parallel fiber LTD (for review see Yuzaki, 2004), but is not expressed in pyramidal cells. Moreover, Purkinje cells lack back-propagating action potentials in the dendrites (Stuart and Häusser, 1994), but fire complex spikes in response to climbing fiber activation (for review see Schmolesky et al., 2002).

Our current understanding of plasticity mechanisms is not yet advanced enough to pinpoint, for example, what consequences the absence of functional NMDARs has for Purkinje cells, or the absence of GluR$\delta 2$ receptors for pyramidal cells. However, we can describe an emerging picture of remarkable differences between hippocampal and cerebellar plasticity, but also of astonishing similarities (see also Hansel, 2005). In several aspects, cerebellar plasticity provides a mirror image of hippocampal plasticity. As described in detail earlier, hippocampal LTP induction requires large Ca transients and the activation of protein kinases (e.g., CaMKII, PKA, PKC), whereas LTD relies on lower Ca transients and the activation of protein phosphatases. In cerebellar plasticity, the Ca and kinase/phosphatase dependencies are inverse to the hippocampal ones (**Figure 7**): LTD induction (by paired parallel fiber and climbing fiber activity) requires larger Ca transients than LTP induction (by parallel fiber activity alone) (Coesmans et al., 2004). Moreover, LTD is PKC dependent (Linden and Connor, 1991; De Zeeuw et al., 1998) and αCaMKII dependent (Hansel et al. 2006), whereas LTP induction requires the activation of protein phosphatases 1, 2A, and 2B (Belmeguenai and Hansel, 2005).

At the level of AMPAR trafficking, the mirror image–like arrangement of induction events is partially confirmed and partially breaks down. Kinase activity promotes the membrane insertion of GluR1 subunits, which dominate trafficking behavior in GluR1/GluR2 heteromers (Song and Huganir, 2002). Three GluR1 phosphorylation sites are discussed in the context of hippocampal LTP induction, namely serine-831 (CaMKII, but for conductance changes), serine-845 (PKA), and most recently, serine-818 (PKC). In GluR1 'phospho-free' mice, NMDAR-dependent LTD is blocked, which has been interpreted as further evidence for the hypothesis that a dephosphorylation at GluR1 promotes GluR1 endocytosis and LTD (Lee et al., 2003). This scenario would indeed provide a mirror image to the phosphorylation events involved in parallel fiber plasticity, which, however, is mediated by GluR2 subunit trafficking. PKCα-mediated phosphorylation at serine-880 causes GluR2 endocytosis and LTD (Leitges et al., 2004), while LTP is phosphatase dependent (PP1/2A/2B; Belmeguenai and Hansel, 2005) and is associated with a membrane insertion of GluR2 subunits (Kakegawa and Yuzaki, 2005).

However, the phosphorylation/AMPAR trafficking events involved in LTP and LTD, particularly at hippocampal synapses, are still not sufficiently well understood to reach such a comprehensive and simplifying view. At the moment it seems safe to say that the cellular events underlying LTP induction indeed differ significantly, simply because there is compelling evidence that hippocampal LTP is mediated in part by a membrane insertion of GluR1 subunits, which are only weakly expressed in Purkinje cells. However, GluR2 subunit trafficking, which clearly mediates parallel fiber plasticity, works similarly in pyramidal cells. GluR2 phosphorylation at serine-880 triggers a clathrin-mediated GluR2 endocytosis at both hippocampal (Man et al., 2000; Seidenman et al., 2003) and cerebellar synapses (Wang and Linden, 2000; Chung et al., 2003). On the other hand, NSF-GluR2 binding promotes GluR2 insertion at both types of synapses (Lüscher et al., 1999; Kakegawa and Yuzaki, 2005). Moreover, recent evidence

suggests that GluR2 rather than GluR1 endocytosis mediates LTD in CA1 pyramidal cells (for review see Malinow, 2003), which would suggest that both types of synapses use the same mechanism for LTD expression. How this scenario fits together with the obviously different Ca signaling requirements and kinase/phosphatase dependences of LTD induction at hippocampal and cerebellar synapses remains to be seen.

4.17.18 Is LTD of the Parallel Fiber–Purkinje Cell Synapse Involved in Motor Learning?

A fruitful approach to testing the hypothesized LTD/motor learning connection has been provided by the generation of mutant mice that lack proteins thought to be required for cerebellar LTD (see Chen and Tonegawa, 1997, for review). Several forms of knockout mice have been reported which have shown impairments in both parallel fiber LTD and motor learning. Mutant mice which lack mGluR1 have severely impaired cerebellar LTD (Aiba et al., 1994; Conquet et al., 1994; Ichise et al., 2000), consistent with previous studies using mGluR1 antagonists or inactivating antibodies. These mice had normal postsynaptic voltage-gated Ca currents and normal paired-pulse facilitation and depression of parallel and climbing fiber synapses, respectively. mGluR1 mutant mice were severely ataxic and showed impairments in several motor coordination tasks. When associative eyeblink conditioning was performed, these animals showed a partial deficit in CR acquisition (Aiba et al, 1994), which may not reflect an inability to produce a CR, but rather an inability to produce the optimal timing of the eyelid closure.

Other knockout mice also have LTD and motor learning deficits. Unfortunately, in some of these cases it is not understood how the missing protein functions in LTD induction. GluRδ2 is a protein which is expressed almost exclusively in Purkinje cell dendritic spines. While a point mutation in this receptor gives rise to a constitutive cation conductance and the *lurcher* phenotype (Zuo et al., 1997), the function of wild-type GluRδ2 is not clear. A GluRδ2 null mouse has impaired cerebellar LTD (Kashiwabuchi et al., 1995), consistent with reports that have used application of antisense oligonucleotides to suppress expression of this protein in culture preparations (Hirano et al., 1995; Jeromin et al., 1996). While the cerebellar cortex appears normal by light microscopy, electron microscopic analysis revealed an ~50% reduction in the number of parallel fiber–Purkinje cell synapses. This mouse is severely ataxic and is retarded in its ability to achieve a form of vestibular compensation: the righting response under a rotation load following unilateral middle ear destruction (Funabiki et al., 1995). These results are supported by more recent observations that injection of an anti-GluRδ2 antibody into the subarachnoidal supracerebellar space caused ataxic gait and poor rotorod test performance (Hirai et al., 2003).

A puzzling LTD defect is found in a mutant mouse which lacks glial fibrillary acidic protein (GFAP), which is, of course, not expressed in neurons. Since at least the early phase of cerebellar LTD may be expressed in reduced preparations that lack glia (Narasimhan and Linden, 1996; Narasimhan et al., 1998), it is likely that this knockout is exerting its effect indirectly. These mice show normal motor coordination, but are severely impaired in parallel fiber LTD and partially impaired in acquisition of associative eyeblink conditioning (Shibuki et al., 1996). Interestingly, these mice show clear improvement with training in a motor coordination task (the rotating rod), indicating that there are forms of motor learning that do not require cerebellar LTD.

There have been several major problems which have complicated the analysis of knockout mice. First, knockout mice have the gene of interest deleted from the earliest stages of development. As a result, these mice often have a complex developmental phenotype. For example, PKCγ, mGluR1 and GluRδ2 (but not GFAP) knockout mice all have cerebellar Purkinje cells that fail to undergo the normal developmental conversion from multiple to mono climbing fiber innervation in early postnatal life (Chen et al., 1995; Kano et al., 1995, 1997; Kurihara et al., 1997). Second, knockout of one gene sometimes produces compensatory upregulation in the expression of other related genes during development. In the CREBa-d (CREB: cAMP response element binding protein) knockout mouse, there is compensatory upregulation of the related transcription factor cAMP response element modulator (Hummler et al., 1994; Blendy et al., 1996). A third complicating factor is that knockout mice have the gene of interest deleted in every cell of the body, not just the cells of interest, making it more difficult to ascribe the knockout's behavioral effects to dysfunction in any one particular structure or cell type. This could be a potential problem for the analysis of behaviors such

as associative eyeblink conditioning and VOR adaptation, which are likely to require use-dependent plasticity at multiple sites, synapses received by both cerebellar Purkinje cells and their targets in the DCN or vestibular nuclei.

To address the latter two complications, an alternative approach has been used (De Zeeuw et al., 1998). Using the promoter of the Purkinje cell-specific gene *pcp-2* (also known as L7; Oberdick et al., 1990), transgenic mice have been created in which a selective inhibitor to a broad range of PKC isoforms (House and Kemp, 1987; Linden and Connor, 1991) is chronically overexpressed. This strategy ensures that, in L7-PKCI mice, PKC inhibition will be restricted to Purkinje cells, and that compensation via upregulation of different PKC isoforms will not succeed in blunting the biochemical effect of the transgene. This transgenic strategy resulted in nearly complete suppression of both cerebellar LTD as assessed in culture and adaptation of the VOR in the intact, behaving animal. In addition, L7-PKCI mice show impaired learning-dependent timing of conditioned eyeblink responses (Koekkoek et al., 2003). The phenotype of these animals was remarkably delimited. They showed normal motor coordination as measured by their ability to display the normal eye movement reflexes, optokinetic reflex, and VOR, as well as by several tests of gross motor coordination (rotorod, thin rod). Basal electrophysiological and morphological features of Purkinje cells were unaltered (with the exception that the Purkinje cells remained multiply innervated by climbing fibers). Similarly, conditional knockout mice lacking cGMP-dependent protein kinase type I (cGKI) selectively in Purkinje cells show impaired LTD and VOR gain adaptation (Feil et al., 2003).

While these mice with Purkinje cell–specific deficits represent a refinement in testing the relationship between parallel fiber LTD and motor learning, there are still caveats and complications which remain. First, the one basal physiological abnormality found in L7-PKCI mice is that about 50% of the Purkinje cells show a persistent multiple climbing fiber innervation, raising the possibility that this could be a cause of their failure to demonstrate VOR adaptation. However, this is unlikely because a PKCγ knockout mouse shows multiple climbing fiber innervation with normal cerebellar LTD and no motor learning deficit (Chen et al., 1995; Kano et al., 1995). Moreover, it has been shown more recently that the elimination of surplus climbing fibers is not entirely blocked in L7-PKCI mice, but only delayed, and that the elimination process is completed in 3- to 6-month-old mice (Goossens et al., 2001) A strategy to alleviate this kind of problem in the future will be to make the L7-PKCI transgene inducible in adult mice. Second, it has recently been reported that the climbing fiber–Purkinje cell synapse undergoes LTD, which, like parallel fiber LTD, requires activation of PKC (Hansel and Linden, 2000). Thus, even inhibition of PKC that is restricted to Purkinje cells cannot have its behavioral effects solely ascribed to parallel fiber LTD. Third, it is clear that PKC does not function in Purkinje cells only to produce LTD of various synapses. For example, it is known that voltage-gated K channels are modulated by PKC in Purkinje cells. Could this or some other function of PKC that is unrelated to LTD underlie the VOR adaptation deficit? While this is not an easy problem to address, future studies will benefit from *in vivo* recording during the appropriate behavioral tasks to distinguish among these possibilities.

While the mutant mouse studies cited here provide strong support for the widely accepted notion that LTD mediates forms of cerebellar motor learning (for review see De Zeeuw and Yeo, 2005), there are also examples of failures to establish such relationship: it has been shown that T-588, a drug that affects Ca release from internal stores, blocks parallel fiber LTD *in vitro* (Kimura et al., 2005), but does not impair associative eyelid conditioning (Welsh et al., 2005). Studies are on the way to examine the effects of disrupting PF-LTP on motor learning. These studies may provide additional insight into how synaptic plasticity contributes to learning and memory. In VOR gain conditioning, LTD is assumed to provide the cellular basis for increasing the VOR gain. When examining properties of reversibility, it has been found that complete reversal can be reached by gain-down training after gain-up training. In contrast, gain-up training after gain-down training led to an incomplete reversal (Boyden and Raymond, 2003; Boyden et al., 2004). It was suggested that this asymmetric reversibility was caused by an involvement of both pre- and postsynaptic LTP in the downregulation of the VOR gain, with only the postsynaptic form of LTP providing a true reversal mechanism. It will also be interesting to study the role of (postsynaptic) LTP in associative eyeblink conditioning. LTP can be observed after parallel fiber stimulation alone, which makes it an ideal candidate mechanism to mediate extinction of conditioned eyeblink responses, as extinction can be obtained by application of the conditioned stimulus alone (for review see Hansel et al., 2001).

4.17.19 Conclusion

In this article, we have provided a portrait of LTD by describing the phenomenon *per se* and by summarizing and explaining the cellular signaling cascades that are involved in LTD induction and expression. At the end of this article, we would like to address a question (once more) that appears particularly crucial for plasticity at large: if LTP provides a cellular correlate of information storage and learning (synaptic memory), why is there a need for LTD?

We would like to discuss this question using again the hippocampal area CA1 (and with it, glutamatergic synapses onto cortical pyramidal cells in general) and the cerebellum as examples.

We hasten to add, however, that the list of possible LTD mechanisms is not exhausted by the study of the two synapses we have highlighted in this article. Inhibitory synapses have also been shown to exhibit LTD (Marty and Llano, 1995; Aizenman et al., 1998). Other forms of LTD have been described in excitatory synapses at other locations, such as the striatum (Lovinger et al., 1993; Calabresi et al., 1999), amygdala (Li et al., 1998; Wang and Gean, 1999), neocortex (Artola et al., 1996; Egger et al., 1999), other parts of the hippocampal formation (Kobayashi et al., 1996; Manahan-Vaughan, 1998), and the olfactory bulb (Mutoh et al., 2005). Time will tell whether these forms of LTD use expression mechanisms which overlap with those we have described here.

By intuition, most researchers in the plasticity field would likely associate LTP with learning and LTD with forgetting. This might be due to the fact that a strengthening of synaptic transmission is easily perceived as information storage, and a weakening of synaptic strength as erasing previously stored information. Moreover, LTP and LTD, as characterized in the adult, have strong overlap with cellular mechanisms involved in strengthening (establishing) and weakening (disconnecting) synapses in the developing brain (e.g., Rittenhouse et al., 1999; for review see Singer, 1995), and synapse elimination certainly qualifies as a form of deleting previously present 'information.' Over the last years, experimental evidence from the hippocampus supports this view; agents that block signaling factors for LTP induction (e.g., NMDARs), or corresponding genetic manipulations, impair hippocampus-dependent learning, for example, in spatial learning tasks (for review see Silva, 2003). Along these lines, mutations that enhance LTP enhance learning. This effect was observed in transgenic mice overexpressing NR2B receptors (Tang et al., 1999), but also in transgenic mice expressing a constitutively active form of H-ras, which facilitates glutamate release through increased extracellular signal-regulated protein kinase (ERK)-dependent phosphorylation of synapsin I (Kushner et al., 2005). Similarly, the protein phosphatases PP1 and PP2B (calcineurin), which are involved in LTD induction, have been shown to constrain LTP and learning (Malleret et al., 2001; Genoux et al., 2002), suggesting that LTD itself constrains learning and promotes forgetting. On the other hand, a forebrain-specific calcineurin knockout resulted in a blockade of LTD, but selectively impaired hippocampus-dependent working and episodic-like memory tasks (Zeng et al., 2001), which suggests that LTD inhibition does not automatically facilitate learning. Moreover, LTD is involved in novelty acquisition (Manahan-Vaughan and Braunewell, 1999; Etkin et al., 2006). It might, therefore, be impossible to generally state that LTP equals learning and LTD equals forgetting. Rather, it will be necessary to look in detail at the type of synapse involved and the particular learning task. At cortical synapses, where the correlation to behavioral outputs is far more difficult to establish, the concept of LTD and LTP as extremes along an array of graded synaptic weights might be more useful. At this synaptic level, the need for reversal mechanisms (e.g., LTD for potentiated synapses) is more obvious, as reversibility prevents saturation and, consequently, the subsequent loss of plasticity. It has to be kept in mind, though, that LTP can act as much as a reversal mechanism for LTD as LTD can be a reversal mechanism for LTP.

In the cerebellum, it is easier to assign a role in motor learning to parallel fiber LTD, although even in the cerebellum this link is not established beyond any doubt (see earlier). A final question then is why the cerebellum uses LTD for learning. A possible answer can be found in the range of spontaneous action potential discharge rates observed in pyramidal cells and Purkinje cells. Recent *in vivo* whole-cell patch-clamp recordings in awake animals show that the spontaneous spike rates in cortical neurons are extremely low; e.g., in the somatosensory cortex they are well below 0.1 Hz (Margrie et al., 2002). In contrast, Purkinje cells have spontaneous discharge rates of 30 Hz, and simple spike rates can transiently exceed 200 Hz upon sensory activation (see Monsivais et al., 2005). These high discharge rates provide a strong, tonic inhibition to the target cells in the

DCN. Parallel fiber LTD leads to a disinhibition of DCN cells, facilitating transmission and/or LTP induction at mossy fiber–DCN synapses (Medina et al., 2002). Thus, it seems that Purkinje cells use different plasticity rules than pyramidal cells, because they are GABAergic projection neurons and because they belong to the few types of neurons with a high spontaneous discharge rate. These features assign different roles to Purkinje cells in the local cerebellar network than those assigned to pyramidal cells in their local networks and projection areas.

Ten years ago, the LTD club was very small, and LTD seemed like an obscure synaptic phenomenon. Indeed, it is probably safe to say that at the end of the 1980s there was little general interest in LTD, or even any strong conviction that homosynaptic depression existed at all outside the cerebellum. This situation has clearly changed. The major challenge for the next 10 years will be to see if the role of LTD in the brain lives up to its lofty theoretical promise.

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4.18 GABAergic Interneurons in Synaptic Plasticity and Information Storage

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4.18.1 Introduction

At the level of the hippocampal network, the net flow of information in the CA1 region is strongly modulated by the action of the nonpyramidal neurons, whose cell bodies are distributed throughout all layers of the hippocampus (for reviews, see Freund and Buzsaki, 1996; McBain and Fisahn, 2001). The vast majority of these cells are glutamic acid decarboxylase (GAD)–positive, inhibitory interneurons whose axons target different domains of the pyramidal cell dendritic tree. This selective targeting is important for the control of principal cell generation of somatic sodium spikes or dendritic calcium spikes (Miles et al., 1996). Moreover, fast γ-aminobutyric acid ($GABA_A$) receptor–mediated synaptic inhibition plays a critical role in determining the net excitability of the hippocampus. The frequency of tonic $GABA_A$-mediated inhibitory postsynaptic currents (IPSCs) received by pyramidal neurons is almost 10 times that of glutamatergic excitatory postsynaptic currents (EPSCs) (McBain and Dingledine, 1992; Soltesz and Mody, 1994). In addition to their control of cell excitability, interneurons also underlie the synchronization of pyramidal neuron firing (Cobb et al., 1995) and have been proposed to be involved in the generation of large-scale network oscillations (Buzsaki and Chrobak, 1995; Klausberger et al., 2003, 2004, 2005; Somogyi and Klausberger, 2005). Therefore, the occurrence of synaptic plasticity in nonpyramidal cells, in addition to that occurring in principal neurons, would greatly increase both the

power and the level of complexity of signal processing and of information storage in the hippocampus.

It is important to point out that interneurons are embedded in complex networks of highly interconnected cells. As such, they receive both excitatory and inhibitory inputs from a variety of sources as well as making inhibitory synaptic outputs onto many distinct structural targets within their particular circuit. Consequently, plasticity of the interneuron circuit may not only arise at the level of excitatory (or inhibitory) drive onto the interneuron but may also occur at the level of inhibitory input onto the cell's target. In this chapter, we consider, first, specific forms of synaptic plasticity of excitatory input onto interneuron populations, which is then followed by a review of the synaptic plasticity of GABAergic output generated by interneurons on their target cells.

4.18.2 Long-Lasting Plasticity of Excitatory Synaptic Input onto Interneurons

Despite rapid progress in elucidating the cellular mechanisms responsible for long-term plasticity at excitatory synapses between principal cells (Bliss and Collingridge, 1993; Nicoll, 2003; Collingridge et al., 2004), relatively little insight has been gained concerning mechanisms of plasticity of excitatory drive onto inhibitory interneurons. Moreover, excitatory transmission typically displays cell-target-specific regulation, indicating that the same rules governing plasticity at principal cell synapses cannot necessarily be applied to equivalent inputs onto interneurons (Maccaferri et al., 1998; Markram et al., 1998; Reyes et al., 1998; Scanziani et al., 1998; McBain et al., 1999; Toth et al., 2000; Toth and McBain, 2000). In an earlier review (McBain and Maccaferri, 1997), we described the technical complexities associated with trying to tease apart 'true' mechanisms of plasticity associated with excitatory synapses transmitted onto identified inhibitory interneurons from network effects from passively propagated forms of plasticity that had their origins at principal cell synapses. Although these issues are still highly relevant, since that time a number of careful studies have directly demonstrated a number of novel plastic mechanisms arising between excitatory synapses and local circuit inhibitory neurons. Interestingly, many of these studies show activity-dependent strengthening or weakening of excitatory synaptic transmission onto interneurons, which occurs via mechanisms distinct from those observed at principal cell synapses within the same subfield. In this section we highlight a number of these studies.

4.18.3 The MF-CA3 Network

In the hippocampus, mossy fiber (MF) axons arising from dentate gyrus granule cells provide excitatory glutamatergic input both to the dentate gyrus/hilar region and to area CA3. Within the CA3 subfield, mossy fiber axons synapse with both CA3 pyramidal cells (PYRs) and stratum lucidum inhibitory interneurons (SLINs). This synaptic arrangement generates a strong feedforward inhibitory circuit, as SLINs, which provide inhibitory inputs to CA3 PYRs, are more readily excited by MFs than are PYRs (Lawrence et al., 2004). Such feedforward inhibitory circuits provide exquisite control over network excitability through surround inhibition and by shaping temporal windows for input summation and spike generation in principal cell targets (Pouille and Scanziani, 2001; Lawrence and McBain, 2003; Mori et al., 2004). Thus, the plasticity of MF feedforward inhibition has the capacity to dramatically influence CA3 information processing.

Interestingly, MF axons innervate their divergent CA3 postsynaptic targets via anatomically distinct presynaptic elements: Inputs onto PYRs are formed by large, complex presynaptic boutons (mossy terminals), while SLINs are predominantly innervated by small en passant varicosities or filopodial extensions (Acsady et al., 1998) (**Figure 1**). Postsynaptically, α-amino-3-hydroxy-5-methyl-isoxazole-4-propionic acid subtype glutamate receptors (AMPARs) at MF-SLIN synapses comprise a continuum ranging from GluR2-lacking, Ca^{2+}-permeable channels (CP-AMPARs) to GluR2-containing, Ca^{2+}-impermeable channels (CI-AMPARs), whereas mature MF-PYR synapses contain only CI-AMPARs (Toth and McBain, 1998; Lei and McBain, 2002).

4.18.3.1 MF–Stratum Lucidum Interneuron Synapses

Interneurons of the stratum lucidum are primarily targeted by the filopodial extensions and en passant MF synapses, but rarely by the larger MF boutons that typically contact principal cells (Acsady et al., 1998) (**Figure 1**). Given that the filopodial extensions originate from the large MF terminal, it might be assumed that any change in presynaptic release

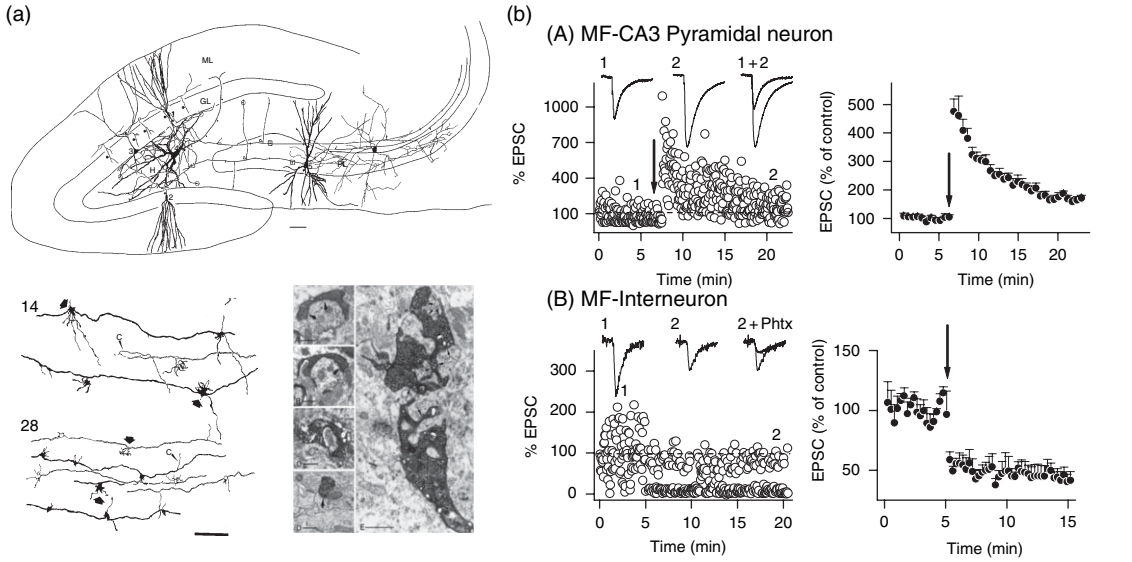

Figure 1 Basic anatomical elements of mossy fiber–CA3 circuitry. (a) The dentate gyrus and proximal portion of the hippocampus (mossy fibers [MFs]). Granule cells (labeled 1) give rise to extensive collateral plexuses, the MFs that are distributed throughout much of the hilus (H) and make synaptic contact with both dentate gyrus basket cells (2) and mossy cells (3). As the parent MF approaches the CA3 pyramidal cell layer (PL), the large presynaptic MF expansions begin to appear and are typically 140–200 μm apart for the entire length of the axon. These expansions form complex synapses on the proximal apical dendrites of pyramidal cells (4). The small filopodial or en passant terminals make synapses onto local circuit interneurons primarily located within the stratum lucidum (5). Scale bar, 100 μm. Left lower panel: Camera lucida drawings of Golgi-impregnated MFs illustrate the filopodial extensions emerging from the large complex MF terminal (large arrows). By postnatal day (P) 14, mature MF expansions are common. At P28 there is an increase in the number of MF expansions that have reached adult shape and size, and the lengths of the individual expansions have decreased to adult levels. Scale bar, 50 μm. Right lower panel: Electron micrographs of different terminal types along MFs in the CA3 region (A–C, E) and of a CA3 pyramidal cell terminal (D). All electron micrographs have the same magnification, for comparison of the relative size of the terminals. (A, B) A small en passant terminal establishes a single asymmetrical synapse on a dendritic shaft with long perforated postsynaptic density (arrows). (C) A filopodial extension of a mossy terminal forms a synapse (arrow) with a substance-P-receptor-immunoreactive interneuron. (D) The postsynaptic target of a pyramidal cell terminal is a simple spine of a CA1 pyramidal neuron. (E) A large, double-headed mossy terminal forms multiple contacts (arrows) with thorny excrescences of a CA3 pyramidal cell. The individual release sites are short. Scale bars, 0.5 μm (A–D) and 1 μm (E). (b) High-frequency stimulation (100 Hz for 1 s) of MF afferents onto CA3 pyramidal cells results in robust NMDAR-independent long-term potentiation, as evidenced by the increase in EPEC amplitude. Left panel represents an individual experiment with associated averaged EPSCs, as indicated by numbers above. Right panel represents pooled data. The identical induction paradigm results in a long-term depression at filopodial/en passant MF synapses onto CA3 stratum lucidum interneurons. These data highlight that, although expression of both forms of plasticity occur within the presynaptic terminal, an opposing change in synaptic strength occurs. Panel (a, top) reproduced from Claiborne et al. (1986) Wiley-Liss, Inc., a subsidiary of John Wiley & Sons, Inc.; the stratum lucidum inhibitory interneuron (cell 5) was added to the original figure by JJ Lawrence and CJ McBain. Lower left panel in (a) reproduced from Amaral and Dent (1981) Wiley-Liss, Inc., a subsidiary of John Wiley & Sons, Inc. Lower right panel in (a) reproduced from Acsady et al. (1998) from the Society for Neuroscience. Panel (b) reproduced from Toth et al. (2000) from the Society for Neuroscience.

probability arising from long-term potentiation (LTP) in the large MF bouton would distribute evenly to all synapses, resulting in long-term changes at both MF–pyramidal cell and interneuron synapses. However, quite the opposite is the case. At MF–CA3 st. lucidum interneuron synapses, the same high-frequency nonassociative protocol that induces LTP at principal cell synapses induces two forms of long-term depression (LTD) at interneuron synapses (**Figure 1**; Lei and McBain, 2004; Pelkey et al., 2005). Postsynaptically, AMPARs at MF-SLIN synapses comprise a continuum ranging from GluR2-lacking, Ca^{2+}-permeable channels (CP-AMPARs) to GluR2-containing, Ca^{2+}-impermeable channels (CI-AMPARs) (Toth et al., 2000; Lei and McBain, 2002). Both CI-AMPAR- and CP-AMPAR-containing MF-SLIN synapses exhibit LTD in response to high-frequency stimulation (HFS) that arises from two distinct mechanisms (**Figure 2**; Maccaferri et al., 1998; Toth et al., 2000; Lei and McBain, 2002, 2004; Pelkey

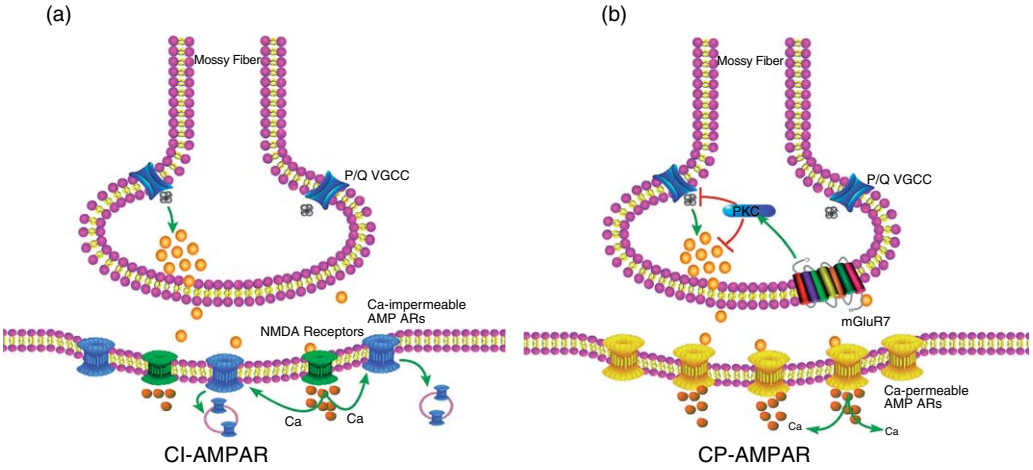

Figure 2 Two forms of mossy fiber-CA3 interneuron long-term depression (LTD). (a) Long-term depression at mossy fiber–interneuron synapses composed of Ca-impermeable α-amino-3-hydroxy-5-methyl isoxazole-4-propionic acid (AMPA) receptors has a postsynaptic locus of induction and expression, is *N*-methyl-D-aspartate (NMDA) receptor dependent, and involves an endocytosis of surface AMPA receptors reminiscent of NMDAR-dependent LTD observed at principal cell synapses. (b) LTD at mossy fiber–interneuron synapses composed of Ca-permeable AMPA receptors has a requirement for a postsynaptic increase in intracellular Ca but is expressed presynaptically. This form of LTD is NMDA receptor independent and requires activation of presynaptic mGluR7 and downstream PKC-dependent cascades to reduce transmitter release probability via a reduction of the voltage-gated Ca transient generated by P/Q Ca channels.

et al., 2005). Induction of each is blocked by inclusion of the Ca^{2+} chelator BAPTA in the postsynaptic compartment, indicating a postsynaptic induction locus for each (cf. MF CA3 pyramidal cell LTP described following). Activity-induced LTD of MF-SLIN transmission at CI-AMPAR synapses is blocked by inclusion of the *N*-methyl-D-aspartate receptor (NMDAR) antagonist, D-AP5, indicating that this form of plasticity is mediated in part by postsynaptic NMDAR activation. Expression of this form of LTD proceeds via subsequent AMPAR internalization by a mechanism similar, but not identical, to postsynaptically expressed LTD observed at excitatory synapses throughout the central nervous system (Lei and McBain, 2002, 2004). Consistent with a postsynaptic expression locus, this form of LTD is not accompanied by changes in the paired pulse ratio, the CV (coefficient of variation), or the synaptic failure rate – parameters typically used to monitor presynaptic mechanisms. Although none of these parameters on their own rigorously supports presynaptic changes (e.g., postsynaptic conversion of silent to active synapses will also change these parameters), an independent test of whether this NMDA receptor–dependent form of mossy fiber plasticity was postsynaptic made use of the low-affinity α-amino-3-hydroxy-5-methyl-isoxazole-4-propionic acid (AMPA) receptor antagonist γ-D-glutamyl glycine (γ-DGG) (Lei and McBain, 2004). In these experiments, Lei and McBain (2004) demonstrated that the magnitude of block of the evoked synaptic event by γ-DGG was unaltered following induction of NMDA-dependent MF-interneuron long-term depression. These data argue that the transmitter-release concentration profile is unchanged following LTD expression. Taken together, these data support the conclusion that this form of plasticity has both its induction and expression locus within the postsynaptic compartment.

In contrast, LTD at CP-AMPAR containing MF-SLIN synapses is NMDAR independent and is expressed presynaptically as a reduction in transmitter release (Lei and McBain, 2004). Again, in contrast to postsynaptic NMDA receptor LTD at CI-AMPARs, this form of plasticity is accompanied by changes in failure rate, paired pulse ratio, and CV, arguing for a presynaptic expression locus. Consistent with this conclusion was the observation that the magnitude of block of the evoked EPSC by γ-DGG was greater following LTD expression. Together, these observations are consistent with NMDAR-independent LTD resulting from a reduction in presynaptic transmitter release through a mechanism that may arise as a reduction in transmitter release probability, by a reduction in multivesicular release, or a mechanism akin to reverse 'kiss and run' (Lei and McBain, 2004; Harata et al., 2006).

Of particular interest is that, whereas MF–CA3 pyramidal cell LTP involves PKA-dependent cascades, transmission and plasticity at either form of the MF-interneuron synapse is largely insensitive to the manipulations that elevate cyclic adenosine monophosphate (cAMP) levels (Maccaferri et al., 1998). In our original experiments, application of forskolin induced a robust potentiation of MF–pyramidal transmission, consistent with MF–principal cell plasticity arising via an adenylyl cyclase–cAMP mechanism involving rab3/Rim1a modulation of synaptic transmission (Nicoll and Schmitz, 2005). The same experiment applied to MF interneuron synapses revealed a complete lack of sensitivity to exogenously applied forskolin, indicating that perhaps cAMP-dependent cascades were either absent or rendered inactive at MF interneuron synapses. This compartmentalization of the biochemical machinery highlights one potential functional utility of the filopodial versus large terminal arrangement of the MF presynaptic complex. How the cAMP-dependent cascade is segregated across the interterminal compartments is presently not understood.

LTD at MF–stratum lucidum Ca^{2+}-permeable AMPA receptor synapses is blocked by antagonists protein kinase C (PKC) (Pelkey et al., 2005) (PKC pathways are also implicated in MF–basket cell synapse LTP; see following for discussion). Furthermore, activation of mGluR7, which specifically localizes to MF terminals opposing stratum lucidum interneurons and not PYRs (Shigemoto et al., 1996, 1997), is an important step in the induction of presynaptic MF-SLIN LTD (**Figure 2(b)**; Pelkey et al., 2005). A brief application of the Group III agonist, L-AP4, at a concentration capable of activating mGluR7 induces a long-lasting depression of excitatory transmission between MFs and CP-AMPAR synapses. This synaptic depression far outlasts the drug application time and is not reversed by subsequent application of a broad-spectrum Group III mGluR antagonist, indicating that L-AP4 induces a chemical form of LTD at these synapses. This chemical form of LTD shares all of the hallmark features of activity-dependent LTD at these synapses, an increase in the paired pulse ratio, increased CV, and failure rate consistent with a presynaptic locus of expression. Interestingly, application of L-AP4 in the absence of continued synaptic stimulation failed to convert a short-term depression into LTD. Furthermore, introduction of the calcium chelator 1,2-bis(*o*-aminophenoxy)-ethane-N,N,N′,N′-tetraacetic acid (BAPTA) into the postsynaptic compartment blocked chemical LTD induction. Taken together, these data suggest that, like HFS-induced LTD at these synapses, there exists a requirement for postsynaptic activity for chemical LTD induction. Finally, of particular interest, blockade of mGluR7 activation during HFS not only prevented LTD induction but also revealed a previously unobserved posttetanic potentiation.

As it turns out, mGluR7 not only functions as a conventional autoreceptor at these synapses but also acts as a metaplastic switch at MF-SLIN synapses. Importantly, mGluR7 activation and cell surface expression governs the direction of plasticity. In naïve slices, mGluR7 activation during HFS generates MF-SLIN LTD, depressing presynaptic release through a PKC-dependent mechanism. Following agonist exposure, mGluR7 undergoes internalization, unmasking the ability of MF-SLIN synapses to undergo presynaptic potentiation or de-depression in response to the same HFS that induced LTD in naïve slices. Thus, the selective mGluR7 accumulation at MF terminals contacting SLINs and not PYRs provides cell-target-specific plasticity and bidirectional control of feedforward inhibition (Pelkey et al., 2005).

Recently one of the downstream targets of mGluR7 activation at these synapses was elucidated. Pelkey et al. (2006) observed that transmission at MF filopodia is supported primarily by P/Q-type voltage-gated calcium channel activity, with only a minor role for N-type channels. This presynaptic calcium channel arrangement is identical to that observed at the large MF bouton inputs into pyramidal cells and ruled out a role for differential voltage-gated calcium channel distribution as a mechanism to explain the functional divergence of the two presynaptic release sites. Of particular importance, they showed that LTD induced by either exogenous application of L-AP4 or HFS resulted in a persistent depression of the presynaptic voltage-gated calcium channel signal monitored by two-photon microscopy. Importantly, this depression of the calcium transient arose through a preferential depression of P/Q calcium channels: Blockade of P/Q channels prevented both the depression of synaptic events and calcium transients by either L-AP4 or HFS. These data indicate that synaptic depression at MF–CP-AMPAR interneuron synapses arises by an mGluR7-dependent persistent reduction in P/Q calcium channel function.

Bidirectional synaptic plasticity of excitatory synaptic transmission onto interneurons has been reported at three other synapses: dentate gyrus granule

cell–basket cell synapses, CA3 pyramidal cell–stratum radiatum interneuron synapses, and CA1 pyramidal cell–stratum oriens–alveus interneuron synapses. In the next section each of these synapses is discussed in turn.

4.18.3.2 MF–Dentate Gyrus Basket Cell Synapses

In experiments employing paired recordings between granule cells and basket cells of the dentate gyrus, or low-frequency stimulation of afferent fibers, Jonas and colleagues (Alle et al., 2001) demonstrated an associative form of MF posttetanic potentiation (PTP) and LTP. Analysis of failures, coefficient of variation, and paired pulse modulation indicated that both PTP and LTP are expressed in the presynaptic compartment. The mechanism was reminiscent, but not identical, to MF–CA3 pyramidal cell LTP. One major discrepancy between MF–pyramidal cell synapse LTP and MF–basket cell LTP was the presynaptic second messenger systems engaged. While MF–CA3 pyramidal cell LTP involves PKA-dependent cascades, MF–basket cell LTP primarily involved a PKC-dependent cascade. In addition, induction of associative LTP was blocked by postsynaptic infusion of the Ca^{2+} chelator BAPTA, indicating a postsynaptic locus of MF–basket cell LTP induction. The induction locus for MF–CA3 pyramidal cell LTP remains somewhat controversial (Nicoll and Schmitz, 2005). Interestingly, by using a nonassociative protocol, in which the postsynaptic cell was held under voltage clamp and incapable of firing action potentials, the same authors demonstrated a long-term depression at MF–basket cell synapses. This observation is consistent with the LTD observed at MF synapses onto stratum lucidum interneurons in the CA3 hippocampus (Maccaferri et al., 1998; Toth et al., 2000) (see earlier for further details). In conclusion, these data again highlight that, although both forms of LTP (MF–CA3 pyramidal cell and MF–basket cell) are expressed presynaptically, there are major differences in both the induction and expression mechanisms that are critically dependent on the identity of the postsynaptic target (Lawrence and McBain, 2003).

4.18.3.3 CA3 Collateral–Stratum Radiatum Interneuron Synapses

McMahon and Kauer (1997) made the important observation that excitatory synapses onto CA1 stratum radiatum interneurons weaken (LTD) in response to a high-frequency stimulation paradigm that typically strengthens (LTP) transmission at the same synapses made onto CA1 pyramidal cell synapses. This was the first demonstration of HFS–induced long-term depression at an excitatory synapse onto a local circuit inhibitory interneuron. Moreover, this LTD was not synapse specific, with depression being triggered at multiple excitatory synapses onto the same interneuron despite HFS of only a single set of synapses. This form of heterosynaptic depression was observed in numerous morphological cell types that were considered to participate in feedforward inhibitory input and suggested that LTD in response to HFS may be a common principal among interneurons.

In a related expansion of this initial observation, Dingledine and colleagues demonstrated that excitatory synaptic transmission between CA3 collateral–stratum radiatum interneuron synapses composed of CP-AMPARs also depressed in response to a HFS induction paradigm (Laezza et al., 1999). This form of LTD was originally reported to arise via a presynaptic mGluR7-dependent form of depression, similar to that observed at MF–stratum lucidum interneuron synapses (Pelkey et al., 2005). However, a subsequent study from the same group then demonstrated a form of bidirectional plasticity at these synapses that depended on postsynaptic membrane potential and NMDAR activation (Laezza and Dingledine, 2004). At synapses expressing CP-AMPARs, HFS of CA3 pyramidal cell collaterals, while voltage clamping the cell at depolarized potentials to relieve the Mg block of the NMDA receptor, resulted in LTP. LTP was prevented by hyperpolarizing the cell to −70 mV, strongly depolarizing the cell to 0 mV (presumably to reduce Ca influx through the NMDAR), or when BAPTA was introduced into the postsynaptic cell. Together these data argued for an entirely postsynaptic induction and expression locus for LTP. This study suggests that stratum radiatum interneurons have an exquisite sensitivity to postsynaptic Ca levels and that only small changes in the Ca driving force are required to convert LTP (induced at −30 mV) into LTD (LTD was observed at synapses clamped at both 0 mV and −70 mV), perhaps reflecting differential Ca entry via both NMDA receptors and CP-AMPARs. Unfortunately, no role was described or considered for the presynaptically expressed mGluR7 in LTP induction or expression, which they had previously implicated as a requirement for LTD at the same synapses. However, the data would

suggest that activation of postsynaptic NMDA receptors is capable of overriding the HFS-induced, mGluR7-dependent changes in presynaptic transmitter release properties associated with LTD.

It is worthwhile pointing out that this novel bidirectional plasticity is fundamentally different from the mGluR7-dependent plasticity described at MF–CP AMPAR stratum lucidum interneuron synapses. When NMDAR signaling is left intact and postsynaptic cells are held close to resting membrane potential, CP-AMPAR containing MF-SLIN synapses exhibit only LTD following HFS (Lei and McBain, 2002), while CP-AMPAR containing collateral–radiatum interneuron synapses give nonuniform responses to HFS (Laezza and Dingledine, 2004). Moreover, rather than NMDAR activation or membrane depolarization, potentiation of MF-SLIN synapses occurs in response to HFS only following the *removal* of mGluR7 from presynaptic terminals.

4.18.3.4 CA1 Pyramidal Cell–Stratum Oriens–Alveus Interneuron Synapses

Excitatory synapses made onto a variety of stratum oriens–alveus interneuron subpopulations express an mGluR1-dependent form of long-term potentiation in response to postsynaptic depolarization and theta burst stimulation of excitatory afferents (**Figure 3**). This form of LTP is NMDAR independent but requires postsynaptic activation of mGluR1a receptors and a consequent postsynaptic Ca elevation (Perez et al., 2001; Lapointe et al., 2004). Confirmation that this form of plasticity is critically dependent of metabotropic glutamate receptor function came from the important observation that LTP is absent in the mGluR1-knockout mouse. Interestingly, like a number of other forms of interneuron plasticity, it occurs only at CP-AMPAR synapses but surprisingly does not appear to require Ca flux through the CP-AMPAR for induction or expression (Topolnik et al., 2005). Although induction required activation of postsynaptic mGluR1a, expression was accompanied by changes in both the paired pulse ratio and coefficient of variation, indicating a role for presynaptic expression. Another interesting feature of this form of LTP was its tight correlation with the presence of presynaptic group II mGluRs but not group III mGluRs (Lapointe et al., 2004). Recently, this same group identified an important piece of the signaling cascade puzzle required for mGluR1a-dependent LTP (Topolnik et al., 2005). Using two-photon imaging of dendritic Ca signals in oriens-alveus interneurons, they convincingly demonstrated that mGluR1a activation induces a Ca elevation that arises in part from transient receptor potential (TRP) channel activation, with a minor component arising from Ca release from ryanodine-sensitive intracellular stores. The mGluR1a/TRP Ca signal depends on both Src tyrosine kinase and

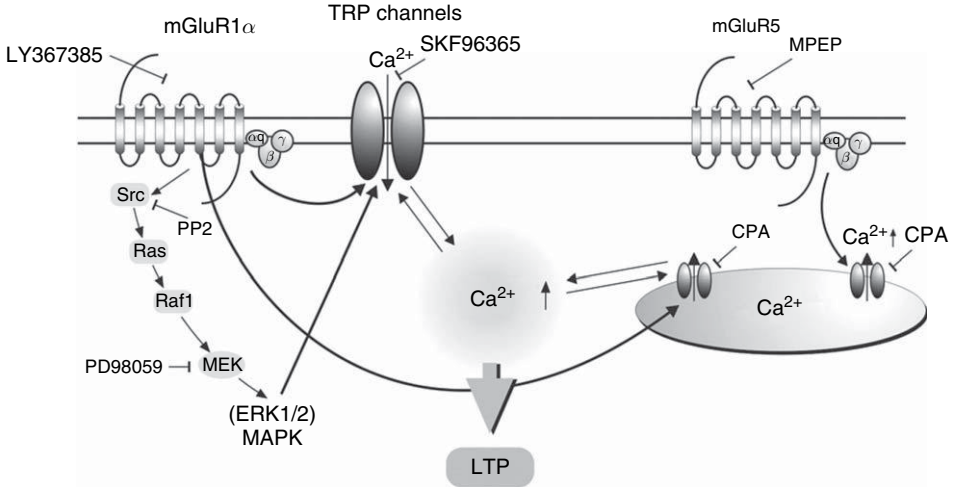

Figure 3 mGluR1-dependent interneuron plasticity. Schematic representation of distinct mGluR1 and mGluR5 Ca^{2+} signaling pathways and their role in long-term potentiation (LTP) induction in O/A interneurons mGluR1 activation is linked to two parallel Ca^{2+} signaling pathways: one leading to Ca^{2+} entry via tryptophan (TRP) channels and a second producing Ca^{2+} release from intracellular stores. Src/ERK-dependent Ca^{2+} entry via TRP channels and intracellular release are necessary for LTP induction. mGluR5 activation causes intracellular Ca^{2+} release that does not require Src/ERK and TRP channels and does not appear to be involved in LTP induction. (Topolnik L, Azzi M, Morin F, Kougioumoutzakis A, and Lacaillc JC (2006) mGluR1/5 subtype-specific calcium signalling and induction of long-term potentiation in rat hippocampal oriens/alveus interneurones. *J. Physiol.* 575: 115–131.)

extracellular signal-regulated protein kinase (ERK) activation (**Figure 3**). This raises an important issue that suggests that ERK signaling is necessary for receptor-activated Ca signaling in interneurons, whereas in principal cells it may produce long-term changes involving gene expression and protein synthesis (Kelleher et al., 2004a, b; Topolnik et al., 2006). One important feature of this plasticity is that the ERK inhibitor PD98059 and the SERCA pump inhibitor CPA (cyclopiazonic acid) not only prevent LTP induction but also uncover a novel form of long-term depression (Topolnik et al., 2006). Similar effects were also observed using SKF96365, a TRP channel inhibitor.

4.18.3.5 NMDAR-Dependent LTP at Schaffer Collateral Stratum Radiatum Interneuron Synapses

Early attempts to identify long-term plastic changes in excitatory synaptic transmission onto interneurons focused on detecting potential NMDAR-dependent mechanisms, since this is the most common form of plasticity observed at hippocampal principal cell excitatory synapses. At principal cell synapses, NMDAR-dependent LTP relies on a transient Ca elevation accompanying NMDA receptor activation, requires activation of Ca-dependent CaMKII, and is thought to arise via a mechanism of 'unsilencing' NMDAR-containing synapses involving the trafficking of AMPA receptor subunits (for reviews, see Nicoll, 2003; Collingridge et al., 2004). Successful attempts to observe a similar NMDAR-dependent form of LTP (or LTD) on interneurons of the CA1 hippocampus were few (Ouardouz and Lacaille, 1995; Cowan et al., 1998; Laezza and Dingledine, 2004). Maccaferri and McBain (1996) performed an extensive search for NMDAR-dependent LTP, using the perforated patch recording configuration to prevent perturbation of the intracellular environment, whose preservation appears critical for LTP induction at principal cell synapses. This approach yielded no evidence for NMDAR-dependent LTP on a number of subpopulations of CA1 interneuron. Given that NMDAR-dependent LTP in principal cells requires activation of CaMKII and that interneurons are generally considered to lack CaMKII, it was perhaps not surprising that this form of LTP was absent from inhibitory interneuron synapses.

In the intervening years, although a number of other forms of LTP and LTD have been described at excitatory synapses on interneurons, evidence for a hebbian form of interneuron LTP requiring NMDA receptors was elusive. However, in 2005, Lamsa et al. (2005) described a hebbian form of NMDAR-dependent LTP at excitatory synapses made onto feedforward inhibitory neurons of the CA1 stratum radiatum. Again, using the perforated patch clamp recording configuration, they demonstrated that, like NMDAR-dependent LTP onto principal cells, pairing-induced plasticity on interneurons was blocked by inclusion of an NMDAR antagonist, was not accompanied by changes in the paired pulse ratio, and was absent in recordings made with whole-cell pipettes. This form of LTP occurred in about 40% of cells tested and, importantly, occurred on cells subsequently identified as spine free, indicating that NMDAR-dependent plasticity is not limited to spines on pyramidal cells. This observation is consistent with the observation that some interneuron types can form spatially restricted Ca domains within their aspiny dendrites (Goldberg et al., 2003). Another important conclusion of this study was the observation that strengthening of excitatory transmission onto interneurons could also influence the degree of feedforward inhibitory drive onto pyramidal cells. This, the authors argued, was a feature important in preventing a potential 'degradation' of the feedforward pathway during NMDAR-dependent LTP of principal cell synapses, and they emphasized that this may be a key feature in ensuring the "fidelity of information processing" (Lamsa et al., 2005).

Several issues here are worthy of further discussion. Although on the surface this form of LTP shares many phenomenological properties with NMDAR-dependent LTP at principal cell synapses, the actual downstream mechanisms of NMDAR activation are completely unexplored. However, given the apparent lack of CaMKII in interneurons (Sik et al., 1998) and the observation that interneurons typically lack silent synapses (Nusser et al., 1998b; although see Wang and Kelly, 2001), it is unlikely that it shares a mechanism identical to NMDAR-dependent LTP at Schaffer collateral synapses onto principal cells. Interestingly, this feature is also true for the NMDAR-dependent long-term depression observed at MF–CI AMPAR interneuron synapses, since interneurons also lack calcineurin (Sik et al., 1998), a key signaling cascade required for NMDAR-dependent LTD (see earlier discussion). Taken together, it would appear that, although interneurons are capable of generating NMDAR-dependent forms of plasticity, the key downstream signaling cascades

responsible for plasticity expression are likely to differ from their principal cell counterparts.

Second, a consideration of the network connectivity of inhibitory interneurons raises an important issue *against* the author's claim that NMDAR LTP on feedforward interneurons is important for the preservation of the "fidelity of information processing." The axons of inhibitory interneurons target hundreds, if not thousands, of principal neuron targets. Furthermore, Schaffer collateral–mediated excitatory transmission onto feedforward interneurons is robust, and in the case of parv albumin (PV)-positive, CB1 receptor–negative interneurons, which provide the bulk of feedforward inhibitory input, the excitatory synaptic event in the naïve slice is on average almost one order of magnitude greater than the concomitant event recorded onto principal cells (Glickfeld and Scanziani, 2006). The authors argue that any mechanism that strengthens Schaffer collateral inputs onto principal cells without a similar strengthening of the inhibitory circuit would lead to a potential imbalance of excitation within the system. However, consideration of the circuit would suggest that any potentiation of excitatory transmission onto feedforward interneurons would result in a massive recruitment of divergent inhibition onto large numbers (potentially thousands) of principal cells, potentially eroding the CA1 excitatory circuit and compromising the temporal fidelity of network signaling (Somogyi and Klausberger, 2005). Interestingly, the original description of plasticity at stratum radiatum interneurons by McMahon and Kauer (1997) demonstrated that stratum radiatum interneuron synapses *weakened* in response to a paradigm that strengthened synaptic transmission onto CA1 pyramidal cells. Moreover, a similar scenario was observed within the MF–CA3 network, where the same paradigm that strengthened MF–CA3 pyramidal cell excitatory synapses *weakened* transmission between MF and interneuron synapses. Clearly, much is left to be uncovered regarding both the mechanism(s) and network consequences of plasticity at excitatory synapses onto interneurons.

4.18.4 Long-Lasting Plasticity of Interneuron-Generated GABAergic Output

As detailed in the introduction to this chapter, GABAergic interneurons are essential elements for shaping cortical circuit dynamics and play critical roles in the regulation of cellular excitability, spike timing, network oscillations, and threshold for the induction of synaptic plasticity. To achieve these functions, GABA is released onto specific postsynaptic domains of target cells by different classes of interneurons (Gulyas et al., 1993; Miles et al., 1996). Therefore, the study of long-term plasticity of interneuronal output, specifically occurring at the level of the GABAergic synapse, is an important element for our understanding of network function dynamics. The integration of synaptic plasticity of excitatory input and GABAergic output will exert profound effects on the overall network function. As a result, although a direct demonstration is still lacking, long-term plasticity of interneuronal output is predicted to have an important impact on brain function and behavior. Furthermore, any long-term decrease in inhibition could also lower the threshold for epileptiform activity *in vitro* (Miles and Wong, 1987; Steltzer et al., 1987) and potentially underlie a number of pathological processes.

Analogous to what we have discussed above for excitatory input, we now consider the evidence for LTP and LTD of GABAergic synaptic transmission in mammalian cortical networks of the developing and mature brain. We discuss their molecular mechanisms and consequences for network processing, and later we briefly examine other forms of GABAergic plasticity relevant for network signaling. An important distinction that needs to be remembered here is that while the previous section considered excitatory input onto interneurons, all of the present discussion reviews recordings of GABAergic input onto pyramidal neurons. Finally, long-term plasticity of GABAergic input to specific types of interneurons is a virtually unexplored field and is not addressed here.

4.18.4.1 Activity-Dependent Long-Lasting Plasticity of GABAergic Input in the Hippocampus and Neocortex: General Considerations

Long-term potentiation and depression of GABAergic synaptic input have been studied in both the mammalian neocortex and hippocampus *in vitro,* with paradigms reminiscent of those used to study conventional forms of plasticity of excitatory input. However, in comparison to classical long-term plasticity of excitatory synaptic transmission, this field is relatively new, and most of the relevant mechanisms are incompletely understood. Furthermore,

the comparison of results from different laboratories is often problematic, since there is a general lack of standard protocols, which complicates the emergence of clear, unifying principles.

Before considering examples of plasticity at specific synapses, a few general points are worth mentioning to avoid confusion. Induction of long-term plasticity at excitatory inputs typically requires activity at the various types of synaptic ionotropic and/or metabotropic glutamate receptors and typically does not require activation of receptors for neurotransmitters other than glutamate. In contrast, induction of synaptic plasticity at GABAergic synapses often requires cross-talk *between* receptors for different neurotransmitters and neuromodulators, further complicating their study. For example, the pharmacological isolation of evoked GABAergic responses that is required for a careful comparison before and after any induction protocol, by necessity, often prevents the activation of glutamate receptors that may be critical for the induction of plasticity itself. Therefore, in some studies, pharmacological isolation of GABAergic test responses is temporarily relieved during the induction protocol, whereas other studies may maintain the blockade throughout the experiment (e.g., compare McLean et al., 1996, with Shew et al., 2000). Such an experimental design may introduce an uncontrollable source of inconsistency, preventing a true comparison of results from different laboratories. Nevertheless, several factors have been recognized as essential in determining the large experimental variability of the results. First, the stage of brain development appears to be especially important. $GABA_A$ receptor–mediated signaling in early neonatal life is excitatory and generates depolarizing IPSPs (dIPSPs, Owens et al., 1996). $GABA_A$ receptors are mostly permeable to chloride (Borman et al., 1989), and chloride homeostasis in neurons is developmentally regulated by the early expression of the Na-K-Cl cotransporter NKCC1 that accumulates chloride intracellularly (Plotkin et al., 1997), in contrast to the later expression of the neuron-specific K-Cl cotransporter KCC2, which results in chloride extrusion and a negative shift in the $GABA_A$ equilibrium potential (Rivera et al., 1999). GABA released onto neonatal pyramidal neurons can depolarize the postsynaptic membrane and hence can directly activate voltage-dependent calcium channels. Furthermore, GABA-mediated dIPSPs can remove the voltage-dependent blockade of NMDARs by Mg^{2+}, a role that is well established as critical for 'classical' NMDAR-dependent LTP of glutamatergic input (Bliss and Collingridge, 1993) and that is mediated by AMPA receptors in mature neurons. Therefore, early in development, GABAergic postsynaptic potentials can directly lead to Ca^{2+} influx or provide the associative signal required for Ca^{2+} entry via NMDARs, in case of simultaneous glutamatergic activity (Ben-Ari et al., 1997). Second, the pattern of stimulation used in the induction protocol appears to be more critical than what is typically observed for glutamatergic synaptic plasticity. For example, both high-frequency tetanization and theta-patterned stimulation are effective induction protocols to trigger LTP at glutamatergic synapses, whereas differential effects have been described with either paradigm at GABAergic inputs. Third, regional brain specificity needs to be taken into careful consideration, and it cannot be assumed that identical molecular mechanisms operating in the hippocampal network are valid for every other cortical region. For example, specific differences have been described in GABAergic plasticity in the hippocampus compared to visual cortex. Fourth, and finally, GABAergic plasticity is often studied by evoking GABAergic input following the stimulation of many fibers originating from undetermined interneuronal types. Yet it is critically important to remember that the cellular diversity of the interneurons generating the GABA release is staggering, and therefore the dynamic properties of averaged events across a number of interneuron subpopulations may blur the results of these types of experiments. Ideally, the specific plastic properties of unitary events originating from a well-identified interneuron should be studied. However, this has been achieved in only a few cases.

4.18.4.2 Activity-Dependent Long-Lasting Plasticity of GABAergic Input in the Neonatal Hippocampus

Bidirectional synaptic plasticity of GABAergic input has been repeatedly described in the neonatal hippocampus. Homosynaptic LTP and LTD of $GABA_A$ receptor–mediated IPSP/Cs have been recorded under a variety of conditions (Gaiarsa et al., 2002). Their induction is generally postsynaptic and is triggered by postsynaptic Ca^{2+} entry. However, the specific sources of Ca^{2+} entry appear critical in determining the direction of plasticity (see **Figure 4**). Ca^{2+} influx via voltage-dependent calcium channels results in LTP, whereas entry via NMDARs or an elevation resulting from Ca^{2+}-induced Ca^{2+} release results in

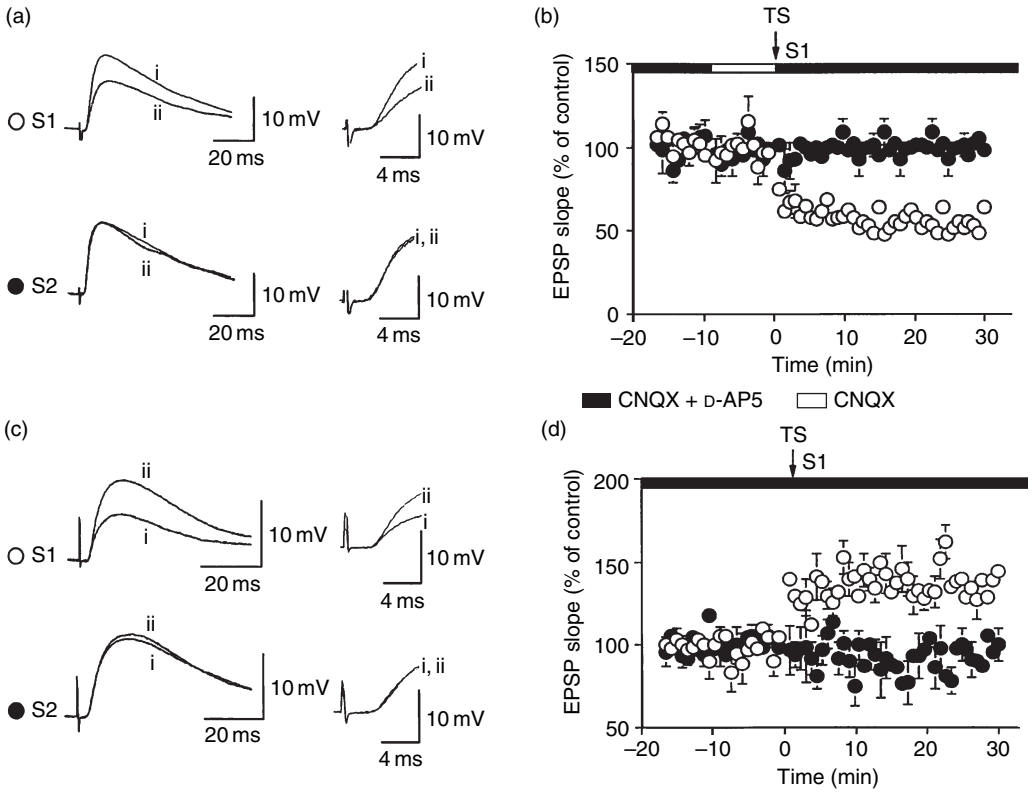

Figure 4 Bidirectional synaptic plasticity of γ-aminobutyricacid (GABA)-ergic inhibitory postsynaptic potentials (IPSPs) in the CA3 neonatal hippocampus. Evoked dIPSPs were recorded in the continuous presence of CNQX and D-AP5 in order to isolated GABAergic input. Notice, however, that the presence/absence of *N*-methyl-D-aspartate receptor (NMDAR) activity during the conditioning protocol determines the direction of the plasticity. Insets show averaged responses from the conditioned (S1) and control (S2) pathway. (a, b) Homosynaptic long-term depression (LTD) is induced by tetanic stimulation (TS) in the absence of the NMDAR blocker D-AP5. Insets in (a) show evoked responses before (i) and after the induction of LTD (ii). (c, d) Homosynaptic long-term potentiation (LTP) is triggered by TS after pharmacological blockade of NMDARs. Reprinted from McLean HA, Caillard O, Ben-Ari Y, and Gaiarsa JL (1996) Bidirectional plasticity expressed by GABAergic synapses in the neonatal rat hippocampus. *J. Physiol. (Lond.)* 496: 471–477, Blackwell.

LTD (McLean et al., 1996; Caillard et al., 2000). The locus of expression in both cases is likely to be presynaptic. LTP in neonatal and juvenile slices was associated with an increase of spontaneous and miniature IPSC frequency, but not amplitude (Caillard et al., 1999a; Shew et al., 2000). Similarly, LTD was associated with an increase in the coefficient of variation of evoked $GABA_A$ receptor–mediated synaptic currents and a decrease in the frequency, but not amplitude, of Sr^{2+}-induced asynchronous $GABA_A$ quantal events (Caillard et al., 1999b). If the release properties of the presynaptic terminals are involved in long-term plasticity, then a retrograde signal is most likely involved. In the case of LTP, neurotrophins acting via TrkB receptor–mediated phosphorylation have been proposed as candidates (Gubellini et al., 2005).

Neonatal long-term plasticity of GABAergic input has been proposed to play a critical role in the refinement of initially coarse patterns of synaptic connections in cortical circuits. Several observations make this hypothesis particularly attractive. First, neonatal plasticity is constrained by a limited period of development, which correlates well with the period of functional maturation of the network examined (Komatsu, 1994; McLean et al., 1996; Gubellini et al., 2001). Second, conditioning protocols that induce long-term plasticity are also effective in transforming silent into functional synapses. For example, the same protocols that are effective in inducing plasticity can turn on synapses in cells that previously displayed a complete absence of activity (Gubellini et al., 2001). Third and last, conditioning protocols used to trigger synaptic plasticity can be

very similar to the types of activity that neurons experience *in vivo*. For example, LTP can be induced by repeated bursts at low frequency that are reminiscent of the firing patterns recorded *in vivo* (Leinekugel et al., 2002).

4.18.4.3 Activity-Dependent Long-Lasting Plasticity of GABAergic Input in the Juvenile/Adult Hippocampus

In the juvenile-adult CA1 hippocampus, LTP of fast IPSPs and IPSCs has been reported following theta-patterned stimulation (Perez et al., 1999; Patenaude et al., 2003), whereas no change, LTD, or LTP (Stelzer et al., 1987; Wang and Stelzer, 1996; Lu et al., 2000; Shew et al., 2000; Chevaleyre and Castillo, 2003; Patenaude et al., 2003) have been reported following tetanic HFS. The induction of both types of plasticity is Ca^{2+} dependent and is prevented by perfusion of Ca^{2+} chelators into the postsynaptic cell (Patenaude et al., 2003). However, theta-pattern stimulation of unitary IPSCs was reported as ineffective in interneuron → pyramidal cell paired recordings, suggesting that a multifiber cooperative action may be required for induction. This is consistent with the observation that theta-patterned induced LTP can be prevented by blockers of either $GABA_B$ receptors or group I/II mGlu receptors (Patenaude et al., 2003), in a manner similar to that previously described in the visual cortex (Komatsu, 1996). The molecular pathway leading to tetanus-induced LTD of IPSPs critically depends on the activation of NMDARs and on the phosphatase calcineurin (Lu et al., 2000). Calcineurin dephosphorylates $GABA_A$Rs through the direct binding of the calcineurin catalytic domain to the second intracellular loop of the $\gamma 2$ subunit (Wang et al., 2003). In contrast to LTD, the molecular pathway leading to LTP remains unclear in these *in vitro* models. However, studies of plastic changes of GABAergic inhibition in the dentate gyrus of control versus kindled rats have provided functional and ultrastructural evidence for modification both of the number and of specific $GABA_A$ receptor subunits expressed at the synapse (Otis et al., 1994; Buhl et al., 1996; Nusser et al., 1998a). Thus, similar mechanisms might potentially be involved in LTP induced *in vitro* by HFS patterns.

In addition to mechanisms of plasticity that are reminiscent of classical LTP and LTD of excitatory synapses onto principal cells, the functional heterogeneity within the hippocampal interneuron population has also revealed novel, cell type–specific forms of plasticity. One specific form of long-term plasticity is expressed at GABAergic connections originating from a subset of interneurons whose presynaptic terminals express type 1 cannabinoid receptors (for a review, see Freund et al., 2003). Endocannabionid-mediated retrograde signaling activates these receptors (Alger, 2002) and reduces neurotransmitter release (Hajos et al., 2000; Katona et al., 1999). Tetanic and theta-burst stimulation of stratum radiatum afferents in the absence of ionotropic glutamatergic synaptic transmission lead to activation of mGluRs located within the pyramidal cell dendritic tree that trigger the retrograde release of endocannabinoids and a consequent selective LTD of transmission from CB1-expressing GABAergic terminals (Chevaleyre and Castillo, 2003). This form of LTD appears to be localized to a restricted subcellular area and affects only a limited subset of GABAergic terminals on pyramidal cell dendrites (Chevaleyre and Castillo, 2004). The complete molecular cascade and molecular mechanisms leading to the expression and maintenance of this form of LTD are presently unknown. This cannabinoid-dependent form of LTD has been suggested to play a critical role in priming LTP of excitatory connections (Carlson et al., 2002; Chevaleyre and Castillo, 2004).

4.18.4.4 Activity-Dependent Long-Lasting Plasticity of GABAergic Input in the Visual Cortex

LTP of fast evoked inhibitory synaptic transmission in the rat visual cortex has also been studied in some depth. Consequently, a detailed picture of the molecular cascades required for its induction is beginning to emerge. In general, visual cortex $GABA_A$ LTP in rats is induced by brief repetitive stimulation at high frequencies in the presence of antagonists of ionotropic glutamate receptors (Komatsu, 1994). Interestingly, when NMDARs were left unblocked during the conditioning protocol, $GABA_A$ LTD was observed (Komatsu and Iwakiri, 1993), indicating the potential for bidirectional plasticity. This type of plasticity is homosynaptic and, therefore, restricted to the conditioned pathway. Furthermore, it is also associative, such that weak conditioning stimulation to one pathway, which alone failed to induce LTP, induced LTP when strong conditioning stimulation was simultaneously applied to another independent pathway

(Komatsu, 1994). LTP can be observed both in neonatal and adult slices, although its threshold appears lower in younger animals (Komatsu, 1994). Importantly, induction is critically dependent on postsynaptic Ca^{2+} loading, mediated by IP3-induced Ca^{2+} release. Although the test responses to single stimuli in these studies were mediated exclusively by $GABA_A$ receptors, HFS used during induction protocols also activated $GABA_B$ and mGlu receptors (Komatsu, 1996). Activation of $GABA_B$ receptors during the induction protocol appeared essential for LTP, whereas mGluR activity was not required. At present, the role played by $GABA_B$ receptors remains unclear. One possibility is that they could be involved in raising postsynaptic calcium concentrations via IP3-induced Ca^{2+} release, but no direct demonstration has been provided yet. Another interesting observation in visual cortex $GABA_A$ LTP is that the activity of adrenalin and serotonin receptors appeared to be required during the conditioning stimuli. Indeed, application of alpha1-adrenoreceptor or 5-HT_2 receptor antagonists reduced the probability of inducing plasticity after a conditioning stimulus (Komatsu, 1996). This raised the possibility that postsynaptic $GABA_B$ receptor activity during the induction protocol may act by facilitating IP3-induced Ca^{2+} release triggered by adrenaline or serotonin (Komatsu, 1996). Maintenance of LTP depends on the activity of presynaptic GABAergic terminals and is mediated by presynaptic Ca^{2+} entry through multiple types of Ca^{2+} channel (Komatsu and Yoshimura, 2000). Furthermore, Yamada et al. (2005) showed that noradrenergic receptors may also play a role in the modulation of the maintenance of HFS-induced LTP. The authors showed that application of noradrenaline after tetanic stimulation in a reduced external calcium concentration that normally does not allow LTP resulted in potentiation of the conditioned, but not the test, pathway. Pharmacological analysis of this result indicated that the effect was mediated by alpha2 and beta adrenergic receptors (Yamada et al., 2005).

Analogous to our previous discussion, a lower threshold for induction of this plasticity exists during a critical developmental period, suggesting a particular role in development. Finally, during development, GABAergic synapses may play a role in the selective responses of visual cortical cells to features of visual stimuli such as orientation, length, or direction of a slit (Hubel and Wiesel, 1962), as suggested by the reduction of selectiveness following application of the $GABA_A$ receptor antagonist bicuculline (Sillito, 1975, but see Nelson et al., 1994). For example, optimal visual stimuli that produce strong spiking activity may result in activation of NMDARs and hence LTD of inhibition, consequently enhancing the optimal visual response. In contrast, nonoptimal stimuli producing only moderate spiking activity may selectively potentiate inhibition and result in an overall depression of the responses triggered by the stimuli.

4.18.4.5 Spike Timing–Dependent Plasticity of GABAergic Input in the Neocortex

In analogy with spike timing–dependent synaptic plasticity of glutamatergic inputs (Markram et al., 1997; for a review see Dan and Poo, 2004), work on reciprocally connected pairs of inhibitory and excitatory neurons in neocortical slices has shown that bidirectional modulation of unitary GABAergic input depends on the relative timing of pre- and postsynaptic activity (Holmgren and Zilberter, 2001). This type of GABAergic spike timing–dependent plasticity is also Ca^{2+} dependent, but the molecular cascade of events following Ca^{2+} elevations has not been elucidated yet. The mechanism proposed by the authors is that Ca^{2+}-dependent protein kinases may directly modulate the activity of postsynaptic $GABA_A$ receptors (Holmgren and Zilberter, 2001). Further work will be required to test this hypothesis. The significance of this form of plasticity is strengthened by the similarity of the induction protocol used *in vitro* to the activity observed *in vivo*. Indeed, bursting patterns of pre- and postsynaptic activity like the ones used in Holmgren and Zilberter (2001) are likely to occur during learning episodes and therefore could trigger long-term plasticity at GABAergic synaptic connections (Buzsaki et al., 1996; Thomas et al., 1998; King et al., 1999; Paulsen and Sejnowski, 2000).

4.18.4.6 Plasticity of $GABA_A$ Receptor Reversal Potential

Activity-dependent long-term plasticity can regulate synaptic transmission in several distinct ways. For example, the transmitter release properties of presynaptic terminals can be modified, or the number and/or the functional properties of postsynaptic receptors can be changed. Above, we have highlighted several examples of plasticity, which are mediated by these two general mechanisms.

However, responses mediated by $GABA_A$ receptors at inhibitory synapses depend also on the transmembrane chloride gradient (Bormann et al., 1987), which determines the polarity of the postsynaptic response, that is, whether the response is depolarizing (excitatory) or hyperpolarizing (inhibitory). Woodin et al. (2003) and Fiumelli et al. (2005) have reported that postsynaptic spiking of principal cells can induce local or global changes in the chloride reversal potential and hence depress the size of postsynaptic currents mediated by $GABA_A$ receptors in hippocampal cell cultures and slices from juvenile animals (**Figure 5**). These effects were mediated by Ca^{2+} entry via L-type Ca^{2+} channels and subsequent PKC activation (Woodin et al., 2003; Fiumelli et al., 2005). This type of plasticity required expression of KCC2, the chloride cotransporter, and significantly, was absent when KCC2 was blocked pharmacologically, suggesting that PKC depresses the activity of the transporter either by direct phosphorylation or by an indirect pathway. A similar modulation of EC1, but opposite in sign, was reported in neonatal slices by Ouardouz et al. (2006).

4.18.4.7 Plasticity of $GABA_B$ Receptor–Mediated Input in the Hippocampus

Synaptic inhibition in the hippocampus is mainly mediated by fast $GABA_A$ and slow $GABA_B$ receptor–mediated postsynaptic potentials (Dutar and Nicoll, 1988). However, in comparison to fast inhibition, studies focusing on the activity-dependent synaptic plasticity of $GABA_B$ receptor–mediated synaptic input are in their infancy. Intriguingly, $GABA_B$ receptors and G protein–activated inwardly rectifying K+ (GIRK) channels, which are responsible for the slow postsynaptic potential (Luscher et al., 1997), have been localized in spines (Drake et al.,

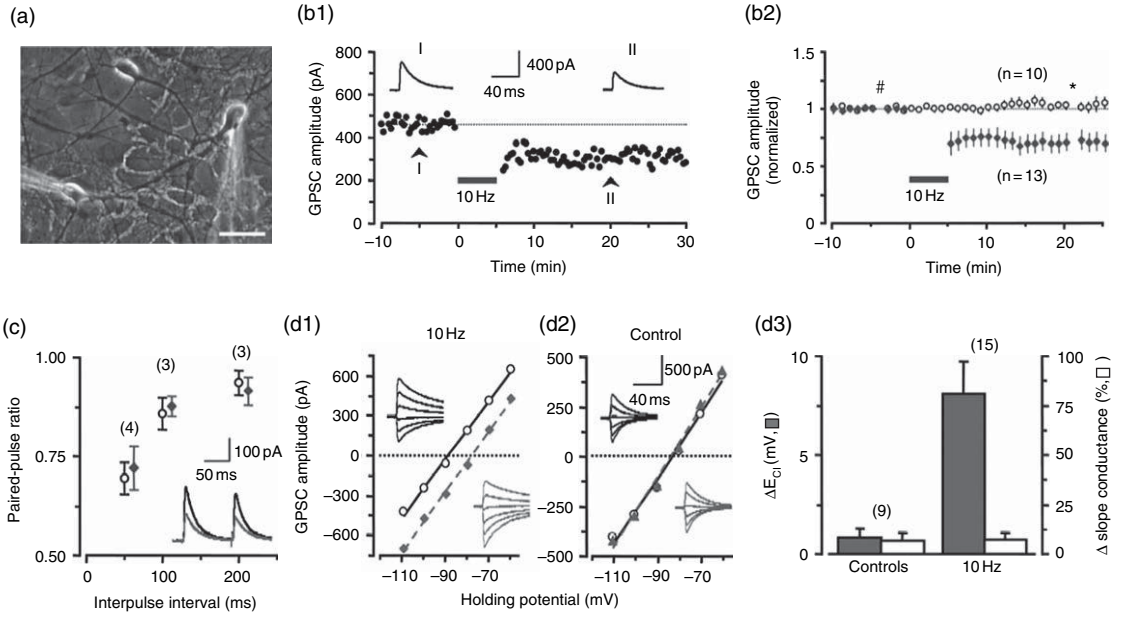

Figure 5 Long-term depression of GABAergic unitary responses recorded in pairs of cultured hippocampal neurons resulting from postsynaptic spiking–induced plasticity of E_{GABAA}. An image of a pair of connected neurons; scale bar, 20 μm. (b1) Single experiment showing that postsynaptic spiking at 10 Hz for 5 min induces long-term depression (LTD) of unitary GABAergic postsynaptic currents (GPSCs). Insets show average traces before (i) and after (ii) the conditioning protocol. (b2) Summary plot from two sets of experiments in which postsynaptic spiking was (filled symbols) or was not (empty symbols) applied. # and ∗ indicate the time selected to measure I/V relationships of the unitary event and estimate their reversal potentials (see panels d1 and d2). (c) Postsynaptic spiking does not alter the mean paired pulse ratio at various interevent intervals, suggesting the lack of presynaptic effects. (d1) I/V relationships of the GABAergic postsynaptic currents before (solid line) and after (dotted line) the conditioning protocol. Notice the shift in the reversal potential. In contrast, d2 shows the stability of I/V relationships and estimated reversal potentials in experiments where no conditioning protocols were used (panel b2, empty symbols). (d3) Summary graph indicating the changes in reversal potential and conductance of unitary GABAergic currents in control experiments (no conditioning protocol) and experiments in which postsynaptic spiking was used to trigger LTD (10 Hz). Reprinted from Fiumelli W, Cancedda L, and Poo MM (2005) Modulation of GABAergic transmission by activity via postsynaptic Ca^{2+}-dependent regulation of KCC2 function. *Neuron* 48: 773–786, © 2005, with permission from Elsevier.

1997; Kulik et al., 2003), suggesting the possibility that the same mechanisms operating in spines and leading to LTP of excitatory synaptic transmission might trigger LTP of slow inhibition. Huang and colleagues (2005) directly tested this hypothesis in the hippocampus and induced $GABA_B$ LTP following conventional stimulation patterns that are effective in the induction of glutamatergic LTP. Furthermore, when the cascade of events involved in LTP of $GABA_B$ receptor–mediated input was examined, it was found to depend on steps identical to the ones described for excitatory LTP. $GABA_B$ LTP was NMDAR and CaMKII dependent (Huang et al., 2005). However, the same authors also showed that $GABA_B$, but not glutamatergic LTP, was absent in mice lacking the RNA binding protein Nova-2 (Ule et al., 2003, 2005), thus indicating that specific control of $GABA_B$ LTP does exist. In the context of network signaling, selective $GABA_B$ LTP could sharpen the coincidence detection of excitatory synaptic input because of the late hyperpolarization, which would reduce the efficacy of late-arriving excitatory synaptic input (Huang et al., 2005).

4.18.4.8 Plasticity of Interneuronal Resting Membrane Potential

Last, we discuss a nonsynaptic form of long-term plasticity, which might nevertheless have important implications for GABAergic interneuron output. Ross and Soltesz (2001) described a long-lasting depolarization of resting membrane potential in hippocampal dentate gyrus interneurons following high-frequency tetanic stimulation. Because the conditioned interneurons now rest at more depolarized membrane potentials, they are more likely to respond to excitatory input by producing action potentials and hence release GABA onto their downstream targets. The induction mechanisms involved in this form of plasticity require activation of Ca^{2+}-permeable AMPA receptors and the consequent rise of intracellular Ca^{2+}, but not NMDAR, activity. Maintenance of this new persistently depolarized membrane potential was suggested to depend on modulation of the rate of Na^+/K^+ ATPase pump function (Ross and Soltesz, 2001).

In conclusion, although the field has received much less attention when compared to excitatory input, activity-dependent long-term plasticity of GABAergic input is an important mechanism that is likely to boost the flexibility of neuronal circuits in the brain. However, the heterogeneity of interneuronal populations and the complexity of the mechanisms involved suggest that considerably more work is required before a clear picture can emerge.

4.18.5 Toward the Future: Plasticity of GABAergic Networks?

Although for many years the focus on synaptic transmission in mammalian cortical system has been almost exclusively concentrated on chemical synapses, ongoing work in the last decade has also clearly shown that interneurons of cortical circuits are functionally organized through electrical coupling in GABAergic networks. In particular, dual recordings made from various types of interneurons have shown that electrical coupling is particularly strong among cells of the same class (reviewed by Hestrin and Galarreta, 2005; but see also Simon et al., 2005; Zsiros and Maccaferri, 2005). Electrical coupling of GABAergic networks appears especially important for the promotion of action potential synchronization between connected cells. Although the direct demonstration of a link *in vivo* is still lacking, this may help explain the stereotyped temporal pattern of activity that specific subsets of interneurons show in the hippocampus *in vivo* during different brain states (Klausberger et al., 2003, 2004, 2005). Long-term plasticity of electrical coupling has been shown in nonmammalian systems (Yang et al., 1990; for a review, see Pereda et al., 2004), and recent work has suggested that mechanisms similar to conventional LTP and LTD might operate at electrical synapses of the mammalian brain (Landisman and Connors, 2005). This possibility is virtually unexplored at present in mammalian circuits of the neocortex and hippocampus, but it is highly likely to become a strong focus of interest in the coming years.

4.18.6 GABAergic Plasticity and Implication for Network Functions

What are the consequences of synaptic plasticity in interneurons for network function? Although many hypotheses exist, few experiments have directly tackled this question. Network activity in a given cortical system is typically mediated by the integration of excitatory input with feedforward and feedback inhibitory loops. Steltzer et al. (1994) tackled this question by recording excitatory and inhibitory postsynaptic responses in hippocampal

pyramidal neurons and interneurons after tetanic stimulation of stratum radiatum. Their results highlighted that LTP of excitatory responses in pyramidal neurons was accompanied by a compartment-specific depression of dendritic, but not somatic, GABAergic input. This is especially intriguing in the light of the fact that pyramidal neurons have multiple action potential initiation sites (Golding and Spruston, 1998; Larkum et al., 2001), and that specific roles for dendritic- and somatic-targeting interneurons have been shown by Miles et al. (1996). Thus, the integration of plasticity in interneurons and principal cells could be related to the switch between two hypothetical modes of operation of pyramidal neurons, which have been proposed to underlie encoding and retrieval of memories (Paulsen and Moser, 1998). Other studies have highlighted LTD of fast inhibition as a critical component of E-S potentiation (Lu et al., 2000; Staff and Spruston, 2003; but see Marder and Buonomano, 2004). E-S potentiation (Bliss and Linch, 1988) is a phenomenon associated with classical LTP, which is described by a change in the input–output curve of a neuron. In other words, E-S spike potentiation associates the same sized excitatory postsynaptic potential (EPSP) with a higher probability of initiating a spike. The establishment of plasticity in feed-forward and feed-back inhibitory circuits may therefore change the EPSP-spike coupling properties of neurons and their precision of firing (Fricker and Miles, 2000, 2001).

4.18.7 General Conclusions

In this albeit brief overview of the state of inhibitory interneuron plasticity, we have tried to emphasize the current state of the field and highlight the complexities associated with plasticity of incoming excitatory afferents as well as discuss the nascent field of plasticity of the inhibitory output from a variety of well-defined interneuron circuits. If there is a single 'take-home' message from the above discussion, it is that no single general principal accounts for either the plasticity of excitatory synaptic transmission onto interneurons or the plasticity of their GABAergic inhibitory output. Moreover, often the same protocols that induce plasticity at principal cell targets either fail to induce plasticity or result in a long-lasting plastic change of opposite polarity at interneuron inputs and outputs. This, in hindsight, is not particularly surprising, given the emergence of the myriad types of cortical interneuron subpopulations, all of which are tuned to a specific function within the cortical network in which they are embedded. It is also important to appreciate that interneurons receive input, often from a wide array of afferent fibers, as well as making their output onto a wide array of diverse cellular targets. We would predict that each and every one of these inputs and outputs will have their own particular set of rules regarding both short- and long-term plasticity. A complete understanding of these plasticity rules will only be obtained by the careful and often arduous task of systemically studying each and every one. However, we hope that this review will help fuel such an endeavor over the coming years, and we predict that we are only on the cusp of truly understanding the role plasticity plays in tuning the role of interneurons in the cortical network.

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4.19 Neurofibromatosis Type I Learning Disabilities

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4.19.1 Learning Disabilities

Learning disabilities are characterized by specific impairments restricted to certain domains of mental function. This is in contrast to the broad impairment seen in mental retardation disorders, which cause generalized impairments in intellectual and social development (Kronenberger and Dunn, 2003; Kelly, 2004). The simplest definition of a learning disorder is a discrepancy between tests of intellectual capability and actual achievement (Kronenberger and Dunn, 2003). Intellectual capability is typically quantified with IQ tests such as the Wechsler scales (WISC-IV, WAIS-III) and the Stanford Binet (SB-IV). These tests provide reliable measures that are standardized across specific age groups (Palumbo and Lynch, 2006). Achievement is also determined using standardized tests that assess performance level in multiple academic areas (e.g., Woodcock-Johnson (WJ-III) (Palumbo and Lynch, 2006). Learning disability is diagnosed when performance in tests of achievement is significantly below predictions based on IQ score.

There are several ways to categorize learning disabilities. The DSM-IV places learning disabilities into three major categories: reading disorder, mathematical disorder, and disorders of written expression (Kronenberger and Dunn, 2003; Kelly, 2004). In addition to these categories, the DSM-IV also recognizes nonverbal learning disorders and learning disorders not otherwise specified. These latter categories represent disorders which lead to academic underachievement but do not fall neatly into one of the first three DSM-IV categories of learning disability (Tramontana, 2006). The DSM-IV categories are also considered distinct from other developmental disorders such as motor skills disorder and communication disorder. Finally, other diagnostic systems, such as those used in neurology, are based on more specific descriptions of learning disabilities such as dyslexia (reading disorder) (Kelly, 2004).

The criteria for diagnosis of learning disabilities are quite complex. Further, they describe patterns of learning problems but may not capture the entire clinical picture or reflect fundamental differences in neuronal mechanisms. For diagnosis and treatment to

be successful, it may be useful to consider a more comprehensive picture that reflects underlying causes and affected cognitive systems. The role of neuroscience is to provide insights into neurological systems and circuits as well as cellular, molecular, and genetic mechanisms that are required for normal function. In this chapter, we focus on investigations of a specific disorder, neurofibromatosis. The integration of basic and clinical neurosciences has led to significant strides in our understanding of learning disabilities associated with NF1. For example, our lab and others have identified specific changes in neuronal function and isolated therapeutic targets that are currently being used to develop novel treatment options for patients with NF1. The integrated approach that led to these advances could lead to similar advances in other cognitive disorders.

4.19.2 Neurofibromatosis Affects Several Brain Systems and Impairs Multiple Cognitive Functions

Neurofibromatosis type I (NF1), also known as von Recklinghausen neurofibromatosis, is an autosomal dominant neurological disorder. Patients with NF1 have widespread clinical symptoms in multiple organ systems (Lynch and Gutmann, 2002). Classic disease characteristics include the presence of neurofibromas, the benign peripheral nerve tumors composed of proliferating Schwann cells, and fibroblasts that give the syndrome its name. Other classic diagnostic features of NF1 are pigmented lesions of the skin called café au lait spots, hamartomas of the iris termed Lisch nodules, and pseudoarthrosis of the tibia. Additional symptoms can range from pain, bone dysplasia, and multiple types of tumors including optic gliomas (Sagar, 2004). Importantly, NF1 also causes high rates of learning disabilities and cognitive symptoms. Cognitive symptoms of NF1 pose one of the most significant sources of lifetime morbidity for patients (North et al., 1997).

Cognitive symptoms in NF1 occur in a specific, domain-restricted pattern, rather than in a globally generalized manner. This is evidenced by the lack of performance differences between NF1 patients and unaffected siblings on many neuropsychological measures considered sensitive to overall cognitive impairment (Kayl and Moore, 2000). For example, patients with NF1 have IQs in the normal range, with only a slight downward shift in distribution. However, their academic and social gains can be limited by learning disabilities and cognitive impairments in four main areas: Reading/vocabulary, attention, executive function and planning, as well as visuospatial function (Hyman et al., 2005).

Learning disability is a common symptom of NF1; it is present in 30%–65% of this population. This wide range is due in part to studies using differing definitions of a learning disorder, as well as the use of small groups without appropriate controls (North et al., 1997; North, 2000). NF1 patients show some characteristics of both nonverbal- and verbal-type learning disability. Components of nonverbal learning disability seen in NF1 include consistently poor performance in tests of visuospatial functioning and spatial learning (North, 2000; Kayl and Moore, 2000), notable impairments in the ability to perceive social cues, poor organizational skills, and increased impulsiveness (North et al., 1997; North, 2000). NF1 patients also show aspects of verbal learning disorder. Specifically, patients with NF1 have deficits in expressive and receptive language, vocabulary, visual naming, and phonologic awareness. In fact, reading and spelling are repeatedly found to be impaired more severely than predicted by IQ in NF1 patients (Hyman et al., 2005). Consistent with these impairments in language-based learning, NF1 patients show poorer academic achievement in reading and writing, compared to their unaffected siblings. Importantly, when IQ is controlled for, children with NF1 do not show a higher incidence of learning problems in mathematics, a core feature of nonverbal learning disability (Kayl and Moore, 2000).

The pattern of learning disabilities seen in NF1 does not fall cleanly into one of the DSM-IV defined categories. Instead, the occurrence of multiple types of learning problems suggests that a more fundamental learning process is impaired in NF1 patients, rather than a deficit in a specialized area such as language or mathematics. Therefore, it is useful to examine the symptoms of NF1 in terms of basic cognitive functions. Learning deficits in NF1 patients suggest dysfunction in the hippocampal memory system. NF1 patients perform poorly on spatial learning and memory tasks that have been shown to depend on the hippocampus and adjacent medial temporal cortex. Damage to these areas is known to produce profound amnesia for spatial information and difficulties navigating through space (Burgess et al., 2002; Squire et al., 2004). Reading comprehension and vocabulary learning impairments seen in NF1 may also be related to hippocampus dysfunction. Learning and remembering new facts and general information

about the world requires the hippocampus and surrounding medial temporal cortex (Schmolck et al., 2002; Bayley and Squire, 2005). For example, patients with damage to this area often have considerable difficulty learning and retrieving new vocabulary words (Verfaellie et al., 2000). A similar impairment could contribute to the learning problems that NF1 patients exhibit in school.

It is important to recognize that in addition to possible hippocampal-dependent learning disabilities, deficits in other behavioral and cognitive domains may play an important role in academic and social achievement problems seen in NF1. This has been suggested by studies showing that up to 40% of children with NF1 who were identified as academic underachievers were also normal on neuropsychological tests for learning disabilities (Kayl and Moore, 2000). In such cases, underachievement is likely due to deficits in attention, planning, and organization skills (Dilts et al., 1996; North, 2000; Kayl and Moore, 2000; Koth et al., 2000; Hyman et al., 2005).

Patients with NF1 consistently show planning impairments that are more severe than would be predicted by IQ (Hofman et al., 1994; Hyman et al., 2005). Further, there is a high comorbidity, ranging from 30% to 50%, between NF1 and attention deficit disorder (Hofman et al., 1994; Mautner et al., 2002; Hyman et al., 2005; Pavol et al., 2006). Comorbid attention deficit disorder affects not only academic but also social development for children with NF1 (Barton and North, 2004). Children with NF1 tend to have social problems and appear socially awkward and withdrawn, in comparison to their siblings and to children with other chronic, life-threatening illnesses (Kayl and Moore, 2000). Some evidence suggests that social deficits are related to poor interpersonal skills, which occur as a result of decreased attention to social cues.

Interestingly, severity of symptoms in planning/organization seems to be independent of attention deficit symptoms. This suggests that attention deficit does not cause symptoms of poor planning and organization in NF1 but, rather, NF1 affects each of these domains of cognitive function (Hyman et al., 2005). Symptoms in these domains are functionally and anatomically related. Attention, planning, and behavioral organization are elements of executive function. Executive functions are a set of higher-order cognitive functions that allow adaptive, goal-directed behavior. Domains of executive function which are impaired in NF1 are thought to critically depend on frontal/subcortical circuits (Ferner et al., 1996; Stuss and Levine, 2002). Specifically, the prefrontal cortex is thought to play a key role in processes such as working memory, control of attention, planning, and rule following (Ferner et al., 1996; Stuss and Levine, 2002; Otani, 2003). Limitations in planning, organization, and control of attention seen in NF1 are also commonly present in syndromes involving impaired prefrontal executive function (Ferner et al., 1996; Stuss and Levine, 2002; Powell and Voeller, 2004). Such evidence implicates prefrontal cortex pathology in NF1. Indeed, functional imaging studies show hypoactivation of prefrontal cortex in NF1 patients during a rhyming task (Billingsley et al., 2003). Improved understanding of how NF1 affects prefrontal cortex function will be essential to understanding the complex cluster of seemingly prefrontal deficits associated with the disorder.

The third major domain of cognitive impairment seen in NF1 is visuospatial. Visuospatial problems are one of the most consistent symptoms seen in NF1. Robust deficits in tests of visuospatial function, such as the Judgment of Line Orientation (JLO) task, occur in over half of patients with NF1 (Hyman et al., 2005). Function in the visuospatial domain is affected more consistently than other neuropsychological domains, and also more severely than predicted by general IQ (Hyman et al., 2003). In fact, combined performance in multiple tests of visuospatial function can discriminate children with NF1 from unaffected children (Schrimsher et al., 2003). Visuospatial processing and spatial working memory required for tasks such as the JLO are thought to be mediated by a network involving the prefrontal, parietal, and visual cortices (Ng et al., 2000; Lanca et al., 2003). Prefrontal cortex dysfunction may contribute to visuospatial impairments in NF1. However, these impairments occur independently of prefrontal cortex-related symptoms such as attention impairments (Schrimsher et al., 2003). Therefore, it is thought that visuospatial processing deficits in NF1 are primarily related to parietal cortex dysfunction.

Finally, motor performance is impaired in NF1 patients. Fine motor coordination and motor speed are decreased in NF1. Visuomotor integration is also impaired so that patients with NF1 have poor hand–eye coordination and balance. As a result, over half of the children with NF1 have problems with tasks such as handwriting that require visuomotor integration and fine motor skills (Hyman et al., 2003). The profile of motor impairments seen in NF1 involves functions classically assigned to the cerebellum (Timmann and

Diener, 2003). Indeed, the principal cells of the cerebellum, the Purkinje cells, express high levels of neurofibromin, the protein encoded by the NF1 gene (Gutmann et al., 1995). Further characterization of cerebellar pathology in NF1 will be important to understand the mechanisms underlying motor impairments associated with the disease.

Large, detailed sibling-controlled studies of patients with NF1 have revealed the extent and high frequency of cognitive symptoms associated with the disease. These studies have demonstrated specific cognitive domains in which symptoms cluster. These cognitive domains are thought to depend on specific brain systems such as hippocampus, prefrontal cortex, and parietal cortex, suggesting that dysfunction in these areas may underlie NF1 symptoms. Examining the genetic causes of NF1 can provide further insight into the type of dysfunction that occurs on a molecular and neuronal level within these brain areas.

4.19.3 Genetic Mechanisms Responsible for Neurofibromatosis

NF1 is caused by mutations to the *Nf1* gene (Wallace et al., 1990; Viskochil et al., 1990), many resulting in loss of function of its protein product, neurofibromin. Affected individuals are heterozygotes, as homozygous mutations are lethal (Friedman, 1999). The *Nf1* gene is one of the largest in the genome, encompassing 60 exons (Marchuk et al., 1991; Li et al., 1995). Interestingly, up to 50% of patients with NF1 have spontaneous mutations of the gene, with no genetic or phenotypic evidence of NF1 in their parents (Huson et al., 1989; Friedman, 1999). The remaining 50% of patients with NF1 inherit it from their parents. Inheritance of NF1 shows an interesting pattern of complete penetrance but variable expressivity (Ward and Gutmann, 2005). Every person with an inactivating mutation in one allele of the *Nf1* gene shows some symptoms of NF1. However, NF1 shows highly variable expressivity. Clinical presentation of NF1 ranges from minimal symptom severity to extremely high symptom load. The reason for this variable expressivity is currently not clear. Explanations for the variable expressivity include variable inheritance of genetic modifiers and differing effects of mutation type and location within the *Nf1* gene. Inheritance of genetic modifiers is thought to account for the majority of variability in NF1 expression. Genetic studies have examined the degree to which inheritance correlates with expression of symptoms, including neurofibromas and referral for remedial education. Monozygotic twins affected by NF1 have a high correlation in symptom severity. The strong genetic component in symptom expression in NF1 is consistent with modulation of disease symptoms by inherited genetic modifiers (Easton et al., 1993). For example, proteins encoded by genetic modifiers may interact with the neurofibromin signaling pathway to exacerbate effects of *Nf1* mutations or confer protection by compensating for the loss of neurofibromin function. Identification of these modifiers could provide additional prognostic information for patients and potentially act as new therapeutic targets.

The type of *Nf1* gene mutation could contribute to the variable expressivity of symptoms in this disorder. Different types of mutations, including point and frame shift mutations, occur throughout the *Nf1* gene. No clear relationship has been identified between the location of the mutation in the gene and the range of symptoms seen (Viskochil, 2002). However, patients who carry large deletions of the *Nf1* gene present with a more severe type of NF1, called the microdeletion syndrome, which involves more extreme symptoms such as mental retardation. This is significant because the occurrence of the microdeletion syndrome may account for the slightly elevated rate of mental retardation seen in the NF1 patient population (4%–5%) compared to the general population (2%–3%) (Venturin et al., 2004).

In addition to the variability in severity of individual symptoms, it is becoming clear that different mechanisms account for the evolution of different symptoms. Generalized symptoms seen in NF1, such as cognitive symptoms, are caused by the heterozygous mutation of one allele of the *Nf1* gene. More focal symptoms, such as formation of neurofibromas, require loss of both alleles of NF1. Reduction to homozygosity (loss of the remaining NF1 wild-type allele in heterozygous tissue) can occur throughout life, due to somatic mutation. This creates localized clusters of cells that show homozygous inactivation of the *Nf1* gene on a background of tissue with heterozygous loss of *Nf1* function. Reduction to homozygosity is thought to underlie a number of serious NF1 symptoms, including peripheral neurofibromas and CNS gliomas (Zhu et al., 2002; Bajenaru et al., 2003; Ward and Gutmann, 2005).

Investigation of the genetics of NF1 has led to improvements in the diagnosis of the disease and extended our understanding of its symptoms. As

NF1 is caused by a single gene with 100% penetrance, diagnosis of the disease can be confirmed by genetic analysis. This is in contrast to diagnosis in other diseases without a known genetic origin, which is purely dependent on symptom expression. For the NF1 patient population, genetic diagnosis improved characterization of the cognitive profile and evaluation of the prevalence of each symptom. For example, prior studies had indicated that mental retardation was a prominent feature of patients with NF1, due to ascertainment biases caused by underdiagnosis of patients without central nervous system (CNS) lesions (North, 2000). However, more comprehensive cognitive assessments in the entire NF1 patient population demonstrated instead specific patterns of cognitive strengths and weaknesses and low normal IQ. Additionally, identification of the *Nf1* gene has allowed the derivation of genetic animal models of NF1. The study of these models has led to significant insights into the molecular, cellular, and system mechanisms affected by mutations in the *Nf1* gene.

4.19.4 Animal Models of Neurofibromatosis

Animal models of genetic disorders have a variety of purposes. Some are made to mimic the human disease as closely as possible, so that disease mechanisms can be studied and potential treatments tested. Other animal models are derived to address specific questions within a complex cluster of biological phenotypes. The study of NF1 has involved a wealth of animal models derived primarily in mouse and *Drosophila*.

Mice with a heterozygous null mutation of the *Nf1* gene ($Nf1^{+/-}$) have compelling genetic and behavioral parallels with human NF1, making them an excellent system in which to study the mechanisms of cognitive deficits associated with NF1. The sequence, transcriptional regulation, and downstream targets of *Nf1* are conserved across species, including mouse and human (Bernards et al., 1993; Hajra et al., 1994). The majority of mutations (70%) to the *Nf1* gene in patients lead to synthesis of a truncated, nonfunctional version of the encoded neurofibromin protein (Thomson et al., 2002; Shen et al., 1996). The $Nf1^{+/-}$ mouse model was made by deleting exon 30 of the *Nf1* gene, which leads to an unstable, quickly degraded transcript (Jacks et al., 1994). Like patients with NF1, the $Nf1^{+/-}$ mouse model is heterozygous for this loss of function mutation.

Interestingly, behavioral analysis of the $Nf1^{+/-}$ mice revealed that they exhibit features of the learning deficits and executive function impairments seen in NF1 patients. Specifically, the $Nf1^{+/-}$ mice show spatial learning impairments and attention deficits. Learning deficits in the $Nf1^{+/-}$ mice were first revealed in a classic test of spatial learning, the hidden platform version of the Morris water maze. In this task, mice learn to navigate to a platform hidden just beneath the water in a round pool. The mice are thought to learn the position of the fixed hidden platform in reference to cues surrounding the pool. Impairments of the $Nf1^{+/-}$ mice in this test are specific to spatial learning and memory, as the $Nf1^{+/-}$ mice display normal visual learning in the visible version of the water maze (Silva et al., 1997; Costa et al., 2002). In this nonspatial control task, the mice learn to navigate to a visible platform. This phenotype is not unlike the profile of learning deficits seen in NF1 patients, where spatial learning is severely affected but visual learning is spared (Hyman et al., 2005).

In addition to spatial learning deficits, NF1 commonly involves attention deficits. Like patients, the NF1 mouse model also displays behavioral attention deficits (Li et al., 2005). Attention is tested in mice using a lateralized reaction time task. This task uses an apparatus originally described for a five-choice serial reaction time task (Robbins, 2002), a standard operant chamber with a curved side wall containing five nose-poke apertures. Mice start a trial with a sustained nose poke at a center aperture. At this point, the far-left or far-right aperture is illuminated for a fixed period (2, 1, or 0.5 s). The illuminated aperture acts as a target, which mice must notice and nose poke for a food reward. Performance of this task requires sustained attention on both spatially separated potential targets (Jentsch, 2003). When the target aperture is illuminated for short intervals of 0.5 s, $Nf1^{+/-}$ mice show a significantly increased rate of omissions, which indicates an inability to sustain visuospatial attention (Li et al., 2005). This task demonstrates behavioral attention deficits in $Nf1^{+/-}$ mice that are similar to attention deficits seen in patients.

Since the $Nf1^{+/-}$ mice model both learning and attention deficits seen in NF1 patients, mechanisms underlying these deficits can be studied using these mice. Spatial learning in both mice and humans is thought to depend on hippocampus while attention is thought to critically involve prefrontal cortex (Dalley et al., 2004). Changes in the biochemistry

and physiology of these brain areas can be examined in the $Nf1^{+/-}$ mice in order to identify dysfunction and to study the contribution of each brain area to the overall pattern of cognitive symptoms seen in NF1. This approach provides insights into the neuronal changes that underlie cognitive symptoms of NF1. Other approaches to the study of NF1 use different animal models which have been developed to address more specific questions about NF1 pathogenesis.

The *Nf1* gene encodes several functional elements, including a ras GTPase–activating domain (Ballester et al., 1990; Xu et al., 1990) and an adenylyl cyclase–activating domain (Tong et al., 2002). To evaluate the behavioral importance of each of these functions, animal models were developed with mutations affecting individual domains. For example, exons 20–27a of the *Nf1* gene encode a ras GTPase–activating domain (GAP domain) (Upadhyaya et al., 1997). A mouse model was made carrying a deletion of exon 23a in the GAP domain of *Nf1*. This deletion modifies the affinity and GAP activity of the mutant neurofibromin. Behavioral analysis of this mutant mouse demonstrates the importance of the GAP domain to cognitive function, including spatial learning (Costa et al., 2001). Importantly, previous studies in humans had identified cognitive deficits in a patient with a point mutation in the GAP domain of the *Nf1* gene (Upadhyaya et al., 1997; Klose et al., 1998; Weiss et al., 1999). These studies suggest that the ras-GAP function of neurofibromin is critical for normal cognition, and they confirm that mice can be used to model the cognitive deficits associated with NF1.

Additionally, studies with *Drosophila* models of NF1 revealed a behavioral role for the interaction between *Nf1* and adenylyl cyclase. In *Drosophila*, homozygous deletion of *Nf1* ($Nf1^{-/-}$) is not lethal, so *Drosophila* lines that are null for *Nf1* can be used. These lines can be engineered to carry partial homozygous deletions of *Nf1*, resulting in flies with truncated neurofibromin proteins. Behavioral studies in such fly lines demonstrate that restoring adenylyl cyclase activation in *Nf1* can rescue biochemical and behavioral deficits of the $Nf1^{-/-}$ flies (Guo et al., 2000; Hannan et al., 2006). Further, *Drosophila* genetics can utilize mutagenesis-based screens to identify novel genes which interact with *Nf1* (i.e., modifiers). *Drosophila* models greatly extend our ability to address the intricate biochemical functions of *Nf1*, examine the behavioral role of each of its biochemical functions, and identify effectors and modifiers of *Nf1*.

Nf1 mutations can affect various signaling pathways, and animal models, such as the ones described above, are being used to identify the contribution of each of these pathways to the phenotype of NF1 mice. By disrupting specific functional domains of the *Nf1* gene, these animal models isolate the contribution of each domain to behavioral and physiologic dysfunction. Like *Nf1*, many other disease-related genes encode multifunctional proteins which participate in complex biochemical cascades. Animal models provide a unique tool to integrate these biochemical properties with cellular and behavioral phenotypes.

Identification of genetic and molecular mechanisms of diseases, such as NF1, leads to another important question: Since the genetic mutation is inherited from birth, are the symptoms of the disease caused by the impact of the disease allele on CNS development, or are the symptoms caused by the effects of the gene mutation on function of the mature brain? Mice or *Drosophila* carrying conditional gene deletions can help to address this question. Conditional mutations can be created in mice using the cre-loxP system, a powerful tool widely used for restricting gene deletions to specific time frames, cell types, or areas. The system utilizes Cre recombinase, a bacteriophage enzyme which mediates site-specific recombination between DNA sequences (loxP sites) flanking the gene of interest (floxed gene). The floxed gene is expressed normally until deleted by Cre recombinase (Yu and Bradley, 2001; Morozov et al., 2003; Ahmed et al., 2004). For NF1, a mouse line was engineered with loxP sites flanking exons 31–32 of the *Nf1* gene ($Nf1^{flox/flox}$). The floxed *Nf1* gene acts like a wild-type allele prior to delivery of Cre recombinase. Mice carrying one floxed *Nf1* allele and one deleted *Nf1* allele ($Nf1^{flox/-}$) show the same phenotypes as $Nf1^{+/-}$ mice; for example, they have the same survival profile. Once the deletion of the floxed *Nf1* allele occurs, following delivery of Cre recombinase, the $Nf1^{flox/-}$;cre+ mice become homozygous knockouts ($Nf1^{-/-}$) (Zhu et al., 2001). Breeding the floxed *Nf1* allele mice to mice with a Cre recombinase gene driven by a specific promoter can result in *Nf1* mice with deletions of the gene in specific tissues and at specific times. For example, promoters can be chosen that are expressed later in development so that the resulting mutants exhibit adult deletion of the *Nf1* gene (Zhu et al., 2001; Morozov et al., 2003). Additionally, viral vectors can be used to deliver Cre recombinase to the $Nf1^{flox/+}$ mouse at specific times and to specific systems (Morozov et al., 2003; Ahmed et al., 2004).

Disease-related genes often encode multifunctional proteins that participate in a range of complex biochemical pathways within cells. This makes it difficult to isolate specific pathways leading to dysfunction. The encoded proteins are also expressed in multiple cell types such as glia, interneurons, and primary neurons that could interact to mediate complex network properties (Gutmann et al., 1995). Therefore, it is often difficult to determine exactly which systems contribute to dysfunction and how. Animal models can be used to identify causal relationships between the biochemical, physiologic, and behavioral effects of disease-causing mutations. Thus, they are an effective tool in understanding which mechanisms underlie specific phenotypes of the disease. In NF1, several types of animal models have been used to understand the cognitive phenotypes of the disease. As stated above, the $Nf1^{+/-}$ mice expresses a nonfunctional allele of the *Nf1* gene, and their phenotype mimics specific behavioral aspects of the human disease (Jacks et al., 1994; Costa et al., 2002). Other mouse and *Drosophila* NF1 models carry mutations targeted to specific functional domains of the *Nf1* gene, allowing the role of these domains to be examined (Costa et al., 2001; Hannan et al., 2006). Finally, animal models with conditional mutations can be used to examine temporal, cell type, or brain system–specific effects of *Nf1* deletion. For example, conditional mutations can be induced in a temporally controlled manner to identify particular times when *Nf1* is critical for function. In combination with clinical data, animal models, such as the ones used to study NF1, are powerful tools in the study of the pathogenesis of the disease.

4.19.5 Biochemical and Physiological Functions of Neurofibromin

The neurofibromin protein has multiple biochemical roles. It acts as a ras-GAP (GTPase activating protein) to negatively regulate ras signaling, and it can also serve as an activator of adenylyl-cyclase. Both pathways play a role in mediating signaling events from the membrane to downstream targets within the neuron. Loss of neurofibromin results in constitutive increases in ras intracellular signaling. Evidence for a role of neurofibromin in the modulation of ras signaling has been obtained in patient and mouse mutant studies. In NF1, ras signaling can be increased to the point that it becomes independent of the constraints of extracellular triggers such as growth factor activation. For example, $Nf1^{-/-}$ sensory neurons no longer require the extracellular growth factor brain-derived neurotrophic factor (BDNF) to survive and mature, presumably due to constitutive level of activity of the ras pathway when it is no longer subject to negative regulation by neurofibromin (Vogel et al., 1995).

The ras protein is part of a large superfamily of GTPases that mediate signaling from membrane receptors to intracellular cascades of kinases. Ras activation requires this protein to be tethered to cell membrane. At the membrane, ras can interact with downstream effectors such as raf kinase, which then activate signaling cascades such as the MEK/MAPK pathway (Boguski and McCormick, 1993; Weiss et al., 1999). These kinases then induce a number of downstream processes, including transcription of immediate early genes leading to short- and long-term changes in neuronal function. The ras protein cycles between the inactive GDP-bound state and the active GTP-bound state. The activity state of ras is determined by a balance of activating proteins (GEFs, guanine nucleotide exchange factors, which allow bound GDP to be released so that GTP can bind) and inactivating proteins (GAPs, GTPase activating proteins, which increase the endogenous GTP hydrolyzing activity of ras) (Bernards and Settleman, 2004). Neurofibromin , as a ras-GAP, is an important negative regulator of ras signaling (Weiss et al., 1999).

Experimental evidence demonstrates that increased ras activity in NF1 plays a critical role in causing impaired learning and cognition. For example, mice in which the ras-GAP activity of *Nf1* was modified by deleting exon 23a of the *Nf1* gene show impairments in behavioral tests of learning and memory similar to those found in the null allele (Costa et al., 2001). Additionally, genetic or pharmacologic normalization of ras activity in $Nf1^{+/-}$ mice rescued behavioral learning and memory deficits. Ras activity was shown to be abnormally increased in the brain of $Nf1^{+/-}$ mice (Li et al., 2005). Genetic rescue was done by breeding these $Nf1^{+/-}$ mice to mice with a heterozygous deletion of *K-ras*. In the resultant double mutants, the decreased total levels of *K-ras* compensate for the increased activation state of the ras pathway caused by the *Nf1* deletion, leading to normalized levels of Ras activity. In contrast to the $Nf1^{+/-}$ mice, these double mutants showed normal performance in the Morris water maze, a test of spatial learning and memory (Costa et al., 2002). Similar results were also obtained by crossing the $Nf1^{+/-}$ mice to mice with heterozygous deletion of N-ras, a different isoform of ras.

Farnesyl-transferase inhibitors (Costa et al., 2002) and lovastatin (Li et al., 2005) can also rescue the learning and memory deficits of the *Nf1*$^{+/-}$ mice. Both of these pharmacologic interventions were shown to decrease ras activity. Therefore, these interventions rescue the biochemical deficits thought to be at the heart of behavioral deficits in *Nf1*$^{+/-}$ mice. For example, lovastatin, a cholesterol synthesis inhibitor that can be safely prescribed to patients, was shown to lower levels of active ras-GTP and its downstream effector, p-MAPK, in the cortex and hippocampus of *Nf1*$^{+/-}$ mice (Li et al., 2005). Both farnesyl-transferase inhibitors (Weiss et al., 1999) and lovastatin (Mendola and Backer, 1990; Xu et al., 1996) are thought to interfere with important aspects of the isoprenylation and activation of ras. Importantly, besides rescuing learning and memory deficits, lovastatin was shown to also reverse the attention impairments of the *Nf1*$^{+/-}$ mice (Li et al., 2005). The ability of ras manipulations to successfully rescue behavioral deficits in the *Nf1*$^{+/-}$ mice demonstrates that the ras-GAP function of neurofibromin is critical for cognitive function.

One of the key functions of Ras is its role as an intracellular mediator of the receptor tyrosine kinases (Bernards and Settleman, 2005). This family of transmembrane receptors is characterized by phosphorylation of intracellular tyrosines upon ligand binding. Tyrosine phosphorylation then leads either to direct binding and inactivation of ras-GAPs such as neurofibromin, or to indirect activation of ras-GEFs (Weiss et al., 1999; Patapoutian and Reichardt, 2001). The growth factor receptors make up a major class of receptor tyrosine kinases, which are likely to be important to NF1 pathology. The formation of peripheral tumors in NF1 is thought to be related to increased growth factor sensitivity. This leads to increased cellular growth, survival, and abnormal function (DeClue et al., 2000; Li et al., 2002; Gottfried et al., 2006). Changes in growth factor signaling may also contribute to the changes in neuronal physiology and resulting cognitive symptoms seen in NF1. BDNF normally signals in neurons through transmembrane receptors such as the TrkB receptors, which activate the ras pathway (Patapoutian and Reichardt, 2001). In neurons with deletion of *Nf1*, constitutive ras activation results in neurons that can survive and extend neurites independently of BDNF (Vogel et al., 1995). Normally, BDNF-dependent signaling protects inactive inhibitory synapses from being pruned (Swanwick et al., 2006). If these pathways are constitutively activated, then activity-dependent shaping of inhibitory synapses and networks may not occur and inappropriately networked inhibitory terminals may persist in the NF1 mice. Additionally, BDNF modulates increases in vesicular release probability at inhibitory terminals through synaptic vesicle–associated proteins such as synapsin 1 (Li et al., 2002). Finally, BDNF enhances mRNA and protein expression of GAD, the rate-limiting enzyme in GABA synthesis (Li et al., 2002; Ohba et al., 2005). It is possible that these functions of BDNF are abnormal in NF1 neurons, thus resulting in changes in GABAergic activity. While the role of neuronal BDNF signaling in NF1 remains speculative, increased GABAergic tone has been suggested in *Nf1*$^{+/-}$ mice. This increased GABAergic release seems to cause deficits in long-term potentiation, a form of synaptic plasticity thought to underlie learning and memory (Costa et al., 2002).

In addition to its role as a ras-GAP, neurofibromin acts as an activator of adenylyl cyclase (Tong et al., 2002). Mutation of the *Nf1* locus in *Drosophila* leads to decreased activity of the cAMP/PKA pathway and to learning and memory deficits. The adenylyl cyclase pathway has been shown to be important for regulation of neuronal development and learning in *Drosophila*. Learning defects in the *Nf1* null flies can be rescued by expression of a constitutively active form of PKA (Guo et al., 2000). This suggests that associative learning impairments in *Nf1* null flies are due to decreased activation of adenylyl cyclase. Interestingly, *Nf1* can increase adenylyl cyclase activity in a ras-dependent manner in *Drosophila*. This form of signaling occurs in response to growth factors such as EGF. *Nf1* can also directly increase adenylyl cyclase activity in a ras-independent manner, through its C-terminal domain. This function of *Nf1* is required for stimulation of adenylyl cyclase by neurotransmitters such as serotonin or histamine (Hannan et al., 2006). *Nf1* loss in *Drosophila* causes some phenotypes, such as small body size, through direct decrease of adenylyl cyclase activity (Tong et al., 2002). Other behavioral functions of *Nf1*, such as circadian rhythm modulation in *Drosophila*, are MAPK dependent and require the GAP domain instead (Williams et al., 2001). It is currently unclear whether these biochemical effects of homozygous *Nf1* deletion are relevant to the behavioral and cognitive deficits associated with NF1, since these deficits are thought to be caused by heterozygous mutations and the effects on adenylyl cyclase are only observed in homozygous mice or flies. In both mouse and *Drosophila* models, heterozygous deletion of *Nf1* did not grossly affect regulation of

the adenylyl cyclase pathway (Tong et al., 2002). Nevertheless, it is conceivable that *Nf1* plays a role in maintaining the relative balance between ras- and cAMP-dependent signaling (Weeber and Sweatt, 2002).

Drosophila models also highlight the behavioral importance of the GAP domain of *Nf1*. Further, the *Drosophila* model demonstrates the complex interplay between the effects of *Nf1* mutation in multiple biochemical pathways, each of which acts in different signaling contexts. Perhaps this biochemical complexity contributes to the highly variable nature of the symptoms seen in NF1.

4.19.6 Interactions Between Different Cell Types in Neurofibromatosis

Symptoms of NF1 are caused not only by disruption of molecular signaling within each cell but also by the effect of *Nf1* mutation on the interaction between different cell types. Although all cells in patients carry the mutant *Nf1* locus, it is possible that the key symptoms in NF1 are caused by the effects of mutation in a subset of these cells. Heterogeneity in the effects of *Nf1* mutation in different cell types may be due to cell-type specific differences in either the expression of *Nf1* or the signaling molecules that interact with *Nf1*. In addition, somatic reduction to homozygosity can create clusters of homozygously deleted cells of one type among tissue that is heterozygous for *Nf1* deletion. As a result of this heterogeneity, symptoms can occur in one cell type as a result of dysfunction in another cell type. For example, some evidence suggests that glial dysfunction in NF1 is not cell autonomous. Glial dysfunction in NF1 patients results in astrocytic gliosis with upregulated glial fibrillary acidic protein (GFAP) expression (North, 2000) and glial-based tumors (Sagar, 2004; Ward and Gutmann, 2005) in the CNS. Data from mouse models suggest that glial dysfunction is caused by an abnormal interaction of glia with neurons expressing mutant *Nf1*. An elegant set of experiments using neuronal specific deletion of *Nf1* have demonstrated that neuronal loss of *Nf1* is sufficient to induce glial changes such as astrogliosis. Mice carrying a floxed *Nf1* gene were crossed to mice where Cre expression was limited to mature neurons by being driven by the synapsin promoter. Offspring from this cross carried a neuronal-specific, homozygous deletion of *Nf1*. *Nf1* deletion from neurons resulted in an increased number of reactive, GFAP-positive astrocytes (Zhu et al., 2001). Glial-derived tumor formation also requires *Nf1* loss in nonglial cell types. Loss of both alleles of *Nf1* in glial cells must occur on an $Nf1^{+/-}$ background in order for tumor formation to occur (Zhu et al., 2002), suggesting that the interaction between $Nf1^{+/-}$ neurons and $Nf1^{-/-}$ glia is permissive for glioma formation.

This example is presented to highlight the importance of identifying primary disease mechanisms. In this case, the changes that occur in astroglia in NF1 CNS may occur as a secondary consequence of *Nf1* loss in neurons. Patients with NF1 commonly demonstrate astrocytic gliosis as well as unidentified bright objects (UBOs). UBOs are areas of T2 magnetic resonance imaging hyperintensity related to edema within the myelin and localized abnormal glial proliferation (North et al., 1997). However, no consistent correlation, and certainly no predictive relationship, has been found between the number of UBOs and scores on cognitive tests. This result is consistent with the idea that neuronal changes, such as increased ras signaling, cause the cognitive deficits associated with NF1.

4.19.7 Increased GABAergic Inhibition in the Neurofibromatosis Mouse Impairs Learning and Memory

Studies in the NF1 mouse suggest that ras-dependent deregulation of inhibitory GABAergic networks is a central mechanism underlying behavioral impairment. Deletion of *Nf1* in the mouse leads to a ras-dependent increase in inhibitory GABAergic currents in the hippocampus. Increased GABAergic tone creates a loss of balance between inhibitory and excitatory processes within hippocampal networks and impairs plasticity. The resulting hippocampal dysfunction is thought to underlie the learning deficits seen in the $NF1^{+/-}$ mice.

In initial studies, learning was assessed in $NF1^{+/-}$ mice using the Morris water maze. This task was chosen because it is extremely sensitive to hippocampus dysfunction (Morris et al., 1982; Morris et al., 1990; Cho et al., 1999). During training, mice were placed in an opaque pool of water and learned to find a submerged platform using spatial cues (Morris, 1984). $NF1^{+/-}$ mice learned this task significantly slower than wild-type animals. For example, in a test after 7 days of training, most wild-type mice had already learned the spatial location of the platform, while most $NF1^{+/-}$ mice still had not. This finding is consistent with hippocampus dysfunction in these animals. A closer

examination of synaptic plasticity in a hippocampal region critical for spatial learning (CA1) found decreased levels of long-term potentiation (LTP). LTP is an increase in synaptic transmission that is observed following high-frequency stimulation (Bliss and Lomo, 1973; Bliss and Collingridge, 1993; Malenka and Nicoll, 1999). In the hippocampus, there is strong evidence that LTP is required for learning of spatial cues and contextual information (Richter-Levin et al., 1995; Martin et al., 2000; Martin and Morris, 2002). The hippocampal LTP impairment in the $NF1^{+/-}$ mice appeared to be caused by increased local inhibitory tone. Input–output curves demonstrated hyperactivity of local inhibitory circuits in CA1, as evidenced by abnormally large evoked inhibitory currents. Further, the $NF1^{+/-}$ LTP deficit can be rescued by lowering hippocampal inhibition using picrotoxin, a GABA(A) antagonist (**Figure 1**).

Interestingly, the LTP impairments seen in the $NF1^{+/-}$ mice are specific to certain strengths and patterns of stimulation. Hippocampal LTP deficits occur in response to theta burst stimulation (TBS), a form of

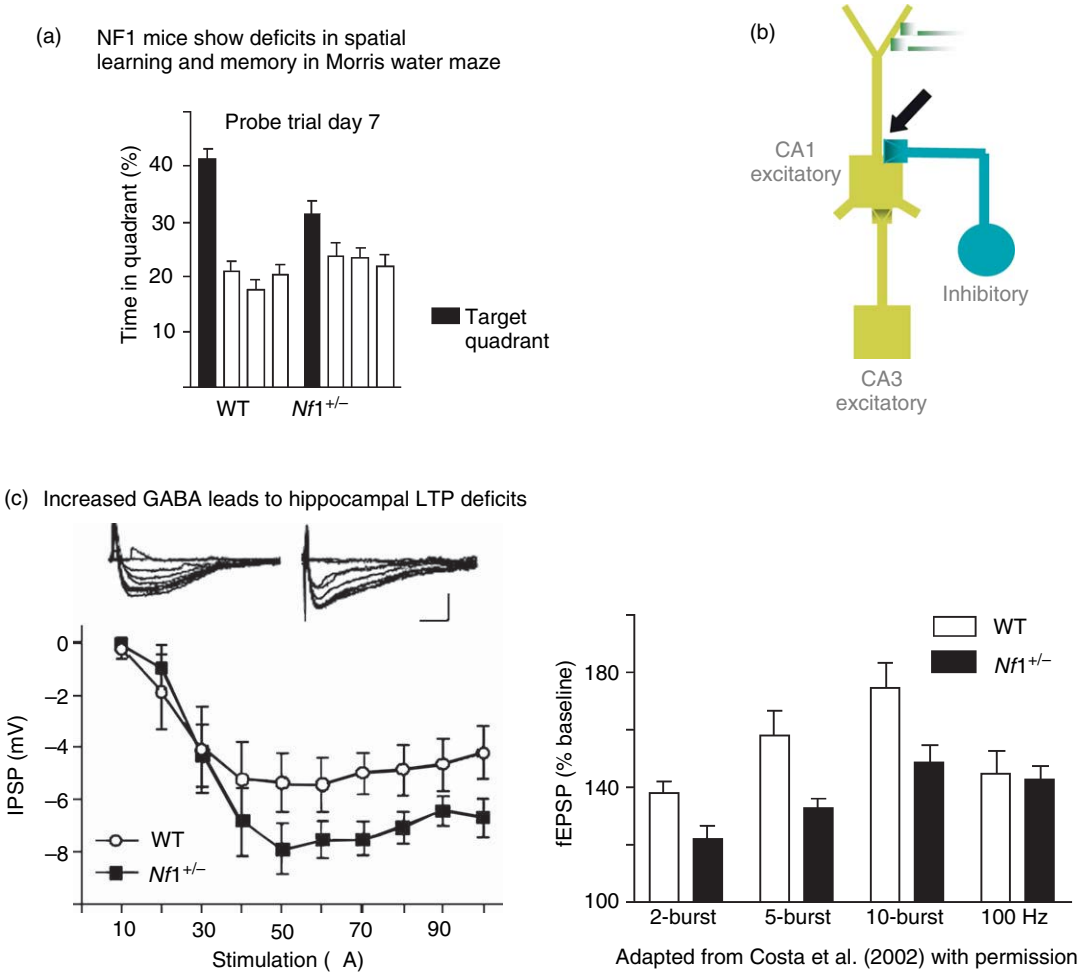

Figure 1 Learning and memory deficits in the NF1 mouse model are caused by increased local inhibition in hippocampal networks. (a) *Nf1*$^{+/-}$ mice show impaired spatial learning, as seen in this day 7 probe trial of the Morris water maze. The percentage of time spent in the target quadrant (*y*-axis), where the hidden platform had been, is significantly less in *Nf1*$^{+/-}$ mice compared to wild-type (WT) littermates. (b) schematic of hippocampal CA3–CA1 circuit, with local inhibitory interneurons. Inhibitory synapses onto CA1 pyramidal neurons (arrow) have abnormally high GABA release in *Nf1*$^{+/-}$ mice. (c) (left) Larger inhibitory potentials (IPSP) are evoked in *Nf1*$^{+/-}$ mice compared to WT littermates. Input–output curves were recorded by whole-cell patch in CA1 pyramidal neurons. Inhibitory potentials were evoked with local field stimulation of increasing amplitude (*x*-axis). (right) long-term potentiation (LTP) deficit in *Nf1*$^{+/-}$ mice at the CA3 to CA1 pathway. LTP deficit is seen when induced by theta burst (2-, 5-, 10-burst), but not 100-Hz stimulation. LTP is measured as the increase in the slope of evoked potentials (EPSPs) 40 min following induction, and is represented as a percentage increase over the baseline EPSP seen prior to induction (*y*-axis).

stimulation that mimics endogenous firing patterns in the hippocampus (Otto et al., 1991; O'Keefe and Recce, 1993; Skaggs et al., 1996). TBS recruits and potentiates synapses from both inhibitory interneurons and excitatory neurons (Chapman et al., 1998; Perez et al., 1999). Since interneurons seem to be overactive in the $NF1^{+/-}$ mice, the increased inhibition prevents normal levels of LTP from being induced. In contrast, LTP deficits in $NF1^{+/-}$ mice do not occur when nonphysiological intense stimulation parameters (e.g., 100 Hz) are used for LTP induction. These intense stimulation parameters strongly potentiate excitatory pathways, which can perhaps overcome the increased inhibition. This type of stimulation is also known to be less effective than theta burst stimulation at recruiting inhibitory activity (Chapman et al., 1998; Perez et al., 1999).

On a behavioral level, the learning deficit exhibited by $NF1^{+/-}$ mice can also be overcome with additional training. Overtraining allows $NF1^{+/-}$ mice to perform spatial learning tasks as well as their wild-type littermates (Silva et al., 1997). Under these conditions, it may be the case that excitatory pathways in $NF1^{+/-}$ mice eventually potentiate enough to compensate for increased inhibition. Physiologic impairments in $NF1^{+/-}$ are limited to specific stimulation paradigms and conditions. This may lead to the specific pattern of behavioral impairments seen in $NF1^{+/-}$ mice. In contrast to these mice, which model a learning disability disorder, plasticity impairments are seen even after 100-Hz stimulation in a rodent model of mental retardation (e.g., the tuberous sclerosis rat) (von der Brelie et al., 2006). It is likely that the severity of the behavioral impairment is related to the severity of the neurophysiological abnormalities.

The NF1 mouse model has clearly demonstrated that neurofibromin is an important regulator of inhibitory tone in neuronal networks. This regulation is functionally important, as its disruption significantly impairs behaviors, such as the acquisition of spatial information. These findings raise the possibility that increased activity in inhibitory networks underlies the learning deficits observed in NF1 patients.

4.19.8 Increased GABAergic Activity and Learning Disabilities in Neurofibromatosis Patients

We found that deletion of NF1 in mice increases hippocampal inhibition, reduces plasticity, and impairs spatial learning (Costa et al., 2002). Spatial learning has been shown to depend on the hippocampus in both humans and rodents (Burgess et al., 2002; Squire et al., 2004). Therefore, learning deficits observed in NF1 patients may also be caused by reduced plasticity and enhanced inhibition in hippocampus. Several aspects of learning deficits in NF1 patients suggest dysfunction in the hippocampal memory system. For example, NF1 patients perform poorly on spatial learning and memory tasks. Lesion and imaging studies in the general population have demonstrated that performance in these tasks depends on the hippocampus and adjacent medial temporal cortex. Damage to these areas produces profound amnesia for spatial information and difficulty in navigating through space (Bohbot et al., 2000; Burgess et al., 2002; Squire et al., 2004). Imaging studies (PET, fMRI) have also found activation of the hippocampus and surrounding cortex when subjects navigate through virtual mazes or when they recall routes they have learned in their natural environment. The most significant effects are often seen on the right side of the brain, although the left hippocampus and surrounding cortex are also thought to contribute (Maguire, 1997; Maguire et al., 1999). It should be noted that the hippocampus is only part of a larger network that supports navigation including the parietal cortex, posterior cingulate cortex, and prefrontal cortex. Its specific function is thought to be the formation and storage of topographical maps of the external environment (O'Keefe, 1978). When the hippocampus is damaged or dysfunctional, these maps cannot be formed or recalled, and spatial navigation is impaired. In NF1 patients, spatial learning and memory deficits suggest partial hippocampal dysfunction. Further, the NF1 animal models predict that hippocampal dysfunction should be caused by increased local inhibition. In this case, we can predict that activation of the hippocampus during spatial memory tasks would be reduced in NF1 patients relative to normal controls. Functional imaging studies can be used to evaluate these predictions.

Hippocampus dysfunction may also underlie the vocabulary and reading problems seen in NF1 patients. Damage to the hippocampus in normal individuals produces multiple memory problems, including an inability to acquire new facts and general information about the world (Schmolck et al., 2002; Bayley and Squire, 2005). For example, amnesic patients have considerable difficulty learning and retrieving new vocabulary words (Verfaellie et al., 2000). Although NF1 patients are clearly not amnesic, a milder hippocampal deficit akin to the one described in $NF1^{+/-}$ mice could contribute to the vocabulary and reading problems associated with

NF1. Imaging studies could be used to explore the possible relationship between deficits in hippocampal activation and vocabulary and reading problems. For example, learning of novel lexicon has been studied in the general population and was found to activate both prefrontal and hippocampal networks. With extended training, the hippocampus shows reduced activity, while the prefrontal cortex shows increased activation. Subjects that have smaller decreases in hippocampal activity with repeated training were found to have better recall of word lists (Breitenstein et al., 2005). Therefore, hippocampal engagement throughout the task appears to maximize learning of word definition associations. Training-dependent changes like these could also be examined in NF1 patients to see how activation in regions such as the hippocampus and prefrontal cortex differs from normal individuals during learning and retrieval of new information. The effects of drug treatment (i.e., lovastatin) on activity in specific brain regions could also be evaluated and correlated with performance in these patients.

4.19.9 Balanced Inhibition and Normal Prefrontal Cortex Function

Previous studies in the $NF1^{+/-}$ mice found high levels of ras activity not only in the hippocampus but also in prefrontal areas (Li et al., 2005). It is likely that ras-dependent increased inhibition is also seen in prefrontal cortex, as in hippocampus. Further, cognitive symptoms seen in NF1 patients are suggestive of prefrontal cortex dysfunction. To better understand the contribution of prefrontal cortex dysfunction to behavioral symptoms of NF1, it is important to consider the known function and physiology of this area. The prefrontal cortex in both mouse and humans plays an important role in mediating executive functions. Executive functions are those required for flexible, adaptive, goal-directed behavior and extrapolation to future goals (Miller, 2000; Stuss and Levine, 2002; Uylings et al., 2003; Dalley et al., 2004; Powell and Voeller, 2004). These include attention, organization, and planning, domains in which significant impairments are seen in NF1 patients (Dilts et al., 1996; Koth et al., 2000; North, 2000; Hyman et al., 2005). In mediating these behaviors, prefrontal cortex encodes representations of task-related information, incorporating cue relevancy, response outcomes, and rewards. This information is available to prefrontal cortex through a broad range of inputs from multimodal sensory areas, memory systems such as hippocampus, and striatal and midbrain systems which encode reward and expectation. Many of these connections are reciprocal, allowing for feedback loops to be established. Additional outputs from prefrontal cortex to motor cortex and striatum allow prefrontal activity to modulate behavior (Miller and Cohen, 2001; Uylings et al., 2003). Activity of prefrontal inhibitory networks is thought to be critical for normal prefrontal function (Goldman-Rakic, 1995; Lewis et al., 2005). In particular, balanced levels of inhibition and excitation in prefrontal cortex are thought to be important for tuning of activity during attention, working memory (Rao et al., 1999; Rao et al., 2000), and behavioral planning (Constantinidis et al., 2002).

Increased prefrontal inhibition may prevent normal control and allocation of attention. Behavioral attention involves cognitive control skills such as inhibition of irrelevant stimuli and inappropriate responses. Tasks that require such cognitive control normally recruit the prefrontal cortex as part of a cortical-striatal network (Ridderinkhof et al., 2004; Chudasama and Robbins, 2006; Arnsten, 2006). Hypoactivation of this network is associated with impairments in attention. For example, impaired performance in cognitive control tasks is seen in attention-deficit/hyperactivity disorder (ADHD). When performing these tasks, subjects with ADHD show decreased activation of prefrontal cortex and striatal structures (Vaidya et al., 2005; Smith et al., 2006). This prefrontal hypoactivity, seen in adolescents and adults independently of previous medication status (Smith et al., 2006), appears to be an intrinsic feature of ADHD thought to underlie behavioral deficits. In particular, the inattentive subtype of ADHD is thought to be caused primarily by prefrontal cortex dysfunction. In NF1, attention deficit, especially of the inattentive subtype, is a common cognitive symptom (Diamond, 2005). It is interesting to speculate that prefrontal hypoactivation may also occur in NF1 patients, leading to the symptoms of ADHD associated with this disorder. Prefrontal hypoactivation might be expected in NF1, as prefrontal increases in inhibition could increase the threshold for activation of local excitatory networks. This could prevent normal engagement of prefrontal cortex. Future studies addressing this hypothesis should examine activation of prefrontal cortex in patients with NF1 during cognitive tests of attention and response inhibition. Such studies

will be important to understand a possible connection between a putative prefrontal hypoactivation and the attention deficits described in NF1 patients.

In addition to attention, prefrontal activity contributes to other executive functions such as working memory, behavioral planning, and response organization (Goldman-Rakic, 1995; Miller, 2000; Miller and Cohen, 2001; Stuss and Levine, 2002; Dalley et al., 2004; Powell and Voeller, 2004). Working memory involves online maintenance and manipulation of information (Goldman-Rakic, 1995). Planning a series of events is a complex task which taxes working memory. Planning requires encoding multiple actions in a manner that bridges the delay between response initiation and eventual attainment of a future goal (Owen, 1997). Performance of working memory and planning tasks requires tuned, persistent activation of prefrontal cortex (Funahashi et al., 1991; Goldman-Rakic, 1995; Mushiake et al., 2006). Some evidence suggests that during planning tasks, ensembles of neurons in prefrontal cortex encode each of the actions being planned. Such evidence, obtained through single-cell recordings in the prefrontal cortex of monkeys, demonstrates that activity of these ensembles appears to be persistent and temporally orchestrated. Thus, neuronal ensembles in prefrontal cortex encode both the direction and order of actions planned through their spatial and temporal tuning (Mushiake et al., 2006). This observation is consistent with a role for this region in encoding an organized sequence of actions to reach a goal.

Both spatial and temporal tuning in prefrontal cortex is thought to be modulated by local inhibitory networks (Rao et al., 1999, 2000; Constantinidis et al., 2002). To get a sharp tuning curve, it is thought that activity of single neurons must be increased over that of their neighbors by local inhibition. This model is supported by data demonstrating that spatial tuning is disrupted quite significantly by modifying inhibitory tone in prefrontal cortex. Increasing local GABA levels leads to ensembles that respond nonspecifically and encode a broad range of movement directions. Blocking local inhibition in prefrontal cortex similarly leads to decreased spatial tuning in some prefrontal neurons. Interestingly, in other prefrontal neurons, disinhibition unmasks spatial tuning, which may reflect previously learned tasks or contexts (Rao et al., 2000). Therefore, prefrontal inhibition may play a role in determining relevancy of a cue to a task as well as sharpening spatial tuning. This is further demonstrated in behavioral data. Pharmacologic disruption of prefrontal inhibition impairs accuracy and increases omissions and inappropriately timed responding in working memory tasks (Sawaguchi et al., 1989). The increased incidence of early responding also demonstrates the importance of inhibition for proper temporal tuning. Prefrontal ensembles representing temporally distinct aspects of working memory tasks are activated in sequence during behavior (Funahashi et al., 1991). Cross-inhibition is seen among prefrontal ensembles during these tasks, often between ensembles that are active at different times (Constantinidis et al., 2002). This potentially allows temporal organization of behavior. For example, at the beginning of a task, cross-inhibition may allow ensembles representing early actions to inhibit those encoding later actions.

Therefore, the precise balance of prefrontal cortex inhibition is crucial to the orchestration of activity required for behavioral organization and planning. In NF1, where increased inhibition is seen in the mouse model, task-relevant tuning of prefrontal cortex neurons may be disrupted. This could cause behavioral impairments in planning and behavioral organization, which are common in NF1 patients. Future studies should utilize the NF1 mouse model to examine the capacity of prefrontal neuronal networks to sustain persistent and properly tuned activity. The mouse model can also be used to determine whether any prefrontal dysfunction seen is caused by increased inhibition. Experiments examining prefrontal cortex in the NF1 mouse offer powerful opportunities to identify the pathogenesis and potential therapeutic avenues for planning and attention deficits seen in NF1.

4.19.10 Parietal Cortex Dysfunction and Visuospatial Deficits in Neurofibromatosis

In addition to attention deficit and planning impairments, patients with NF1 show visuospatial deficits. In fact, one of the most robust impairments seen in NF1 is a visuospatial processing deficit commonly demonstrated in the JLO task. Deficits in complex visuospatial tasks are classically referred to parietal cortex dysfunction (Goldstein and Silverman, 2005). Indeed, fMRI studies show that the JLO task recruits parietal cortex (Ng et al., 2000). Like other cortical areas, function of parietal cortex requires balanced inhibition and excitation. As enhanced inhibition causes functional and behavioral deficits in a mouse

model of NF1, it is important to consider whether abnormal inhibition in parietal cortex could lead to visuospatial impairments such as deficits in JLO.

Parietal cortex is a multimodal area of the brain that creates integrated spatial representations of the external world. Parietal representations combine multiple sensory aspects of the environment and goal-directed motor outputs (Culham and Valyear, 2006). Transformation between different sensory modalities, such as touch and vision, and modulation of information from one sensory modality by another also involves the parietal cortex. Therefore, this area plays a critical role in putting together all the information that is being processed about a surrounding to create an integrated, whole representation of that environment (Grefkes and Fink, 2005). These representations are then available for other brain areas to use for manipulations in working memory such as rotations or integration with motor responses such as eye movement or grasping. This interaction with other brain areas means that in addition to pure sensory processing, parietal cortex function is important for online monitoring of the interaction of the individual with the environment (Andersen and Buneo, 2002; Culham and Valyear, 2006). Parietal activity is thought to maintain a stable and updated representation of space while the individual moves through and manipulates it. Individuals with NF1 show generalized visuospatial and visuomotor impairments, which may be caused by broad parietal cortex dysfunction.

Like other cortical areas, information is arranged in the parietal cortex so that subregions of this area are concerned with specific functions. Visuospatial deficits seen in the JLO are consistent with dysfunction of a specific compartment of the parietal cortex, the caudal intraparietal cortex. The caudal intraparietal cortex (CIP) receives extensive projections from visual cortex and processes three-dimensional representations of objects, in particular encoding orientations of object surfaces (Shikata et al., 2001; Grefkes and Fink, 2005) The anterior intraparietal cortex also responds to orientation of objects but is thought to be downstream of CIP processing. In the CIP, neurons are tuned to the orientation of the longitudinal axis of objects, with a topographic organization of neurons which preferentially fire to specific orientations. Further, these neurons show activity during delay periods, which may be a short-term memory trace of surface orientation (Grefkes and Fink, 2005). Activity of a subset of these neurons therefore encodes the presence of a set of object features. In this way, combined activity of ensembles of CIP neurons allows a visual reconstruction of an object in the environment. The JLO test asks individuals to distinguish between lines of different orientations and to make a response following a delay. Therefore, this task may depend on CIP orientation encoding.

Orientation coding in the CIP may be disrupted by increased activity of local inhibitory networks. In other cortical areas, parts of the cortex that are maximally activated by and therefore represent a stimulus feature also maximally recruit lateral inhibition, which represses activity of neighboring areas of cortex. In this way, the borders of neuronal ensembles or columns become more sharply defined. In visual cortex, manipulations that increased inhibition were shown to broaden orientation columns (Hensch and Stryker, 2004). Increased inhibition in parietal cortex, such as that seen in hippocampal areas of the $Nf1^{+/-}$ mouse, may also broaden ensembles. These larger ensembles could disrupt the specificity of object feature encoding in parietal cortex. Redundancy in encoding could lead to impairments such as those seen in the JLO task, where the specific orientation of a line must be distinguished from test lines of similar orientations. Such hypotheses regarding dysfunction of parietal cortex in NF1 can be explored using animal models of NF1.

While impaired parietal function in NF1 is thought to underlie JLO and general visuospatial processing deficits, it is important to consider that this is based on imaging studies in normal subjects and brain damage effects observed in otherwise normal patients. In NF1, however, other brain areas which interact intimately with the parietal cortex may also be dysfunctional. *Nf1* is expressed throughout cortex, and so inhibition is potentially increased throughout the brain, leading to deficits across multiple cortical areas which participate in visuospatial processing. For example, in areas such as visual cortex, encoding of orientation information also depends on tuning of orientation columns by balanced inhibition (Hirsch and Martinez, 2006). Therefore, in NF1 it is possible that poorly tuned orientation columns in visual cortex could also induce or exacerbate deficits in classically parietal cortex dependent tasks such as the JLO. Further, some of the multimodal associations mediated by parietal cortex also involve prefrontal cortex and hippocampus, areas which are suspected to function abnormally in NF1.

When an individual navigates through space toward a goal, parietal cortex interacts with the

hippocampus to manage external and self-motion cues. Parietal cortex encodes a series of representations of body-centered movements as well as associated visual changes as these movements are executed. This information is then converted to a representation of a single, fixed route relative to environmental cues. This integrated representation of a route through space can then be encoded and learned by the hippocampus. Since the hippocampus interacts closely with parietal cortex, seemingly parietal visuospatial and visuomotor integration deficits seen in NF1 can also be caused by changes in hippocampal activity (Save and Poucet, 2000). This possibility is particularly relevant since the mouse model of NF1 is known to have impaired hippocampal function. To fully understand which brain systems drive visuospatial and visuomotor deficits associated with NF1, it will be important to supplement classic neuropsychological testing. Neuropsychological testing batteries are not designed to isolate the individual contributions of brain structures that are recruited together during a task such as the JLO. Therefore, it may be productive to additionally use tools that systematically probe multiple aspects of parietal function to determine whether this structure is impaired in NF1 patients. Functional imaging studies provide another important tool to examine parietal cortex dysfunction in NF1 patients. Given the potential for impairment in multiple brain areas, it is particularly important to examine visuospatial impairments by carrying out imaging studies in the NF1 patient population, rather than extrapolating from imaging studies carried out in the general population. Ultimately, findings from human studies can be integrated with results from animal models to better understand cognitive symptoms associated with NF1 and to identify potential treatments.

4.19.11 Developmental Aspects of NF1

During early brain development, experience and skill acquisition shapes normal organization of neuronal networks. Genetic disorders such as NF1 are present from birth and therefore may affect early development. NF1 mutations impair experience-dependent plasticity in the adult (LTP learning) and so may also cause abnormal organization of neuronal networks early in development.

Early in development, the brain goes through a series of sensitive periods, windows of time during which experience-dependent learning has a particularly strong impact on the organization of neural networks – and therefore behavior. It is thought that during these sensitive periods, experience is more likely to induce plasticity and can shape the physical organization and connectivity of neural networks. After the sensitive period, later learning and plasticity has a lower probability of induction and is constrained by the networks established during development (Knudsen, 2004; Hensch, 2004) Sensitive periods are most easily demonstrated in primary sensory processes such as visual acuity, which depends on early binocular visual experience. Sensitive periods for complex sensory processes depend on acquisition of more simple processing abilities. For example, the visual system progresses through several sensitive periods, with the development of higher-order functions such as motion detection having a very short sensitive period that occurs after the normal development of visual acuity. Sensitive periods also occur for very complex functions such as language, music, and social interaction. For each of these functions, the ability to acquire and develop new skills throughout life is shaped by early experience during the sensitive period. Development of language skills requires early exposure to language, as is demonstrated by the limited proficiency often seen in languages learned later in life. Normal social interactions later in life require early exposure to healthy social relationships in the home and are dramatically influenced by inappropriate social interaction during this time (Knudsen, 2004; Hensch, 2004; Johnson, 2005).

It is currently unknown whether patients with NF1 have abnormal sensitive periods. However, the $NF1^{+/-}$ mice demonstrate impairments in physiological properties that are thought to be important to sensitive-period regulation. For example, work in primary cortical areas such as visual and barrel cortices in rodent has demonstrated the importance of the maturation and activity of inhibitory networks in modulating cortical sensitive periods. As local inhibitory networks mature and become increasingly active, local excitation induced by visual experience becomes less effective at causing plasticity. Therefore, the maturation and increased activity of inhibitory cortical networks is thought to contribute to the closure of sensitive periods (Fagiolini and Hensch, 2000; Hensch, 2005). Consistent with this hypothesis, tonic increases in GABAergic activity

correlate with the closure of a sensitive period in the visual cortex which is important for visual acuity. Expression of cortical BDNF at abnormally early time points in a mouse model leads to accelerated maturation of inhibitory networks during development and earlier closure of a sensitive period (Huang et al., 1999). In NF1, hyperactivity of the ras pathway may similarly lead to early, constitutive increases in signaling downstream of BDNF receptors, to enhancements of GABAergic tone, and consequently to early closure of sensitive periods. This hypothesis can be tested with animal models of NF1. It is important to determine whether NF1 alters the properties of sensitive periods because it may be critical to treat patients during these periods.

Developmental aspects of NF1 are important to explore; however, mounting evidence suggests that cognitive symptoms of NF1 can be treated even in the adult. The ongoing effects of *Nf1* mutation in the mature brain (higher ras signaling and subsequent increased inhibition) drive the behavioral impairments seen in the adult $NF1^{+/-}$ mouse. Indeed, normalizing ras levels in the adult $NF1^{+/-}$ mouse leads to rescue of attention and learning deficits. Further, current pharmacologic treatments are successful in treating ADHD symptoms in children and adults with NF1. The effectiveness of these interventions in treating symptoms of NF1 in the adult brain emphasizes the importance of neurofibromin as a modulator of neuronal function in the mature brain, independent of its effects during development.

4.19.12 Mechanism-Driven Treatment: Pharmaceutical Targets for Treatment of Neurofibromatosis

Hyperactivation of the ras pathway as a result of *Nf1* mutation has been shown to be a critical pathology causing learning and behavioral deficits in the NF1 mouse model. Downregulation of ras activity in the CNS, therefore is an important therapeutic goal (Weiss et al., 1999).Targeting ras signaling can potentially reverse the functional effects of *Nf1* mutation at its direct downstream effector. Therapeutic interventions that manipulate the ras pathway have the potential to specifically treat NF1 symptoms with limited disruption of behaviors and cognitive processes that are unaffected in NF1.

Activation of ras requires its association with the membrane. This association occurs through isoprenyl groups that are added to the ras protein following translation. Isoprenyl groups, including farnesyl groups, have a lipid moiety that can insert into the membrane lipid bilayer. This tethers ras to the plasma membrane, where it can be activated (Bernards and Settleman, 2004). Isoprenyl groups are synthesized as a byproduct of the cholesterol synthesis pathway (Casey et al., 1989; Weiss et al., 1999). A class of therapeutics, the statins, inhibits the cholesterol synthesis pathway by inhibiting its rate limiting enzyme, HMG-CoA reductase. In addition to inhibiting cholesterol synthesis, statins lower production of isoprenyl groups. This lowers the levels of ras at the plasma membrane (Mendola and Backer, 1990; Xu et al., 1996). The statins are a relatively safe drug class, which is currently widely prescribed to people as a cholesterol-lowering agent (Corvol et al., 2003). This makes the statin class an attractive candidate for treatment of NF1.

Studies of the effect of statins in $NF1^{+/-}$ mice have been promising. Indeed, statins can normalize the total levels of active ras in the hippocampus and neocortex of $NF1^{+/-}$ mice. Statin treatment also normalizes activation of downstream targets of ras, such as p-MAPK. Importantly, adult $Nf1^{+/-}$ mice that were given a brief treatment of lovastatin, a statin that penetrates the blood–brain barrier (Duggan et al., 1989; Botti et al., 1991), performed normally in behavioral tests of attention and spatial learning. Lovastatin also reversed the deficits in hippocampal plasticity seen in the $Nf1^{+/-}$ mice. These effects of lovastatin were seen at a dose which did not affect ras/MAPK signaling in wild-type controls (Li et al., 2005), demonstrating that ras/MAPK signaling in NF1 may be more sensitive to the effects of statins. Heightened sensitivity to lovastatin in $NF1^{+/-}$ mice is an important characteristic for a potential therapeutic. This suggests that despite their wide action spectrum, statins may be used at low doses to selectively improve specific disease-causing abnormalities in biochemical signaling.

These results demonstrating the effectiveness of lovastatin in treating $NF1^{+/-}$ mice have led to the initiation of clinical trials. Lovastatin and simvastatin are currently being tested for safety and efficacy in several NF1 patient populations. Statin clinical trials for NF1 are an exciting example of rational pharmacologic therapy being designed to target the functional effects of a disease-causing mutation. While such therapeutics are being developed, other pharmaceuticals specific to certain cognitive symptoms are currently being used successfully in children with NF1.

Methylphenidate can be used to treat ADHD symptoms of NF1. In fact, patients with NF1 respond to much lower doses than are used to treat ADHD not associated with NF1 (Mautner et al., 2002). The effect of treatment on attention deficits was assessed quantitatively in children with ADHD compared to children with comorbid NF1 and ADHD using the Test of Variables of Attention (TOVA). The TOVA is a computerized test that measures sustained attention and impulsivity. Improvements in general cognition as well as academic and social performance by methylphenidate treatment were assessed using the child behavioral checklist (CBCL). This test is based on a standardized series of questions that elicit feedback from the child with ADHD, their parents, and their teachers. The CBCL is used to assess effects of attention deficit relevant to function in daily life (Palumbo and Lynch, 2006). In children with NF1 and ADHD, low-dose methylphenidate treatment improved TOVA scores immediately and improved CBCL scores after 1 year of treatment (Mautner et al., 2002). While the mechanism of methylphenidate activity in ADHD is unclear, methylphenidate is known to modulate excitability of prefrontal inhibitory networks (Andrews and Lavin, 2006). Inappropriate, increased inhibition is critical to some of the behavioral deficits seen in the NF1 mouse model. Therefore, it is possible that the ability of methylphenidate to regulate inhibitory networks is important to its effectiveness in treating ADHD associated with NF1.

4.19.13 Implications for Other Learning Disabilities

The approach to NF1 taken here has attempted to integrate multidisciplinary aspects of NF1 research in order to build a more complete framework for understanding mechanisms of disease. As NF1 is a single-gene disorder, all patients with the disease can be identified and the symptoms of the disease fully described using carefully carried out neuropsychological batteries. In NF1, cognitive symptoms include learning deficits, attention and planning impairments, and visuospatial impairment. Insights into the neurosystems that mediate these cognitive functions are drawn from functional imaging studies and basic neuroscience. Particularly, dysfunction in hippocampus, prefrontal cortex, and parietal cortex may contribute to the cognitive symptoms associated with NF1. Importantly, animal models of the disease have been utilized to study these systems in a manner that integrates genetics, biochemistry, physiology, and behavior. This approach has yielded powerful insights into mechanisms of pathology in NF1. In mouse models of NF1, increased inhibitory tone was found to cause deficits in hippocampal plasticity required for behavioral learning. These increases in inhibitory tone were caused by constitutively increased activity of the ras signaling pathway, which is normally negatively modulated by the *Nf1* gene product, neurofibromin. These insights into the neuronal mechanisms of NF1 have led to exciting prospects for therapeutic intervention using statins to decrease ras pathway activity. In NF1, integration of current genetics and neuroscience with psychology and medicine has allowed a unified approach to cognitive disease and successfully identified potential biochemical targets that can restore cognitive health.

As this integrative approach is applied to other diseases affecting cognitive function, it will hopefully expand our understanding of critical physiological processes and systems that mediate cognition. Some of the genes mutated in these diseases are involved in similar pathways to those modulated by NF1. For example, Coffin-Lowry syndrome is an X-linked disorder involving severe mental retardation caused by disruption of the gene encoding Rsk2, a ribosomal S6 serine-threonine kinase-2. Rsk2 lies downstream of pMAPK, the target of ras which is upregulated in $Nf1^{+/-}$ mice. Rsk2 activation by ras/pMAPK leads to CREB activation. Neurofibromin and Rsk2 are both part of the ras signaling pathway, with neurofibromin being a negative regulator of the pathway, while Rsk2 is a downstream target that connects ras activity to CREB activation of transcription. In a mouse model made by disruption of Rsk2, loss of CREB activation in the hippocampus caused severe learning deficits (Weeber and Sweatt, 2002). It will be interesting to compare the effects of *rsk2* and *Nf1* mutations in different cell types and systems and on behavior and physiology. Modification of GABA function has also been implicated as a mechanism of cognitive dysfunction by other genetic neurodevelopmental disorders. Abnormal inhibition was found in a mouse model of Down syndrome that shows hippocampal LTP deficits (Kleschevnikov et al., 2004; Costa and Grybko, 2005) and spatial learning impairments (Reeves et al., 1995). Comparison of the physiology and systems level pathology seen in Down syndrome and NF1 may yield interesting insights about the contribution of inhibition to multiple cognitive processes.

As these data accumulate, a synthesized picture will gradually unfold of the critical molecular pathways and systems level interactions that underlie normal and abnormal cognitive function.

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4.20 The NMDA Receptor

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4.20.1 Introduction

In 1983, Graham Collingridge made the breakthrough discovery that induction of tetanus-induced forms of long-term potentiation (LTP) are blocked by blockade of a specific subtype of glutamate receptor, the *N*-methyl-D-aspartate (NMDA) receptor (Collingridge et al., 1983). Collingridge's fascinating discovery was based on the observation that the glutamate analog aminophosphonovaleric acid (APV), an agent that selectively blocks the NMDA subtype of glutamate receptor, could block LTP induction while leaving baseline synaptic transmission entirely intact.

This was the first experiment to give a specific molecular insight into the mechanisms of LTP induction. The properties of the NMDA receptor that allow it to function in this unique role of selectively triggering long-term synaptic potentiation, and indeed memory (Morris, 1989; see **Figure 1**), are important, and in this chapter I will present a detailed analysis of regulation of the NMDA receptor.

The NMDA receptor is both a glutamate-gated channel and a voltage-dependent one. The simultaneous presence of glutamate and a depolarized membrane is necessary and sufficient (when the co-agonist glycine is present) to gate the channel (**Figure 2**). Pairing synaptic stimulation with membrane depolarization provided via either alpha-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA)-subtype glutamate receptors or neuronal action potential firing (plus the low levels of glycine always normally present) opens the NMDA receptor channel. Channel gating in this fashion leads

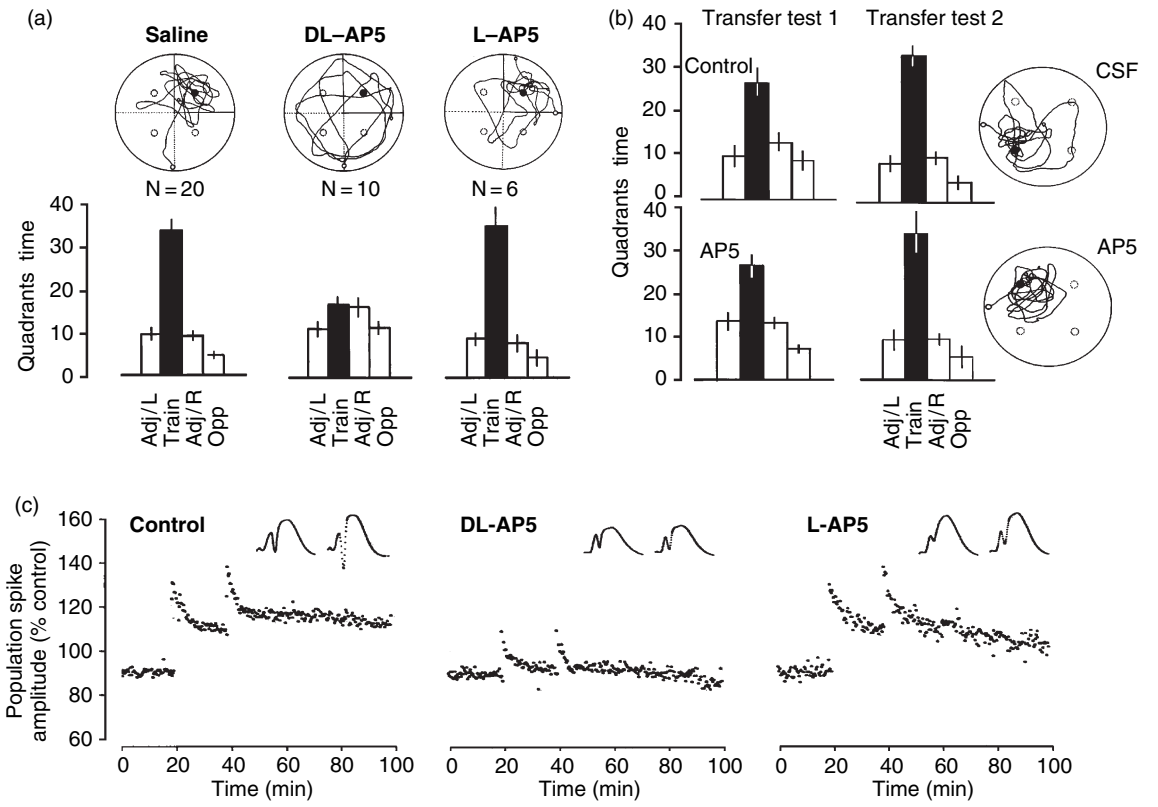

Figure 1 The NMDA receptor antagonist D-AP5 (i.e., APV) blocks learning in the Morris water maze. These data are from the landmark paper by Richard Morris (Morris, 1989), demonstrating that infusion into the central nervous system of DL-AP5 (an active mixture) but not the control, inactive enantiomer L-AP5, blocks learning in the water maze task. (a) Pretraining infusion of DL-AP5 blocks learning of a spatially selective search strategy for locating the hidden platform. Adj/L, adjacent left of training quadrant; Adj/R, adjacent right of training quadrant; Train, training quadrant; Opp, opposite from training quadrant. (b) Posttraining blockade of NMDA receptors does not affect memory recall. (c) The same infusion protocol leads to effective blockade of NMDA receptor-dependent LTP in the dentate gyrus.

to the induction of long-term changes in synaptic strength and contributes to long-term memory formation in vivo.

The NMDA receptor is a calcium channel and its gating leads to elevated intracellular calcium in the postsynaptic neuron. It is this calcium influx that triggers lasting changes in synaptic function, and indeed many subsequent chapters in this volume deal with the various processes this calcium influx triggers. It is important to remember that it is not necessarily the case that every calcium molecule involved in synaptic plasticity and memory formation actually comes through the NMDA receptor. Calcium influx through voltage-gated calcium channels in the membrane and calcium released from intracellular stores are also involved, but for this chapter we will focus exclusively on the NMDA receptor.

The gating of the NMDA receptor/channel involves a voltage-dependent Mg^{++} block of the channel pore. Depolarization of the membrane in which the NMDA receptor resides is necessary to drive the divalent Mg^{++} cation out of the pore, which then allows calcium ions to flow through. Thus, the simultaneous occurrence of both glutamate in the synapse and a depolarized postsynaptic membrane is necessary to open the channel and allow LTP-triggering calcium into the postsynaptic cell.

These properties, glutamate dependence and voltage dependence, of the NMDA receptor allow it to function as a coincidence detector. This is a critical aspect of NMDA receptor regulation and this allows for a unique contribution of the NMDA receptor to information processing at the molecular level. Using the NMDA receptor, the neuron can trigger a unique

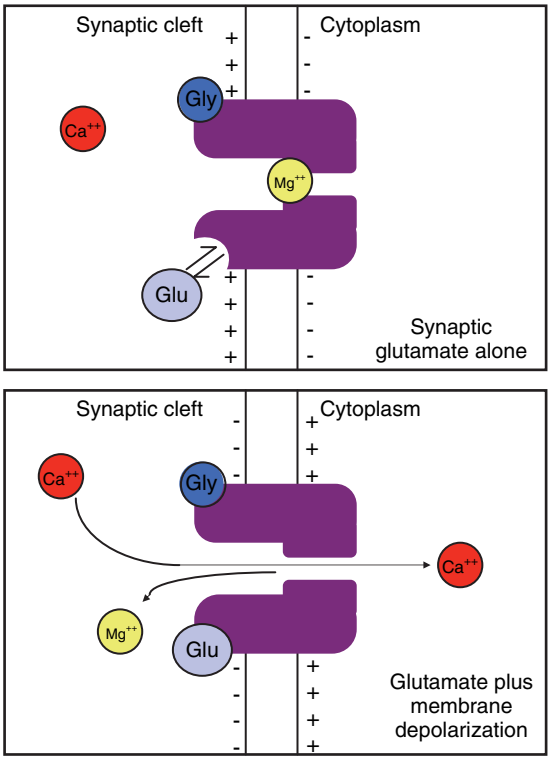

Figure 2 Coincidence detection by the NMDA receptor. The simultaneous presence of glutamate and membrane depolarization is necessary for relieving Mg^{++} blockade and allowing calcium influx. Figure by J. David Sweatt and Sarah E. Brown.

event, calcium influx, specifically when a particular synapse is both active presynaptically (glutamate is present in the synapse) and postsynaptically (when the membrane is depolarized).

This confers a computational property of associativity on the synapse. This attribute is nicely illustrated by pairing LTP where low-frequency synaptic activity paired with postsynaptic depolarization can lead to LTP (*See* Chapter 4.16). The associative property of the NMDA receptor allows for many other types of sophisticated information processing as well, however. For example, activation of a weak input to a neuron can induce potentiation, provided a strong input to the same neuron is activated at the same time (Barrionuevo and Brown, 1983). These particular features of NMDA receptor regulation have stimulated a great deal of interest as they are reminiscent of classical conditioning, with depolarization and synaptic input roughly corresponding to unconditioned and conditioned stimuli, respectively.

The associative nature of NMDA receptor activation allows for synapse specificity of the induction of long-term changes in synaptic strength as well. For example, if one pairs postsynaptic depolarization with activity at one specific set of synaptic inputs to a cell, while leaving a second input silent or active only during periods at which the postsynaptic membrane is near the resting potential, then selective potentiation of the paired input pathway occurs.

Thus we see that the NMDA receptor is a critical molecular locus for triggering lasting changes, which has unique biophysical properties that allow it to perform a coincidence detection role in the neuron. For these intriguing reasons, the most-studied molecule in the short history of molecular studies of learning and memory is the NMDA subtype of glutamate receptor.

4.20.1.1 Structure of the NMDA Receptor

The NMDA receptor is a glutamate-gated cation channel and as such is a multisubunit transmembrane protein. Current models hypothesize that it is a tetrameric hetero-oligomeric protein with more than one glutamate binding site. It is of course voltage dependent and this arises from the voltage-dependent Mg block of the pore that we discussed above. The protein has binding sites for zinc, polyamines, and glycine (a co-agonist necessary for activity).

Abundantly expressed individual subunits of the receptor are named NR1, NR2A, NR2B, NR2C, and NR2D (the somewhat unusual nomenclature arose for historical reasons related to different groups that cloned the first NMDA receptor subunits). One functional NMDA receptor comprises one or more NR1 subunits plus one or more NR2-type subunits. The NR2 subunits determine the calcium permeability of the channel and can influence the voltage dependence of its activation, kinetics of opening, and other biophysical properties. NR1 and NR2A and 2B are phosphorylated at a number of different sites: NR1 by protein kinase C (PKC) and PKA, NR2A by cyclin-dependent kinase 5, and NR2A and NR2B by various tyrosine kinases such as src and fyn.

Indeed, the NMDA receptor is subject to a wide variety of direct modulatory influences, some of which are listed in **Table 1**. Moreover, recent work by Grant and colleagues has shown that the NMDA receptor is in fact a large multiprotein complex (Husi et al., 2000; Husi and Grant, 2001). This complex includes a striking representation of many different types of scaffolding proteins and signal transduction molecules (see **Figure 3**). In fact, the NMDA receptor

Table 1 Direct modulators of the NMDA receptor

Modulator	*Mechanism*	*Effect*
Src family tyrosine kinases (src, fyn)	Tyrosine phosphorylation, loss of Zn inhibition	Enhancement
Scaffolding proteins		
Receptor for activated C kinase	Binding	Inhibition
Polysynaptic density-95	Scaffolding	Modulation
Protein kinase C	Ser/thr phosphorylation (direct)	Enhancement
	Src activation (indirect)	Enhancement
Protein kinase A/protein phosphatase 1/Yotiao	Phosphorylation	Enhancement
	Dephosphorylation	Inhibition
Cyclin-dependent kinase 5	Ser/thr phosphorylation	Enhancement
Nitric oxide/reactive oxygen species	Sulfhydryl nitrosylation Or oxidation	Inhibition
Polyamines (e.g., spermine, spermidine)	Direct binding to a modulatory Site	Augmentation
Casein kinase II	Ser/thr phosphorylation Modulation of polyamine effects	Enhancement

Molecule	Mr (kD)
Glutamate receptors	
NR1	120
NR2A	180
NR2B	180
GluR6 + 7	117
mGluR1a	200
Scaffolding and adaptors	
PSD-95	95
ChapSyn110/PSD-93	110
Sap102	115
GKAP/SAPAP	95-140
Shank	200
Homer	28/45
Yotiao	200
AKAP150	150
NSF	83
PKA	
PKA catalytic subunit	40
PKA-R2β	53
PKC	
PKCβ	80
PKCγ	80
PKCε	90
CaM kinase	
CaM kinase II β	60
phosph-CaM kinase	60

Molecule	Mr (kD)
Phosphatases	
PP1	36
PP2A	36
PP2B (calcineurin)	61
PPs	50
PTPID/SHP2	72
Tyrosine kinases	
Src	60
PYK2	116
MAP kinase pathway	
ERK (pan ERK)	42/44
ERK1	42/44
ERK2	42
MEK1	45
MEK2	46
MKP2	43
Rsk	90
Rsk-2	90
c-Raf1	74
Small G proteins and modulators	
Rac1	21
Rap2	21
SynGAP	10,12,35,60
NF1	60,101

Molecule	Mr (kD)
Other signaling molecules	
Calmodulin	15
nNOS	155
PI3 kinase	85
PLCγ	130
cPLA2	110
Citron	183
Arg3.1	55
Cell adhesion and cytoskeletal proteins	
N-cadherin	150
Desmoglein	165
β-Caternin	92
LI	200
pp120cas	120
MAP2B	280
Actin	45
α-actinin 2	110
Spectrin	240/280
Myosin (brain)	205
Tubulin	50
Coractin	80/85
CortBP-1	180/200
Clathryn heavy chain	180
Dynamin	100
Hsp-70	70

Figure 3 Summary of molecular composition of the NMDA receptor supramolecular complex. The component molecules and their molecular weights are listed. Adapted from Husi H, Ward MA, Choudhary JS, Blackstock WP, and Grant SG (2000) Proteomic analysis of NMDA receptor-adhesion protein signaling complexes. *Nat. Neurosci.* 3: 661–666, with permission from Elsevier; and Husi H and Grant SG (2001) Proteomics of the nervous system. *Trends Neurosci.* 24: 259–266, with permission from Elsevier.

supramolecular complex includes a number of proteins whose function has been directly implicated in human learning, including neurofibromatosis type 1 protein (NF1), PKA, raf-1, mitogen and extracellular signal-related kinase (MEK), extracellular signal-regulated protein (ERK), and ribosomal S-6 kinase type 2 (RSK-2). These gene products have all directly or indirectly been implicated in human learning, as they are associated with various types of human mental retardation syndromes: as was commented upon by Grant and colleagues in their report, the presence of gene products linked to human mental retardation within the NMDA receptor complex was intriguing, and consistent with a role for this complex in human cognition.

Given the dozens of individual molecular events that have been reported as being involved in NMDA receptor regulation in the literature, how can one begin to organize this immense molecular system into a coherent picture? In order to do this, in this chapter regulation of the NMDA receptor will be broken down into the following three basic components:

1. Mechanisms upstream of the NMDA receptor that directly regulate NMDA receptor function,
2. Mechanisms upstream of the NMDA receptor that control membrane depolarization, and
3. The components of the synaptic infrastructure that are necessary for the NMDA receptor, and the associated synaptic signal transduction machinery, to function normally.

These various components of the NMDA receptor regulatory machinery are schematized in **Figure 4**. This remainder of this chapter will fill in the molecular details necessary to flesh out this general model.

4.20.2 NMDA Receptor Regulatory Component 1: Mechanisms Upstream of the NMDA Receptor that Directly Regulate NMDA Receptor Function

The NMDA receptor is a biochemical signal integrator. Its capacity for signal integration and coincidence detection is not limited to determining the simultaneous presence of glutamate and depolarization. It senses biochemical signals as well, that are used in computing the degree of calcium influx. This section will describe many of the biochemical mechanisms known to directly regulate the NMDA receptor. In general, the discussion will be limited to those processes which have been directly implicated in LTP induction, memory formation, or both.

These biochemical processes generally are not all-or-none like the glutamate/depolarization mechanism, but rather serve to modulate the magnitude of postsynaptic calcium influx. The section titled 'NMDA receptor regulation component 2: Mechanisms upstream of the NMDA receptor that control membrane depolarization' will describe biochemical processes that are used to control the NMDA receptor in an all-or-none fashion, indirectly through controlling the membrane potential.

As described, the NMDA receptor is a coincidence detector, but in addition the NMDA receptor is also a temporal integrator. These temporal integration mechanisms operate on a longer time frame and are limited to biochemical processes, as opposed to biophysical processes. The time frame in which the NMDA receptor can detect the simultaneous presence of depolarization and glutamate is of course quite limited because the membrane depolarization

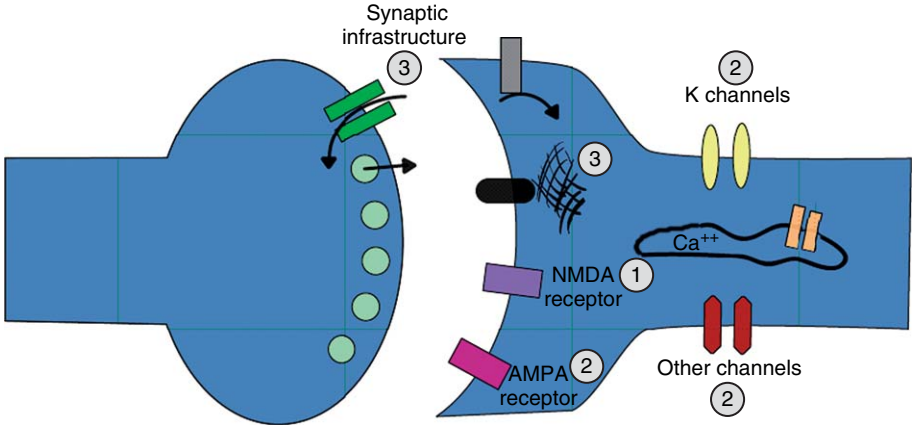

Figure 4 LTP induction machinery. Schematic diagram summarizing examples of the three different components of NMDA receptor regulation discussed in this chapter. See text for explanation. Figure by J. David Sweatt and Sarah E. Brown.

is so brief. In contrast, a biochemical signal such as elevation of a second messenger or increased protein kinase activity has a much longer half-life. Thus a temporal integration mechanism on the seconds (or longer) timescale must use these types of processes. The capacity of the NMDA receptor to be modulated by protein kinases and other messenger molecules allows for this sort of temporal integration.

4.20.2.1 Kinase Regulation of the NMDA Receptor

One of the oncogene products produced by the Rous sarcoma virus, which also has a homolog in the mammalian genome, is the tyrosine kinase src. Src family tyrosine kinases such as src and fyn directly phosphorylate the NMDA receptor (Raymond et al., 1994; Suzuki and Okumura-Noji, 1995), increasing calcium flux through the receptor. Tyrosine phosphorylation of the NMDA receptor increases current flow through the ion channel by reducing a tonic, zinc-dependent inhibition (Zheng et al., 1998).

It is important to bear in mind that there are a number of different mechanisms that can cause increased current flow through a ligand-gated ion channel:

1. The probability that the channel will open can be increased, which is referred to as increased channel open probability.
2. The conductance, that is, the rate at which ions flow through the channel, can be increased.
3. The number of channels in the membrane can increase.
4. The affinity of the channel for its ligand can increase, a mechanism that of course can only operate at subsaturating ligand concentrations.

This chapter will not go into detail on which of these mechanisms is involved in channel modulation, except where the mechanism is directly relevant to the molecular mechanisms that are involved (for example, increased membrane insertion of a channel implies the involvement of specific molecular processes). In addition, in many cases the specifics are not known or the channel modulation involves multiple mechanisms. As one example, src modulation of the NMDA receptor at a minimum involves increased channel open probability.

Protein tyrosine kinase phosphorylation of the NMDA receptor may be required for LTP induction, and at a minimum this mechanism serves an important modulatory role controlling the likelihood of LTP induction (Lu et al., 1998). The activities of Src and Fyn in neurons are controlled by a number of upstream signal transduction cascades. One important regulator of Src is the focal adhesion kinase (FAK) CAKbeta, also known as pyk2, and this cascade has been shown to be involved, through Src, in regulating LTP induction (Huang et al., 2001). In addition, the extracellular signal-regulated kinase/mitogen-activated protein kinase (ERK/MAPK) cascade and the PKC cascades, which will be discussed below, also can activate Src family kinases and these pathways may also modulate NMDA receptor function and LTP induction via Src (Grosshans and Browning, 2001). Dephosphorylation of the src/fyn sites on NMDA receptors likely occurs through the action of the tyrosine phosphatase STEP.

A number of interesting cell surface receptors modulate NMDA receptor function, and thus potentially LTP induction, acting through the src cascade (see **Figure 5**). The ephrins, which have been mostly studied in the context of nervous system development, modulate NMDA receptors in cultured neurons (Takasu, 2002). Ephrin B2, acting through its receptor EphB2, activates src and modulates NMDA receptors via this mechanism. Genetic deletion of EphB2 leads to an attenuation of LTP in area CA1 (Grunwald et al., 2001; Henderson et al., 2001). Apolipoprotein E receptors in hippocampus also modulate LTP induction via a src/NMDA receptor pathway (Weeber et al., 2002). Finally, the *obese* gene product leptin acts through its cell surface receptor and a phosphatidyl-inositol-3 (PI3)-kinase/MAPK/src pathway to modulate NMDA receptors and LTP induction in the hippocampus (Shanley et al., 2001). Thus, the Src/Fyn pathways serve an important role in funneling cell surface signals to the NMDA receptor itself, modulating its activity and regulating LTP induction.

Src tyrosine kinase potentiation of NMDA receptors is also subject to a variety of other influences. RACK1 (receptor for activated C kinase 1) promotes formation of a Fyn/RACK1/NR2B complex that actually inhibits fyn phosphorylation of the NMDA receptor and diminishes current through the receptor (Yaka et al., 2002). Also, the postsynaptic density (PSD) core protein PSD-95 modulates src phosphorylation of NMDA receptors (NMDARs) and src potentiation of NMDAR currents appears to require the presence of PSD-95 (Liao et al., 2000).

PKC not only can act indirectly through Src to modulate the NMDAR, but PKC also can also directly phosphorylate the receptor on serine/

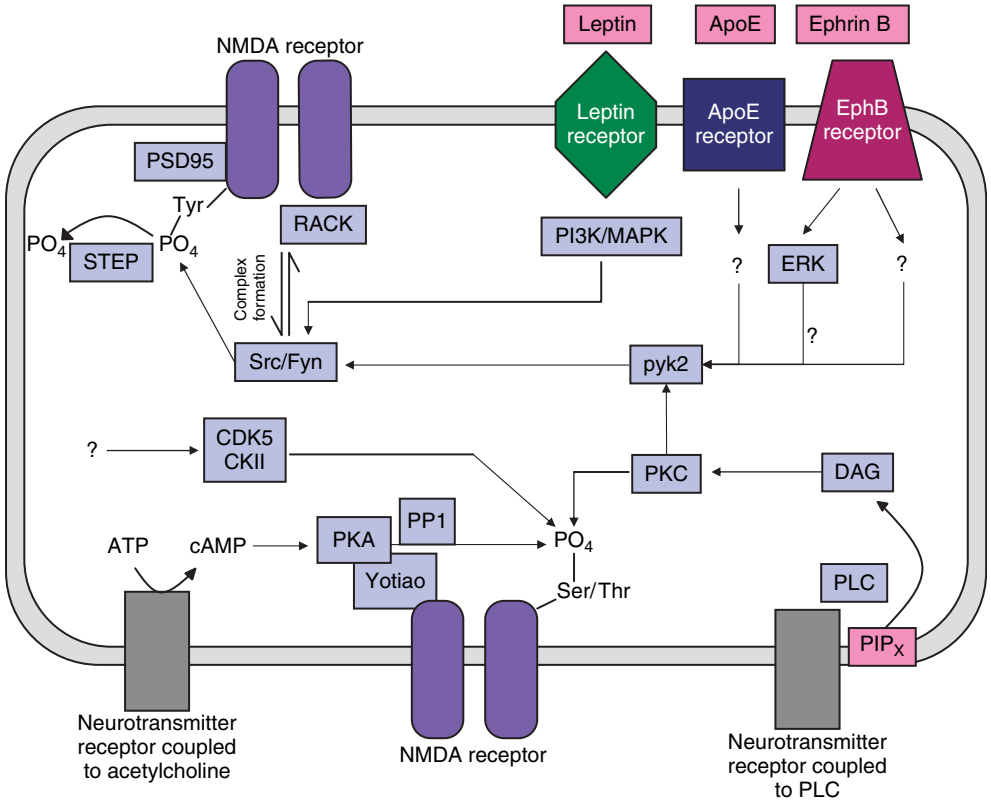

Figure 5 Receptor modulation of the NMDA receptor by the Src, PKA, and PKC cascade. These kinase cascades are represented here in relation to modulation of NMDA receptors, which could potentially lead to regulating LTP induction. See text for discussion and definitions. Figure by J. David Sweatt and Sarah E. Brown.

threonine residues and affect its function (Logan et al., 1999; Liao et al., 2001). Phosphorylation of the NMDAR by PKC causes increased calcium flow through the receptor (Ben-Ari et al., 1992). The potential import of this is quite straightforward: any cell surface receptor coupled to a phospholipase C cascade can modulate the likelihood of LTP induction through direct regulation of the NMDA receptor complex (see **Figure 5**).

The cyclic adenosine monophosphate (cAMP)-dependent protein kinase (PKA) also can augment NMDA receptor function, although the mechanism is complex and not entirely worked out (Westphal et al., 1999). PKA binds to the NMDAR via an associated protein, Yotiao (Yotiao is a specific isoform of A kinase anchoring protein, a category of protein which we will discuss again in section 4.20.3 of this chapter). Yotiao binds both PKA and protein phosphatase 1 (PP1) to the NMDA receptor, and when all three are bound together the PP1 activity predominates and keeps the NMDA receptor phosphorylation (and activation) low. PKA activation by cAMP leads to enhancement of NMDA currents, although it is not entirely clear if this is due to PKA phosphorylation of the NMDA receptor, loss of tonic dephosphorylation by PP1, or both. Again, in the context of the hippocampal pyramidal neuron this mechanism represents a basis for any neurotransmitter receptor coupled to adenylyl cyclase to be able to modulate NMDA receptor function and the induction of LTP.

The cyclin-dependent kinases (CDKs) are key regulators of cell division, controlling progression through the cell cycle. However, this role is of course not germane to understanding the function of nondividing neurons in the adult central nervous system (CNS). However, one cdk isoform, cdk5, is selectively expressed in postmitotic neurons and functions in regulating neuronal migration and neurite outgrowth in development. Moreover, recent work has shown that this kinase is involved in synaptic plasticity and learning in adult animals (Fischer et al., 2002). Specifically, inhibition of cdk5 blocks NMDAR-dependent LTP in area CA1 and blocks contextual fear conditioning. One possible mechanism for this

effect is cdk5 regulation of NMDA receptor function, as cdk5 phosphorylates the NR2A subunit and cdk5 inhibitors reduce NMDA-induced currents in hippocampal neurons (Li et al., 2001). Thus, cdk5 may modulate NMDA receptor function in a manner reminiscent of src, PKC, etc. The mechanisms controlling cdk5 regulation in hippocampal pyramidal neurons have yet to be worked out.

4.20.2.2 Redox Regulation of the NMDA Receptor

Modulation of the NMDAR is of course not restricted to posttranslational modifications involving phosphorylation. An interesting and novel type of regulation that is gaining increased attention is redox modulation of protein function. In the context of NMDAR function, there are two specific examples of this type of regulation, both of which elicit inhibition of NMDAR function. The reactive nitrogen species nitric oxide (NO), a free radical, can react with sulfhydryl moieties in cysteine side chains, a reaction leading to S-nitrosylation of the side chain. This reaction occurs in NR2A subunits at reasonably low levels of free NO and leads to decreased channel opening (Choi et al., 2000). A second example of redox regulation of NMDA receptors involves reactive oxygen species (ROS) such as superoxide and peroxynitrite, the product of the reaction of superoxide plus NO (Choi and Lipton, 2000). ROS inhibition of the NMDAR likely occurs via cysteine oxidation in a fashion reminiscent of the effects of NO, although the mechanisms of this effect are not clear at present.

The physiologic role of NO and ROS inhibition of NMDAR function is not clear. One interesting speculation is that oxidative inhibition of NMDARs might serve to lock the synapse in a particular state after plasticity had been triggered, or the mechanism might serve as a basis for inhibitory crosstalk limiting the capacity of a synapse to undergo LTP.

4.20.2.3 Polyamine Regulation of the NMDA Receptor

Finally, polyamine compounds such as spermine, spermidine, and putrescine can modulate NMDAR function. Polyamines are synthesized normally in cells and are essentially long aliphatic chains with several amino moieties covalently attached. Polyamines have diverse modulatory effects on NMDA receptors *in vitro* and *in vivo*, but one effect that they have is augmentation of NMDAR function. This effect is through the unusual mechanism of relief of tonic proton inhibition of the channel (Traynelis et al., 1995, Gallagher et al., 1997). Polyamine co-application with NMDA leads to an enhancement of NMDA-induced synaptic potentiation in area CA1. Casein kinase II (CKII), a calcium-independent protein kinase whose function has not been widely investigated in the CNS, augments NMDAR function by acting in concert with polyamine binding to the receptor intracellular domain (Lieberman and Mody, 1999). The role of this mechanism in LTP induction in the intact cell is unknown. However, CKII is activated by LTP-inducing stimulation (Charriaut-Marlangue et al., 1991) and evidence exists suggesting activity-dependent increased polyamine synthesis in the hippocampus (Ingi et al., 2001). These mechanisms might serve a role in temporal integration with repeated stimulation or in setting a baseline likelihood of LTP induction.

4.20.3 NMDA Receptor Regulatory Component 2: Mechanisms Upstream of the NMDA Receptor that Control Membrane Depolarization

As was discussed in Chapter 4.16, recent discoveries have highlighted the importance of mechanisms for controlling membrane depolarization in LTP induction. Although the potential importance of modulating the level of membrane potential in regulating LTP induction was first pointed out in 1987 (Byrne, 1987), the recent emphasis was catalyzed by the discovery of back-propagating action potentials and of their involvement in providing the depolarization of the synaptic membrane necessary for LTP induction (Stuart and Sakmann, 1994; Magee and Johnston, 1995; Spruston et al., 1995). A second important development was the emergence of the silent synapse model of LTP induction, wherein there are synapses that contain NMDA receptors but no AMPA receptors. Obviously, in the silent synapse model the membrane depolarization necessary for NMDA receptor activation cannot come from local AMPA receptors, but must be propagated via the membrane from a distal site. Taken together, these two considerations bring into focus the necessity of understanding the mechanisms that control the electrical properties of the dendrite and dendritic spines, and their role in regulating the NMDA receptor through controlling the depolarization envelope that the receptor experiences.

Table 2 lists a number of the important molecules contributing to regulation of membrane depolarization in neuronal dendrites. Progress in this area has been greatly facilitated by relatively recent technical advances that allow direct cell-attached patch recording from the distal dendritic regions of CA1 pyramidal neurons. These studies have identified a number of relevant membrane currents that control dendritic membrane depolarization and excitability, and in most cases there are reasonable hypotheses about the molecules underlying these currents. However, keep in mind that linking a specific molecule with a specific ionic current involves some degree of speculation at present.

Finally, please note that in the next section, wherever possible, the term current will be used to refer to an entity identified in physiology experiments, and use the term channel to refer to specific molecules. Also, in some cases the channel molecules can be referred to by their own specific subtype names (e.g., Kv4.2).

4.20.3.1 Dendritic Potassium Channels

4.20.3.1.1 A-type currents

A-type, or voltage-dependent, rapidly inactivating K^+ channels localized in the dendrites of hippocampal pyramidal neurons play a critical role in shaping the local electrical responses of the dendritic membrane and dendritic tree. The functions of A-type channels in general are to repolarize the membrane after an action potential, contribute to the resting membrane potential (modestly), and regulate firing frequency. Johnston and coworkers have proposed a model in which A-type channels in distal dendrites of the hippocampus are critical regulators of back-propagating action potentials, regulating LTP induction through controlling voltage-dependent NMDA receptor activation (Hoffman et al., 1997; Yuan et al., 2002; Watanabe et al., 2002).

Moreover, this type of regulatory mechanism is subject to modulation by cellular signal transduction cascades. Activation of PKA or PKC shifts the activation curve of A-type K^+ currents recorded in hippocampal area CA1 dendrites (Hoffman et al., 1997). The voltage-dependence of their activation is shifted in the depolarizing direction, leading to increases in dendritic excitability and increased back-propagating action potentials in dendrites. More recent work has shown that the alterations in A-current voltage dependence caused by application of PKA, PKC, or β-adrenergic receptor activators are secondary to activation of ERK/MAPK (Yuan et al., 2002; Watanabe et al., 2002). Overall, these observations indicate that K channel regulation of dendritic membrane properties is regulated by cell surface neurotransmitter receptors coupled to ERK activation. The implication of this, as will be discussed in more detail, is that neuromodulation of K channel function could serve a critical role in controlling action potential back-propagation and local membrane electrical properties. This mechanism would then allow indirect but critical control over the membrane depolarization necessary for NMDA receptor activation.

What is the molecular basis for this regulation of voltage-dependent K channel function? The A-type potassium channel pore-forming subunit Kv4.2 is localized to subsynaptic compartments of dendrites in CA1 pyramidal neurons and is likely the pore-forming

Table 2 Mechanism upstream of the NMDA receptor involved in membrane depolarization

Ionic current	*Molecules involved*	*Role*	*Mechanisms of modulation*
K currents			
Voltage-dependent "A" currents	Kv4.2 (and Kv4.3)	Limit bAPs Limit EPSP magnitude	ERK, PKA, CaMKII
"H" currents	NCN channels (HCN)	Regulate excitability	Cyclic nucleotides (direct)
Na currents			
AMPA receptors	GluR1, GluR2 aka GluR-A,B	Depolarize membrane	PKA, CaMKII, PKC
Voltage-dependent Na^+ currents	Na(v)1.6, 1.1, 1.2	AP propagation	PKC (decreased inactivation)
Ca currents	? – Likely many	AP propagation (hypothetical)	PKA
Cl currents			
GABA receptors	All GABA-A receptor subunits	AP firing Excitability	Numerous

bAPs, back-propagating action potentials; EPSP, excitatory postsynaptic potential.

subunit of dendritic A-type channels in these regions. Moreover, Kv4.2 is a substrate for ERK in hippocampal pyramidal neurons (Adams et al., 2000), and activation of PKA and PKC, as well as stimulation of β-adrenergic receptors, leads to ERK activation and Kv4.2 phosphorylation by ERK in hippocampal area CA1 (Yuan et al., 2002). Furthermore; modulation of A currents by PKA, PKC, and β-adrenergic receptors are secondary to ERK activation, and this mechanism is a basis for controlling back-propagating action potentials in pyramidal neuron dendrites.

Thus, one contemporary hypothesis is that ERK phosphorylation of Kv4.2, the K^+ channel pore-forming subunits likely to mediate A currents in hippocampal dendrites, decreases the probability of channel opening or the number of channels in the membrane. Once these channels in a particular region of a dendrite are rendered nonfunctional due to phosphorylation, the ability of a back-propagating action potential to invade that particular dendrite increases. This will allow, or increase the likelihood of, NMDA receptor activation and Ca^{2+} influx locally, and thus control the induction of LTP at that synapse.

Available data suggest the particular importance of this mechanism in theta-type LTP induction protocols (Winder et al., 1999; Watabe et al., 2000). Theta-frequency stimulation causes complex spike bursting in area CA1 cells, which can back-propagate into the dendrites and depolarize synapses. Several groups, including the laboratories of Kandel, Winder, and O'Dell, have shown that blocking ERK activation in mouse area CA1 blocks not only the complex spike bursting seen with the theta-frequency stimulation protocol, but also the LTP that is so induced (Winder et al., 1999; Watabe et al., 2000). It seems likely that blocking ERK in these experiments decreases the phosphorylation of Kv4.2, leading to an increase in the probability of current flux through these channels. Moreover, Winder's group has found that β-adrenergic receptor-mediated modulation of LTP induced with theta-frequency stimulation is blocked by inhibitors of ERK activation. Again, these findings are consistent with a model wherein ERK regulation of membrane electrical properties, via control of Kv4.2 channels, regulates back-propagating action potentials and controls NMDA receptor activation.

4.20.3.1.2 The H current

The H in H current stands for hyperpolarization, and this is a cation-mediated (sodium + potassium) current that is partially active at the resting membrane potential and that is further activated with membrane hyperpolarization. Thus, the channels underlying the H current are voltage-dependent, hyperpolarization-activated channels. They also are directly gated by cyclic nucleotides (cAMP and cyclic guanosine monophosphate (cGMP)), which open the channels, but there also is good evidence that some of the enhancing effects of cAMP on Ih are mediated by PKA-dependent phosphorylation. The net effect of H channel function in dendrites is to dampen membrane excitability like other potassium channels do, at least in CA1 pyramidal neurons. However, they dampen membrane excitability without significantly attenuating the peak depolarization of back-propagating action potentials. Because they are hyperpolarization activated, they shut down when the membrane is strongly depolarized: this means that they do not significantly affect peak depolarization.

As a first approximation, enhancing H channel function can be thought of as leading to sharpening of the back-propagating action potential, narrowing the window of membrane depolarization (Magee, 1998). H channels are open and counteracting membrane depolarization when the membrane is modestly depolarized, but inactive when the membrane is at the peak of the action potential. This effect might play an important role in timing-dependent plasticity mechanisms, restricting the time frame over which associative actions such as NMDA receptor activation might occur. A second, more tonic effect of augmenting H channels is to limit the ability of modest depolarization to penetrate a dendritic region. Overall, one can think of H channels as a cyclic nucleotide/PKA-dependent filtering mechanism for limiting membrane depolarization, a mechanism for enhancing the signal-to-noise ratio for depolarization-dependent associative events. cAMP/PKA, acting through H channels, might serve to assure that associative events such as NMDA receptor activation occur only in the presence of sufficiently robust electrical signals.

4.20.3.2 Voltage-Dependent Sodium Channels (and Calcium Channels?)

As in axons, propagation of action potentials along dendrites depends on voltage-gated sodium channels. In situations where back-propagating action potentials provide the depolarization necessary for NMDA receptor activation, this effect is of course dependent on the function of voltage-gated sodium channels.

Work from Colbert and colleagues has demonstrated that this is a potential site of plasticity for the regulation of LTP induction (Colbert and Johnston, 1998). Specifically, PKC can regulate the rate of inactivation of sodium channels in pyramidal neuron dendrites, a mechanism allowing PKC control of the extent of action potential back-propagation. Mechanistically, PKC decreases the extent of sodium channel inactivation, allowing for repetitive action potentials (where Na channel inactivation is relevant) to more effectively penetrate the dendrites (Spruston et al., 1995; Tsubokawa, 2000). By this mechanism PKC activation can promote the induction of LTP.

Back-propagating action potentials also activate voltage-dependent calcium channels in the dendritic membrane (Spruston et al., 1995; Andreasen and Nedergaard 1996). This is a critical source of dendritic calcium influx, but under certain conditions voltage-gated calcium channels (VGCCs) can contribute to the action-potential-associated membrane depolarization as well. In various experimental circumstances, VGCCs can even propagate a dendritic calcium spike, a regenerative action potential mediated by calcium flux across the membrane. Thus, modulation of calcium channels, aside from being a mechanism for regulating calcium influx, also can theoretically contribute to controlling local membrane depolarization.

4.20.3.3 AMPA Receptor Function

Three chapters in this volume (*See* Chapters 4.28, 4.29) will cover in detail a variety of mechanisms by which protein kinases augment AMPA receptor function. This is one of the principal mechanisms by which synaptic strength is enhanced during the expression of early-phase LTP (E-LTP). However, it should not escape our attention that these mechanisms could play a critical role in the induction of LTP as well. In this context, it is important to note that Ca^{2+}/calmodulin-dependent protein kinase II (CaMKII), PKC, and PKA all enhance AMPA receptor function; any cell surface receptor or calcium influx process that leads to activation of these kinases could influence the likelihood of LTP induction through augmenting AMPA receptor membrane depolarization.

This amplification of AMPA receptor function could be important in several contexts. First, AMPA receptors provide the initial depolarization of the membrane that brings the cell to threshold for firing an action potential. An augmentation of AMPA receptor function means that for any given level of glutamate at the synapse there is greater depolarization, at least until the concentration of glutamate reaches a saturating level. Thus, the cell is more likely to reach threshold for firing an action potential, and thus is more likely to trigger NMDA receptor activation. In non-silent synapses where AMPA receptors and NMDA receptors are both present, AMPA receptor augmentation will directly lead to greater local membrane depolarization and enhanced NMDA receptor function. This is an appealing model in the context of second messenger-coupled neurotransmitter receptors modulating LTP induction.

Second, enhanced AMPA receptor function can serve as a temporal integration mechanism. Protein kinase activation and substrate phosphorylation typically are much longer lasting (relatively) than glutamate elevation at the synapse. Depolarization-associated calcium influx or second messenger elevation can set in motion a positive feedback mechanism over time, such that subsequent stimulation of AMPA receptors is enhanced relative to an initial response. This could occur via calcium-activated kinases phosphorylating AMPA receptors or even through AMPA receptor insertion into the membrane. Although this idea is quite speculative at this time, these mechanisms nicely fit the classical definition of a temporal integration system.

4.20.3.4 GABA Receptors

As described in Chapter 4.18, gamma-aminobutyric acid-gated chloride ion channels (GABA-A receptors) control numerous processes relevant to the triggering of LTP. As these processes were described in Chapter 4.18 in the context of hippocampal circuit information processing mechanisms, they will not be covered here. Moreover, at present the role of GABA-A receptors has largely been studied in the context of controlling the likelihood of action potential firing and membrane depolarization in the cell body region, as opposed to local control of the membrane electrical properties in the distal dendritic regions. While it is clear that dendritic GABA-A receptor activation could play a powerful role in controlling NMDA receptor activation locally, at present the specific molecular mechanisms that might operate in the plasticity of this system have not been well defined.

4.20.4 NMDA Receptor Regulation Component 3: The Components of the Synaptic Infrastructure that Are Necessary for the NMDA Receptor and the Synaptic Signal Transduction Machinery to Function Normally

The components of the synaptic infrastructure, such as scaffolding proteins, cytoskeletal proteins, and cell surface adhesion molecules (**Table 3**), are now coming to be appreciated as important signaling components in the cell, which respond rapidly and with great variety to extracellular and intracellular signals. However, placing them in a scheme for NMDA receptor regulation is fairly speculative at this point, and in some cases the most that can be said is that they are known to interact with other proteins known to be involved in NMDA receptor regulation. With these caveats in mind, what follows is a brief listing of the more notable components of the synaptic infrastructure that have been implicated as potentially contributing to NMDA receptor regulation. While this section will delve into several specific categories of molecules, it is important to keep in mind that the overall take-home message of this section is that the NMDA receptor does not function in isolation. It is a component of a richly complicated physical structure that is itself subject to regulation.

4.20.4.1 Cell Adhesion Molecules and the Actin Matrix

A prominent category of synaptic adhesion molecules is the integrins (Martin et al., 2002). Integrins are cell surface molecules that transduce signals from the extracellular matrix to the inside of the cell. They are single-transmembrane-domain proteins that usually function as heterodimers of alpha and beta subunits. Knockout mice deficient in alpha5 and beta3 integrin exhibit hippocampus-dependent learning deficits and deficits in NMDA receptor-dependent LTP in area CA1, as is described in more detail in Chapter 4.32 by Davis and colleagues.

Table 3 Components of the synaptic infrastructure necessary for NMDA receptor function

Component	*Targets*	*Role*
Cell adhesion molecules		
Integrins	src, rho, rac, ras/MAPKs	Transmembrane signaling Interactions with extracellular matrix NMDAR regulation
	MLCK, FAK?	Spine morphology?
Syndecan-3	fyn, NMDAR	Signaling from matrix heparan sulfates to the NMDA receptor
N-cadherin	Other cadherins, Cytoskeleton	Spine morphology? Pre–post adhesion?
Actin cytoskeleton/associated proteins		
Rho	Membrane–cytoskeleton interactions	Regulate synaptic structure
Cdk5	NMDA receptor	Increase NMDA receptor function
Filamin	K channels	K channel localization
Presynaptic processes		
Glutamate release	Synaptic glutamate	NMDA receptor activation
Glutamate re-uptake	Synaptic glutamate	Limiting NMDA receptor desensitization
Anchoring/interacting proteins		
PSD-95	Receptors signal transduction mechanisms nNOS, SynGAP, GKAP	Postsynaptic organization
RACK1/fyn	NMDA receptor	Direct regulation of NMDA receptor
Shank/HOMER	Metabotropic receptors	Effector localization, cytoskeleton
GRIP	AMPA receptors, PICK-1/PKC	Postsynaptic organization
AKAP	PKA, PP2B	Kinase and phosphatase localization
CaMKII	Signal transduction	Regulate likelihood of LTP induction

Integrins interact with a wide variety of intracellular effectors, three categories of which are clearly important to keep in mind in terms of LTP induction in general and regulating NMDA receptor function specifically (see **Figure 6**). First, integrins couple to src activation in many cells, and as we discussed in the section titled 'NMDA receptor regulatory component 1: Mechanisms upstream of the NMDA receptor that directly regulate NMDA receptor function,' this is a mechanism for directly augmenting NMDA receptor function. Second, integrins couple to ras and via this mechanism can lead to ERK activation: this might play a role in K channel regulation (and regulating other effectors), as was discussed in the section titled 'NMDA receptor regulatory component 2: Mechanisms upstream of the NMDA receptor that control membrane depolarization.' Finally, the prototype function of integrins is in regulating the actin cytoskeleton. This potential role of integrins has taken on especial significance given findings by a number of laboratories, principally among them Lisman's and Lynch's, that normal dynamic regulation of the actin cytoskeleton is necessary for LTP induction. Exactly how the actin cytoskeleton regulates LTP induction is unclear at present, but there are many examples of candidate effector mechanisms that will be discussed in the remainder of this section.

Integrin regulation of the actin cytoskeletal matrix is complex. One principal role is linking the extracellular matrix to sites of actin matrix adhesion on the cytoplasmic side of the membrane. Integrin cytoplasmic tails bind to alpha-actinin and talin, which in turn recruit actin-binding proteins, such as vinculin, to the complex. This complex serves to anchor the cytoskeleton to the perisynaptic plasma membrane and synaptic zone.

Integrins also regulate the small G proteins rho (ras homolog, first identified in *Aplysia*) and rac, which regulate actin dynamics; this dynamic regulation of the actin matrix may contribute to activity-dependent changes in spine morphology. Consideration of integrin regulation of rho activity is especially appealing in this context because the classic role of rho is in regulating actin–myosin-based movement through activating myosin light-chain kinase. Another potential

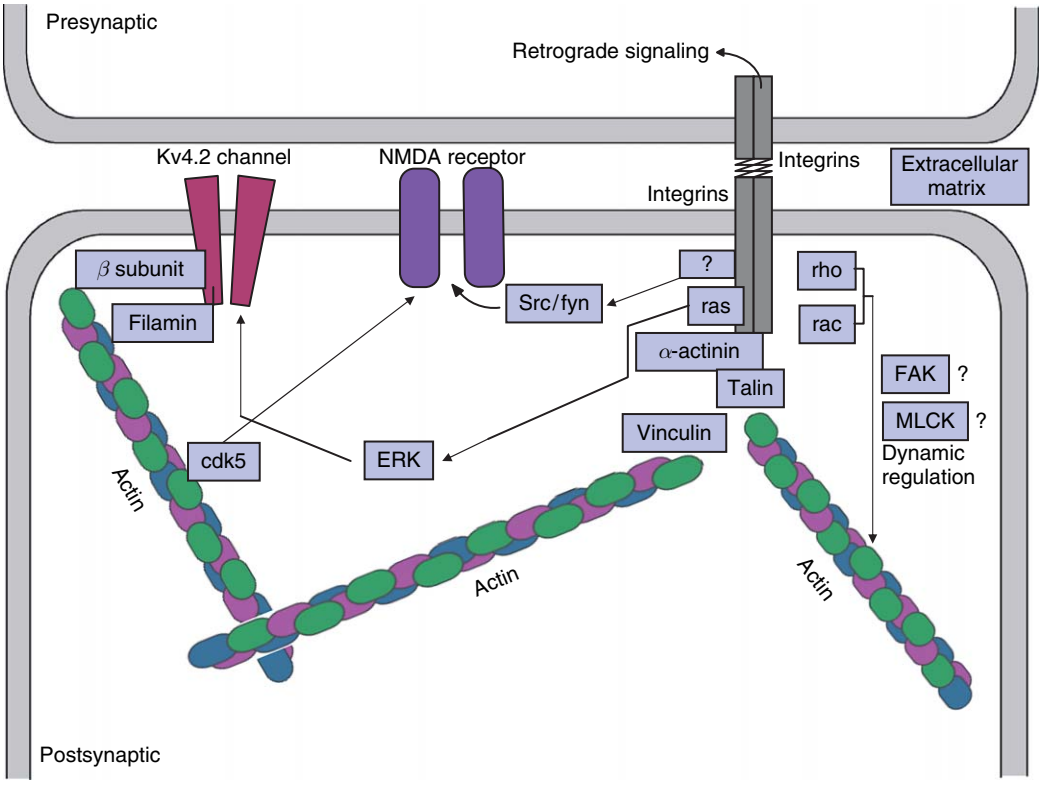

Figure 6 Interactions among integrins and intracellular effectors that regulate NMDA receptor function. See text for discussion and definitions. Figure by J. David Sweatt and Sarah E. Brown.

integrin effector in this context is FAK, which also serves to control cell morphology via the actin cytoskeletal matrix in many cells.

The actin cytoskeletal matrix may also contribute to NMDA receptor regulation in fairly direct ways. For example, actin microfilaments can serve as anchors for signaling components that affect the NMDA receptor directly. One example of this is actin filaments serving as the anchor for cdk5, which, as was discussed earlier, can phosphorylate and activate the NMDA receptor. Also, A-type potassium channels interact with the actin-binding protein filamin via their cytoplasmic c-terminal domain, and potassium channel beta subunits couple these channels to the actin cytoskeleton as well. These interactions certainly help localize A-channels appropriately in the dendritic spine. Perhaps more importantly, disruption of these interactions can cause attenuation of potassium channel function, and as we discussed above, A-channel inhibition promotes increased membrane excitability and enhanced NMDA receptor function.

An additional transmembrane, extracellular-matrix-binding protein that has been directly implicated in LTP induction is Syndecan-3. Inhibition of this molecule using various approaches leads to deficits in LTP (Lauri et al., 1999). Syndecan-3 binds heparan sulfates in the extracellular space, which are components of the glycosaminoglycan family of molecules present there. Syndecan-3 associates with the tyrosine kinase fyn, which might regulate NMDA receptor activity through direct tyrosine phosphorylation. The cell adhesion molecules L1 and N-CAM have also been implicated in the expression of LTP in some studies; however, recent results from knockout mice have suggested that loss of these molecules does not lead to LTP deficits (Luthl et al., 1994; Holst et al., 1998; Bliss et al., 2000). Finally, the N-cadherin subtype of cell adhesion molecule has been implicated in LTP induction and maintenance (see Huntley et al., 2002, and Chapter 4.31 for reviews). A likely role for the cadherins is in stabilizing strong connections between the presynaptic and postsynaptic membranes, although like other cell adhesion molecules, the cadherins also interact with and can regulate the actin cytoskeleton.

4.20.4.2 Presynaptic Processes

It is a statement of the obvious that any presynaptic process that regulates glutamate release can impinge upon NMDA receptor function, through controlling the level of synaptic glutamate that is attained. A number of specific examples of this type of mechanism are discussed in Chapter 4.33, so I will not reiterate the details here. However, please note that these are potentially relevant mechanisms for regulating NMDA receptor function indirectly.

4.20.4.3 Anchoring and Interacting Proteins of the Postsynaptic Compartment

4.20.4.3.1 Postsynaptic density proteins

The postsynaptic density is a multiprotein assembly that is the organizing center for many receptors and effectors, and the cytoskeleton, in the postsynaptic compartment (Sheng and Pak, 2000; Sheng, 2001).

PSD-95 is a protein enriched in the postsynaptic density and a prominent player in this context (Migaud, 1998). Identification of this protein by Mary Kennedy helped launch great progress in our understanding of the molecular basis for the organization of the complex postsynaptic infrastructure. PSD-95 binds to NMDA receptors (specifically the NR2 subunit) postsynaptically and serves as a multidomain anchoring protein for a large number of scaffolding and structural proteins postsynaptically (see **Figure 7**). PSD-95 helps anchor nitric oxide synthase (NOS), localizing this source of the reactive nitrogen species NO. PSD-95 also binds the ras GTPase (GTP: guanosine triphosphate) activating protein synGAP, whose function is still under investigation but which may regulate the ras/ERK cascade locally at the synapse. PSD-95 also anchors the cytoskeleton through its interactions with a protein termed SPAR, which is a GTPase activating protein (GAP) for rap and also an actin-interacting protein in its own right. Rap helps control the cytoskeleton indirectly through its target signal transduction processes such as the MAPK cascades.

PSD-95 also binds to proteins that seem to be more explicitly structural, such as the scaffolding protein Shank. Shank is another multidomain molecule that links the PSD-95-binding protein GKAP to the actin skeleton through cortactin. Shank also binds to HOMER, a metabotropic receptor-binding protein. Thus, via Shank and HOMER, group I metabotropic receptors coupled to phospholipase C can be localized near the NMDA receptor. HOMER also binds the inositol triphosphate (IP3) receptor, which may help localize the endoplasmic reticulum close to the NMDA receptor, allowing for proximity of intracellular calcium release mechanisms to the

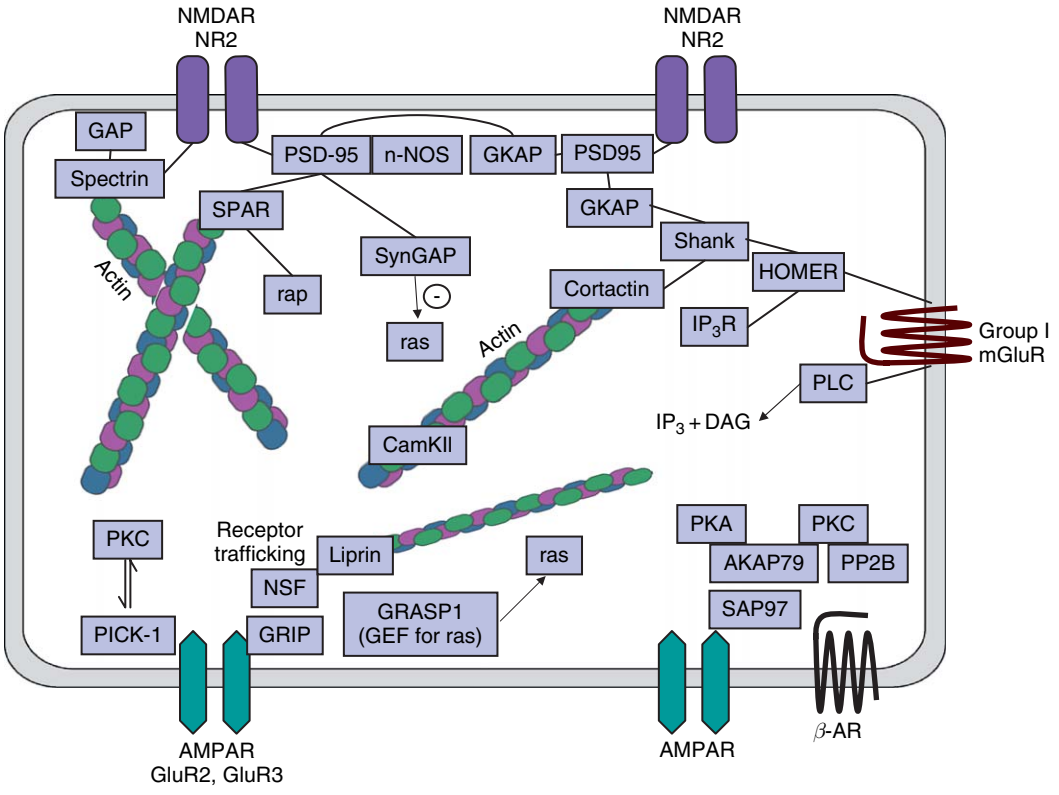

Figure 7 PSD-95 as an anchoring protein for NMDA receptors. See text for explanation. Figure by J. David Sweatt and Sarah E. Brown.

principal pathway for extracellular calcium influx, the NMDA receptor.

Genetic deletion of PSD-95 leads to striking alterations in LTP in area CA1: LTP is enhanced (Migaud et al., 1998). This is associated with learning deficits. It is unclear how the loss of PSD-95 leads to the augmentation of LTP; interpretation of the result is difficult because of the many complex roles of PSD-95 at the synapse, as outlined earlier. Overall, the data suggest that PSD-95 is somehow altering the threshold for LTP induction, a result consistent with the idea that PSD-95 and its associated proteins serve as dynamic modulators of NMDA receptor function.

4.20.4.3.2 Additional direct interactions with the NMDA receptor

The NMDA receptor NR1 and NR2 subunits also bind spectrin, the actin-binding protein. This may serve as an additional cytoskeleton anchoring site postsynaptically. Moreover, this interaction is subject to regulation by phosphorylation: tyrosine phosphorylation of NR2B leads to decreased interactions of spectrin with the receptor, and NR1 interaction with spectrin is modulated by serine/threonine phosphorylation. However, the role of these effects in synaptic plasticity and memory formation is not clear at this point. Finally, as described in the section titled 'Kinase regulation of the NMDA receptor,' the scaffolding protein RACK1 promotes formation of a Fyn/RACK1/NR2B complex that actually inhibits fyn phosphorylation of the NMDA receptor and diminishes current through the receptor (see **Figure 5**). Also, PSD-95 modulates src phosphorylation of NMDARs and src potentiation of NMDAR currents appears to require the presence of PSD-95.

Consideration of the complicated structure and regulation of the postsynaptic density complex highlights the importance of thinking of the entire postsynaptic domain as a large functional unit. The NMDA receptor is embedded in a dynamic multiprotein complex that it regulates and in turn that regulates it. While many details of the structural components of the PSD complex are still being worked out and their roles in LTP induction and memory formation are being actively investigated,

it is clear that disrupting one or more of the cogs in this machine can lead to disruption of the proper function of the NMDA receptor.

4.20.4.3.3 AMPA receptors

AMPA receptors of course provide the initial depolarization, either locally or distally in the neuron, that ultimately results in NMDA receptor activation. As such, alterations in the AMPA receptor protein or its associated interacting proteins can lead to loss of proper regulation of NMDA receptor activation. However, in this section we will focus on the AMPA receptor as a structural component of the synapse.

AMPA receptors, like NMDAR, also reside postsynaptically but are in much more of a state of flux than NMDA receptors. In fact, the average half-life for an AMPA receptor in the postsynaptic membrane is 15 min. Also, AMPA receptor membrane insertion can be activity-dependent. Thus the AMPA receptor should probably not be thought of like the NMDA receptor: the NMDA receptor likely serves a frankly structural role in addition to its function as a ligand-gated ion channel, while the AMPA receptor is more peripherally associated with the PSD (see Passafaro et al., 2001).

The AMPA receptor binds at least two structural proteins: PICK-1, which binds PKC, and GRIP (see Sheng and Pak, 2000). GRIP is a multidomain scaffolding protein that likely functions in AMPA receptor trafficking. GRIP also binds to GRASP1, a guanine nucleotide exchange factor for ras (see Sweatt, 2001; Vetter, 2001); the functional role of GRASP1 at the synapse is unclear at present. AMPA receptors can also bind NSF, a vesicle-associated protein that may also be involved in receptor membrane insertion is a fashion reminiscent of its role presynaptically in neurotransmitter vesicle fusion.

AMPA receptors also bind the A kinase anchoring protein AKAP79, an interaction that appears to be mediated by the PSD-95 homolog SAP-97 (Coghlan et al., 1995; Colledge, 2000; Dodge and Scott, 2000). As the name implies, AKAPs bind and localize PKA through interacting with the regulatory subunits of the kinase. The general role of AKAPs is to help localize PKA near relevant targets such as the AMPA receptor postsynaptically. The story is actually more complicated than that, as AKAP79 in the hippocampus also binds and localizes a protein phosphatase, PP2B (aka calcineurin). As a first approximation, it is useful to think of proteins such as AKAPs serving a role to increase the signal-to-noise ratio for signal transduction, localizing kinases close to their substrates to increase the efficacy of phosphorylation, but also localizing phosphatases to those same substrates in order to keep their basal phosphorylation low and to allow for rapid reversal of phosphorylation events once the kinase activation is over (Dodge and Scott, 2000). AKAP79 may also serve specifically to localize the calcium-sensitive phosphatase PP2B to the AMPA receptor in order to facilitate calcium-dependent AMPA receptor dephosphorylation and downregulation (Tavalin et al., 2002).

4.20.4.3.4 Ca^{2+}/calmodulin-dependent protein kinase II

CaMKII is highly enriched at the postsynaptic density complex. This enrichment occurs through CaMKII binding to the actin cytoskeleton, and the anchor for the cytoskeleton is the NMDA receptor, as we have discussed extensively. Thus, one purpose of the NMDA receptor/PSD-95/cytoskeleton complex is to help localize CaMKII to the PSD domain. This keeps a critical effector of the NMDA receptor, CaMKII, tightly bound and localized for effective responsiveness to NMDA receptor activation. Interaction of CaMKII with the PSD also can be regulated by CaMKII autophosphorylation; this is discussed in more detail in Chapter 4.23.

While the various scaffolding proteins, PSD-95, etc., that have been discussed are involved upstream of the NMDA receptor, regulating its function, CaMKII is downstream of the NMDA receptor. However, it is listed as a component of the synaptic infrastructure necessary for proper NMDA receptor function because it is such an important and direct target of the NMDA receptor; in essence loss of CaMKII function may functionally translate as equivalent to loss of NMDA receptor function. In addition, CaMKII binding to the PSD complex may play a structural role in concert with the actin cytoskeleton to serve as part of the infrastructure necessary for the NMDA receptor to function appropriately.

4.20.5 Summary

This discussion makes clear that the NMDA receptor does not reside in isolation in the membrane. In fact, Seth Grant's laboratory has clearly established that the NMDA receptor is the anchor for a large multiprotein

complex of structural proteins and signal transduction components (see **Figure 3**). This consideration serves as an important caveat for interpreting results from NMDA receptor knockout mice. This apparently clean experimental manipulation, wherein the NMDA receptor is entirely lost, likely results in a large number of secondary effects on molecules normally associated with the NMDA receptor postsynaptically. In fact, experiments using various deletion mutants missing the cytoplasmic anchoring domains of the NMDA receptor have allowed dissection of the role of the NMDA receptor as a scaffolding protein versus its role as a ligand-gated ion channel (Sprengel et al., 1998). Deletion of the intracellular domain of the NMDA receptor appears to be sufficient to account for essentially all of the physiologic and behavioral deficits observed in NMDA receptor knockout mice. An important take-home message of this finding is that the role of the NMDA receptor as a component of the PSD infrastructure is just as important as its role as a ligand-gated ion channel.

In this chapter, we have discussed three major categories of molecular components and processes that are involved in NMDA receptor regulation. It is very important not to think of these in isolation from each other: they are functional categories to help organize the complex biochemical machinery of NMDA receptor regulation, not compartmentalized biochemical processes in the cell. Thus, we need to begin to think of the NMDA receptor as an immensely complicated information processing machine. It integrates a plethora of biochemical signals and computes, based on a number of molecular inputs, whether to trigger a lasting molecular change. The interactions of these various processes are what allow the NMDA receptor to serve in its role as a molecular decision-maker, and allow for at least part of the necessary sophistication required for deciding when to trigger memory formation in the animal in vivo.

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4.21 Second Messengers: Calcium and cAMP Signaling

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4.21.1 Introduction

Second messenger systems provide incredibly powerful mechanisms for the transformation of stimuli from merely electrical and biochemical signals into stable, long-lasting, and memory-specific neuronal representations. The brain uses the second messengers, calcium and cyclic adenosine monophosphate (cAMP), to effectively transmit synaptic stimulation into stored information. The use of second messengers for this process provides several benefits. First of all, the primary message does not necessarily have to enter into the cell, a process that can be energy-consuming and temporally restrictive because of the rapidity with which neurons must respond to stimuli. Secondly, second messengers are typically small molecules that can easily diffuse within the cell. Calcium and cAMP fit these criteria. These messengers allow amplification of a primary signal as well as the integration of multiple signaling pathways at different locations within the cell. This chapter focuses on the ways in which neurons use second messenger systems, with particular emphasis on calcium and cAMP, in order to provide localized, amplified, and specific responses to memory-inducing stimuli. In this chapter, we illustrate the ways in which second messenger systems converge to produce long-term changes in synaptic plasticity.

4.21.2 The Role of Calcium in Synaptic Plasticity and Memory Processing (Introduction)

Hebbian plasticity, thought to be the basis for memory encoding, depends on the generation of a localized calcium pool that is large enough to cause presynaptic neurotransmitter release. In turn, the presynaptic release must be sufficient to evoke a postsynaptic response that is strong enough to cause

persistent modifications at synapses. Calcium signals of this magnitude can be generated by a number of mechanisms, including entry through voltage-gated calcium channels (VGCCs) or ligand-gated channels. An additional source of calcium comes from intracellular calcium stores including the endoplasmic reticulum (ER). Calcium signals affect the activity of many proteins including kinases, phosphatases, and membrane receptors. The calcium-binding protein calmodulin (CaM) mediates many of calcium's effects in the cell and plays a central role in the convergence of cAMP and calcium signaling.

4.21.2.1 Mechanisms of Generating a Calcium Signal

Neurons use a variety of mechanisms to generate localized calcium signals that are large enough to generate long-term plasticity. Cytosolic free calcium concentrations (less than 100 nM) are much lower than in the extracellular space, and therefore calcium influx at the membrane is driven by a 1000-fold concentration gradient. The primary mechanisms for calcium entry into neurons are *N*-methyl-D-aspartate (NMDA) receptors (*See* Chapter 4.20) and calcium channels, of which multiple types are expressed throughout the brain. When activated by glutamate, NMDA receptors produce localized calcium increases at the synapse. However, calcium passed via NMDA receptors is not always sufficient to drive long-term potentiation (LTP) or the downstream transcriptional and translational events required for long-term memory. For example, tetanus-induced long-lasting LTP (L-LTP) in area CA1 of the hippocampus can be expressed even in the presence of NMDA receptor inhibitors. This expression instead depends on calcium entry through L-type calcium channels (Impey et al., 1996). L-type calcium channels are one of several VGCCs expressed in neurons. Other VGCCs are the P/Q-, N-, L-, R-, and T-type channels. VGCCs are composed of four repeating pore proteins, each containing six transmembrane regions. One of these transmembrane domains contains several positively charged residues, the presence of which permits rapid activation of the channel in response to changes in membrane potential (**Figure 1**).

Calcium entry through calcium channels activates nearby potassium channels that release potassium from the cell, propagating a cascade of VGCC activation within the vicinity of the original calcium signal. Although L-type channels are distributed primarily (though not exclusively) in proximal dendrites, N- and P/Q-type calcium channels are found at presynaptic terminals and participate in neurotransmitter release after strong depolarization. That there are at least some cases in which LTP occurs in spite of NMDA receptor blockade, indicating that the calcium signal is input specific (Grover and Teyler, 1990; Moosmang et al., 2005). Deviant temporal input at neurons can also produce different forms of LTP, probably because of the localization of the calcium source and the magnitude of the calcium signal.

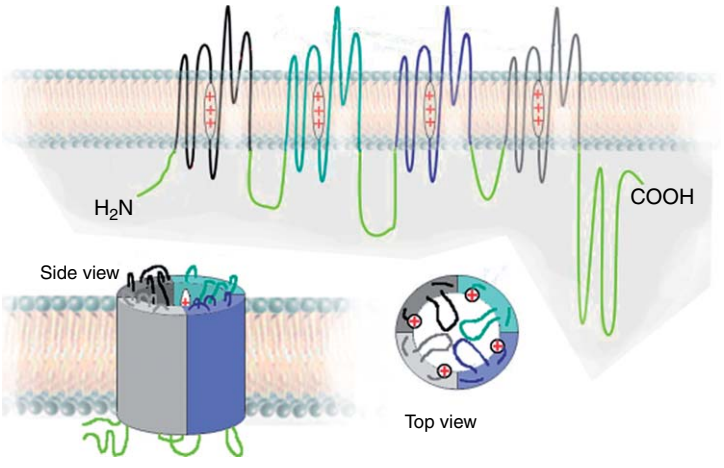

Figure 1 Voltage-gated calcium channel (VGCC) structure. VGCCs are composed of four repeating pore domains. Each pore domain consists of six transmembrane segments, the fourth of which acts as a voltage sensor, allowing rapid opening of the channel pore in response to changes in membrane voltage. Original figure, courtesy of T. Jacob; revised image courtesy of D. Trapp. Adapted from Jacob T, Olfaction: A tutorial on the sense of smell and taste – A brief tutorial, School of Biosciences, Cardiff University, UK, with permission from D. Trapp.

Not all of the calcium signal originates from extracellular sources. Inositol-1,4,5-triphosphate (IP3), derived from phosphatidyl inositol bisphosphate by phospholipase C (PLC), mediates the release of calcium from intracellular stores. Levels of IP3 increase when receptors coupled to PLC (e.g., metabotropic and glutamatergic receptors) are activated (Ross et al., 2005). Once produced and mobilized, the large diffusion constant of IP3 as well as the distribution of IP3 receptors (IP3R) within the ER provide input-specific IP3 receptor binding and calcium release from the ER. Although IP3 functions more globally than calcium because of its large diffusion constant, IP3-mediated diffusion is not the only way for intracellular calcium to participate in synaptic plasticity. ER calcium is also controlled by calcium-sensitive ryanodine receptors (RyR) localized at the ER membrane. Which ER receptors get activated appears to be input specific. For example, activation of cholinergic neurons via adenosine involves IP3-induced release of ER calcium, whereas RyRs remain nonparticipants (Basheer et al., 2002). The contribution of these ER receptors to LTP and memory formation remains elusive. Knockout animals of the RyR type three receptor actually display a facilitation of hippocampal CA1 LTP that is not blocked by NMDAR inhibition, suggesting that the calcium release from the ER may actually inhibit LTP in area CA1 (Futatsugi et al., 1999). IP3R knockout animals are reported to have a similar enhancement in LTP at hippocampal mossy fiber synapses (Itoh et al., 2001), underscoring the unique function of distinct calcium pools in plasticity.

4.21.2.2 Calcium Effector Molecules

The localization and strength of the calcium signal can determine the fate of a synapse, for example, by favoring LTP or long-term depression (LTD). This can be accomplished by direct or indirect activation or inhibition of effector proteins. The effects of calcium on target proteins are often mediated through the high-affinity calcium-binding protein, CaM. CaM, when bound to calcium, binds to and activates a number of enzymes that are critical for long-term synaptic plasticity. The following sections discuss some of the calcium-sensitive proteins that are central to synaptic plasticity.

4.21.2.2.1 Calcium-activated proteins

Calcium activates many proteins including specific adenylyl cyclases, calcium/phospholipid-dependent protein kinases (PKCs), the Ca^{2+}/calmodulin-dependent protein kinases (CAMKs), phosphodiesterases, and phosphatases. Calcium stimulation of these enzymes is mediated by the Ca^{2+} binding protein, CaM. In the 1960s, CaM was first discovered as an activator of cyclic 3′5′-nucleotide phosphodiesterase (PDE). CaM is especially abundant in the brain. CaM is a 17-kD protein that contains no intrinsic enzyme activity but, rather, functions to regulate a wide variety of enzymes in the presence of Ca^{2+}. Containing the prototypical calcium-binding E-F motifs, calmodulin contains four binding sites for calcium. As a result of heterotropic positive cooperativity, enzymes respond to Ca^{2+} with greatly different sensitivities. Free energy coupling between CaM and its different binding proteins varies, allowing Ca^{2+}/CaM effector molecules to respond with slightly different kinetics to increases in free Ca^{2+} (Xia and Storm, 2005). CaM's influence on neuronal functions is widespread, with protein partners that include the calcium-sensitive adenylyl cyclases 1 and 8, protein kinases, calcineurin, nitric oxide synthase (NOS), Ca^{2+} channels, RASGRF1 (a mitogen-activated protein kinase [MAPK] upstream activator), neuromodulin, and neurogranin. **Figure 2** outlines some of the proteins that control free CaM within in the cell. The proteins neuromodulin, neurogranin, and regulator of calmodulin signaling (RCS) are all proteins that determine the levels of free CaM in the resting neuron. Neuromodulin, a neuronal-specific binding protein for CaM, is inhibited from binding to calmodulin in the presence of calcium. In addition, PKC phosphorylation of neuromodulin blocks CaM binding.

The serine/threonine protein kinase C family is highly expressed in brain and contains several isoforms that are responsive to Ca^{2+}. The PKC family of proteins is composed of 11 isozymes that make up three subclasses: the conventional, novel, and atypical groups. Ca^{2+} and diacylglycerol both activate the alpha, beta, and gamma members, all conventional isoforms of PKC, but the novel and atypical groups appear to be calcium-insensitive. When not associated with the plasma membrane, PKC binds Ca^{2+} very inefficiently. PKC can bind at least eight calcium ions per protein (Bazzi and Nelsestuen, 1990) in the presence of a phospholipid bilayer. Translocation of PKC from the cytosol to the membrane occurs following certain types of hippocampus-dependent training, presumably a requirement for interaction and modification of synaptic proteins at relevant circuit synapses (Bank et al., 1989). Although the temporal aspects of this translocation are debated, disruptions in PKC isoforms indicate the importance

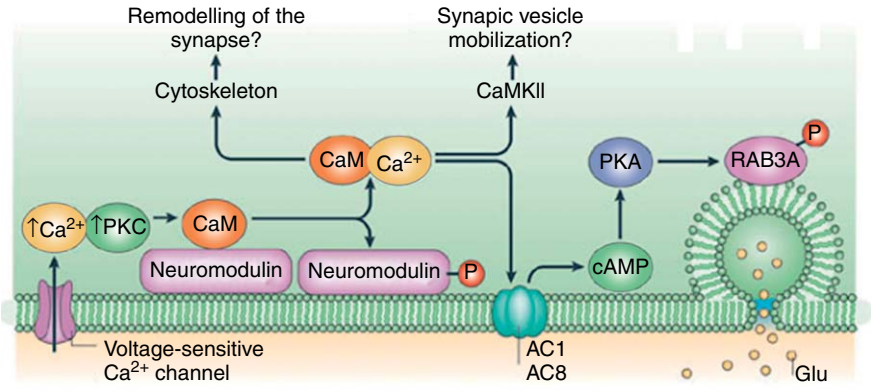

Figure 2 Neuromodulin participation in presynaptic events. Neuromodulin controls the amount of presynaptic calmodulin (CaM) available for calcium binding. Calcium activation of calcium/phospholipid-dependent protein kinase (PKC) causes a PKC-dependent phosphorylation of neuromodulin and the subsequent release of CaM results in direct phosphorylation of the small G protein, Rab3A, which is throught to assist in facilitation of presynaptic vesicle release. Reproduced from Xia Z and Storm DR (2005) The role of calmodulin as a signal integrator for synaptic plasticity. *Nat. Rev. Neurosci.* 6: 267–276.

of this kinase family as calcium effectors in LTP and memory formation. PKC gamma knockout animals assayed for synaptic plasticity and memory perturbations show normal LTD and short-term plasticity; however, LTP is severely diminished except when the LTP tetanus is preceded by a low-frequency stimulus (Abeliovich et al., 1993a). This indicates that PKC gamma contributes to the induction, but not maintenance, of LTP. (*See* Chapter 4.22 for more on the role of the PKC family.)

A second group of Ca^{2+}-sensitive kinases that are central players in synaptic plasticity are the Ca^{2+}/CaM kinases, collectively known as CaM kinases (CAMK). These kinases consist of CAMK1–CAMKVI. Calcium/calmodulin-dependent protein kinase II (CAMKII) is ubiquitously expressed within the brain and is heavily localized to postsynaptic densities. The binding of Ca^{2+}/CaM to CAMKII regulates the activity of the kinase by allowing it to autophosphorylate its own inhibitory domain. This phosphorylation event causes CAMKII to function in a constitutively active state. Phosphorylated CAMKII appears to interact with the NR2B subunit of the NMDA receptors (Strack and Colbran, 1998), an association critical for the CAMKII-dependent LTP at Schaffer collateral-CA1 synapses (Barria and Malinow, 2005). (A thorough presentation of CAMKII in synaptic plasticity is addressed in Chapter 4.23.) In addition to activation of CAMKII, CaM also activates CAMK1, a cytosolic CAMK that probably affects synaptic strength by phosphorylating synapsin I. CAMKIV is also activated by Ca^{2+}/CaM and directly phosphorylates cAMP-responsive element binding protein (CREB). Activation of the predominantly nuclear CAMKIV is critical for late-phase LTP and also for consolidation of the hippocampus-dependent spatial memory (Kang et al., 2001). This correlates with the observations that CREB-induced transcription is critical for several forms of experience-dependent plasticity (Barth et al., 2000; Athos et al., 2002; Kida et al., 2002).

Although Ca^{2+}-activated kinases are critical mediators of the calcium signal, the calcium-sensitive adenylyl cyclases are central players in transmitting the Ca^{2+} message at activated synapses to downstream signaling pathways. There are ten adenylyl cyclases expressed in mammals, and at least three of these – AC1 and AC8, and AC3 – are strongly calcium sensitive, although some others have been reported to respond to calcium as well (Hanoune and Defer, 2001; Cooper, 2003). AC1 and AC8 are both calcium activated and highly expressed in the brain. Whereas AC1 is neurospecific, AC8 is expressed in several peripheral tissues in addition to the brain (Xia et al., 1993; Muglia et al., 1999). The neurospecific expression of AC1 makes it an excellent pharmacological target for increasing cAMP specifically in the CNS for treatment of memory and neurodegenerative disorders. AC1, which is considerably more sensitive to calcium than AC8, is activated half-maximally *in vitro* at approximately 150–200 nM free Ca^{2+}, levels that hover just above the resting intracellular calcium concentration in neurons. Calcium-sensitive adenylyl cyclases contain

a CaM-binding domain that mediates the calcium sensitivity of the enzyme (Wu et al., 1993). Mutation of this calmodulin-binding site strongly inhibits its calcium sensitivity. Because the second half of the chapter focuses on cAMP as a second messenger, the role of the adenylyl cyclases as mediators of the calcium signal is reviewed there in greater detail (see the section titled 'cAMP as a second messenger in plasticity and memory').

Calcium's effects on kinases and cyclases result in phosphorylation of target proteins and the production of cAMP, respectively. However, Ca^{2+}-modulated proteins also include phosphatases and cAMP hydrolyzing proteins. The Ca^{2+}-stimulated serine/threonine phosphatase calcineurin (also known as PP2B), for example, is the most calcium-sensitive of the phosphatases ($Kd = 0.1 - 1$ nM) and the only calcium-sensitive phosphatase found in the brain (Mansuy, 2003). Calcineurin regulates synaptic plasticity both presynaptically (by restraining vesicle release) and postsynaptically, where it complexes with the postsynaptic density (PSD) and, in general, orchestrates the suppression of downstream signaling. Because of its calcium sensitivity and its association with the PSD, calcineurin can respond quickly to calcium influxes at the membrane and negatively regulate further calcium increases by dephosphorylating VGCCs, NMDA receptors, and AMPA receptors. Concomitant to inhibiting calcium influx at the membrane, calcineurin can also reduce inositol-induced calcium release (IICR) and calcium-induced calcium release (CICR) by negatively regulating both IP3 and RyRs. This negative regulation comes in the form of calcineurin-mediated dephosphorylation of PKC target sites on the receptors themselves (Mikoshiba, 1997; Bandyopadhyay et al., 2000). Mice with forebrain-specific calcineurin ablation have been engineered and tested for LTP and memory phenotypes. Animals without forebrain calcineurin display impaired LTD but normal LTP at Schaffer collateral-CA1 synapses. No deficits in several hippocampal-dependent memory tasks have been observed in these animals; however, working memory is impaired (Zeng et al., 2001). Although NMDA receptor function in calcineurin-deficient animals appears normal, it is hypothesized that an overall elevation in postsynaptic calcium and, perhaps, a deficit in AMPA receptor endocytosis might cause the observed LTD deficits. (For other mouse models with aberrant calcineurin expression, see **Table 1**.) Interestingly, calcineurin has recently been discovered to activate adenylyl cyclase activity (Chan et al., 2005), a property that may antagonize its other functions at the synapse.

4.21.2.2.2 Calcium-inhibited proteins

Not all calcium-sensitive proteins are activated by calcium. As mentioned earlier, the protein neuromodulin is one of a number of proteins inhibited by Ca^{2+}. Neuromodulin is expressed presynaptically and presides over the availability of free calmodulin by sequestering it at the membrane until PKC-mediated phosphorylation of neuromodulin releases calmodulin (Andreasen et al., 1983; Xia and Storm, 2005). Phosphorylation of neuromodulin by PKC occurs during LTP and probably functions presynaptically to allow maximal Ca^{2+}/CaM binding and vesicle release. Modulating postsynaptic CaM availability is the postsynaptically expressed protein neurogranin (also called RC3). Neurogranin is concentrated in dendritic spines and is also phosphorylated by PKC. Like neuromodulin, neurogranin is inhibited from binding CaM in the presence of Ca^{2+}.

Intracellular calcium levels must not be tonically elevated, however, or excitotoxicity can ensue. One way in which this can be prevented is the inhibition of calcium entry by calcium itself. An example of such inhibition is the calcium-mediated inhibition of NMDA receptor currents in hippocampal neurons (Legendre et al., 1993), a process that can be blocked by intracellular buffering agents. Under certain conditions, calcium inhibits further CICR by inhibiting ER-mediated calcium release. Specifically, both RyRs and IP3Rs can be inhibited by Ca^{2+}/CaM binding to the receptors. Although the situations in which this occurs are still somewhat unclear, CaM-mediated inhibition of intracellular calcium release appears to tightly control calcium concentrations, balancing the inhibition of release with ATP and phosphorylation-induced activation of these receptors (for a review, see Bardo et al., 2006). CaM binding is not the only way in which IP3 and RyR receptors can be inhibited by calcium, however. As already addressed, calcineurin-mediated dephosphorylation of PKC sites on these receptors provides a second mechanism by which neurons can regulate the optimal cytoplasmic Ca^{2+} concentrations required for synaptic plasticity and memory encoding.

4.21.2.3 Localization of the Calcium Signal

Efforts to determine the range of diffusion for intracellular calcium and other second messengers, such as IP3, produced evidence that calcium has a very

Table 1 Mouse genetic approaches to studying the role of calcium signaling in memory

Gene modified	*Alteration*	*Affected area of expression*	*Effect on plasticity*	*Effect on memory*
PKCβ[a]	Knockout	Global	Normal short-term and long-term plasticity	Deficits in cued and contextual fear
αCAMKII[b]	Knockout	Global	Deficit in STP, LTP and LTD	Deficits in spatial memory
αCAMKII$^{+/-}$[c]	Heterozygote	Global	Impaired cortical LTP	Deficient in remote memory maintenance
CAMKIV[d]	Transgenic, dominant negative	Forebrain	Deficits in L-LTP	Deficits in cued and contextual fear consolidation
NR2B[e]	Transgenic, overexpression	Forebrain	Enhanced LTP, lower threshold required	Enhanced auditory and contextual fear
AC1[f]	Knockout	Global	Decreased cerebellar LTP	Deficits in rotarod performance and spatial memory deficits in Morris water maze
AC1/AC8[f]	Double knockout	Global	Deficits in CA1 LTP	Deficient in hippocampus-dependent fear memory
RasGRF1[g,h]	Knockout	Global	Small impairment in Schaffer collateral LTP after two 100-Hz stimuli, severe impairments in LTD	Deficits in hippocampal but not amygdala-dependent long-term memory
RasGRF1/2[h]	Double knockout	Global	Impaired Schaffer collateral-CA1 theta burst LTP and LTD; deficits in L-LTP	No memory deficits reported
PKCγ[i]	Knockout	Global	Required for LTP induction, not maintenance	Mild hippocampal memory deficits
CaV1.2[j]	Transgenic, IQ domain mutation	Forebrain	No NMDAR-independent LTP	Deficient in spatial memory
Calcineurin (regulatory subunit CNB1)[k]	Knockout	Forebrain	LTD deficit, modest increase in LTP at CA1 synapses	Working, episodic memory impairments (eight-arm radial maze and delayed matching-to-place)
Calcineurin[l]	Transgenic, inducible inhibition	Forebrain	Enhanced one-train LTP and *in vivo* LTP in CA1	Enhanced spatial memory
Calcineurin (catalytic subunit CNAα)[m]	Transgenic, constitutively active	Forebrain	Deficits in PKA-dependent intermediate-LTP	Spatial memory deficits in Barnes maze
αCAMKII[n]	Transgenic, overexpression	Forebrain	Enhanced one-train LTP	Spatial memory deficits
AC1[o]	Transgenic, overexpression	Forebrain	Increased Schaffer collateral-CA1 LTP; 100-Hz, two-train LTP	Increased recognition memory
NR1[p]	Transgenic, tet-inducible; knockout	Forebrain, CA1 specifically	Deficits in CA1 LTP and LTD	Deficits in hippocampal memory consolidation
Neurogranin/RC3[q]	Knockout	Global	Reduced thresholds for LTP, but baseline transmission reduced	Deficits in Morris water maze acquisition, spatial learning deficits

STP, short-term potentiation, LTP, long-term potentiation, LTD, long-term depression; PKC, protein kinase C.
[a](Weeber et al., 2000); [b](Silva et al., 1992a,b); [c](Frankland et al., 2004); [d](Kang et al., 2001); [e](Tang et al., 1999; Wong et al., 1999); [f](Wong et al., 1999); [g](Giese et al., 2001); [h](Li et al., 2006); [i](Abeliovich et al., 1993a; Abeliovich et al., 1993b); [j](Moosmang et al., 2005); [k](Zeng et al., 2001); [l](Malleret et al., 2001); [m](Mansuy et al., 1998); [n](Wang et al., 2003); [o](Wang et al., 2004); [p](Krucker et al., 2002; Shimizu et al., 2000); [q](Krucker et al., 2002; Miyakawa et al., 2001).

low range of diffusion (particularly unbuffered calcium) compared with IP3. This makes calcium functional within restricted domains (Allbritton et al., 1992). The specificity generated by localized calcium pools allows transduction of synaptic signals that take place at postsynaptic densities far from the nucleus. Membrane pumps, the presence or absence of localized Ca^{2+}-binding proteins, and the stored calcium present in intracellular organelles all function to produce changes in local calcium concentrations. The smooth endoplasmic reticulum (SER) sequesters most of the intracellular calcium, a process mediated by sarco/endoplasmic reticulum Ca^{2+} ATPase (SERCA) pumps. SERCA pumps help maintain Ca^{2+} concentrations in the ER much higher than the intracellular Ca^{2+} concentrations. Because of this discrepancy between SER calcium and intracellular calcium, IICR and CICR provide neurons mechanisms to rapidly produce localized increases in Ca^{2+}. Because the SER membrane flexibly innervates even synaptic spine regions (Cooney et al., 2002), IICR and CICR can produce Ca^{2+} increases at synaptic densities.

Localization of the Ca^{2+} signal is extremely important and probably accounts for NMDA receptor-independent LTP versus NMDA-receptor-dependent LTP. For example, the temporal information in a 100- versus 200-Hz stimulation at Schaffer-collateral synapses in CA1 can shift LTP dependency from NMDA receptors to VGCCs. This type of temporal information may only be interpreted intracellularly through a second messenger such as Ca^{2+}, which, because of its limited diffusion, is restricted to local domains. Perhaps the Ca^{2+} signal generated by very rapid stimuli allows a sufficient number of VGCCs to be activated, in effect producing a Ca^{2+} concentration that overrides the Ca^{2+} signal provided by NMDA receptor activation. NMDA receptor-independent LTP has long been studied at the hippocampal mossy fiber inputs into the CA3 region. Here, NMDA receptor inhibition has been reported not to have any effect on mossy fiber LTP (Harris and Cotman, 1986). Similar reports of NMDA-independent LTP have been reported at Schaffer-collateral CA1 synapses (Impey et al., 1996; Moosmang et al., 2005) and also in the amygdala (Weisskopf et al., 1999).

4.21.2.3.1 Synaptic calcium

Among the Ca^{2+}-activated and -inhibited proteins, what effectors are critical for presynaptic vesicle release and long-lasting, input-specific, postsynaptic responses? Ca^{2+} influences both presynaptic properties such as vesicle release and postsynaptic properties, including activation of kinases that serve as moderators of membrane channels and receptors. Although Ca^{2+} affects the synapse in many ways, we review some of the central presynaptic and postsynaptic targets for calcium.

Synaptic vesicle release occurs within microseconds of the presynaptic action potential and is dependent on Ca^{2+} entry through VGCCs. The ways in which calcium moderates presynaptic terminal vesicle release are diverse and moderated by a number of presynaptic Ca^{2+} buffering agents including calretinin, calbindin D-28K, and parvalbumin. The distinct mobility, localization, solubility, and Ca^{2+}-sensitivity of these buffering proteins provide specific presynaptic responses in different areas of the brain and probably contribute to the type of synaptic connections made following specific stimuli. Of course, CaM also has specific roles in presynaptic activity. Ca^{2+} activation of PKC at presynaptic terminals allows phosphorylation of the presynaptic CaM-binding protein, neuromodulin. As already mentioned, this phosphorylation releases CaM for activation of its target proteins (see **Figure 2**).

Presynaptic increases in Ca^{2+} not only release CaM from its synaptic captor, neuromodulin, but lead to protein kinase A (PKA)-mediated vesicle mobilization through the activation of the Ca^{2+}-sensitive adenylyl cyclases. **Figure 2** pictorially demonstrates the cascade of molecular events leading from cAMP increases to facilitation of presynaptic neurotransmitter release. PKA directly phosphorylates RAB3A, a Ras-associated protein, which increases vesicle release probability. Rab3A is one of several presynaptic PKA targets; PKA phosphorylation of SNAP25 and its binding protein snapin probably sustains pools of primed and release-ready vesicles (Leenders and Sheng, 2005). Also effective in increasing presynaptic release is the expression of CAMKII, the inhibition of which blocks glutamate-stimulated boutons and miniature excitatory postsynaptic currents in neurons of the hippocampus (Ninan and Arancio, 2004). CAMKII is also thought to assist in vesicle release by phosphorylating synapsin I, a phosphorylation event that occurs and persists for several hours following LTP induction. It is hypothesized that synapsin I phosphorylation increases the number of vesicles available for release.

Because Ca^{2+} affects postsynaptic responses in numerous ways, we describe a few of the key postsynaptic Ca^{2+}-dependent events that are required for

long-term synaptic plasticity or memory formation. A central target for postsynaptic Ca^{2+} signal transduction is activation of the Ca^{2+}/CaM-sensitive CAMKII. CAMKII is thought to be the primary regulator of LTP while, in general, calcineurin determines the fate of a neuron for LTD. Once activated, postsynaptic CAMKII phosphorylates the AMPA receptor subunit GluR1, enhancing AMPA current conductance and, perhaps, translocation of the receptor to the membrane. A second way that calcium mediates AMPA receptor activation during LTP is by activating AC1 and AC8. This mechanism for LTP is commonly observed during early postnatal development. In this case, PKA phosphorylation of GluR4 is necessary for hippocampal LTP (Yasuda et al., 2003). While CAMKII activation affects ion channel activity, it can also function postsynaptically by downregulating proteins following calcium activation. For example, in olfactory sensory neurons, CAMKII phosphorylation of AC3 results in cAMP transients that allow sensitization in response to odorant stimulation. The interplay between PDE activation and CAMKII-induced AC3 inhibition are required for this process (Wei et al., 1998). Although both processes are calcium mediated, the temporal aspect of calcium activation is probably crucial for the olfactory responses in an animal.

The Ras-guanine nucleotide-releasing factors (RasGRF) – RasGRF1 and RasGRF2 – are Ca^{2+}/CaM-sensitive proteins that activate the small G proteins such as Ras and Rac and assist in the transmission of NMDAR calcium signals to activation of the Ras/MEK/MAPK family (see **Figure 5**). Glutamate activation of NMDA receptors causes a calcium-mediated binding of RasGRF1 to the NR2 subunit of NMDA receptors. RasGRF1 acts as a calcium sensor for NMDA receptors (Krapivinsky et al., 2003). RasGRF1 binding to NR2 causes activation of the MAPK pathway in the postsynaptic neuron and probably mediates LTD and, to some extent, LTP at Schaeffer collaterals in the hippocampus (Giese et al., 2001). Animals deficient in RasGRF1 expression have severe impairments in low-frequency stimulation-induced LTD (see **Table 1**).

Neurogranin, the postsynaptic member of the IQ domain family, is highly expressed in dendritic spines and modulates postsynaptic responses to calcium that are critical for postsynaptic plasticity. It is clear that neurogranin contributes to postsynaptic plasticity, as rodents that lack neurogranin expression have reduced thresholds for hippocampal LTP and are unable to undergo LTD in response to low-frequency stimulation probably because basal transmission is already depressed (Krucker et al., 2002). It is proposed that the neurogranin knockout animals have excess levels of free calmodulin present in the brain, and that therefore, greater sensitivity to calcium results in LTD at baseline calcium concentrations. However, when low-frequency stimuli that would normally cause LTD in wild-type animals are administered to neurogranin knockout hippocampal slices, LTP occurs.

4.21.2.3.2 Calcium's effects in the nucleus

The roles of Ca^{2+} in the nucleus during synaptic plasticity and memory processing are complex, often integrating several signaling cascades that converge in nuclear events. Like cytosolic effector specificity, the specificity of the Ca^{2+} signal is crucial for determining activity in the nucleus. For example, a Ca^{2+}-sensitive site (CaRE2) in the promoter of brain-derived neurotrophic factor (BDNF) can be activated by upstream regulatory factors 1 and 2 (USF1, USF2), but only in response to specific calcium signals, such as those generated through the L-type VGCCs. Glutamate activation of NMDA does not result in a Ca^{2+} signal sufficient to drive USF-activated BDNF gene expression (Chen et al., 2003). This distinction is critical in light of the fact that BDNF is upregulated transcriptionally during the consolidation of certain forms of hippocampus-dependent memory. Contributing to the complexity of Ca^{2+}-activated transcription are the different modes of activation by Ca^{2+}. For example, Ca^{2+} can activate transcription factors in the cytoplasm that then translocate to the nucleus, or it can induce translocation of proteins that activate transcription factors, such as CaM or CREB kinases. In fact, Ca^{2+}-activated CaM can physically translocate to the nucleus within seconds after neuronal activation (Mermelstein et al., 2001). Ca^{2+} can also bind to transcription activators or repressors directly (such as the transcriptional repressor DREAM) controlling the transcription of genes such as prodynorphin and the immediate early gene, c-fos (Carrion et al., 1999; Deisseroth et al., 2003). This translocation is critical for CAMK-mediated CREB activation and the transcription of some genes under the control of CRE-containing promoters.

As membrane and cytosolic proteins respond to localized Ca^{2+} signals, so do nuclear events. For example, CREB activation depends on specific and localized

Ca^{2+} signals. Mice with mutations in the IQ domain of the L-type calcium channel have significantly reduced Ca^{2+}-dependent CREB phosphorylation and transcriptional activity (Dolmetsch et al., 2001). Sustained CREB phosphorylation in the nucleus depends upon the binding of CaM to the IQ domain of L-type channels, and transduction of this signal is mediated through activation of the Ras/Raf/MAPK pathway. Although pharmacological inhibitors of NMDA receptors and N-, P/Q-, and L-type calcium channels are all capable of decreasing intracellular calcium to a similar concentration in wild-type neurons, only L-type channel inhibition blocks depolarization-induced, sustained CREB phosphorylation in neuronal nuclei. Ca^{2+} regulation of CREB activity is complex, because Ca^{2+}-mediated dephosphorylation of this protein also occurs. Ca^{2+} activation of calcineurin causes the dephosphorylation of CREB at serine 133, reducing CREB's transcriptional activity. In fact, inhibition of calcineurin can reduce the threshold of stimulus intensity that is usually required for CREB-mediated transcription in hippocampal neurons (Bito et al., 1996).

4.21.2.4 Calcium Signaling During Memory Processing

In attempts to address the requirement of Ca^{2+} and the specificity of the Ca^{2+} signal required for memory formation and synaptic plasticity, many pharmacological and genetic studies have been conducted. The next sections address a few of the genetic and pharmacological perturbations of Ca^{2+} signaling that have been carried out to more clearly define the role of Ca^{2+} in synaptic plasticity and memory processing.

4.21.2.4.1 Mammalian genetic models of calcium in memory processing

The generation of transgenic or knockout mice with perturbations in calcium signaling has greatly increased our knowledge of calcium's contribution to different forms of plasticity, learning, and memory. Conventional knockout animals have been gene targeted so as to eliminate the function of a specific gene within the genome. Although compensatory mechanisms during development are cause for caution when interpreting data from conventional knockout animals, much can be learned by gene knockout technology and verified by alternative means. Furthermore, conditional knockouts, which involve tissue-specific and, sometimes, inducible gene knockdown properties (accomplished through a site-specific DNA recombination event), have been extremely useful in determining the tissue-specific attributes of certain proteins in synaptic plasticity. Some of the mouse models central to defining a role for Ca^{2+} signaling in synaptic plasticity are listed in **Table 1**.

Collectively, the calcium-sensitive kinases including PKC and the CAMK have been central targets for genetic manipulations. In general, the abolition or reduction of kinase activity has correlated with deficits in LTP and memory, although this is not always the case. For example, there are mouse models in which a reduction in calcium-sensitive kinase activity critically impairs LTP but produces only mild behavioral phenotypes in tasks that are dependent on the same brain region as the observed LTP impairment. Such is the case for the PKCγ knockout animal, for example. Of course, isozyme compensation is always a consideration, especially when perturbation of a kinase isozyme occurs in a family of proteins represented by a large number of isozymes. Proteins of the CAMK family have also been studied using transgenic and gene knockout technologically because of their contribution to synaptic stabilization as well as nuclear events such as CREB-mediated gene transcription. Complete ablation of αCAMKII results in plasticity deficits; specifically, short-term potentiation, and therefore LTP and LTD are completely absent in these animals (Giese et al., 1998). However, animals heterozygous for αCAMKII demonstrate a remarkable phenotype. Interestingly, αCAMKII heterozygotes have impaired cortical LTP and severe remote memory deficits. The remote memory deficits observed in these animals are consistent with the observation that hippocampus-dependent memories undergo systems consolidation (Debiec et al., 2002), a process that provides hippocampal memories with a cortical dependence over time. It is possible that a full dose of CAMKII activity is required to retain synaptic configurations responsible for remote but not hippocampus-dependent memories. Animals in which CAMKIV has been knocked out show severe deficits in both contextual and auditory fear conditioning at 1 and 7 days following training, whereas short-term memory remains intact (Wei et al., 2002). The activation of transcription by CAMKIV is cohesive with the long-term memory phenotype, as long-term but not short-term memory requires *de novo* transcription and translation.

In addition to genetic perturbations of Ca^{2+}-sensitive kinases, receptors and channels that pass Ca^{2+} have been central genetic targets for studying plasticity and memory. For example, mice with mutations in CaV1.2,

the most abundant L-type calcium channel in the hippocampus, show no NMDA-receptor-independent LTP in the hippocampus and also have impairments in spatial memory (Moosmang et al., 2005). As might be expected, genetic manipulations of NMDA receptors demonstrate that the Ca^{2+} signal specific to NMDA-receptor activation plays a key role in both hippocampal LTP and LTD. For example, NR1 subunit ablation in the CA1 area of the hippocampus interferes with both Schaffer collateral LTP and LTD, and these results are mirrored by deficits in the consolidation phase of several hippocampal forms of memory. Conversely, NR2B overexpression reduces the threshold required for CA3-CA1 LTP and also results in behavioral facilitation of auditory and cued fear (see **Table 1**). These and other mammalian genetic models are referenced in **Table 1** and, although an incomplete list, are some of the key models that have resulted in a more concise understanding of how calcium-sensitive proteins (or the lack thereof) affect synaptic plasticity and memory.

4.21.2.4.2 Pharmacological advancements in calcium signaling during memory formation

In general, studies using pharmacological inhibition of intracellular Ca^{2+} strongly corroborate the genetic evidence that Ca^{2+} is central to both synaptic plasticity and memory processing. However, as developmental compensation is to knockout gene technology, so is drug specificity to pharmacological inhibition. For example, inhibition of VGCCs such as the L-type calcium channel has produced confounding results in the synaptic plasticity and memory fields. Although in some cases, inhibition of L-type channels actually enhances contextual fear memories (Cain et al., 2002), inhibition has also been reported to cause deficits in amygdala and hippocampal-dependent memory or LTP (Bauer et al., 2002). Because only low doses of antagonists, such as nifedipine, have resulted in behavioral augmentation, it has been hypothesized that channel modification, rather than complete inhibition, occurs at low doses; memory enhancements are not observed at high doses of antagonists. In spite of these contradictions, within-study comparisons can be made that underscore the specificity of the calcium signal for particular phases of memory processing. For example, systemic administrations of VGCC inhibitors can block extinction of fear memory while having no effect on acquisition or on retrieval-induced reconsolidation. Inhibition of NMDA receptors, on the other hand, results in not only extinction blockade but also blockade of consolidation and reconsolidation phases of memory (Suzuki et al., 2004). The disparate effects of these inhibitors on memory processing underscore the level of specificity required in the calcium signal for these different levels of memory processing. Although less is known about intracellular calcium's contribution to memory-encoding events, pharmacological inhibitors to SER pumps and receptors can and have been used to determine the contribution of SER calcium to events such as vesicle release and the strength of the postsynaptic response. The developments of SERCA pump inhibitors, such as thapsigargin and cyclopiazonic acid (CPA), and the RyR inhibitors (ryanodine red and dentrolene) have assisted in our knowledge of IICR and CICR in synaptic plasticity. CPA application, as well as high doses of ryanodine, for example, can reduce Ca^{2+} transients following synaptic stimulation in hippocampal neurons (Emptage et al., 1999). Inhibition of SERCA pumps by bath application of thapsigargin inhibits LTP at Schaffer collateral-CA1 synapses. Heparin-mediated inhibition of IP3 receptors also interferes with potentiation of CA3 neurons (Bardo et al., 2006).

Whereas inhibitors that block calcium entry and release have proved quite useful in clarifying the temporal and spatial roles for different phases of LTP or memory formation, pharmacological inhibition of calcium-sensitive proteins has also been instrumental in elucidating calcium's plieotropic effects in synaptic plasticity. Pharmacological inhibition of calcineurin, for example, by bilateral ventricular administration of calcineurin antisense oligonucleotides, causes an increase in contextual fear conditioning (Ikegami and Inokuchi, 2000). This study is cohesive with the conditional forebrain calcineurin knockout mouse that showed enhancements in LTP and spatial memory. However, early studies in chicks using intracranial infusions of calcineurin inhibitors cyclosporine A or FK506 resulted in acquisition deficits in a single-trial passive avoidance task (Bennett et al., 1996, 2002). Species differences could account for these discrepancies, or the contribution of calcineurin could be dependent on the type of memory for which the organism was trained and tested. Of course, it is also possible that calcineurin contribution varies according to both the type and location of pharmacological intervention. In short, the role of calcineurin in different memory tasks is complex, and much remains to be clarified about its activity during memory formation. As can be noted in **Table 1**, several calcineurin mouse models

have been made and may lead to more clarification of the role of calcineurin in the different phases of synaptic plasticity and memory processing.

As previously discussed in the section titled 'Calcium's effects in the nucleus,' one of the nuclear effects of synaptic Ca^{2+} is the transcription of BDNF, which is mediated by calcium-sensitive sites in the promoter region of the gene. BDNF strongly potentiates dentate granule cell LTP when synaptic stimuli are coapplied with dendritic BDNF application (Kovalchuk et al., 2002). (There is probably also a presynaptic component to BDNF, as viral rescue of BDNF expression in the hippocampal area CA3 of $BDNF^{-/-}$ mice can recover Schaffer collateral LTP deficits that are induced by 100-Hz stimulus (Zakharenko et al., 2003)). BDNF expression is induced following training for contextual fear conditioning, and BDNF antisense oligonucleotides have been used to study the role of this protein in consolidation and reconsolidation of this task. Infusion of BDNF antisense oligonucleotides into the hippocampus of rats prior to memory training disrupts the consolidation of contextual fear memory, whereas short-term memory is preserved (Lee et al., 2004). As oligonucleotide (such as siRNA and antisense) and viral pharmacological approaches have become more commonly used, the study of Ca^{2+} in synaptic plasticity has been enhanced by the ability to alter protein expression in distinct areas of the brain.

4.21.3 cAMP as a Second Messenger in Plasticity and Memory

The role of cyclic adenosine monophosphate will now be more closely addressed, as it is critical for activation of both cytosolic and nuclear components of synaptic plasticity and memory formation. Adenylyl cyclases convert adenosine triphosphate (ATP) to cyclic adenosine monophosphate. The structure of ATP and its conversion to cAMP are depicted in **Figure 3**.

cAMP functions as a second messenger by coupling extracellular signals to intracellular effectors and integrating specific stimuli into the production of long-lasting neuronal configurations or representations. Two major classes of proteins control cellular concentrations of cAMP: adenylyl cyclases (synthesis) and phosphodiesterases (degradation). Although adenylyl cyclases control the rate of cAMP synthesis, PDE proteins control the rate of its degradation, and these two groups of enzymes tightly regulate intracellular levels of cAMP. Here we focus primarily on the convergence of Ca^{2+} with cAMP signaling at the level of adenylyl cyclases and the effect of this signaling crosstalk on learning and memory.

4.21.3.1 Synthesis and Degradation of cAMP

4.21.3.1.1 Adenylyl cyclases

Ten adenylyl cyclases have been identified to date. Most of these enzymes share several properties, including transmembrane and cytoplasmic domains, the latter being the site of catalysis (Dessauer and Gilman, 1997; Hurley, 1999). With the exception of a soluble adenylyl cyclase, adenylyl cyclases are integral membrane proteins that consist of two groups of six transmembrane domains, a structure that has prompted speculation that these enzymes might have transport activity. The highly conserved cytoplasmic loops form

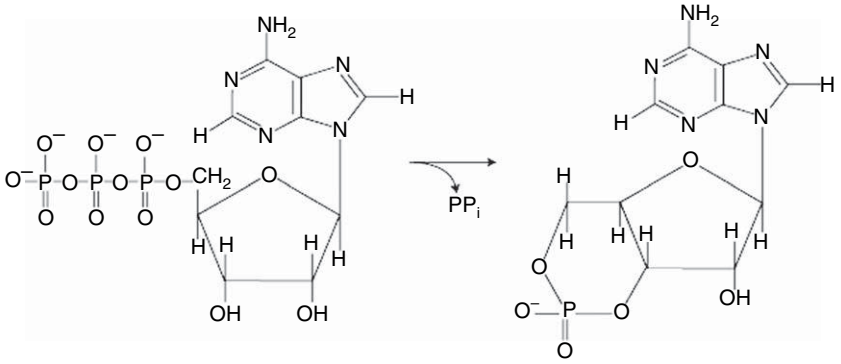

Figure 3 Catalysis of cAMP by adenylyl cyclases. Adenylyl cyclases catalyze the conversion of adenosine triphosphate (ATP) on the left, to cyclic adenosine monophosphate (cAMP) on the right. Adenylyl cyclases catalyze this conversion by attacking the 3′ hydroxyl group on the ribose of the nucleoside triphosphate, ATP.

the catalytic domains of the enzyme and must both be present for adenylyl cyclase activity. Although all isoforms of adenylyl cyclases are expressed in the nervous system, only AC1 is neurospecific. AC2 (a calcium-insensitive cyclase) and the calcium-inhibited AC9 are also highly enriched in the brain (Antoni et al., 1998; Xia et al., 1991). AC8 and AC3, both expressed in the nervous system, are also Ca^{2+} sensitive, with AC8 being Ca^{2+} activated, whereas AC3 is Ca^{2+} inhibited. The presence of distinct classes of adenylyl cyclases (Ca^{2+} sensitive and Ca^{2+} insensitive) was discovered by the application of brain extracts to Ca^{2+}-dependent CaM-Sepharose affinity columns (Westcott et al., 1979). Although most adenylyl cyclases are membrane bound, there is at least one soluble, bicarbonate ion-activated cyclase that appears to be important for several physiological processes, including axon outgrowth (Wu et al., 2006; Zippin et al., 2001).

The structure of adenylyl cyclases and the molecular machinery involved in adenylyl cyclase activation by heterotrimeric G proteins is schematically portrayed in **Figure 4**.

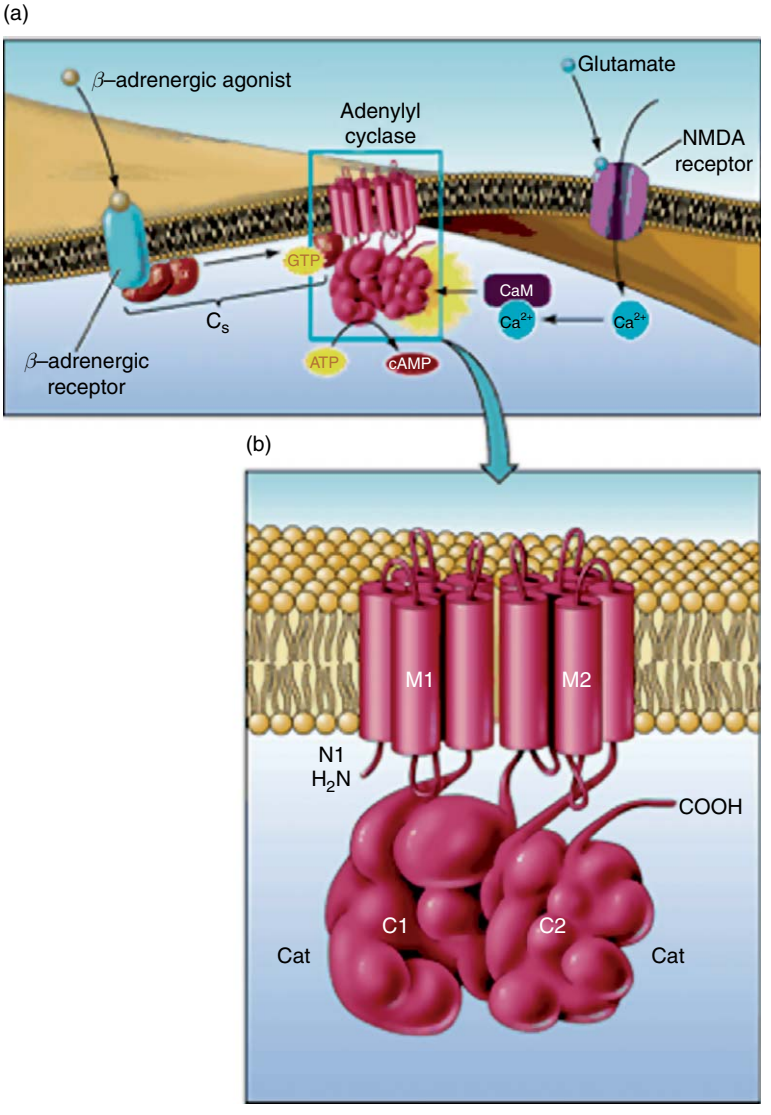

Figure 4 Structure of adenylyl cyclases and activation by G protein–coupled receptors. Activation of G protein–coupled receptors results in a direct association of GTP-bound Gα subunits with adenylyl cyclases in the membrane (a). (b) The catalytic subunits for adenylyl cyclases (cat), composed of the intracellular loops, mediate Ca^{2+}/CaM sensitivity for multiple adenylyl cyclases and are required for enzymatic catalysis of cAMP. Reproduced with permission from Ferguson GD and Storm DR (2004) Why calcium-stimulated adenylyl cyclases? *Physiology* 19: 271–276.

The most common form of adenylyl cyclase regulation is via G protein–coupled receptors, which convey extracellular signals to intracellular signaling pathways. (See Video Clip 1.) Some examples of G protein–coupled receptors include adrenoceptors, metabotropic glutamate, acetylcholine, dopamine, histamine, serotonin, PACAP, somatostatin, vasopressin, melatonin, cannabinoid, opioid, rhodopsin, prokineticin, pheromone, and olfactory receptors. G protein–coupled receptors regulate membrane adenylyl cyclases via direct interaction with the alpha subunits of heterotrimeric G proteins, although in some cases the G$\beta\gamma$ subunits can also activate adenylyl cyclases. Gα subunits are comprised of two domains, a GTPase and an alpha-helical region. These can be subcategorized into the following families: G_s (stimulatory), G_i (inhibitory), G_{olf}, G_q (which couples to phospholipase C), G_t (transducin), and $G_{12/13}$ (motility-activating) proteins. Adenylyl cyclases can be inhibited by binding to the alpha subunit of inhibitory G proteins ($G_{i\alpha}$). There are three $G_{i\alpha}$ isozymes in the hippocampus, and $G_{i\alpha1}$ functions as the primary isozyme for adenylyl cyclase inhibition. $G_{i\alpha1}$ mutant mice demonstrate the importance of this inhibition in normal memory processing and are discussed in the section titled 'Mammalian genetic models of cAMP in memory processing.' Not exempt from affecting cyclase activity, however, are the Gβ,γ protein subunits that can activate adenylyl cyclases II and IV in the presence of $G_{\alpha S}$.

4.21.3.1.2 Phosphodiesterases

Although adenylyl cyclases control the rate of cAMP production in response to external stimuli, PDE proteins are involved in enzymatic catalysis of cAMP. Cyclic nucleotide PDEs comprise a family of ten protein types (from 19 genes) that function to inactivate cAMP (Beavo, 1995; Soderling and Beavo, 2000). PDE proteins were first identified as hydrolyzing enzymes for cAMP by Dr. Earl Sutherland (Butcher and Sutherland, 1962). The PDEs also degrade guanine 3′5′-cyclic monophosphate (cGMP). The family of PDE enzymes provides an important convergence between the Ca^{2+} and cAMP second messenger systems. For example, some PDE proteins are phosphorylated in a Ca^{2+}/CaM-dependent manner and participate in direct interactions with calmodulin. PDE1 is expressed at high levels in the brain and is activated by Ca^{2+}/CaM (Cooper, 2005; Kakkar et al., 1999). To date, there are no known calcium-inhibited PDE proteins.

The *Drosophila* phosphodiesterase *dunce* mutant provided the first evidence that cAMP affects memory formation. The *dunce* mutation caused deficiencies in memory, and since that discovery, the PDEs have become a central object of study in plasticity-related fields. The PDE4 inhibitor rolipram, commonly used as an antidepressant, has been administered to rodents *in vivo*, yielding memory-enhancing tasks in certain forms of memory (Bourtchouladze et al., 2003). This and other studies using pharmacological inhibition of PDE proteins are addressed in the section titled 'Pharmacological advancements in cAMP signaling during memory formation.'

4.21.3.2 cAMP Effector Molecules

Protein kinase A is the primary mediator of cAMP signaling in the cell; however, it is now known to be one of several cAMP effector molecules expressed in the brain. PKA was first discovered by Fischer and Krebs who studied the protein's actions in context of glycogen breakdown (Sutherland and Wosilait, 1955; Walsh et al., 1968). The PKA protein contains two catalytic and two regulatory sites that form what is referred to as the holoenzyme in the absence of cAMP. In the presence of cAMP, the regulatory and catalytic protein subunits disassemble, allowing the catalytic subunits to function as a serine/threonine protein kinase. This kinase activity ultimately affects neurotransmitter release, channel conductance, translocation of proteins to the nucleus, and orchestration of nuclear events such as the activation of gene transcription. The cell maintains tight control over PKA-mediated cAMP signaling; when the catalytic subunits are overexpressed, for example, the regulatory subunit 1 (R1) undergoes posttranscriptional upregulation, producing a more stable protein subunit (Amieux and McKnight, 2002). Animals that aberrantly express some portion of the PKA holoenzyme demonstrate the power of this cAMP-binding protein in transmitting the second messenger signal into lasting, synapse-specific information. For example, animals that express a dominant-negative regulatory subunit R(AB) have deficits in L-LTP and contextual memory (Abel et al., 1997), and animals that do not express one of the catalytic (C1β) or one of the regulatory (RIβ) subunits of PKA have deficits in hippocampal mossy fiber LTP (Brandon et al., 1995). Because PKA is a MAPK activator and appears to mediate its translocation to the nucleus, it is not surprising that deficits in PKA subunit expression result in impairments in synaptic plasticity and memory. Furthermore, PKA is involved in direct binding and activation of several CREB kinases, which will be discussed further.

Although cAMP has traditionally been thought to participate in downstream signaling primarily through the activation of PKA, it can, however, activate MAPK by alternative mechanisms. Specifically, cAMP activation of the small G protein, Rap1, originally believed to be mediated exclusively by PKA phosphorylation (Hata et al., 1991), has now been discovered to occur in a PKA-independent manner. Kawasaki et al. identified two Epac (exchange protein directly activated by cAMP) proteins, both Rap1-activating, cAMP-binding guanine nucleotide exchange factors (GEFs), that activate Rap1 independent of PKA (de Rooij et al., 1998). Considering the high expression of Epacs in areas of the brain known to participate in memory processing, it is likely that these proteins allow coding of information in a way distinct from PKA. Epac proteins generally serve as Ras-activating GEFs, and their effects on Ras and Rap-1 appear to be dependent on both the upstream stimuli presented to the cell and the localization of Ras GTPase-activating protein (GAP) proteins within the cell. Because these exchange proteins have been recently discovered, their role in memory formation and synaptic plasticity remains to be established. Epac proteins have been reported to regulate neurotransmitter release at the crayfish neuromuscular junction, a process that is completely independent of PKA activity (Zhong and Zucker, 2005). New technological advancements in studying Epac proteins are being developed that should allow a delineation of cAMP roles distinct from PKA action.

One of the central targets of cAMP that affects presynaptic plasticity is the family of cyclic nucleotide-gated ion channels (CNGs). CNG channels are nonselective cation channels that bind both cGMP and cAMP. These channels are perhaps most widely studied in the context of photoreceptor cells and olfactory sensory neurons where cGMP and cAMP activate these channels by direct binding. The role of cAMP in activating CNG channels has also been well studied at the crayfish neuromuscular junction, where presynaptic facilitation has a temporal dependence on cAMP modulation of these channels. cAMP activation of CNG channels tags the synapse in such a way that an additional stimulus provides an enhancement of neurotransmission independent of CNG activation. It is unclear exactly how this synaptic tagging occurs, but cAMP activation of CNG channels and Epac proteins appears to increase the number of vesicles ready for release. Epac interaction with the vesicle protein, Rab3a, may be a plausible explanation for this phenomena, but it is unclear why this process requires activation of CNG channels (Zhong and Zucker, 2005).

4.21.3.2.1 Synaptic and Cytoplasmic cAMP Signaling Cascades

Impingement of cAMP on the MAPK pathway has been studied in a variety of cells with cAMP, sometimes activating and sometimes depressing MAPK activation (for review, see Burgering and Bos, 1995). Here we focus on the effects of cAMP signaling in various pathways in the brain, particularly during memory processing. The contribution of MAPK proteins in memory formation is addressed in much greater depth in Chapter 4.25. It is necessary, however, to discuss the Ras/Raf/MAPK pathway here in order to underscore the importance of cAMP-mediated activation of this pathway during memory processing.

Figure 5 illustrates the key interactions important for coupling of cAMP increases to activation of the Ras/MEK/MAPK pathway.

The Raf/MEK/MAPK pathway is critical for the consolidation and reconsolidation of many forms of memory, and inhibition of this pathway interferes with both LTP and long-term memory formation. The small G protein, Ras, is localized by Ras exchange protein, Sos, and the adapter protein, Grb2. Once activated (often by receptor tyrosine kinases) it binds to Raf-1, which mediates its localization and phosphorylation by PKC or other kinases (Kolch et al., 1993). Raf-1 activates the dual-specificity kinase MEK1, which in turn phosphorylates the ERK/MAPK serine/threonine kinases. An alternative mechanism for MAPK pathway activation is through Epac proteins. Rap1, also a small G protein, is activated by Epac proteins and can subsequently activate MEK1/2 through B-raf proteins. Activation of the Raf/MEK/MAPK pathway results in transcriptional and translational activation required for the consolidation of long-term memory. Specifically, inhibition of MAPK kinases in the lateral amygdala will block consolidation of cued fear conditioning while allowing acquisition of the task (Schafe et al., 2000). Activation of the MAPK pathway is also critical in the amygdala for reconsolidation of auditory fear memory (Duvarci et al., 2005). Retrieval of the auditory conditioning results in a molecular cascade of events in the amygdala that includes activation of MAPK during the reconsolidation period. Reconsolidation of the original auditory fear memory is blocked by amygdalar infusion of MAPK inhibitors. Although activation of the Raf/MEK/MAPK

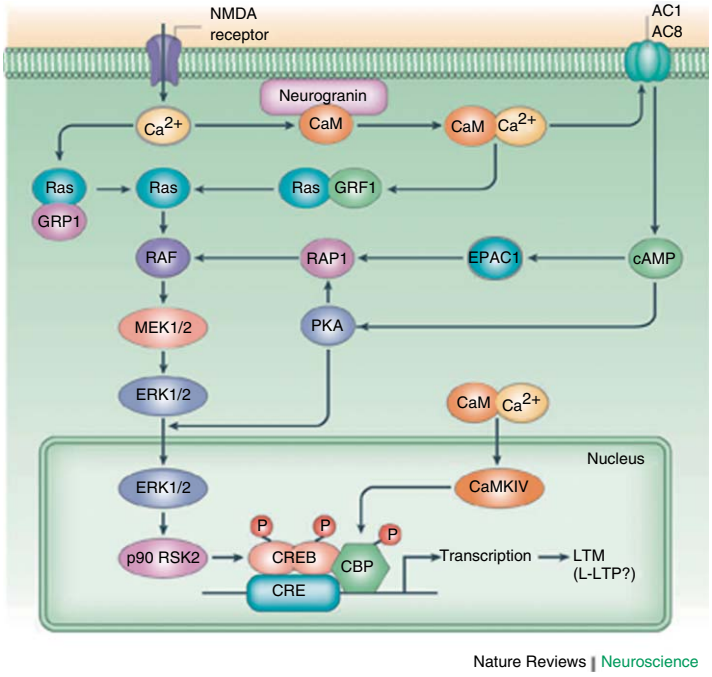

Figure 5 Activation of the ERK/endoplasmic reticulum kinase (ERK)/mitogen-activated protein kinase (MAPK) pathway of calcium and cyclic adenosine monophosphate (cAMP). The postsynaptically expressed protein, neurogranin, controls the level of free calmodulin available for adenylyl cyclase activation. cAMP mediates activation of the MAPK pathway through EPAC proteins, or through protein kinase A (PKA), which can mediate the translocation of ERK/MAPK from the cytosol to the nucleus. Reproduced with permission from Xia Z and Storm DR (2005) The role of calmodulin as a signal integrator for synaptic plasticity. *Nat. Rev. Neurosci.* 6: 267–276.

pathway is critical for auditory fear memories, it is also a requirement for the consolidation of contextual fear memories; training an animal for contextual fear results in an increase in activation of MAPK. Inhibition of MAPK kinases (MEK1/2) with PD98059, UO126, or SL327 inhibits consolidation of contextual fear memories (Atkins et al., 1998).

Phosphatases also play a critical role in cAMP signaling. Two phosphatases that participate in cAMP signaling are the protein tyrosine phosphatase STEP and calcineurin. cAMP impinges on the Raf/MEK/MAPK pathway via the aforementioned Epac proteins, or it can indirectly facilitate persistent MAPK activation by PKA-mediated regulation of STEP (Saxena et al., 1999). Increases in cAMP result in a direct phosphorylation of STEP by PKA, a phosphorylation event that increases MAPK signaling because it interferes with STEP binding to its substrates, such as MAPK (Braithwaite et al., 2006). Whereas STEP is phosphorylated and inactivated by elevations in cAMP, the phosphatase calcineurin functions upstream of cAMP and, through a second mechanism, probably contributes to increases in MAPK activity. It is thought that this phosphatase is responsible for the residual calcium-sensitive cyclase activity in AC1 and AC8 DKO animals. This residual activity can be inhibited by blockade of NMDA receptors; however, general inhibitors of protein serine/threonine kinases do not inhibit depolarization-induced calcineurin activity in hippocampal neurons depleted of AC1 and AC8 (Chan et al., 2005). Pharmacological inhibition of calcineurin indicates that the residual calcium sensitivity in AC1/AC8 double-knockout animals is dependent on this phosphatase. This dependency could arise by a couple of mechanisms: calcineurin might directly dephosphorylate adenylyl cyclases, relieving inhibition of activity, or calcineurin might activate protein phosphatase 1 (PP1) by dephosphorylating inhibitor 1 (I-1), the net result being the dephosphorylation of adenylyl cyclases by PP1.

Calcineurin's participation in cAMP and calcium signaling are not so simple, however. For example, although calcineurin can activate cAMP production through one or more adenylate cyclases, it can reverse PKA phosphorylation of STEP, enabling it to inactivate MAPK and its other targets such as Fyn kinase and the NR2B subunit of NMDA receptors.

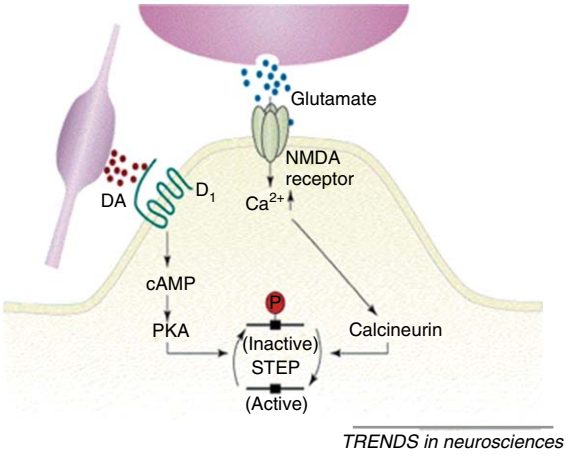

Figure 6 Activity of the phosphatase STEP is controlled by the phosphatase calcineurin. Intracellular increases in cAMP by dopamine (DA) binding to its receptor, D1, induces the phosphorylation of STEP by PKA. Inactive, phosphorylated STEP is incapable of binding to its substrates such as ERK/MAPK. Calcium-activated calcineurin can reverse this phosphorylation, allowing the subsequent activation of STEP and inhibition of MAPK signaling. Reproduced with permission from Braithwaite SP, Paul S, Nairn AC, and Lombroso PJ (2006) Synaptic plasticity: One STEP at a time. *Trends Neurosci.* 29: 452–458.

Therefore, although calcineurin can increase intracellular cAMP levels, it can also serve as a brake for MAPK pathway activation by negatively regulating STEP (see **Figure 6**). Studies are currently in progress looking at the ways in which STEP and calcineurin interact after memory-specific stimuli. One thing is clear, however: cAMP increases are required for activation of the Raf/MEK/MAPK pathway during memory consolidation. Neurons temporally orchestrate the activities of cAMP effectors in such a way that permits the transfer of memory stimuli into long-lasting synaptic configurations that serve as the basis of information storing.

4.21.3.2.2 Functions of cAMP in the nucleus

One of the central nuclear targets (and, perhaps, one of the more thoroughly studied transcription factors) affected by intracellular increases in cAMP is CREB and its family members, cAMP response element modulator (CREM) and activating transcription factor 1 (ATF1). These proteins contain DNA-binding domains in the C-terminal end of the protein that specify their binding to an 8-base pair palindromic region of the DNA, TGACGTCA (Lonze and Ginty, 2002). CREB and CREM both contain a domain known as the KID domain, which contains the serine-133 phosphorylation site that induces binding to the transcriptional coactivator, CREB-binding protein (CBP) (Chrivia et al., 1993). Disruptions in CREB binding to CBP prevent CREB-mediated transcription, and mice expressing CBP that cannot bind to CREB show impairments in long-term memory and LTP (see **Table 2**). This is not surprising, as training induces the transcription of a large number of CRE-regulated genes. Among these are immediate early genes c-fos, zif268, C/EBPbeta, and BDNF. Many of these CREB targets have subsequently been demonstrated to be critical for the consolidation or reconsolidation processes (e.g., see **Table 2** for the effects of genetic disruption of zif268). A later chapter (Chapter 4.27) focuses on transcriptional regulation in plasticity and reviews CREB-mediated gene transcription in detail. A useful tool for analysis of CREB-mediated gene expression during contextual learning is the transgenic CRE-beta-galactosidase mouse, a mouse engineered to express beta-galactosidase under the control of a promoter containing six CRE consensus sites (Impey et al., 1996). Training these animals for hippocampal-dependent memory tasks produces large increases in hippocampal beta-galactosidase gene expression. Contextual memory and passive avoidance memory both stimulate increases in CRE-mediated gene expression in the hippocampus, particularly in area CA1 (Impey et al., 1998). Following auditory fear conditioning, increases in CREB-induced transcription are also observed in the basolateral amygdala (a region that is well established to be required for auditory fear conditioning) but not the hippocampus, indicating that CREB is activated in a stimulus-specific way.

Although cAMP induces the transcription of some genes, cAMP can actually inhibit gene expression in certain cases. For example, the promoter of AC1 has been shown to be inhibited by cAMP, a property that probably explains why AC1 mRNA oscillates in pinealocytes of the pineal gland (Chan et al., 2001). The oscillation of AC1 mRNA and protein in the pineal is important, as it appears to be critical for the activation of enzymes important in the oscillatory release of melatonin.

4.21.3.3 cAMP in Memory Processing (Introduction)

The dependence of memory processing on cAMP signaling extends from *Aplysia* to mammalian systems. Although other chapters focus in detail on the role of

Table 2 Mouse genetic approaches to studying the role of cyclic adenosine monophosphate signaling in memory

Gene modified	*Alteration*	*Affected area of expression*	*Effect on plasticity*	*Effect on memory*
AC1[a]	Knockout	Global	Decreased cerebellar LTP	Deficits in rotarod performance and Morris water maze memory
AC1+[b]	Transgenic, overexpressed	Forebrain	Increased Schaffer collateral LTP, 100-Hz, two-train LTP	Increased recognition memory
AC8[c,18]	Knockout	Global	No LFS-induced LTD	No observable deficits
AC1/AC8[c]	Double knockout	Global	L-LTP deficit, 100 Hz, 4-train LTP	Deficits in passive avoidance and contextual fear memories
Giα1[d]	Knockout	Global	Lower threshold for L-LTP, single 100-Hz stimulus	Impaired passive avoidance, contextual fear, and object recognition memory
PAC1[e,p]	Knockout[e,p] Transgenic[p]	Global or forebrain	Impaired mossy fiber LTPP	Impaired contextual fear memory or context memory persistance but unimpaired in Morris water maze and cued fear
α/ΔCREB[f]	Knockout	Global	Impaired Schaffer collateral LTP, no deficits in STP	Impaired spatial and contextual fear memory but gene dosage and genetic background contribute[m] also; see[n]
αCREB^{1R}[g]	Transgenic, dominant negative	Forebrain	None reported	Deficits in conditioned taste aversion
CBP $^{+/-}$ (RTS model)[h]	Knockout	Global	L-LTP deficit, 100-Hz, 4-train LTP	Deficits in object recognition and fear memory
Zif268$^{-/-,+/-}$[i]	Knockout Heterozygote	Global	L-LTP deficits in dentate gyrus *in vivo*	Deficits in conditioned taste aversion, olfactory discrimination, and spatial memory
R(AB)[j]	Transgenic, dominant negative PKA regulatory subunit	Forebrain	L-LTP deficit in Schaffer collateral-CA1	Spatial and contextual fear memory deficits
PKA (catalytic subunit C1β)[k]	Knockout	Global	Deficits in Schaffer collateral L-LTP	No observable deficits
PKA (regulatory subunit R1β)[k]	Knockout	Global	LTD deficits at Schaffer collateral-CA1, impaired LTP, LTD, and PPF in layers II/III of visual cortex[17]	No observable memory deficits
VP16-CREB[l]	Transgenic, expression of constitutively active CREB	Forebrain	Enhanced Schaffer collateral L-LTP	No behavioral deficits reported

PKA, protein kinase A: LTP, long-term potentiation; LTD, long-term depression.
[a](Storm et al., 1998; Wu et al., 1995); [b](Wang et al., 2004); [c](Wong et al., 1999); [d](Pineda et al., 2004); [e](Sauvage et al., 2000); [f](Bourtchuladze et al., 1994); [g](Josselyn et al., 2004); [h](Bourtchouladze et al., 2003); [i](Jones et al., 2001); [j](Abel et al., 1997); [k](Huang et al., 1995); [l](Barco et al., 2002); [m](Gass et al., 1998); [n](Balschun et al., 2003); [o](Hensch et al., 1998); [p](Otto et al., 2001).

cAMP in synaptic plasticity in specific organisms (*See* Chapters 4.02, 4.03, 4.04, 4.05, 4.06, 4.07, 4.08, 4.09, 4.10), it is worth noting the universality of cAMP signaling in memory. *Drosophila* screens have revealed mutations in cAMP signaling molecules that affect memory (Livingstone et al., 1984). The *Drosophila rutabaga* mutation, for example, has been confirmed to be an X-linked recessive point mutation in an calcium-sensitive

adenylyl cyclase that results in reduced Ca^{2+}/CaM cyclase activity (Levin et al., 1992). Also discovered through *Drosophila* screens is the *dunce* learning mutant (Byers et al., 1981), *dunce* being a mutation that results in loss of phosphodiesterase activity. Similar to the *rutabaga* mutation, the *dunce* mutation results in a net increase in cAMP. Kandel and colleagues pioneered much of the work demonstrating the central role of cAMP signaling in plasticity in the marine snail, *Aplysia californica*. The *Aplysia* sensory to motor synapse has been a central tool in our understanding of cAMP in both pre- and postsynaptic events. In this system, serotonin-induced increases in cAMP lead to CREB-mediated gene expression, a pathway that is necessary for long-term facilitation (Otto et al., 2001) (*See also* Chapters 4.02, 4.03 and 4.10).

4.21.3.3.1 Mammalian genetic models of cAMP in memory processing

In efforts to determine to what extent the cAMP signaling pathways contribute to learning and memory, transgenic and knockout technology has been used to disrupt cAMP signaling. **Table 2** lists some of the animals generated with specific mutations or deletions in cAMP signaling molecules in order to address their function in LTP and memory processing. The neurospecific expression of AC1 makes it an especially appealing drug target for increasing cAMP, specifically in the brain. AC1 knockout animals were created to determine to what extent this enzyme contributes to various forms of LTP or memory formation (Wu et al., 1995). The absence of AC1 results in a 50% reduction in calcium-sensitive adenylyl cyclase activity in both the hippocampus and the cerebellum (Wong et al., 1999). Although spatial memory is impaired in AC1 knockouts (Wu et al., 1995), memory for contextual and passive avoidance training is normal (Wong, 1999). AC8 knockouts also show normal memory for contextual and passive avoidance training. However, AC1/AC8 double knockouts (DKO) exhibit severe deficiencies in spatial, passive avoidance, and contextual long-term memory. Although these animals exhibit impairments in long-term memory, they learn normally and exhibit short-term memory lasting 5–10 min. Interestingly, these memory defects are completely reversed when forskolin, a general activator of adenylyl cyclases, is administered to the hippocampus of DKO mice right before training. Because the memory deficits in DKO mice can be reversed by a general increase in cAMP across the hippocampus, memory deficits exhibited by the mutant mice are attributable to decreased cAMP signaling and not to developmental defects in the CNS. DKO mice also exhibit no long-lasting LTP (L-LTP), whereas the AC1 and AC8 single knockouts do. These data indicate that calcium-stimulated adenylyl cyclases are required for two transcriptionally dependent processes, L-LTP and LTM. The cAMP signal required for these long-lasting processes can be generated by AC1 or AC8. Recently, it was discovered that DKO mice show no training-induced increase in ERK/MAPK activity or the CREB kinase, MSK1, indicating that Ca^{2+}-stimulated adenylyl cyclase activity is required for increases in MAP kinase activity and CREB phosphorylation during memory consolidation.

The contribution of AC1 has also been studied in the context of the anterior cingulated cortex (ACC), a region well known for its contribution to remote fear memory maintenance (Frankland et al., 2004). Unlike AC8, which only contributes to forskolin-induced potentiation, LTP in the ACC requires AC1 activity (Liauw et al., 2005).

To determine whether genetic increases in AC1 activity can improve memory formation, a transgenic mouse strain that overexpresses AC1 in the forebrain ($AC1^{+}$) was used to assess the effect on plasticity and memory. An αCAMKII promoter-driven overexpression of AC1 in the forebrain of mice results in an approximately twofold increase in hippocampal Ca^{2+}-stimulated adenylyl cyclase activity. These mice show an enhancement in novel object memory and also display an enhancement in L-LTP. A 100-Hz, 1-s LTP protocol, normally sufficient for only E-LTP at hippocampal Schaffer-collateral CA1 synapses in wild-type mice, produces L-LTP in $AC1^{+}$ animals (Wang et al., 2004). Contextual fear memory is normal in these animals; however, contextual memory extinction is severely impaired. The decrease in the rate of contextual memory extinction suggests that increased cAMP may impair extinction and is consistent with the observation that $CB1^{-/-}$ mice also show impaired extinction (Marsicano et al., 2002). CB1 receptors couple to inhibition of adenylyl cyclase through G_i proteins, implying that endocannabinoids may mediate extinction through adenylyl cyclase inhibition.

As already mentioned, one of the primary modes for stimulation of adenylyl cyclase is via Gs protein-coupled receptors. One of these receptors, PAC1, is a receptor for pituitary adenylyl cyclase activating peptide (PACAP). PACAP is a circadian-regulated peptide that increases cAMP in target tissues by binding to PAC1, a heterotrimeric G protein-coupled

receptor. The presence of PAC1 in hippocampal presynaptic mossy fiber terminals suggests that it may play a role in LTP or memory processing that uses these synaptic circuits. PAC1 knockout mice show a mild memory phenotype in one-trial contextual fear conditioning as well as in a contextual discrimination task (Sauvage et al., 2000). PAC1 signaling appears to be critical for memory processing in the hippocampus but not the amygdala. Amygdala-dependent memory is unimpaired in PAC1-deficient mice.

4.21.3.3.2 Pharmacological advancements in cAMP signaling during memory formation

Although transgenic technology has proved vital to understanding the importance of adenylyl cyclase activity in long-term memory formation, there are alternative methods to modulate cAMP levels in brain. For example, a direct demonstration of the importance of cAMP signaling for long-term memory formation was a study in which CRE decoy oligonucleotides (nucleotides containing CRE consensus sequences) were directly infused into the CA1 region of the hippocampus after training for contextual fear. These decoys interfered with CRE-mediated gene transcription and memory consolidation, whereas the infusion of scrambled oligonucleotides that did not compete with CREB binding had no effect on behavior (Athos et al., 2002).

Other means of pharmacological interference with cAMP signaling underscore the necessity of cAMP signaling for long term memory formation. For example, the inhibition of PKA by Rp-cAMP (a diastereoisomer of cAMP) infusions into the amygdala accelerates extinction for taste aversion (Koh and Bernstein, 2003). It is worthwhile noting that PKA inhibition, CB1 receptor deficiency, and AC1 overexpression studies all indicate that cAMP may be a molecular restraint on extinction learning.

Forskolin has provided a powerful pharmacological agent by which to stimulate adenylyl cyclases enzymes *in vitro* and *in vivo*. This diterpene activates all of the known adenylyl cyclases except AC9. Forskolin-activated adenylyl cyclase activity can rescue memory deficits in the AC1/AC8 DKO animal. Because PDEs depress cAMP signaling, another way to increase cAMP signaling is through inhibition of PDEs. Phosphodiesterases are strong candidates for memory-enhancing pharmacological targets as interfering with specific PDE proteins can rescue drug-induced memory deficits. For example, the PDE4 antagonist, rolipram, has been used to reverse UO126-induced deficits in radial arm maze retrieval (Zhang et al., 2004). Furthermore, rolipram can also rescue memory deficits in the heterozygous CREB-binding protein (CBP) mice, a mouse with cognitive impairments that have often been compared with those in patients with Rubinstein-Taybi syndrome (Bourtchouladze et al., 2003).

4.21.4 Concluding Remarks

Although it was not possible to describe all of the experiments implicating crosstalk between cAMP and Ca^{2+} in memory formation in this chapter, the examples discussed provide ample evidence for the importance of these second messengers in memory. Crosstalk between cAMP and Ca^{2+} signaling defines the extent to which Hebbian plasticity can occur and, thereby, regulates memory formation.

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4.22 PKMζ, LTP Maintenance, and Long-Term Memory Storage

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4.22.1 Introduction: Protein Kinase Mζ, a Memory Storage Molecule

How long-term memory persists over time is a fundamental question in neuroscience, yet very little is known about the molecular mechanisms that maintain this persistence. In contrast, a vast literature has been written on the molecular mechanisms that trigger learning and the early consolidation of memory. Because this initial phase of memory acquisition is immensely complex, it has been natural to assume that the maintenance of memory storage is similarly complex. In this chapter, however, I would like to present the evidence that a simple molecular mechanism underlies the storage of at least some forms of long-term memory. This core storage mechanism centers around the function of an unusual, persistently active protein kinase C (PKC) isoform, protein kinase Mzeta (PKMζ).

PKMζ's unique mnemonic function was initially characterized through its role in information storage at synapses during long-term potentiation (LTP), the leading candidate for the physiological substrate of memory (Bliss and Collingridge, 1993; Ling et al., 2002; Bliss et al., 2006). LTP can be divided into an early, protein synthesis-independent form and a late, protein synthesis-dependent form (Bliss and Collingridge, 1993). Each of these forms can be further subdivided at the mechanistic level into an induction phase, triggering plasticity, and a maintenance phase, sustaining the plasticity over time (Roberson et al., 1996; Sanes and Lichtman, 1999; Sweatt, 1999). PKMζ's function is specific to one form and phase of LTP: The maintenance phase of

the late, protein synthesis-dependent form of LTP (Serrano et al., 2005).

Late LTP induction is triggered by strong afferent synaptic stimulation that releases glutamate, activating postsynaptic *N*-methyl-D-aspartate receptors (NMDARs) (Bliss and Collingridge, 1993). Postsynaptic Ca^{2+} influx through the receptors then stimulates a complex network of signaling molecules, including many protein kinases, that induces new protein synthesis (Roberson et al., 1996; Sanes and Lichtman, 1999; Sweatt, 1999). Disrupting many of these molecules from the NMDAR to the components of this initial molecular network blocks the formation of LTP. In contrast, inhibiting these molecules after late LTP has been established for approximately 1 h has no effect on the potentiation (Ling et al., 2002). Thus, strictly speaking, these molecules are important for the induction, but not for the maintenance of late LTP.

Because LTP induction initiates the new synthesis of many gene products (Park et al., 2006), one might think that many of these newly expressed molecules would have been shown to be required for maintaining the persistence of LTP, perhaps through the formation of new synapses, and that blocking the effects of these proteins could reverse LTP. But this is not the case. Although it is only one of many proteins synthesized during LTP, PKMζ is the only molecule found to be both necessary and sufficient for the persistence of synaptic potentiation, i.e., introducing PKMζ into neurons causes synaptic potentiation without tetanization, and inhibiting PKMζ reverses established late LTP (Ling et al., 2002). Thus, whereas LTP induction is exceedingly complex, involving a network of dozens, if not hundreds of molecules, LTP maintenance appears to be mediated by a relatively simple molecular mechanism involving the persistent action of PKMζ.

Because the kinase activity of PKMζ is readily inhibited, its role in maintaining the persistence of memory is testable (Pastalkova et al., 2006). Analogous to the ability of PKMζ inhibition to reverse late LTP, PKMζ inhibition eliminates the retention of established long-term memory. Several different forms of memory can be erased, without altering the ability later to establish new long-term memories. Memories up to 1 month after training can be eliminated by the inhibitor, indicating that even remote memories are maintained by PKMζ's persistent action.

PKMζ is thus the first known molecule whose function is to maintain experience-dependent modifications of behavior – a memory storage molecule. In this chapter, we will begin by reviewing the experimental path that led to the discovery of PKMζ, focusing on its unique structure that is key to its ability to store information at synapses. Second, we will examine the enzyme's function in LTP maintenance. Third, we will examine the role of PKMζ in memory persistence and its implications for a new, dynamic view of long-term memory storage.

4.22.2 Protein Kinase Mζ, a Persistently Active Protein Kinase C Isoform Synthesized During Long-Term Potentiation

4.22.2.1 The Discovery of Protein Kinase Mζ, a Constitutively Active Atypical Isoform of Protein Kinase C

The biochemical property of PKMζ that allows it to maintain long-term memory is its persistent kinase activity (Sacktor et al., 1993). Most of the approximately 500 protein kinases in a typical mammalian genome are activated only transiently by second messengers and thus transduce information, but not store it. This is because most kinases are autoinhibited in their basal states in cells, often by a pseudosubstrate sequence within the kinase itself. Second messengers, produced in response to ligand–receptor interactions, bind to the kinases and activate them by producing conformational changes that relieve their autoinhibition. However, because second messengers are usually then rapidly eliminated, the kinases quickly return to their inactive basal states, awaiting the next round of extracellular signaling. Thus most kinases are activated by posttranslational modifications and their effects are transient.

PKMζ, in contrast, is activated through *de novo* synthesis and its effects are persistent (Hernandez et al., 2003). These unusual properties of PKMζ are the result of its unique structure as an independent PKC catalytic domain gene product that completely lacks autoinhibition (Sacktor et al., 1993; Kelly et al., 2007), allowing it to be a constitutively active form of PKC.

PKC is a gene family of closely related serine-threonine kinases, each consisting of a single polypeptide (Dekker et al., 1995; Nishizuka, 1995; Newton, 2001). All full-length PKCs consist of an N-terminal regulatory domain and a C-terminal catalytic domain. The regulatory domain of PKC contains both second messenger binding sites and a pseudosubstrate, which

binds to and inhibits the catalytic domain. Second messengers stimulate PKC by binding to the regulatory domain and causing a conformational change that relieves the autoinhibition. Because lipid membrane is an obligatory cofactor for the binding of these second messengers to the PKC regulatory domain, activation of full-length PKCs is associated with translocation from cytosolic to membranous compartments within a cell. The three classes of PKC isoforms are distinguished by the different second messengers that activate the isoforms. Conventional PKCs (cPKCs: α, βI, βII, γ) are activated by Ca^{2+} and/or diacylglycerol (DAG); novel PKCs (nPKCs: δ, ε, η, θ) by DAG, but not Ca^{2+}; and atypical PKCs (aPKCs: ζ, ι/λ) are stimulated not by DAG or Ca^{2+}, but by other lipid second messengers, including arachidonic acid, phosphatidic acid, and ceramide, and by specific proteins that bind to its regulatory domain (Zhou et al., 1994; Hirai and Chida, 2003).

The translocation of PKC to membrane by second messengers is generally short-lived, because increases in lipid second messengers or intracellular Ca^{2+} are usually transient (Nishizuka, 1995). However, Nishizuka, who discovered PKC, in his very first papers on the enzyme, described an *in vitro* mechanism for permanently activating the kinase (Inoue et al., 1977; Takai et al., 1977). He and his colleagues found that proteases, in particular the Ca^{2+}-dependent calpains, could cleave PKC at its hinge, separating the regulatory from the catalytic domain, thus permanently removing the enzyme's autoinhibitory constraint to form a constitutively active kinase (Inoue et al., 1977; Kishimoto et al., 1983). The resulting independent catalytic domain was called PKM (M standing for the Mg^{2+} in Mg^{2+}-adenosine triphosphate (ATP), the sole requirement for the enzyme's activity) (Inoue et al., 1977). Although an attractive mechanism for sustaining long-term cellular responses, particularly as a potential biochemical basis for long-term memory, PKM formation had never been observed under physiological conditions.

The notion of a persistent kinase as a possible mechanism of memory storage came largely from the work of James Schwartz (1933–2006), who pioneered the biochemical analysis of memory in a model system, the mollusk *Aplysia* (Schwartz, 1993; Schwartz and Greenberg, 1987). Following speculation by Francis Crick (Crick, 1984), Schwartz had formulated the idea that protein kinases critical for short-term memory might be transformed into persistently active forms for storing long-term memory. Specifically, Schwartz proposed that certain kinases, which normally depend upon second messengers for activation, could, through extensive or repetitive stimulation during behavioral training, become partially or fully independent of second messenger stimulation, and thus persistently active to maintain memories. In support of this notion, Schwartz found that two kinases in *Aplysia* neurons, the cyclic adenosine monophosphate (cAMP)-dependent protein kinase (PKA), which, together with Eric Kandel and colleagues had been shown to be critical for short-term memory (Kandel and Schwartz, 1982), and the Ca^{2+}/calmodulin-dependent protein kinase II (CaMKII), became less dependent on second messengers after stimulation by the facilitory neurotransmitter serotonin. PKA became autonomously active through a protein synthesis-dependent loss of its autoinhibitory regulatory subunit (Greenberg et al., 1987; Bergold et al., 1990); CaMKII was converted into an autonomously active form through posttranslational mechanisms – autophosphorylation and translocation from cytosol to a membranous compartment of neurons (Saitoh and Schwartz, 1985).

Schwartz also examined PKC, and, together with the author, found the kinase was activated by translocation to membrane, its standard mechanism of activation, during short-term facilitation and behavioral sensitization in *Aplysia* (Sacktor et al., 1993). This work was continued by Wayne Sossin, who later showed that the Ca^{2+}-independent, novel *Aplysia* PKC was important specifically for activity-independent short-term facilitation, particularly for facilitation from a previously depressed state, whereas the Ca^{2+}-dependent, conventional PKC was important for activity-dependent short-term facilitation (Zhao et al., 2006). Sossin also showed that novel *Aplysia* PKC could be persistently activated, perhaps through a sustained phosphorylation (Lim and Sossin, 2006), which, he proposed, might be important for an intermediate form of facilitation (Sossin et al., 1994).

Around the time of the initial work of PKC in *Aplysia*, there was also growing interest in PKC in LTP. Malenka, Madison, and Nicoll found that a PKC activator, phorbol esters, increased synaptic transmission (Malenka et al., 1986), and Malinow et al. (1988) and Routtenberg and colleagues (Colley et al., 1990) showed that H7, a PKC catalytic domain inhibitor, both prevented LTP induction and reversed LTP maintenance. Furthermore, Routtenberg had also detected a persistent translocation of PKC during *in vivo* LTP (Akers et al., 1986), and, in 1991, Klann and Sweatt showed that the constitutive activity of

PKC persistently increased in LTP maintenance in hippocampal slices (Klann et al., 1991, 1993). Subsequently, phosphorylation (Klann et al., 1993; Sweatt et al., 1998) and oxidative activation (Klann et al., 1998) were found to contribute to these persistent effects of PKC. The functional role of PKC in maintaining LTP, however, was soon questioned, both because the effects of H7 were found to be relatively nonspecific (Muller et al., 1990), and because other PKC inhibitors that blocked the enzyme's regulatory domain (e.g., sphingosine (Malinow et al., 1988) and calphostin C (Lopez-Molina et al., 1993) and a second inhibitor of the PKC catalytic domain (staurosporine)), while preventing LTP induction, did not reverse established LTP maintenance (Denny et al., 1990; Muller et al., 1992). Thus, interest in persistently active PKC as a mechanism for sustaining LTP began to decline.

However, around the time that these doubts were raised, PKC was found to be a family of kinases, derived from nine separate genes (Dekker et al., 1995; Nishizuka, 1995). Therefore, the apparent discrepancies in the initial inhibitor studies could have been due to isoform-specific effects. (Indeed, both regulatory domain inhibitors and staurosporine (McGlynn et al., 1992; Kochs et al., 1993; Ling et al., 2002) do not effectively inhibit PKMζ.) The possibility that specific isoforms might be important in LTP was explored in detail by our laboratory.

When we began our investigations into PKC in LTP, our initial hypothesis was that some PKC isoforms might be important for induction, and others for maintenance, either through persistent translocation of PKC or formation of a PKM. Because we did not know which isoforms might be important for which phase, we generated antisera specific to all known isoforms of PKC (Sacktor et al., 1993). The antisera were made to epitopes in each isoform's C-terminal. This site was chosen for two reasons: first, the C-terminals were specific for each PKC known at that time, and second, because the PKC catalytic domain is C-terminal, we could detect the formation of the smaller PKM fragment by Western blot.

We found that, although hippocampus expressed nearly all the PKCs, only the single PKCζ isozyme (~72 kDa) was expressed as a PKM form at approximately 55 kDa (Sacktor et al., 1993; Naik et al., 2000). Immunoprecipitation of PKMζ from hippocampal extracts, followed by autophosphorylation, demonstrated the autonomous activity of the kinase (Sacktor et al., 1993).

By comparing PKC levels from tetanized CA1 regions to control CA1 regions from adjacent hippocampal slices that had received only test stimulation, we found that most of the isoforms were activated by translocation to membrane (Sacktor et al., 1993). However, this translocation was observed 15 s after the tetanus and rapidly reversed by 1 min. The only form of PKC to increase at a later time point, 40 min after the tetanization, was PKMζ (Sacktor et al., 1993; Osten et al., 1996b).

The increase in PKMζ correlated well with the persistence of LTP. A time course showed that the increase occurs 10 min after the tetanization and persists for at least 2 h (Osten et al., 1996b). The level of the persistent increase in PKMζ correlated significantly with the level of the persistent increase in synaptic transmission (Osten et al., 1996b). The increase in PKMζ was blocked by NMDAR antagonists (Sacktor et al., 1993; Osten et al., 1996b) and was specific for LTP, because we observed that PKMζ did not change with test stimulation alone, short-term potentiation (Osten et al., 1996b), or supramaximal tetanic stimulation that produced spreading depression (Osten et al., 1996a). Conversely, PKMζ decreased after a 3-Hz stimulation that produced long-term depression (LTD) (Hrabetova and Sacktor, 1996, 2001).

Because the only mechanism known at the time to form PKM was through proteolysis of PKC, we assumed in these early studies that PKMζ was formed from PKCζ by calpains (Sacktor et al., 1993), Ca^{2+}-dependent proteases that become active during LTP (Vanderklish et al., 1995) and could generate PKM *in vitro* (Kishimoto et al., 1983). Curiously, however, while we observed that PKMζ increased during LTP, we did not detect a concomitant decrease in what we believed to be PKCζ, i.e., we did not observe a precursor–product relationship (Osten et al., 1996b). We therefore guessed that there might be a newly synthesized pool of PKCζ that replaces the cleaved PKCζ. To test this hypothesis, we applied protein synthesis inhibitors, anisomycin or cycloheximide, during the LTP to reveal the precursor–product relationship (Osten et al., 1996b). As expected, the inhibitors blocked the persistent increase in synaptic potentiation. Unexpectedly, however, the inhibitors also prevented the persistent increase of PKMζ (Osten et al., 1996b). This experiment was the first indication that the formation of PKMζ required new protein synthesis and, therefore, was generated by a novel, nonproteolytic mechanism.

4.22.2.2 Protein Kinase Mζ Synthesis from an Internal Promoter Within the Protein Kinase Cζ Gene

The formation of PKMζ during LTP requires new protein synthesis because PKMζ in brain is produced not by proteolysis, but by the direct synthesis of the independent catalytic domain of PKCζ from the ζ gene (Hernandez et al., 2003). Because PKC isoforms are defined as distinct PKC gene products (Dekker et al., 1995; Nishizuka, 1995; Newton, 2001), PKMζ is now classified as an atypical PKC isoform (Hernandez et al., 2003).

The ζ gene produces two sets of RNAs from two distinct promoters (Marshall et al., 2000): a full-length PKCζ mRNA (Ono et al., 1989) and a PKMζ mRNA (Ono et al., 1988) (**Figure 1**). The 5′ end of the PKMζ mRNA is a unique sequence not present in the PKCζ mRNA, whereas its 3′ end is identical to that of PKCζ mRNA. The open reading frame (ORF) for the kinase domain of PKMζ mRNA begins in the hinge and extends to the end of the catalytic domain, thus encoding the PKMζ protein (Hernandez et al., 2003). By screening a panel of tissues, we found that both PKMζ mRNA and protein are expressed specifically in brain (except for a low abundant PKMζ mRNA in kidney that does not express detectable levels of protein) (Hernandez et al., 2003). (The only other brain-specific PKC isozymes are PKCγ (Kikkawa et al., 1987) and a form of PKCη (Sublette et al., 1993).) PKMζ is expressed in most, if not all regions of the brain, with twice as much of the protein present in forebrain structures, such as neocortex, striatum, and hippocampus, relative to hindbrain structures, cerebellum, and tegmentum (Naik et al., 2000).

Translational regulation is believed to be the primary mechanism for the initial increase of PKMζ in LTP. The PKMζ mRNA has a long 5′ untranslated region (UTR) and multiple short ORFs prior to

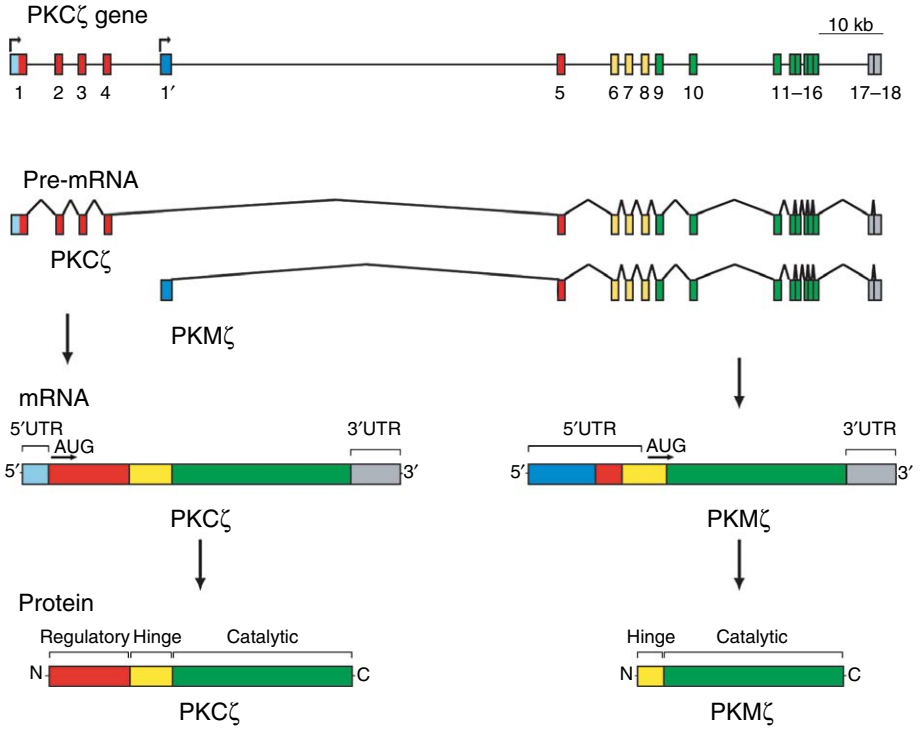

Figure 1 PKMζ mRNA formation from an internal promoter within the PKCζ gene. (Top) The intron–exon structure of the human PKCζ gene shows two exon clusters separated by a large intron: exons 1–4, encoding the PKCζ 5′UTR (light blue) and regulatory domain (red), and exons 5–18, encoding the remaining regulatory domain, hinge (yellow), catalytic domain (green), and 3′UTR (grey). The unique 5′PKMζ mRNA sequence is in a single exon (exon 1′, dark blue) within the large intron. PKCζ mRNA transcription begins at exon 1, resulting in full-length PKCζ (bottom left). Transcription from exon 1′ and splicing to exon 5 generates PKMζ mRNA, translation of which begins in the hinge to generate PKMζ (bottom right). From Hernandez AI, Blace N, Crary JF, et al. (2003) Protein kinase Mζ synthesis from a brain mRNA encoding an independent protein kinase Cζ catalytic domain. Implications for the molecular mechanism of memory. *J. Biol. Chem.* 278: 40305–40316; used with permission.

the kinase ORF, consistent with an inhibitory constraint of the 5′UTR on the translation of the message (Hernandez et al., 2003). This inhibitory mechanism was confirmed by sequential truncation of the 5′UTR, which greatly increased PKMζ translation *in vitro* (Hernandez et al., 2003). Furthermore, metabolic labeling of hippocampal slices with ^{3}H-methionine/cysteine followed by immunoprecipitation of PKMζ showed a large increase in the rate of PKMζ synthesis between 10 and 45 min after tetanization (Hernandez et al., 2003). Since the rate of transcription is 1–2 kb/min (Shilatifard, 1998) and the length of DNA encoding the PKMζ mRNA is approximately 100 kb (Hernandez et al., 2003), new transcription of PKMζ mRNA would begin only as early as roughly 1 h after tetanization.

Because the PKMζ message is dendritic (Muslimov et al., 2004), local translational regulation may provide a mechanism for the synapse specificity that is a characteristic feature of LTP, which would be important for its ability to store memory (Bliss and Collingridge, 1993). *In situ* hybridization with a specific probe to the 5′UTR of PKMζ mRNA showed PKMζ mRNA both in the pyramidal cell body layer and in dendritic layers of the hippocampus (Muslimov et al., 2004). The dendritic location of PKMζ mRNA was confirmed by observations of primary cultures of hippocampal neurons where individual neuritic processes could be clearly identified. Furthermore, injection of truncated versions of the PKMζ mRNA revealed two dendritic localization signals, one in the kinase ORF and a second in the 3′UTR. In addition to local dendritic synthesis, other mechanisms may also contribute to the synapse-specific effects of PKMζ in LTP, including synaptic tagging and capture (Sajikumar et al., 2005) (see the section titled 'Protein kinase Mζ maintains potentiation after synaptic tagging').

Although translational regulation is important for the initial increase of PKMζ in LTP, transcriptional upregulation may also contribute to more persistent increases in PKMζ. Indeed, an *in vivo* LTP study suggested a trend upward in the level of ζ mRNA at 2 and 24 h after tetanization (Thomas et al., 1994). The promoter region of the PKMζ mRNA in the mouse, rat, and human PKCζ genes contains a canonical cAMP response element (CRE), as well as putative sites for nuclear factor-κB (NF-κB) and CCATT/enhancer binding protein (C/EBP) (Hernandez et al., 2003). Each of these transcription factors has been implicated in memory formation (Dash et al., 1990; Alberini et al., 1994; Yin and Tully, 1996; Silva et al., 1998; Merlo et al., 2002; Yeh et al., 2002; Meffert et al., 2003). Interestingly, both the human and chimpanzee PKMζ promoters contain an additional partial CRE duplication immediately adjacent to the canonical mammalian CRE (Hernandez et al., 2003). Whether this duplication enhances CREB binding and aids the transcription of PKMζ during memory formation in primates is speculative, but the strong conservation of putative activity-dependent transcription factor binding regions in the PKMζ mRNA promoter strongly suggests that PKMζ mRNA transcription may be functionally important.

4.22.2.3 Regulation of Protein Kinase Mζ Synthesis by Multiple Protein Kinases and Actin Filaments in Long-Term Potentiation Induction

Following NMDAR activation, several multifunctional protein kinases are critical for inducing LTP, in particular CaMKII, mitogen-activated protein kinase (MAPK), and PKA (Roberson et al., 1996; Sweatt, 1999). Other kinases important for translational regulation, the lipid kinase, phosphatidylinositol 3-kinase (PI3-kinase) (Kelly and Lynch, 2000; Sanna et al., 2002; Opazo et al., 2003), and mammalian target of rapamycin (mTOR) (Tang et al., 2002; Cammalleri et al., 2003; Cracco et al., 2005) are also important for LTP induction. We found that selective inhibitors of each of these kinases block the synthesis of PKMζ in LTP (Kelly et al., 2007). Thus the actions of many disparate signaling molecules converge to regulate PKMζ synthesis.

Another important effector mechanism for LTP is the actin-based cytoskeleton. Although usually thought of as part of structural changes assumed to contribute to the expression of potentiation (Fukazawa et al., 2003; Lang et al., 2004; Okamoto et al., 2004; Huang et al., 2005; Ouyang et al., 2005; Kramar et al., 2006), the function of actin in LTP is unknown. Inhibition of actin filament formation by latrunculin B (which binds to monomeric G-actin and prevents its formation into filaments) blocks LTP induction (Kim and Lisman, 1999; Krucker et al., 2000; Fukazawa et al., 2003). Inhibition of actin filament formation with latrunculin also blocked PKMζ synthesis (Kelly et al., 2006). This is consistent with the notion that actin filaments serve as a scaffolding for assembling proteins important for translation (Stapulionis et al., 1997), particularly elongation factor 1A (Kandl et al., 2002), or perhaps as a local trafficking mechanism for targeting dendritic mRNAs into spines. In contrast to

the agent's effect when applied around the time of tetanization, when latrunculin was applied 1 h after tetanization, the drug did not reverse LTP maintenance (Kelly et al., 2006). Furthermore, latrunculin had no effect on PKMζ-mediated potentiation when placed together with the kinase in a whole-cell recording pipette (Kelly et al., 2006). Thus, actin filament formation regulates the synthesis of PKMζ and therefore is part of the signaling network of induction, rather than maintenance.

Although the induction period of LTP lasts only roughly 30–60 min after a tetanus, actin filament formation can be long-lasting, even weeks in the case of *in vivo* LTP at perforant pathway-dentate gyrus granule cell synapses (Fukazawa et al., 2003). This suggests the possibility that actin filament formation may persistently upregulate local PKMζ synthesis for maintaining increased levels of PKMζ in the face of its turnover.

Interestingly, PKMζ activity itself is also necessary for the *de novo* synthesis of PKMζ in LTP, because both chelerythrine, a PKC inhibitor that appears selective for PKMζ at low doses, and the PKMζ-selective inhibitor ζ-inhibitory peptide (ZIP) block PKMζ synthesis after tetanization and prevent LTP (Kelly et al., 2006). These results suggest the possibility of a positive feedback loop at the level of translation that might maintain the persistent increased PKMζ. Further characterization of this and other potential positive feedback mechanisms will be critical topics for future studies of long-term and remote memory persistence.

4.22.2.4 Phosphatidylinositol-Dependent Protein Kinase 1 Forms a Complex with Protein Kinase Mζ to Maintain Autonomous Kinase Activity in Long-Term Potentiation

Because PKMζ is an independent PKC catalytic domain without an autoinhibitory regulatory domain, it does not require stimulation by second messengers that are necessary for the activation of full-length PKCs, but instead is regulated by activity-dependent protein synthesis (Hernandez et al., 2003). In order for this increase in the amount of PKMζ to translate into an increase in phosphotransferase activity, the kinase must be enzymatically active. Many protein kinases, including PKCs, however, are inactive (or with relatively little activity) immediately after synthesis. This is because their kinase domains are initially in an inactive conformation that inefficiently binds protein substrates. These kinases require an additional kinase, phosphatidylinositol-dependent protein kinase 1 (PDK1), to phosphorylate the activation loop of their catalytic domain to obtain a maximally active conformation (Newton, 2003; Biondi, 2004).

The specific cellular role of PDK1 phosphorylation is different for the various classes of PKC. For cPKCs, PDK1 phosphorylation is constitutive, rendering the kinases competent for subsequent regulation by second messengers (Newton, 2003). In contrast, for full-length aPKCs, like PKCζ, PDK1 phosphorylation is regulated and is an important part of aPKC activation by extracellular signals (Chou et al., 1998; Le Good et al., 1998). Growth factors or hormones, most notably insulin, stimulate PI3-kinase, producing phosphatidylinositol-(3,4,5)-triphosphate (PIP3) that can bind to both the regulatory domain of PKCζ and PDK1. A PDK1-binding site in the catalytic domain of PKCζ can also serve to direct interactions between the two kinases (Balendran et al., 2000). PDK1 then phosphorylates the activation loop of PKCζ, increasing its enzymatic activity.

We found that the function of PDK1 phosphorylation of PKMζ is different from that of the other PKCs and may be essential for PKMζ's role in memory persistence (Kelly et al., 2007). In brain, basal PKMζ directly binds to PDK1, which maximally phosphorylates the PKC on its activation loop. Within 1 h of LTP, the newly synthesized PKMζ is also maximally phosphorylated. Thus, in contrast to full-length PKCζ, in which activation loop phosphorylation is regulated, PKMζ is constitutively phosphorylated, ensuring maximal autonomous activity of the kinase for sustaining responses in neurons.

To summarize this section, PKMζ is a unique, autonomously active isoform of PKC derived from an internal promoter within the PKCζ gene. The PKMζ mRNA is transported to dendrites, and strong afferent tetanization that produces LTP increases the *de novo* synthesis of PKMζ. This initial step is regulated by NMDAR activation, as well as by many signaling molecules and pathways critical for LTP induction, including CaMKII, MAP, PKA, mTOR, and actin filament formation. The newly synthesized PKMζ rapidly binds to PDK1 and is phosphorylated on its activation loop to obtain maximal autonomous activity. In hippocampal slices, the increase in PKMζ protein persists for hours after tetanization and correlates with the degree of synaptic potentiation. The next section examines the function of this persistent increased activity in maintaining LTP.

4.22.3 Protein Kinase Mζ Is Both Necessary and Sufficient for Long-Term Potentiation Maintenance

4.22.3.1 Protein Kinase Mζ Potentiates Synaptic Transmission by Persistently Upregulating Postsynaptic AMPAR Trafficking

Does the persistent kinase activity of PKMζ maintain LTP? Is it necessary and sufficient for the persistent enhancement of synaptic transmission? To examine the question of sufficiency, PKMζ was recombinantly expressed and purified from the baculovirus/*Spodoptera frugiperda* (*Sf*9) cell overexpression system, in which endogenous PDK1 of the *Sf*9 insect cells phosphorylates the activation loop of the overexpressed PKMζ (Ling et al., 2002). We then patched CA1 pyramidal cells in hippocampal slices with low concentrations (1–3 nmol l^{-1}) of the purified kinase in the whole-cell recording solution (**Figure 2(a)**), thus bypassing the synthesis of the kinase that occurs during LTP induction (Ling et al., 2002). Diffusion of PKMζ into cells rapidly doubled the amplitude of the evoked alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionate receptor (AMPAR)-mediated excitatory postsynaptic current (EPSC), which then stabilized for the length of the recordings. Bath application of the PKC catalytic domain inhibitor chelerythrine (Herbert et al., 1990) reversed the potentiation to baseline, indicating that PKMζ activity was continually required for the synaptic potentiation. Bath applications of the PKMζ-selective inhibitor ZIP also prevented the potentiation (Serrano et al., 2005) (**Figure 2(a)**).

PKMζ is by far the most potent potentiating substance known. In comparison, postsynaptic perfusion of 100–1000 times more CaMKII produces approximately half the potentiation of AMPAR responses (Lledo et al., 1995; Shirke and Malinow, 1997). PKMζ-mediated increases in EPSCs completely occluded LTP induced by tetanization paired with postsynaptic depolarization, indicating that PKMζ and LTP enhance transmission through the same mechanism (Ling et al., 2002).

We next determined the site of synaptic transmission enhanced by PKMζ (Ling et al., 2006). LTP affects several sites of synaptic transmission, both pre- and postsynaptic (Bliss and Collingridge, 1993; Malenka and Bear, 2004). It is important to note, however, that almost all the studies of LTP expression have focused on its early phase, not its late phase. As we will see in the section titled 'Protein kinase Mζ, maintains late long-term potentiation,' however, PKMζ functions specifically in the late phase. As all possibilities were

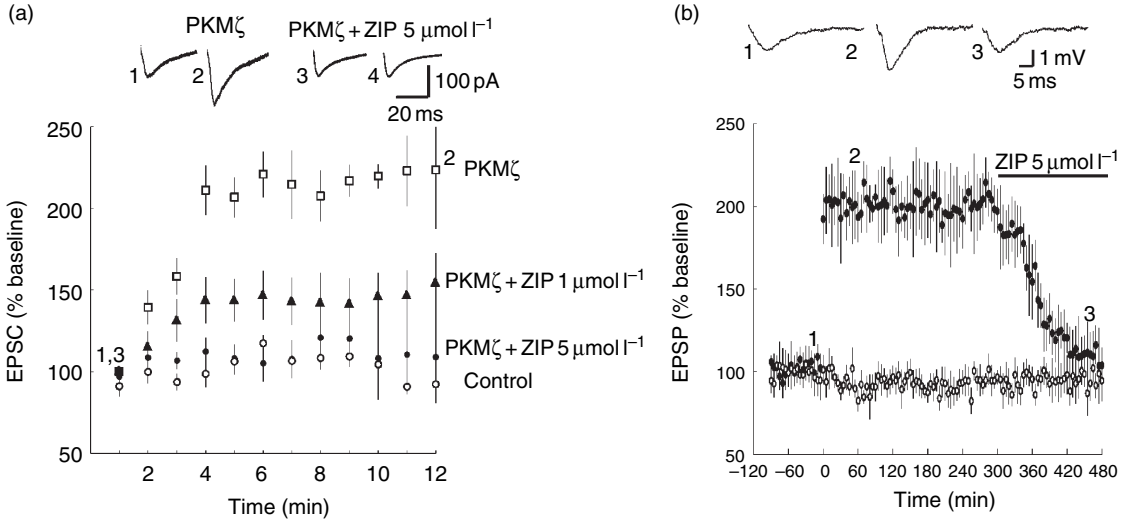

Figure 2 PKMζ is necessary and sufficient for LTP maintenance. (a) Whole-cell perfusion of PKMζ into a CA1 pyramidal cell potentiates AMPAR-mediated synaptic transmission. A total of 1 μmol l^{-1} ZIP applied to the bath partially blocks and 5 μmol l^{-1} ZIP fully blocks the PKMζ-mediated potentiation of AMPAR responses. (b) A total of 5 μmol l^{-1} ZIP applied to the bath 5 h after tetanization reverses LTP maintenance, but does not affect synaptic transmission of a control pathway simultaneously recorded within the hippocampal slice. EPSC, excitatory postsynaptic current; EPSP, excitatory postsynaptic potential. From Serrano P, Yao Y, and Sacktor TC (2005) Persistent phosphorylation by protein kinase Mζ maintains late-phase long-term potentiation. *J. Neurosci.* 25: 1979–1984; used with permission.

open, we examined multiple potential sites for the action of PKMζ on AMPAR-mediated synaptic transmission (Ling et al., 2006). Analysis of the coefficient of variance of evoked responses recorded from CA1 pyramidal cells before and after PKMζ-mediated potentiation showed no evidence of retrograde presynaptic enhancement. Consistent with this result, postsynaptic PKMζ perfusion doubled the amplitude of AMPAR-mediated, miniature excitatory postsynaptic currents (mEPSCs), without affecting their frequency, confirming that PKMζ enhanced synaptic transmission at a postsynaptic site. These results also indicate that PKMζ enhances synaptic transmission without unsilencing NMDAR-only, silent synapses, which would also have led to changes in the coefficient of variance of evoked responses and an increase in mEPSC frequency. The kinetics of the mEPSCs during PKMζ-mediated potentiation, dendritic filtering, and input resistance of the cell also did not change with PKMζ-mediated synaptic enhancement.

This left two possible sites for PKMζ-mediated potentiation of AMPAR responses: channel unit conductance and the number of active channels (defined as the product of the number of physical channels and their open probability). To distinguish between these two sites of action, we used a statistical technique: peaked-scale, nonstationary fluctuation analysis (Traynelis et al., 1993) (NSFA), also referred to as noise analysis, to examine the effect of PKMζ on mEPSCs. NSFA showed that PKMζ did not affect unit conductance, but doubled the number of active AMPAR channels. The doubling was rapidly reversed by applying chelerythrine to PKMζ-potentiated mEPSCs (Ling et al., 2006).

These results indicated that PKMζ enhances synaptic transmission exclusively by increasing the number of functional postsynaptic AMPARs. As mentioned, however, peaked-scale NSFA does not distinguish between an increase in the number of physical channels and an increase in open probability. The open probability of AMPAR channels on CA1 pyramidal cells, however, has been suggested to be already high in its basal state (~0.9) and not to increase with LTP (Andrasfalvy and Magee, 2004), suggesting that a change in the number of receptors was the more likely possibility. Therefore, we explored further the mechanisms of AMPAR trafficking that PKMζ might regulate to increase the numbers of postsynaptic receptors.

AMPARs rapidly move into and out of the synapse through several distinct pathways. One of these pathways involves the trafficking protein *N*-ethylmaleimide-sensitive factor (NSF) that interacts directly with the AMPAR subunit glutamate receptor 2 (GluR2) in order to maintain the receptors at postsynaptic sites (Nishimune et al., 1998; Osten et al., 1998; Song et al., 1998). Inhibiting NSF–GluR2 interactions by a peptide, called pep2m (Nishimune et al., 1998), that mimics the NSF binding site on GluR2 and prevents their interaction, blocked PKMζ-mediated potentiation of AMPARs (Yao and Sacktor, 2006). Furthermore, bath applications of a cell-permeable myristoylated version of the peptide both blocked PKMζ-mediated potentiation and reversed the late phase of LTP (as observed for the PKMζ kinase inhibitor ZIP; see the section titled 'Protein kinase Mζ maintains late long-term potentiation'). In contrast, inhibiting a second AMPAR trafficking pathway that involves vesicle-associated membrane protein (VAMP)/synaptobrevin-mediated, constitutive exocytosis of receptors from internal stores to the plasma membrane (Luscher et al., 1999) had no effect on PKMζ-potentiation of AMPARs. The NSF/GluR2-dependent pathway may involve lateral movement of plasma membrane receptors into the synapse (Gardner et al., 2005). Consistent with this mechanism in LTP, the AMPAR subunits GluR2 and 3 were found to increase in synaptosomes through the action of PKMζ and NSF/GluR2-dependent trafficking, whereas the total level of surface GluR2 (and its total level overall) did not change (Kelly and Sacktor, 2006). Thus PKMζ enhances synaptic transmission through a specific molecular mechanism – persistently increasing NSF/GluR2-mediated trafficking of AMPARs from extrasynaptic to postsynaptic sites.

4.22.3.2 Atypical Protein Kinase M Increases Synaptic Size

Although persistent enhancement of AMPAR trafficking to the synapse is the mechanism of synaptic potentiation by PKMζ, it may not be the only PKMζ-mediated mechanism important for the maintenance of LTP and long-term memory. One indication that PKMζ also affects the structure of synapses comes from the study of the *Drosophila* atypical PKM. Unlike vertebrates that have two aPKC genes, PKCζ and PKCι/λ, *Drosophila* has a single gene, DaPKC (Drier et al., 2002). DaPKC also comes in a PKM form, which, as we will see in the section titled 'Protein kinase Mζ enhances the persistence of associative memory in *Drosophila*,' has a critical role in persistence of memory in the fly (Drier et al., 2002).

A well-characterized model system for synapse development in *Drosophila* is the neuromuscular junction, a glutamatergic synapse for which there are techniques for overexpression of genes at either the pre- or postsynaptic side (Ruiz-Canada et al., 2004). On the presynaptic side, overexpression of DaPKC regulates the stability of microtubules by promoting their association with the microtubule-associated protein 1B (MAP1B)-related protein, Futsch (Ruiz-Canada et al., 2004). On the postsynaptic side, which corresponds to the site of the increase of PKMζ in LTP, overexpression of DaPKM increases the size of the synapse by regulating the synaptic cytoskeleton through control of the extent of actin-rich and microtubule-rich areas (Ruiz-Canada et al., 2004). Interestingly, both the post- and presynaptic sides appeared to increase in size after postsynaptic overexpression, indicating a retrograde effect across the synapse. Conversely, postsynaptic overexpression of a dominant negative form of DaPKM decreased postsynaptic as well as presynaptic size.

Curiously, overexpression of postsynaptic DaPKM decreased the density of glutamate receptors and attenuated synaptic transmission at the neuromuscular junction, opposite to the effect of postsynaptically perfused PKMζ at hippocampal synapses. One possible explanation for this difference is that the overexpression of the kinase in the fly was relatively slow and in a developmental context, whereas the perfusion of PKMζ was rapid into a mature synapse. Indeed, rapid overexpression of PKMζ in a mature fly had no effect on motor responses to shock (Drier et al., 2002). Another interesting possibility is that the NSF/GluR2-mediated trafficking mechanism for driving AMPARs into a hippocampal synapse is absent in the fly neuromuscular junction.

Recent work suggests that the size of hippocampal synapses in culture may also be increased by overexpression of PKMζ and be decreased through inhibition of endogenous PKMζ (Sondhi et al., 2006). This regulation appears to be mediated through actions on the state of postsynaptic density 95 (PSD-95) aggregation.

Thus there may be two arms to PKMζ's effects at synapses: an AMPAR trafficking pathway that increases synaptic strength and a structural modification pathway that increases the size and, perhaps, the longevity of synapses, which correlates with synaptic size (Holtmaat et al., 2006). In the next section we will examine when these PKMζ actions on synapses are critical for maintaining LTP.

4.22.3.3 Protein Kinase Mζ Maintains Late Long-Term Potentiation

If PKMζ is sufficient for synaptic potentiation, is it necessary for the maintenance of LTP? As mentioned in the introduction, although many inhibitors block LTP induction when applied around the time of tetanization, none had reversed established late-phase LTP prior to our work on PKMζ (Ling et al., 2002). In our initial investigation, we examined LTP maintenance 1 h after tetanization by comparing the effects of applications of two PKMζ inhibitors: chelerythrine, an inhibitor of the catalytic domain of PKC, which selectively inhibits PKMζ at low concentrations relative to conventional/novel PKCs (c/nPKCs) and CaMKII (Ling et al., 2002); and the myristoylated ζ-pseudosubstrate peptide (ZIP), a cell-permeable selective PKMζ inhibitor (Bandyopadhyay et al., 1997; Ling et al., 2002; Braun and Mochly-Rosen, 2003). As a control, we examined staurosporine, a general kinase inhibitor that blocks c/nPKCs, CaMKII, and PKA, but not atypical PKCs such as PKMζ (McGlynn et al., 1992; Kochs et al., 1993; Ling et al., 2002). Consistent with several earlier studies (Denny et al., 1990; Muller et al., 1992), staurosporine, while blocking LTP induction when applied prior to tetanization, had no effect on maintenance when bath-applied 1–5 h after tetanization (Ling et al., 2002). Therefore, CaMKII, c/nPKCs, PKA, and the many other protein kinases that are blocked by staurosporine are not required for LTP maintenance. In contrast, both chelerythrine and ZIP reversed maintenance when applied 1 h after tetanization. The reversal was specific to potentiated synapses because a nontetanized pathway, simultaneously recorded in each experiment, remained stable.

Because the ability to reverse established LTP is very unusual, we explored the effect of PKMζ inhibition by ZIP on LTP maintenance in more detail (Serrano et al., 2005). In general, in order to avoid nonspecific effects of a drug, it is important to use the lowest concentration that effectively blocks the relevant function of the target in the cellular context of interest. We therefore determined the lowest concentration of ZIP that, when applied to the bath of hippocampal slices, would block the ability of PKMζ to enhance synaptic transmission by intracellular perfusion of the kinase. We found that 1 $\mu mol\,l^{-1}$ attenuated the enhancement, whereas 5 $\mu mol\,l^{-1}$ ZIP completely blocked PKMζ-mediated potentiation of synaptic transmission (**Figure 2(a)**).

We then bath-applied 5 μmol l^{-1} ZIP continuously throughout an LTP experiment, and found that there was a residual potentiation in the presence of the inhibitor that was indistinguishable from the early, protein synthesis-independent phase of LTP (Serrano et al., 2005). Therefore, PKMζ did not contribute to early LTP.

We then examined the ability of 5 μmol l^{-1} ZIP to reverse established LTP when applied 1, 3, and 5 h after tetanization (**Figure 2(b)**) (Serrano et al., 2005). The agent reversed LTP at all time points, but had no effects on control untetanized pathways, simultaneously recorded within each slice. Furthermore, the IC50 of ZIP for reversal of established late LTP at 3 h was nearly identical to its IC50 for the ability to block PKMζ-mediated potentiation of AMPAR responses. These results indicated that the persistent activity of PKMζ is required for maintaining late LTP.

4.22.3.4 Protein Kinase Mζ Maintains Potentiation after Synaptic Tagging

PKMζ is synthesized by strong afferent stimulation of synapses that produces late LTP, but not weak afferent stimulation that produces only transient potentiation (Osten et al., 1996b; Hernandez et al., 2003) and maintains late but not early LTP (Serrano et al., 2005). How does newly synthesized PKMζ affect only specific, activated synapses? One possibility is that the kinase might be involved in the process of synaptic tagging and capture (Frey and Morris, 1997; Sajikumar et al., 2005). Synaptic tagging is the hypothesis, first proposed by Frey and Morris, to explain how newly synthesized plasticity-related proteins (PRPs), which are presumably synthesized away from synapses in the soma or dendrites, can maintain potentiation specifically at recently activated synapses but not at neighboring synapses that had not been activated. They proposed that a tag could be set at activated synapses to which the PRPs might bind or be captured. To test their hypothesis, they showed that a weak tetanization, which normally would produce only early, decremental LTP, could nonetheless result in persistent late LTP, if late LTP had been produced elsewhere in the neuron by an independent synaptic pathway (Frey and Morris, 1997). In their model, the weak tetanization, although not strong enough to stimulate protein synthesis, could still set synaptic tags that could capture the PRPs that had been synthesized by the strongly tetanized input.

Sajikumar and Frey (Leibniz Institute for Neurobiology, Magdeburg, Germany) tested the hypothesis that PKMζ was a PRP that could be captured to maintain potentiation at tagged synapses (Sajikumar et al., 2005). They first showed that ZIP reversed late LTP, as expected, but did not affect the maintenance of the transient early LTP. Then during a tagging experiment, when the early LTP pathway would normally have been converted to late LTP, they found that ZIP reversed potentiation at both the strongly and the weakly tetanized synapses (Sajikumar et al., 2005). These results demonstrated that persistent PKMζ activity maintains potentiation not only at strongly tetanized synapses, but also is critical for the conversion of early into late LTP at tagged synapses.

Sajikumar and Frey had also discovered another type of tagging phenomenon known as cross-tagging, which revealed a surprising relationship between LTP and LTD. They found that strong LTD, just like strong LTP, could allow a subsequent weak tetanus to produce late LTP. Conversely, strong LTP could allow a subsequent weak LTD stimulus to produce a late, long-lasting depression. Is PKMζ's role to produce the persistence of any change in synaptic strength, regardless of its direction, or is it specific to the persistence of potentiation? Sajikumar and Frey first found that ZIP had no effect on reversing LTD maintenance (although it did block LTD induction). They then showed that ZIP could nonetheless reverse the persistent synaptic potentiation produced by cross-tagging after strong LTD. On the other hand, ZIP did not affect the persistent depression produced by cross-tagging after strong LTP, even though the agent reversed the persistent potentiation produced by the strong tetanization. These results demonstrated that PKMζ specifically maintains synaptic potentiation, not depression, and were consistent with our results showing that the postsynaptic perfusion of PKMζ caused potentiation of synapses.

How is PKMζ's role in synaptic tagging commensurate with the possibility that the kinase is produced from a dendritic mRNA during LTP? One notion is that activity-dependent synthesis of PKMζ is local in dendrites, but that tagging is required for finer compartmentalization of the synthesized PKMζ to only activated synapses. Alternatively, the phenomenon of tagging may be caused by metaplastic changes in the efficiency of dendritic local synthesis (Tsokas et al., 2006). Regardless of which is correct, the results of this section showed that the inhibition of PKMζ

rapidly reverses late LTP maintenance. This meant that, for the first time, there was a tool to test the hypothesis that the molecular mechanism of LTP maintenance, the persistent activity of PKMζ, stores information in behaving animals.

4.22.4 Protein Kinase Mζ and Memory Storage

4.22.4.1 Protein Kinase Mζ Enhances the Persistence of Associative Memory in *Drosophila*

PKMζ's role in behavior was first examined by Jerry Yin, Eric Drier, and colleagues at the University of Wisconsin in *Drosophila*, a model system with several advantages for the study of the molecular pathways of learning and memory. One advantage is the ability to rapidly express transgenes through the heat shock promoter, allowing expression of proteins in less than 1 h. A second advantage is that a relatively simple behavior, associative olfactory conditioning (Quinn et al., 1974; Aceves-Pina et al., 1983), has been well characterized, with various stimulation protocols producing different forms of memory: A single training session produces short-term memory lasting a few hours, mass training (i.e., multiple training sessions with short between training intervals) produces memory lasting around a day, and spaced training produces long-term memory lasting several days.

Either DaPKM or mouse PKMζ overexpressed by the heat shock promoter shortly after short-term memory training converted the behavioral response into 24-h memory (Drier et al., 2002). Similarly, overexpression of either transgene could convert a mass-trained memory lasting 1 day into a memory lasting 4 days, the phenotype of long-term memory (Drier et al., 2002). Conversely, overexpression of a dominant negative version of the mouse PKMζ (Lallena et al., 1999; Romanelli et al., 1999; Drier et al., 2002) (which also blocked LTP (Ling et al., 2002)), or feeding flies the PKC inhibitor chelerythrine, prevented the persistence of memory after mass training, but did not affect initial learning (Drier et al., 2002). The ability of mouse PKMζ to substitute for fly DaPKM demonstrates strong evolutionary conservation of PKMζ function.

Surprisingly, the overexpression of PKMζ has to occur in a narrow time window of less than 1 h after the training. Overexpression prior to training or 2 h after had no effect on memory enhancement. This suggests that exogenous PKMζ has to be overexpressed at the time when endogenous DaPKM would have been synthesized in response to synaptic activity, if flies generated DaPKM during memory formation as hippocampal neurons do in LTP. Perhaps just as remarkable, the enhancement of memory by the transgenic expression of PKMζ by the heat shock promoter occurs in all tissues of the fly. Therefore being at the right time appears essential for the ability of PKMζ to prolong memory, but being at the right place is not. As noted by Yin and colleagues (Drier et al., 2002), these results are consistent with the notion of synaptic tagging, in which PKMζ is a newly synthesized PRP that is captured by a synaptic tag, set in the fly by behavioral short-term memory, rather than in hippocampal slices by early LTP.

PKM forms of PKC may also be important in other forms of memory in invertebrates. A constitutively active form of PKC, i.e., PKC activity not requiring regulatory domain activators such as Ca^{2+} or DAG, was observed to increase after long-term olfactory conditioning in the antennal lobes of the honeybee (Grunbaum and Muller, 1998). A transient increase required proteolysis, whereas a persistent increase lasting for days required new protein synthesis, like PKMζ formation in LTP. In *Aplysia*, a PKM form of PKC may be generated by proteolysis, rather than protein synthesis, to mediate an intermediate form of activity-dependent facilitation (Sutton and Carew, 2000).

This sampling of various species of different phyla suggests that PKM might be formed from PKC by either proteolysis or synthesis to mediate memory, but that mammals (at least as assayed by hippocampal LTP) had selected the synthetic pathway. This suggested that the persistent activity of PKMζ might maintain a form of memory in mammals.

4.22.4.2 Protein Kinase Mζ Maintains Long-Term Memories in Multiple Regions of the Brain

In order to translate our findings from hippocampal slices to behavior, we began by focusing on the role of PKMζ in spatial memory that is initially encoded and stored in the hippocampus (Pastalkova et al., 2006). Because the synapses that are thought to encode spatial information are widely distributed in the hippocampus (Moser and Moser, 1998; Kubik and Fenton, 2005), to test the hypothesis that PKMζ maintained this information it was important to inhibit PKMζ widely in the hippocampus. We

therefore examined *in vivo* LTP at perforant path-dentate gyrus synapses, using an array of recording electrodes spaced up to 2 mm away from the injection site in CA3. We determined that 10 nmol ZIP (in a 1-μl bolus) rapidly reversed LTP when injected 22 h after LTP induction. Labeling the ZIP peptide with biotin showed that the agent diffused approximately 3–4 mm within the hippocampus but not to other brain regions.

We then were in a position to examine the role of PKMζ in maintaining hippocampus-dependent spatial memory. We chose active place avoidance (Cimadevilla et al., 2000a,b, 2001; Wesierska et al., 2005), a behavior with several experimental advantages for the initial study of PKMζ function in spatial memory. Hippocampus inactivation by tetrodotoxin blocks both initial learning and short-term memory, as well as long-term memory retention of active place avoidance (Cimadevilla et al., 2000a, 2001). This would allow us to distinguish between an effect of PKMζ inhibition on short-term and long-term retention of the conditioned responses. Learning is rapid (optimal performance observed in roughly 2.5 h of training) and remains hippocampus-dependent for at least 1 month, allowing us also to examine the role of PKMζ in remote spatial memory.

The apparatus for active place avoidance consists of a slowly rotating platform, open to the room environment, within which a nonrotating 60-degree sector is a shock zone (Cimadevilla et al., 2000b). The rotation brings the animal into the shock zone, and the animal rapidly learns to avoid the shock by actively moving to the nonshock areas of the platform. After an initial 10-min exposure to the apparatus without shock, rats were trained in eight 10-min sessions with the shock on, separated by 10-min rest intervals in their home cages.

Twenty-two hours after the last training session, we injected either ZIP or saline into both hippocampi. Two hours later, retention testing on the apparatus without shock (i.e., extinction testing) showed that the saline-injected animals exhibited long-term memory by avoiding entry into the shock zone. In contrast, the ZIP-injected animals failed to avoid the shock zone and actively explored the entire apparatus as if naive. Whereas the ZIP-injected animals did not recall the long-term memory, spending time in the shock zone at the level of chance, immediate reconditioning and then retesting without the shock showed that the animals could nonetheless recall the short-term memory of the conditioned response. These results indicate that ZIP produces retrograde amnesia specifically for long-term memory, not short-term memory of place avoidance. Staurosporine, the nonspecific kinase inhibitor that blocks conventional and novel PKCs, CaMKII, and PKA, and which blocks LTP induction but not maintenance (Ling et al., 2002), had no effect on long-term memory retention at a dose that completely prevented learning when injected prior to training.

A critical question concerning the effect of PKMζ inhibition of memory retention is whether ZIP disrupts information retrieval or information storage. One way to distinguish between these possibilities is to inject ZIP after long-term memory has been established, wait for the drug to be eliminated, and then test whether the memory returns. If the conditioned behavior returns, this would indicate an effect on information retrieval; if the memory were persistently eliminated, this would indicate an effect on storage. We therefore trained animals and then 1 day later injected ZIP without testing. One week later (when the ZIP had been eliminated), the previously ZIP-injected animals had no recall of the long-term memory, whereas the saline-injected animals avoided the shock zone. Then to test whether ZIP caused permanent damage to memory function, we immediately retrained the previously ZIP-injected animals and found that they learned and could remember place avoidance as a new long-term memory. Thus ZIP erases previously acquired memory, but does not affect the ability to subsequently store new long-term memory.

We also examined whether memories older than 1 day were also sustained by PKMζ and found that 1-month-old place avoidance memory could be eliminated by ZIP.

Subsequent to our initial study, other forms of long-term memory have also been shown to be maintained by PKMζ. Spatial accuracy in the water maze and in the appetitively conditioned eight-arm radial maze are strongly affected by intrahippocampal injections of ZIP after training (Serrano et al., 2007). In the latter, the deficit resulted from increases of reference memory errors and not working memory errors, indicating a specific effect on spatial information.

Other areas of the brain in which memories are thought to be stored have also been examined. ZIP injection in the basolateral amygdala complex inhibited memory retention for auditory fear conditioning (Friedman et al., 2007). Injection in the insular neocortex erased memory for conditioned taste preference (Shema et al., 2007) either days or weeks after training, yet ZIP had no anterograde effect on long-term

memory formation when injected immediately prior to training. As in place avoidance, the memory for conditioned taste aversion did not return even 1 month after the agent's injection. Because the neocortex is thought to be the ultimate repository of many long-term memories, these results indicate that the persistent activity of PKMζ is a general brain mechanism for long-term memory storage.

4.22.4.3 Protein Kinase Mζ and Alzheimer's Disease

Because of PKMζ's critical function in memory storage, we examined the role of the kinase in the most common disorder of memory, Alzheimer's disease (AD) (Crary et al., 2006). The characteristic features of the pathology of AD are extracellular amyloid plaques and intracellular neurofibrillary tangles (Braak and Braak, 1991). Using antisera specific to each of the PKC isoforms, we found that only PKMζ and the other atypical PKCι/λ were present in a subpopulation of tangles. Aggregates of AMPARs GluR1 and GluR2 were also associated with the aggregates of PKMζ in perisomatic granules, another neuropathological feature of AD (Aronica et al., 1998; Probst et al., 2001). Several other neurodegenerative diseases also are characterized by tangles, frontotemporal dementia (Pick's disease), corticobasal degeneration, and progressive supernuclear palsy, collectively known as tauopathies. These tangles contain PKCι/λ, but not PKMζ (Shao et al., 2006).

PKMζ has two unique features compared to all other known components of the tangles in AD. Tangles are primarily composed of hyperphosphorylated tau, as well as smaller amounts of other molecules, including ubiquitin and several protein kinases, notably glycogen synthase kinase-3β. Tangles are found widely in the brains of patients with AD, and most of its molecular components are present in the tangles from all of these regions (Papasozomenos, 1989). PKMζ, in contrast, is found in tangles only in the limbic system and medial temporal lobe, i.e., hippocampus, entorhinal cortex, and amygdala. PKMζ is not found in tangles in regions of the neocortex, despite the widespread expression of PKMζ in human neocortex. PKMζ is also not in the tangles present in nonlimbic subcortical regions, including nucleus basalis of Meynert and locus ceruleus. This distribution of aggregated PKMζ only to limbic structures suggests the possibility that high levels of PKMζ may be generated as a compensatory mechanism in some remaining healthy neurons in memory-encoding regions, as synaptic plasticity generally fails in affected neurons because of the burden of amyloid and neurofibrillary degeneration.

The second unusual feature of PKMζ is that it is found only in the tangles of patients with dementia. Some tangles in the limbic areas are a common finding in the elderly without dementia. These tangles had previously been found to be indistinguishable at the molecular level from the tangles in the brains of patients with AD. PKMζ, however, was not present in the tangles of the elderly individuals without known dementia. This suggests that the aggregation of PKMζ may signal the exhaustion of the compensatory increased plasticity, mentioned earlier, thus leading to memory loss, the characteristic symptom of AD.

4.22.5 Conclusions and Future Prospects

The unique structure and function of the PKMζ gene, mRNA, and protein provides a simple molecular mechanism of memory storage through synthesis of a persistently active protein kinase (Sacktor et al., 1993; Hernandez et al., 2003). This mechanism is radically different from the standard notion of memory storage as a passive change in the large-scale structure of synaptic connections between neurons. Although the standard model may yet prove to be important for memory storage in invertebrates and in some forms of memory in the mammalian brain, the persistent activity of PKMζ maintains spatial memory in hippocampus, fear memory in the amygdala, and associative memory in the neocortex.

Thus PKMζ is a fundamental biological molecule for information storage in the brain. How did this mechanism of storage evolve? A clue to its origin may be in the general function of atypical PKC in other cellular contexts. In many different cell types, from *Caenorhabditis elegans* (Izumi et al., 1998; Tabuse et al., 1998; Wu et al., 1998) to *Drosophila* (Wodarz et al., 2000) to mammalian species (Lin et al., 2000; Qiu et al., 2000), atypical PKC is critical for the establishment and maintenance of cell polarity within cells and junctional complexes between cells (Ohno, 2001), including neurons early in development (Shi et al., 2003). At many of these polarized regions of the cell and junctions, the activity of atypical PKC regulates the assembly of various receptors and structural

proteins. Because of PKCζ's role in the regulation of protein trafficking at junctions in general, the kinase may have been easy to adapt to a similar role in the synaptic junctions between neurons. The key development for the formation of long-term memory, the formation of the internal promoter within the PKCζ gene that generates the persistent activity of PKMζ, may have been an evolutionary accident. At synapses, the formation of PKMζ may lead to persistently enhanced transmission, but outside neurons, the constitutive atypical PKC activity of PKMζ would most likely have been developmentally lethal. Therefore, only a promoter capable of neuron-specific regulation might benefit the organism. How the expression of PKMζ then came to be under the control of strong but not weak synaptic activity is a mystery, but as Francis Jacob famously said, "Evolution is a tinkerer" (Jacob, 1977). One may surmise, however, that any animal with even a rudimentary capacity for long-term memory as a mechanism to adapt to its environment would have considerable evolutionary advantage. In this context, the recently evolved, tandem CREB-binding site in the PKMζ promoter of chimpanzees and humans is of particular interest (Hernandez et al., 2003).

Several important avenues are now open for future investigation. First, what are the substrates of PKMζ that mediate its function at synapses? Although both AMPAR trafficking through NSF/GluR2-mediated mechanisms and structural changes by PSD-95 may be downstream of PKMζ, the specific substrates mediating these processes are unknown. Unfortunately, identifying functionally important substrates of any kinase is a hit-or-miss affair, and there is no straightforward path other than informed guesswork.

Second, because the effects of PKMζ are essential for long-term memory maintenance yet are rapidly reversible, memory storage can now be viewed as a much more dynamic process than conceived of in the purely structural growth model, and one that is more amenable to experimental, even therapeutic, manipulation. Injections of ZIP eliminate previously established LTP maintenance but spare short-term synaptic plasticity and basal synaptic transmission. Thus for the first time, the information storage function of a brain region can be distinguished from its information processing function, including its role in transferring information to another brain region. Can other dynamic features of long-term memory, such as reconsolidation, now be explained by the degradation and resynthesis of PKMζ? Can we visualize the engram by monitoring changes in PKMζ after conditioned behavior? Can human disorders thought to be mediated by pathophysiologically enhanced LTP, such as neuropathic pain, posttraumatic stress, even addictive behavior, now be reversed by local injections of ZIP?

Third, what do the basic properties of PKMζ tell us about memory? What is the half-life of PKMζ? Presuming it is shorter than 1 month, a time when memories are still maintained by the persistent activity of the kinase, how then could PKMζ sustain memory beyond the half-life of the PKMζ protein? Are there multiple transcriptional, translational, and posttranslational positive feedback mechanisms that maintain persistently increased PKMζ at specific synapses? Perhaps there will be a network of molecules maintaining PKMζ, just as there is a network of molecules initiating its formation in LTP induction. Whether such a putative maintenance network is large, involving persistent transcriptional or translational upregulation, or small, involving perhaps only a single synaptic substrate of PKMζ with which it interacts, we are only at the beginning of our understanding of PKMζ, LTP maintenance, and memory storage.

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4.23 CaMKII: Mechanisms of a Prototypical Memory Model

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4.23.1 Introduction

Elucidating the molecular basis for complex behaviors like learning and memory is one of the largest challenges currently facing neuroscientists. It is generally accepted that such complex behaviors reflect changes in connectivity in neuronal circuits, which are believed to arise due to the intrinsic ability of individual synapses to adapt their properties in the face of changes in the ongoing activity. Consequently, synaptic plasticity in several brain regions has been studied as a more tractable surrogate for learning and memory, and many molecules implicated in synaptic plasticity have been linked to behavioral consequences.

The rodent hippocampus is the most widely studied model system for synaptic plasticity, particularly the glutamatergic synapses formed by Schaffer collateral projections of CA3 neurons to dendrites of CA1 pyramidal neurons. In general, strong stimulation (e.g., brief high-frequency stimulation) results in long-term potentiation (LTP), whereas more prolonged weaker stimulation paradigms (e.g., low-frequency stimulation) result in long-term depression (LTD). Both LTP and LTD at these synapses require Ca^{+2} influx via postsynaptic *N*-methyl-D-aspartate (NMDA)-type glutamate receptors (NMDARs) and generally are restricted to the same synapses that receive modified input, rather than spilling over to modulate nearby unstimulated synapses. These fundamental properties imply that postsynaptic Ca^{2+} signaling responds in frequency-dependent and spatially restricted manners to effect opposing modifications of synaptic properties. Dendritic spine morphology undoubtedly provides a spatial constraint on postsynaptic Ca^{2+} signals, but compartmentalization of signaling proteins is also likely to be important for precise synaptic modulation.

Central among known Ca^{2+}-sensitive signaling molecules is Ca^{2+}/calmodulin-dependent protein kinase II (CaMKII). Overwhelming amounts of biochemical, electrophysiological, and genetic data suggest that CaMKIIα is essential for normal hippocampal-based synaptic plasticity, learning, and memory, and that CaMKIIα autophosphorylation at both Thr^{286} and Thr^{305}/Thr^{306} is critical (see Lisman et al., 2002; Colbran and Brown, 2004; Elgersma et al., 2004). However, the molecular basis of CaMKII actions to regulate excitatory synaptic transmission

during induction or maintenance of hippocampal LTP are less clear. The complexity of these underlying mechanisms is demonstrated by recent findings that autophosphorylation of CaMKIIα at Thr^{305}/Thr^{306} is essential for hippocampal metaplasticity (Zhang et al., 2005) and that CaMKIIα is required for LTD, and not LTP, in the cerebellum (Hansel et al., 2006). Moreover, mutation of Thr^{305} and Thr^{306} to prevent autophosphorylation rescued both behavioral and electrophysiological deficits in a mouse model of Angelman Syndrome (van Woerden et al., 2007), a mental retardation disorder resulting from mutation in the Ube3a ubiquitin ligase. These studies underscore the importance of understanding the molecular basis of CaMKII regulation.

This chapter will discuss the intrinsic regulatory properties of CaMKII that are ideally suited to integrate postsynaptic Ca^{2+} signals and recent studies describing CaMKII interactions with other synaptic proteins, providing insight into the compartmentalization of CaMKII actions in neuronal dendrites. These findings suggest new ways in which CaMKII may fulfill its pleiotropic roles in modulating synaptic transmission.

4.23.2 The Molecular Nature of CaMKII

CaMKII was originally identified as an abundant neuronal protein kinase that is highly enriched in subcellular fractions containing postsynaptic densities (Kennedy et al., 1983; Goldenring et al., 1984; Kelly et al., 1984). Mammalian genomes encode four CaMKII isoforms, and each mRNA is subject to alternative splicing to yield multiple transcripts. Consequently, at least 25 distinct CaMKII proteins may be differentially expressed in cells and during development. In neurons, CaMKIIα and CaMKIIβ predominate. CaMKIIβ is expressed in most brain regions throughout development, but with a changing representation of multiple splice variants (Brocke et al., 1995). In contrast, CaMKIIα is expressed predominantly in the forebrain beginning at about postnatal day 5 in rodents, and expression levels increase in parallel with ongoing postnatal synaptogenesis (Brocke et al., 1995; Bayer et al., 1999). CaMKIIα transcripts, but not those of CaMKIIβ, contain an extended 3′ untranslated region that directs the mRNA to dendritic protein translation sites (Mayford et al., 1996). Indeed, NMDAR activation stimulates the local dendritic translation of CaMKIIα, possibly via a CaMKII-mediated pathway (Atkins et al., 2004, 2005), and dendritic translation of CaMKIIα is essential for the stabilization of synaptic plasticity and memory consolidation (Miller et al., 2002).

Almost all CaMKII isoforms contain highly similar amino-terminal catalytic and regulatory domains, which interact to suppress kinase activity in the basal state. The carboxyl-terminal association domain assembles subunits into the dodecameric holoenzyme structure (see **Figure 1**). Recent studies provide structural insight into the nature of the CaMKII holoenzyme. (Kolodziej et al., 2000; Morris and Torok, 2001; Hoelz et al., 2003; Gaertner et al., 2004; Rosenberg et al., 2005, 2006). Unresolved discrepancies in the interpretation of data from different laboratories may represent different naturally occurring conformations. The holoenzyme structure is essential for normal regulation by autophosphorylation and CaMKII association with other synaptic proteins, as will be described.

4.23.3 Regulation of CaMKII: Formation of a Molecular Memory

Residues 281–302 within the regulatory domain interact with the catalytic domain to interfere with the binding of both ATP and protein substrates (Colbran et al., 1989). Recent x-ray crystal structures of an inactive mutant of *Caenorhabditis elegans* CaMKII suggest that the regulatory domain interacts with the substrate binding lobe of the catalytic domain to displace helix D from the active conformation seen in many other kinases, distorting the nucleotide binding site (Rosenberg et al., 2005). Ca^{2+}/CaM interacts with residues 294–302 of the regulatory domain (Meador et al., 1993), disrupting the autoinhibitory interactions. This presumably allows helix D to relax into an active conformation that allows nucleotide and protein substrate binding (Rosenberg et al., 2005).

Ca^{2+}/CaM randomly binds to and activates individual CaMKII subunits within the holoenzyme. Simultaneous activation of adjacent subunits allows for trans-autophosphorylation at Thr^{286} within the regulatory domain (Hanson et al., 1994; Mukherji and Soderling, 1994). Thr^{286} autophosphorylation slows Ca^{2+}/CaM dissociation by over 100-fold and interferes with the autoinhibitory interaction to generate CaMKII with Ca^{2+}-independent (autonomous) activity (Colbran et al., 1989; Meyer et al., 1992). These dual effects are generally thought to prolong

CaMKII-mediated signaling initiated by transient changes in intracellular Ca^{2+}. Thus, the Thr^{286} autophosphorylated CaMKII serves as a molecular memory of a transient Ca^{2+} signal and must be dephosphorylated to return CaMKII to the basal state (reviewed in Hudmon and Schulman, 2002).

Binding of Ca^{2+}/CaM also blocks autophosphorylation at Thr^{306} and Thr^{305}. In the absence of Ca^{2+}/CaM, inactive CaMKII is slowly autophosphorylated, predominantly at Thr^{306}, blocking Ca^{2+}/CaM binding and preventing CaMKII activation (Colbran, 1993). Prior Thr^{286} autophosphorylation greatly enhances Ca^{2+}/CaM-independent autophosphorylation at Thr^{305} or Thr^{306}; although this dually phosphorylated CaMKII cannot bind Ca^{2+}/CaM, it remains active due to the prior autophosphorylation at Thr^{286} (Hashimoto et al., 1987; Hanson and Schulman, 1992). Thus, CaMKII that is autophosphorylated at Thr^{305} or Thr^{306} is Ca^{2+}/CaM-insensitive, but may exist in inactive or partially active forms depending on the level of Thr^{286} autophosphorylation.

In addition to the well-established effects of autophosphorylation at Thr^{286} and $Thr^{305/306}$ on CaMKII activity, several additional *in vitro* autophosphorylated sites have been identified (Miller et al., 1988; Dosemeci et al., 1994; Patton et al., 1990). Most of these additional sites are not known to regulate CaMKII activity directly, but recent studies found that Thr^{253} autophosphorylation in intact neurons (Migues et al., 2006) may play a role in controlling CaMKII subcellular localization.

Since autophosphorylation profoundly regulates CaMKII, it follows that protein phosphatases (PPs) affect CaMKII signaling. PP1, PP2A, and PP2C can dephosphorylate CaMKII *in vitro* (reviewed in Colbran, 2004a). In forebrain extracts, soluble Thr^{286}-phosphorylated CaMKII is primarily dephosphorylated by PP2A, but Thr^{286}-phosphorylated CaMKII associated with postsynaptic densities (PSDs) is predominantly dephosphorylated by PP1 (Strack et al., 1997). However, PP2C was suggested to play a significant role in CaMKII dephosphorylation in cerebellar granule cells (Fukunaga et al., 1989, 1993). Thus, the identity of specific enzymes responsible for CaMKII dephosphorylation likely depends on the specific cell type and the subcellular localization of CaMKII. Moreover, regulation of the expression and/or activity of these phosphatases during physiological stimulation, in diseased states, or during aging may critically modulate CaMKII activation and downstream signaling.

4.23.4 Integration of Dynamic Ca^{2+} Signals by CaMKII

The *in vitro* regulatory properties of CaMKII are ideally suited to respond to dynamic Ca^{2+} signals according to the amplitude, duration, and frequency of intracellular Ca^{2+} transients. In an idealized *in vitro* model, CaMKII is not significantly autophosphorylated following stimulation with short duration pulses (80 ms) delivered at <3 Hz, but Thr^{286} autophosphorylation acutely increases as the frequency of Ca^{2+} transients increases to 10 Hz (De Koninck and Schulman, 1998). Modification of the duration of the individual Ca^{2+} transients dramatically modifies the frequency-response relationship. Interestingly, the 80-ms Ca^{2+} pulses approximate the duration of postsynaptic Ca^{2+} transients during normal synaptic transmission, and 3–10 Hz is similar to the natural hippocampal theta frequency. Thus, this hypersensitivity to stimulation frequency may provide the fundamental basis for the frequency-dependent activation of CaMKII to induce LTP. More recent work showed that CaMKIIβ is similarly regulated, but that the relationship between stimulation frequency and autophosphorylation is different than for CaMKIIα and is modulated by alternative splicing (Bayer et al., 2002). In part, this may reflect the fact CaMKIIβ has a higher affinity for Ca^{2+}/CaM than does CaMKIIα (Brocke et al., 1999). Thus, it is likely that cells expressing different ratios of CaMKII isoforms and splice variants exhibit different stimulation-response properties in CaMKII signaling.

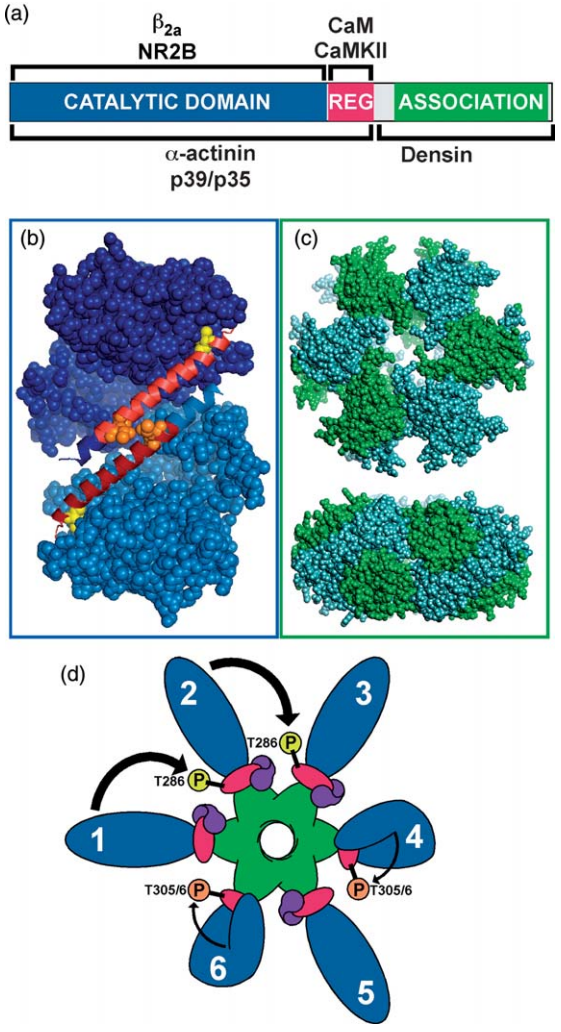
It also should be noted that the experiments described were conducted in the absence of opposing phosphatase activities. PP1 and PP2A appear to be the major activities responsible for CaMKII dephosphorylation, depending on the localization. *In vitro* studies showed that PP1 enhances the cooperativity for CaMKII activation by Ca^{2+}/CaM, providing ultrasensitive responsiveness to increasing Ca^{2+} concentrations (Bradshaw et al., 2003). PP1 has been hypothesized to couple with CaMKII to form a bistable molecular switch controlling synaptic plasticity (Zhabotinsky, 2000; Lisman and Zhabotinsky, 2001). The inhibition of hippocampal PP1 is necessary for increased Thr^{286} autophosphorylation of CaMKII, LTP induction, and memory, at least in some paradigms (Blitzer et al., 1998; Genoux et al., 2002). In addition, PP2A is inhibited following LTP induction (Fukunaga et al., 2000), possibly contributing to prolonged CaMKII autophosphorylation following LTP. However, it is not yet clear whether phosphatases

modify the frequency responsiveness to repeated Ca^{2+} transients.

Aside from Ca^{2+} transient frequency, Ca^{2+} transient dynamics are discretely regulated in the spines and shafts of neuronal dendrites (Sabatini et al., 2001), likely playing an important role in modulating CaMKII activation. In addition, small microdomains close to individual Ca^{2+} channels may contain substantially higher concentrations of Ca^{2+}, allowing associated pools of CaMKII to be preferentially activated at submaximal stimulation intensities (see below). Thus, CaMKII localization to discrete cellular domains may have a profound impact on the dynamics of CaMKII activation by Ca^{2+}.

Several studies have shown that CaMKII activity/autophosphorylation is stimulated by Ca^{2+} entry via NMDARs or voltage-gated Ca^{2+} channels (Fukunaga et al., 1989, 1992; Molloy and Kennedy, 1991; Ocorr and Schulman, 1991) and during LTP induction in hippocampus (Fukunaga et al., 1993; Ouyang et al., 1997, 1999). In cultured hippocampal neurons, brief depolarization was shown to induce compartmentalized CaMKII activation (Menegon et al., 2002). In immature neurons, CaMKIIα was activated in the somatodendritic compartment, but not in the axonal compartment. However, inclusion of PP1/PP2A inhibitors revealed axonal CaMKII activation. CaMKII activation remained somatodendritic in more mature neurons, but the activated kinase is localized to dendritic spines rather than dendritic shafts in the more distal dendrites (Menegon et al., 2002). This may reflect the translocation of activated kinase into spines.

CaMKII autophosphorylation is coupled to extracellular Ca^{2+} concentrations in hippocampal slices (Molloy and Kennedy, 1991). A recent study in



dorsal root ganglion neurons may provide additional insight into this coupling (Cohen and Fields, 2006). Under standard culture conditions the level of Thr^{286} autophosphorylation was substantial, but removal of extracellular Ca^{2+} resulted in almost complete Thr^{286} dephosphorylation within 20 min. Interestingly, removal of extracellular Ca^{2+} had no detectable effect on bulk cytoplasmic Ca^{2+}, but it depleted intracellular Ca^{2+} stores. Depletion of intracellular stores using caffeine similarly reduced CaMKII autophosphorylation. Thus, CaMKII autophosphorylation at Thr^{286} is tightly coupled to Ca^{2+} entry via intracellular Ca^{2+} stores, at least in dorsal root ganglion neurons (Cohen and Fields, 2006).

In combination, these observations suggest that Ca^{2+} changes in small subcellular microdomains due to specific mechanisms of Ca^{2+} mobilization are tightly coupled to CaMKII activation and Thr^{286} autophosphorylation. Uncovering these mechanisms will require the ability to resolve changes in activation in distinct subcellular compartments, as promised by recent development of fluorescence resonance energy transfer (FRET)-based reporters of CaMKII activation (Takao et al., 2005).

4.23.5 Synaptic Targeting of CaMKII

CaMKII is highly abundant in neurons, particularly in the forebrain, where it represents 1–2% of the total protein (Erondu and Kennedy, 1985), which calculates to an average concentration of 10–20 $\mu mol\,l^{-1}$ CaMKII subunit. The size of dendritic spines is highly variable, ranging from 0.004 to 0.6 μm^3 (Harris and Kater, 1994), but there would be ≈50 holoenzymes per average dendritic spine (volume of 0.05 μm^3) if CaMKII were randomly distributed. However, early studies showed that CaMKII is tightly associated with a variety of cytoskeletal components, preparations of plasma, synaptic and vesicular membranes, and especially with PSDs (Kennedy et al., 1983; Goldenring et al., 1984; Kelly et al., 1984; Ouimet et al., 1984; Fukunaga et al., 1988). Recent analyses suggest that there are ≈80 CaMKII holoenzymes per average isolated PSD, representing approximately 6% of the total PSD protein (Chen et al., 2005). This estimate is in broad agreement with quantitative mass spectrometry analyses (Cheng et al., 2006) and represents a substantial enrichment over the number expected based on random distribution (3 CaMKII holoenzymes per average

Figure 1 Structure-function of CaMKII holoenzymes. (a) Domain structure of a CaMKII subunit. Each CaMKII subunit contains an N-terminal catalytic domain, a central regulatory domain (REG), and a C-terminal association domain. The approximate domains needed for interaction with CaMKAPs and other proteins are indicated above and below the bar. (b) Structure of CaMKII catalytic/regulatory domain. The figure was generated using MacPyMol from the protein database file 2BDW. This is an x-ray crystal structure of the *Caenorhabditis elegans* CaMKII catalytic/regulatory domain (residues 1–340) containing an inactivating mutation. The protein appears monomeric in solution but forms a dimer in the crystal. In this orientation, the approximate dimensions of this structure are 90 Å by 50 Å. Each catalytic domain in the dimeric unit is shown as a space-filling model in different shades of blue. A critical component of dimer formation appears to be interactions between the adjacent regulatory domains, which formed long α-helices (shown as ribbon structures in different shades of red). In this inactive structure, the Thr^{286} autophosphorylation sites (yellow) are buried in pockets on the same catalytic domain, whereas Thr^{305}/Thr^{306} autophosphorylation sites in the Ca^{2+}/CaM-binding domain (orange) are at the dimerization interface. Generated from protein database from Rosenberg OS, Deindl S, Sung RJ, Nairn AC, and Kuriyan J (2005) Structure of the autoinhibited kinase domain of CaMKII and SAXS analysis of the holoenzyme. *Cell* 123: 849–860. (c) Structure of the dodecameric CaMKII holoenzyme hub. The figure was generated using MacPyMol from a structural model of the dodecameric association domain (residues 336–478). The subunits are arranged as a stacked pair of hexameric rings, and alternating subunits are shown in different shades of green when viewed from above (top panel) and from the side (bottom panel). The dimensions of the holoenzyme hub are approximately 145 Å in diameter by 60 Å high. Structural model of the dodecameric association domain is available online as supplementary material to Rosenberg OS, Deindl S, Comolli LR, et al. (2006) Oligomerization states of the association domain and the holoenyzme of Ca^{2+}/CaM kinase II. *FEBS J.* 273: 682–694. (d) Model for regulation of CaMKII by autophosphorylation. The diagram illustrates a top view of a single hexameric ring from the dodecameric holoenzyme. In the absence of Ca^{2+}/CaM, the regulatory domain (red) interacts with the catalytic domain (blue) to suppress activity (subunits 4 and 6) (see panel (b)). Ca^{2+}/CaM-binding (purple) activates individual CaMKII subunits by disrupting this interaction (e.g., subunits 1, 2, 3, 5). Activation of adjacent subunits in the holoenzyme results in the rapid trans-autophosphorylation at Thr^{286} (yellow circles) (subunits 2, 3). It is unknown whether trans-autophosphorylation occurs within (as shown) or between hexameric rings in the dodecameric holoenzyme. The Thr^{286}-autophosphorylated kinase has increased affinity for Ca^{2+}/CaM and retains autonomous kinase activity after dissociation of Ca^{2+}/CaM because the interaction of catalytic and regulatory domains is disrupted. 'Inactive' subunits (4 and 6) undergo slow intra-subunit autophosphorylation at Thr^{305} or Thr^{306} (orange circles) to block binding of Ca^{2+}/CaM, inhibiting CaMKII activation. Adapted from Colbran RJ (2004b) Targeting of calcium/calmodulin-dependent protein kinase II. *Biochem. J.* 378: 1–16; used with permission from The Biochemical Society.

PSD with 0.36 μm diameter, 30 nm depth). However, it should be emphasized that there is considerable variability between individual PSDs (Chen et al., 2005), as estimated in isolated PSDs and by immunoelectron microscopy in brain slices (Ouimet et al., 1984; Fukunaga et al., 1988).

Variability of CaMKII abundance in PSDs suggests that this interaction may be dynamically regulated. Initial studies showed that CaMKII interactions with PSD-enriched proteins are dependent on Thr^{286} autophosphorylation (or CaMKII activation) (McNeill and Colbran, 1995). Moreover, isolated PSDs containing substantial amounts of endogenous CaMKII were shown to have spare binding capacity for activated CaMKII (Strack et al., 1997; Yamauchi and Yoshimura, 1998). Consistent with these *in vitro* studies, CaMKII activation in hippocampal slices was shown to result in increased CaMKII association with PSD-enriched fractions (Strack et al., 1997). Subsequent studies examined the localization of green fluorescent protein (GFP)-tagged CaMKII isoforms expressed in cultured neurons. GFP-CaMKIIα initially adopts a freely diffusible cytosolic localization in the dendrites and soma, but translocates to dendritic spines following Ca^{2+} mobilization via NMDARs or voltage-dependent Ca^{2+} channels, where it colocalizes with PSD95, the classical PSD marker. Translocation of CaMKIIα can occur independent of the kinase activity and is dependent on binding of Ca^{2+}/CaM (Shen and Meyer, 1999; Shen et al., 2000). However, it seems likely that under physiological conditions activated cytosolic kinase would bind ATP and undergo Thr^{286} autophosphorylation before it could diffuse and bind to the PSD. Mutagenesis studies suggest that Thr^{286} autophosphorylation stabilizes CaMKII localization to spines, increasing the time constant for dissociation from spines from ≈10 s to ≈50 s. Similarly, inhibition of PP1 also results in more sustained synaptic translocation of CaMKIIα (Shen et al., 2000). In contrast, autophosphorylation at Thr^{305} and Thr^{306} appears to destabilize the punctate localization of CaMKII in spines, because a mutated kinase lacking these sites had an increased time constant for spine dissociation of ≈300 s (Shen et al., 2000).

Certain splice variants of GFP-CaMKIIβ associate with F-actin filaments under basal conditions when expressed in neurons and other cells (Shen et al., 1998; Fink et al., 2003). Binding of Ca^{2+}/CaM to GFP-CaMKIIβ following Ca^{2+} mobilization displaces the kinase from F-actin, allowing translocation to synapses. However, GFP-CaMKIIβ accumulates at synapses more slowly than does GFP-CaMKIIα, presumably because of the requirement for dissociation from F-actin. Thus, the relative expression of these two isoforms dictates the kinetics of synaptic translocation (Shen and Meyer, 1999).

In recent studies using more mature neuronal cultures, intrinsic neuronal activity resulted in synaptic accumulation of GFP-CaMKIIα, which could be dispersed by blocking NMDARs, but not by removal of extracellular Ca^{2+} (Bayer et al., 2006). Similarly, transient translocation of endogenous CaMKII to spines following Ca^{2+} mobilization induced by glutamate-, NMDA-, or KCl-induced depolarization was demonstrated using immunocytochemical methods (Dosemeci et al., 2001; Merrill et al., 2005). This endogenous synaptic CaMKII is highly autophosphorylated at Thr^{286}, and inhibition of PPs results in a more sustained synaptic localization (Dosemeci et al., 2002), as seen with GFP-CaMKIIα.

The localization of GFP-CaMKIIα also has been investigated in hippocampal slice cultures. In this system, chemical LTP induction resulted in persistent accumulation of GFP-CaMKIIα in dendritic spines in parallel with increases in PSD-associated CaMKII, as determined by immuno-gold labeling (Otmakhov et al., 2004). Moreover, sensory stimulation of zebrafish induces the sustained translocation of GFP-CaMKII to PSDs *in vivo* (Gleason et al., 2003). Presumably, induction of bona fide LTP recruits additional signaling processes that stabilize CaMKII binding to PSDs, perhaps by inhibiting phosphatases that are active toward Thr^{286}. Investigation of these apparently physiological mechanisms for CaMKII translocation is complicated by observations that a variety of pathological conditions also induce CaMKII translocation to spines. Some of these synaptic punctae may result from activity-dependent clustering of CaMKII into higher-order assemblies of the holoenzyme, as will be discussed below. It will be important to examine the localization of CaMKII *in vivo* using mammalian models in order to investigate the relationship between learning, memory, synaptic activity, and CaMKII translocation.

The idea that targeting of CaMKII to the PSD might potentiate phosphorylation of nearby substrates was supported by the initial observation that binding of exogenous CaMKII to PSDs potentiates phosphorylation of many proteins *in vitro,* including alpha-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA) receptor glutamate receptor 1 (GluR1) subunits (Strack et al., 1997). However, recent studies suggest considerable nuance to the role of PSD-associated CaMKII. A fragment of

vimentin containing a specific CaMKII phosphorylation site was fused to four different proteins that were themselves phosphorylated by CaMKII *in vitro* and in human embryonic kidney 293 (HEK293) cells and that were targeted to synapses by different mechanisms (Tsui et al., 2005). When expressed in neurons, all of these proteins were targeted to synapses. However, phosphorylation of the vimentin model site could be detected with a phospho-vimentin antibody only when this domain was fused to stargazin or PSD95, but not when it was fused to NMDAR 2B (NR2B) or GluR1. Moreover, phosphorylation of the PSD95 and stargazin model substrates appeared to be coupled to Ca^{2+} entry via the NMDAR, but not to Ca^{2+} entering via voltage-gated Ca^{2+} channels. These data are surprising because CaMKII is only known to bind directly to NR2B, and this interaction potentiates phosphorylation of the vimentin model site in HEK293 cells. In combination, these studies suggest that CaMKII is targeted to specific microdomains within the PSD where it can access only a subset of the total CaMKII substrates in the PSD (Tsui and Malenka, 2006). Unfortunately, co-localization of CaMKII with the different substrates was not monitored in these experiments, and the authors were unable to detect CaMKII-dependent phosphorylation of the endogenous sites in GluR1 or NR2B in these experiments (Tsui and Malenka, 2006). Nevertheless, taken at face value, the data suggest that multiple mechanisms (perhaps involving several other proteins) may be required to direct CaMKII to specific PSD microdomains that are coupled to different substrates and distinct routes of Ca^{2+} entry.

4.23.6 Interactions of CaMKII with CaMKAPS

It has been increasingly recognized over the last several years that intracellular signaling pathways are tightly compartmentalized, often by assembly of large multi-protein signaling complexes that are sometimes referred to as signalosomes. Perhaps the best characterized system is the interaction of protein kinase A (PKA) with a family of A-kinase anchoring proteins (AKAPs), which nucleate complexes containing PKA and a diverse array of additional signaling proteins (Smith et al., 2006). Based on this paradigm and emerging evidence that CaMKII localization is dynamically regulated, several laboratories have investigated the association of CaMKII with other cellular proteins, which we have termed CaMKAPs (CaMKII-associated proteins). Here, I will focus on CaMKAPs that may play a role in postsynaptic targeting of CaMKII and/or modulate functions likely to be relevant for synaptic plasticity, learning, and memory. **Figure 2** shows domain structures and interacting proteins for these CaMKAPs.

4.23.6.1 NMDA-Type Glutamate Receptor Subunits

As discussed above, CaMKII is partially localized to excitatory synapses that contain NMDARs. The NR2B subunit was initially identified as a CaMKAP that binds only to the activated Thr^{286}-autophosphorylated kinase (Strack and Colbran, 1998). Recent studies have shown that CaMKII activation by Ca^{2+}/CaM binding coupled with nucleotide binding to the active site is sufficient for formation of a stable complex with NR2B (Robison et al., 2005a). CaMKII interacts with two distinct domains in the C-terminal tail of NR2B: a central domain (residues 1290–1309) appears to have higher affinity for CaMKII than a less-well defined membrane-proximal domain (Strack and Colbran, 1998; Leonard et al., 1999, 2002; Strack et al., 2000a; Bayer et al., 2001) (**Figure 2**). NR2A contains an amino acid sequence with similarity to the high-affinity binding site in NR2B, but insertion of two additional amino acids disrupts CaMKII binding (Strack et al., 2000a; Barria and Malinow, 2005) (**Figure 3**). CaMKII co-localizes only with NMDARs that contain NR2B in heterologous cells, and mutations within the central high-affinity CaMKII-binding domain that disrupt *in vitro* interactions of CaMKII with NR2B also disrupt co-localization (Strack et al., 2000a).

Recently, NR2B was shown to interact close to the CaMKII active site, possibly in two different binding modes that mediate a transition from transient to more sustained interaction (Bayer et al., 2006). These data are interesting in light of studies showing that NR2B can have two distinct effects on CaMKII activity. On one hand, binding of NR2B has been shown to prop open the regulatory domain, trapping an autonomously active form of CaMKII in the absence of Thr^{286} autophosphorylation (Bayer et al., 2001). In contrast, under different conditions NR2B inhibits CaMKII activity (Robison et al., 2005a), perhaps not surprisingly because the CaMKII binding domain of NR2B shares considerable amino acid sequence similarity with the CaMKII regulatory domain (Strack et al., 2000a) (**Figure 3**). The impact of these two modes of binding in intact cells remains unclear.

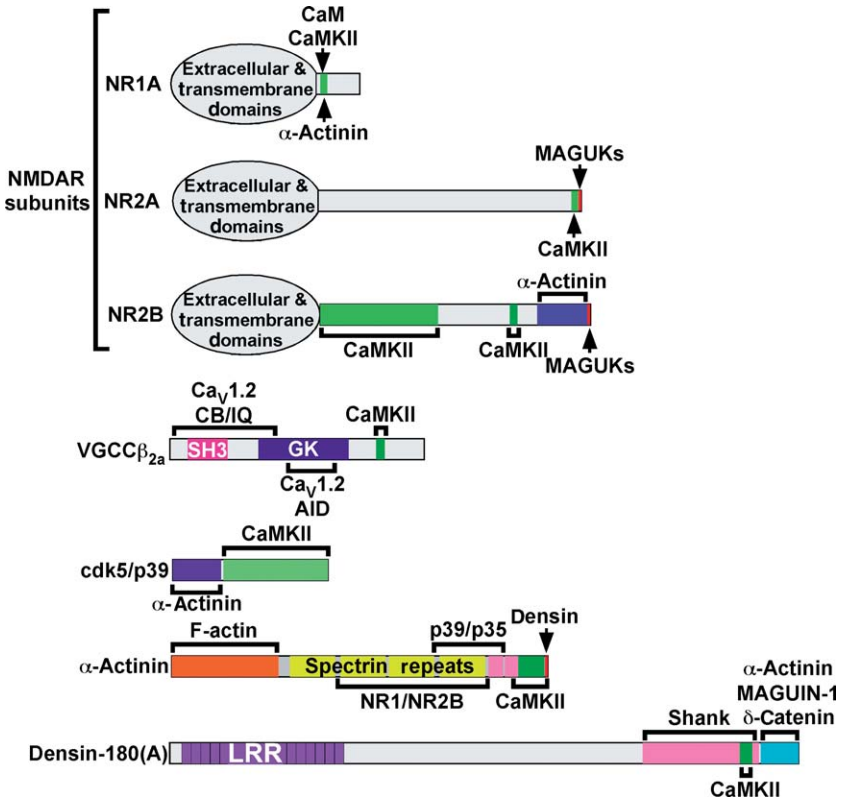


Figure 2 CaMKII-associated proteins (CaMKAPs) domain structures and known interacting proteins. Linear domain maps of CaMKAPs showing conserved protein interactions domains. Domains known to interact with CaMKII and selected additional proteins are indicated above and below each protein. SH3, Src-homology 3 domain; GK, guanylyl kinase-like domain; LRR, leucine-rich repeats; cdk5; cyclin-dependent kinase 5; NR, NMDA receptor; CB/IQ, a domain in the C-terminal tail of $Ca_V1.2$ containing multiple Ca^{2+}/CaM-binding motifs; AID, alpha-interacting domain in the I-II domain linker of $Ca_V1.2$; MAGUKs, membrane-associated guanylate kinases, a family of proteins. See text for details.

Activated CaMKII has also been shown to interact with the NR2A and NR1 subunits of the NMDAR (see **Figure 2**). An alternatively spliced region of NR1 binds CaMKII, and both calmodulin and α-actinin compete for this interaction (Leonard et al., 1999, 2002). CaMKII also competes with PSD95 for binding near the extreme C-terminus of NR2A (Gardoni et al., 1998, 1999, 2001b). However, interactions of CaMKII with NR1 and NR2A are not detected in all laboratories (e.g., see Strack and Colbran, 1998; Leonard et al., 1999), and CaMKII does not appear to significantly colocalize with NR1/NR2A NMDARs in heterologous cells (Strack and Colbran, 1998). Thus, interactions with NR1 and NR2A subunits may be too weak to mediate CaMKII targeting to NMDARs. Perhaps targeting of activated CaMKII to the NMDAR complex via NR2B facilitates these interactions.

Several studies have shown that CaMKII is dynamically associated with neuronal NMDARs using coimmunoprecipitation approaches. Interpretation of these data is complicated by the assembly of NMDAR subunits into functional receptor ion channel complexes with many other proteins unless they are fully solubilized using harsh ionic detergents, and also depends on the specificity of the antibodies for the NMDAR subunits. Initial studies in hippocampal slices found that activation of NMDARs promotes CaMKII association with the entire NMDAR complex (Leonard et al., 1999). Association of CaMKII with the C-terminus of NR2A appears to be inhibited by protein kinase C (PKC) phosphorylation at Ser^{1416} (Gardoni et al., 2001a), allowing for increased binding of PSD95 that may be critical for LTP induction (Gardoni et al., 2001b). However, more recent experiments suggested that CaMKII is preferentially associated with the NR2B subunit and not with NR2A (Kim et al., 2005). Taken together, these findings are consistent with biochemical data demonstrating binding of activated CaMKII to NMDAR

Figure 3 Alignment of amino acid sequences from CaMKII-binding domains in NMDA receptor 2B (NR2B) and β_{2a} with the CaMKIIα regulatory domain. Residues in green boxes are identical, and residues in grey boxes are conserved. The yellow circle indicates CaMKII phosphorylation sites in NR2B and β_{2a} and the Thr^{286} autophosphorylation site in CaMKII. These sites are highly efficient CaMKII substrates, and the domains surrounding these sites all form stable interactions with the CaMKII catalytic domain. Despite strong sequence similarity, the regulatory domain of CaMKII competes with ATP for binding to and inhibiting CaMKII (Colbran RJ, Smith MK, Schworer CM, Fong YL, and Soderling TR (1989) Regulatory domain of calcium/calmodulin-dependent protein kinase II and synaptic plasticity. *Curr. Opin. Neurobiol.* 14: 318–327), whereas Ca^{2+}/CaM-dependent binding of CaMKII to NR2B and β_{2a} requires that ATP first bind to the kinase (Robison AJ, Bartlett RK, Bass MA, and Colbran RJ (2005a) Differential modulation of Ca^{2+}/calmodulin-dependent protein kinase II activity by regulated interactions with NMDA receptor NR2B subunits and alpha-actinin. *J. Biol. Chem.* 280: 39316–39323). The arrow above the NR2B sequence indicates the location of an isoleucine-asparagine insertion in the corresponding domain of NR2A that appears to disrupt CaMKII binding (Barria A and Malinow R (2005) NMDA subunit composition controls synaptic plasticity by regulating binding to CaMKII. *Neuron* 48: 289–301).

subunits, but more studies are needed to establish the precise mechanisms and role for this interaction in neurons.

The original CaMKAP hypothesis suggested that CaMKAPs target CaMKII to particular subcellular compartments to facilitate signaling to specific downstream substrates. This raises the question of whether interactions with NMDARs can account for the known enrichment of CaMKII in PSDs. A CaMKII-binding glutathione-S-transferase-NR2B fusion protein blocked ≈75% of CaMKII binding to PSDs *in vitro* (Strack et al., 2000a), suggesting that NR2B makes a significant contribution to binding, although a role for other CaMKII-binding proteins sharing a similar interaction mechanism cannot be excluded. Excitatory synapses may contain about 50 NMDARs (reviewed in Kennedy, 2000), in the same range as estimated numbers of PSD-associated CaMKII holoenzymes (Chen et al., 2005), suggesting that NMDARs may be quantitatively important for PSD targeting if each receptor complex targets one CaMKII holoenzyme. In contrast, a recent mass spectrometry study found that PSDs contain a substantial (>20-fold) excess of CaMKII holoenzymes over NMDAR channels (Cheng et al., 2006). These data suggest that bulk CaMKII targeting to PSDs must involve interactions with additional proteins or the formation of higher-order clusters of CaMKII holoenzymes. More studies are needed to understand the quantitative contribution of NMDARs to PSD targeting of CaMKII.

High local concentrations of Ca^{2+} near cytoplasmic domains of the NMDAR would be predicted to potentiate activation of associated CaMKII to enhance the phosphorylation of nearby substrates. Consistent with this hypothesis, phosphorylation of a membrane-bound model CaMKII substrate (a vimentin fragment) in HEK293 cells was shown to be facilitated by binding of CaMKII to NR2B localized to the same membrane compartment (Tsui et al., 2005). However, this effect is independent of Ca^{2+} influx, because the NR2B was not present in a functional NMDAR, and is perhaps more consistent with the possibility that NR2B is trapping an autonomously active form of CaMKII. More studies are needed to determine the effect of disrupting the CaMKII-NR2B interaction on phosphorylation of PSD proteins, LTP induction, learning, and memory.

An additional function for CaMKII binding to NMDARs may be to provide feedback modulation of NMDAR activity. Activation of CaMKII in isolated PSDs enhanced the binding of MK801, an open channel blocker of the NMDAR, suggesting that CaMKII increases channel opening (Kitamura et al., 1993). Consistent with these findings, CaMKII increased NMDAR currents in spinal dorsal horn neurons (Kolaj et al., 1994). However, these studies did not address contributions of NMDARs with different subunit combinations to the CaMKII response. CaMKII potently phosphorylates NR2B at Ser^{1303} within the high-affinity CaMKII-binding domain, and this residue is phosphorylated *in situ* (Omkumar et al., 1996). In heterologous cells, CaMKII enhanced the desensitization of NMDARs formed from NR2B and one of multiple NR1 subunit slice variants but modestly reduced the desensitization of NMDARs containing NR2A in place of NR2B (Sessoms-Sikes et al., 2005). Effects of CaMKII on NMDARs containing both NR2A and NR2B were not investigated. Thus, CaMKII may function as a negative feedback regulator of Ca^{2+} entry via NMDARs containing NR2B, but a positive feedback regulator of those containing NR2A. Interactions of CaMKII with NMDAR subunits may facilitate specific CaMKII actions on NMDARs with different subunit compositions. It will be important to elucidate the roles of

these NR2 subunit–specific effects of CaMKII in modulating synaptic and extrasynaptic NMDARs and synaptic plasticity.

Initial insights into the impact of CaMKII binding to NR2B on synaptic plasticity have been provided recently. Insertion of two amino acids into the CaMKII-binding domain of NR2B significantly reduced Ser^{1303} phosphorylation *in vitro* (Mayadevi et al., 2002). This mutation disrupts CaMKII binding to NR2B, and overexpression of the mutant protein interfered with LTP induction in organotypic hippocampal slice cultures (Barria and Malinow, 2005). Although the effects of this mutation on NR2B phosphorylation *in situ* were not investigated, these data suggest that CaMKII binding to NR2B is important for LTP induction. These findings appear at odds with pharmacological studies suggesting that synaptic NR2A-containing NMDARs are critical for LTP, whereas extra- or perisynaptic NR2B NMDARs are critical for normal LTD (Liu et al., 2004; Massey et al., 2004). However, the specificity of the pharmacological reagents has been questioned (Weitlauf et al., 2005), and a recent study suggests that the subunit dependence of synaptic plasticity varies with the developmental stage (Bartlett et al., 2007). The precise relationships between CaMKII interactions with, and phosphorylation of, specific NMDAR subunits and different forms of synaptic plasticity clearly warrant further study.

4.23.6.2 The Densin-180/α-Actinin/cdk5 Complex

Prompted by the realization that NMDARs may not fully account for PSD targeting of CaMKII (see above), and by the development of yeast two-hybrid strategies for identifying novel protein–protein interactions in unbiased screens, CaMKII was shown to interact directly with densin-180, α-actinin, and the p35/39 subunit of cyclin-dependent kinase 5 (cdk5) (Strack et al., 2000a; Walikonis et al., 2001; Dhavan et al., 2002). In addition, α-actinin also binds to densin-180 and to p35/39 independently of CaMKII. Characterization of these interactions revealed a diversity of new mechanisms by which CaMKII interacts with other cellular proteins to regulate synaptic function.

4.23.6.2.1 Densin-180

Densin-180 was originally identified as a relatively abundant 180-kDa PSD protein containing a leucine-rich repeat (LRR) domain at the N-terminus and a single PDZ domain at the C-terminus (Apperson et al., 1996) (see **Figure 2**). It became a founding member of the LAP protein family, which contain **L**eucine-rich repeats (LRRs) **A**nd (1 or more) **P**DZ domains (Bilder et al., 2000; but see Wilson et al., 2001). Initial studies suggested that densin-180 had a single transmembrane-spanning domain with a large extracellular N-terminal domain containing the LRRs and some glycosylation sites and only a short cytosolic C-terminal domain. However, other LAP protein family members are exclusively intracellular, and cell surface biotinylation of dissociated hippocampal neurons failed to modify densin-180 (Izawa et al., 2002). Thus, the entire densin-180 protein may be cytosolic. Additional studies are needed to clarify this issue.

In part because of the putative transmembrane structure of densin-180, most studies focused on protein interactions mediated by the C-terminal domain of densin-180. Biochemical and yeast two-hybrid studies showed that CaMKII holoenzymes bound to a 29-amino-acid domain adjacent to the PDZ domain (Strack et al., 2000a; Walikonis et al., 2001; Robison et al., 2005b). This CaMKII-binding domain exhibits no amino acid sequence similarity to CaMKII-binding domains in the NMDAR subunits. Interestingly, multiple mRNA splice variants are differentially expressed during embryonic and postnatal development, and one variant lacks the CaMKII-binding domain (Strack et al., 2000b). The major adult densin-180 splice variant (densin-A) interacts with nonphosphorylated inactive CaMKIIα (Walikonis et al., 2001; Robison et al., 2005b), and this interaction is insensitive to Ca^{2+}/calmodulin or to $Thr^{305/306}$ autophosphorylation (Robison et al., 2005a). However, autophosphorylation at Thr^{286} potentiates the interaction to a varying extent depending on assay conditions (Strack et al., 2000b; Walikonis et al., 2001; Robison et al., 2005a). Densin-A is unable to bind CaMKIIα monomers generated by C-terminal truncation (Robison et al., 2005b), and the oligomeric C-terminal association domain of CaMKIIα binds densin-A in the yeast two-hybrid assay (Walikonis et al., 2001). Thus, it seems that densin-A interacts with the C-terminal association domain of CaMKIIα and/or requires the holoenzyme structure for interaction. In contrast, an embryonic densin splice variant (densin-D) binds to monomeric, C-terminal truncated forms of CaMKIIα and also to CaMKIIβ, CaMKIIγ, and CaMKIIδ isoforms (Robison and Colbran, unpublished observations). Thus, this embryonic form of densin-180 can interact with CaMKII isoforms that are expressed at similar developmental stages. In combination, these data suggest that densin-180 splice variants can have different roles during development, some independent

of CaMKII. Additional cellular studies are required to further explore the role of alternative splicing of densin-180.

In addition to CaMKII, the C-terminal domain of densin-180 has also been shown to interact with α-actinin (Walikonis et al., 2001), δ-catenin (Izawa et al., 2002), Shank (Quitsch et al., 2005), and MAGUIN-1 (Ohtakara et al., 2002), providing links to many other PSD proteins. Densin-180 overexpression induces branching of neuronal dendrites, and this effect is antagonized by coexpression of Shank, which competes with δ-catenin, but not CaMKII, for binding to densin-180 (Quitsch et al., 2005). In combination, these data suggest that densin-180 serves as a scaffolding protein, assembling postsynaptic signaling complexes to modulate dendritic morphology. However, although the LRR domain appears sufficient for membrane targeting (Quitsch et al., 2005), it remains unclear whether the N-terminal domain is intracellular or extracellular, and its specific role remains largely unknown. It will be important to identify additional binding partners for densin-180, determine the effects of CaMKII on these interactions, and elucidate their biological role.

4.23.6.2.2 α-Actinin

Dendritic spines are highly enriched in F-actin and F-actin-binding proteins, such as α-actinin. All four mammalian α-actinin isoforms contain an N-terminal F-actin-binding domain, a central spectrin-repeat domain, and a C-terminus containing an EF-hand divalent metal ion–binding domain that may not be functional in all isoforms (see **Figure 2**). The spectrin repeat domains form antiparallel dimers in a long, rodlike structure, with single F-actin-binding and C-terminal domains at each end (reviewed in Broderick and Winder, 2005). Yeast two-hybrid screens identified interactions between the extreme C-terminus of α-actinin-4 and the densin-180 PDZ domain (Walikonis et al., 2001), the C-terminal domain of α-actinin-1 and the N-terminal domain of the cdk5 regulatory subunits p35 and p39 (Dhavan et al., 2002), and the central rod domain of α-actinin-2 and NMDAR NR1 and NR2B subunits (Wyszynski et al., 1997).

CaMKII was shown to bind to residues 713–892 of α-actinin-1 and residues 638–879 of α-actinin-4 using the yeast two-hybrid assay (Walikonis et al., 2001; Dhavan et al., 2002). Biochemical experiments found that residues 819–894 constitute the smallest CaMKII-binding fragment of α-actinin-2, which is thought to be the major isoform present in dendritic spines (Robison et al., 2005b). This domain is highly conserved in all the α-actinin isoforms, but is considerably larger than, and shares no homology with, CaMKII-binding domains in NMDARs or densin-180. All CaMKII isoforms, as well as monomeric catalytic/regulatory domain fragments, can bind α-actinin. Interestingly, Ca^{2+}/calmodulin competes with α-actinin for binding to CaMKII. Moreover, $Thr^{305/306}$ autophosphorylation of CaMKII appears to block α-actinin binding, but Thr^{286} autophosphorylation has little effect (Robison et al., 2005a). Coimmunoprecipitation and colocalization experiments are consistent with the interaction of CaMKII with the α-actinins at synapses *in situ* (Walikonis et al., 2001; Dhavan et al., 2002; Robison et al., 2005b). Together, these data suggest the intriguing hypothesis that α-actinin localizes inactive CaMKII under basal conditions, and that localized elevations of Ca^{2+} would displace CaMKII and activate the kinase.

4.23.6.2.3 p35/p39 regulatory subunits of cyclin-dependent kinase 5

CaMKII was identified as a direct binding partner for p35/p39 in a yeast two-hybrid screen (Dhavan et al., 2002). A monomeric catalytic/regulatory domain fragment of CaMKII interacts with the C-terminal domain of p35/p39, separate from the α-actinin-binding site in the N-terminal domain of p35/p39 (**Figure 2**). Notably, CaMKII colocalizes with α-actinin, cdk5, and p35 in cultured neurons, especially in dendritic spines. Coimmunoprecipitation experiments isolated complexes containing CaMKII, α-actinin, and p35 from brain extracts, and pretreatment of cultured neurons or hippocampal slices with NMDAR agonists increased the amount of coimmunoprecipitation in a manner that required CaMKII activity (Dhavan et al., 2002). These data suggest an intriguing regulatory relationship between CaMKII and cdk5. Given recent progress in elucidating critical roles for cdk5 in regulating synaptic morphology and transmission (Cheung et al., 2006), it will be important to determine the role of α-actinin as a scaffolding protein to facilitate this potential cross talk.

4.23.6.3 Voltage-Gated Calcium Channels

Voltage-gated calcium channels are essential both presynaptically for release of neurotransmitter and postsynaptically in synaptic plasticity. They are formed from a pore-forming α subunit, encoded by multiple genes in three distinct families (Ca_V1, Ca_V2, and Ca_V3), which associate with β subunits (four genes, β_1, β_2, β_3, and β_4, that encode many mRNA splice variants) and other regulatory subunits/

proteins, yielding a tremendous diversity of neuronal calcium channels. There are multiple mechanisms for the feedback regulation of voltage-gated calcium channels, including binding of heterotrimeric G protein subunits and phosphorylation by multiple kinases. Ca^{2+}-dependent feedback is also an important mechanism for regulation of voltage-gated Ca^{2+} channels. For example, direct binding of Ca^{2+}/CaM to α subunit C-terminal domains controls Ca^{2+}-dependent inactivation of several calcium channel subtypes (reviewed in Catterall, 2000). More complex Ca^{2+}-dependent mechanisms are also important for integrating the actions of calcium channels, such as the activation of dendritic L-type calcium channels, which activates a CaMKII-dependent pathway to inhibit Ca^{2+} entry via R-type channels, interfering with LTP induction under some conditions (Yasuda et al., 2003). In addition, contributions of T-type and L-type channels to Ca^{2+} transients in spines and dendritic shafts of striatal medium spiny neurons are dynamically modulated by up/down-state transitions between two resting membrane potentials (Carter and Sabatini, 2004). Association of CaMKII with the calcium channels may be important for modulation of these activities.

4.23.6.3.1 L-type calcium channels

Neuronal L-type calcium channels (LTCCs) contain $Ca_V1.2$ or $Ca_V1.3$ α subunits associated with diverse β subunits, which exert differential effects on the trafficking and biophysical properties of the channels (reviewed in Dolphin, 2003). LTCCs were initially localized to the soma and proximal dendrites (Hell et al., 1993), but they are also enriched at more distal synapses (Obermair et al., 2004). LTCCs play a key role in modulating neuronal gene transcription and synaptic plasticity (Deisseroth et al., 2003), with $Ca_V1.2$ and $Ca_V1.3$ subunits making distinct contributions to transcriptional responses in the hippocampus (Zhang et al., 2006). Recent studies found that the loss of dendritic spines on striatal medium spiny neurons following dopamine depletion *in vivo* requires LTCC activity that is specifically mediated by $Ca_V1.3$ (Day et al., 2006).

CaMKII can facilitate LTCC current via actions on α subunits. The cytosolic N- and C-terminal domains, as well as the intracellular linker domains, of $Ca_V1.2$ can be phosphorylated by CaMKII, and CaMKII also binds to the N- and C-terminal domains (Hudmon et al., 2005). Interaction of CaMKII with the C-terminal domain was suggested to be important for facilitation of a mutated $Ca_V1.2$ channel in oocytes (Hudmon et al., 2005). In addition, a genetic mutation associated with Timothy syndrome creates a CaMKII phosphorylation site (Ser^{439} in the I-II linker domain) that mediates an increase in channel open probability (Erxleben et al., 2006). CaMKII may also act to increase channel activity by phosphorylating the membrane-proximal region of the C-terminal domain (Erxleben et al., 2006; Lee et al., 2006). However, the physiological relevance of these forms of LTCC regulation by CaMKII is unclear.

LTCCs are also facilitated in cardiomyocytes during trains of action potential stimulation, in part due to an increase in channel open probability mediated by CaMKII (Dzhura et al., 2000). CaMKII is specifically targeted to the cardiac LTCC complex because the open probability of channels presented in isolated membrane patches is increased by an associated CaMKII activity (Dzhura et al., 2002). The regulation of channel open probability by CaMKII can be reconstituted in heterologous cells coexpressing $Ca_V1.2$ with the β_{2a} subunit, but not in cells lacking β_{2a} (Grueter et al., 2006). CaMKII binds to β_{2a} and efficiently phosphorylates Thr^{498}. Interestingly, the amino acid sequence surrounding Thr^{498} is very similar to those surrounding Ser^{1303} in the CaMKII-binding domain of NR2B and Thr^{286} in the regulatory domain of CaMKII itself (**Figure 3**). Mutation of Thr^{498} to alanine essentially prevents CaMKII-dependent increases in both single LTCC open probability and whole-cell LTCC current in cardiomyocytes. These studies define the β subunit as a key locus for physiological modulation of LTCCs by CaMKII-mediated protein phosphorylation (Grueter et al., 2006), in contrast to the direct actions of Ca^{2+}/calmodulin and PKA to modulate channel activity via the α subunit (see Catterall, 2000).

The actions of CaMKII on the α and β subunits to facilitate LTCCs are not necessarily mutually exclusive. Thus, it will be interesting to investigate whether specific β subunits are required for CaMKII actions via the α subunits and to determine their role in modulating neuronal LTCCs. The four β subunit genes exhibit distinct but overlapping expression patterns in the brain (reviewed in Dolphin, 2003). Whereas the β_1 and β_3 subunits contain amino acid sequences similar to those surrounding Thr^{498} in β_{2a}, similar sequences cannot be identified in β_4. Thus, one prediction is that CaMKII will differentially modulate LTCCs in neurons, depending on the identity of the β subunit present

in the channel complex. Moreover, β subunits associate with non-L-type calcium channel α subunits, and evidence is beginning to emerge that CaMKII can also regulate Ca_V2 channels (Jiang et al., 2004). Thus, specific β subunits may be involved in CaMKII modulation of N-, P/Q, and/or R-type channels.

4.23.6.3.2 T-type calcium channels

T-type channels are somewhat distinct from other calcium channels in that they are activated at lower voltages and are thought to operate independent from β subunits. Three genes encoding T-type channel variants ($Ca_V3.1$, $Ca_V3.2$, and $Ca_V3.3$) are differentially expressed in different brain regions. Single-nucleotide polymorphisms in the $Ca_V3.2$ gene have been linked to a form of epilepsy (reviewed in Perez-Reyes, 2006). Neuronal T-type channels are dynamically regulated by diverse mechanisms, including feedback modulation by intracellular Ca^{2+} (reviewed in Chemin et al., 2006). Intracellular Ca^{2+} facilitates T-type calcium channels by shifting the voltage dependence for channel activation to more negative potentials via the activation of CaMKII (Lu et al., 1994; Barrett et al., 2000; Welsby et al., 2003). CaMKII directly regulates recombinant $Ca_V3.2$ T-type channels by phosphorylating Ser^{1198} in the II-III loop (Welsby et al., 2003; Yao et al., 2006). Recent studies have shown that CaMKII binds to the II-III loop of $Ca_V3.2$, but there is little amino acid sequence similarity with CaMKII-binding domains in other proteins. Notably, CaMKII does not regulate $Ca_V3.1$ channels and does not bind to or phosphorylate the II-III loop of $Ca_V3.1$. Phosphorylation of the adrenal T-type channels at Ser^{1198} is dynamically regulated by angiotensin II *in vivo* (Yao et al., 2006). It will be important to investigate Ser^{1198} phosphorylation in neurons and determine the contribution of this phosphorylation to modulation of dendritic Ca^{2+} signaling.

4.23.6.4 SynGAP/MUPP1

Guanosine triphosphate (GTP)ase activating proteins (GAPs) inhibit G proteins by stimulating GTP hydrolysis. SynGAP binds to PSD95 and is enriched at synapses where it regulates ras, thereby controlling glutamatergic signaling via extracellular signal-regulated protein kinase (ERK) and p38 MAP kinase pathways. Reductions in synGAP expression activate ERK pathways and inhibit the p38 MAP kinase pathway (Rumbaugh et al., 2006). Recent studies specifically linked NMDARs containing NR2B to the modulation of synGAP and synaptic MAP kinases (Krapivinsky et al., 2003; Kim et al., 2005). Selective interactions of CaMKII with NR2B (see Kim et al., 2005) may be important for this selectivity, because CaMKII phosphorylates and activates synGAP, thereby downregulating the ERK kinase pathway following Ca^{2+} influx (Oh et al., 2004). Modulation of synGAP is an important control point for AMPA-type glutamate receptor (AMPAR) trafficking, contributing to synaptic plasticity (Krapivinsky et al., 2004; Rumbaugh et al., 2006).

In other studies, synGAP was shown to be significantly phosphorylated by CaMKII under basal conditions (Krapivinsky et al., 2004). Binding of CaMKII and synGAP to distinct PDZ domains in **mu**ltiple-**P**DZ **p**rotein-1 (MUPP-1) appeared to promote this basal phosphorylation. In these studies, Ca^{2+} influx via the NMDAR disrupted the interaction of CaMKII with MUPP-1, reducing synGAP phosphorylation at the same time that the calcineurin phosphatase was activated to dephosphorylate and inhibit synGAP (Krapivinsky et al., 2004). These data suggest that MUPP-1 mediates assembly of synGAP and CaMKII and is an important mechanism for modulating synGAPα isoforms. However, a more minor synGAPβ isoform appears to bind CaMKII directly (Li et al., 2001). The existence of multiple mechanisms for assembly of CaMKII-synGAP complexes may allow for diversity in the regulatory interactions and contribute to some of the discrepancies in this literature.

4.23.7 Roles for CaMKII in Synaptic Plasticity

Abundant evidence suggests that CaMKII activation is important for LTP induction, and many investigators have studied relevant molecular mechanisms. The most prominent substrate for CaMKII is the Thr^{286} autophosphorylation site in CaMKIIα, which is essential for normal synaptic plasticity, learning, and memory (reviewed in Elgersma et al., 2004). Several interesting postsynaptic CaMKII substrates have been identified. Most attention has focused on the regulation of the activity and subcellular localization of subunits of the AMPAR. CaMKII and PKC phosphorylate the GluR1 subunit of AMPARs at Ser^{831} to increase unitary conductance of GluR1 homomeric channels (Barria et al., 1997a,b). Indeed, Ser^{831} and a nearby PKA phosphorylation

site (Ser^{845}) in GluR1 are essential for normal synaptic plasticity (Lee et al., 2003). However, it was recently shown that Ser^{831} phosphorylation does not affect the conductance of heteromeric GluR1/GluR2 channels that are physiologically relevant (Oh and Derkach, 2005), so the significance of Ser^{831} phosphorylation to LTP induction is unclear. In addition, CaMKII promotes GluR1 trafficking to synapses by a mechanism that is independent of GluR1 phosphorylation and requires binding of the C-terminus of GluR1 to a PDZ domain in an unidentified protein (Hayashi et al., 2000). Thus, with the exception of Thr^{286} autophosphorylation, the CaMKII substrates relevant to LTP induction are unclear.

There is even greater uncertainty about the role of CaMKII activity during maintenance of LTP. Early studies focused on the hypothesis that LTP induction would increase Thr^{286} autophosphorylation of CaMKII, resulting in a sustained increase of autonomous CaMKII activity that played a critical role in maintenance. However, this hypothesis was confounded by many studies showing that CaMKII inhibitors cannot reverse preexisting LTP, even though they effectively block LTP induction (discussed in Chen et al., 2001). A potential explanation for this conundrum was suggested in a recent study that found that LTP induction rapidly increased both Thr^{286} autophosphorylation and autonomous CaMKII activity, but that the activity returned to basal levels within a few minutes even though Thr^{286} autophosphorylation was maintained for at least 1 h (Lengyel et al., 2004). Possibly the autonomous kinase activity of the Thr^{286} autophosphorylated kinase is suppressed by interactions with other proteins, such as NR2B. Thus, CaMKII and sustained Thr^{286} autophosphorylation may be important for maintenance of LTP without an essential role for ongoing kinase activity. The temporal distinction between requirements for Thr^{286} autophosphorylation and autonomous kinase activity may extend to certain learning/memory paradigms, because experiments using a chemical-genetic approach to inhibit CaMKII activity at various times after a fear conditioning paradigm showed that kinase activity is not required indefinitely (Wang et al., 2003).

Investigators have long questioned why a highly efficient enzyme like CaMKIIα is expressed in forebrain at levels that are more comparable with cytoskeletal proteins such as actin or tubulin (Erondu and Kennedy, 1985). The high expression levels were suggested to imply some sort of structural role. Intriguing insight into this possibility was provided by recent analyses of isolated PSDs by electron microscopy (Petersen et al., 2003). Variable amounts of CaMKII were found on the cytosolic face of PSDs, as expected based on the dynamic regulation of CaMKII binding to PSDs. However, rather than being randomly distributed, the CaMKII appeared to be present in an orderly array, forming 'towers' projecting from the PSD into the cytosol. The CaMKII towers may be related to hypothesized 'slots' for synaptic insertion of AMPARs (Lisman and Zhabotinsky, 2001), and their dimensions were reasonably consistent with the possibility that they are formed from stacks of CaMKII holoenzymes (Petersen et al., 2003). Whatever the nature and function of the CaMKII towers, the orderly distribution of the kinase on the cytosolic face hints at a structural role.

If CaMKII indeed plays a structural role, one might then ask why a structural protein needs to be an enzyme, especially one with such intricate regulatory mechanisms. In this regard, two possible interrelated mechanisms might be involved. First, CaMKII holoenzymes may assemble into higher-order complexes in response to Ca^{2+}-mobilizing stimuli in intact cells. Second, CaMKII may coordinate the assembly of multiple CaMKAPs in a complex. Ca^{2+}/calmodulin-binding and autophosphorylation modulates both of these processes, suggesting that CaMKII may function as an autoregulated scaffolding protein. Data supporting these two mechanisms are reviewed in turn.

4.23.7.1 Higher-Order Assemblies of CaMKII Holoenzymes

Early *in vitro* studies showed that autophosphorylation of CaMKII at pH 6.5 using low concentrations of ATP resulted in clustering of CaMKII holoenzymes into loosely packed, large branched complexes which then associated to form larger complexes (Hudmon et al., 1996, 2001). Electron microscopy subsequently revealed that spherical clusters of CaMKII contaminated isolated PSD preparations (Dosemeci et al., 2000). The reversible formation of these spherical clusters could be induced in intact cultured neurons and hippocampal slices by glutamate and calcium mobilizing stimuli (Tao-Cheng et al., 2001, 2002). CaMKII clustering may be a neuroprotective mechanism to sequester the kinase away from critical substrates when abnormally activated, perhaps under excitotoxic conditions that result in reduced ATP

levels and cytosolic pH. Recent studies found that in intact cells CaMKII holoenzymes reversibly oligomerized by a mechanism involving interaction of regulatory and catalytic domains in different holoenzymes (Hudmon et al., 2005). Formation of CaMKII clusters under more pathological conditions may be related in some way to the perhaps more physiologically relevant CaMKII 'towers' observed in PSDs (Petersen et al., 2003) (discussed earlier). An understanding of the structural basis for formation of the towers will likely provide insight into their function.

4.23.7.2 Binding to CaMKAPs

CaMKII interactions with the CaMKAPs are differentially regulated by Ca^{2+}/calmodulin-binding and autophosphorylation. Moreover, different domains in CaMKII appear to be involved in binding to CaMKAPs (**Figure 1(a)**), as reflected by the lack of competition between CaMKAPs for binding to CaMKII and by the fact that C-terminal truncation of CaMKII has different effects on each interaction (Strack et al., 2000a; Robison et al., 2005b). Consistent with these observations, Thr^{286} autophosphorylated CaMKII can bind simultaneously to fragments of NR2B, densin-180, and α-actinin *in vitro,* suggesting that under appropriate conditions CaMKII can nucleate complexes containing these three proteins in intact cells (Robison et al., 2005b). Despite the distinct regulation of their interactions with CaMKII and their different effects on CaMKII activity, NR2B, densin-180, and α-actinin are at least partially colocalized in dendritic spines. Moreover, multiprotein complexes containing these proteins can be immunoprecipitated from brain extracts (Wyszynski et al., 1997; Dunah et al., 2000; Walikonis et al., 2001; Robison et al., 2005b). However, the role of CaMKII in formation of these multiprotein complexes in cells is unclear, and it is not clear how these protein assemblies relate to the regularly spaced arrays of CaMKII towers observed in PSDs. However, one can speculate that regulated interactions of CaMKII with different CaMKAPs might induce structural rearrangements of these complexes that may be important for ultrastructural or functional changes at the synapse.

4.23.8 Concluding Comments

Studies over the last 20 years have revealed tremendous complexity in the biochemical properties of CaMKII. Thr^{286} autophosphorylation provides an intuitively appealing mechanism for a molecular memory, and there is abundant evidence that this process is critical for synaptic plasticity and learning. However, the exact molecular basis for modulation of synaptic transmission by CaMKII remains unclear. Ongoing studies are revealing new ways in which CaMKII signaling can be modulated by its interactions with a plethora of additional synaptic proteins. These interactions are differentially regulated and can have diverse effects on CaMKII activity. These findings are revealing considerable nuance to CaMKII regulation that suggest new ways in which CaMKII may modulate synaptic transmission. The challenge will be to link these biochemical mechanisms to specific aspects within the continuum of physiological responses that are collectively described as synaptic plasticity. The reward for meeting this challenge will likely be new strategies for therapeutic intervention that might target disrupted excitatory synaptic transmission that is associated with numerous neurological diseases and disorders.

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4.24 Angelman Syndrome

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4.24.1 Introduction

The ability to perceive our environment, store information about our experiences, and retrieve that information when needed is a fundamental necessity for human cognition. Neuroscience research has only recently begun to unravel the mysteries of learning and memory by identifying the elemental molecules, important proteins, and signal transduction pathways that underlie the formation of long-lasting memories. The identification of specific genes and their products involved in disorders associated with human mental retardation has greatly facilitated our overall understanding of the mechanisms underlying human cognition. Recent research of Angelman Syndrome (AS) represents a quintessential example of this process at work.

Angelman syndrome is a devastating human mental retardation condition that affects specific areas of the central nervous system, including the hippocampus. The discovery of this brain region–specific disorder and subsequent production of a mouse model has allowed researchers the unique opportunity to explore the molecular mechanisms at work in the adult hippocampus that directly underlie human memory processes. This chapter recounts the important discoveries surrounding this disorder and the implications of these discoveries for our current knowledge of the molecular players present in the hippocampus that are thought to facilitate learning and memory.

4.24.2 Understanding the Genetics of AS

4.24.2.1 The Prevalence of AS

The prevalence of AS among children and young adults is between 1/10,000 and 1/20,000 and is commonly accompanied by severe mental retardation, epilepsy, a puppet-like gait, dysmorphic facial features, a happy disposition with bouts of inappropriate laughter, hyperactivity, sleep disorders, and lack of speech. The etiology of AS arises from an absence of genetic contribution from a localized region on chromosome 15. Nearly 70% of AS cases involve cytogenetic deletion of q11-q13 (~4 Mb) on the maternally inherited chromosome 15 (15_{mat}).

Interestingly, 15q11 deletions also occur in Prader-Willi syndrome (PWI); however, the deletion is found on the paternally inherited chromosome 15 (15_{pat}), and the two disorders are characterized by distinct phenotypic differences (Cassidy et al., 2000). This suggests that there is differential expression of the genes in the homologous chromosome 15 and that the etiology of AS is specifically the consequence of cytogenetic disruption of the maternal chromosome (first to postulate this were Magenis in 1990 and Williams in 1990).

Understanding the genetic anomaly that defines AS begins in understanding how genes can be inherited in an active state from one parent and, alternatively, in an inactive state from the other parent. This phenomenon, referred to as genomic imprinting, is a poorly understood epigenetic mechanism affecting a relatively few known autosomal mammalian genes (Redei et al., 2006). Usually, autosomal genes are present in duplicate (i.e., two alleles), with one inherited from the father and one from the mother. For the majority of genes, both alleles are transcribed (or expressed) equally. However, for a small subset of genes, known as imprinted genes, only one allele is expressed in a parent-of-origin-dependent manner. Note that the 'imprint' here refers to the epigenetic mechanism through which one allele is silenced and is completely unrelated to the classical 'filial imprinting' manifest at the behavioral level.

4.24.2.2 Maternal Imprinting and AS

The mechanism of imprinting involves the biochemical marking of DNA by methylation of CpG-rich domains in association with particular chromatin conformations. This epigenetic mark allows the molecular machinery of each cell in the progeny to recognize and appropriately express only one allele at a particular locus. For example, during maternal expression, paternally allelic DNA methylation silences the paternal transcript, and the remaining functional genes in the locus are exclusively expressed maternally. Chromosome 15 contains a cluster of imprinted genes in the q11-q13 region, many of which are involved in brain development and function and normally undergo exclusively maternal expression. Therefore, if cytogenetic deletion of q11-q13 occurs on 15_{mat}, none of the imprinted gene products are expressed. There are additional genetic mutations that can occur to produce null expression of these imprinted genes. For example, 7–9% of AS cases are the result of imprinting mutations in which both the maternal and paternal copies undergo paternal-like methylation, and 2–3% show paternal unpaired disomy (UPD), whereby two copies of the paternal chromosome region are inherited with no maternal copies present (**Figure 1**). Finally, there is a subpopulation of patients with clinically diagnosable AS who do not fall into the aforementioned three groups but, rather, represent familial-derived mutations of one or more genes in the maternal 15q11-q13 critical region.

The discovery that 15q11-q13 represents the AS critical region narrowed the search for candidate genes that give rise to AS. In 1997, a gene within the AS critical region named *Ube3a* was found to be mutated in approximately 5% of AS individuals. These mutations can be as small as 1 base pair. The product of the *Ube3a* gene was initially identified following viral studies of p53 tumor suppressor protein degradation by the oncogenic human papilloma virus (HPV). Researchers interested in HPV *E6* gene expression following infection found that the HPV E6 protein alone had no effect on p53; however, in the presence of an associated protein p53, degradation was observed (Huibregtse et al., 1991). This associated protein, referred to as E6-associated protein, or E6-AP, was found to be the 100-kDa protein encoded by the *Ube3a* gene. The *Ube3a* gene has a spatially restricted imprinted expression pattern.

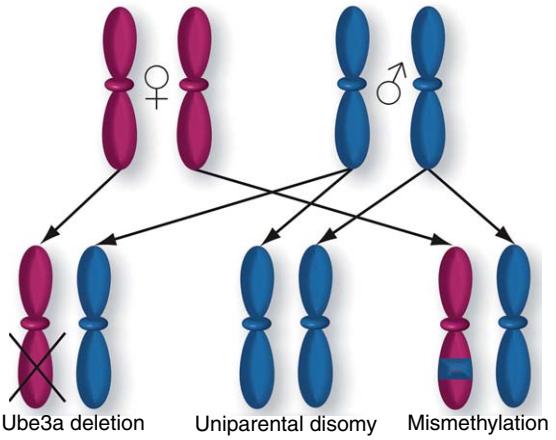

Figure 1 Maternal imprinting disorders resulting in Angelman Syndrome (AS). Genomic imprinting results in parent-specific epigenetic differentiation and monoallelic gene expression. Parental imprints are established during gametogenesis, where alleles of imprinted genes are maintained as either paternal or maternal origin. AS can arise from maternal deletion, uniparental disomy, or mismethylation of the AS critical region.

Ube3a shows imprinted expression in the brain but biallelic expression in other tissues. Each of the four genetic mechanisms described above that cause AS – large deletion, imprinting mutation, UPD, and point mutation – also cause inactivation or absence of the *Ube3a* gene.

4.24.2.3 The Ubiquitin Ligase Pathway

E6-AP is an enzymatic component of a complex protein degradation system termed the ubiquitin-proteosome pathway. This pathway is located in the cytoplasm of all cells. The pathway involves a small molecule, ubiquitin, which can be attached to proteins causing them to be degraded. Multiple steps are involved in the ubiquitination process (**Figure 2**). In the initial enzymatic step, the ubiquitin-activating enzyme E1 activates ubiquitin in an ATP-dependent reaction that links the E1 with the C terminus of a ubiquitin molecule. The ubiquitin-conjugating E2 enzymes then accept and transfer the activated ubiquitin moiety from E1 either to an E3 ligase or directly to a target substrate. The E3 ligase confers specificity to substrate recognition for ubiquitination. E6-AP ubiquitin ligase falls into the E3 category of ubiquitin lipases; therefore, it was believed that identifying the protein target(s) of E6-AP would unlock the mystery surrounding the AS disorder. To date, only four proteins have been identified as targets of E6-AP-dependent ubiquitination. These include the p53 tumor suppressor protein; the HHR23A protein, a human homolog to the yeast DNA repair protein Rad23 (Kumar et al., 1999); the multicopy maintenance protein (Mcm) 7 subunit involved in the initiation of DNA replication (Kuhne and Banks, 1998); and E6-AP, which is a target for itself (Nuber et al., 1998). Unfortunately, no obvious connection can be made between these identified targets and the etiology of AS.

4.24.3 Modeling AS in a Mouse

4.24.3.1 Production of the AS Mouse Model

The lack of a clear connection between E6-AP function and AS, coupled with the scarce availability of

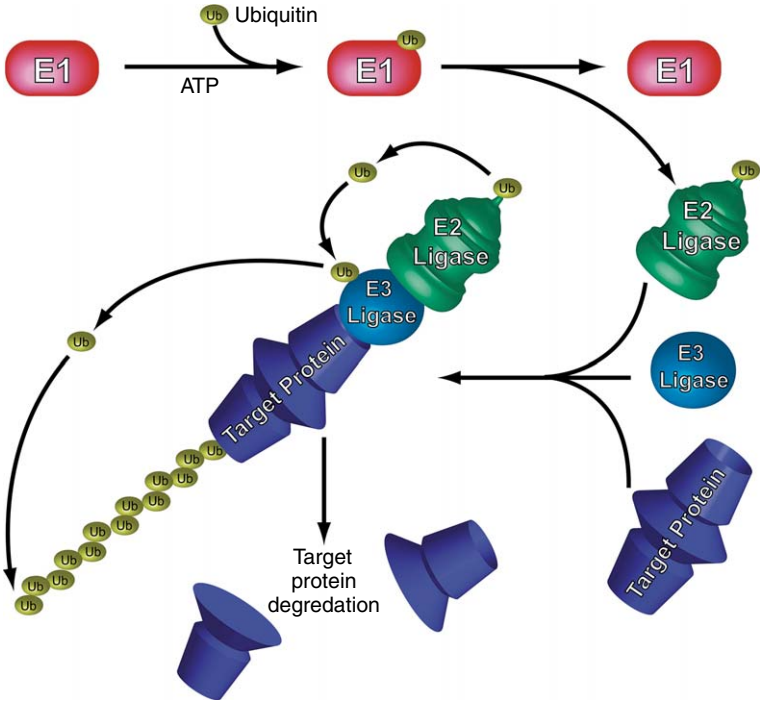

Figure 2 Ubiquitin ligase pathway. Association of an E1 ubiquitin ligase with a single ubiquitin (Ub) molecule results in an ATP-dependent manner. Ub is subsequently transferred to an E2 ligase. The E2 then associates with the E3, in this case Ube3a, and the target protein specified by the E3 ligase. The E3 ligase transfers successive Ub molecules to the target protein forming poly-Ub chains. When a critical length of the Ub chain is reached, the targeted protein associates with the proteosome and undergoes degradation.

AS postmortem brain tissue, necessitated the production of a mouse model for AS. Two mouse models were developed, each with a different strategy to disrupt the murine homolog of *Ube3a*. The mouse chromosome 7 contains a region exhibiting a similar gene compliment to the human AS critical region. The first mouse line, created by Gabriel and colleagues, utilized an Epstein-Barr virus latent membrane protein 2A (LMP2A) transgenic insertion that resulted in the deletion of the entire murine equivalent AS critical region. The rational for this method lies in mimicking what is seen in humans that exhibit the most common genetic defect leading to AS: an approximately 4-Mb deletion in the 15_{mat}q11-q13 region. Alternatively, the mouse model developed by Jiang and colleagues utilized a null mutation in *Ube3a* via a single gene knockout (Jiang et al., 1998). While both of these mouse models effectively disrupt *Ube3a*, there exists some controversy over whether *Ube3a* deficiency in and of itself is responsible for the manifestation of AS, or if the full phenotype is due to the combined affect of disrupting several genes in the human chromosome 15_{mat}q11-q13 critical region (Lee and Wevrick, 2000).

A phenotypic assessment in patients with the cytogenetic deletion (class I) exhibit a profile more true to what is commonly considered to be clinically classical AS. These patients show the full realm of AS characteristics including microcephaly and hypopigmentation. In contrast, patients for whom AS arises from specific *Ube3a* mutation (class II) or *Ube3a* mismethylation (class III) result in patients with fewer instances of hypopigmentation, microcephaly, and seizure. This indicates that AS caused by *Ube3a* disruption is exacerbated by the disruption of additional genes located at the same locus. Among these genes is the $GABA_A$ receptor $\beta 3$ subunit (*GABRB3*). There is evidence that the inhibitory neurotransmitter GABA is involved in the suppression of seizures, which may explain why class II and III AS patients experience fewer occurrences of seizure. In support of this hypothesis, a lower instance of seizure activity in the Jiang et al. mouse model compared to the other mouse model was observed. Because nearly all available evidence points to *Ube3a* as the locus for AS, and given the inherent difficulties in interpreting the effects of multiple gene deletions in mice, the consensus was that the single *Ube3a*-null mutation mouse would provide a better instrument to investigate the molecular mechanisms underlying the AS etiology. The following section discusses experiments conducted using the Jiang et al. AS mouse model and relates the results to the human condition.

4.24.3.2 Characterization of the AS Mouse Model

The initial characterization of the maternal deficient *Ube3a*-null mouse mutant (*Ube3a* m^-/p^+) revealed striking similarities to the human phenotype (Jiang et al., 1998). These phenotypic similarities are more easily discernible when they are subdivided into categories of physical and physiologic characteristics.

4.24.3.2.1 Physical Similarities of AS and the Maternal Deficient Ube3a-Null Mouse

The brains of *Ube3a*-m^-/p^+ mice showed an overall normal morphology, with no obvious abnormalities in any particular brain regions, suggesting that maternal E6-AP deficiency does not effect neuronal development or organization. However, by 18 days of age *Ube3a*-m^-/p^+ mice were observed to have smaller bodies and brains than wild-type mice. This size difference was seen in both the cortex and cerebellum of the brain. Imprinted expression analysis revealed that, similar to the human *Ube3a* gene, the murine *Ube3a* gene also exhibited a spatially restricted imprinted expression pattern in which exclusively maternal expression was observed in the brain. In adult wild-type mice, the detection of *Ube3a* mRNA revealed high expression levels in areas CA1 and CA3 of the hippocampus, basal ganglia, cerebellum, and cerebral cortex and the periglomerular, granual and mitral cells of the olfactory bulb. This was compared to maternal deficient mice that showed no detectable hippocampal expression, reduced cerebellar expression with no detectable expression in the Purkinje cell layer, and an overall reduced expression in the olfactory region associated with a lack of detectable expression in the mitral cell layer.

Motor function and coordination was assessed using hind-paw footprint analysis, the bar crossing test and the accelerating Rotorod test. Each of these tests confirmed a distinct and significant motor deficit in the *Ube3a*-m^-/p^+ mice, which mimics the tremor, ataxia and motor coordination defect described in human AS patients. In addition, seizure in AS patients is very prominent and found to affect greater than 90% of diagnosed AS patients. Likewise, seizures in *Ube3a*-m^-/p^+ mice could easily be induced through audiogenic means by simply running an object vigorously over the metal grate lid of

the mouse's home cage. Physical changes are more easily quantifiable and tend to be a more convincing argument for the recapitulation of a human mouse model. However, there are several ways to measure the behavioral correlates to human cognitive ability in the mouse by assessing its learning ability through behavioral testing and its function at a synaptic level through electrophysiologic techniques.

4.24.3.2.2 Cognitive Similarities of AS and the Maternal Deficient Ube3a-Null Mouse

The hippocampus is intimately involved in spatial memory processes and is linked to numerous cortical regions. Acquisition of explicit memory involves the hippocampus as part of a polymodal sensory integration scheme that processes visual, auditory, and somatosensory inputs. A great deal of evidence now implicates the hippocampus as an important brain structure involved in cognitive processing in humans. Although determining cognition is less straightforward in mice than it is in humans, we can call upon Pavlovian conditioning paradigms to asses the associative learning ability of *Ube3a*-m^-/p^+ mice.

The cognitive ability of the *Ube3a*-m^-/p^+ mice was assessed with the fear conditioning learning paradigm. The training paradigm consisted of an auditory conditioned stimulus (CS) paired twice with an unconditioned aversive stimulus (US: a mild foot shock) (*See* Chapter 4.11). The mice demonstrated their ability to associate the CS as a cue for the US or the training chamber context and the US by exhibiting a freezing behavior when re-presented with the CS or the training chamber. Although both contextual and cued fear-conditioned learning is dependent on the proper function of the amygdala, only the contextual learning is hippocampus dependent. The *Ube3a*-m^-/p^+ mutant mice exhibited robust freezing when re-presented with the CS 24 h after training, but significantly less freezing when reintroduced to the training chamber. These results indicate that hippocampus-dependent associative learning is disrupted in the *Ube3a*-m^-/p^+ mutant mice and is concordant with the imprinted expression pattern of *Ube3a*.

4.24.3.2.3 Physiologic Similarities of AS and the Maternal Deficient Ube3a-Null Mouse

The lack of hippocampal *Ube3a* expression coupled with the severe hippocampus-dependent learning deficit made the characterization of hippocampal synaptic function essential. The hippocampus is separated into three distinct synaptic pathways: the perforant path, the mossy fiber synapse, and the Schaffer collateral synapses. Of these three major synaptic connections, the Schaffer collaterals in area CA1 of the hippocampus are by far the most well characterized. It is in this region that synaptic transmission and plasticity were characterized in the *Ube3a*-m^-/p^+ mice. Jiang and colleagues found that basal synaptic transmission was unaltered in the AS mouse. This indicated that overall connectivity and single-stimulus synaptic function were essentially normal. Next, synaptic plasticity induced with high-frequency stimulation (HFS) was tested. It was shown that *Ube3a*-m^-/p^+ mice exhibited a severe deficit in long-term potentiation (LTP) induction. Although controversial, parallels can be drawn between the mechanisms of synaptic strengthening that occur during hippocampal LTP and those postulated to occur during hippocampus-dependent learning and memory events. Thus, a deficit in hippocampal LTP is consistent with a deficit in learning and memory. Perhaps most importantly, these studies were the first to implicate a deficit in hippocampal LTP in a human learning disability.

4.24.3.3 AS Mouse Hippocampal Physiology

It took nearly 3 years to identify a specific biochemical alteration in the AS mouse. Initially, the strategy employed identification of proteins that exhibited (1) a lack of ubiquitination in the AS mouse compared to normal mouse brain protein complement and (2) increased levels due to lack of degradation through the ubiquitin proteosome pathway. Although sound in design, identifying short-lived ubiquitinated proteins is problematic, and no gross changes in protein levels were observed in the AS mouse imprinted brain regions. It was not until a more in-depth analysis of hippocampal synaptic plasticity was performed that new and interesting implications of maternal Ube3a deficiency in the hippocampus were revealed.

Multiple stimulation patterns will elicit LTP in area CA1 of the hippocampus. To better understand the basis of the LTP deficit in the AS mouse, it is prudent to consider the LTP response to different patterns of stimulation (*See* Chapter 4.16). A standard LTP-inducing stimulation consisting of a 1-s, 100-Hz

stimulation elicits a long-lasting increase in synaptic plasticity. Alternatively, increasing the number of high-frequency trains of stimulation can produce a potentiation that is both substantially higher in magnitude and longer lasting. Manipulating these variables in the LTP induction protocol is often used to determine the efficacy or potency of an applied drug or, as in this case, to evaluate the severity or penetrance of the Ube3a deficiency. In order to test the possibility that the LTP deficits in *Ube3a*-m^-/p^+ mutants were due to an increase in the threshold of LTP induction, LTP was induced with a saturating amount of HFS designed to give the maximum potentiation in a given hippocampal slice. It was found that the saturating HFS could rescue the LTP deficit in the AS mouse.

What was the mechanism by which the LTP deficit could be overcome? The induction of LTP is highly dependent upon an influx of postsynaptic Ca^{2+}. Increasing the number of stimulations would increase the amount of Ca^{2+} influx significantly and could explain the LTP rescue. The question then would be whether the derangement of LTP induction was upstream (receptor activation) or downstream of calcium influx. The major neurotransmitter-activated calcium channel at the CA1 hippocampal synapse is the *N*-methyl-D-aspartate receptor (NMDAR) (*See* Chapter 4.20). Slices stimulated with repeated very high frequency stimulation in the presence of the NMDA receptor antagonist DL-2-Amino-5-phosphonopentanoic acid (AP5) would determine whether the increase in LTP threshold observed in *Ube3a* m^-/p^+ mutants was due to insufficient postsynaptic NMDAR-dependent Ca^{2+} influx. Under these conditions, the LTP deficit in the *Ube3a*-m^-/p^+ mice remained. The inability of *Ube3a*-m^-/p^+ mutants to achieve NMDAR-independent LTP induction suggested that the LTP deficit, and importantly, the potential site of hippocampal synaptic dysfunction, resided downstream of calcium influx. The studies described earlier set the stage for the investigation into the molecular derangements that could be responsible for the cognitive loss in AS patients.

4.24.4 Molecular Changes in the AS Mouse

It has been hypothesized that the deficit in LTP and learning was biochemical in origin. The lack of any morphological changes in the CNS, coupled with the ability to rescue LTP, suggested that a disruption in a biochemical signal transduction pathway was the explanation. The rescue of LTP also suggested that the mechanisms responsible for proper synaptic function were present but that the system needed to be pushed, (i.e., during repeated HFS) in order for the system to function at a level for LTP induction to occur. More importantly, the in-depth electrophysiology described earlier reduced the number of proteins to be examined from the greater than 10 000 present in the central nervous system to just a handful. The proteins chosen to be examined closer had to meet the following specific criteria: (1) known to be involved in LTP induction processes, (2) known to be involved in learning and memory processes, and (3) known to be activated or modified in the presence of Ca^{2+}. Extensive research showed no changes in the major synaptic-associated protein kinases including the phosphorylation levels of PKC, PKA, or ERK (P42) isolated from hippocampal homogenates of *Ube3a*-m^-/p^+ mice. However, a significant increase was detected in phospho-αCaMKII at the autophosphorylation site threonine 286 (Thr^{286}) (**Figure 3**) and threonine 305/306 (Thr^{305}/Thr^{306}). This single observation represented the first identified biochemical alteration in the AS mouse model that could potentially explain the learning and LTP deficits in the mouse and the cognitive deficits in human AS (Weeber et al., 2003). To help explain the implications of CaMKII misregulation, the next section addresses what is currently known about CaMKII regulation and its importance in normal signaling in the mammalian CNS.

4.24.5 CaMKII in Synaptic Plasticity and Memory Formation

4.24.5.1 Activation and Regulation of CaMKII

Initial studies of CaMKII's involvement in memory formation were conducted using an *in vitro* model for memory formation: LTP of the Schaffer-collateral synapses in area CA1 of the hippocampus. The Schaffer-collateral synapses are excitatory and use L-glutamate as their excitatory neurotransmitter. It has long been appreciated that induction of LTP requires high-frequency synaptic activity (Bliss and Lomo, 1973; Alger and Teyler, 1976) that results in activation of two types of glutamate receptors on postsynaptic CA1 pyramidal neurons: alpha-amino-3-hydroxy-

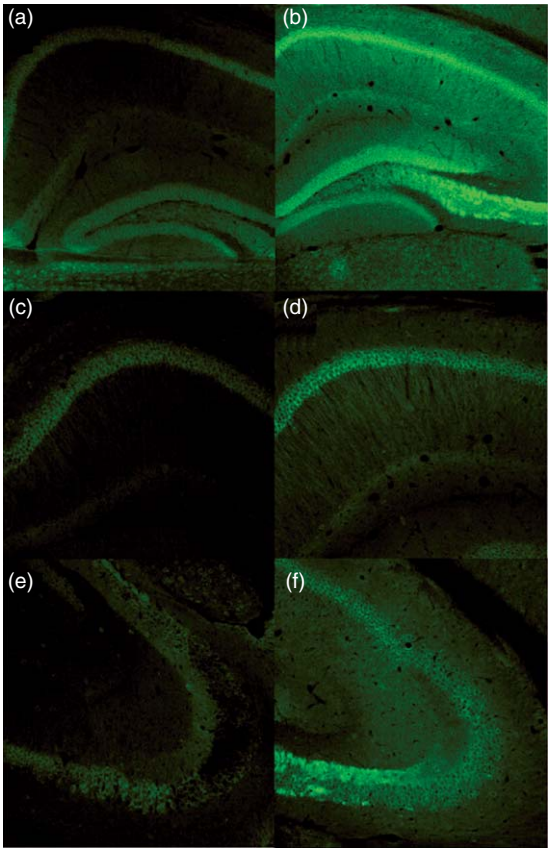

Figure 3 Increased threonine 286 Thr^{286} CaMKII phosphorylation in the Angelman Syndrome mouse model. Increased immunoreactivity to phosphorylated CaMKII at Thr^{286} in Angelman mouse hippocampus. Phosphorylated CaMKII at Thr^{286} was detected immunohistochemically in the hippocampi of wild type (a, c, e) and Angelman (b, d, f) mice. Increases in immunoreactivity are seen in the stratum pyramidale, stratum oriens, and stratum radiatum of CA1 and CA3 as well as the molecular layer and granule cell layer of the dentate gyrus. a, b, 100× magnification of the hippocampus. c, d, 200× magnification of area CA1. e, f, 200× magnification of area CA3.

5-methyl-4-isoxazole propionic acid (AMPA) and NMDA receptors. AMPA receptors have an ion channel that, when activated by glutamate, allows Na^+ to enter the cell, resulting in depolarization of the postsynaptic membrane (*See* Chapter 4.30). NMDARs are also coupled to an ion channel; however, under resting conditions this ion channel is blocked by a molecule of Mg^{2+} (*See* Chapter 4.20). Once the membrane is depolarized, the Mg^{2+} block is removed and Ca^{2+} ions flow through the NMDAR ion channel into the cytoplasm. The resulting increase in intracellular Ca^{2+} mediated by the NMDAR is the primary signal for induction of LTP (Lynch et al., 1983; Harris et al., 1984; Morris et al., 1986; Malenka et al., 1988). The increase in cytoplasmic Ca^{2+} concentration mediated by NMDARs is relatively short-lived, and therefore it cannot account for the long-term increase in synaptic efficacy observed after induction of LTP (*See* Chapter 4.21). Therefore, it has been proposed that the initial increase in Ca^{2+} leads to the activation of several downstream kinases, which modify the function of the various synaptic proteins and induce long-lasting changes in synaptic efficacy. This intimate relationship between NMDAR-mediated Ca^{2+} influx and CaMKII activation brings us back to the LTP deficit seen in the AS mouse. Saturating amounts of high-frequency stimulation, causing an optimum amount of Ca^{2+} influx, were used to overcome the LTP deficit. The signaling and autoregulatory aspects of Ca^{2+} on CaMKII will become more apparent in the following section.

The regulation of CaMKII through autophosphorylation mechanisms is extraordinarily dynamic and is a subject of review in a corresponding chapter of this volume (*See* Chapter 4.23). Briefly, Ca^{2+}-calmodulin activates CaMKII by disrupting the association between the catalytic and inhibitory domains (Colbran et al., 1989) (**Figure 4**). Once bound to Ca^{2+}-calmodulin, CaMKII undergoes autophosphorylation at amino acid residue Thr^{286}, a residue located within the inhibitory domain. Phosphorylation of Thr^{286} has three consequences for CaMKII function: the kinase becomes autonomously active (Saitoh and Schwartz, 1985), the affinity of Ca^{2+}-calmodulin for CaMKII increases 1000-fold, and a site becomes exposed, allowing CaMKII to bind NMDA glutamate receptors (Strack and Colbran, 1998; Leonard et al., 1999; Bayer et al., 2001). There exists another autophosphorylation site within the regulatory domain of the enzyme at threonine 305 and 306 ($Thr^{305/306}$) of the alpha and beta subunits, respectively. The potential functional consequences of phosphorylation at $Thr^{305/306}$ are poorly understood. However, it has been shown that $Thr^{305/306}$ phosphorylation can render the CaMKII enzyme inactive (Colbran, 1993). In addition, the $Thr^{305/306}$ resides in the Ca^{2+}-calmodulin binding site of CaMKII and inhibits subsequent binding of Ca^{2+}-calmodulin to CaMKII. Phosphorylation of $Thr^{305/306}$ can also occur in the absence of activation of CaMKII and possibly prevents subsequent activation by Ca^{2+}-calmodulin. This management of activation, alterations in Ca^{2+} and calmodulin sensitivity, and autophosphorylation is highly orchestrated.

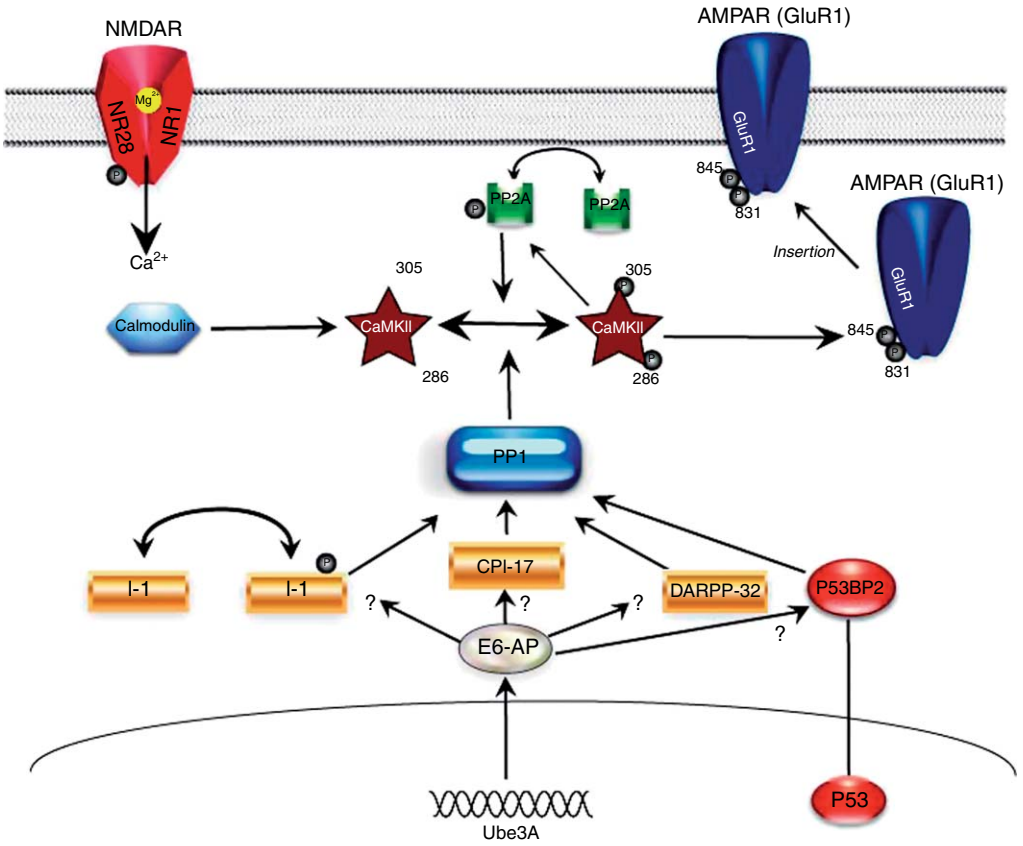

Figure 4 Schematic model of CaMKII regulation in the hippocampus. Inactivated CaMKII is located in the cytoplasm. Once activated by calcium-calmodulin and phosphorylated at threonine 286 (Thr^{286}), CaMKII translocates to the postsynaptic density, where it associates with *N*-methyl-D-aspartate receptors and is involved in alpha-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid receptor insertion. Autophosphorylation at $Thr^{305/306}$ decreases the affinity of CaMKII for *N*-methyl-D-aspartate receptors and signals for CaMKII translocation away from the postsynaptic density. Dephosphorylation of CaMKII by phosphatase activity once again allows sensitivity of CaMKII to future signaling via calcium influx.

Thus, even slight disruptions of this process can have devastating affects on synaptic function and cognitive ability (Colbran and Brown, 2004).

4.24.5.2 Regulation of CaMKII Activity in Synaptic Plasticity and Memory Formation

How is autophosphorylation tied to the regulation of synaptic plasticity? Recent studies have examined the role of the Thr^{286} and $Thr^{305/306}$ autophosphorylation in CaMKII-dependent synaptic function. Genetically modifying the Thr^{286} site to an alanine (T286A), which prevents phosphorylation at the Thr^{286} site, results in complete loss of LTP induction. Genetically modifying the Thr^{286} site to an aspartate (T286D), which mimics phosphorylation, also leads to impairment of LTP induction. More refined studies suggest that low levels of the autonomously active kinase facilitate, while high levels of autonomously active kinase inhibit, LTP induction. Alternatively, mutating the $Thr^{305/306}$ sites from a threonine to an alanine (T305/306A) increases the amount of CaMKII in the postsynaptic density (PSD) (*See* Chapter 4.32) and enhances LTP induction (Elgersma et al., 2002). Furthermore, mutating the threonine to an aspartate (T305/306D) decreases the amount of CaMKII in the PSD and blocks induction of LTP (Elgersma et al., 2002). Together, these results indicate that CaMKII is critical in the induction of LTP and must be active in a precise spatiotemporal pattern. Synaptic plasticity in the hippocampus is believed to be involved in the formation of spatial memories. Disruption of CaMKII function has profound effects on hippocampal LTP; therefore, one would expect disruption of CaMKII to have dramatic effects on long-term memory

formation. Animals lacking the CaMKII gene show profound deficits in spatial learning tasks (Silva et al., 1992; Bach et al., 1995). In addition, both sites of autophosphorylation appear to be involved in the formation of long-term memory. Deficits in spatial learning tasks are observed in animals that have either the T286A or T286D mutation. Moreover, animals possessing the T305/6A mutation exhibited enhanced spatial learning, while animals with the T305D mutation had severe deficits in spatial learning. All of these observations indicate that CaMKII is critical for the proper formation of hippocampus-dependent spatial memory.

4.24.6 Genetic Rescue of the AS Phenotype

The identified alterations in CaMKII raise the question of which alteration in phosphorylation, Thr^{286}, $Thr^{305/306}$, or both, is responsible for the observed AS phenotype. Due to the severe detrimental effects of CaMKII $Thr^{305/306}$ phosphorylation on synaptic plasticity and memory formation, as well as the similarities in the phenotypes with the AS mouse, the $Thr^{305/306}$ site was hypothesized to be responsible for the major phenotypes seen in AS. Studies in the laboratories of Weeber and Elgersma utilized female AS mice crossed with heterozygous αCaMKII males that carried the targeted *αCaMKII-T305V/T306A* mutation (CaMKII-TT305/6AV). This point mutation essentially prevents CaMKII inhibitory phosphorylation , but the use of heterozygotes allows a subset of endogenous CaMKII to be present without the mutation. Thus, the resulting F1 offspring yielded mutants with four different genotypes: wild-type (WT) mice, mutants carrying the single $\alpha CaMKII\text{-}305/6^{+/-}$ or AS mutation, and mice carrying the double AS/CaMKII-$305/6^{+/-}$ mutation. Hippocampal-dependent learning was assessed using the contextual fear-conditioning paradigm (*See* Chapter 4.11), utilizing the same protocol of two US–CS pairings employed in the original AS mouse characterization. The genetic prevention of $Thr^{305/306}$ phosphorylation is sufficient to rescue the defect in learning and memory. The CaMKII-$305/6^{+/-}$ mice can learn to associate the context and the foot shock to the same extent as wild-type littermates tested 24 h or 7 days following training (**Figure 5**). These results support the hypothesis that the underlying hippocampal learning deficit is due primarily to αCaMKII inhibitory phosphorylation.

Interestingly, αCaMKII inhibitory phosphorylation can influence the threshold for the induction of LTP. This is nicely demonstrated in the homozygous CaMKII-TT305/6AV mouse that exhibits increased LTP induction when a subthreshold stimulation is given, but in which normal LTP is induced when a strong stimulation is applied (*See* Chapter 4.16). In contrast, AS mice exhibit a significant LTP deficit utilizing a modest stimulation protocol, which is rescued using multiple trains of HFS. Consistent with the associative learning rescue experiments, AS mice showed a severe LTP deficit compared to wild-type mice and CaMKII-$305/6^{+/-}$ mice. However, the AS/CaMKII-$305/6^{+/-}$ double mutants show comparable LTP to that of wild-type mice and lack the LTP deficit seen in their AS mouse littermates (**Figure 5**). These studies dramatically show that the synaptic plasticity deficits established in the AS mice are the result of increased CaMKII inhibitory phosphorylation. Thus, genetic manipulation of the phosphorylation state of the inhibitory site of CaMKII effectively rescues both the synaptic plasticity deficit and cognitive disruption in the *Ube3a*-null mutation mouse model of AS.

The studies described here strongly suggest that the increased inhibitory phosphorylation of CaMKII is the site of molecular dysfunction underlying the cognitive and synaptic plasticity deficits associated with AS. This validates the great amount of research showing CaMKII dependence for normal synaptic plasticity and memory formation. Astonishingly, AS represents the lone human disorder that to date presents with a disruption in cognition that is associated with CaMKII dysfunction. This is likely attributed to both the importance of CaMKII substrates in peripheral cellular function and the number of developmental pathways in which CaMKII is enlisted. If not for the limited region-specific deletion of Ube3a, it is likely AS would result in embryonic lethality. This consideration raises the obvious question of whether the genetic rescue with the CaMKII-$305/6^{+/-}$ mutation is due to the normalization of the CaMKII signaling capacity in the adult, the ability for normal CaMKII-dependent development of the central nervous system, or both. The observation of occasional abnormalities in AS patients including scoliosis, hyperreflexia, hypopigmentation, strabismus, myopia or hypermetropia, and nystagmus would suggest a development component resulting from a CaMKII dysfunction.

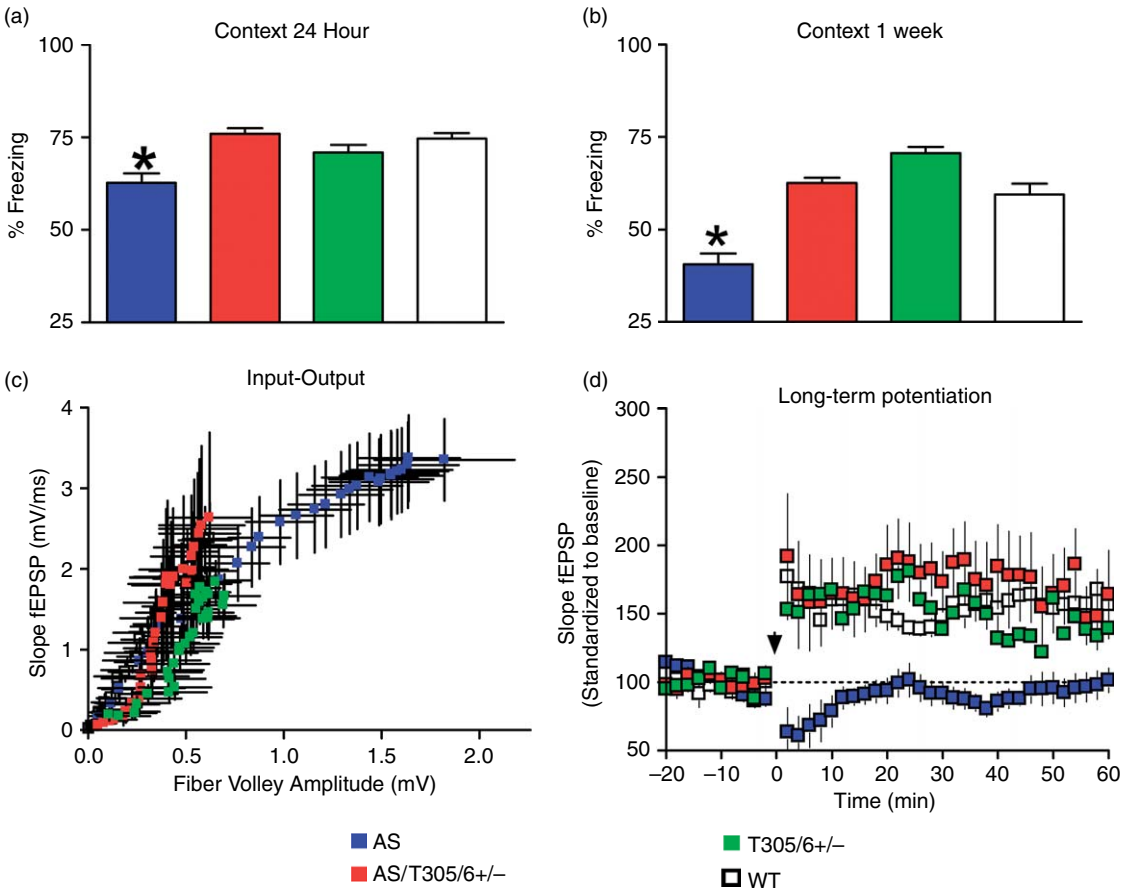

Figure 5 Hippocampal learning and synaptic plasticity in AS mice is rescued by the T305/6-CaMKII mutation. Mice trained in a conditioning chamber through the association with the context and two mild foot shocks were later reintroduced to the context. Freezing was assessed during a 3-min time period, and the amount of freezing represents the strength of the associative memory formed. Angelman Syndrome (AS) mutants show impaired contextual conditioning 24 h (a) and 1 week (b) after training, which is rescued in the AS/T305/6-CaMKII$^{+/-}$ mutants. Increased synaptic transmission (c) and disruption of LTP (d) is rescued in *Ube-3a*-m^{-}/p^{+}/T305/6-CaMKII$^{+/-}$ double mutants compared to AS mice. LTP was induced with two trains of 100-Hz stimulation delivered 20 s apart (represented by arrow) in hippocampus area CA1.

4.24.7 Proposed Mechanisms Underlying CaMKII Misregulation

The genetic rescue of the AS phenotype is a tremendous step forward, but the molecular connection between ubiquitin deficiency and alterations in CaMKII phosphorylation remains unclear. As discussed above, CaMKII cycles between phosphorylated and nonphosphorylated states. The cycling state of CaMKII is not unlike many other proteins involved in signal transduction processes that undergo endless cycles of phosphorylation and dephosphorylation. Evolution has perfected this process in the CNS in order to provide an exceptionally rapid and potent mechanism for the regulation of protein function. The phosphorylation of numerous proteins is kept in check by three major protein phosphatases (PP) found in the brain: PP1, PP2A, and PP2B (calcineurin). PP1 and PP2A appear to be primarily responsible for dephosphorylation of CaMKII at Thr^{286}, with PP2A regulating CaMKII phosphorylation in the cytoplasm and PP1 in the postsynaptic density (Mansuy, 2003) (**Figure 4**). Through the actions of PP1 and PP2A, the activity of CaMKII is held in check and allows the enzyme to return to a state where Ca^{2+} influx can again activate the proper signal transduction cascades involving CaMKII. The presence of PP1 and PP2A causes an apparent conundrum in the regulation of CaMKII. CaMKII is a Ca^{2+}-dependent kinase; however, under

conditions conducive to induction of long-term synaptic plasticity or memory formation, CaMKII undergoes autophosphorylation and becomes a Ca^{2+}-independent autonomous enzyme. How does this occur in the presence of PP1 and PP2A? Several proteins have been identified that act as inhibitors of PP activity. One example is the Inhibitor 1 (I1) protein (Huang and Glinsmann, 1976). When phosphorylated via protein kinase A, I1 can bind to and inhibit the activity of PP1. Inhibition of PP1 via I1 facilitates the transition of CaMKII from a Ca^{2+}-dependent to an autonomous state. The functional consequences of PPs on CaMKII activity are not solely negative. As noted above, CaMKII contains an inhibitory autophosphorylation site at $Thr^{305/306}$. Phosphorylation at this site downregulates CaMKII activity and prevents binding by Ca^{2+}-calmodulin. PP1 can dephosphorylate $Thr^{305/306}$, which then allows for reactivation of CaMKII by Ca^{2+}-calmodulin (Patton et al., 1990). **Figure 4** shows the highly regulated action of protein phosphatases, and the proteins that control their activity as well. Thus, the changes in the phosphorylation state of AS mouse hippocampal CaMKII could be due to changes in protein levels in one or more of these proteins. Interestingly, PP1 and/or PP2A phosphatase activity is significantly reduced in the AS mutants by a prodigious 2.5-fold decrease (Weeber et al., 2003). This finding strongly suggests that the aberrant state of P-Thr^{286} and P-Thr^{305} αCaMKII phosphorylation is due to changes in the activity of one or both of these important phosphatases. This also suggests that the increase in Thr^{305} αCaMKII is due to both the increase in P-Thr^{286} αCaMKII, since phosphorylation at Thr^{286} precedes phosphorylation at Thr^{305} (Colbran and Soderling, 1990; Hanson and Schulman, 1992), and reduced PP1 and/or PP2A activity, which are known to dephosphorylate P-Thr^{305} αCaMKII *in vitro* (Patton et al., 1990).

4.24.8 Concluding Remarks

The studies discussed here have set the stage for the next phase of research that will work toward uncovering the molecular pathways and mechanisms linking Ube3a maternal deficiency and CaMKII regulation. Future discoveries of E6-AP targets will likely shed the most light on this puzzling connection. Regardless, important lessons have been learned from the discovery of AS allele and its regulation. First, the production of the AS mouse model reemphasizes the utility and importance of mouse models for human disorders (*See* Chapters 4.19, 4.33, 4.42). Second, AS was one of the first human mental retardation disorders to bring to light the potential deleterious effects of epigenetic changes. The identification that the AS gene expresses a housekeeping-type protein dramatically illustrates the unexpected importance of proteins such as ubiquitin ligases once considered minor for normal synaptic function. Finally, these studies illustrate a compelling example of a human learning and memory disorder associated with deficits in hippocampal synaptic plasticity and CaMKII function.

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4.25 Mitogen-Activated Protein Kinases in Synaptic Plasticity and Memory

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4.25.1 Introduction

Mitogen-activated protein kinases (MAPK) make up an evolutionarily conserved family that regulates cellular growth and stress responses in mitotic cells throughout eukaryotic phyla (reviewed in Chang and Karin, 2001; Pearson et al., 2001). Mammalian cells express three major subfamilies of MAPK: The extracellular-regulated kinases (ERKs), the c-Jun amino-terminal kinases (JNKs), and p38 MAPKs. Each of these subfamilies of MAPKs forms part of a modular kinase cascade in which a MAPK is activated by dedicated MAPK kinases (MAPKKs or MAP2Ks, also designated MKKs or MEKs), which are in turn activated by multiple MAPK kinase kinases (MAPKKKs or MAP3Ks, also designated MKKKs or MEKKs). Sequential phosphorylation within these modules allows for integration of extracellular signals as well as increased specificity and amplification of MAPK-mediated downstream responses. Scaffold proteins that facilitate the interaction of components of these tripartite MAPK modules further enhance specificity with respect to both activating stimuli and substrates, allowing for precise spatial and temporal control of signaling activity.

Each MAPK subfamily or module is characterized by the existence of multiple isoforms at each hierarchical level: ERK1-2 are activated by MEK1-2, JNK1-3 are activated by MEK4 and MEK7, and p38α-δ are activated by MEK3 and MEK6. In contrast to the essentially monogamous relationship between MAP2Ks and their substrate MAPKs,

MAP3Ks can activate multiple MAP2Ks, and conversely, a given MAP2K can be activated by multiple MAP3Ks, depending on the nature of the extracellular signal. MAPKs are proline-directed serine/threonine kinases that phosphorylate substrates preferentially at (S/T)P target sequences. MAP2Ks are dual specificity kinases that phosphorylate both threonine and tyrosine residues in a conserved TXY target sequence in the activation loop of MAPK substrates. Dual phosphorylation by a MAPKK is absolutely required for MAPK activity under most conditions, and experimental detection of dually phosphorylated MAPK therefore provides a useful marker for MAPK activity. MAPK activation is antagonized by a group of protein phosphatases that dephosphorylate the critical threonine and/or tyrosine residues in the activation loop (reviewed in Keyse, 2000; Barr and Knapp, 2006).

The prototype and best-studied of the mammalian MAPK subfamilies is the ERK cascade. ERK1/2 are activated downstream of Ras-coupled tyrosine kinase receptors (RTKs) in response to growth, proliferation, or differentiation signals. GTP-bound Ras activates the MAP3Ks B-Raf and Raf-1, which leads to sequential activation of MEK1/2 and ERK1/2. The signaling activity of Ras is activated by guanine nucleotide exchange factors (GEFs), which stimulate replacement of Ras-bound GDP with GTP; conversely, Ras activity is suppressed by GTPase-activating proteins (GAPs), which stimulate the intrinsically weak GTPase activity of Ras. Consistent with the role of the ERK pathway in cell growth and proliferation, activating mutations in multiple components of this cascade, including Ras, Raf, and MEK, confer oncogenic properties and have been associated with human cancers (Schubbert et al., 2007). Furthermore, germline mutations in Ras, Raf, and MEK, which are associated with upregulation of ERK activity, are responsible for several developmental disorders, including Noonan, Costello, and cardiofaciocutaneous syndrome (Schubbert et al., 2007).

In addition to the well-described role of RTKs and Ras in activating the Raf-MEK-ERK module, ERK activity can also be regulated by seven-transmembrane domain G protein-coupled receptors (GPCRs) through G protein-dependent and -independent mechanisms. Agonist binding to GPCRs results in G protein activation and stimulation of adenylyl cyclase. Elevated intracellular cyclic adenosine monophosphate (cAMP) levels activate the small GTPase Rap1, and activated Rap1 can either stimulate or inhibit Ras-dependent ERK activation in a cell type-specific manner (Stork and Schmitt, 2002). Agonist binding to GPCRs also induces phosphorylation of the receptors by G protein–coupled receptor kinases (GRKs), promoting recruitment of β-arrestins and receptor desensitization. β-arrestins uncouple GPCRs from G proteins by blocking their interaction, but at the same time β-arrestins provide a scaffold for assembly of Src tyrosine kinases with components of the Raf-MEK-ERK family, resulting in ERK activation (Miller and Lefkowitz, 2001; Morrison and Davis, 2003). In contrast to the strong, transient ERK activation induced by Ras-coupled RTKs, which often elicits nuclear translocation of ERKs, GPCR-dependent ERK activation exhibits a more modest, sustained and localized profile.

Transcriptional regulation is a major downstream effector mechanism by which the ERK pathway transduces mitogenic signals into growth responses (Treisman, 1996). Two principal routes to transcriptional regulation by the ERK pathway have been characterized. First, activated ERK translocates to the nucleus and directly phosphorylates a number of transcription factor substrates, including Elk-1 and c-Myc. Phosphorylation of Elk-1 by the ERK pathway has been shown to stimulate serum response element (SRE)-dependent transcription. Second, ERK can activate several intermediary kinases, which then phosphorylate transcription factors and modulate their activity. For example, the ERK substrates Rsk2, MAPKAP kinase 2, and Msk1/2 have all been reported to phosphorylate the cAMP response element-binding protein (CREB) on a serine residue (Ser133) critical for its transcriptional activation function. Expression of the immediate-early gene *c-fos* is highly responsive to ERK activation due to the presence of both SRE and cAMP response element (CRE) sequences in its proximal promoter.

Although there is some overlap among the ERK, JNK, and p38 pathways with respect to activating stimuli in mitotic cells, the JNK and p38 cascades respond primarily to physical and chemical stress. The JNKs, which are also known as stress-activated kinases (SAPKs), are activated in response to ultraviolet (UV) irradiation, growth factor deprivation, cytokines, and inhibition of macromolecular synthesis. The p38 MAPKs can be activated by cytokines, UV irradiation, and osmotic and oxidative stress. Transcriptional regulation is a major effector mechanism by which the JNK and p38 cascades induce specific cellular responses, such as JNK-dependent apoptosis and p38-dependent cytokine production and inflammatory responses.

Components of MAPK cascades exhibit widespread and abundant neuronal expression throughout the central nervous system (Fiore et al., 1993a). In view of the well-characterized role of the ERK cascade in cellular growth and proliferation, it was initially unclear what functional role they might serve in postmitotic neurons. Fundamentally, however, MAPK cascades enable cells to produce appropriate and adaptive responses to their extracellular environment. Therefore, it is perhaps not surprising that MAPK cascades have been co-opted in neurons to mediate cellular responses to synaptic stimulation. In particular, MAPK signaling plays a central role in regulating modifications of synaptic strength and structure, which may represent the neuronal analog of growth in mitotic cells.

A number of excellent reviews have previously summarized the accumulating evidence for essential roles of MAPK in synaptic plasticity, learning, and memory in both mammals and invertebrates (Sweatt, 2001, 2004; Sharma and Carew, 2004; Thomas and Huganir, 2004; Davis and Laroche, 2006). Our objective here is to provide an up-to-date picture of the involvement of MAPK pathways in these processes, highlighting emerging evidence for a selective contribution of the ERK pathway to protein synthesis-dependent synaptic plasticity and memory consolidation. Owing to constraints of space, we focus on the role of the ERK pathway in synaptic plasticity and memory in mammalian systems while touching on important findings relating to the other MAPK cascades.

4.25.2 Mitogen-Activated Protein Kinase Activation in Neurons

The ERK cascade is activated in neurons in response to a wide variety of stimuli associated with synaptic plasticity. The mechanism of ERK activation most reminiscent of its action in mitotic cells occurs through stimulation of the tyrosine kinase Trk receptors by neurotrophins (Marsh et al., 1993; Segal and Greenberg, 1996). Treatment of cortical neurons with brain-derived neurotrophic factor (BDNF) elicits tyrosine autophosphorylation of the trkB receptor, recruitment of Ras, and activation of the canonical Raf/MEK/ERK cascade. Stimulation of *N*-methyl-D-aspartate (NMDA) receptors elicits ERK activation in hippocampal neurons (Bading and Greenberg, 1991; Kurino et al., 1995; English and Sweatt, 1996). Overexpression of dominant-negative Ras or a Ras GAP suppresses NMDA-induced ERK activation (Iida et al., 2001; Zhu et al., 2002), implying that calcium influx through NMDA receptors (NMDARs) impinges on mechanisms that increase the levels of GTP-bound Ras. Similarly, membrane depolarization and calcium influx through voltage-gated calcium channel (VGCC) stimulate Ras-dependent ERK activation in neuronal cells (Rosen et al., 1994; Farnsworth et al., 1995; Rosen and Greenberg, 1996; Iida et al., 2001).

At least five routes leading from elevated neuronal calcium levels to Ras activation have been characterized. First, calcium-dependent activation of Ras can be mediated by two highly related neuron-specific GEFs (also known as GRFs, guanine-nucleotide releasing factors), Ras-GRF1, and Ras-GRF2. Binding of calcium-calmodulin to an IQ motif in the Ras-GRFs strongly enhances their ability to activate Ras. Analysis of single and double Ras-GRF knockout mice demonstrated that Ras-GRF2 makes the major contribution to ERK activation in response to NMDAR activation in mature hippocampal slices, while Ras-GRF1 makes a relatively minor contribution (Li et al., 2006). The use of pharmacologic inhibitors specific for NMDARs containing either the NR2A subunit or NR2B subunit further suggested that Ras-GRF2 selectively couples NR2A-containing receptors to ERK activation in the mature hippocampus. In contrast, Ras-GRF1 couples to NR2B-containing NMDARs to activate p38 MAPK (Li et al., 2006). Consistent with these findings, an independent study employing NR2 subunit-specific inhibitors and RNA interference identified a dominant role for NR2A NMDARs in mediating NMDA-induced ERK activation in mature neurons, whereas NR2B NMDARs functioned to inhibit ERK activation, shortening its duration (Kim et al., 2005).

The relative contributions of Ras-GRFs, and NR2A- and NR2B-containing NMDAR, to NMDA-induced activation of the Ras-ERK pathway are subject to significant developmental regulation. Ras-GRFs are present at low levels in the neonatal mouse brain and gradually increase during the first few postnatal weeks, with persistently high levels of expression in the adult brain. Accordingly, Shc- and GRB2-mediated recruitment of Son-of-Sevenless (SOS) GEFs may support NMDA-induced activation of the Ras-ERK cascade in immature neurons (Tian et al., 2004). Similarly, NR2B appears to play a more important role in mediating NMDAR-induced ERK activation in immature neurons, followed by a developmental shift in which NR2B acts to limit ERK activation (Krapivinsky et al., 2003; Kim et al.,

2005). Several lines of evidence suggest that SynGAP, a neuron-specific Ras GAP, may act to limit ERK activity in response to NR2B NMDAR activation. Both Ras-GRF1 and SynGAP associate with NR2B, Ras-GRF1 through a direct interaction and with SynGAP through indirect interaction mediated by PSD-95 (Krapivinsky et al., 2003; Kim et al., 2005). The GAP activity of SynGAP is reportedly stimulated by phosphorylation by calcium/calmodulin-dependent protein kinase II (CaMKII), which also interacts with the NR2B subunit, suggesting a mechanism for calcium-induced Ras inhibition following NR2B NMDAR activation (Oh et al., 2004). Consistent with this notion, NMDAR-mediated ERK activation is depressed by SynGAP overexpression and accentuated by reduced SynGAP expression (Kim et al., 2005; Rumbaugh et al., 2006). In contrast, p38 MAPK activation is potentiated by SynGAP overexpression and depressed by SynGAP inactivation, but these effects are observed only in the absence of synaptic activity (Rumbaugh et al., 2006). Interestingly, it has also been observed that ERK is activated by stimulation of the synaptic pool of NMDARs, which contain both NR2A and NR2B subunits, whereas ERK is inactivated by stimulation of extrasynaptic NMDARs, which contain primarily NR2B subunits (Ivanov et al., 2006). Parallel examination of p38 MAPK has suggested opposing regulation of ERK and p38 activity mediated by SynGAP (Rumbaugh et al., 2006).

A second calcium-dependent route to Ras-ERK activation involves the cytoplasmic tyrosine kinases Src and Pyk2. Pyk2 is highly enriched in brain, and its tyrosine kinase activity is strongly stimulated by membrane depolarization and calcium influx through VGCC, as well as by activation of protein kinase C (PKC) (Lev et al., 1995). Autophosphorylated PYK2 can recruit the Grb2-Sos1 complex directly and indirectly through Shc, leading to calcium- and PKC-induced activation of Ras and MAPK. Tyrosine autophosphorylation of PYK2 also promotes direct interaction with Src, which activates Src kinase activity (Thomas and Brugge, 1997). Src has been implicated in Ras activation and downstream neuronal events in response to calcium influx through VGCCs (Rusanescu et al., 1995), suggesting an additional mechanism by which calcium-induced PYK2 activation can recruit Ras-ERK signaling.

Third, calcium signals can be relayed to the Ras-ERK cascade through calcium-calmodulin-dependent kinases. Distinct roles for both CaMKI and CaMKII in Ras-ERK activation have been proposed. NMDAR-induced ERK activation in hippocampal neurons and slices is blocked by inhibition of CaMKK and its downstream target CaMKI, whereas inhibition of CaMKIV and CaMKII activity had no effect (Schmitt et al., 2005). ERK activation in response to membrane depolarization in neuronal cells has also been reported to require CaMKK and CaMKI acting upstream of Ras (Schmitt et al., 2004). NMDAR-dependent long-term potentiation (LTP) induced by TBS activates ERK in a CaMKK-dependent manner, and inhibition of CaMKK and MEK cause mutually occlusive impairments in LTP. These findings suggest that activation of Ras-ERK signaling by calcium influx through NMDARs or VGCCs occurs through sequential activation of CaMKK and CaMKI. However, other findings suggest that NMDAR-mediated calcium influx during LTP induction can activate the Ras-ERK cascade through CaMKII. In cultured rat hippocampal slices, expression of a constitutively active form of CaMKII produces a Ras-dependent potentiation of AMPAR-mediated responses that occludes pairing-induced LTP (Zhu et al., 2002). The mechanism linking CaMKII to Ras in this context, which is presently undefined, would seem to oppose the inhibition of Ras-ERK signaling mediated by the action of CaMKII on SynGAP. A fourth mechanism by which calcium influx is coupled to ERK activation relies on the calcium-stimulated adenylyl cyclases AC1 and AC8 (Ferguson and Storm, 2004). Overexpression of AC1 enhances levels of ERK activation in hippocampal neurons, and hippocampal ERK activation is impaired by deletion of AC1 and AC8 (Wang et al., 2004; Sindreu et al., 2007). Finally, a fifth pathway conveying calcium signals to ERK involves the activation of Ras and Rap1 by a family of calcium and diacylglycerol-regulated GEFs, known as CalDAG-GEFs (Ebinu et al., 1998; Kawasaki et al., 1998).

Adenylyl cyclase activation and elevated levels of cAMP have been widely reported to activate ERK in PC12 cells and hippocampal neurons (Vossler et al., 1997; Impey et al., 1998; Roberson et al., 1999; Ambrosini et al., 2000; Iida et al., 2001; Patterson et al., 2001). However, the mechanism coupling elevated levels of cAMP to the ERK cascade remain unsettled, with evidence in neuronal cells supporting either Ras- or Rap1-dependent pathways, which can function in protein kinase A (PKA)-dependent or PKA-independent manners. The complexity of ERK activation by cAMP appears to derive from the differing subcellular localizations of Ras and Rap1 and their respective GEFs, as well as their

differential ability to activate Raf isoforms (reviewed in Stork, 2003). Ras can activate both of the neuronal Raf isoforms, Raf-1 and B-Raf. In contrast, Rap1 is capable of activating B-Raf, whereas it binds to but does not activate Raf-1, which may account for its ability to antagonize Ras function in some contexts. Ras is primarily localized to the plasma membrane, while Rap1 localizes to a variety of membrane compartments, including Golgi, endosomes, lysosomes, plasma membrane, and perinuclear structures. PKA-independent activation of Rap1 by cAMP is mediated by Epac1/2, Rap1-GEFs that directly bind cAMP. Epacs activate a perinuclear pool of Rap1 that does not result in activation of B-Raf or ERK. In contrast, PKA-dependent activation of Rap1 by the Rap1-GEF C3G enables activation of plasma membrane-localized Rap1 and B-Raf, which appears to be the major pathway responsible for forskolin-induced ERK activation in PC12 cells (Wang et al., 2006). The mechanisms linking cAMP to ERK have been widely found to exhibit cell type-specific differences; for example, cAMP-induced ERK activation is accompanied by Ras activation in cortical neurons but not in PC12 cells (Ambrosini et al., 2000). Highlighting the significance of restricted patterns of subcellular localization, inhibition of hippocampal Rap1 activity in transgenic mice causes a selective reduction in the activation of a membrane-associated pool of ERK, while overall levels of cytosolic ERK activation remain unaffected (Morozov et al., 2003).

A role for Rap1 and Rap2 in activation of the p38 and JNK cascades, respectively, has emerged from studies of LTP and AMPAR trafficking in cultured rat hippocampal slices. Overexpression of constitutively active Rap1 stimulates p38 but not ERK activation, and overexpression of dominant-negative Rap1 suppresses p38 but not ERK activation (Zhu et al., 2002). Similarly, overexpression of constitutively active Rap2 stimulates JNK but not ERK or p38 activation, and overexpression of dominant-negative Rap2 suppresses JNK but not ERK or p38 activation (Zhu et al., 2005).

The ERK pathway is also potently activated in cultured neurons and hippocampal slices by agonist binding to several classes of neuromodulatory receptors that play important roles in synaptic plasticity, including group 1 metabotropic glutamate receptors, muscarinic acetylcholine receptors, dopamine receptors, and β-adrenergic receptors (Fiore et al., 1993b; Roberson et al., 1999; Ambrosini et al., 2000; Rosenblum et al., 2000). Consistent with the role of the cAMP/PKA second messenger pathway in transducing signals from G-protein coupled receptors, ERK activation in hippocampal slices in response to dopamine and the β-adrenergic agonist isoproterenol is blocked by a PKA inhibitor. Activation of PKC by phorbol ester elicits ERK activation, and ERK activation in response to specific agonists of group I mGluRs requires PKC. The role of PKC in conveying signals from muscarinic acetylcholine receptors to the ERK pathway is less clear. Stimulation of muscarinic receptors with carbachol reliably induces ERK activation in hippocampal slices and cultured cortical neurons, but variable blockade of carbachol-induced ERK activation by PKC inhibitors has been reported (Roberson et al., 1999; Rosenblum et al., 2000). The scaffolding protein KSR1 (kinase suppressor of Ras 1), which promotes association of Raf-1 with MEK and ERK, specifically couples type 1 mGluRs and PKC to activation of a membrane-associated pool of ERK, which appears to target only a subset of ERK substrates in the hippocampus (Shalin et al., 2006). In contrast, KSR1 does not influence the coupling of cAMP/PKA to hippocampal ERK activation.

Induction of CA1 LTP and hippocampal long-term memory are accompanied by NMDAR-dependent ERK activation (English and Sweatt, 1996; Atkins et al., 1998). A large number of studies subsequently confirmed and extended these findings by documenting ERK activation in response to the induction of various forms of ERK-dependent LTP, long-term depression (LTD) and long-term memory in the hippocampus, amygdala, and neocortex (e.g., Berman et al., 1998; Blum et al., 1999; Winder et al., 1999; Kanterewicz et al., 2000; Schafe et al., 2000; Dudek and Fields, 2001; Patterson et al., 2001; Thiels et al., 2002; Ying et al., 2002; Kelly et al., 2003; Gallagher et al., 2004; Kelleher et al., 2004a; Levenson et al., 2004; Wang et al., 2004; Kushner et al., 2005; Banko et al., 2006; Chen et al., 2006; Paul et al., 2007; Sindreu et al., 2007). Where examined, ERK activation in NMDAR-dependent forms of synaptic plasticity and learning is blocked by NMDAR antagonists. Similarly, ERK activation in NMDAR-independent forms of synaptic plasticity is blocked by VGCC antagonists (e.g., Kanterewicz et al., 2000; Dudek and Fields, 2001). In addition, an important role for action potential generation in recruitment of ERK activation during LTP induction has been proposed, based on the observation that action potentials are sufficient for ERK activation in the absence of synaptic activity (Dudek and

Fields, 2002; Zhao et al., 2005). Interestingly, stimulation with TBS and varying frequencies (5–100 Hz) of tetanus strongly activates ERK throughout dendrites, soma, and nucleus of CA1 neurons, suggesting that ERK-dependent processes may be activated on a cell-wide basis during LTP (Dudek and Fields, 2001; Patterson et al., 2001).

4.25.3 Mitogen-Activated Protein Kinases in Long-Term Potentiation and Long-Term Depression

Considerable evidence now supports the importance of bidirectional modifications of synaptic strength as cellular correlates of learning and memory. The opposing processes of synaptic strengthening and weakening, embodied experimentally by LTP and LTD, have been most intensively studied at hippocampal area CA1 synapses. Given the wealth of experimental evidence concerning synaptic plasticity at CA1 synapses, and the likely importance of plasticity at this synapse for hippocampal learning and memory, our discussion will be largely confined to CA1 LTP and LTD. Both LTP and LTD exhibit a transient early phase (E-LTP, E-LTD), which is independent of new protein synthesis, and a longer-lasting late phase (L-LTP, L-LTD), which requires new protein synthesis (reviewed in Kelleher et al., 2004b). In addition, NMDAR-dependent and NMDAR-independent forms of both LTP and LTD have been described (*See* Chapter 4.16). The expression of early-phase LTP and LTD is mediated by synaptic activity-dependent insertion or removal of AMPARs into the postsynaptic membrane (reviewed in Malinow and Malenka, 2002; Bredt and Nicoll, 2003; Malenka and Bear, 2004). As outlined below, the ERK pathway has been implicated in essentially all of these processes.

The availability of highly specific pharmacologic inhibitors has allowed the consequences of acute inhibition of MAPK activation on various forms of synaptic plasticity to be determined. In the case of the ERK cascade, cell-permeable inhibitors of MEK1/2 have been widely used as specific probes of ERK function, revealing an important role for ERK in many, but not all, forms of LTP and LTD. Genetic methods of interfering with MAPK function provide an important complement to pharmacologic approaches, allowing inhibition of cascade components for which pharmacologic agents are not available, and also permitting analysis of the effects of partial or complete inactivation of one or more isoforms of a particular component.

4.25.3.1 Essential Roles for Extracellular-Regulated Kinase in *N*-Methyl-D-Aspartate Receptor–Dependent and –Independent Long-Term Potentiation

A requirement for ERK activation in synaptic plasticity was first documented for NMDAR-dependent LTP induced by high-frequency tetanic stimulation (100 Hz) in acute hippocampal slices (English and Sweatt, 1997; Atkins et al., 1998; Impey et al., 1998). While MEK inhibitors significantly attenuated expression of LTP induced by modest or strong tetanization protocols, MEK inhibition had no effect on basal synaptic transmission, short-term forms of synaptic plasticity, or LTP maintenance. Inhibition of tetanus-induced CA1 LTP has been observed with multiple, structurally distinct MEK inhibitors (PD98059, U0126, SL327) (English and Sweatt, 1997; Atkins et al., 1998; Impey et al., 1998; Rosenblum et al., 2002; Selcher et al., 2003). Comparison of results obtained in rat and mouse hippocampal slices, however, has revealed a curious species-specific difference in the ERK dependence of LTP induced by modest tetanic stimulation. Specifically, LTP induced by a pair of closely spaced tetanic trains is inhibited by MEK antagonists in the rat hippocampus but not in the mouse hippocampus (English and Sweatt, 1997; Atkins et al., 1998; Watabe et al., 2000; Selcher et al., 2003). Similarly, LTP induced at mouse CA1 synapses by a single tetanic train is unaffected by MEK inhibition (Winder et al., 1999). The differing ERK requirements of various forms of CA1 LTP induced by patterns of 100-Hz tetanization may relate to their dependence on protein synthesis, with ERK activation selectively required for protein synthesis–dependent LTP (see Section 4.25.3.4).

ERK activation also contributes to other well-characterized forms of NMDAR-dependent LTP. CA1 LTP can be effectively induced by stimulation at the frequency of the hippocampal theta rhythm (3–12 Hz). This form of LTP is thought to be particularly relevant to behavior, since CA1 pyramidal cells in the rodent hippocampus fire spikes or complex spike bursts at theta frequency during spatial exploration (O'Keefe, 1993). MEK inhibitors suppress CA1 LTP induced in the mouse hippocampus

by 5-Hz stimulation (theta frequency stimulation, TFS) or brief high-frequency bursts of stimulation delivered at 5 Hz (theta burst stimulation, TBS) (Winder et al., 1999; Watabe et al., 2000; Patterson et al., 2001; Selcher et al., 2003). Increases in postsynaptic excitability and the firing of complex action potential bursts have been implicated in the induction of LTP by TFS and TBS, and ERK activation can modulate these neuronal properties in the context of TFS and TBS (Thomas et al., 1998; Winder et al., 1999; Watabe et al., 2000; Selcher et al., 2003). One mechanism through which ERK may influence postsynaptic excitability during theta-like stimulation, particularly with regard to dendritic backpropagation of action potentials, is through regulation of A-type potassium channel function (Adams et al., 2000; Yuan et al., 2002).

Induction of NMDAR-dependent LTP can be facilitated or enhanced by neuromodulatory agents under certain conditions (e.g., when combined with subthreshold afferent stimulation). Activation of β-adrenergic receptors, either alone or in conjunction with muscarinic acetylcholine receptors, stimulates ERK activation and facilitates CA1 LTP induction in an ERK-dependent manner (Winder et al., 1999; Watabe et al., 2000; Giovannini et al., 2001; Gelinas and Nguyen, 2005).

Using intracellular recordings, NMDAR-dependent CA1 LTP can be induced by pairing postsynaptic depolarization with low-frequency (typically 2 Hz) presynaptic stimulation. A MEK inhibitor (PD98059) blocks such pairing-induced LTP at CA1 synapses in acute and cultured rat hippocampal slices (Bolshakov et al., 2000; Zhu et al., 2002). Moreover, virus-mediated expression of dominant-negative or constitutively active forms of Ras in slice culture demonstrated that Ras activation is necessary and sufficient to induce LTP (Zhu et al., 2002). Synaptic potentiation produced by constitutively active Ras occludes pairing-induced LTP and is prevented by MEK inhibitors, indicating that ERK activation downstream of Ras is essential for LTP.

Investigation of the molecular mechanisms underlying LTP has focused largely on NMDAR-dependent forms, since hippocampal learning and memory characteristically depend on NMDAR activation (Morris et al., 1986, 1990; Miserendino et al., 1990; Kim et al., 1991). However, synapses in the rodent hippocampus and other brain regions also exhibit NMDAR-independent LTP, in which postsynaptic calcium influx depends on activation of voltage-gated calcium channels (Grover and Teyler, 1990; Aniksztejn and Ben-Ari, 1991; Aroniadou and Teyler, 1992; Huang and Malenka, 1993; Weisskopf et al., 1999). Although the behavioral significance of NMDAR-independent LTP is somewhat unclear, VGCCs have been implicated in certain forms of learning and memory, particularly aspects of amygdala-dependent fear memory (Bauer et al., 2002; Cain et al., 2002; Moosmang et al., 2005; McKinney and Murphy, 2006). Consistent with the potent activation of the ERK cascade evoked by calcium influx through VGCC, two well-studied forms of NMDAR-independent CA1 LTP have been shown to depend on ERK activation. High-frequency tetanization (200 Hz) recruits a substantial component of NMDAR-independent LTP, and treatment with the potassium channel blocker tetraethylammonium (TEA) induces LTP (termed TEA-LTP or LTP_K) that is insensitive to NMDAR antagonists (Grover and Teyler, 1990; Aniksztejn and Ben-Ari, 1991; Huang and Malenka, 1993). Induction of CA1 LTP with either 200-Hz tetanization or TEA treatment activates ERK in a VGCC-dependent manner, and MEK inhibitors block the induction of both forms of LTP (Kanterewicz et al., 2000). BDNF treatment also induces an NMDAR-independent form of LTP at CA1 synapses in acute hippocampal slices and at perforant path-dentate granule cell synapses *in vivo* (Kang and Schuman, 1995; Messaoudi et al., 2002); in the latter case, BDNF-induced LTP has been shown to require ERK activation (Ying et al., 2002). Enhanced presynaptic release of neurotransmitter has been implicated in the expression of BDNF-dependent LTP and LTP induced by TEA or 200-Hz stimulation (Zakharenko et al., 2001, 2003), suggesting that postsynaptic ERK activation may contribute to the generation of a retrograde messenger.

4.25.3.2 Different Forms of Long-Term Depression Mediated by Extracellular-Regulated Kinase and p38 Mitogen-Activated Protein Kinase at CA1 Synapses

Several distinct forms of LTD have been characterized at CA1 synapses in hippocampal slices (Dudek and Bear, 1992; Bolshakov and Siegelbaum, 1994; Oliet et al., 1997; Palmer et al., 1997; Kemp and Bashir, 1999; Huber et al., 2000; reviewed in Malenka and Bear, 2004). NMDAR-dependent LTD can be induced in the neonatal hippocampus by prolonged periods of low-frequency stimulation (LFS, typically at 1 Hz). In comparison with the

higher frequencies of stimulation required to induce NMDAR-dependent LTP, the lower levels of calcium influx generated in response to low-frequency stimulation are thought to activate protein phosphatases and favor the production of LTD. Analysis of the mechanisms underlying NMDAR-dependent LTD induced by LFS in neonatal hippocampal slice culture demonstrated an essential role for p38 MAPK activation downstream of Rap1 (Zhu et al., 2002). Viral expression of dominant-negative Rap1 blocked LTD, while expression of constitutively active Rap1 depressed AMPA-mediated synaptic transmission and occluded LTD. Furthermore, LTD and the synaptic depression induced by constitutively active Rap1 were blocked by a p38 MAPK inhibitor (SB203580), but not by MEK inhibitor (PD98059) or a JNK inhibitor (SP600125).

At least two forms of NMDAR-independent LTD that depend on the activation of group I mGluRs have been described at CA1 synapses, with the mechanistic features apparently differing depending on the developmental stage and/or induction protocol. mGluR-dependent LTD induced in neonatal hippocampal slices by prolonged 5-Hz stimulation is suppressed by a p38 MAPK inhibitor (SB203580), but not by a MEK inhibitor (PD98059) (Bolshakov et al., 2000). LTD induction is associated with mGluR-dependent p38 MAPK activation (though possible concomitant ERK activation was not assessed in this study). Further supporting an essential role for p38 MAPK, intracellular dialysis of CA1 neurons with activated p38 MAPK induces LTD, which occludes subsequent LTD in response to 5-Hz stimulation. The induction of this form of LTD depends on postsynaptic mGluR activation, while its expression appears to be mediated presynaptically through a decrease in transmitter release (Bolshakov and Siegelbaum, 1994). These mechanistic features suggest that p38 MAPK is recruited downstream of mGluR activation in postsynaptic neurons and participates in the production of a retrograde messenger.

In contrast, mGluR-dependent LTD induced in more mature hippocampal slices by paired-pulse low-frequency stimulation or direct application of a type I mGluR agonist (e.g., DHPG) requires postsynaptic protein synthesis and is expressed postsynaptically through internalization of synaptic AMPARs (Huber et al., 2000; Snyder et al., 2001; Xiao et al., 2001). This form of mGluR-dependent LTD is inhibited by antagonists of MEK (PD98059, U0126) but not p38 MAPK (SB203580) (Gallagher et al., 2004; Banko et al., 2006). In addition, mGluR stimulation sufficient to induce LTD activates ERK but not p38 MAPK. Thus, both NMDAR-dependent and mGluR-dependent forms of LTD in the neonatal hippocampus appear to depend on p38 MAPK, while the mGluR-dependent LTD that predominates at later developmental stages depends instead on ERK. The available evidence suggests that both p38 MAPK and ERK can promote the synaptic removal of AMPARs in different contexts, with ERK possibly playing a specific role in protein synthesis–dependent LTD.

A role for the remaining MAPK family pathway, JNK, has been described in NMDAR-dependent synaptic depotentiation. LTD and depotentiation share many features and are generally thought to represent closely related processes mechanistically. However, one possible asymmetry pertains to the identities of the synaptic AMPARs that are removed by each process, since LTP is mediated by insertion of GluR1- and GluR4-containing AMPARs, while LTD is mediated by removal of GluR2/3-containing AMPARs (Malinow and Malenka, 2002; Bredt and Nicoll, 2003; Malenka and Bear, 2004). In cultured rat hippocampal slices, Rap2-dependent activation of JNK mediates depotentiation of pairing-induced LTP and removal of synaptic GluR1-containing AMPARs (Zhu et al., 2005).

4.25.3.3 Coupling of NR2A to Extracellular-Regulated Kinase and NR2B to p38 in Long-Term Potentiation and Long-Term Depression

As outlined above, analysis of LTP and LTD in cultured rat hippocampal slices, combined with pharmacologic inhibition and viral expression of mutant forms of Ras and Rap, has indicated specific roles for Ras-ERK signaling in NMDAR-dependent LTP and Rap1-p38 MAPK signaling in NMDAR-dependent LTD at CA1 synapses (Zhu et al., 2002). Other data have suggested opposing patterns of recruitment of ERK and p38 MAPK activation by NMDARs containing the NR2A and NR2B subunits. Specifically, association of NR2B-containing NMDARs with Ras-GRF1, CaMKII, and SynGAP appears to limit ERK activation and promote p38 MAPK activation (Zhu et al., 2002; Krapivinsky et al., 2003; Oh et al., 2004; Kim et al., 2005; Rumbaugh et al., 2006). Conversely, Ras-GRF2 and NR2A-containing NMDARs are functionally coupled to ERK activation. Interestingly, germline deletion of Ras-GRF1 and Ras-GRF2 produces opposing effects on

NMDAR-dependent LTP and LTD at CA1 synapses (Li et al., 2006). Ras-GRF1 inactivation significantly impairs LFS-induced LTD but has no effect on LTP induced by TBS and a minor effect on LTP two trains of 100-Hz tetanization separated by 20 s. In contrast, inactivation of Ras-GRF2 impairs LTP induced by both TBS and paired 100-Hz tetanization but leaves LTD unaffected. Although the apparent requirement for ERK in LTP induced with a pair of closely spaced 100-Hz tetanic trains is at odds with other data showing this form of LTP to be ERK independent in the mouse hippocampus (Selcher et al., 2003; Kelleher et al., 2004a), these findings nevertheless suggest a specific role for NR2B and p38 in LTD and for NR2A and ERK in LTP. The foregoing observations are further consistent with the reported dependence of CA1 LTP on NR2A NMDARs and CA1 LTD on NR2B NMDARs (Liu et al., 2004). However, this model has been challenged by data from several groups demonstrating that induction of CA1 LTP can be mediated by NMDARs containing either NR2B or NR2A (Kiyama et al., 1998; Kohr et al., 2003; Berberich et al., 2005, 2007; Weitlauf et al., 2005). Several groups have similarly shown that activation of NR2B-containing NMDARs is not required for CA1 LTD. These data argue that NR2 subunit composition does not directly specify the induction of LTP or LTD (Morishita et al., 2007). Rather, they support an alternate model in which NR2 subunit composition regulates the threshold for induction of LTD versus LTP – a form of 'metaplasticity' – by modulating the degree of postsynaptic calcium influx and the activation of signaling mechanisms favoring either LTD or LTP.

4.25.3.4 A Specific Role for Extracellular-Regulated Kinase in the Protein Synthesis-Dependent Phase of Synaptic Plasticity

Increasing evidence suggests a specific role for ERK activation in the protein synthesis–dependent phases of LTP and LTD. Genetic inhibition of ERK activation in the postnatal hippocampus impairs L-LTP but not E-LTP, and the effect of ERK inhibition on L-LTP is indistinguishable from the effect of the translational inhibitor anisomycin (Kelleher et al., 2004a). Surprisingly, transcriptional inhibition with actinomycin-D exerts a distinct, delayed kinetic pattern of inhibition of L-LTP. ERK inhibition also abolishes the enhancement of translation and translation factor phosphorylation in CA1 neurons that accompanies the induction of L-LTP. Taken together, these findings suggest that upregulation of ERK-dependent translation plays a dominant role in the establishment of protein synthesis–dependent LTP, and transcriptional induction is required for the subsequent maintenance of these synaptic changes (Kelleher et al., 2004a). ERK is also required for a translation-dependent and transcription-independent form of LTD induced by mGluR activation (Huber et al., 2000; Gallagher et al., 2004; Banko et al., 2006). This selective involvement of the ERK pathway in protein synthesis–dependent synaptic plasticity likely underlies its selective role in the protein synthesis–dependent process of memory consolidation (see following).

Further arguing for a specific role of ERK in protein synthesis–dependent LTP, the differing ERK dependence of tetanus-induced forms of LTP in the mouse hippocampus parallels the distinction between E-LTP and L-LTP, suggesting a selective contribution of ERK to L-LTP. MEK inhibition blocks LTP induced by multiple 100-Hz tetanic trains separated by several minutes, a protocol which has been shown to produce protein synthesis–dependent LTP. In contrast, LTP induced by a single or by a pair of 100-Hz tetanic trains separated by 10–20 seconds is unaffected by MEK inhibition, and both of these induction protocols have been shown to evoke protein synthesis–independent LTP (Huang and Kandel, 1994; Huang et al., 1996; Winder et al., 1998). The sensitivity of LTP induced by a pair of closely spaced tetanic trains to MEK inhibition in the rat hippocampus could be reconciled with this hypothesis if this protocol induced a protein synthesis–dependent form of LTP in the rat (but not the mouse). Data suggesting that this is in fact the case have recently been presented: LTP induced by a pair of 100-Hz tetanic trains separated by 20 s persists for more than 3 h and is sensitive to transcriptional inhibition (Levenson et al., 2004). Moreover, early-phase LTP induced with a standard TBS protocol exhibits a protein synthesis–dependent component that is entirely blocked by MEK inhibition with U0126, while the protein synthesis–independent component is unaffected by MEK inhibition (Schmitt et al., 2005). These observations suggest that the ERK pathway exerts its effects on TBS-induced LTP primarily through a protein synthesis–dependent mechanism. Further supporting the generality of this proposed role for the ERK pathway, ERK activation is required for forskolin-induced LTP, which is entirely dependent on new protein synthesis (Frey et al., 1993; Patterson et al., 2001), and protein synthesis–dependent L-LTP in the amygdala requires ERK activation (Huang et al., 2000). It should be noted,

however, that some forms of TBS can produce a protein synthesis–independent LTP that is nevertheless sensitive to MEK inhibition, indicating that ERK can facilitate induction of TBS-LTP through additional mechanisms, such as modulation of neuronal excitability (Winder et al., 1999; Selcher et al., 2003).

4.25.4 Mitogen-Activated Protein Kinase in Learning and Memory

4.25.4.1 ERK Activation in Hippocampus- and Amygdala-Dependent Learning and Memory

Consistent with the central role of the ERK pathway in many forms of synaptic plasticity, a variety of studies in experimental rodents over the past decade have established the importance of the ERK pathway in mammalian learning and memory. As with its role in synaptic plasticity, the responsiveness of the ERK pathway to excitatory and neuromodulatory synaptic transmission, and particularly its position as a convergence point for a variety of neuronal activity–induced signaling mechanisms, makes it well-suited to function as a key mediator of associative memory. A requirement for ERK in associative memory was first demonstrated in fear conditioning, in which rodents learn to associate an unconditioned stimulus (an experimental chamber in contextual conditioning or an experimental tone in cued conditioning) with a conditioned stimulus (an aversive foot shock). Formation of an associative fear memory in cued conditioning depends on the integrity of the amygdala, whereas associative memory in contextual conditioning depends on both the hippocampus and the amygdala (Kim and Fanselow, 1992; Phillips and LeDoux, 1992). Training of rats in contextual fear conditioning elicits heightened ERK activation in the hippocampus within 1 h, while exposure to the unconditioned or conditioned stimuli without formation of an associative memory fails to activate ERK (Atkins et al., 1998). Pretraining systemic administration of an NMDAR antagonist, which is known to block the acquisition of conditioned fear memory (Miserendino et al., 1990; Kim et al., 1991), suppressed the specific increase in hippocampal ERK phosphorylation. Similarly, ERK activation is transiently and specifically increased in the amygdala 1 h after fear conditioning (Schafe et al., 2000). Systemic administration of a MEK inhibitor (SL327) 1 h prior to training causes a dose-dependent reduction in associative memory for both contextual and cued conditioning in rats and mice when tested 24 h later (Atkins et al., 1998; Selcher et al., 1999). Contextual and cued fear conditioning are also impaired in a dose-dependent manner following pretraining intraventricular infusion of a MEK inhibitor (PD98059) (Schafe et al., 1999). A specific requirement for ERK activation in the amygdala was subsequently demonstrated by targeted infusion of MEK inhibitor in the amygdala, which caused a dose-dependent impairment in long-term memory for cued fear conditioning (Schafe et al., 2000). Similarly, intraamygdalar infusion of a cell-permeable form of the ERK phosphatase STEP causes amnesia for cued fear conditioning (Paul et al., 2007).

These findings made the important initial demonstration that ERK activation downstream of NMDARs is essential for the formation of associative memory. The impairment in both cued and contextual conditioning imposed by MEK inhibition indicates a requirement for ERK activation in the amygdala, but its possible role in the hippocampus could not be distinguished. Subsequent studies using a hippocampus-dependent task, the hidden platform Morris water maze, demonstrated an essential role for ERK activation in the formation of hippocampal memory. In this task, rodents learn through a series of trials to locate a submerged platform in a pool using distal visual cures, thereby acquiring NMDAR-dependent spatial reference memory for the platform location (Morris et al., 1982). Acquisition of spatial reference memory in rats is associated with transient ERK activation in the dorsal hippocampus (Blum et al., 1999). Systemic administration of a MEK inhibitor (SL327) in mice prior to training on 5 consecutive days interferes with acquisition of spatial reference memory in the hidden platform water maze; control experiments excluded effects of the drug on memory recall and sensorimotor performance (Selcher et al., 1999). The role of hippocampal ERK activation in spatial reference memory was addressed more directly using a stereotactic approach (Blum et al., 1999). Intrahippocampal infusion of a MEK inhibitor (PD98059) 20 min prior to or immediately following training significantly impaired memory retention when tested 48 h later. In contrast, posttraining infusion of a p38 MAPK inhibitor (SB203580) did not interfere with memory retention (Blum et al., 1999). Spatial reference memory following training in the hidden platform water maze and long-term memory for contextual but not cued fear conditioning are

impaired by genetic inhibition of ERK activation in the postnatal hippocampus (Kelleher et al., 2004a). Contextual fear conditioning was also impaired in another transgenic mouse with neuron-specific inhibition of ERK activation (Shalin et al., 2004).

4.25.4.2 Upstream Regulators of Extracellular-Regulated Kinase in Learning and Memory

Studies of mice bearing mutations in upstream regulators of the ERK cascade have provided supporting evidence that ERK is an essential component of the molecular machinery underlying hippocampus- and amygdala-dependent memory. Heterozygous disruption of the K-Ras isoform causes deficient hippocampus-dependent memory in the hidden platform Morris water maze (Costa et al., 2002). Conditional inactivation of the B-Raf isoform in excitatory neurons of the postnatal mouse forebrain (which bypasses the embryonic lethality caused by germline disruption) attenuates hippocampal ERK activation following contextual fear conditioning and impairs spatial reference memory in the hidden platform Morris water maze, but surprisingly leaves contextual and cued fear conditioning unaffected (Chen et al., 2006). Analysis of two independent lines of knockout mice lacking Ras-GRF1 have been analyzed; in one case, hippocampus-dependent but not amygdala-dependent memory was found to be impaired, while in the other case, essentially the reverse pattern was observed (Brambilla et al., 1997; Giese et al., 2001). The basis for this discrepancy is not clear but may relate to methodological differences.

Several studies have examined the consequences of upregulation of Ras-ERK signaling. Transgenic mice overexpressing an activated form of H-Ras display enhanced hippocampus-dependent learning (Kushner et al., 2005). Acquisition of reference memory in the hidden platform water maze was accelerated, and memory for contextual fear conditioning was enhanced both 2 h and 24 h following training. In wild-type mice, contextual fear conditioning induced parallel increases in ERK-mediated phosphorylation of synapsin I. Synapsin I phosphorylation was potentiated in H-Ras transgenic mice, and inactivation of synapsin I prevented enhancement of contextual fear memory by H-Ras overexpression. In conjunction with electrophysiological findings indicating augmented presynaptic function and plasticity in H-Ras mice, these observations implicate Ras-ERK signaling in the activity-dependent regulation of neurotransmitter release (Kushner et al., 2005). The specificity of this genetic manipulation for presynaptic function may relate, at least in part, to the subcellular distribution of Ras-ERK activation, as both endogenous and transgene-derived H-Ras were found to be predominantly localized to excitatory presynaptic terminals, with no detectable dendritic localization. In contrast to the enhancement of hippocampal LTP and memory observed with H-Ras overexpression, other genetic manipulations that elevate Ras-ERK signaling activity, such as heterozygous disruption of the Ras GAPs Syn-GAP and NF1, appear to have a deleterious effect (Silva et al., 1997; Komiyama et al., 2002). Furthermore, restoration of normal levels of Ras-ERK signaling in *NF1* mutant mice (through mutation of K-Ras or N-Ras isoforms) rescues the hippocampal LTP and memory defects (Costa et al., 2002). These findings are consistent with the presence of cognitive impairment in human developmental disorders caused by hyperactive Ras-ERK signaling (Schubbert et al., 2007).

Two recent studies have highlighted the importance of the subcellular localization of ERK activation for synaptic plasticity and memory. Transgenic overexpression of a dominant-negative form of Rap1 in excitatory forebrain neurons decreased the basal activities of B-Raf and a membrane-associated pool of ERK in the hippocampus, while increasing basal levels of Raf-1 and leaving total and cytosolic hippocampal ERK activity unaltered (Morozov et al., 2003). This reduction in basal membrane-associated ERK activity was sufficient to impair hippocampal memory and two ERK-dependent forms of CA1 LTP (theta frequency– and forskolin-induced LTP). Impaired ERK-dependent LTP was associated with reduced basal and tetanus-induced phosphorylation of the ERK substrate Kv4.2 and reduced complex spiking of CA1 neurons during LTP induction. The activity of a membrane-associated pool of ERK was also compromised by germline disruption of KSR1, a scaffold protein that facilitates interaction of components of the ERK cascade (Shalin et al., 2006). Interestingly, KSR1 inactivation interfered with the coupling of PKC to membrane-associated ERK activation, without altering the responsiveness of membrane-associated ERK to adenylyl cyclase/cAMP. Moreover, ERK activation in the cytosol in response to stimulation of either PKC- or adenylyl cyclase/cAMP pathways was unaffected. A corresponding dissociation of ERK-dependent forms of LTP was observed, with impaired PKC-mediated LTP (TBS-induced LTP) but normal cAMP-mediated LTP (LTP induced

by TFS plus isoproteronol). Long-term memory formation was impaired in the hidden platform water maze as well as contextual and cued conditioning, suggesting that PKC-dependent recruitment of membrane-associated ERK activity is essential for hippocampus- and amygdala-dependent memory, possibly acting *via* regulation of synaptic activity-dependent neuronal excitability (Shalin et al., 2006).

4.25.4.3 A Specific Role for Extracellular-Regulated Kinase Activation in Memory Consolidation

Analysis of the contribution of ERK activation to the temporal phases of memory has highlighted a specific role in the process of memory consolidation. Short-term memory is insensitive to inhibitors of protein synthesis, whereas long-term memory is disrupted by such agents. The transition from short-term to long-term memory, or memory consolidation, thus depends on new gene expression (reviewed in Davis and Squire, 1984). Analysis of the role of ERK activation in fear conditioning has revealed that intraventricular or intraamygdalar infusion of MEK inhibitor blocks long-term memory assessed 24 h after training, but short-term memory assessed 30–60 min after training remains intact (Schafe et al., 1999, 2000). Similarly, infusion of a cell-permeable ERK phosphatase into the amygdala selectively disrupts consolidation of fear memory (Paul et al., 2007). Genetic inhibition of hippocampal ERK activation through expression of a dominant-negative form of MEK1 causes a significant impairment of long-term memory for contextual fear conditioning 24 h after training, while short-term memory 1 h after training is unaffected (Kelleher et al., 2004b). The temporal pattern of memory impairment in fear conditioning caused by MEK inhibition in these studies corresponds closely to the temporal pattern observed with inhibition of protein synthesis (Abel et al., 1997; Schafe and LeDoux, 2000), suggesting that ERK activation plays an important role in the new gene expression required for memory consolidation. Investigation of conditioned taste aversion (CTA) memory, which is encoded in the insular cortex, has demonstrated the importance of ERK activation in protein synthesis–dependent memory consolidation beyond the hippocampus and amygdala. The formation of conditioned taste aversion memory for an unfamiliar taste is accompanied by ERK activation in the insular cortex, and infusion of a MEK inhibitor (PD98059) into the insular cortex interferes with long-term CTA memory, but not short-term CTA memory, similar to the effect of translational inhibition (Rosenblum et al., 1993; Berman et al., 1998). In addition to its role in memory consolidation, the ERK cascade also appears to be required for the related protein synthesis–dependent phenomenon of memory reconsolidation (Kelly et al., 2003; Duvarci et al., 2005).

Recent evidence suggests that ERK-mediated translational upregulation is a crucial mechanism underlying memory consolidation (Kelleher et al., 2004b). The impaired memory consolidation caused by genetic inhibition of hippocampal ERK activation in the postnatal hippocampus is associated with reduced site-specific phosphorylation of translation initiation factors (eIF4E and S6), consistent with a reduced translational response during long-term memory formation. MEK inhibition in this study caused parallel reductions in neuronal activity–dependent translation (including dendritic protein synthesis) and translation factor phosphorylation in cultured hippocampal neurons and hippocampal slices. Transcriptional regulation is an alternate mechanism by which ERK may regulate the new gene expression needed for memory consolidation. Fear conditioning elicits coincident activation of ERK, CREB, and the CREB kinase MSK1 in CA1 neurons downstream of calcium-stimulated adenylyl cyclases, and MSK1 activation is blocked by systemic administration of a MEK inhibitor (SL327) (Sindreu et al., 2007). Fear conditioning also induces ERK-dependent acetylation of histone H3 in CA1 neurons, and increased histone acetylation is associated with enhanced long-term memory for contextual fear conditioning (Levenson et al., 2004).

There has been comparatively little investigation of the role of the other MAPK families, p38 and JNK, in learning and memory. Inhibition of p38 MAPK in the hippocampus has been reported to have no effect on hippocampal learning and memory in the hidden platform Morris water maze. However, requirements for all three MAPK families – ERK, p38 MAPK, and JNK – have been reported in another hippocampus-dependent task, one-trial inhibitory avoidance learning (Walz et al., 1999; Alonso et al., 2003; Bevilaqua et al., 2003). Definition of the role played by the stress-induced MAPK pathways in learning and memory, relative to the widely demonstrated importance of the ERK pathway, awaits further exploration.

4.25.5 Mitogen-Activated Protein Kinase Effector Mechanisms in Synaptic Plasticity and Memory

4.25.5.1 Neuronal Excitability and A-Type Potassium Channels

Modulation of membrane excitability, particularly in dendrites, makes an important contribution to the extent of membrane depolarization and calcium influx during LTP induction, thereby influencing the probability of LTP. For example, complex bursting of CA1 pyramidal neurons and dendritic backpropagation of action potentials have been implicated in efficient induction of LTP using particular stimulation protocols (Magee and Johnston, 1997; Thomas et al., 1998; Watanabe et al., 2002).The ERK cascade appears to regulate both of these manifestations of neuronal excitability. During TBS, complex spike bursting of CA1 pyramidal neurons is augmented, and MEK inhibition suppresses both LTP and increased spiking induced by TBS (Winder et al., 1999; Selcher et al., 2003). The ability of backpropagating action potentials to invade dendrites and amplify excitatory postsynaptic potentials (EPSPs) is regulated primarily by dendritic A-type potassium channels (reviewed in Johnston et al., 2000). A gradient of increasing density from proximal to distal dendrite of the A-type channel, particularly the Kv4.2 subunit, effectively dampens backpropagation of action potentials and limits dendritic excitability.

Treatment of hippocampal slices with isoproterenol or activators of PKA and PKC increases the amplitude of action potentials in distal dendrites of CA1 pyramidal neurons, consistent with downregulation of dendritic A-type currents (Yuan et al., 2002). These increases in action potential amplitude are abolished by MEK inhibition, indicating that downregulation of A-type channel function by PKA and PKC is mediated by the ERK pathway. Moreover, isoproterenol, PKA activation, and PKC activation all produced ERK-dependent increases in the phosphorylation of the Kv4.2 subunit. ERK can directly phosphorylate Kv4.2, and phosphorylation of one of these sites appears to suppress the A-type current (Adams et al., 2000; Schrader et al., 2006). Thus, direct phosphorylation of Kv4.2 by ERK is likely responsible for the heightened dendritic excitability associated with ERK activation, which may in turn account for the dual dependence of TBS-LTP on ERK activation and complex spike bursting. Increased ERK-mediated susceptibility of dendrites to backpropagation of action potentials generated by complex spiking may enhance activation of NMDARs and VGCCs and facilitate LTP induction.

4.25.5.2 AMPAR Trafficking

Parallel roles for all three MAPK families in the synaptic activity–dependent trafficking of AMPARs that underlies bidirectional synaptic modifications have emerged from a series of recent studies. The expression of NMDAR-dependent LTP and LTD is mediated by the synaptic insertion and removal, respectively, of AMPARs, which account in large part for the resulting changes in synaptic strength (reviewed in Malinow and Malenka, 2002; Bredt and Nicoll, 2003; Malenka and Bear, 2004). Thus, LTP is associated with the conversion of silent synapses lacking AMPARs to active synapses, while LTD is associated with synaptic silencing due to net loss of AMPARs. These dynamic changes in the synaptic content of AMPARs occur as a result of the endocytosis and exocytosis of specific populations of AMPARs, defined by their subunit composition. LTP is mediated by the synaptic insertion of AMPARs containing GluR1, which contains a long cytoplasmic tail, whereas LTD is mediated by the synaptic removal of AMPARs containing GluR2 and GluR3, which contain short cytoplasmic tails. Synaptic depotentiation appears to occur through a reversal of LTP, namely, synaptic removal of GluR1-containing AMPARs.

As described above, Ras was found to control synaptic potentiation in an ERK-dependent manner in cultured hippocampal slices, while Rap1 controlled synaptic depression in a p38-dependent manner (Zhu et al., 2002). Furthermore, constitutively active Ras mimics LTP by driving the synaptic insertion of GluR1-containing AMPARs, and constitutively active Rap1 mimics LTD by removing GluR2-containing AMPARs (Zhu et al., 2002). Consistent with the selective coupling of ERK activation to NR2A-containing NMDARs, NR2A promotes the surface expression and delivery of GluR1 in cultured hippocampal neurons, whereas NR2B opposes synaptic delivery of GluR1 (Kim et al., 2005). Consistent with the proposed role of SynGAP acting through NR2B to antagonize ERK activity, SynGAP overexpression inhibits surface delivery of GluR1 in hippocampal neurons (Kim et al., 2005; Rumbaugh et al., 2006). Complementary electrophysiological studies in cultured hippocampal neurons showed that SynGAP overexpression

depresses AMPAR-mediated synaptic transmission, whereas SynGAP deficiency enhances synaptic transmission (Rumbaugh et al., 2006). In addition, SynGAP negatively regulates ERK activation and positively regulates p38 activation.

Taken together, these findings suggest a model in which Ras-ERK signaling acts downstream of NR2A NMDARs to drive GluR1 insertion and LTP, and Rap1-p38 signaling operates downstream of NR2B NMDARs to promote loss of AMPARs and LTD. This model is consistent with the reported dependence of CA1 LTP on activation of NR2A-containing NMDARs, and CA1 LTD on activation of NR2B-containing NMDARs (Liu et al., 2004). However, the hypothesis that NR2A or NR2B containing AMPARs directly specify LTP or LTD is challenged by data from several groups showing that activation of NR2A-containing NMDARs is not obligatory for CA1 LTP, nor is activation of NR2B-containing NMDARs necessary for CA1 LTD (Kiyama et al., 1998; Kohr et al., 2003; Berberich et al., 2005; Weitlauf et al., 2005; Berberich et al., 2007; Morishita et al., 2007). As one possible resolution of this apparent discrepancy, differential coupling of NMDARs containing NR2A or NR2B to distinct MAPK cascades, which can in turn promote the synaptic insertion or removal or AMPARs, may contribute to metaplastic regulation of the threshold for induction of LTD or LTP.

However, there is some apparent uncertainty concerning the AMPAR subtype whose synaptic levels are reduced by NR2B NMDARs, SynGAP, and p38. Some data suggest that Rap1-p38 promotes Ras-ERK-independent synaptic removal of GluR2 (Zhu et al., 2002), whereas other results suggest that NR2B and SynGAP, which appear to inhibit ERK activity and stimulate p38 activity, instead antagonize GluR1 insertion (Kim et al., 2005; Rumbaugh et al., 2006). Synaptic depotentiation would appear to be the process properly associated with reversal of LTP-induced AMPAR trafficking and removal of surface GluR1. Recent data suggest that depotentiation of pairing-induced LTP in a hippocampal slice culture system is mediated by activation of a Rap2-JNK pathway downstream of NR2A NMDARs, leading to internalization of GluR1 (Zhu et al., 2005). In this study, depotentiation was induced using the same LFS protocol that induces LTD in naïve slices, combined with a p38 inhibitor to block LTD and isolate depotentiation. The situation is further complicated by the observation that AMPARs containing subunits with long cytoplasmic tails, such as GluR1, can undergo activity-independent synaptic exchange with AMPARs containing short cytoplasmic tails, such as GluR2/3. Further investigation will help to clarify the relative roles of NR2 subunits and MAPK pathways in AMPAR trafficking mechanisms in these systems.

4.25.5.3 Transcriptional Regulation

MAPK pathways, principally the ERK pathway, influence the transcriptional response to neuronal activity by regulating the activity of the transcription factors Elk-1 and CREB. Transcriptional regulation is a major mechanism by which MAPKs, especially ERK, mediate the growth responses of mitotic cells to growth factor stimulation (Treisman, 1996). Transcription of so-called immediate early genes is rapidly upregulated in response to extracellular stimulation as a result of the interaction of transcriptional activator proteins with *cis*-acting elements located upstream of minimal promoter sequences. One of the best characterized immediate-early genes encodes the c-Fos protein, which together with c-Jun makes up the AP-1 transcription factor. The *c-fos* gene contains two types of upstream *cis*-acting element that mediate its transcriptional induction in response to cellular stimulation, designated the serum response element (SRE) and the cAMP-response element (CRE). SRE-dependent transcription is mediated by the formation of a ternary complex in which serum response factor (SRF) and members of the TCF (ternary complex factor) family of transcription factors simultaneously interact with the SRE. ERK directly phosphorylates TCFs, such as Elk-1, in response to growth factor stimulation, resulting in enhanced ternary complex formation and SRE-dependent transcription (Wasylyk et al., 1998). p38 MAPK and JNK have also been reported to phosphorylate TCFs under some conditions. CRE-dependent transcription in response to elevated intracellular levels of cAMP and calcium is mediated by CREB, which binds the CRE and activates transcription (Shaywitz and Greenberg, 1999; Mayr and Montminy, 2001). The ability of CREB to activate CRE-dependent transcription is dependent on the inducible phosphorylation of a specific residue (Ser133), as well as its interaction with the co-activator CBP (CREB-binding protein), which possesses histone acetylase (HAT) activity. ERK activation enhances Ser133-phosphorylation of CREB and CRE-dependent gene

expression through MAPK-activated protein kinases such as ribosomal S6 kinase (RSK) and MSK1, ERK substrates that can phosphorylate CREB on Ser133 (Xing et al., 1996; Arthur et al., 2004).

Neuronal activity–dependent gene expression plays an essential role in many long-lasting physiological and morphological responses to experience in the developing and adult nervous systems. CREB is the transcription factor most widely implicated in such responses, particularly synaptic plasticity and learning and memory (Lonze and Ginty, 2002). CREB function is essential for long-term forms of synaptic facilitation and memory in invertebrate systems, and mutant mice bearing loss-of-function CREB mutations display variable defects in LTP and memory (Silva et al., 1998). CREB is activated via Ser133 phosphorylation in response to diverse neuronal stimuli, including calcium influx through NMDARs and VGCCs, activation of Trk receptors by neurotrophins, and increases in cAMP levels through activation of G protein–coupled receptors (Shaywitz and Greenberg, 1999).

The Ras/ERK pathway has been implicated in CREB phosphorylation and CRE-dependent transcription in response to neurotrophins and calcium influx. For example, induction of LTP with both NMDAR- and VGCC-dependent components by repeated high-frequency tetanization produced ERK-dependent stimulation of CRE-dependent gene expression (Impey et al., 1996, 1998). BDNF-induced LTP in the dentate gyrus *in vivo* requires ERK activation and is associated with ERK-dependent CREB phosphorylation and transcription of the immediate-early gene *Arc* (Ying et al., 2002). The neurotrophins nerve growth factor (NGF) and BDNF stimulate CREB phosphorylation and CRE-dependent transcription through a Ras-ERK-RSK pathway in neuronal cells (Ginty et al., 1994; Xing et al., 1996, 1998; Finkbeiner et al., 1997; Pizzorusso et al., 2000). Following neurotrophin stimulation of neuronal cells, activated ERK directly phosphorylates RSK isoforms (RSK1/2/3), which translocate to the nucleus and act as Ser133 CREB kinases. An alternate ERK-dependent pathway from neurotrophins to CREB phosphorylation involves the nuclear ERK substrate MSK1 (mitogen- and stress-activated kinase 1), which also functions as a CREB kinase (Arthur et al., 2004). In this study, neurotrophin-induced CREB phosphorylation and CRE-dependent transcription in cortical neurons were not affected by inhibition of RSK1 and RSK2 activity but were markedly reduced by inhibition or genetic disruption of MSK1 activity.

CREB phosphorylation and CRE-dependent transcription in response to calcium influx through NMDAR and VGCC have been reported to involve both CaMKIV and ERK (Bito et al., 1996; Impey et al., 1998; Hardingham et al., 1999; Dolmetsch et al., 2001; Wu et al., 2001; Impey et al., 2002). The ERK cascade appears to be the major kinase pathway conveying calcium signals to the nucleus and CREB following activation of both NMDARs and VGCCs. Calcium influx through VGCCs evokes a rapid and transient CaMKIV-dependent increase in CREB phosphorylation, as well as a slower and more persistent ERK-dependent increase (Wu et al., 2001). ERK activation occurs in response to localized increases in calcium in the vicinity of VGCCs, followed by nuclear translocation of ERK and/or RSK (Dolmetsch et al., 2001). Similarly, NMDAR-mediated ERK activation leads to persistent CREB phosphorylation and appears to involve local microdomains of elevated calcium (Hardingham et al., 2001). Calcium-dependent ERK activation is necessary but not sufficient for CRE-induced transcription, which additionally requires elevated nuclear calcium, activation of CaMKIV, and CaMKIV-mediated phosphorylation of CBP (Hardingham et al., 1997; Chawla et al., 1998; Impey et al., 2002).

Much less is known about the mechanisms regulating Elk1 phosphorylation and Elk1-dependent transcription in neurons. Stimulation of Elk1-dependent transcription in PC12 cells by glutamate and NGF has been reported to require ERK activation (Xia et al., 1996; Johnson et al., 1997). Similarly, glutamate-induced Elk1 phosphorylation in striatal slices is blocked by a MEK inhibitor (Vanhoutte et al., 1999). LTP in the dentate gyrus *in vivo* stimulates phosphorylation of ERK, CREB, and Elk1, as well as transcription of the immediate-early gene *zif268*; all of these effects are prevented by infusion of a MEK inhibitor, which produces a rapid decay of LTP (Davis et al., 2000). ERK activation is also required for LTD and LTD-induced enhancement of Elk1 phosphorylation in hippocampal area CA1 *in vivo* (Thiels et al., 2002). A possible role for Elk1 in synaptic plasticity and learning and memory has not yet been investigated.

Chromatin modification provides an additional mechanism through which ERK may regulate transcription. Acetylation and phosphorylation of histones, particularly histone 3, facilitate transcriptional activation (Cheung et al., 2000; Nowak and

Corces, 2004). Recent studies have implicated the ERK pathway in neuronal activity–induced acetylation and phosphorylation of histone H3. Contextual fear conditioning stimulated H3 acetylation and phosphorylation in an NMDAR- and ERK-dependent manner in hippocampal area CA1, and ERK activation similarly mediated enhanced H3 phosphoacetylation in area CA1 *in vitro* (Levenson et al., 2004; Chwang et al., 2006). In striatal slices, glutamate acts through the ERK pathway and MSK1/2 to stimulate H3 phosphorylation (Brami-Cherrier et al., 2007).

4.25.5.4 Translational Regulation

It has long been known that long-term memory formation and persistent forms of synaptic plasticity in both vertebrate and invertebrate systems can be disrupted by inhibitors of mRNA and protein synthesis (Davis and Squire, 1984; Kandel, 2001; Kelleher et al., 2004b). Investigation of the mechanisms enabling these processes focused for many years on transcriptional regulation. However, several lines of evidence support a crucial role for translational control in the consolidation of synaptic plasticity and memory. First, the observation that transcriptional and translational inhibitors impair L-LTP with significantly different kinetics highlighted a role for translational control independent of transcription in protein synthesis–dependent LTP (Kelleher et al., 2004a). Translational inhibition causes an early and progressive impairment of L-LTP, whereas transcriptional inhibition has a delayed effect, leaving L-LTP unaffected for the initial 60–90 min, and thereafter causing a progressive inhibition. These observations imply that the initial expression of L-LTP is mediated by the enhanced translation of preexisting mRNAs, and translation of newly synthesized mRNAs contributes to the subsequent maintenance of protein synthesis–dependent LTP. Second, the recognition that a subset of neuronal mRNAs is localized to dendrites, and that dendrites can support translation independent of the cell body, suggested that local translation could provide a mechanism for rapid synthesis of new protein products in response to synaptic activity (Sutton and Schuman, 2006). Third, dendritic translation is sufficient for certain forms of protein synthesis–dependent LTP, such as mGluR-dependent LTD and BDNF-induced LTP (Kang and Schuman, 1996; Huber et al., 2000).

Translational control by the ERK pathway has recently emerged as a central mechanism in the establishment of long-lasting forms of synaptic plasticity and memory (reviewed in Kelleher et al., 2004b; Klann and Dever, 2004). Eukaryotic translation is primarily regulated at the level of initiation through a cap-dependent mechanism. Recognition of the 5′ mRNA cap by eIF4E promotes the formation of a translation initiation complex and ribosomal recruitment. The rate-limiting step of cap recognition is regulated by the availability of eIF4E, which is in turn regulated by a family of inhibitory eIF4E binding proteins, 4E-BPs. 4E-BPs and eIF4E are phosphorylated in response to mitogenic stimulation in mitotic cells, which results in increased availability of eIF4E and an increased rate of translation initiation. Mitogen-induced activation of S6 kinases and phosphorylation of ribosomal protein S6 are also highly correlated with increased translation rates, although it is unclear how these events may contribute to translational induction. In mitotic cells, ERK has been implicated in mitogen-induced eIF4E phosphorylation, which is mediated by the ERK substrates and eIF4E kinases, Mnk1/2 (Waskiewicz et al., 1997; Wang et al., 1998). The mTOR and ERK pathways appear to cooperate to effect sequential hyperphosphorylation of 4E-BPs in response to mitogenic stimulation (Gingras et al., 2001; Herbert et al., 2002). However, until recently, little was known about translational regulation in response to neuronal activity or its possible role in synaptic plasticity and memory.

A recent study demonstrated a crucial role for translational control by the ERK pathway in the consolidation of LTP and memory (Kelleher et al., 2004a). Translation of transfected reporter mRNAs and endogenous mRNAs in hippocampal neurons was stimulated in an ERK-dependent manner by multiple forms of neuronal activity, including BDNF, membrane depolarization, and excitatory synaptic activity. Investigation of the underlying mechanisms revealed that BDNF, membrane depolarization, and excitatory synaptic activity elicited ERK-dependent site-specific phosphorylation of components of the translational machinery, specifically eIF4E, 4EBPs, and ribosomal protein S6. Excitatory synaptic activity also induced ERK-dependent translation of endogenous mRNAs and phosphorylation of eIF4E, 4E-BPs, and ribosomal protein S6 in synaptoneurosomes, indicating that similar ERK-dependent mechanisms operate at local dendritic and neuron-wide levels. Supporting the physiological relevance of neuronal activity–dependent translation mediated by the ERK pathway

to synaptic plasticity and memory, induction of L-LTP in area CA1 of hippocampal slices was accompanied by a rapid increase in the translation of endogenous mRNAs and the phosphorylation of eIF4E and ribosomal protein S6, and these translational stimulatory effects were abolished by inhibition of ERK activation in CA1 neurons by conditional expression of a dominant-negative form of MEK1. Similarly, contextual fear conditioning likewise stimulated phosphorylation of hippocampal eIF4E and ribosomal protein S6, and these effects were abolished by conditional genetic inhibition of hippocampal ERK activation. These biochemical defects were correlated with specific impairments of L-LTP and hippocampal long-term memory, while E-LTP and short-term memory were unaffected.

Additional lines of evidence have further substantiated a central role for the ERK pathway in control of neuronal activity–dependent translation. Treatment of cortical neurons with BDNF stimulated ERK-dependent translation of endogenous mRNAs and phosphorylation of eIF4E (Takei et al., 2001). Activation of NMDARs, adenylyl cyclase (forskolin), PKC (PDA) or group I mGluRs (DHPG) in hippocampal slices stimulated ERK-dependent phosphorylation of Mnk1 and eIF4E in area CA1 (Banko et al., 2004, 2006). Interestingly, p38 MAPK appears to contribute to basal levels of Mnk1 and eIF4E phosphorylation in hippocampal area CA1, but not to DHPG-induced increases, which are mediated instead by ERK (Banko et al., 2006). Phosphorylation of eIF4E and 4E-BPs is also stimulated in an ERK-dependent manner by induction of a form of TBS-LTP that requires both ERK activation and protein synthesis (Schmitt et al., 2005). Stimulation of the phosphorylation of ERK, Mnk1, and eIF4E by NMDAR activation in area CA1 requires PKA, while stimulation of ERK, eIF4E, and 4E-BP phosphorylation by induction of TBS-LTP requires CaMKK (Banko et al., 2004; Schmitt et al., 2005). These observations suggest that ERK-dependent translational control during some forms of LTP can occur downstream of cAMP/PKA and CaMKK/CaMKI second messenger pathways.

4.25.5.5 Implications for Synaptic Tagging and Capture

To account for the preservation of input specificity in protein synthesis–dependent forms of synaptic plasticity, an attractive model has been proposed in which stimulated synapses are marked by an immobile synaptic tag, which can function to capture newly synthesized gene products needed for the expression of the appropriate synaptic change (Frey and Morris, 1997). In essence, the model implies that the expression of protein synthesis–dependent synaptic plasticity involves two parallel processes operating downstream of NMDAR activation: (1) protein synthesis–independent generation of a synaptic tag, and (2) protein synthesis–dependent generation of new protein products. On the basis of the evidence outlined above, it has been suggested that the ERK pathway participates primarily in the second process, and specifically that ERK-dependent translation supplies the new products needed for the capture of L-LTP or L-LTD by tagged synapses (Kelleher et al., 2004b). In view of their similar requirements for ERK-dependent translation, L-LTP and L-LTD are likely distinguished by the setting of LTP- or LTD-specific synaptic tags, respectively, rather than the synthesis of a pool of proteins dedicated to either LTP or LTD. These LTP- and LTD-specific tags may then recruit from the available pool of proteins the components needed for the consolidation of the corresponding synaptic change. According to this proposal, the spatial extent of activation of ERK-dependent translation will define a local domain within which tagged synapses can share and compete for the available pool of new proteins, which will determine the ultimate pattern of consolidated synaptic weight changes. Such a mechanism may be advantageous for the efficient storage and reactivation of memory engrams (Govindarajan et al., 2006).

4.25.6 Conclusions

As described in this review, research over the past decade has revealed that MAPK signaling cascades are key regulators of synaptic plasticity and memory consolidation in the adult brain. Regulation of long-term synaptic modifications in nondividing, mature neurons reprises the general role for these cascades in controlling committed changes during development, such as cell division and terminal differentiation. Moreover, MAPK cascades in the adult brain are pluripotent regulators of diverse biochemical processes, including transcription, translation, receptor trafficking and ion channel function. These neuronal functions recapitulate the broader biological role of MAPK signaling mechanisms as high-order coordinators of cellular responses to extracellular signals.

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4.26 Proteolysis and Synaptic Plasticity

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4.26.1 Introduction

Understanding mechanisms underlying synaptic plasticity has been an intense area of research in neuroscience. As a result of many years of research, we now know that short-term synaptic plasticity requires posttranslational modification of existing proteins, whereas long-term synaptic plasticity entails new gene expression and protein synthesis. In recent years, proteolysis by the ubiquitin–proteasome pathway has attained prominence as a new molecular mechanism underlying synaptic plasticity.

4.26.2 The Ubiquitin–Proteasome Pathway

Although the ubiquitin–proteasome pathway was originally thought to function in degradation of abnormal proteins, a large number of studies indicate that this pathway plays a major role in regulated proteolysis of short-lived regulatory protein substrates under physiological conditions. In this pathway, the process of degradation of a substrate protein can be divided into two steps: (1) covalent attachment of ubiquitin to the substrate protein, forming the polyubiquitin chain attached to the substrate (ubiquitin conjugation), and (2) degradation of the polyubiquitinated substrate and disassembly of the polyubiquitin chain and recycling of free ubiquitin (**Figure 1**).

When a protein is to be specifically degraded in the cell, it is marked by covalent attachment of ubiquitin to an ε amino group of lysine residues. The ubiquitin conjugation step is a highly regulated step catalyzed by the action of three classes of enzymes called E1, E2, and E3. E1, the ubiquitin-activating enzyme, activates the free ubiquitin, a small protein of 76 amino acids, in an adenosine triphosphate (ATP)-dependent reaction. Activated ubiquitin is then transferred to an E2, which is generally referred to as a ubiquitin-carrier enzyme. An enzyme belonging to a class of enzymes called E3s (ubiquitin ligases) then ligates the activated ubiquitin to the substrate. A second ubiquitin is attached to an internal lysine residue in the first ubiquitin (Lys-48) and thus by sequential linkages of monoubiquitins, a polyubiquitin chain grows (Glickman et al., 2002; Hegde, 2004).

The polyubiquitinated substrate is then recognized by a large multisubunit proteolytic complex called the 26S proteasome and is degraded to small peptides and amino acids (Hegde and DiAntonio, 2002). Ubiquitination also plays a role in endocytosis. Attachment of a single ubiquitin to a lysine residue (monoubiquitination) or single ubiquitin molecules to multiple lysine residues in a substrate (multiple monoubiquitination) usually marks the protein substrates in the plasma membrane for endocytosis. The disassembly of polyubiquitin chains or removal of monoubiquitins is carried out by deubiquitinating enzymes (DUBs) (Hegde, 2004).

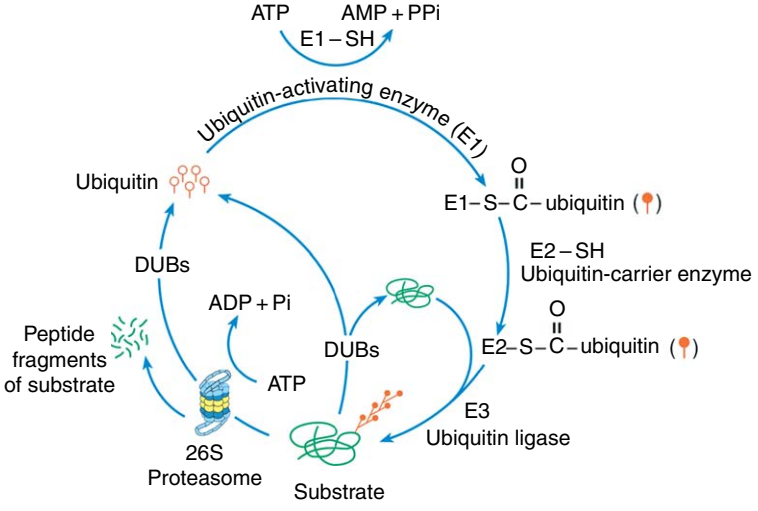

Figure 1 The ubiquitin–proteasome pathway. In this proteolytic pathway, ubiquitin (a single ubiquitin molecule is represented by open circles with straight tails) is selectively and covalently linked to the substrate. The enzymatic process of attaching ubiquitin to substrates is called ubiquitination, or ubiquitin conjugation, and depends on the action of three different classes of enzymes: E1, E2, and E3. First, ubiquitin is activated by E1 to form a ubiquitin-adenosine monophosphate (AMP) intermediate. Activated ubiquitin (closed circles with straight tails) is passed onto E2 (ubiquitin carrier enzymes). E2s transfer ubiquitin to an E3 (ubiquitin ligase), which ligates the activated ubiquitin to the substrate. To the ubiquitin attached to substrate, another ubiquitin is attached and thus through successive linkages of ubiquitin a polyubiquitin chain forms. Polyubiquitinated substrates are degraded by a proteolytic complex called the 26S proteasome in an adenosine triphosphate (ATP)-dependent reaction. Ubiquitin is not degraded but the polyubiquitin chain is disassembled and ubiquitin is recycled by deubiquitinating enzymes (DUBs). Before being committed to being degraded by the proteasome, ubiquitination is reversible. DUBs can disassemble the polyubiquitin chain if a substrate is ubiquitinated erroneously and prevent the degradation of the substrate.

4.26.3 Ubiquitin-Conjugating Enzymes: E1, E2, and E3

Among the three classes of ubiquitin-conjugating enzymes, E1 is the least physiologically regulated. E1 activates ubiquitin in an ATP-dependent step. Activated ubiquitin is then attached to all the substrates degraded by the ubiquitin–proteasome pathway; there is no substrate specificity in this process. E2s are more selective and are believed to interact with specific E3s. Based on our knowledge of ubiquitination reaction, it can be asserted that E3s are the enzymes that possess substrate specificity.

Originally, E2s were believed only to carry the activated ubiquitin and transfer it onto the E3s. Recent studies, however, suggest that at least some E2s can directly conjugate ubiquitin to substrates. E2s are structurally and functionally diverse. Simple eukaryotes such as yeast (*Saccharomyces cerevisiae*) have 13 genes potentially encoding E2s. The number of E2s in mammals is estimated to be in the range of 25–30. Most E2s have a core domain of around 14 K that is approximately 35% conserved between different E2s. The other part of the enzymes appears to be variable. Although most E2s are small, some notable exceptions exist. For example, an E2 called BIR-repeat-containing ubiquitin-conjugating enzyme (BRUCE) is a gigantic 528-K protein. The diversity of E2s generates some degree of specificity in the ubiquitin-conjugating reaction. E2s bind to E3s selectively. Since the diversity of E3s is even greater than that of E2s, the combination of E2s and E3s potentially can generate a high degree of specificity. The heterogeneity of E2s is reflected in their subcellular localization as well. Although several E2s are present in the cytosol, some E2s are localized to other subcellular compartments. For example, BRUCE is localized to the Golgi apparatus and a yeast E2 called Ubc6 is anchored to the membrane of endoplasmic reticulum (Glickman et al., 2002).

E3s are the enzymes that specifically recognize the substrates. E3s can be single proteins or a complex of proteins. Single-subunit E3s can accept ubiquitin in a thioester linkage from E2s and ligate ubiquitin to the substrate. When E3 contains multiple subunits, it is generally believed that the enzyme brings the E2 and the substrate together and facilitates the transfer of ubiquitin to substrate. However, E3s are the most diverse among the ubiquitin-conjugating enzymes and the least characterized. Therefore, it is possible that E3 catalytic mechanisms other than those described below might exist in nature. There are two major classes of E3s: (1) HECT (homologous to E6-AP carboxyl-terminus) domain E3s and (2) RING (really interesting new gene) finger E3s (**Figure 2**).

4.26.3.1 HECT Domain E3s

The typical example in this class of E3 is the ubiquitin ligase called E6-AP, which ligates ubiquitin to the tumor suppressor protein p53. Viral protein E6 associates with a cellular protein called E6-AP (E6-associated protein). The C-terminal region of E6-AP contains the catalytic domain of the ubiquitin ligase. E6-AP ligase can function with either of the E2s called UbcH5 and UbcH7 (**Figure 2**). Later studies found that a family of proteins, ubiquitin ligases, with homology to the catalytic domain of E6-AP exists. These ubiquitin ligases are called HECT domain E3s. In addition to the HECT domain, there is another domain in many E3s called the WW domain. The WW domain-containing E3s also tend to have a C2 domain. The presence of the C2 domain is highly relevant to nervous system function because the C2 domain responds to elevation of intracellular Ca^{2+} and helps in translocation to the plasma membrane. Therefore, presence of this domain in neuronal HECT E3s might be critical in ligating ubiquitin to neurotransmitter receptors or proteins associated with them (Hegde, 2004).

4.26.3.2 RING Finger E3s

These E3s are called RING finger E3s because they contain a RING finger domain, which consists of seven cysteine residues and one histidine residue, forming a single folded domain binding two zinc ions. The arrangement of metal binding residues in the RING finger domain contrasts with the tandem arrangement in the zinc finger domain found in many proteins. Although numerous other proteins were found to have the RING finger motif, the biological function of these proteins remained elusive. During the past few years, several ubiquitin ligases were found to contain the RING finger. The RING finger motif in ubiquitin ligases is critical for transfer of ubiquitin to substrates or to RING finger proteins themselves. The RING finger category of E3s can be subdivided into: (1) single-subunit RING finger E3s, and (2) multisubunit RING finger E3s.

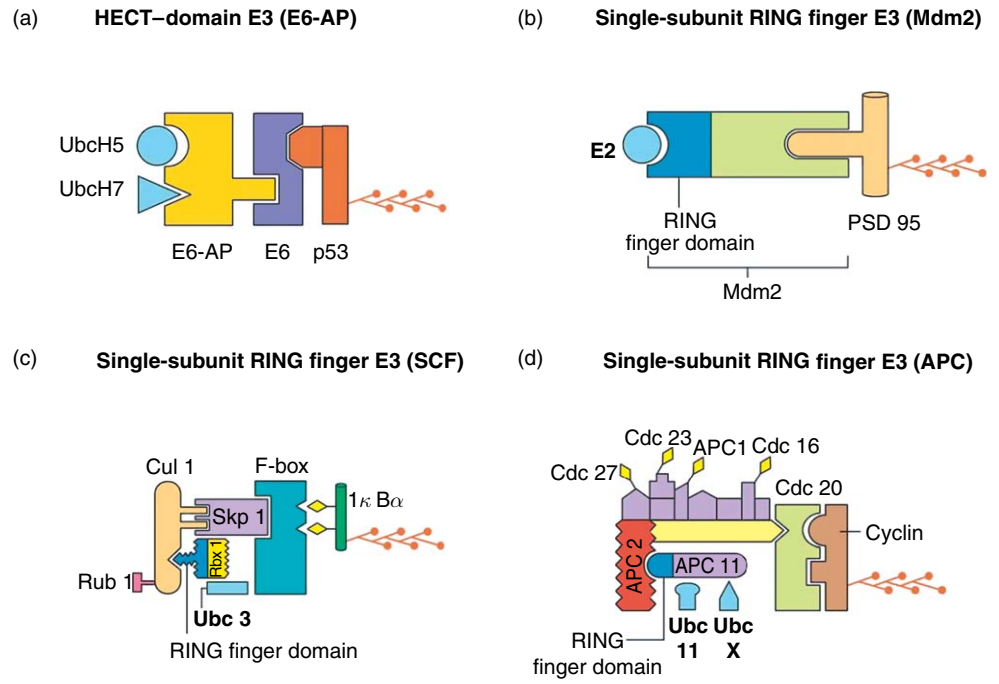

Figure 2 Classes of ubiquitin ligases (E3s). (a) HECT-domain E3. E6-AP ubiquitin ligase in combination with E6 protein and one of the two E2s (UbcH5 or UbcH7) ligates ubiquitin to the p53 tumor suppressor protein. (b) Single-subunit RING finger E3. Mdm2 ligates ubiquitin to postsynaptic density 95 (PSD 95) with the help of an E2 enzyme. (c) Multisubunit RING finger E3. SKP1-cullin-F-Box protein (SCF) ligases contain the substrate recognition site on an F-box protein. Skp1 is an adaptor that joins the F-box protein to Cul1. Ring finger domain is on Rbx 1. The E2 is Ubc3. Cul 1 is modified by Rub1, another ubiquitin-like protein, leading to an increase in the activity of the ligase complex. The substrate is phosphorylated (diamonds) IκBα. (d) Multisubunit RING finger E3. Anaphase-promoting complex (APC) is a more complex example of multisubunit RING finger E3s and has a subunit composition distinct from that of SCF. Cdc20 protein in APC has the substrate (Cyclin) recognition site. The RING finger domain is on APC11. The E2s Ubc 11 or UbcX can function with the APC ligase. In addition, several adaptor proteins, some labeled (Cdc27, Cdc23, APC 1, Cdc16) and some unlabeled, interact with Cdc20 and APC11. Diamonds on the adaptor subunits indicate phosphorylation. The polyubiquitin chain is shown on the substrates in each panel.

4.26.3.2.1 Single-subunit RING finger E3s

Single-subunit RING finger E3s contain the RING finger domain and the substrate recognition site in the same protein. One of the well-characterized single-subunit RING finger E3s is Mdm2, which ubiquitinates p53 in normal cells. As discussed above, E6-AP, a HECT ubiquitin ligase, ubiquitinates p53 in human papilloma virus (HPV)-infected cells. A recent study showed that in HPV-infected cells, E6-AP ubiquitinates p53. Although Mdm2 is present in HPV-infected cells, it does not mediate ubiquitination of p53. Other studies using antisense oligonucleotides directed against E6-AP showed that E6-AP is essential for degradation of HPV-positive cells but not in HPV-negative cells. Conversely, decreasing Mdm2 expression or expression of Mdm2-inactivating peptides decreased p53 degradation in HPV-negative cells but not in HPV-positive cells. Interestingly, the structural determinants on p53 that are recognized by E6-AP and Mdm2 are different from each other. For example, p53 that is a substrate for E6-AP has an asparagine at position 268, whereas p53 that is a substrate for Mdm2 has an aspartate at position 268 (Hengstermann et al., 2001).

4.26.3.2.2 Multisubunit RING finger E3s

4.26.3.2.2.(i) The SKP1-cullin-F-Box protein complex The SKP1-cullin-F-Box protein (SCF) complex contains at least four proteins: Skp1, Cul1, Roc1/Rbx1/Hrt1, and an F-box protein (**Figure 2**). In the center of the SCF complex is the RING finger domain-containing protein Rbx1. The SCF type ligases have another invariant protein called cullin. The theme appears to be that the cullins interact with linker proteins such as Skp1 to recruit substrate-interacting proteins such as the F-box proteins. There are at least five different cullins in mammals. There are several F-box proteins as well. Although it

is not clear how many F-box proteins exist in mammals, the budding yeast genome comprises 17 F-box proteins. Therefore, with just with the cullin and F-box combination alone, it would be theoretically possible to generate close to 100 E3s with differing specificities. Although the regulation of SCF ligases is not completely understood, two mechanisms of posttranslational regulation have been discovered thus far. One is the covalent linkage of ubiquitin-related protein Rub1 to a cullin (Cul1 in **Figure 2**). The second mode of regulation appears to be regulation of levels of F-box proteins through ubiquitin-mediated degradation via an autocatalytic mechanism (Deshaies, 1999). A well-characterized substrate of the SCF complex is IκBα.

4.26.3.2.2.(ii) Anaphase-promoting complex

Although anaphase-promoting complex (APC) has a subunit with a RING finger domain (APC 11), this ubiquitin ligase is distinct from the SCF ligase in overall subunit combination (**Figure 2**). For example, instead of one adaptor found in SCF ligases (such as Skp1), APC has multiple subunits that serve as adaptors. Also, unlike SCF ligases, substrate phosphorylation is not an important determinant for specific substrate recognition by the APC ligase. Rather, substrate specificity of APC ligases appears to be modulated by incorporation of specificity factors into the ligase complex. For example, Cdc20 (**Figure 2**) enables APC to degrade substrates at the onset of anaphase such as the anaphase inhibitor Pds1p, whereas substitution of Cdc20 with another specificity factor called Hct1 enables APC to degrade a different set of substrates such as mitotic cyclins late in the anaphase. APC acts together with an E2 Ubc11 or Ubc X. One of the most studied substrates of APC is mitotic cyclin. This substrate has a short stretch of nine amino acids called the destruction box, which is critical for recognition by the APC ubiquitin ligase (Page and Hieter, 1999).

4.26.3.2.2.(iii) Von Hippel-Lindau-elongin B-elongin C complex

The VBC (VHL-elongin B-elongin C) complex was identified in association with the VHL (von Hippel-Lindau) tumor-suppressor protein. The structure of this complex is similar to that of the SCF complex. The substrate (transcription factor hypoxia-inducible factor 1α, HIF1-α) binds to the VHL protein or SOCS box containing proteins. The VHL protein binds to the Skp1 homolog elongin C and a ubiquitin homolog elongin B. Cul2 and the Roc1/Rbx1 RING finger protein are associated with the complex (Jackson et al., 2000).

Another class of proteins called E4s that elongate the polyubiquitin chain has been discovered (Koegl et al., 1999). The protein product of a gene previously known as ubiquitin fusion degradation protein 2 (UFD2) in yeast was found to catalyze ubiquitin chain assembly along with E1, E2, and E3 and was named E4. E4s contain a modified version of the RING finger, designated as U-box. Since there have not been many studies on E4s and the first E4 discovered functions as cofactor for an E3, it might be premature to conclude that E4s belong to a special class of E3s.

4.26.4 The Proteasome

The term proteasome is used to describe two kinds of multisubunit proteolytic complexes, the 26S and 20S, based on their sedimentation coefficient. The 26S proteasome degrades ubiquitinated protein substrates. The 26S complex contains the 20S as a core and regulatory caps on either end like a dumbbell. Each cap of the 26S proteasome is called the 19S regulatory complex (19S RC). The 20S core is a cylindrical structure consisting of the catalytic part of the proteasome (Hegde, 2004; Pickart and Cohen, 2004).

4.26.4.1 The Catalytic 20S Core

Our knowledge of the proteasome organization comes from the studies on the crystal structure of the proteasome from the archaebacterium *Thermoplasma acidophilum* (Lowe et al., 1995) and the yeast *S. cerevisiae* (Groll et al., 1997). It appears that the proteasome is more ancient than ubiquitin because archaebacteria have the proteasome but not ubiquitin. *T. acidophilum* has two genes encoding α and β subunits. The subunits are arranged in four stacked rings to form the catalytic cylinder with the two middle rings consisting of β subunits, which are sandwiched between two rings of α subunits. In the *T. acidophilum* proteasome, both the α and β subunits are present in seven copies each and assembled in a symmetrical fashion $\alpha_7\beta_7\beta_7\alpha_7$. This general structure is preserved in eukaryotes but the α and β subunits have diverged into seven different subunits each. In yeast, the 20S core is made up of two outer rings with seven α subunits (α1–α7) in each ring and two inner rings consisting of seven β subunits each (β1–β7).

The catalytic core of the proteasome is a threonine protease. Based on the crystal structure of the proteasome, it was concluded that proteasome functions through a new kind of proteolytic mechanism. In this mechanism, the active site nucleophile is the hydroxyl group on the threonine residue at the N-terminus of the β subunit. This catalytic mechanism seems to be conserved across evolution. The antibiotic lactacystin and the active form β-lactone, which are specific and irreversible inhibitors of the proteasome, bind to the N-terminal threonine residue in the β subunit of the mammalian proteasome. The 20S proteasome can exist not only as a core of 26S, but also as a separate population that cannot degrade ubiquitinated proteins. However, the 20S proteasome by itself has chymotrypsin-like, trypsin-like, and postglutamyl peptidase activities which cleave after hydrophobic, basic, and acidic residues, respectively. The peptide hydrolyzing activity of the 20S proteasome can itself be modulated by an 11S regulatory cap (Glickman et al., 2002; Hegde, 2004).

4.26.4.2 19S Regulatory Complex

The 19S RC recognizes the polyubiquitinated substrate, and channels the substrate into the catalytic 20S core of the proteasome. It also has the capacity to regulate the activity of the catalytic core and determine the nature of the degradation process. Usually one 19S RC is attached to either end of the catalytic core. The subunits of the 19S RC are highly conserved across evolution. Two subcomplexes can be recognized within the 19S RC called the base and the lid.

4.26.4.2.1 The base of the proteasome

The base consists of six ATPase subunits (Rpt1–Rpt6) and two non-ATPase subunits (Rpn1 and Rpn2). The Rpt subunits are highly conserved through evolution (up to 75% identical between yeast and humans). The ATPase subunits have a domain called the AAA (ATPases associated with different cellular activities) domain in the center. These subunits are homologous to each other, with the highest degree of homology in the AAA domain. Some of the Rpt subunits contact the α ring in the catalytic core of the proteasome and are believed to channel the substrate into the catalytic chamber for degradation (Hegde, 2004).

4.26.4.2.2 The lid of the proteasome

The lid, which comprises eight subunits, can attach itself to the base of the 19S RC or detach as a complex from the base. All the subunits in the lid are non-ATPase subunits. The exact function of the lid subunits is not known. Degradation of polyubiquitinated proteins requires lid attachment, indicating that the lid performs an essential function in ubiquitin–proteasome-mediated degradation. Also, in archaebacteria that lack ubiquitin, the proteasome is devoid of the lid. The lid subunits of the proteasome share a characteristic sequence of 200 amino acids with COP9 signaling complex and translation initiation factor 3. This sequence is called the PCI (proteasome, COP9, initiation factor 3) domain. In addition, the subunits of the lid have a 120-amino-acid-long MPN (Mpr1p/Pad1p N-terminus) domain that is important for the structure of Rpn8 and Rpn11 subunits. In addition, a subset of MPN domain-containing proteins have a motif of five polar residues called the MPN+ motif. The MPN+ motif has been shown to be critical for the function of Rpn11 (Hegde, 2004).

4.26.5 Deubiquitinating Enzymes

Ubiquitination reaction is reversible before the ubiquitinated protein is committed to degradation by the proteasome. The reversibility is less clear with respect to endocytotic degradation, i.e., internalization of plasma membrane proteins through endocytosis and their degradation through the lysosome. Based on knowledge of the endocytotic pathway, it is reasonable to assume that ubiquitination is reversible until the endocytosed membrane proteins such as neurotransmitter receptors are routed to the multivesicular body (MVB) for lysomal degradation or prior to its entry into invaginating vesicles of MVB. However, if the machinery responsible for recognizing ubiquitinated cargo and initiating its entry into the MVB vesicles is defective, recycling of activated receptors might occur because of the action of DOA4, a DUB required for the removal of ubiquitin from the MVB cargo proteins.

Ubiquitin is removed from substrates by enzymes called DUBs. Based on protein sequence and the molecular size, DUBs can be classified into two general classes: (1) Low-molecular-weight (20–30 K) ubiquitin C-terminal hydrolases (UCHs), and (2) high-molecular-weight (approximately 100 K) ubiquitin-specific proteases (UBPs; also called USPs) (Hegde, 2004). There are numerous DUBs in almost every eukaryotic organism studied. Among the DUBs, UBPs belong to a large family containing diverse genes, whereas the UCH family has fewer genes. For example, in yeast

S. cerevisiae there are 17 UBPs and one UCH (Amerik et al., 2000). In the human genome there are 63 genes encoding UBPs and four genes that code for UCHs. UCHs and UBPs subserve different functions in the eukaryotic cell. Although the current name for these enzymes – DUBs – emphasizes the removal of ubiquitin from substrates, some DUBs, particularly UCHs, function to process linearly linked ubiquitin precursors and generate monoubiquitin. UCHs are cysteine proteases in that the critical residue in the catalytic site is a cysteine. In addition, histidine and aspartate residues are critical for catalytic activity. All UCHs contain these residues even if they do not share a high degree of homology elsewhere in the sequence. UCHs cleave small peptide chains linked to the carboxyl-terminus of ubiquitin. UBPs can cleave the isopeptide bond between ubiquitins in a polyubiquitin chain as well as the isopeptide bond between ubiquitin and the substrate. DUBs are important for generating free ubiquitin at various steps of the ubiquitin–proteasome pathway. Ubiquitin is encoded by the tandemly linked polyubiquitin gene. In the cell, linear polyubiquitin protein molecules are not detected. When polyubiquitin and UCH-L1 are co-expressed in bacterial cells, polyubiquitin is co-translationally processed to generate monoubiquitin. Therefore it is possible that polyubiquitin is processed by UCH or other DUBs to generate monoubiquitin in eukaryotic cells as well. In addition to the polyubiquitin gene, ubiquitin is also encoded by fusion to two ribosomal subunits called L40 and S27. These gene products are also believed to be processed by DUBs. Cleavage of isopeptide bond in the ubiquitin chains linked through Lys-48 of ubiquitin serves two purposes. One is to recycle ubiquitin after it has been used for marking a substrate for ubiquitination. Another function is to edit the errors made by the ubiquitin-conjugating enzymes and reverse the ubiquitination reaction so that the substrate is no longer degraded (Wilkinson, 2000). Also, the editing function of DUBs probably serves to reverse the monoubiquitin attachment that marks membrane proteins for endocytosis.

4.26.6 Regulation of the Ubiquitin–Proteasome Pathway

Proteolysis by the ubiquitin–proteasome pathway can be regulated at the ubiquitin-conjugation step or at the proteasome step. Since the specificity of ubiquitination lies at the conjugation step, clearly regulation of the conjugation process is important in determining whether or not a substrate is targeted for degradation. Regulation of proteasome has a global effect on degradation of cellular substrates.

4.26.6.1 Regulation of Ubiquitin Conjugation

Ubiquitin needs to be conjugated to the right substrate, at the right place in the cell, and at the right time in order to control physiological processes properly. Commitment of a substrate protein to ubiquitin–proteasome-mediated degradation is regulated by (1) modification of the substrate; (2) modulation of ubiquitin ligase activity; and (3) removal of ubiquitins.

4.26.6.1.1 Modification of the substrate

Protein substrates are degraded in the cell at specific times in response to physiological stimuli. In addition, degradation of substrates is likely to be spatially restricted within a cell. Based on the accumulated evidence, it appears that the vulnerability or resistance to ubiquitin–proteasome-mediated degradation is regulated usually by a posttranslational modification. The protein substrates are modified in two main ways: (1) by posttranslational modification such as phosphorylation or (2) by allosteric modifications.

4.26.6.1.1.(i) Phosphorylation of the substrate

Phosphorylation of a substrate can make it vulnerable to ubiquitination or resistant to ubiquitination. For example, yeast cyclins Cln2 and Cln3, the cyclin-dependent kinase inhibitor p27Kip1, and transcriptional regulators IκBα and β-catenin are ubiquitinated after phosphorylation. In neurons, ubiquitination of p35, a neuronal-specific activator of cyclin-dependent kinase 5 (Cdk5), is stimulated when the protein is phosphorylated by Cdk5 within the active kinase complex (Patrick et al., 1998).

It is instructive to consider examples of some substrates as to how the regulation at the level of substrate works. The transcription factor nuclear factor kappa B (NF-κB) is inhibited by IκBα, which binds to NF-κB and keeps it in an inactive form in the cytosol. NF-κB is activated by numerous external stimuli such as cytokines, ionizing radiation, or neuronal injury. NF-κB is activated by proteolysis of IκBα, which releases NF-κB to be translocated to the nucleus, where it initiates transcription. Ubiquitination of IκBα requires phosphorylation on Ser-32 and Ser-36. Upon phosphorylation, IκBα is recognized by a specific

multisubunit RING finger ligase complex called $SCF^{\beta\text{-TrCP}}$. Activity of $SCF^{\beta\text{-TrCP}}$ ligase seems to be constitutive, while the kinases that phosphorylate IκBα are activated by the stimuli known to induce NF-κB-mediated transcription. It must be noted that phosphorylation of a substrate can make a stable protein vulnerable to degradation, as we saw above, or phosphorylation can have the opposite effect of stabilizing a short-lived protein. In the *Aplysia* nervous system, for example, CCAAT/enhancer binding protein (C/EBP), a transcription factor critical for long-term synaptic plasticity, is made resistant to ubiquitin–proteasome-mediated degradation when it is phosphorylated by mitogen-activated protein kinase (MAPK) (Yamamoto et al., 1999).

4.26.6.1.1.(ii) Allosteric modification of the substrate Although less well studied, a mechanism for making a substrate susceptible to ubiquitination is allosteric modification by ligands. A physiological example is that of degradation of regulatory (R) subunits of cyclic adenosine monophosphate (cAMP)-dependent protein kinase (PKA) (Hegde et al., 1993). R subunits are substrates for ubiquitination and degradation by the proteasome. Degradation of R subunits leads to persistent activation of PKA without persistence in cAMP elevation and bridges the short-term action of the neurotransmitter serotonin (5-hydroxytryptamine, 5-HT) to gene expression (Hegde et al., 1997). The R subunit has two cAMP-binding sites. Without cAMP binding, R subunits are resistant to ubiquitination. Mutation studies have reveled that for ubiquitination of R subunits, binding of cAMP to both sites is essential. For example, R subunit mutants that bind cAMP to only one site are not efficiently degraded (Chain et al., 1999).

4.26.6.1.2 Modulating the activity of ubiquitin ligases

Ubiquitin ligases largely control the substrate specificity of the ubiquitin conjugation reaction. The temporal specificity of ubiquitin conjugation to substrates by these enzymes is provided by regulation of the ligase activity. The activity of ubiquitin ligases can be modulated by posttranslational modification such as phosphorylation and by allosteric modification of the enzyme, or by attachment to ubiquitin-like (UbL) proteins.

4.26.6.1.2.(i) Modulation of ubiquitin ligases by phosphorylation Regulation of ubiquitin ligase activity by phosphorylation has been shown in the studies on a multisubunit ligase, APC. As the name implies, this complex is critical for cell cycle progression into anaphase. A recent study shows that a form of APC is also expressed in postmitotic neurons.

APC can be activated by Cdc2 kinase, which appears to exert its effect by activating another protein kinase called polo-like kinase. In *Xenopus* and humans, phosphorylation of four different APC subunits, APC1, CDC16, CDC23, and CDC27, has been shown to be increased during mitosis. In neurons, APC might have a role in ubiquitinating different substrates from the ones ubiquitinated during cell cycle progression. An observation that lends credence to this notion is that levels of polo-like kinases Fnk and Snk dramatically increase with stimuli that produce long-term potentiation (LTP) and other forms of synaptic plasticity (Kauselmann et al., 1999). Activity of at least one single-subunit RING finger ubiquitin ligase, c-Cbl, is known to be regulated by phosphorylation. c-Cbl ubiquitinates the epidermal growth factor receptor (EGFR). Tyrosine phosphorylation of c-Cbl stimulates the ligase to ubiquitinate the EGFR at a site next to the RING finger domain (Glickman et al., 2002).

Phosphorylation of ubiquitin ligases could have an inhibitory effect as well. In the fission yeast *Schizosaccharomyces pombe*, PKA blocks APC activity. Moreover, the inhibitory effect of PKA seems to be dominant over the stimulatory effect of the polo-like kinase. Even if APC has been activated by polo-like kinase, addition of mammalian PKA to APC-containing fractions inhibits ubiquitination of the substrate cyclin B. Phosphorylation of ubiquitin ligases is also regulated by phosphatases. For example, type I protein phosphatases (PP1) are necessary for progression into anaphase. Also, in *S. pombe*, mutations in *dis2+*, a gene that encodes a catalytic subunit of PP1, have a deleterious effect. Although the exact mechanism of phosphatase action in promoting activity of APC is not clear, perhaps the phosphatases act by counteracting the negatively regulating protein kinases such as PKA (Hegde, 2004).

4.26.6.1.2.(ii) Allosteric modification of ubiquitin ligases In addition to phosphorylation, ubiquitin ligase activity can be stimulated by allosteric activation. A ubiquitin ligase called Ubr1 targets transcription factor Cup9, which is a negative regulator of di/tri peptide transporter *Ptr1* gene. Ubr1 has three sites at which it can bind other molecules. It is believed that site III on Ubr1 binds to the substrate

Cup9. Peptides that bind to site I or II can allosterically stimulate ligase activity of Ubr1 toward Cup9 (Turner et al., 2000).

4.26.6.1.2.(iii) Modulation of ubiquitin ligase activity by attachment of ubiquitin-like proteins

Activity of the ligases is also modified by posttranslational modification by covalent linkage of UbL proteins. Linkage of a UbL protein to an E3 ubiquitin ligase appears to modulate the activity of the ligase. For example, a UbL protein called Rub1 (related to ubiquitin 1) is conjugated to proteins of the cullin family, which are part of the SCF ligase complex. Conjugation to Rub1 is required for maximal activity of the SCF ligase 14 (Hegde, 2004).

4.26.6.1.3 Removal of ubiquitins

Ubiquitin–proteasome-mediated degradation can be regulated by removal of ubiquitin. When a protein is polyubiquitinated, it is targeted to the proteasome for degradation unless the ubiquitin chains are removed by the action of DUBs. Deubiquitination by DUBs serves two purposes: (1) Reversing ubiquitination of a protein or (2) disassembling the polyubiquitin chains before the ubiquitinated proteins are channeled to the 26S proteasome. Disassembly of polyubiquitin chains at the proteasome step is likely to be a rate-limiting step for degradation. Since the pore of the proteasome catalytic chamber is small (13 Å), the polyubiquitin tag needs to be removed before the substrate is fed into the catalytic core. Otherwise the catalytic chamber is likely to be clogged, thereby reducing the rate of degradation. The DUBs, called UBP6 (called USP14 in yeast) and UCH37, are associated with the proteasome. Also, a subunit in the lid of the 19S RC, Rpn 11, has been shown to possess deubiquitinating activity (Yao and Cohen, 2002).

4.26.7 Achieving Specificity of Ubiquitin Linkage: Combinatorial Coding by E2s and E3s

Specificity of conjugation of ubiquitin to a substrate requires the action of three enzymes: E1, E2, and E3. E1 is common to all ubiquitination reactions because this enzyme activates ubiquitin. There is some degree of specificity at the E2 step. The E3s are the most specific to a given substrate, however. Initially it was thought that there was a specific E3 for each substrate. This situation would be untenable because of the coding burden it places on the genome. Rather, it is likely that the specificity is derived from combination of recognition modules. A given single or multisubunit E3 has a domain specific to a given substrate. In some instances, an E3 ligates ubiquitin to only one substrate. In other cases, an E3 ligates ubiquitin to more than one substrate. When an E3 has activity toward more than one substrate, it is generally thought that the domain on an E3 that interacts with one substrate is distinct from the domain that an E3 uses to interact with another substrate. In some cases, however, all of the different substrates bind to the same site through the same interaction motif. It is estimated that the human genome contains approximately 1000 genes encoding E3s (Ciechanover and Brundin, 2003). The estimate for number of genes coding for E2s is at least 25 (Weissman, 2001). Considering that there are roughly 30,000 genes in the human genome, theoretically E2s and E3s together could potentially generate a unique combination for every protein encoded by the human genome! Besides the unique E2–E3 combinations, specificity can be generated by the state of the substrate (vulnerable or resistant to degradation) as well as regulation of E3s in many ways, as described. Thus, the ubiquitin conjugation machinery can be highly specific to a given substrate (Hegde, 2004).

4.26.8 Regulation of the Proteasome

Proteasome activity can be regulated in two main ways. One is regulation by cofactors or proteins that are loosely associated with it and by induction or phosphorylation of the subunits, particularly those of the 19S RC. The proteasome can also be regulated by a change in the composition of the intrinsic subunits.

4.26.8.1 Regulation by Cofactors and Loosely Associated Factors

In addition to the intrinsic subunits, proteins that interact with the proteasome complex regulate its activity. Often the cofactors are components of the ubiquitin pathway. Proteins such as chaperones and heat shock proteins also assist in proteasome-mediated degradation of proteins. Both E2s and E3s have been found to interact with the proteasome. For example, ubiquitin conjugating enzymes (E2s) Ubc1, Ubc2, and Ubc4 coimmunoprecipitate with the proteasome. E3s such as Ubr1 and Ufd4 have been shown to physically interact with subunits of the 19S RC.

APC and SCF ubiquitin ligases co-purify with the 19S RC. A ubiquitin ligase called Hul5 is associated with the proteasome as well. The cofactors interact with the proteasome directly or via other proteins such as Cic1 and hPLICs that recruit the cofactors to the proteasome. For example, hPLIC-1 and hPLIC-2, the human counterparts of yeast Dsk2, interact with the proteasome as well as specific E3s E6-AP and β-TrCP. Two other proteins, Rad23 and BAG1, are also known to interact with the proteasome (Hegde, 2004).

In addition, the DUBs interact with the proteasome. A DUB called Ubp6 is known to interact with the regulatory complex of the proteasome. Since binding to the proteasome increases the activity of Ubp6 300-fold, Ubp6 is thought to assist in the removal of polyubiquitin chain just before the substrate is degraded and aid in recycling ubiquitin for further use. Other DUBs have also been reported to associate with the proteasome. A yeast DUB which is related to the human tre-2 oncogene associates with the proteasome and assists in the disassembly of the polyubiquitin chain. The *Aplysia* homolog of UCH-L1 (Ap-uch) associates with the proteasome and improves proteolytic activity. In *in vitro* experiments, it was shown that Ap-uch cleaves the first ubiquitin attached to the substrate (Hegde et al., 1997). Among the factors that interact with the proteasome are heat shock proteins and chaperones. It has been known for a long time that the ubiquitin–proteasome pathway degrades misfolded or unfolded proteins. The general belief is that the heat shock proteins refold the misfolded proteins into the right conformation. Misfolded proteins not rescued by the heat shock proteins are thought to be substrates for the ubiquitin–proteasome pathway. It was not clear, however, which ubiquitin ligase (E3) specifically recognizes misfolded proteins. Recently, a chaperone called BAG1 has been shown to interact both with the proteasome and the heat shock protein Hsp70. CHIP (carboxyl terminus of the Hsc70-interacting protein) is known to ubiquitinate unfolded proteins. CHIP contains a protein sequence motif called the U-Box and a RING finger domain. CHIP can interact with either Hsp70 or Hsp90, and together with either of the heat shock proteins can ligate ubiquitin to misfolded proteins. The cooperation between heat shock proteins and the proteasome is also required to degrade aberrant membrane proteins. For example, the cystic fibrosis transmembrane conductance regulator (CFTR) is a membrane protein which is often misfolded. CHIP and Hsc70 together recognize the CFTR protein and target it to proteasome-mediated degradation (Hegde, 2004).

Proteasome activity can also be regulated by substitution of subunits of the 20S core with subunits such as the ones induced by interferon γ (IFNγ). Furthermore, alternative regulatory complexes with different composition than that of the 19S RC can be attached to the 20S core, which alters the substrate specificity and activity of the proteasome (Glickman et al., 2002).

4.26.8.2 Regulation of the Proteasome by Induction and Phosphorylation of Subunits and Subcellular Distribution

The subunits of the 26S proteasome are not fixed but change in response to the physiological condition of the cell. The capacity of the 26S proteasome to degrade ubiquitinated proteins can be regulated by changes in (1) the total amount of the proteasome, (2) its subunit composition, and (3) its subcellular distribution. The changes in the total amount of 26S can be brought about by the extent of 19S cap binding to the 20S core. This could occur by an increase in the amount of 19S as well as by increased association of the existing 19S with the 20S core. During metamorphosis of *Manduca*, flight muscles develop and intersegmental muscles are destroyed. The destruction of intersegmental muscles is brought about by an increase in ubiquitin-dependent proteolysis as a result of extensive hormone-dependent reprogramming of the 19S RC. It has been shown that the multiubiquitin binding subunit (MBP/S5a) and the ATPases MSS1 (S7) and S4 are induced significantly during this period. An increase in the amount of 26S by enhanced binding of 19S to 20S without an increase in the total amount of 20S occurs during the metaphase-anaphase transition in the meiotic cell cycle. In addition to the regulation of total proteasome content, the activity of the proteasome also appears to be regulated by alterations in its subcellular distribution. For example, during ascidian (marine animals commonly called sea squirts) embryonic development, the distribution of the proteasome changes in a cell-cycle-dependent manner. The proteasome localized in the nucleus during interphase disappears from the nucleus during prophase, and in telophase the proteasome is again localized in the newly formed nucleus (Hegde, 2004).

Proteasome activity can also be regulated by phosphorylation. For example, it has been shown that phosphorylation events are necessary for the

assembly of the 26S proteasome. Several subunits, including MSS1, S4, S6, and S12 of the 19S RC, have been shown to be phosphorylated. Recently it was demonstrated that assembly of the proteasome requires phosphorylation of Rpt6, an ATPase subunit. Our recent studies showed modulation of the proteasome activity by protein kinases in the nucleus and synaptic terminals. Interestingly, we observed that protein kinases differentially modulate the proteasome activity in the two subcellular compartments of the neuron (Upadhya et al., 2006).

4.26.9 Ubiquitin–Proteasome Pathway and Synaptic Plasticity

Although ubiquitin was used a marker for brain pathology, no physiological or pathological role for ubiquitin in the nervous system was found until a little over a decade ago. The first discovery of ubiquitin–proteasome-mediated degradation of a physiologically relevant substrate in the nervous system was that of R subunits of PKA (Hegde et al., 1993). Since then several substrates of the ubiquitin–proteasome pathway in the nervous system have been identified (**Table 1**).

4.26.9.1 Degradation R Subunits of PKA and Proteolytic Removal of a cAMP Response Element Binding Protein Repressor

A role for the ubiquitin–proteasome pathway in synaptic plasticity was discovered during the investigation of persistent activation of PKA. Studies on the biochemical mechanism of long-term facilitation (Greenberg et al., 1987) in *Aplysia* indicated that PKA was persistently activated in the absence of elevated cAMP. How is PKA activated in the absence of sustained increase in cAMP? It was found that the R subunits of PKA were decreased without any change in the catalytic (C) subunit during induction of long-term facilitation. Since there was no change in mRNA for either the R subunit or the C subunit, it was concluded that R subunits were diminished perhaps through proteolysis. What is the mechanism of R subunit degradation? In a

Table 1 Components of the ubiquitin–proteasome pathway with known physiological function in the nervous system[a]

Molecule	*Description*	*Reference*
R subunit	Subunit of cAMP-dependent protein kinase—crucial for synaptic plasticity in *Aplysia*	Hegde et al., 1993
CREB1b	A transcription repressor that antagonizes CREB during long-term facilitation in *Aplysia*	Upadhya et al., 2004
GluR1	Transmitter receptor critical for regulating synaptic strength	Burbea et al., 2002
β2-adrenergic receptor	Receptor that functions to modulate synaptic activity	Shenoy et al., 2001
PSD-95	A structural protein in PSD important for synaptic plasticity	Colledge et al., 2003
Dunc-13	Presynaptic protein in *Drosophila* that primes synaptic vesicle release	Speese et al., 2003
Epsin15	Monoubiquitination of epsin bridges transmembrane receptors to clathrin-coated pits for endocytosis	Carbone et al., 1997
E6-AP	Ubiquitin ligase (E3) important for synaptic plasticity, contextual memory	Jiang et al., 1998
Ap-uch	Deubiquitinating enzyme with a role in long-term synaptic plasticity in *Aplysia*	Hegde et al., 1997
Bendless	Ubiquitin-conjugating enzyme (E2) that controls synapse formation in *Drosophila*	Muralidhar and Thomas, 1993; Oh et. al., 1994
APC	A multisubunit RING finger ligase critical for regulating axonal morphogenesis, synaptic size and activity	Juo and Kaplan, 2004; van Roessel et al., 2004; Stegmuller et al., 2006
LIN-23	The substrate-binding part (F-Box) of SCF ligase that regulates glutamate receptor abundance	Dreier et al., 2005
Fat facets	Deubiquitinating enzyme that regulates glutamate receptor abundance	DiAntonio et al., 2001

[a]This list includes only some examples and is not meant to be comprehensive.

series of biochemical experiments, Hegde et al. (1993) found that R subunits were substrates for ubiquitination and proteasome-mediated degradation. Moreover, a UCH (Ap-uch) that interacts with the proteasome was found to be induced by 5-HT, the neurotransmitter that induced long-term facilitation. Ap-uch was found to be critical for induction of long-term facilitation (Hegde et al., 1997) (**Figure 3**). Subsequently, Chain et al. (1999) showed that at sensorimotor neuron synapses, injection of lactacystin, a specific proteasome inhibitor, blocked induction of long-term facilitation. Since the R subunit inhibits the activity of C subunits of PKA, the results were interpreted to suggest that the ubiquitin–proteasome pathway operates to remove inhibitory constraints on formation of long-term memory. This has been corroborated by work carried out on the rat hippocampus. Lopez-Salon and coworkers (Lopez-Salon et al., 2001) demonstrated that bilateral infusion of lactacystin to the CA1 region of the rat hippocampus caused total retrograde amnesia for a one-trial avoidance learning. They also showed that total ubiquitination increases in the hippocampus 4 h after the training. These results are consistent with the idea that a decrease in some critical inhibitory proteins during long-term memory formation is mediated by the ubiquitin–proteasome pathway.

Additional evidence that the ubiquitin–proteasome pathway might function to degrade proteins that normally inhibit long-term synaptic plasticity has also been obtained using the *Aplysia* model. Stimulation protocols that induce long-term facilitation in *Aplysia* cause ubiquitination and degradation of a cAMP response element binding protein (CREB) repressor called CREB1b. Both ubiquitination and degradation of CREB1b are increased by protein kinase C (**Figure 3**). It remains to be seen whether CREB1b or the ligase that targets CREB1b for ubiquitination is modulated by PKC (Upadhya et al., 2004).

4.26.9.2 Modulation and Essential Function of a Deubiquitinating Enzyme in Synaptic Plasticity

Subsequent to the finding on ubiquitin–proteasome-mediated degradation of R subunits of PKA, a crucial role in long-term facilitation for a neuronal-specific UCH was discovered. *Aplysia* UCH (Ap-uch) is the homolog of human UCHL1 and is induced by stimuli that induce long-term facilitation but not stimuli that induce short-term facilitation. Injection of antibodies or antisense oligonucleotides specific to Ap-uch into sensory neurons that synapse onto motor neurons in

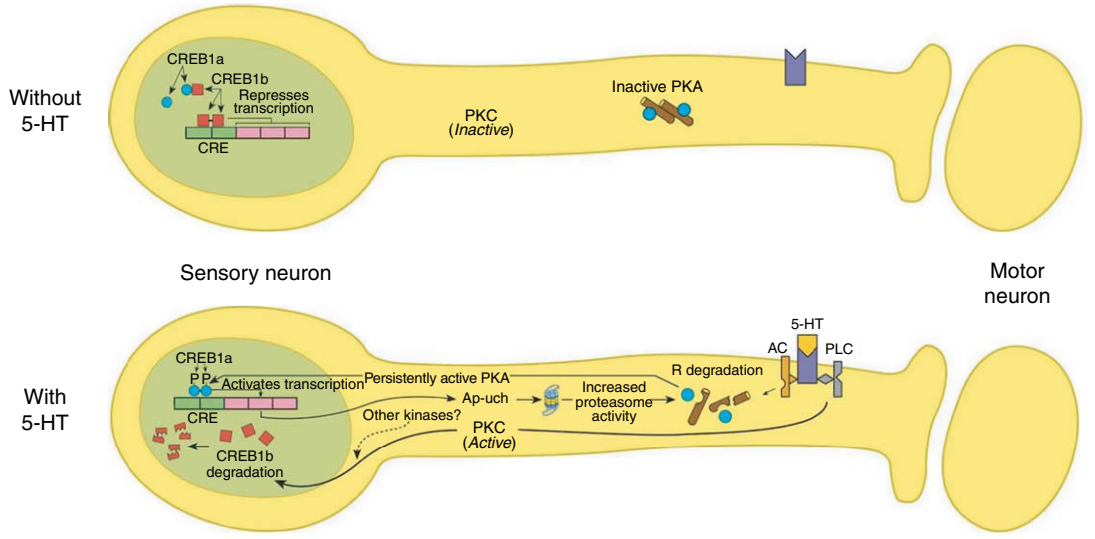

Figure 3 Role of the ubiquitin–proteasome pathway in long-term facilitation. When sensory neurons are stimulated with the neurotransmitter serotonin (5-HT), which induces long-term facilitation, R subunits of protein kinase A (PKA) are ubiquitinated and degraded by the proteasome, making the kinase persistently active. The catalytic subunit of PKA (blue circle) translocates to the nucleus and phosphorylates cAMP response element binding protein 1a (CREB1a), the activator form of CREB. Concomitantly, the repressor form of CREB, CREB1b, is degraded by the ubiquitin–proteasome pathway. Protein kinase C (PKC), which is also activated by 5-HT-mediated signaling, stimulates ubiquitin conjugation to CREB1b and subsequent degradation. AC, adenylyl cyclase; PLC, phospholipase C.

culture blocked induction of long-term facilitation (Hegde et al., 1997). Investigation of biochemical functions of Ap-uch indicated that Ap-uch is capable of cleaving small attachments to linearly attached ubiquitin molecules such as ubiquitin–ubiquitin–cysteine but not large attachments such as glutathione-S-transferase (GST) in substrates such as ubiquitin–GST. More interestingly, additional biochemical analyses showed that Ap-uch associates with the proteasome. The association of Ap-uch increases the rate of degradation by the proteasome. For example, addition of recombinant Ap-uch to *in vitro* degradation systems showed that there was approximately double the degradation of the R subunit of PKA. Since persistent activation of PKA has been shown to be critical for induction of long-term facilitation and R subunits of PKA were found to be substrates for the ubiquitin–proteasome pathway, the experiments on Ap-uch provided some molecular explanation for the role of regulated proteolysis in long-term facilitation (Hegde et al., 1997). How does Ap-uch increase the rate of degradation by the proteasome? Using recombinant ubiquitin with its Lysine-48 mutated to Arg, which can support multiple monoubiquitin attachments to the protein substrate, it was shown that Ap-uch stimulates the release of ubiquitin from substrates (Hegde et al., 1997). Therefore, it can be inferred that Ap-uch perhaps cleaves the first ubiquitin in the polyubiquitin chain attached to the substrate. The function of UCHs in synaptic plasticity appears to be evolutionarily conserved. Recently, the mammalian homolog of Ap-uch, UCH-L1, has been shown to play a role in long-term memory in mice.

4.26.9.3 Possible Differential Role of the Proteasome in Different Compartments of Neurons

Although previous investigations found that proteasome inhibitors block induction of long-term facilitation, recent analysis of long-term facilitation at *Aplysia* synapses obtained different results. Bath application of the active form of lactacystin, *clasto*-lactacystin β-lactone, to sensorimotor neuron synapses resulted in strengthening the synapses and increasing neurite outgrowth in isolated sensory neurons. Furthermore, application of β-lactone increased 5-HT-induced long-term facilitation. The increase in neurite elongation is consistent with results obtained in PC12 and Neuro2A cells in which lactacystin induces neurite outgrowth. Interestingly, the increase in synaptic strength using the *Aplysia* sensorimotor neuron synapses observed by application of β-lactone was blocked in the presence of the translation inhibitor anisomycin but not by the transcription inhibitor actinomycin D (Zhao et al., 2003). This result is highly surprising, since innumerable studies have documented the requirement for transcription in the induction of long-term facilitation in *Aplysia* as well as several vertebrate forms of long-term memory (Chen and Tonegawa, 1997; Kandel, 2001). Both sets of results can be reconciled if one postulates that proteasome has different roles in different cellular compartments. In the same neuron, the proteasome is likely to carry out different tasks in different subcellular compartments, resulting in different physiological consequences at different loci. Therefore, blocking different roles of the proteasome during induction of memory would lead to distinct and even opposite effects on synaptic strength. For example, the proteasome is known to degrade transcription repressors. Degradation of transcription repressors should allow transcription activators to induce gene expression, which in turn leads to development of long-term facilitation. If the proteasome is inhibited only in the nucleus before the repressors are degraded, gene expression and hence induction of long-term facilitation should be blocked. On the other hand, if the degradation of proteins needed at the synapse for developing long-term facilitation is inhibited by the proteasome, long-term facilitation should be enhanced. Although it might sound radical, I think the main purpose of transcription during induction of long-term facilitation or other forms of long-term memory is to provide mRNAs for synthesis of the rapidly turning over proteins needed for memory formation. These proteins are likely to be degraded by the ubiquitin–proteasome pathway. If the degradation of these proteins is prevented, then long-term memory formation becomes independent of transcription. In support of this idea, Zhao et al. (2003) found that proteasome inhibitor-induced synaptic strengthening depends on translation but not transcription. Differential temporal regulation of the proteasome could occur within the same subcellular compartments. In the nucleus, the proteasome has to degrade transcription repressors to allow transcription activators to induce gene expression, which in turn leads to development of long-term facilitation. If the proteasome is inhibited during the time the repressors are degraded, gene expression and hence induction of long-term facilitation should be blocked. In support of this idea, we have found that a transcription repressor called CREB1b is degraded by the ubiquitin–proteasome pathway in response to long-term facilitation-inducing protocols (Upadhya et al., 2004).

Our biochemical experiments on the proteasome also support differential function of the proteasome in different neuronal compartments. Our results showed that in both *Aplysia* nervous system and mouse brain, proteasome activity in the synaptic terminals is significantly higher than that of the nuclear proteasome. Moreover, the proteasome activity in the two compartments is differentially regulated by protein kinases relevant to synaptic plasticity such as PKA, PKC, and MAPK (Upadhya et al., 2006).

As discussed, differential activity of the proteasome in *Aplysia* might explain conflicting results obtained in different studies. Does differential proteasomal activity affect synaptic plasticity differentially in vertebrates? Our preliminary studies indicate that proteasome has differential roles during induction and maintenance phases of the late phase of long-term potentiation (L-LTP). We have found that proteasome inhibition enhances the early phase of L-LTP but inhibits the late, maintenance phase of L-LTP (Hegde et al., 2006). Although two other studies have tested proteasome inhibition on LTP, neither study reported differential function of proteasome on LTP (Fonseca et al., 2006; Karpova et al., 2006). Both of these studies only showed inhibition of LTP with proteasome inhibitor. The study by Karpova et al. (2006) used MG132, which is not a highly specific proteasome inhibitor. MG132 has been known to strongly inhibit other proteases such as calpain and cathepsin B (Chain et al., 1999; Tang and Leppla, 1999). Because calpain inhibition is known to block LTP (del Cerro et al., 1990; Denny et al., 1990), the results of experiments that use only MG132 are not strictly attributable to proteasome inhibition. In the study by Fonseca et al. (2006), I think the main reason the authors failed to discover the enhancement of the early phase of LTP with proteasome inhibition is because they used nanomolar concentrations of lactacystin and epoxomycin. Numerous studies, including the one cited in the aforementioned study (Dick et al., 1996), have established that micromolar concentration of lactacystin is required for efficient proteasome inhibition. All three catalytic activities of the proteasome are inhibited only with 100 μmol l^{-1} lactacystin or 50 μmol l^{-1} β-lactone (Fenteany et al., 1995).

4.26.10 Roles of the Ubiquitin–Proteasome Pathway at the Synapse

Ubiquitin–proteasome-mediated proteolysis has been found to have both presynaptic and postsynaptic roles at the synapse.

4.26.10.1 Presynaptic Roles of Proteolysis: Degradation of Synaptic Vesicle Proteins

The earlier observations on the role of the ubiquitin–proteasome pathway in synaptic plasticity mainly focused on long-term effects. Recent studies, however, indicate that the ubiquitin–proteasome pathway also functions in regulating short-term synaptic plasticity. For example, a protein, Dunc-13, which is critical in priming the synaptic vesicles, is ubiquitinated and degraded by the proteasome in *Drosophila* neuromuscular synapse. Application of proteasome inhibitors and the dominant-negative mutation in a core subunit (β6) of the *Drosophila* proteasome both lead to an increase in presynaptic accumulation of Dunc-13 protein. Also, application of the proteasome inhibitors lactacystin and epoxomycin cause an increase in the excitatory junctional current, suggesting that accumulation of Dunc-13 and the resultant increase in the net Dunc-13 quantity leads to enhanced synaptic transmission (Speese et al., 2003).

Is it likely that the quantity of other synaptic proteins with a key role in neurotransmission is regulated by the ubiquitin–proteasome pathway? If so, regulated proteolysis may have a wider role in controlling short-term synaptic plasticity. As of now, two other synaptic vesicle proteins, synaptophysin and syntaxin, have been shown to be substrates for ubiquitin–proteasome-mediated degradation. Vulnerability of synaptophysin for ubiquitin–proteasome-mediated degradation was discovered indirectly by looking for proteins that bind to synaptophysin in the yeast two-hybrid system. Two genes encoding for proteins Siah-1A and Siah-2 were isolated from the rat brain two-hybrid cDNA library. These two genes are closely related to each other and are homologs of *Drosophila seven in absentia* gene. Siah proteins are single-subunit RING finger ubiquitin ligases with the RING finger domain in the N-terminal region. It is not clear which ligase (Siah1-A or Siah-2) is responsible for degradation of synaptophysins *in vivo* in neurons. Because the experiments were carried out in nonneuronal cells (Chinese hamster ovary cells) and PC12 cells, the physiological consequences of synaptophysin degradation remain to be determined.

Another presynaptic substrate for the ubiquitin–proteasome pathway is syntaxin 1. The group that isolated the Siah ligases also investigated ubiquitin–proteasome-mediated degradation of syntaxin 1 using the yeast two-hybrid strategy. The ligase that ubiquitinates syntaxin 1 is called staring (syntaxin 1-interacting

RING finger protein). Co-expression of staring with syntaxin 1 in HeLa cells increases the degradation of syntaxin 1, which can be inhibited by the proteasome inhibitor MG132. Again, the physiological effect of ubiquitin–proteasome-mediated degradation of syntaxin 1 remains to be determined.

4.26.10.2 Modulation of Postsynaptic Structure and Function by Proteolysis

Ubiquitin and proteasome appear to regulate proteins that control synaptic transmission such as neurotransmitter receptors and proteins that are part of the synaptic structure such as postsynaptic density proteins. Investigations in *Caenorhabditis elegans* revealed a role for ubiquitin in endocytosis of GLR-1 type of glutamate receptor (Burbea et al., 2002). Later studies in *C. elegans* showed a role for APC in regulating the abundance of GLR-1 glutamate receptor, although the APC substrate was not identified (Juo and Kaplan, 2004). Intriguingly, SCF ligase also appears to control GLR-1 glutamate receptor quantity in *C. elegans* (Dreier et al., 2005). In mammalian hippocampal neurons, treatment with the proteasome inhibitor MG132 blocks agonist-induced endocytosis of alpha-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA)-type glutamate receptors (Patrick et al., 2003). The exact mechanism of AMPA receptor endocytosis in mammals is not clear. Patrick et al. (2003) argue that the AMPA receptor itself is not the target of proteasome action. Furthermore, apart from AMPA-induced internalization, *N*-methyl-D-aspartate (NMDA)-induced internalization is also prevented by application of the proteasome inhibitor. The authors speculate that a protein(s) that interacts with AMPA receptor might be the target (Patrick et al., 2003). Given that both NMDA and AMPA agonists stimulated internalization that is inhibited by the proteasome, I think that this is a case of passive internalization and not an instance of regulated AMPA receptor endocytosis. In support of this idea, a thorough study by others (Colledge et al., 2003) showed that postsynaptic density-95 (PSD-95) is regulated by ubiquitin–proteasome-mediated degradation, and degradation of PSD-95 leads to AMPA receptor internalization. In this study as well, proteasome inhibitors prevent AMPA receptor internalization. Furthermore, Colledge et al. (2003) demonstrated that application of the proteasome inhibitor MG132 to hippocampal slices reduces the magnitude of hippocampal long-term depression. A direct role for ubiquitin in regulated endocytotic degradation of AMPA receptors is still an open question. Given the *C. elegans* data of Burbea et al. (2002), it is possible that targeting of AMPA receptors for degradation after internalization depends on ubiquitination. Endocytosis of other neurotransmitter receptors might be directly or indirectly regulated by ubiquitination. Glycine receptor has been shown to be internalizedupon ubiquitination. A protein associated with gamma-aminobutyric acid A (GABAA) receptors, Plic-1, indirectly controls removal of GABAA through endocytosis. Interestingly, proteasome inhibitors prevent degradation of internalized GABAA receptors. Ubiquitin–proteasome-mediated proteolysis appears to regulate several other proteins in addition to PSD-95 in the PSD proteins, including several structural proteins. For example, Shank, GKAP, and AKAP79/150 are degraded through the ubiquitin–proteasome pathway (Ehlers, 2003). Unlike the degradation of PSD-95, the physiological relevance of proteolytic removal of Shank, GKAP, and AKAP79/150 is not clear because the studies were correlative and a direct link between ubiquitin–proteasome-mediated degradation of the PSD proteins and structural remodeling was not established. There is also evidence that the ubiquitin–proteasome pathway controls a protein that regulates spine shape. SPAR controls dendritic spine shape by reorganizing the actin cytoskeleton (Pak et al., 2001). During activity-dependent remodeling of synapses, SPAR was found to be degraded by the ubiquitin–proteasome pathway (Pak and Sheng, 2003). Degradation of SPAR is stimulated by serum-inducible kinase (SNK). Neuronal activity induces SNK mRNA in the cell body and the induced SNK is targeted to the dendritic spines. Because of the time required for SNK mRNA to travel to the spines, Pak and Sheng (2003) speculate that SPAR may function to oppose synaptic remodeling after elevated activity.

4.26.11 The Ubiquitin–Proteasome Pathway and Developmental Synaptic Plasticity

Support for a role for the ubiquitin–proteasome pathway in neuronal connectivity was obtained through investigations on a *Drosophila* mutation called bendless, which causes abnormal neuronal connectivity. The bendless gene encodes a ubiquitin-conjugating enzyme (Muralidhar and Thomas, 1993; Oh et al., 1994). Additional evidence for a function of the ubiquitin–proteasome pathway in synapse formation

came from a screen in *Drosophila* designed to identify the genes controlling size and strength of synapses. This screen found that fat facets (faf), a gene that encodes a DUB, functions in synapse formation in the neuromuscular junction. Overexpression of faf in the developing *Drosophila* nervous system causes synaptic overgrowth and perturbs synaptic transmission. Another genetic screen for identifying the mutants that enhance the faf overexpression phenotype found that a loss of function in a gene called highwire (hiw) causes a significant increase in number of synapses. The hiw gene encodes a huge protein with 5233 amino acids. The hiw protein contains a RING finger domain, a characteristic feature of some ubiquitin ligases. Therefore, it is conceivable that hiw and faf promote or inhibit the degradation of the same substrate by ubiquitinating or deubiquitinating it, respectively (Wan et al., 2000; DiAntonio et al., 2001). How does hiw regulate synaptic growth? It has been shown to negatively regulate signaling by bone morphogenetic protein (BMP)/transforming growth factor β (TGFβ), which stimulates presynaptic growth in *Drosophila* neuromuscular junction. Highwire binds to a protein in the BMP/TGFβ signaling pathway called Med. Although there is no direct evidence yet for ubiquitination of Med by hiw, the genetic data suggest that hiw functions to ubiquitinate Med for degradation. Moreover, synaptic overgrowth produced by overexpression of faf is suppressed by disrupting BMP signaling (McCabe et al., 2004). Regulation of the BMP/TGFβ signaling pathway may be only one way in which hiw controls synaptic growth. Another study using a screen for suppression of synaptic growth caused by hiw mutation identified mutation in wallenda, a MAP kinase kinase kinase. Hiw protein was shown to regulate synaptic levels of endogenous wallenda protein. It has been suggested that hiw might have multiple functions because of its vast size and multiple domains and therefore might be responsible for regulating BMP/TGFβ signaling as well as the MAPK pathway.

The ubiquitin–proteasome pathway also seems to regulate synaptic size. In *Drosophila* neuromuscular junction (NMJ), a mutation in APC2, a subunit of APC, greatly increases the number of boutons. APC in *Drosophila* NMJ regulates levels of Liprin-α in neurons. Liprin-α is a protein that interacts with tyrosine phosphatases. In *Drosophila*, Liprin-α is required for control of synaptic number by Dlar, a receptor tyrosine phosphatase (van Roessel et al., 2004). The exact mechanism by which regulation of Liprin-α by APC regulates synaptic size remains to be elucidated.

Protein degradation has been found to play a role in other major processes that establish neuronal connectivity such as axon guidance and dendrite sculpting. In neurons of *Xenopus* retina, netrin-1 causes the growth cones to turn and lysophosphatidic acid (LPA) causes the growth cone to collapse. Both netrin-1 and LPA effects on the growth cone are blocked by proteasome inhibitors. In addition, in cerebellar granule neurons, APC regulates axonal morphogenesis through its action on a transcription co-repressor called SnoN (Stegmuller et al., 2006). The evidence for ubiquitin–proteasome-mediated proteolytic molding of the dendrites came from work on *Drosophila*. During metamorphosis, specific sensory neurons in *Drosophila* larvae undergo extensive dendritic pruning and remodel their dendrites. Mutation in a ubiquitin-conjugating enzyme called UbcD1 blocks dendritic pruning. Additional experiments revealed that UbcD1 is likely to degrade a ubiquitin ligase called DIAP1 that antagonizes caspases. This study provides an elegant explanation for local activation of caspases which enables removal of dendrites while preserving the neurons.

4.26.12 Ubiquitination and Endocytosis

As a result of earlier research on nonneuronal cells and recent studies on neurons, it has become clear that ubiquitination plays a vital role in targeting membrane proteins for endocytosis. Some general principles regarding the role of ubiquitin in endocytosis have emerged. A membrane protein, say, a neurotransmitter receptor, becomes a target for endocytosis when it is ubiquitinated. Unlike the polyubiquitin chain that marks substrate for proteasome-mediated degradation, endocytosis appears to be mainly mediated by monoubiquitination. The ubiquitinated receptor binds to adaptor proteins called epsins that couple the receptor to the clathrin-coated pits (Carbone et al., 1997). The ubiquitinated receptor then undergoes endocytosis and is incorporated into endosomes, which in turn are sequestered into the multivesicular body. The membrane of the multivesicular bodies becomes continuous, with lysosomes leading to degradation of the receptor. It has been shown that targeting the internalized receptor to the multivesicular body also requires ubiquitination. Moreover, a 350-kDa complex called endosomal sorting complex that is

required for transport (ESCRT-I) and recognizes the ubiquitinated receptors has been identified. Function of ESCRT-I is essential for sorting the endocytosed receptor into the multivesicular body (**Figure 4**) (Katzmann et al., 2001). Two other complexes, ESCRT-II and ESCRT-III, are thought to be necessary for the continued sorting into the multivesicular body (Babst et al., 2002a,b). If the ubiquitin from the endocytosed receptor is removed the receptor recycles back to the plasma membrane. Although deubiquitination of endocytosed receptors is less well studied, UBPs are likely to carry out the ubiquitin removal function (**Figure 4**).

4.26.12.1 Endocytosis and Synaptic Function

Internalization through ubiquitin-mediated signaling and sorting into the multivesicular body is likely to play a critical role in controlling the neurotransmitter receptor number on the plasma membrane (hence synaptic function) in the nervous system. In support of this idea, Burbea et al. (2002) found in *Caenorhabditis elegans* that GLR-1, a homolog of the mammalian GluR1 which is part of the AMPA-type glutamate receptor, is internalized through a ubiquitin-mediated mechanism. GLR-1 was found to be ubiquitinated *in vivo*. When ubiquitin was overexpressed, the quantity of GLR-1 on the neuronal surface was reduced. The effect of ubiquitin overexpression was blocked by mutations in the unc-11 gene, which encodes a clathrin adaptin protein (AP180). Mutation of a specific lysine residue to arginine in the cytoplasmic tail of GLR-1 reduced internalization of GLR-1. All these results together suggest that GLR-1 is endocytosed through a ubiquitin- and clathrin-dependent mechanism. Furthermore, mutation of the lysine residues that prevent ubiquitination of GLR-1-affected locomotion in *C. elegans*. These observations indicate that prevention of ubiquitin-mediated GLR-1 endocytosis leads to an increase in synaptic strength resulting from

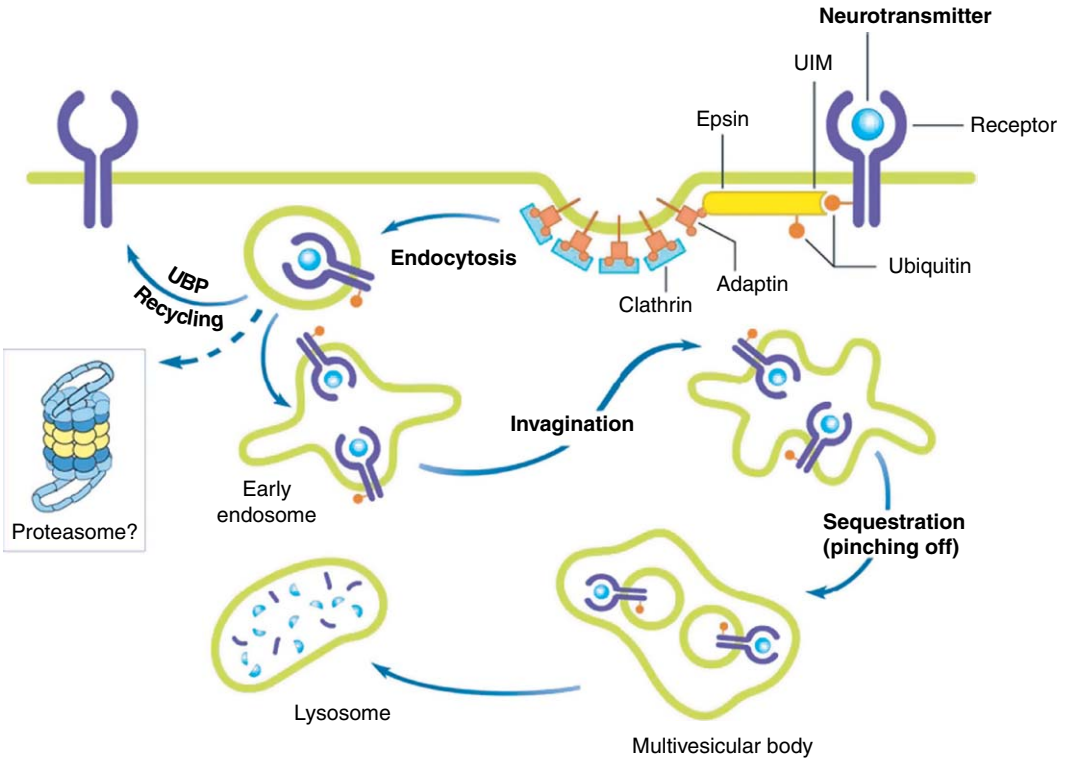

Figure 4 Ubiquitin and endocytosis. Receptors on the plasma membrane undergo monoubiquitination as a result of ligand (e.g., neurotransmitter). Ubiquitinated receptors bind to proteins called epsins, which in turn interact with adaptor proteins (adaptin) bound to clathrin-coated pits. Ubiquitination also functions to sort the internalized membrane protein into early endosomes, which directs them to degradation by lysosome via the multivesicular body. If ubiquitin from the endocytosed receptors is removed by a UBP, the receptor recycles back to the membrane. Proteasome inhibitors block endocytotic degradation of some proteins such as glutamate receptor subunits, indicating a possible role for the proteasome. UIM, ubiquitin-interacting motif.

a higher number of GLR-1 on the neuronal surface. Since the cytoplasmic tails of four mammalian AMPA receptor subunits (GluR1–GluR4) and *C. elegans* GLR-1 have a stretch of 16 conserved amino acids homologous to the region in the yeast Ste2p and Ste6p proteins that have been shown to be signals for ubiquitination and endocytosis, it is highly likely that GluR subunits in other species are endocytosed through a ubiquitin-dependent mechanism as well. Moreover, it has been found that internalization of GluR1 and GluR2 subunits of the AMPA receptor is inhibited by introduction of ubiquitin chain elongation mutant (Lys-48 mutated to Arg-48) (Patrick et al., 2003). Intriguingly, it was observed that a proteasome inhibitor, MG132, reduced internalization of AMPA receptors. One would expect the ubiquitinated GluRs to be either recycled back to the plasma membrane or to be routed to the lysosome for degradation. Involvement of the proteasome, if proven, would add a new twist to the process of endocytosis. A similar effect of MG132 on degradation of internalized vasopressin receptors (Martin et al., 2003) as well as the quantity of GABA receptors (Bedford et al., 2001) has been reported (see following for description). Other neurotransmitter receptors have been shown to be internalized through a ubiquitin-dependent process as well. Using the *Xenopus* oocyte expression system, Buttner and colleagues (Buttner et al., 2001) demonstrated that inhibitory glycine receptors are ubiquitinated at the plasma membrane and internalized. The internalization process generates fragments of 35 and 13 kDa. In their experiments, application of concanamycin, which blocks acidification of lysosomal and endosomal compartments by inhibiting vesicular H^+-ATPases, prevents cleavage, whereas the proteasome inhibitor lactacystin has no effect on generation of the smaller fragments of glycine receptor. The GABAA receptor number on the neuronal membrane appears to be determined by a ubiquitin-mediated process as well. Evidence for a possible role of ubiquitin in GABAA receptor internalization is indirect. The GABAA receptor interacts with the UbL protein Plic-1, which stabilizes the GABAA receptor. Application of the proteasome inhibitor lactacystin leads to a significant increase in the steady-state levels of a1 and b3 subunits of the GABAA receptor (Bedford et al., 2001). The inference drawn with respect to GABAA internalization is that association with Plic-1 prevents the receptor from being ubiquitinated and routed to either the lysosome or the proteasome for degradation. It is not clear what roles proteasome and lysosome play in GABAA receptor degradation (Luscher and Keller, 2001). The Plic-1 UbL domain contains a proteasome-interacting motif (Upadhya et al., 2003). Since Plic-1 also binds the GABAA receptor a and b subunits through a different domain (ubiquitin-associated [UBA] domain, which is different from UbL domain), it is possible that the UbL domain in Plic-1 is utilized for routing the internalized GABAA receptor subunits for degradation through the proteasome.

Ubiquitination of plasma membrane receptors is likely to have a widespread role in the brain. Recently, mammalian G-protein-coupled receptors (GPCRs) have been shown to be endocytosed through a ubiquitin-mediated mechanism. Two receptors, the β2-adrenergic receptor (β2-AR) and the V2-type vasopressin receptor (V2-VR), have been studied in detail (Shenoy et al., 2001; Martin et al., 2003). The endocytosis of GPCRs mediated by the ubiquitin signal differs from the endocytosis of other receptors in that an adaptor protein called β-arrestin plays a role. β-arrestin is ubiquitinated as well. The endocytosed β2-AR is routed to the lysosome for degradation if the β-arrestin is deubiquitinated or dissociated from β2-AR. If β-arrestin remains ubiquitinated, β2-AR recycles back to the plasma membrane. In contrast to the β2-AR example, endocytosis of V2-VR typifies the regulated ubiquitin-mediated endocytotic removal and destruction of the receptor, which effectively reduces the receptor number on the plasma membrane. In response to simulation by the agonist, arginine-vasopressin V2-VR is internalized. Agonist stimulation induces ubiquitination of V2-VR as well as ubiquitination of β-arrestin2. Ubiquitination of β-arrestin2 as well as ubiquitination of V2-VR is persistent. Unlike other receptors, V2-VR appears to be polyubiquitinated. How is endocytosed V2-VR degraded? Is it routed to the lysosome? Although earlier studies showed that V2-VRs were delivered to the lysosome after internalization, it was found that ubiquitinated V2-VR could only be detected in the presence of a proteasome inhibitor, MG132 (Martin et al., 2003), thus suggesting that the ubiquitinated V2-VR was degraded by the proteasome. Is it possible, however, that both the proteasome and lysosome have a role in degradation of V2-VR? Requirement for both proteasome- and lysosome-mediated degradation has been reported for other plasma membrane receptors such as insulin-like growth factor receptor (Vecchione et al., 2003), estrogen receptor (Nawaz et al., 1999), and growth hormone receptor (van Kerkhof et al., 2000).

4.26.12.2 Fate of Proteins after Endocytosis: Lysosomal Versus Proteasomal Degradation

The traditional view of endocytotic degradation is that the degradation occurs in the lysosome. Also, as discussed, ubiquitin appears to target the endocytosed proteins to the multivesicular body for eventual degradation in the lysosome. A role for the proteasome is also indicated based on several studies. For example, endocytotic degradation of interleukin-2 receptor complex (Yu and Malek, 2001), growth hormone receptor (van Kerkhof et al., 2000), δ opioid receptor (Chaturvedi et al., 2001), vasopressin receptor (Martin et al., 2003), and GABAA receptor subunits (Bedford et al., 2001) is blocked by proteasome inhibitors. In addition, proteasome inhibitors have been shown to block endosomal sorting of membrane proteins to the lysosome (van Kurkhof et al., 2001). There is a caveat to these studies, however. Prolonged application of proteasome inhibitors leads to depletion of the ubiquitin pool (Patrick et al., 2003). Therefore, proteasome inhibitors might have an adverse effect on ubiquitination of receptors and hence indirectly block lysosomal degradation of receptors. If there is a role for the proteasome, it might be to partially degrade some proteins which otherwise block routing to the lysosome through the multivesicular body. Since proteasome is known to act on retrotranslocated endoplasmic reticulum proteins, it might act on the proteins in the early endosome in a similar fashion. In support of this idea, the investigations by others suggest that proteasome inhibitors might have a global effect on intracellular trafficking (Rocca et al., 2001). The least likely possibility is that endocytosed proteins are degraded through the lysosome or the proteasome, with the choice determined by differential ubiquitination.

4.26.13 Unanswered Questions and Future Directions

The research on the role of the ubiquitin–proteasome pathway in synaptic plasticity and other physiological functions in the nervous system has only begun to scratch the surface. One of the major tasks is to understand how the spatial and temporal regulation of substrate stability occurs in the nervous system. It is likely that the local protein degradation may be responsible for strengthening or weakening specific synapses (Hegde, 2004). It would also be important to elucidate how protein degradation works hand-in-hand with protein synthesis during induction and maintenance of synaptic plasticity. In addition, researchers need to develop precise tools for manipulating specific components of the ubiquitin–proteasome pathway in the nervous system. We can look forward to many exciting future discoveries on how protein degradation helps sculpt synapses.

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4.27 Transcription Regulation of Memory: CREB, CaMKIV, Fos/Jun, CBP, and SRF

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4.27.1 Introduction

Memories can persist for dramatically different lengths of time, from seconds and minutes to a lifetime. These different forms of memory have distinct molecular requirements. Short-term memory (STM) persists for minutes to hours and is thought to be mediated by covalent modifications of existing synaptic molecules, such as phosphorylation or dephosphorylation of enzymes, receptors, or ion channels (Stork and Welzl, 1999). In contrast, long-term memory (LTM) persists for days or longer and is thought to be mediated by both the growth of new synapses and restructuring of existing synapses (Bailey and Chen, 1989). There is extensive evidence from a range of species demonstrating that, unlike STM, LTM requires the transcription and translation of new proteins. Therefore, regulatory mechanisms that direct transcription may be important in the formation of LTM (Davis and Squire, 1984; Matthies, 1989). Here we examine the role of several transcriptional regulators (including transcription factors, a coactivator, and an upstream protein kinase) in LTM formation in rodents.

Transcription factors are proteins that activate or suppress transcription of a particular gene by binding to specific sets of short conserved sequences contained in the gene's promoter region. In general, transcription factors are composed of two domains: a DNA-binding domain that interacts with these gene-specific regulatory sites and a domain that exhibits transcriptional activation potential (for review, see Schwabe and Rhodes, 1991; Brivanlou and Darnell, 2002). The DNA-binding domain consists of amino acids that recognize specific DNA bases near the start of transcription. The activator domain of a transcription factor typically interacts with regulatory proteins and components of the transcriptional apparatus (RNA polymerase), thereby affecting the efficiency of DNA binding and regulating transcription (Agalioti et al., 2000). There are three main types of transcription factors: inducible transcription factors that are expressed as immediate-early genes in response to a particular stimulus (such as c-fos, c-jun, and Krox), constitutive transcription factors that are always expressed and are regulated by posttranslational modifications (such as Ca^{2+}/cAMP (cyclic adenosine 3′,5′-monophosphate); cAMP response element binding protein (CREB); and serum response factor (SRF)), and ligand-activated transcription factors that are activated by binding to a particular ligand (such as an estrogen receptor).

Transcription factors do not function in isolation to control transcription but work in concert with other transcription factors, upstream signaling pathways, and transcriptional cofactors. In this way, LTM probably requires the coordinated action of many transcriptional regulators. Nevertheless, this chapter focuses on the role of six well-studied transcriptional regulators, namely, CREB, c-fos, c-jun, CaMKIV (Ca^{2+}-calmodulin kinase type IV), CBP (CREB binding protein), and SRF in LTM formation.

4.27.1.1 Techniques to Examine the Role of Transcriptional Regulators in Memory

4.27.1.1.1 Correlating transcriptional activation with memory: Imaging studies

The role of transcriptional mechanisms in memory may be studied in two complementary ways. The first method uses imaging techniques to visualize activation of the transcription factor or a reporter of transcription following various types of behavioral training that normally induce LTM (see following). Although the data from these studies are correlative in nature, they are invaluable because they provide a powerful combination of systems and molecular approaches. They can identify where and when activation of a transcription factor occurs following stimulation that induces memory.

4.27.1.1.2 Altering transcription mechanisms

The second approach manipulates the function of a transcriptional regulator and assesses the impact of this manipulation on LTM. Several techniques have been used to disrupt or enhance the function of a

given transcriptional regulator. For instance, the gene of interest can be deleted constitutively throughout the body (classical knockout mice) or in specific brain regions (conditional knockout mice), or the function of the gene may be antagonized by the expression of a dominant negative form of the gene (through a transgenic mouse) or disrupted using antisense or, more recently, RNAi. Alternatively, viral vectors may be used to express different constructs (a wild-type, dominant negative, or dominant active version of the gene of interest) in specific brain regions at specific times. Each method alters the function of the gene of interest in a distinct way, and there are advantages and disadvantages associated with each approach. These are briefly reviewed below.

In a classical knockout mouse, the gene of interest is constitutively deleted throughout the body throughout development into adulthood. Although the analysis of mice in which transcriptional regulators have been constitutively knocked out have provided important information regarding the role of transcriptional regulators in memory formation, these constitutive knockout mice may have developmental deficits that interfere with the interpretation of the results from behavioral tests. In addition, because of the chronic nature of the disruption, there may be a compensatory upregulation of related transcriptional regulators.

Conditional gene knockouts, in which a gene of interest may be knocked out in a time-dependent and tissue-specific manner, may alleviate some of these interpretive difficulties. At present, most conditional knockouts are generated using the Cre-loxP system. Cre recombinase is an enzyme that excises a gene that is flanked by two target loxP recombination sites. Mice with the 'floxed' gene of interest are bred with a transgenic mouse expressing Cre recombinase. In the offspring, the floxed gene is excised only in tissue expressing sufficient Cre recombinase. Tissue-specific expression of Cre recombinase may be achieved by using different promoters. Two commonly used promoters are αCaMKII (which directs gene expression in postnatal excitatory neurons of the forebrain) and nestin (which directs gene expression specifically in neural precursor cells, such that both glia and neurons throughout the central nervous system (CNS) will express Cre recombinase). It is important to note that the efficiency of the gene knockout depends on the efficiency of recombination, and this may vary among different mouse lines. In other words, a gene may not be deleted in all cells of a given region in some conditional knockouts lines.

To manipulate gene function in a temporally and region-specific manner, antisense, RNAi, or viral vectors may be microinjected into target brain regions at specific times. Although these techniques are powerful, there may be between-animal variability in the manipulation. Further, some of these manipulations may produce cytotoxicity or off-target effects (knockdown of genes other than the intended target).

Because each method of manipulating gene function is associated with potential interpretive limitations, it is important that converging data are obtained from multiple methods before firm conclusions regarding the importance of a particular transcriptional regulator in memory formation are drawn.

4.27.1.2 Paradigms Used to Examine Memory

Just as there are multiple methods to alter the function of transcriptional regulators, there are several techniques commonly used to assess memory in rodents.

4.27.1.2.1 Conditioned fear memory

In a typical conditioned fear experiment, mice or rats are placed in a conditioning chamber, and a footshock is delivered. In the discrete cue version of this task, a tone (or light) that coterminates with the footshock is presented. Memory is commonly assessed as the percentage of time rodents spend freezing (defined as the cessation of all movements except respiration) when placed back in the conditioning chamber (referred to as contextual fear conditioning) or when the tone is presented in a novel chamber (tone or discrete cue fear conditioning). One advantage of this paradigm is that the neural systems critical for mediating fear conditioning have been well characterized. The amygdala, and in particular the lateral nucleus, is crucial for both tone and contextual fear conditioning. Lesioning or inhibiting protein synthesis in the lateral nucleus of the amygdala disrupts the formation of LTM (Davis, 1992; LeDoux, 2000; Schafe and LeDoux, 2000; Fanselow and Gale, 2003). The role for the hippocampus in fear conditioning is not as straightforward (see following).

4.27.1.2.2 Conditioned taste aversion memory

In the conditioned taste aversion (CTA) paradigm, ingestion of a novel taste is paired with transient sickness (produced by injection of lithium chloride). Memory for this association is evident when the animal avoids that taste on subsequent presentations (Garcia et al., 1955). In addition to brainstem (nucleus of the solitary tract) and pontine (parabrachial nucleus (PBN)) regions, several forebrain areas have been shown to be important for conditioned taste aversion (Schafe and Bernstein, 1996; Tokita et al., 2004). Furthermore, LTM for conditioned taste aversion requires protein synthesis in the amygdala (Yamamoto and Fujimoto, 1991; Josselyn et al., 2004) and insular cortex (Rosenblum et al., 1993).

4.27.1.2.3 Recognition memory (object and social recognition)

Recognition memory tests rely on the natural exploratory behavior of rodents. In a typical object recognition task, training consists of exposing mice or rats to two objects for a short period of time (e.g., 15 min). In the test phase, the subject is presented with one of the objects used during training (familiar object) and a novel object. The time the subject spends interacting with each object is compared. Memory is shown by animals spending more time exploring the novel object rather than the familiar object. Testing may occur at any time after training, allowing for the evaluation of both STM and LTM. Object recognition memory has been linked to the hippocampus (Clark et al., 2000; Ainge et al., 2006) and cortex, and especially the perirhinal cortex (Brown and Aggleton, 2001).

The social recognition task is based on similar principles. During the training phase, the subject is presented with a conspecific mouse. During the test phase, the subject is allowed to interact with the mouse previously used in the training (familiar) and a novel mouse. Again, memory is shown by the subject interacting longer with the novel mouse.

4.27.1.2.4 Social transmission of food preference memory

In the social transmission of food preference task, rodents develop a preference for foods recently smelled on the breath of other rodents (Galef et al., 1983, 1988; Bunsey and Eichenbaum, 1995). When subsequently given a choice between this food and a novel food, the subject demonstrates its memory for the odor through a preference for the same-scented food (Galef, 1985). This task can be used to assess memory for the sampled food at various intervals such that both STM and LTM may be tested. Previous research shows that this memory depends on the hippocampus (Winocur, 1990; Winocur et al., 2001). Therefore, object and social recognition, as well as the social transmission of food preference task, are memory tests that critically rely on the hippocampus but that are not spatial in nature.

4.27.1.2.5 Spatial memory

Spatial memory is most commonly assessed using the Morris water maze. In the hidden platform version of the water maze, rodents learn to find a platform submerged in a pool of opaque water by using spatial cues in the experimental room (Morris et al., 1982). Typically, spatial memory is assessed during a probe trial in which the platform is removed and the percentage of time the animals spend searching in the spatial location where the platform was previously positioned (target quadrant) is measured. This form of spatial memory is sensitive to hippocampal lesions (Morris et al., 1982; Sutherland et al., 1982). The Barnes maze is a dry land task that also measures spatial memory. In this task, animals escape from a brightly lit open arena into a small recessed chamber located under the arena. Animals learn to navigate toward this target escape box by using spatial cues (Barnes, 1979). The hippocampus is similarly important for this form of spatial memory (Koopmans et al., 2003).

These behavioral tests tap into different aspects of memory and place unique performance demands on the subject. Although there is not strict one-to-one mapping, in general, these tasks rely to a lesser or greater degree on different brain regions. For instance, tone fear memory and conditioned taste aversion both depend on the amygdala, among other regions, while the recognition and spatial memory tasks heavily depend on intact hippocampal function. It is conceivable that different molecular mechanisms may mediate memory formation in different brain regions. For these reasons, it is also important that the results from multiple memory tests are compared.

4.27.2 CREB

CREB is perhaps the best-studied transcription regulator in terms of memory. CREB refers to a family of structurally related transcription factors that modulate the transcription of genes that contain cAMP responsive elements (CRE) in their promoter regions.

4.27.2.1 Structure

In mammals, at least three genes encode CREB-like proteins: CREB, CREM (cAMP response element modulator), and ATF-1 (activating transcription factor 1) (Hoeffler et al., 1988; Rehfuss et al., 1991; Foulkes and Sassone-Corsi, 1992). The mouse and human CREB gene is composed of 11 exons (Waeber et al., 1991; Cole et al., 1992; Hoeffler, 1992), and alternative splicing generates the three major activator isoforms of CREB: α, δ, and β (Gonzalez and Montminy, 1989; Yamamoto et al., 1990; Blendy et al., 1996). In addition to these transcriptional activators, the CREB family also includes transcriptional repressors. For example, the CREM gene codes several isoforms that repress CRE-dependent transcription, the CREM α, β, and γ isoforms, as well as the inducible cyclic-AMP early repressor (ICER) (Foulkes et al., 1991; Molina et al., 1993).

CREB, CREM, and ATF1 proteins share a conserved basic leucine zipper (bZip) domain in the C-terminus region that is responsible for dimerization between CREB family members and binding to the CRE site (Busch and Sassone-Corsi, 1990). The kinase-inducible transactivation domain (KID) is located in the N-terminal region and contains a key phosphorylation site that was initially characterized as being regulated by cAMP-dependent protein kinase A (PKA). CREB is constitutively expressed and is thought to be constitutively bound to DNA in the nucleus. Transactivation of CREB, however, largely depends on phosphorylation.

Although CREB may be phosphorylated at a number of residues (such as Ser129 or Ser142; see Sun et al., 1994; Giebler et al., 2000; Kornhauser et al., 2002), activation of transcription requires phosphorylation at Ser133 in the KID domain (for review, see Fimia et al., 1998). Mutation of this site (to a nonphosphorylatable alanine) abolishes the stimulus-induced transcriptional activation of CREB (Gonzalez and Montminy, 1989). The phosphorylation state of Ser133 is regulated by the balance between protein phosphatases and kinases, which remove or add phosphate groups, respectively. Numerous kinases transmit signals from the plasma membrane to the nucleus to phosphorylate CREB at Ser133 (Dash et al., 1991; Chen et al., 1992; Bacskai et al., 1993; Hagiwara et al., 1993; Bito et al., 1996; Xing et al., 1996; Finkbeiner et al., 1997; Hardingham et al., 2001; Wu et al., 2001). Perhaps the best-characterized of these kinases are PKA, p38 mitogen-activated protein kinase (MAPK), and CaMKIV. In addition, a potent CREB coactivator, transducer of regulated CREB (TORC2), has recently been identified (Conkright et al., 2003a). TORC2 may mediate the cooperativity between cAMP and calcium signals (Screaton et al., 2004).

Phosphorylation of CREB at Ser133 alters the affinity of the KID for the KIX domain of the coactivators CBP and p300, resulting in enhanced transcriptional activity (Chrivia et al., 1993). On the other hand, protein phosphatase 1 (PP1) and PP2A dephosphorylate Ser133 (Hagiwara et al., 1992; Wadzinski et al., 1993; Bito et al., 1996), which decreases transcriptional activity. In addition to this mechanism, recent evidence suggests that the transcriptional activity of CREB may also be regulated by mechanisms in the bZip domain (Katoh et al., 2006).

4.27.2.2 Correlating Transcriptional Activation with Memory: Results from Imaging Studies

CREB activation may be monitored by examining the levels of activated CREB (CREB phosphorylated at Ser133) or products of CRE-mediated transcription that are endogenous (such as C/EBP) (Taubenfeld et al., 2001a; Desmedt et al., 2003) or engineered (CRE-reporter mice) (Impey et al., 1996, 1998). For instance, training that induces LTM for conditioned fear was correlated with an increase in the levels of phosphorylated CREB (pCREB) (Impey et al., 1998; Stanciu et al., 2001) and CRE-reporter in the amygdala and hippocampus (Impey et al., 1998; Athos et al., 2002). Additional studies have shown that behavioral training that normally induces LTM in other tasks is also associated with increased levels of pCREB and CRE-regulated genes (such as C/EBP) (Impey et al., 1998; Bevilaqua et al., 1999; Taubenfeld et al., 1999; 2001b; Cammarota et al., 2000; Viola et al., 2000; Stanciu et al., 2001). The results of these studies converge to show that training that induces LTM in a variety of paradigms is associated with CREB activation, as measured by several techniques.

4.27.2.3 Effects of Manipulating CREB Function on Memory

The role of CREB in memory has been well studied. Indeed, the effects of a range of CREB manipulations have been assessed in a variety of memory paradigms.

4.27.2.3.1 Conditioned fear memory

There are a variety of ways to manipulate CREB function. The homozygous deletion of the three major CREB isoforms (α, β, and δ) produced by a deletion in the bZIP domain (exon 10) of the CREB gene results in fully formed mice ($CREB^{null}$) that die at birth because of atelectasis of the lung (Rudolph et al., 1998). However, mice that lack the two main isoforms of CREB ($CREB^{\alpha\delta}$ mice) develop into adults and were tested for memory.

$CREB^{\alpha\delta}$ mice generally showed disrupted LTM but intact STM for both contextual and cued fear conditioning (Bourtchuladze et al., 1994; Kogan et al., 1997; Graves et al., 2002; Frankland et al., 2004). This LTM deficit in the $CREB^{\alpha\delta}$ mice was replicated using fear-potentiated startle to measure conditioned fear (Falls et al., 2000). It is important to note that the LTM deficit in these CREB mutants may be overcome by additional spaced training trials, highlighting the importance of the intensity and temporal dynamics of the training protocol to the memory phenotype (Kogan et al., 1997).

Using $CREB^{\alpha\delta}$ mice from a different genetic background (FVB/N x C57BL\6), Gass and colleagues (1998) found normal STM and mild LTM deficits in both cued and contextual fear conditioning (Gass et al., 1998). However, as pointed out by Graves and colleagues (2002), mice from this genetic background are generally poorer learners, potentially masking a greater effect of the $CREB^{\alpha\delta}$ mutation. These results highlight the importance of genetic background in behavioral studies.

Of interest is the finding that $CREB^{comp}$ mice (with only one CREBβ allele, obtained by crossing a $CREB^{\alpha\delta}$ mouse with a mouse heterozygous for the $CREB^{null}$ mutation) showed severe LTM deficits in both context and cued fear conditioning (Gass et al., 1998). This finding indicates that gene dosage is another important variable in the LTM phenotype produced by disrupting CREB function. That is, mice with a greater disruption in CREB function ($CREB^{comp}$ vs. $CREB^{\alpha\delta}$ mice) show a greater impairment in LTM for conditioned fear.

Conditioned fear memory has also been probed using transgenic mice overexpressing a dominant negative form of CREB. Three lines of mice that constitutively express a dominant negative form of CREB (mCREB) in the amygdala and hippocampus were generated. Of these, only one line showed impaired LTM for cued fear conditioning as measured by conditioned suppression of ongoing behavioral activity (Rammes et al., 2000). However, as the CREB repressor is constitutively expressed in these transgenic mice, compensatory upregulation of other CREB family members may confound the interpretation of the role of CREB in memory.

To overcome this potential limitation, transgenic mice were developed that allowed precise temporal control over the expression of the dominant negative CREB repressor (Kida et al., 2002). Importantly, these CREB inducible repressor ($CREB^{IR}$) transgenic mice developed with normal CREB levels. CREB function was acutely and reversibly disrupted by administering the inducer (tamoxifen) 6 h before training for conditioned fear. Mice were tested 24 h later. In this way, mice were trained with impaired CREB function but tested when CREB function had presumably returned to normal. Acutely disrupting CREB during training impaired LTM, but not STM, for both cued and contextual fear conditioning (Kida et al., 2002). Consistent with these findings, acutely disrupting CREB function in the CA1 region of the hippocampus through the use of antisense oligonucleotides also disrupted LTM for contextual fear (Athos et al., 2002). As the disruptions of CREB function are temporary, these converging data also provide compelling evidence that the LTM phenotype observed in mutant mice with a chronic disruption of CREB ($CREB^{\alpha\delta}$ or $CREB^{comp}$ mice) cannot be solely attributed to developmental abnormalities.

Therefore, the finding of normal LTM for contextual fear conditioning found in three additional lines of CREB mutant mice may be initially surprising. Normal LTM for contextual fear conditioning was observed in dCA1-KCREB mice, which express a dominant negative form of CREB in the CA1 region of the dorsal hippocampus (Pittenger et al., 2002), and in two lines of conditional CREB knockout mice, in which a floxed CREB mouse was crossed with two lines of transgenic mice expressing Cre recombinase under different promoters ($CREB^{CaMKCre7}$ and $CREB^{NesCre}$) (Balschun et al., 2003). However, these findings may not be entirely unexpected in light of results showing that the hippocampus is not strictly required for contextual fear conditioning. Whereas posttraining lesions of dorsal hippocampus dramatically impaired contextual fear conditioning, pretraining lesions did not (Maren et al., 1997; Frankland et al., 1998). It has been hypothesized that rodents with pretraining lesions of the hippocampus use elemental, rather than contextual, strategies to produce normal LTM for contextual fear conditioning (Maren et al.,

1997; Frankland et al., 1998). Importantly, the lines of CREB mutant mice that show normal LTM for contextual fear have chronic disruptions in CREB that could be analogous to 'pretraining lesions' of CREB. Thus, unlike antisense that acutely disrupts CREB function, CREB function was chronically impaired in both the $CREB^{CaMKCre7}$ and $CREB^{NesCre}$ mice. Even in the inducible dCA1-KCREB mice, the dominant negative CREB transgene was expressed for a significant period of time before training. Taken together, these findings suggest that contextual fear conditioning (in which mice may acquire the task by using nonhippocampal, elemental-based strategies) may not be sufficiently sensitive to show potential LTM deficits in mice with chronic pretraining perturbations in hippocampal CREB function.

To address this potential shortcoming, a variation of the context fear conditioning task that critically relies on the hippocampus was used to evaluate the role of CREB in contextual LTM. In the context preexposure task, the acquisition of context information is temporally separated from its association with footshock (Wiltgen et al., 2001; Frankland et al., 2004; Matus-Amat et al., 2004). Unlike animals given a chance to explore a novel environment before footshock (as in the standard context fear conditioning paradigm), animals given a footshock immediately after placement in a novel environment showed low levels of conditioned fear. This phenomenon is sometimes referred to as the immediate shock deficit. Animals preexposed to the training context 24 h before the immediate shock, however, showed robust conditioned fear (Wiltgen et al., 2001; Frankland et al., 2004; Matus-Amat et al., 2004). Thus, context preexposure rescued the immediate shock deficit. Protein synthesis in the hippocampus is crucial for the rescue of the immediate shock deficit by context preexposure, as infusion of anisomycin into the dorsal hippocampus following context preexposure produced low levels of freezing following the immediate shock (Barrientos et al., 2002). Similarly, $CREB^{\alpha\delta}$ mice showed low levels of freezing following an immediate shock despite being preexposed to the training context the day before. Indeed, freezing levels in the $CREB^{\alpha\delta}$ mice were similar to those observed in wild-type (WT) mice that were not preexposed to the training context (Frankland et al., 2004). Therefore, by using a task that critically depends on protein synthesis in the hippocampus, CREB was shown to be important for contextual LTM.

Together, the majority of these findings indicate that decreasing CREB function disrupts LTM for conditioned fear. But what are the effects of increasing CREB function on LTM for conditioned fear? To answer this question, Josselyn and colleagues (2001) used viral vectors to increase CREB levels and function in a subpopulation of amygdala neurons (roughly 20–30% of neurons in the lateral/basolateral nucleus of the amygdala) (Josselyn et al., 2001). Rats were trained for cued fear conditioning using a massed training protocol that typically produces weak STM but no, or weak, LTM. However, rats infused with viral vectors encoding CREB in the amygdala showed robust LTM at levels similar to those produced by more intense, spaced training. The finding of enhanced cued fear memory following infusion of herpes simplex virus encoding CREB into the amygdala was replicated using freezing as a measure of conditioned fear (Wallace et al., 2004) and extended to include memory for social defeat in hamsters (Jasnow et al., 2005). These findings in rodents are consistent with results from *Drosophila* (Yin et al., 1995) and *Aplysia* (Bartsch et al., 1995), showing that increasing CREB function enhances LTM.

4.27.2.3.2 Conditioned taste aversion memory

Data from multiple studies have shown that CREB is important in LTM for conditioned taste aversion. Thus, $CREB^{\alpha\delta}$ (Josselyn et al., 2004), $CREB^{IR}$ (Josselyn et al., 2004), and $CREB^{NesCre}$ (Balschun et al., 2003) mice showed disrupted LTM for conditioned taste aversion. Other lines of CREB mutant mice ($CREB^{CaMKCre7}$ mice and $CREB^{comp}$ mice) have not been tested in this paradigm. Acutely disrupting CREB function in the amygdala through the use of antisense oligonucleotides similarly disrupted LTM, but not STM, for conditioned taste aversion (Lamprecht et al., 1997). Furthermore, training that produced conditioned taste aversion (pairing the novel taste with lithium chloride) also induced robust CREB activation (phosphorylation) in the lateral nucleus of the amygdala (Swank, 2000). Similar increases were not observed if rats were exposed to the taste or lithium chloride alone, indicating that activation of CREB is related to associative learning. Together, these data from rats and mice show that CREB is important in CTA memory. Unlike some other memory tasks, conditioned taste aversion places few performance demands on the subject. Therefore, conclusions regarding the role of CREB in memory may

be drawn independently of potential confounding deficits in other behavior, such as motor deficits.

4.27.2.3.3 Recognition memory (object and social recognition)

Several studies have shown that decreasing CREB function specifically disrupted LTM, but not STM, in both the object and social recognition tasks. Thus, dCA1-KCREB mice (Pittenger et al., 2002) and $CREB^{IR}$ mice (Bozon et al., 2003) have disrupted LTM for object recognition. In addition, viral-mediated transfer of a dominant negative form of CREB to the perirhinal cortex similarly impaired LTM for object recognition in rats (Warburton et al., 2005). CREB has also been shown to be important in memory for the social recognition task. LTM, but not STM, for social recognition was impaired in both the $CREB^{\alpha\delta}$ (Kogan et al., 2000) and $CREB^{IR}$ mice (Bozon et al., 2003).

Consistent with previous data using a variety of learning tasks and species, spaced training produces maximal LTM whereas massed training produces weak LTM for object recognition (Genoux et al., 2002). Furthermore, spaced but not massed training in the object recognition task has been associated with increased levels of phosphorylated CREB and CRE-mediated transcription in the hippocampus and cortex (Genoux et al., 2002). However, in transgenic mice with decreased protein phosphatase-1 (PP1) activity (PP1 dephosphorylates CREB at Ser133, thereby decreasing transactivation), massed training alone was sufficient to produce both an increase in CRE-mediated transcription and robust LTM (Genoux et al., 2002). A similar pattern of results was also observed in transgenic mice that overexpress type 1 adenylyl cylase (Wang et al., 2004). These transgenic mice, but not WT littermates, showed both elevated levels of pCREB and strong LTM following training that normally induces weak object recognition memory. Therefore, increasing CREB function through indirect manipulations also enhances LTM.

4.27.2.3.4 Social transmission of food preference memory

The role of CREB in memory has also been tested in another task that critically involves the hippocampus: social transmission of food preference. Training that produced LTM for social transmission of food preference was associated with increased levels of pCREB in the hippocampus (Countryman et al., 2005b). Furthermore, disrupting CREB function by expression of a dominant negative form of CREB (S133A) in the dorsal hippocampus through viral vectors blocked LTM, but not STM, for this task (Brightwell et al., 2005). Consistent with this, $CREB^{\alpha\delta}$ mice showed intact STM, but impaired LTM, in this task (Kogan et al., 1997). However, using a more intense training protocol (a longer 'training time' (10 min) that permitted an observer mouse to smell the breath of a demonstrator mouse for a longer period of time), Gass and colleagues (1998) found normal LTM in both $CREB^{\alpha\delta}$ and $CREB^{comp}$ lines of mutant mice. Together, these results indicate that the effects of disrupting CREB are dependent on the training conditions used to induce LTM for social transmission of food preference.

4.27.2.3.5 Olfactory memory

Memory may also be assessed using an olfactory conditioning task in neonatal rats. In this task, an odor is paired with an appetitive (such as a stroke of the back) or an aversive (footshock) stimulus. Both appetitive and aversive olfactory conditioning were associated with an increase in pCREB levels in the olfactory bulbs (McLean et al., 1999; Zhang et al., 2003). Moreover, disrupting CREB function (via infusion of CREB antisense oligonucleotides or viral vectors expressing mCREB) produced a specific LTM deficit for olfactory conditioning (Yuan et al., 2003; Zhang et al., 2003).

4.27.2.3.6 Spatial memory

In the first study to assess the role of CREB in spatial memory, $CREB^{\alpha\delta}$ mice were trained in the water maze using a low-intensity training protocol (one trial a day for 15 days) (Bourtchuladze et al., 1994). At the beginning of training, WT and $CREB^{\alpha\delta}$ mice showed long latencies to reach the platform. As training progressed, the time to reach the platform was reduced in WT mice (indicating learning), whereas the latencies of $CREB^{\alpha\delta}$ mice remained significantly longer. As predicted from these training latencies, WT mice searched selectively in the target quadrant during the probe test, while $CREB^{\alpha\delta}$ mice searched randomly (Bourtchuladze et al., 1994). Moreover, WT mice crossed the exact location of the platform more often than the $CREB^{\alpha\delta}$ mice. This initial spatial memory deficit in $CREB^{\alpha\delta}$ mice was replicated using similar, as well as more intense, training schedules (Bourtchuladze et al., 1994; Kogan et al., 1997). However, similar to other memory tests discussed above, the role of CREB in spatial memory is sensitive to the training parameters. Thus, increasing the time between trials to 10 min or 1 h (rather than

1 min) rescued the spatial memory deficit in CREB$^{\alpha\delta}$ mice (Kogan et al., 1997; Gass et al., 1998).

Spatial memory deficits have also been observed using two different techniques to significantly disrupt CREB function specifically in the hippocampus. Transgenic mice that express a dominant negative form of CREB in the dorsal CA1 region of the hippocampus (dCA1-KCREB) also showed impaired spatial memory as measured by several variables (percentage time spent in the target quadrant, proximity to the training location of the platform, and platform crossing) (Pittenger et al., 2002). A critical control experiment showed that dCA1-KCREB transgenic mice fed doxycycline (thereby turning off transgene expression) for 2 weeks before the start of water maze training showed normal spatial memory. This result indicated that the spatial memory phenotype in these mutant mice cannot be attributed to developmental deficits. In addition, infusion of CREB antisense into the dorsal hippocampus of adult rats (Guzowski and McGaugh, 1997) and mice (Florian et al., 2006) disrupted spatial memory in the water maze. Furthermore, disrupting CREB function in aged mice using a viral vector expressing the CREB inhibitor ICER disrupted spatial memory on a Barnes maze (Mouravlev et al., 2006). In accordance with these findings, prenatal administration of morphine, a treatment that significantly decreased the level of phosphorylated CREB in the hippocampus of adult mice, also produced a deficit in spatial memory (Yang et al., 2003). Collectively, these findings indicate that intact CREB function in the hippocampus is critical for spatial memory.

In an overall analysis of the impact of various CREB mutations on spatial memory, Balschun and colleagues (2003) compared the water maze performance of various lines of CREB mutant mice (using all CREB mutant mice lines studied by this group, including those of Gass et al., 1998). They found that spatial memory varied in the different lines of CREB mutant mice; the percentage time spent in the target quadrant during the probe trial for WT mice = 35.78%, CREB^{comp} = 26.39%, CREB$^{\alpha\delta}$ = 28.5%, CREB^{NesCre} = 31.11%, and CREB^{CaMKCre7} = 40.87%. These authors interpreted this pattern of results as evidence that CREB is dispensable for spatial memory. However, a more parsimonious explanation of these data is that the spatial memory phenotype is determined by the degree of CREB disruption. In support of this interpretation, the CREB^{comp} mice (with a loss of both CREBα and δ alleles as well as one CREBβ allele) showed poorer spatial memory than the CREB$^{\alpha\delta}$ mice (with a loss of both CREBα and δ alleles but two intact CREBβ alleles) (see also Gass et al., 1998). Similarly, the CREB^{NesCre} mice (with a virtually complete deletion of CREB throughout the CNS) showed poorer spatial memory than the CREB^{CaMKCre7} mice (with a loss of CREB in 70–80% of CA1 hippocampal neurons only). Together, these results suggest that transgenic overexpression of a dominant negative form of CREB (as in dCA1-KCREB transgenic mice) or CREB antisense may disrupt CREB function in a larger portion of hippocampal neurons than the 'mosaic' pattern of CREB deletion observed in the CREB^{CaMKCre7} mice.

4.27.2.4 Conclusion

The majority of evidence suggests that CREB is critically involved in consolidation of LTM. In tasks ranging from conditioned fear to conditioned taste aversion, various recognition tasks, and spatial memory, most manipulations that disrupt CREB function similarly disrupt LTM. On the other hand, treatments that enhance CREB function generally enhance LTM. This loss- and gain-of-function pattern of results is also produced by CREB manipulations in invertebrate species (Dash et al., 1990; Yin et al., 1994, 1995; Bartsch et al., 1995).

This conclusion, however, has two important caveats. First, the memory phenotype is sensitive to the training parameters (specifically the spacing between successive trials). The sensitivity was observed across several paradigms and may be related to the fundamental differences between the types of memory produced by massed and spaced training. In many instances, multiple spaced trials rescued the LTM deficit resulting from decreased CREB function, suggesting that spaced training protocols may recruit additional molecular mechanisms, including other transcription regulators.

The second important caveat is that the extent of CREB disruption determines the presence and degree of LTM impairment. In general, greater disruptions in CREB function produced greater disruptions in LTM. For instance, the CREB^{comp} (with only one CREB β allele) mice showed a larger impairment than the CREB$^{\alpha\delta}$ mice (with two CREB β alleles) in many memory tasks (Gass et al., 1998). Furthermore, CREB^{NesCre} mice (with a brainwide deficit in CREB) showed larger spatial memory impairments than CREB^{CaMKCre7} mice (with a

deletion of CREB in only 70–80% of CA1 neurons) (Balschun et al., 2003). Indeed, these results show that the remaining 20–30% of CA1 neurons with normal CREB levels in the $CREB^{CaMKCre7}$ mice are sufficient to support normal spatial memory. This result is consistent with the observation that increasing CREB function in a small proportion of neurons in the lateral amygdala was sufficient to enhance LTM for conditioning fear (Josselyn et al., 2001; Wallace et al., 2004) and social defeat (Jasnow et al., 2005).

4.27.3 c-Fos

4.27.3.1 Structure

Inducible transcription factors regulate the delayed onset of effector genes that may code for structural, growth-related, and synaptic proteins, and as such, they have been implicated in LTM (Herdegen and Leah, 1998). Two of the best-studied inducible transcription factors are Fos and Jun. c-fos codes for a 380 amino acid protein that belongs to a family of transcription factors that also includes FosB and the Fos-related antigens 1 and 2 (Fra-1 and Fra-2) (Karin et al., 1997; Herdegen and Leah, 1998). The transcriptional regulatory region of c-fos contains recognition sequences for CRE, SRE, c-Sis inducible element (SIE), TCF (ternary complex factor), and AP-1 (Treisman, 1992).

Fos proteins activate late-effector genes by forming heterodimeric complexes with Jun (c-Jun, JunB, JunD) proteins and the related activating transcription factor (ATF2, ATF3/LRF1, B-ATF) subfamilies to form the activating protein 1 (AP-1) transcription factor (Morgan and Curran, 1991). The AP-1 complex binds to a palindromic DNA binding sequence (5′-TGACGTCA-3′), referred to as the TRE (TPA response element) site, in the regulatory region of several target genes, including nerve growth factor, proenkephalin, prodynorphin, endorphin, neurotensin, tyrosine hydroxylase, and neuropeptide Y (Greenberg and Ziff, 1984; Karin et al., 1997; Herdegen and Leah, 1998).

4.27.3.2 Correlating Transcriptional Activation with Memory: Results from Imaging Studies

The first hint that c-fos played an important role in memory came from expression studies in which Fos induction was observed following behavioral training that induced LTM (Tischmeyer and Grimm, 1999; Guzowski, 2002). Fos protein was upregulated in various brain regions following training in many behavioral tasks, including conditioned taste aversion (Schafe and Bernstein, 1996), Morris water maze (Guzowski et al., 2001), socially transmitted food preference (Countryman et al., 2005a), and conditioned fear (Stanciu et al., 2001). However, because c-fos is induced by a variety of physiological and pathological stimuli (Morgan et al., 1987), the functional relevance of the increase in Fos protein was equivocal without corresponding studies examining the effects of manipulating c-fos function on memory.

4.27.3.3 Effects of Manipulating c-fos Function on Memory

4.27.3.3.1 Spatial memory

To manipulate c-fos function, several techniques have been used. One of the first studies to examine the impact of c-fos manipulations on memory took advantage of a global c-fos knockout mouse in which the c-fos gene was deleted throughout the body throughout the lifetime of the mouse. Although these c-fos knockouts were viable, they lacked tooth eruption, showed altered hematopoiesis, and were smaller than their wild-type littermates (Wang et al., 1992). These mice showed severe impairments in the hidden version of the water maze (Paylor et al., 1994). However, they also showed significant deficits in the visible (nonhippocampal) version of the water maze, suggesting that the behavioral deficit may be a result of nonspecific sensorimotor and/or motivational dysfunction. Therefore, the strong developmental abnormalities in the global c-fos knockout mouse make it difficult to discriminate putative mnemonic disruption from confounding nonspecific impairments.

To overcome the limitations of the c-fos global knockout, two lines of conditional c-fos knockout mice in which c-fos function was disrupted in the brain only have been developed. In this way, the impact of disrupting c-fos function in the brain could be assessed free from gross developmental deficits. Mice with a floxed c-fos gene were crossed with transgenic mice expressing Cre recombinase under the control of two different promoters. Crossing the floxed c-fos mice with transgenic mice in which Cre recombinase was driven by the αCaMKII promoter (Tsien et al., 1996) produced a mouse line in which the disruption of c-fos was largely limited to the hippocampus (Zhang et al., 2002a). Using an intense

training protocol (6 days, four trials a day, with a 1-h intertrial interval), these mice showed normal spatial memory as assessed in the water maze (Zhang et al., 2002a). In addition, these mice showed normal memory on the Barnes maze (Zhang et al., 2002a). However, a different result was obtained when a less intense training protocol was used to train mice with a greater disruption of c-fos.

Deleting c-fos throughout neurons and glia of the central nervous system using transgenic mice in which Cre recombinase was driven by the nestin promoter produced c-fos^{NesCre} or c-fos$^{\Delta CNS}$ mice. These c-fos^{NesCre} mice showed spatial memory deficits using a less intense training protocol in the water maze (one trial per day for 11 days; Fleischmann et al., 2003). Consistent with this observation, Guzowski and colleagues also showed that disrupting c-fos function in the hippocampus impaired spatial memory in the water maze. Injection of c-fos antisense oligonucleotides into the hippocampus had no effect on short-term spatial memory but did disrupt LTM (Guzowski, 2002). It is important to note that the c-fos^{NesCre} mice also showed deficits in LTM for contextual fear conditioning (Fleischmann et al., 2003).

4.27.3.3.2 Social transmission of food preference memory

In addition to spatial memory tests, the role of hippocampal c-fos has also been examined in the social transmission of food preference memory task. Training that produced robust LTM for this task was also associated with increased levels of Fos protein in the hippocampus (Countryman et al., 2005a). Moreover, disrupting c-fos function in the hippocampus using antisense impaired LTM but not STM for socially transmitted food preferences (Countryman et al., 2005a).

On balance, these data suggest that disrupting c-fos function disrupts LTM for two different hippocampal-dependent tasks. However, memory disruption was not observed in all lines of mice under all training conditions. Specifically, the mice with a disruption of c-fos function only in the hippocampus (c-fos^{CaMKCre}) showed intact spatial memory following intensive training. The levels of Fos protein in these mice were reduced by roughly 95% in CA1, CA2, and CA3 neurons but by only 70% in dentate granule cells following kainic acid administration (a potent inducer of c-fos) (Zhang et al., 2002b). It is possible that the remaining unaffected neurons with intact c-fos function in the dentate gyrus are sufficient to support normal spatial memory following intense training. In contrast, the deletion of c-fos was nearly complete in the CNS-specific (c-fos^{NesCre}) mice in that kainic acid administration produced no observable Fos. These mice also showed a more pronounced memory deficit. In order to truly compare the results of different levels of c-fos disruption on memory, these different lines of mutant mice should be trained using the same training protocol.

4.27.3.3.3 Conditioned taste aversion memory

The role of c-fos in memory has also been assessed using the conditioned taste aversion paradigm. Training that induced conditioned taste aversion was associated with increased Fos levels in the amygdala (basolateral and central nuclei), insular cortex, and intermediate nucleus of the solitary tract (Wilkins and Bernstein, 2006). Whether Fos is increased in the PBN is unclear (Wilkins and Bernstein, 2006; Yamamoto and Sawa, 2000). Indeed, the type and strength of conditioned taste aversion training may be an important factor in determining the precise brain regions that express Fos (Yamamoto and Sawa, 2000). Overall, however, these data indicate that training in this task reliably increases Fos expression in the brain.

Several studies have examined the impact of disrupting c-fos function on memory for conditioned taste aversion. Recently, Yasoshima and colleagues (2006) examined the effects of microinjecting c-fos antisense oligonucleotide into various brain regions, including the amygdala (targeted to the border between the basolateral amygdala and central amygdala), insular cortex, and PBN. They found that infusions of c-fos antisense into the PBN (but not insular cortex or amygdala) blocked LTM for conditioned taste aversion. Surprisingly, these researchers also found normal taste aversion memory in global c-fos knockout mice. In addition, infusion of c-fos antisense into the PBN, amygdala, and insular cortex disrupted the retrieval of conditioned taste aversion when injected before testing (Yasoshima et al., 2006). These findings are not consistent with a previous report from Lamprecht and Dudai (1996), who found that injection of c-fos antisense into the central amygdala impaired LTM but not retrieval of conditioned taste aversion memory. These discrepancies could be a result of the different efficiencies of the c-fos antisense or the precise brain regions targeted, as well as procedural differences in the conditioned taste aversion task (one bottle vs. multiple bottles).

4.27.4 c-Jun

4.27.4.1 Structure

c-jun is a related immediate early gene that encodes a protein of 334 amino acids in the rat (Herdegen and Leah, 1998). The c-jun promoter contains binding sites for the transcription factors CREB, ATF-1, TFIID, Sp1, CTF, AP-1, NF, SRF, and ATF-2 (Angel et al., 1988; de Groot et al., 1991; Rozek and Pfeifer, 1993). c-jun is induced by a diverse array of stimuli, including serum, TPA, and various growth factors, cytokines, and second messengers (Angel et al., 1988; de Groot et al., 1991; Rozek and Pfeifer, 1993).

Transactivation of Jun is tightly regulated by phosphorylation in two distinct regions. C-terminal phosphorylation sites lie proximal to the DNA binding domain and prevent DNA binding. These sites are dephosphorylated in response to growth stimulation and likely function to repress the activity of the transcription factor under resting conditions (de Groot et al., 1991; Rozek and Pfeifer, 1993). Since Jun protein is present in many types of resting cells, this may provide a mechanism for rapid induction of Jun activity. Jun is also phosphorylated at two residues proximal to the major transactivation domain, and phosphorylation of Ser 63 and 73 is required for efficient transactivation. The kinases primarily responsible for this modification in vivo are the Jun N-terminal kinases (JNKs) (Smeal et al., 1992).

4.27.4.2 Correlating Transcriptional Activation with Memory: Results from Imaging Studies

Similar to c-fos, several studies have shown that training that normally induced LTM also induced c-jun expression. In fact, in most cases enhanced expression of Fos is not a solitary event but is often accompanied by an induction of Jun. For instance, neurons in the amygdala show increased levels of both Fos and Jun proteins following conditioned taste aversion training (Swank, 1999). This corresponding increase in both Fos and Jun would allow for the formation of AP-1.

4.27.4.3 Effects of Manipulating c-jun Function on Memory

Although the role of c-fos in LTM has been widely studied, fewer studies have examined the impact of disrupting c-jun function on LTM. One of the few studies showed that training that produces memory in a shock-motivated brightness discrimination paradigm increased the levels of hippocampal c-jun mRNA (Grimm et al., 1997). Consistent with this, intrahippocampal infusion of c-jun antisense oligodeoxynucleotides blocked LTM for this task (Tischmeyer et al., 1994). However, another study reported no effects of c-jun disruption on memory. Although training in the hidden platform version of the Morris water maze increased levels of Jun protein in the hippocampus (Teather et al., 2005), mice with a CNS-wide disruption of c-jun (produced by crossing a floxed c-jun mouse with a nestin-Cre recombinase transgenic mouse) showed normal spatial memory following intense training (six trials per day) in the water maze (Raivich et al., 2004). It is possible that memory impairment would be observed if these mice were trained with a less intense protocol. Overall, the results from the studies that manipulate c-jun function are inconclusive as to the importance of c-jun function in memory formation.

4.27.4.4 Conclusion

c-fos and c-jun are closely related immediate early genes. The results from these studies clearly suggest that the c-fos is important in memory. However, the precise role of c-jun is unclear, and additional research is necessary before firm conclusions may be reached.

4.27.5 CaMKIV

4.27.5.1 Structure

CaMKIV is a multifunctional serine/threonine kinase present throughout the forebrain (Ohmstede et al., 1989; Jensen et al., 1991a,b; Means et al., 1991; Anderson et al., 2004). The CaMKIV gene is composed of 13 exons that extend over roughly 42 kb of DNA (Sun et al., 1995). Through alternative transcription initiation and splicing, three isoforms are generated, the α and β isoforms of CaMKIV and calspermin (Ohmstede et al., 1991; Sun et al., 1995). Similar to other CaM kinases, the active site of CaMKIV is sterically blocked by an autoinhibitory domain that prevents substrate binding to the enzyme. Upon a transient rise in intracellular calcium, calcium-bound calmodulin (Ca^{2+}/CaM) binds to the autoregulatory domain of CaMKIV, which relieves this intrasteric inhibition and allows intramolecular

phosphorylation of multiple serine residues (Cruzalegui and Means, 1993; McDonald et al., 1993; Okuno et al., 1995). Unlike other CaM kinases, however, CaMKIV may be localized in the cytoplasm or nucleus (Jensen et al., 1991a; Gao et al., 2004). CaMKIV is imported into the nucleus by importin α (Kotera et al., 2005), where it directly activates several transcription factors, including CREB, ATF-1, the MADS-box family members SRF and MEF2D, and the transcriptional coactivators CBP and p300 (Sun et al., 1994; Enslen et al., 1995; Impey et al., 2002). CaMKIV has been implicated in regulation of transcription of a number of genes including those encoding interleukin 2, c-fos, c-jun, BDNF, and orphan members of the steroid receptor superfamily such as ROR and COUP-TF (Sun et al., 1994; Enslen et al., 1995; Impey et al., 2002).

4.27.5.2 Effects of Manipulating CaMKIV Function on Memory

The role of CaMKIV in LTM has been examined using two lines of mutant mice that employ different strategies to disrupt CaMKIV function. The first line of mice has a targeted deletion of the α and β isoforms of CaMKIV (Ho et al., 2000). CaMKIV$^{\alpha\beta}$ mice showed normal spatial memory in the water maze (four trials a day with an intertrial interval of roughly 35 s) and radial arm maze tasks (Ho et al., 2000). However, these mice showed disrupted LTM (measured 1 and 7 days following training) in both the contextual and cued fear conditioning tasks (Wei et al., 2002). It is interesting to note that a decrease in pCREB levels in the amygdala was observed following fear conditioning, suggesting that the observed memory deficit may be partly attributed to decreased CREB function in this region.

A different pattern of results was found using a line of transgenic mice expressing a dominant negative form of CaMKIV in postnatal forebrain regions (Kang et al., 2001). These mice showed spatial memory deficits in the water maze that were partially overcome with extensive training and LTM deficits in contextual fear conditioning (measured 7 days but not 1 day following training) (Kang et al., 2001). However, normal memory for cued fear conditioning was observed, perhaps due to the differential pattern of transgene expression (highest expression in the hippocampus and lower levels in the amygdala).

Although the majority of findings suggest that CaMKIV is important in memory, perhaps through a CREB-related process, more research is required. It would be ideal if the two lines of CaMKIV mutant mice could be tested side-by-side in the same behavioral task. In addition, the effects of other techniques that disrupt CaMKIV function on memory would be informative.

4.27.6 CBP

4.27.6.1 Structure

CBP codes for a 2442–amino acid protein that was first identified by a physical interaction with CREB (Chrivia et al., 1993). In addition to CREB, CBP is an essential coactivator protein for many transcription factors, including c-jun, c-fos, SRF, and many others (Chrivia et al., 1993). CBP is composed of different domains, including three cystidine-histidine rich domains, a bromodomain, a PKA phosphorylation site, two zinc finger motifs, an N-terminal nuclear receptor binding domain, and a histone acetyltransferase (HAT) domain (Kalkhoven, 2004). CBP facilitates transcription by coupling transcription factor recognition to chromatic remodeling. Specifically, CBP serves as a physical link between DNA-binding transcription factors and the basal transcriptional machinery (McManus and Hendzel, 2001). Through intrinsic HAT activity, CBP also facilitates transcription by opening up condensed DNA, thereby allowing greater access by the transcriptional machinery (McManus and Hendzel, 2001).

4.27.6.2 Effects of Manipulating CBP Function on Memory

Several mouse models have been generated in which CBP function is compromised. The first is a conventional knockout of CBP. Although the homozygous mutation is lethal, heterozygous mice with a null mutation of CBP (CBP$^{+/-}$) develop into adults (Tanaka et al., 1997). Despite showing deficits in skeletal patterning, growth retardation, and motor coordination, these mice show normal activity, working memory, motivation, and anxiety levels (Alarcon et al., 2004; Tanaka et al., 1997). Furthermore, CBP$^{+/-}$ mice showed normal STM but impaired LTM for conditioned fear (both cued and context), as well as object recognition (Alarcon et al., 2004). In contrast, spatial memory was intact following spaced training in the water maze (four trials a day with a 15-min intertrial interval) (Alarcon et al., 2004).

Second, a line of mice that have an insertional mutation in the cbp allele that produces a truncated form of CBP protein ($CBP^{+/-C\text{-}term}$) was developed (Oike et al., 1999). This truncated protein functions as a dominant negative (Petrij et al., 1995). Mice homozygous for this mutation die prenatally, but heterozygous mice survive into adulthood. These mice showed abnormal growth, osseous maturation retardation, hypoplastic maxilla, and changes in locomotor activity (Oike et al., 1999). Spatial memory in the water maze and memory for contextual fear conditioning were intact in these mice (Oike et al., 1999). However, $CBP^{+/-C\text{-}term}$ mice showed impaired LTM but normal STM in passive avoidance, cued fear conditioning (Oike et al., 1999), and object recognition tests (Bourtchouladze et al., 2003).

Transgenic mice expressing a dominant negative form of CBP (CBPΔ1) in postnatal forebrain neurons were also tested for memory (Wood et al., 2005). These CBPΔ1 transgenic mice showed severe deficits in spatial memory (using a low-intensity training protocol in which they were given two training trials per day). Importantly, normal performance was observed in the visible version of the water maze, indicating that the motor abilities and motivation aspects required to perform the task were intact (Wood et al., 2005). Furthermore, CBPΔ1 transgenic mice showed a specific deficit in LTM for contextual fear conditioning. Perhaps surprisingly, no impairment was observed in cued fear conditioning. This may be a result of low expression levels of the truncated CBP protein in the amygdala.

Finally, to gain spatial and temporal control over CBP function, transgenic mice were developed that inducibly express CBP with a point mutation in HAT domain (Korzus et al., 2004). Expression of this dominant negative form of CBP was observed in the hippocampus, caudate, and cortex. These transgenic mice showed intact STM but impaired LTM for object recognition (Korzus et al., 2004). In addition, mice receiving moderate (two trials per day) training in the water maze showed a spatial memory deficit, whereas more intense training rescued the spatial memory deficit.

4.27.6.3 Conclusions

In general, most of the behavioral results from the various lines of CBP mutant mice converge to indicate that CBP is critical for LTM. Moreover, the finding that postnatal disruption of CBP function impaired memory suggests that the LTM deficits observed in mice with more chronic disruptions of CBP cannot be solely attributed to developmental deficits. Similar to the memory phenotypes in CREB and c-fos mutant mice, the amount and type of training is an important factor in determining the presence or extent of memory impairment in the lines of CBP mutant mice.

4.27.7 SRF

4.27.7.1 Structure

SRF is a 67-kDa protein that belongs to the MADS box family of transcription factors (Treisman, 1986). These proteins, which include the MEF2 family of transcription factors, all share a MADS box at their N terminus that is composed of a basic DNA-binding domain, a dimerization domain, and an interface for protein–protein interactions (Norman et al., 1988). SRF is a constitutive transcription factor that binds to the 10-bp sequence CC(A/T)6GG (called CArG box) found in the regulatory regions of dozens of genes, including c-fos, c-jun, and egr-1 (Chai and Tarnawski, 2002).

4.27.7.2 Effects of Manipulating SRF Function on Memory

SRF is important during development; mice with complete deletions of SRF die 3 weeks postnatally (Arsenian et al., 1998). Therefore, to examine the effects of SRF manipulations on memory, strategies must be employed that overcome the developmental necessity of SRF. To date, two such strategies have been used. First, conditional SRF knockout mice in which SRF was excised in postnatal forebrain regions (obtained by crossing a mouse with floxed srf alleles with a mouse expressing Cre recombinase driven by an αCaMKII promoter) were generated (Etkin et al., 2006). The resulting conditional SRF knockout mice had a near total elimination of SRF in the CA1 and dentate gyrus of the hippocampus and decreased SRF levels in CA3 of the hippocampus, cortex, and striatum. These mice showed abnormal habituation in a novel environment and impaired spatial memory in the water maze. A second strategy used infusions of SRF antisense to disrupt SRF function in a temporally and spatially restricted manner. Infusion of SRF antisense oligonucleuotides impaired spatial memory in the water maze (Dash et al., 2005). Although there are only two experiments that examined the role of SRF in memory, the available data support the

conclusion that SRF is important in LTM formation. The finding that two different methods of disrupting SRF produced similar memory impairments bolsters this conclusion.

4.27.8 Overall Conclusions

Long-term changes in synaptic strength and structural remodeling are thought to underlie the formation of LTM. It is widely accepted that changes in gene expression mediate some aspects of this process. Here we examined the role of six transcriptional regulators in memory. The preponderance of evidence indicates that CREB, CaMKIV, c-fos, c-jun, CBP, and SRF are important in mammalian LTM. In many cases, a variety of techniques were used to disrupt the function of the transcriptional regulator, and the impact of this disruption was measured using multiple tests. It is important to point out that few studies have reported effects of these manipulations on learning or STM. Therefore, the effects of disrupting these transcriptional regulators seem to be specific for LTM. These findings, however, do not preclude the importance of other transcriptional regulators in memory formation.

It is perhaps not surprising that similar memory disruptions were produced by manipulations of the transcriptional regulators reviewed here, because they are closely linked. For instance, although CBP was initially identified based on its physical interaction with CREB, it also serves as an essential transcriptional cofactor for c-fos, c-jun, and SRF. CaMKIV phosphorylates both CREB and CBP, thereby enhancing CBP-mediated transcription (Impey et al., 2002). Finally, both CRE and SRE recognition sites are found in the promoter regions of c-fos and c-jun. Indeed, these transcriptional regulators likely work in concert to coordinate gene expression necessary for memory. This close relationship between these transcriptional regulators also suggests that there could be functional redundancy. This could account for the subtle phenotypes observed in some instances where a single transcriptional regulator is disrupted.

Two interesting themes emerged from the overall pattern of the data examined in this chapter. The first is that the memory phenotype is sensitive to the training parameters used to induce LTM (particularly the spacing between successive trials). This sensitivity was observed in mice with mutations in CREB, c-fos, c-jun, CaMKIV, and CBP and across several memory paradigms. More intense training (typically spaced training trials) generally produces stronger memory. Moreover, some of the memory deficits in the mutant lines of mice are overcome with intense training protocols. This may be because a stronger memory is less likely to be disrupted, or it could be that intense training may recruit additional molecular mechanisms.

The second theme is that the extent of gene disruption determines the memory phenotype. This pattern was observed in both CREB mutants and in conditional c-fos knockouts. In general, the greater the gene disruption, the greater the memory impairment.

The ultimate products of transcription mediated by these transcriptional regulators may support the structural and functional processes necessary for LTM in species ranging from snails to flies to rodents and, perhaps, humans. The identity of these genes, however, is currently unknown, and the list of candidate genes is long. For instance, a genome-wide analysis of CREB binding motifs resulted in 1349 sites in the mouse genome and 1663 'hits' in the human genome (Conkright et al., 2003b). Application of proteomic strategies may assist in confirming important targets.

4.27.8.1 Transcriptional Regulators and Memory Disorders in Humans

The importance of transcriptional regulation in memory formation has been highlighted by recent findings showing that some cognitive disorders in humans are associated with molecular lesions in several transcriptional regulators, including some reviewed earlier (see Weeber and Sweatt, 2002; Hong et al., 2005). For instance, mutations in the gene encoding RSK2, a protein kinase that phosphorylates CREB, is thought to be responsible for Coffin-Lowry Syndrome, a syndrome that produces cognitive deficits (Trivier et al., 1996). Rett syndrome, an X-linked disorder that is characterized by arrested neurological development and cognitive decline, is associated with a disruption in the transcription factor methyl-CpG-bindign protein 2 (MeCP2) (Amir et al., 1999). Mutations in CBP are thought to be responsible for Rubinstein-Taybi Syndrome (Petrij et al., 1995). Rubinstein-Taybi Syndrome is an autosomal dominant disorder that produces, among other deficits, mental retardation. Finally, dysregulation of CREB function (Asanuma et al., 1996; Foster et al., 2001; Brightwell et al., 2004),

PKA (Karege et al., 2001), and CBP (Chung et al., 2002) has also been implicated in age-related cognitive decline. Together, these examples highlight the importance of transcriptional regulation in human memory and cognition. They also raise the intriguing possibility that some forms of cognitive dysfunction in humans may be caused by deficits in the transcription regulation necessary for memory formation. Therefore, targeting the transcriptional regulators, including those reviewed in this chapter, may provide novel therapeutic targets for cognitive and memory disorders in humans.

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4.28 The NF-κB Family in Learning and Memory

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4.28.1 Overview of NFκB Signaling

The nuclear factor kappa B (NFκB) family of transcription factors was first discovered as transcriptional regulators of the immunoglobulin loci (Sen and Baltimore, 1986) and has received widespread attention in many fields for roles in inflammation, host defense, and programmed cell death. NFκB transcription factors function as dimers that regulate gene expression by binding to consensus κB-binding sites of target genes. The mammalian NFκB family (also known as the Rel family) consists of five members which can hetero- and homodimerize: p50 (product of the NFκB1 gene), p52 (product of the NFκB2 gene), p65 (also known as Rel A), c-Rel, and Rel B. All Rel family members contain a conserved Rel homology domain (RHD), a 300-amino-acid amino-terminal region containing an immunoglobulin fold DNA-binding domain, a dimerization domain, and a nuclear localization sequence (NLS). The carboxyl-terminal domains of Rel family members differ, with only p65, c-Rel, and RelB containing transactivation domains (TADs). Although p50 and p52 do not contain TADs and therefore do not function as transcriptional activators by themselves, they may activate gene transcription through binding interactions with other transcription factors. In addition, homo- or heterodimers of p50 or p52 may repressively regulate transcription by competing for DNA binding sites with NFκB dimers that do contain TADs.

Dimers of NF-κB are held latent in the cytoplasm by noncovalent interactions with a class of inhibitors called inhibitor of NFκB (IκB) proteins. Activation of NFκB does not require *de novo* synthesis of the transcription factor and is mediated by the IκB kinase complex (IKK) containing IKKα, IKKβ, and IKKγ subunits. The IKK complex is activated by a plethora of upstream signals including cytokines, neurotransmitters, and growth factors, as well as numerous cytotoxic stimuli (Scheidereit, 2006). The canonical pathway of NFκB activation proceeds through phosphorylation of IκB mediated by IKK (**Figure 1**). Phosphorylated IκB is targeted for ubiquitination and subsequent degradation by the 26S proteasomal complex. This removes IκB from the NFκB dimer, exposes both the NLS and DNA-binding domains of NFκB, and permits stable nuclear translocation and DNA binding by the NFκB dimer.

The p50 and p52 members of the NFκB family are initially synthesized as the precursor molecules p105 and p100, respectively. The carboxyl-terminal domains of these precursor molecules contain ankyrin repeats which resemble IκB. Like IκB molecules, the carboxyl-termini of p105 and p100 are capable of binding and inhibiting the NFκB dimer and causing the cytoplasmic localization of dimers containing p105 or p100. Activation of RelB:p100 dimers occurs through what is known as the noncanonical or alternative NFκB activation pathway. Phosphorylation of p100 by a homodimer of IKKα is essential in this activation pathway and serves to target p100 for subsequent ubiquitination. Ubiquitination is followed by an unusual process of limited proteolytic degradation mediated by the 26S proteasomal complex that removes the C-terminus of p100 to generate active p52 proteins (**Figure 1**).

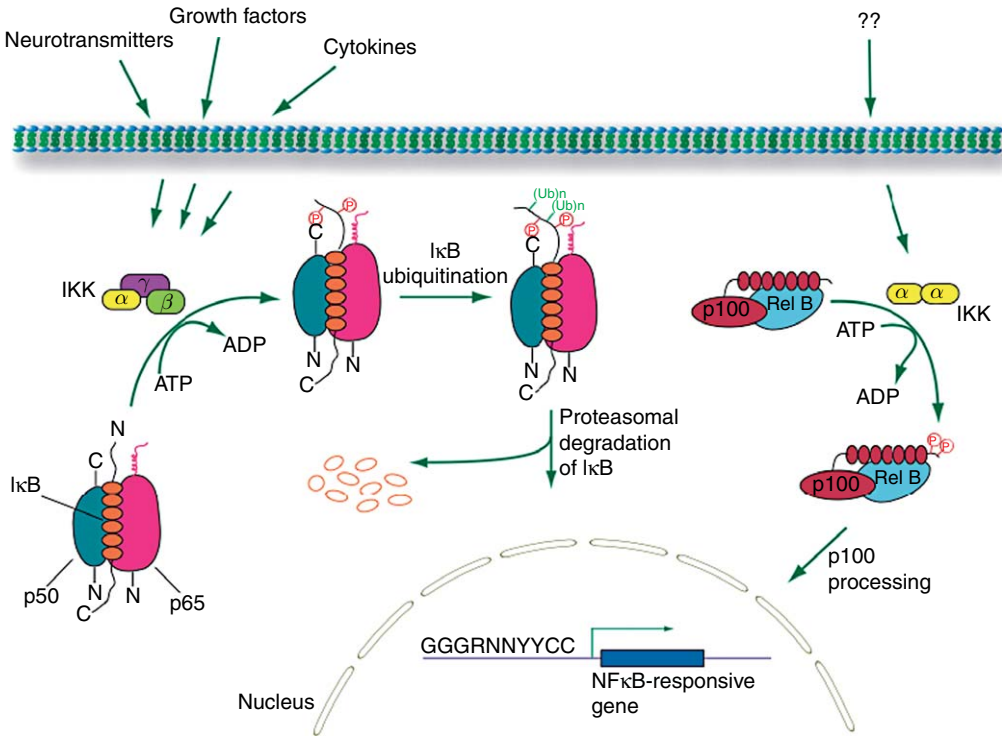

Figure 1 Activation pathways of nuclear factor kappa B (NFκB). Dimers of NFκB family members (shown as p50:p65) can be activated by the canonical pathway within the central nervous system. This pathway depends upon the ubiquitination and degradation of inhibitor of NFκB (IκB) by the 26S proteasome to permit the stable nuclear translocation of activated NFκB. The alternative pathway of NFκB activation, in which ankyrin repeats of p100 are removed by proteasomal processing to generate an active p52:RelB dimer, has not yet been clearly demonstrated in neurons. Activated NFκB can bind to exposed consensus sequences in the regulatory regions of target genes to alter transcription. IKK, IκB kinase complex.

Activated nuclear NFκB regulates transcription at many promoters containing variations in a highly divergent consensus DNA-binding sequence (the κB site). The general loose consensus sequence for the decameric κB site is: GGGRNNYYCC (R = purine, Y = pyrimidine). Variations in the κB site appear to confer regulatory specificity for dimers of different NFκB family members by two general mechanisms. The exact sequence of the κB site can determine which coactivators are capable of forming productive interactions with the bound NFκB dimer (Leung et al., 2004). This mode of regulatory specificity exists independently of any inherent differences in binding affinities of individual κB sites for discrete NFκB dimers. Specificity in transcriptional regulation can also be conferred by preferential binding of distinct NFκB dimer combinations to divergent κB sites. The finding of a variation in the κB site which preferentially binds RelB:p52 heterodimers provides one example of this type of regulatory specificity (Bonizzi et al., 2004).

4.28.2 Activation Pathways in the CNS

Expression of multiple Rel family members has been documented in the central nervous system (CNS), including p50, p65, and c-Rel. The most commonly reported neuronal species is the p65:p50 heterodimer, but dimers containing c-Rel have also been described. Neuronal NFκB can be activated by a wide variety of stimuli occurring in both physiological settings, such as glutamate and nerve growth factor (NGF), and pathological settings, such as β-amyloid and viral infection. Inducers of NFκB with particular relevance to normal brain function are listed in **Table 1**. Many stimuli capable of activating NFκB were first discovered and characterized in cells of the immune system. Interestingly, some of these immune modulators, including cytokines, chemokines, and their receptors, are now known to have additional functions in regulating neuronal development and

Table 1 Activators of NFκB with potential relevance to plasticity and cognition

Gene	*Tissue/cell type*	*Methods used*	*Reference*
Neurotransmitters			
Adenosine	Rat basal forebrain	1. EMSA (electrophoretic mobility shift assay) – NFκB activation inhibited by an A1 adenosine receptor antagonist 2. IκB degradation	Basheer et al., 2001
Glutamate	Murine cerebellar granule neurons and astrocytes	EMSA, supershift – NFκB activation in neurons but not astrocytes; inhibited by NMDA or AMPA glutamate receptor antagonists	Guerrini et al., 1995
	Rat cerebellar granule neurons	IHC – diminished staining with AMPA glutamate receptor antagonist	Kaltschmidt et al., 1995
	Rat cerebellar granule neurons	EMSA	Grilli et al., 1996
	Rat cerebellar granule neurons	1. EMSA, supershift – NMDA activation of p50:p65 2. Immunoblot – cytoplasmic levels of IκBα and phospho-IκBα	Lipsky et al., 2001
	Murine hippocampal cultures	1. EMSA, supershift – activation of p65:p50 and p50:p50 in neurons but not astrocytes through NMDA receptors, L-type Ca^{2+} channels, and Ca^{2+} ionophore. 2. Reporter assay – tests specificity with mutated NFκB consensus site	Meffert et al., 2003
	Murine cerebellar granule neurons	1. Reporter assay 2. EMSA – Ca^{2+}-dependent basal activation of p50:p65 and p50:p50	Lilienbaum and Israel, 2003
	Murine cortical neurons and neuronal SK-N-SH cells	1. EMSA – NFκB activation with Group I metabotropic receptor agonist 2. Increased DNA-binding activity of dimers containing p65, p50, and c-rel through mGlu5 receptor using NFκB ELISA-based assay kit	Pizzi et al., 2005
	Rat piriform cortex and hippocampus	EMSA – following *in vivo* kainate injection	Rong and Baudry, 1996
	Murine hippocampal slices	Increased DNA binding activity of p65, p50, and c-rel via group I metabotropic glutamate receptors using NFκB ELISA-based assay kit	O'Riordan et al., 2006
Nitric oxide	Rat striatal neuronal culture	EMSA	Simpson and Morris, 1999
Serotonin	Chinese hamster ovary cells	1. Immunoblot – cytoplasmic levels of IκBα 2. Reporter assay – serotonin 1A agonist, uses HIV-LTR which also contains an SP1 site	Cowen et al., 1997
	Chinese hamster ovary cells	1. NFκB activation through serotonin 1A receptor using NFκB ELISA-based assay kit 2. Immunoblot – cytoplasmic levels of IκBα and phospho-p65	Hsiung et al., 2005
Cytokines/chemokines			
IL-1α	Rat glioma C6 cell line	EMSA – NFκB activation inhibited by IL-1 receptor antagonist	Moynagh et al., 1993
	Astrocytes	EMSA	Guerrini et al., 1995
IL-1β	Rat glioma C6 cell line	1. EMSA – NFκB activation inhibited by IL-1 receptor antagonist, specificity tested by competitor oligonucleotides 2. Reporter assay – NFκB activation inhibited by IL-1 receptor antagonist	Moynagh et al., 1993

(*Continued*)

Table 1 (Continued)

Gene	*Tissue/cell type*	*Methods used*	*Reference*
	Rat astrocytes and cerebellar granule neurons	Reporter assay	Krushel et al., 1999
	Rat cerebellar granule neurons and SH-SY5Y neuroblastoma cells line	1. EMSA 2. Reporter assay	Grilli et al., 1996
	Rat cerebellar granule neurons	1. EMSA, supershift 2. Immunoblot – cytoplasmic levels of IκBα and IκBβ	Pizzi et al., 2002
	Murine hippocampal slices	IHC – increased p65 and c-rel nuclear localization	
SDF-1α	Murine cortical astrocytes	1. EMSA, supershift – activation of p65:p50 dimer; specificity tested by competitor wild-type and mutant κB oligonucleotides 2. Reporter assay	Han et al., 2001
TNFα	Neuroblastoma cell line	EMSA, supershift	Drew et al., 1993
	Hippocampal cultures	EMSA	Barger et al., 1995
	Astrocytes	EMSA	Guerrini et al., 1995
	Astrocytes and cerebellar granule neurons	Reporter assay	Krushel et al., 1999
	Cerebellar granule neurons	Reporter assay	Lilienbaum and Israel, 2003
TNFβ	Hippocampal cultures	EMSA	Barger et al., 1995
	SH-SY5Y neuroblastoma cell line	CAT reporter assay	Barger and Mattson, 1996
Neurotrophins/growth factors			
ADNF	Hippocampal cultures	1. EMSA, supershift – shows p65 and p50 activation 2. IHC – increased p65 nuclear localization	Glazner et al., 2000
BDNF	Cerebellar granule neurons	EMSA	Lipsky et al., 2001
	Murine P19 neurons	EMSA, supershift	Burke and Bothwell, 2003
IGF-1	Rat cerebellar granule neurons and GT1-7 neuronal cell line	1. EMSA, supershift – activation of p65:p50 dimers, specificity tested by competitor oligonucleotides 2. Reporter assay 3. Immunoblot – cytoplasmic levels of IκBα and nuclear levels of p65	Heck et al., 1999
NGF	Rat and murine Schwann cells	EMSA, supershift – activation of p65, p50, c-Rel	Carter et al., 1996
	Sympathetic neuronal cultures	EMSA, supershift – activation of p65, p50, and c-Rel	Maggirwar et al., 1998
	Rat cortical oligodendrocyte cultures	1. EMSA 2. IHC – increased p65 nuclear localization	Yoon et al., 1998
	Murine P19 neurons	EMSA, supershift	Burke and Bothwell, 2003
NT-3	Murine P19 neurons	EMSA, supershift	Burke and Bothwell, 2003

(*Continued*)

Table 1 (Continued)

Gene	*Tissue/cell type*	*Methods used*	*Reference*
NT-4	Murine P19 neurons	EMSA, supershift	Burke and Bothwell, 2003
PEDF	Rat cerebellar granule cell culture	1. EMSA, supershift – activation of p65:p50 and p50:50 dimers, specificity tested by competitor oligonucleotides 2. Immunoblot – cytoplasmic levels of IκBα and phospho-IκBα 3. IHC – increased p65 nuclear localization	Yabe et al., 2001
TGF-β1	Murine hippocampal cultures	1. Reporter assay 2. Immunoblot – cytoplasmic levels of IκBα, IκBβ and phospho-IκBα 3. IHC – increased p65 nuclear localization	Zhu et al., 2004
VEGF	Murine HN33 neuronal cell line	Immunoblot – cytoplasmic levels of phospho-IκBα and nuclear levels of p65	Jin et al., 2000
Cell adhesion molecules			
N-CAM	Astrocytes and cerebellar granule neurons	1. EMSA and supershift – shows p65 and p50 activation 2. Reporter assay 3. Immunoblot of cytoplasmic IκBα levels	Krushel et al., 1999
Miscellaneous			
Apotransferrin (aTf)	Oligodendroglial cell lines	1. EMSA 2. Immunoblot of cytoplasmic IκBα levels	Paez et al., 2006
Bradykinin	Murine astrocytes	1. EMSA, supershift – activation of p65:p50 and p50:p50, specificity tested by competitor mutant κB oligonucleotides 2. Reporter assay – activation inhibited by DNIκBα	Schwaninger et al., 1999

Abbreviations: ADNF, activity-dependent neurotrophic factor; AMPA, alpha-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid; BDNF, brain-derived neurotrophic factor; CAT assay, chloramphenicol acetyltransferase reporter assay; ELISA, enzyme-linked immunosorbent assay; HIV-LTR, long terminal repeat from HIV; IGF-1, insulin-like growth factor; IHC, immunohistochemistry; N-CAM, neural cell adhesion molecule; NMDA, *N*-methyl-D-aspartate; NT-3, NT-4, neurotrophins 3 and 4; PEDF, pigment epithelium-derived factor; SDF-1α, stromal-derived factor 1α; TGF, tumor growth factor; TNF, tumor necrosis factor; VEGF, vascular endothelial growth factor.

synaptic plasticity. For example, the CD40 receptor, which activates NFκB during B cell development, is also expressed in neurons of the CNS, where it can regulate neuronal survival and may also promote neuronal differentiation (Tan et al., 2002). Likewise, tumor necrosis factor (TNF) and its receptors are well-characterized activators of NFκB in the immune system and are also expressed in the CNS. The functions of TNFα in the brain remain incompletely characterized, but one role appears to be the regulation of neuronal responses to the excitatory neurotransmitter glutamate (Albensi and Mattson, 2000; Beattie et al., 2002; Stellwagen et al., 2005; Stellwagen and Malenka, 2006). In most cases, the role that NFκB activation might play in the effects of these immune modulators in neurons is as yet unknown. In addition, some activators of NFκB, such as glutamate, depolarization, NGF, and activity-dependent neurotrophic factor (ADNF) (Glazner et al., 2000), have been most completely characterized in cells of the nervous system or neuronal cell lines and may play unique roles in the neuronal regulation of this transcription factor.

The canonical pathway of NFκB activation involving phosphorylation of the IKK complex and proteasomal degradation of the IκB inhibitor is well documented in neurons of the CNS (**Figure 1**). The upstream signaling pathways leading to IKK activation in the brain appear to be as diverse as they are outside the CNS. For example, the major excitatory neurotransmitter of the hippocampus, glutamate, can activate NFκB in hippocampal neurons through both an *N*-methyl-D-asprate (NMDA) receptor–mediated pathway dependent upon Ca^{2+} elevation (Lilienbaum and Israel, 2003; Meffert et al., 2003), and a metabotropic receptor-mediated pathway, which is likely to be independent of Ca^{2+} elevation and

involves the activities of phosphatidylinositol 3-kinase (PI3K), mitogen-activated protein kinase/extracellular signal-related kinase (MEK), and p38-mitogen-activated protein kinase (p38-MAPK) (Lubin et al., 2005; O'Riordan et al., 2006). Potential functions of NFκB activation by the alternative pathway (IKKα-regulated cleavage of the p100 precursor) have not been clearly determined in the brain. Although both the p100 and p105 precursors are present in brain tissue, the expression pattern of essential components of the alternative activation pathway, including the NFκB-inducing kinase (NIK), await characterization. NIK-deficient mice are reported to display no obvious defects in brain function and are capable of normal growth, behavior, reproduction, and nursing (Yin et al., 2001); however, a detailed analysis of the CNS in NIK-deficient mice has not been published. In a neuron-like cell line (PC12), overexpression of wild-type NIK but not kinase-dead NIK enhances the formation of neurites (Foehr et al., 2000a). Whether the alternative and canonical pathways of activation subserve separate or overlapping functions in the CNS remains to be determined.

Neurotrophins are essential for many aspects of cognition and participate in regulating differentiation and synaptogenesis during brain development as well as influencing growth and plasticity in the mature brain. NGF is the most well-characterized neurotrophic activator of neuronal NFκB. NGF can activate NFκB through signaling pathways using either the tyrosine kinase A (TrkA) receptor or the $p75^{NTR}$, both of which converge to activate IKK. Stimulation of chimeric NGF receptors has revealed that the $p75^{NTR}$ activates dimers containing p65, p50, or p52 subunits of NFκB, while trkA activates only dimers containing p50 or p65. This selective NFκB dimer activation may also have functional consequences, as certain genes are specifically induced by stimulation through one receptor or the other, in addition to a significant overlap in gene regulation (Carter et al., 1996; Foehr et al., 2000b). The $p75^{NTR}$ NGF receptor is a member of the TNF superfamily of receptors and, like other family members, is coupled to both Jun N-terminal kinase (JNK) and NFκB activation through the tumor necrosis factor receptor associated factor-6 (TRAF6) protein. A dominant negative TRAF6 eliminates NFκB activation through the $p75^{NTR}$ while sparing activation through TrkA (Foehr et al., 2000b). The loss of neurotrophin-induced apoptosis in sympathetic neurons from TRAF6-null mice suggests that TrkA stimulation alone could mediate prosurvival functions of neurotrophins (Yeiser et al., 2004). These potential roles for neurotrophin-induced NFκB activation have yet to be validated in neurons of the CNS, however.

Among the numerous stimuli capable of activating NFκB, one of the most interesting from the standpoint of plasticity and cognition is the regulation of this transcription factor by synaptic transmission. The application of exogenous glutamate and analogs such as NMDA and kainate were first shown to activate NFκB in cerebellar granular neurons (Guerrini et al, 1995; Kaltschmidt et al., 1995). The finding that endogenous synaptic activity could also activate NFκB made it clear that this pathway could have physiological relevance apart from a role in the response to excitotoxicity. Ca^{2+} influx through NMDA receptors and L-type Ca^{2+} channels appears to play an important role in glutamate-mediated NFκB activation in both the hippocampus and cerebellum (Lilienbaum and Israel, 2003; Meffert et al., 2003; Scholzke et al., 2003). Blockade of these receptors significantly depresses NFκB activity in cultured cells and *in vivo,* indicating that basal levels of synaptic transmission effectively regulate this transcription factor (Meffert et al., 2003). A study using cerebellar neurons in culture for 4 days (likely prior to synapse formation) has demonstrated that release of intracellular Ca^{2+} stores following glutamate stimulation could also contribute to NFκB activation (Lilienbaum and Israel, 2003).

Multiple neurotransmitters including glutamate, dopamine, adenosine (Basheer et al., 2001), and serotonin (Hsiung et al., 2005) have now been reported to activate NFκB. Stimulation of dopamine D2-like receptors in a neuroblastoma cell line (NG108) (Takeuchi and Fukunaga, 2004a) as well as intrastriatal injection of dopamine have been reported to activate NFκB (Luo et al., 1999). A deletion mutation analysis of the D2 receptor and pharmacological manipulation indicate that D2 receptor activation of NFκB proceeds through members of the MAPK family, including MAPK and the extracellular signal-regulated kinase (ERK) (Takeuchi and Fukunaga, 2004b). Adenosine-stimulated NFκB activation is mediated by the A1 receptors in basal forebrain neurons and may participate in regulating wakefulness and the response to sleep deprivation. Regulation by more than one neurotransmitter suggests that NFκB has the potential to participate in a variety of cognitive functions in distinct brain regions.

4.28.3 NFκB at Synapses

An unusual subcellular localization was one of the first intriguing aspects reported for neuronal NFκB (Kaltschmidt et al., 1993; Guerrini et al., 1995; Suzuki et al., 1997). The presence of NFκB in dendritic processes and at synapses was suggested by studies using immunohistochemistry and electron microscopy of brain regions containing synapses in discrete cell layers. These observations were strengthened by the finding of NFκB in isolated preparations of synapses termed synaptosomes (Kaltschmidt et al., 1993; Meberg et al., 1996). Interestingly, not all dimers of NFκB have been found to be equally represented at synapses. Isolated synapses from the hippocampus and cortex of wild-type mice contain primarily p65:p50 dimers, while synapses from p65-deficient mice are devoid of NFκB (Meffert et al., 2003). It is possible that neuronal stimulation could alter the complement of NFκB dimers that is targeted to synapses, although this type of regulation has not yet been reported. NFκB appears to be present at both the postsynaptic side of the synapse (in preparations of the postsynaptic density, PSD) as well as in the presynaptic compartment.

After isolation by subcellular fractionation, synaptosomes can reseal to form biochemically functional synaptic compartments. As reported for intact neurons, the latent NFκB found within these isolated synapses can be activated by glutamate or by Ca^{2+} elevation alone (Meffert et al., 2003). This finding implies that all of the components required for the NFκB activation pathway are located at synapses or in adjacent dendritic regions. Neuronal studies of the ubiquitin proteasomal degradation pathway, which is critical for activation of NFκB by IκB degradation, support this implication. Like NFκB activation, ubiquitination and activity of the 26S proteasome are also regulated by excitatory neurotransmission (Ehlers, 2003; Patrick et al., 2003). A recent analysis of the 26S proteasome in hippocampal dendrites has revealed that this complex uses the actin cytoskeleton to move into dendritic spines in response to synaptic input. In addition, the level of local proteasomal degradation is enhanced by neuronal depolarization in an NMDA receptor–dependent manner (Bingol and Schuman, 2006). These studies provide an upstream correlate for the finding that synaptic activity and localized Ca^{2+} elevations lead to neuronal NFκB activation. At hippocampal synapses, the Ca^{2+}-dependent pathway of NFκB activation is also inhibited by NMDA receptor blockade and appears to require the activity of Ca^{2+}/calmodulin-dependent protein kinase II (CaMKII), which is concentrated in PSDs (Meffert et al., 2003).

4.28.4 Translocation from Cytoplasm to Nucleus

Imaging of neurons expressing a green fluorescent protein–tagged p65 (GFPp65) subunit of NFκB has confirmed the localization of NFκB in neuronal processes. The architectural complexity of neurons means that NFκB activated at synapses can be distant from the site of transcriptional regulation in the nucleus. The potential exists that synaptic NFκB might not effectively translocate long cytoplasmic distances following stimulation and might instead have local nontranscriptional functions. To date, however, nontranscriptional functions for NFκB have not been demonstrated, and studies in living neurons have revealed that NFκB in distal neuronal processes is capable of translocating to the nucleus following stimulation (Wellmann et al., 2001; Meffert et al., 2003). Little is known about the molecular machinery involved in the cytoplasmic phase of NFκB translocation to the nucleus. However, upon reaching the nucleus, an NFκB interaction with the importin/karyopherin class of chaperone molecules has been found to mediate its translocation across the nuclear pore (Fagerlund et al., 2005), most likely through a direct binding interaction (Cunningham et al., 2003). While importin molecules are found in the dendrites and axons of mature neurons (Hanz et al., 2003; Thompson et al., 2004), a requirement of importins for cytoplasmic transport of neuronal NFκB has not been reported to date. Involvement of the NLS of p65 in the redistribution of NFκB from neuronal cytoplasm to nucleus has been suggested by imaging studies evaluating the distribution of fluorophore-tagged wild-type and NLS mutant p65 (Wellmann et al., 2001). Interestingly, synthetic NLS peptides undergo active retrograde transport mediated through interactions with importins in *Aplysia* axons subjected to injury (Ambron et al., 1992; Schmied and Ambron, 1997; Hanz et al., 2003). Loss of an active *Aplysia* NFκB-like molecule along the axons near sites of axonal crush injury has also been reported (Povelones et al., 1997). Since levels of activated nuclear NFκB were not assessed

in this study, it remains uncertain whether the activated NFκB might have undergone nuclear translocation or whether it may have been degraded in the crushed axon.

4.28.5 NFκB in *In Vitro* Assays of Plasticity

The activation of NFκB by neurotransmission and, in particular, its regulation by the excitatory neurotransmitter glutamate suggest the potential participation of NFκB in plasticity requiring activity-dependent transcriptional regulation. Several investigators have examined the role of NFκB in *in vitro* paradigms of plasticity in which changes in gene expression are essential for long-lasting alterations of synaptic function. Long-term potentiation (LTP) and long-term depression (LTD) occur in many brain regions and are commonly studied in the Schaffer collateral pathway between CA1 and CA3 neurons in hippocampal slices. LTP is the use-dependent lasting enhancement of synaptic connections, while hippocampal LTD is the long-term weakening of synaptic connections in response to persistent low-frequency stimulation. Recent work has firmly established the often-proposed link between LTP and the cognitive functions of learning and memory (Whitlock et al., 2006). Initial studies in which NFκB was inhibited by bath application or microinjection of DNA oligos containing consensus binding sites for NFκB (called 'decoy' DNA) reported a deficit in LTP both in the hippocampus (Albensi and Mattson, 2000) and in the amygdala (Yeh et al., 2002).

These early studies have subsequently been confirmed by genetically manipulating the NFκB pathway using either transgenic expression of a dominant inhibitor of NFκB or by knockout of Rel family members. Expression of a dominant negative nondegradable mutant of IκBα (DNIκBα) is believed to result in broad spectrum inhibition of all Rel family members. Specific transgenic expression of the DNIκBα in neurons of the forebrain produced a modest but significant reduction in the late (≥100 min post potentiating stimulus), transcription-dependent phase of hippocampal LTP (Kaltschmidt et al., 2006). In addition, forebrain neuronal expression of the DNIκBα also appeared to impair the induction of hippocampal LTD (Kaltschmidt et al., 2006). A form of hippocampal LTD mediated by activation of metabotropic glutamate receptors (mGluRs) has also been examined in knockout mice selectively deficient in the c-Rel subunit of NFκB. Early LTD was found to be normal in c-Rel-deficient mice, however, a late (≥70 min post mGluR stimulus) transcription-dependent phase of LTD was disrupted with synaptic efficacy returning to predepression levels by 180 min after LTD simulation in c-Rel-deficient mice (O'Riordan et al., 2006). An analysis of frequency and amplitude of miniature synaptic currents in the same mice revealed no significant difference compared to c-Rel wild-type littermates.

Consistent with the inhibition of LTP and LTD produced by disrupting NFκB function, the generation of LTP by itself may also enhance the function of the NFκB pathway through two mechanisms. The high-frequency stimulation used to generate LTP is reported to activate latent NFκB (Freudenthal et al., 2004) and may also mediate increases in the transcription of the p65 subunit of NFκB (Meberg et al., 1996). A gel shift analysis of the hippocampus following *in vivo* perforant path LTP or LTD indicated that heterodimers of p65:p50 as well as p50:p50 homodimers were activated by high-frequency stimulation, while the low-frequency stimulation associated with LTD had no significant effect on NFκB activity (Freudenthal et al., 2004). The enhancement of p65 and p50 mRNA production was reported 60–120 min following LTP induction in hippocampal pyramidal and granule neurons. While increased production of p65 and p50 subunits might enhance the function of the NFκB pathway, a caveat to this interpretation is that NFκB is not typically regulated by transcription, and as the authors point out, a similar increase in the mRNA level of a classical NFκB target gene, IκBα, was not observed.

4.28.6 NFκB in Behavioral Assays of Learning and Memory

4.28.6.1 Crustaceae

The excitement of finding NFκB activation by synaptic activity and its role in *in vitro* assays of plasticity has led to the investigation of this transcription factor's potential involvement in cognition. Some of the first reports suggesting a potential cognitive role for NFκB came from habituation studies in the marine crab, *Chasmagnathus,* which contains a homolog of mammalian NFκB. Repeated presentation of a visual danger signal simulating an overhead predator leads to eventual habituation of the crab's escape response. This predator avoidance paradigm

has been characterized to function as a model of long-term memory (LTM) mediated by a conditioned association between contextual cues and the predator-eliciting stimulus. A growing body of work suggests that the crab NFκB homolog is critical both to the formation of this LTM and to the process of memory reconsolidation.

Initial reports demonstrated that spaced training resulting in habituation and LTM increased the levels of activated NFκB present in crab neuronal synapses and nuclei, while training which did not result in LTM also did not activate NFκB (Freudenthal et al., 1998; Freudenthal and Romano, 2000). In subsequent studies, injection of a nonspecific inhibitor of IKK, sulfasalazine, was reported to block NFκB activation and also inhibit LTM formation (Merlo et al., 2002). Sulfasalazine contains 5-aminosalicylic acid (the main ingredient in aspirin) in combination with a sulfa antibiotic and can also inhibit cyclooxygenase. However, control injection of alternative cyclooxygenase inhibitors did not block LTM. The process of memory retrieval is thought to initiate a second process of memory consolidation, termed reconsolidation, which invokes some of the same cellular mechanisms used in initial memory formation. A study of reconsolidation using the same predator avoidance paradigm in *Chasmagnathus* suggests that memory retrieval, activated by reexposure to the training context, also activates NFκB. IKK inhibition by sulfasalazine impaired reconsolidation when administered prior to memory retrieval, but had no apparent effect on the LTM when preceding exposure to an alternative training context not expected to activate memory retrieval (Merlo et al., 2005). Work by other researchers has implicated another activity-dependent transcription factor, cyclic adenosine monophosphate (cAMP) response element binding protein (CREB), as well as the immediate early gene and transcription factor Zif268, in reconsolidation (Kida et al., 2002; Bozon et al., 2003). Collectively, these findings provide evidence for an emerging consensus that transcription is required for memory reconsolidation in addition to the previously established role for new protein synthesis in this process.

4.28.6.2 Vertebrates

Evaluation of the function of NFκB in vertebrate cognitive functions has focused largely on behavioral paradigms testing either fear conditioning or spatial memory. A role for NFκB in these types of learning appears to be consistent in both mammalian and invertebrate systems. Studies using knockout mice indicate that deletion of the p65, p50, and c-Rel subunits can affect the processes of learning and memory. Deficits in contextual fear memory and a long-term passive avoidance task utilizing both the hippocampus and amygdala occur in mice lacking the c-Rel subunit of NFκB (Levenson et al., 2004; O'Riordan et al., 2006). Mice deficient in p50 are impaired in short-term active avoidance paradigms and may show reduced anxiety responses (Kassed et al., 2002). In addition, mice lacking p50 are reported to have increased exploratory activity and less apparent anxiety in open field and novel object tests (Kassed and Herkenham, 2004). Mice lacking the p65 subunit of NFκB are rescued from embryonic lethality if pro-apoptotic stimulation through the tumor necrosis factor receptor (TNFR) is abrogated. Behavioral assays comparing double knockout mice ($TNFR^{-/-}$,$p65^{-/-}$) to single knockout ($TNFR^{-/-}$,$p65^{+/+}$) littermates revealed that p65 deficiency results in a spatial learning deficit. Mice lacking p65 took approximately twice as long to reach peak performance in a spatial version of the radial arm maze compared to p65 wild-type littermates; no differences in performance were observed in a cued version of the radial arm maze which did not require the use of spatial information (Meffert et al., 2003). While differences in the behavioral phenotypes of NFκB knockout mice are intriguing, it remains too early to assign any subunit-specific functions in cognition.

Inhibition of the NFκB pathway using either pharmacological approaches or transgenic overexpression of a mutant nonphosphorylatable form of IκBα (DNIκB) which functions as a dominant negative can also disrupt the processes of learning and memory. Several studies in rats have used intra-amygdalar or intrahippocampal infusion of small double-stranded decoy oligonucleotides for NFκB to impair either long-term memory in paradigms involving fear conditioning (Yeh et al., 2002; Freudenthal et al., 2005) or long-term spatial memory in the Morris water maze (Dash et al., 2005), respectively. Decoy oligonucleotides resemble the consensus DNA binding site for NFκB and are hypothesized to function by competitively interfering with the ability of NFκB to bind to its target sequences upstream of regulated genes. In both cases, oligonucleotides containing a scrambled NFκB sequence were ineffective, but ruling out any

potential side effects of an NFκB DNA-binding sequence present in the cytoplasm is not possible.

Two lines of transgenic mice expressing the DNIκB either through the CaMKIIα promoter (Fridmacher et al., 2003; Kaltschmidt et al., 2006) or the prion promoter (O'Mahony et al., 2006) have been reported. While studies using knockout mice have permitted an analysis of subunit specificity, these are the first studies allowing an assessment of tissue or cell type–specific effects of NFκB deficiency. In the first study, tetracycline-regulated DNIκB was predominantly expressed in forebrain excitatory neurons in a double transgenic mouse with the tetracycline transactivator under control of the CaMKIIα promoter (TetOFF) (Fridmacher et al., 2003). Inhibition of NFκB by the DNIκB compromised spatial learning as assessed by the Morris water maze (Kaltschmidt et al., 2006). The effects of DNIκB expression were modest, however, and the authors also found that doxycycline itself compromised memory formation or retention and may have contributed to the lack of significant differences between double transgenics in the absence or presence of doxycycline. Single transgenics, harboring nonexpressed DNIκB, did perform significantly better in the spatial learning task than double transgenic mice expressing DNIκB (in the absence of doxycycline). Experiments by a separate team of investigators using a transgenic line expressing DNIκB under control of the prion promoter yielded disparate results. In this study, NFκB inhibition by the DNIκB actually enhanced spatial memory formation using both Morris water maze and radial arm maze tasks (O'Mahony et al., 2006). Opposing results in an assay of *in vitro* plasticity were also observed, with NFκB inhibition generating a modest enhancement of hippocampal LTP. The mechanisms underlying the opposing results found in this study may be complicated, but could potentially shed light on the function of NFκB in the CNS. The authors suggest that the prion promoter directs expression of the DNIκB to inhibitory GABAergic neurons and that this might enhance gamma-aminobutyric acid (GABA) release by upregulating the GAD65 GABA synthetic enzyme (see the section titled 'Transcriptional regulation by NFκB'). Differences in CNS GABA levels were not demonstrated, however. Evidence is presented that the prion promoter in this transgenic line does not drive glial expression but does direct DNIκB expression to both excitatory and inhibitory neurons. This is unlike CaMKIIα promoter-driven gene expression, which occurs predominantly in excitatory neurons. Spatial and temporal differences also exist in the brain gene expression patterns directed by these two promoters. The prion promoter is active during embryonic development and adulthood (Miele et al., 2003), while the CaMKIIα promoter becomes active only postnatally. In addition the prion promoter generates broad expression in neurons (and sometimes other brain cells such as endothelial cells, epithelial cells, glia, and microglia (Moser et al., 1995)) throughout the cortex, cerebellum, and brainstem, while the CaMKIIα promoter is active only in forebrain neurons. It remains to be determined whether any of these differences in gene expression patterns driven by the prion or CaMKIIα promoters account for the behavioral discrepancies between the two studies.

4.28.7 Transcriptional Regulation by NFκB

DNA microarrays have been successfully used to identify genes that are differentially regulated during events requiring plasticity, such as learning and memory. Recently, a bioinformatics analysis used microarray data to identify transcription factors involved in LTM by scanning for potential regulatory elements in the upstream regions (1 kb) of identified target genes associated with memory consolidation (Levenson et al., 2004). This open-ended approach used an NMDA receptor–dependent contextual fear conditioning paradigm to screen a significant fraction of the murine genome for transcripts with altered expression levels in area CA1 of the hippocampus. Analysis of these transcripts revealed that binding sites for NFκB were selectively overrepresented in the subset of genes regulated by LTM formation. Interestingly, while binding sites for other activity-regulated transcription factors such as CREB and serum response factor (SRF) were present in genes regulated by LTM, this study did not find them to be enriched in this population. NFκB-regulated gene expression has also been explicitly linked to learning in eyeblink conditioning, a form of associative learning that is well conserved across species (Park et al., 2006). In this learning paradigm the conditioned response (CR, eyeblink) is evaluated following the pairing of a conditioned stimulus (CS, tone) with an unconditioned stimulus (US, airpuff). A CS–US associative memory results in the generation of the CR in response to the CS. A recent study found that the prototypical NFκB-dependent gene,

IκBα, was differentially upregulated by microarray and *in situ* hybridization following paired training in the murine eyeblink response.

NFκB is a potent transcription factor, which can robustly regulate the expression of a broad range of target genes including growth factors, adhesion molecules, chemokines and cytokines, and components of inflammatory pathways. This review focuses primarily on genes regulated by NFκB with potential relevance to processes of cognition and neuronal plasticity (see **Table 2**). Many NFκB targets identified outside of the nervous system are likely to also be

Table 2 NFκB gene targets with potential relevance to plasticity and cognition

Gene	*Tissue/cell type*	*Methods used*	*Reference*
Cytokines/chemokines			
Interleukin-6	Murine astrocytes	Reporter assay – effects of DNIκB expression on promoter activity	Schwaninger et al., 1999
	Rat retinal cells	siRNA knockdown of p65 reduces mRNA levels by RT-PCR	Wang et al., 2006
Interleukin-1α	Hippocampus	κB decoy DNA downregulates mRNA levels by RPA	Qiu et al., 2004
TNFα	Hippocampus	κB decoy DNA downregulates mRNA levels by RPA	Qiu et al., 2004
TNFβ	Hippocampus	κB decoy DNA downregulates mRNA levels by RPA	Qiu et al., 2004
Cell adhesion molecules			
NCAM	Rat cultured neurons	1. NFκB binding site present in promoter region 2. EMSA 3. p50 antisense decreases protein levels by IHC and immunoblot	Simpson and Morris, 2000
Receptors			
Metabotropic receptor – mGlu2		NFκB binding site present in promoter region	Chiechio et al., 2006
Metabotropic receptor – mGlu3		NFκB binding site present in promoter region	Chiechio et al., 2006
Metabotropic receptor – mGlu5	Neuroblastoma and glioblastoma cell lines	1. NFκB binding site present in promoter region 2. EMSA – data not shown	Corti et al., 2003
NMDA receptor – NR2A	Rat, Human	Conserved NFκB binding site present in promoter region	Richter et al., 2002
NMDA receptor – NR1	Human	Polymorphism of NFκB binding site present in promoter region	Begni et al., 2003
	Murine P19 cell line	Reporter assay – modest effect of DNIκB or κB decoy DNA; SP1 factors show cooperativity and activation at this promoter[a]	Liu et al., 2004
AMPA receptor – GluR1	Human NT2-N cell line, murine brain	NFκB binding sites present in promoter region	Borges and Dingledine, 2001; Yu et al., 2002
		1. Diminished protein expression in hippocampus and cortex of p50 knockout mice by immunoblot 2. Decreased protein expression in NT2-N cells by immunoblot after p50 anti-sense treatment 3. Overexpression of p50 increases expression in NT-2 by IHC	
μ-Opioid receptor	Human Rajii, U937 immune cell lines	1. NFκB binding site present in promoter region 2. EMSA, supershift	Kraus et al., 2003

(*Continued*)

Table 2 (Continued)

Gene	*Tissue/cell type*	*Methods used*	*Reference*
δ-Opioid receptor	Rat PC12 cell line	1. Reporter assay – effects of p65 and DNIκB overexpression on promoter activity 2. NLS peptide reduces mRNA levels by RT-PCR 3. EMSA, supershift 4. siRNA knockdown of p65 reduces mRNA levels by RT-PCR	Chen et al., 2006
Dopamine D_2 receptor	Human prolactinoma, COS-7 cell lines	1. NLS peptide reduces mRNA levels by RT-PCR 2. Reporter assay – effects of p50 and/or c-rel overexpression on promoter activity	Fiorentini et al., 2002
Serotonin (5-HT) 1A receptor	Murine P19, monkey COS-1 and CV1-β cell lines	1. NFκB binding sites present in promoter region 2. Reporter assay – effects of p50 and p65 expression on promoter activity; deletions of NFκB sites in promoter 3. EMSA with p50, p65 overexpression; deletions of NFκB sites	Wissink et al., 2000
Adenosine A1 receptor	Rat PC12 cell line	Receptor upregulation inhibited by DNIκB expression using radioligand binding assay	Jhaveri et al., 2006
CD40–TNFR super family	Murine microglia and macrophage cell line	1. Reporter assay – effects of wtIKKα, dnIKKα, wtIKKβ, or dnIKKβ expression on promoter activity; deletion and mutations of NFκB site in promoter 2. Chromatin immunoprecipitation assay shows bound p65, p50	Qin et al., 2005
Growth factors			
NGF	Rat astrocyte cultures	κB decoy DNA and IκB overexpression decrease mRNA levels by RT-PCR	Zaheer et al., 2001
BDNF	Rat astrocyte cultures	1. NFκB binding site present in promoter region 2. κB decoy DNA and IκB overexpression decrease mRNA levels by RT-PCR	Zaheer et al., 2001
	Rat astrocyte cultures	1. NFkB binding site present in promoter region 2. EMSA, supershift 3. κB decoy DNA downregulates mRNA induction by RT-PCR	Lipsky et al., 2001
Inflammatory response genes			
Cox-2	Neuroblastoma, HeLa cell lines	1. NFκB binding site present in promoter region 2. EMSA 3. Reporter assay – test effects of IκBα overexpression and deletion of NFκB site on promoter activity	Kaltschmidt et al., 2002
	Neuroblastoma cell line	Reporter assay – test effects of p65 or IκBα overexpression, and deletion of NFκB sites on promoter activity	Alvarez et al., 2005
iNOS	Rat neuronal culture	p50 antisense decreases protein levels	Simpson and Morris, 2000
	Rat cerebral cortex	EMSA	Madrigal et al., 2001
nNOS		NFκB binding site present in promoter region	Boissel et al., 1998

(*Continued*)

Table 2 (Continued)

Gene	*Tissue/cell type*	*Methods used*	*Reference*
Enzymes			
PKA catalytic subunit α	Neuro2a cells, murine brain sections	1. NFκB binding site present in promoter region 2. Downregulation in hippocampus of DNIκB expressing mice by microarray analysis 3. Downregulation in hippocampus of DNIκB expressing mice by *in situ* hybridization 4. Decreased mRNA levels in Neuro2a expressing DNIκB by Northern blot 5. EMSA, supershift in Neuro2a 6. Reporter assay – effects of DNIκB expression on promoter activity in Neuro2a	Kaltschmidt et al., 2006
Na+ K+ ATPase γ subunit	Murine hippocampus	Analysis in p50 knockout mice by microarray and RT-PCR	Kassed et al., 2004
CaMKII δ	Murine hippocampus	Analysis in p50 knockout mice by microarray and RT-PCR	Kassed et al., 2004
Transcription factors			
Oct-6	DRG neuron and Schwann cell cocultures	Decreased activation of Oct-6 activation with DNIκB expression by EMSA, may or may not be transcriptionally mediated	Nickols et al., 2003
Hormones			
Human prolactin	Pituitary GH3 cells	Reporter assay – effects of wtIKKβ, dnIKKβ, dnNEMO, truncated IkBα, or dnIKKα expression on promoter activity	Friedrichsen et al., 2006
Pituitary proopiomelanocortin (POMC)	Pituitary corticotroph cell line (AtT20)	Upregulation of mRNA levels by IκBα overexpression using Northern blot	Karalis et al., 2004
Miscellaneous			
Amyloid precursor protein	Rate cerebellum extracts, H9 and HeLA cell lines, embryonal carcinoma F9 cells	1. NFκB binding site present in promoter region 2. EMSA, supershift 3. Reporter assay – effects of p65 and p50 overexpression on promoter activity	Grilli et al., 1995
Tissue-type plasminogen activator (t-PA)	Neuroblastoma and glioblastoma cell lines	1. NFκ B binding site present in promoter region 2. EMSA, supershift 3. Reporter assay – test wt and NFκB site deletion in promoter	Lux et al., 2005
Myelin basic protein	Human oligodendroglioma cell line	1. NFκB binding site present in promoter region 2. EMSA, supershift (with HeLa cell extracts) 3. Reporter assay – test wt and mutated NFκB site in promoter	Huang et al., 2002
	DRG neuron and Schwann cell co-cultures	1. DNIκB expression decreases protein levels by immunoblot 2. Blocking NFκB activity or using murine cells lacking p65 attenuates myelination	Nickols et al., 2003
SNAP-25A	Rat brain	κB decoy DNA downregulates mRNA levels by microarray analysis	Qiu et al., 2004

[a]A precedence for direct binding interactions and cooperative transcriptional activation between NFκB and SP1 factors bound to closely adjacent sites (within 10 bp) exists at the HIV and IL-2R promoters (Pomerantz et al., 1989; Perkins et al., 1993).
Abbreviations: AMPA, alpha-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid; BDNF, brain-derived neurotrophic factor; Cox-2, cyclooxygenase-2; DRG, dorsal root ganglion; EMSA, electrophoretic mobility shift assay; IHC, immunohistochemistry; iNOS, induced nitric oxide synthase; nNOS, neuronal NOS; RPA, ribonuclease protection assay; RT-PCR, real-time polymerase chain reaction.

regulated within neurons, but have not yet been verified. In addition, several new targets of NFκB have been identified by studies within the CNS. These include the catalytic alpha subunit of cyclic AMP-dependent protein kinase A (PKA_{CA}). A promoter analysis indicates that PKA_{CA} could be a direct transcriptional target of NFκB, and the physiological relevance of regulation by NFκB is attested to by a decrease in forskolin-activated PKA_{CA}-dependent CREB phosphorylation observed in mice expressing the DNIκB inhibitor of NFκB (Kaltschmidt et al., 2006). Interestingly, the regulation of PKA_{CA} expression could also have important feedback implications for NFκB activity. PKA_{CA}-dependent phosphorylation of the p65 subunit strongly regulates the transactivation potential of NFκB by promoting its association with the CREB binding protein (CBP)/p300 (Zhong et al., 1997, 1998, 2002). Another potential target of NFκB is the enzyme glutamate decarboxylase (GAD65) that is involved in synthesis of the inhibitory neurotransmitter GABA. Both protein and mRNA for GAD65 were reported to be decreased in hippocampal lysates from mice expressing the DNIκB (O'Mahony et al., 2006). At present, it remains unknown whether transcriptional regulation of GAD65 expression is mediated directly by NFκB or is an indirect outcome of NFκB inhibition. The GAD65 promoter is TATA-less and contains multiple transcription initiation sites and transcription factor binding sites, but a putative NFκB consensus sequence has not yet been reported (Pinal et al., 1997)

Many additional genes which are reported to be regulated by NFκB appear likely to have significance in mechanisms of plasticity (see **Table 2**). Glutamate-responsive receptors and channels are known to contribute to multiple forms of plasticity, including LTP and LTD, and homeostatic and distance-dependent synaptic scaling. Several glutamate receptor subtypes are reported to be targets of NFκB. The regulatory regions of the gene encoding the GluR1 subunit of α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA)-responsive glutamate receptors contains a consensus binding site for NFκB and GluR1 protein levels can be upregulated by stimuli that activate NFκB. In addition, protein levels of GluR1 are substantially reduced in the cortex and hippocampus of mice lacking the p50 subunit of NFκB (Borges and Dingledine, 2001; Yu et al., 2002). While comparisons of gene expression in wild-type mice versus mice lacking the p50 subunit of NFκB have been very useful in demonstrating NFκB regulation of genes, it should be kept in mind that these experiments do not define which NFκB dimers (e.g., p50:p50 vs. p50:p65) are most critical for the endogenous regulation of the gene. Brain-derived neurotrophic factor (BDNF) has been implicated in many processes involving plasticity both in the mature nervous system and during CNS development. The expression of this growth factor is regulated by several activity-dependent transcription factors, including CREB and NFκB. Interestingly, BDNF is not only transcriptionally upregulated by NFκB, but also serves as an activator of NFκB (**Tables 1** and **2**). This type of feedforward loop is a recurring theme in NFκB regulation, with a classic example being the transcriptional induction of TNFα and TNFβ by NFκB and activation of NFκB through the TNF receptor. An additional example with relevance for the CNS is the transcriptional regulation of nitric oxide synthetase (NOS) by NFκB and the activation of NFκB by nitric oxide (**Tables 1** and **2**).

4.28.8 Neurological Disorders with Cognitive Deficits

Dysregulation of the NFκB activation pathway has been implicated in a number of CNS disorders that exhibit cognitive impairment (for a recent review see Mattson and Meffert, 2006). These include Alzheimer's disease, Huntington's disease, and Parkinson's disease, as well as depression and age-related cognitive decline. Whether the dysregulation of NFκB is a causative agent in the cognitive deficits associated with these diseases, or simply a by-product of activating neuronal cell survival pathways, is not yet understood. In some cases, as with the mutant huntingtin protein (Khoshnan et al., 2004), activated NFκB is reported to contribute to the neurotoxicity, whereas in many other examples NFκB activation appears to preserve neuronal function.

Increases in basal NFκB activation associated with aging have been observed in multiple regions of the CNS (Korhonen et al., 1997). Age-associated increases in NFκB activation have also been observed outside the CNS, including in liver and skin, where they have been linked with accelerated inflammatory processes. An exacerbation of age-related neural degeneration has also been reported in the cortex, hippocampus, and caudate-putamen of mice deficient in the p50 subunit of NFκB (Lu et al., 2006). Mice deficient in p50 also exhibit an acceleration of both age-related and noise-induced hearing loss. The hearing loss occurs concomitantly with

enhanced degeneration of the auditory nerve spiral ganglion neurons (SGNs) in p50 knockout mice compared to wild-type controls (Lang et al., 2006).

It is plausible that a role for NFκB in cell growth and plasticity could imply a complementary function in cell survival and the maintenance of cognitive function. A potential role for NFκB in disorders of cognitive function in which neurodegeneration is not the most prominent component is also emerging. A serial analysis of frontal cortex gene expression in patients with bipolar disorder has revealed that the expression of NFκB pathway components are elevated compared to an unaffected control population (Sun et al., 2001). A link between NFκB signaling pathways and Down syndrome (trisomy 21) has also been established by a group studying the human Down syndrome critical region 1 gene (DSCR1) (Kim et al., 2006). Overexpression of the DSCR1.4 isoform was found to increase basal levels of the IκBα inhibitor of NFκB, likely by diminishing the steady-state degradation of the inhibitor. This resulted in an attenuation of NFκB activation and a downregulation of the pro-inflammatory NFκB target genes, cyclooxygenase-2 and interleukin1β in a glioblastoma cell line. Interestingly, increased mRNA levels of DSCR1 are reported not only in patients with trisomy 21, but also in postmortem brain samples from Alzheimer's patients (Fuentes et al., 2000; Ermak et al., 2001) and in the penumbra area of mouse brain after focal cerebral ischemia (Kim et al., 2006).

4.28.9 Conclusions

The regulation of gene expression by the NFκB family of transcription factors is emerging as a widespread mechanism involved in shaping cognitive processes such as learning, memory, and reconsolidation within the CNS of both vertebrates and invertebrates. A definitive role for NFκB in *in vitro* assays of plasticity, primarily within the hippocampus, has also been established. Whether NFκB might participate in additional aspects of cognition such as awareness, judgment, and problem solving remains to be defined. The molecular mechanisms by which NFκB modulates plasticity is an exciting area that remains to be explored. However, as the list of NFκB-regulated genes within the CNS continues to grow, some attractive potential targets have already been described (**Table 2**). Because NFκB is ubiquitously expressed within the CNS, experiments directed at cell type–specific functions of NFκB should also be illuminating. Future research toward understanding the transcriptional requirements essential to cognitive processes will undoubtedly necessitate investigating the ways in which activity-regulated transcription factors such as NFκB, CREB, immediate early genes, and other transcriptional regulators function independently and in cooperation.

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4.29 Dendritic Transport of mRNA, the IEG Arc, and Synaptic Modifications Involved in Memory Consolidation

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4.29.1 Introduction

It is widely believed that persistent modifications of synaptic properties induced by activity underlie long-term memory storage in the brain (Kandel, 2001; Dudai, 2002; Martin and Morris, 2002). Understanding the mechanisms underlying such synaptic modifications at a molecular level is a prominent goal of modern neuroscience. Two assumptions guide current thinking regarding memory storage

mechanisms. First, synaptic modifications that are triggered by activity and underlie memory storage are thought to occur in a synapse-specific fashion based on the history of activity in that particular synapse. Thus, to understand synaptic plasticity, one must understand how synaptic weights can be stably modified independent of the state of neighboring synapses. Although synapse specificity is generally postulated, there is an alternative view that synapses are not independently modified during the storage of information in the brain, but instead, clusters of co-active synapses are jointly regulated, independent of the history of activity of individual synapses (Govindarajan et al., 2006). In either case, modifications occur selectively at sets of synapses that are in close proximity. Second, enduring synaptic modifications, including the ones that underlie long-term memory storage, are thought to require gene transcription and mRNA translation (Kandel, 2001). These assumptions motivate searches for mechanisms that could allow activity-dependent alterations of individual synapses or small clusters of synapses in a way that involves gene transcription and mRNA translation. Here, we review one potential mechanism, which involves activity-induced expression of the immediate early gene (IEG) Arc, delivery of Arc mRNA to dendritic domains contacted by synapses that have experienced particular patterns of activation, and local synthesis of Arc protein. This mechanism is of particular interest because recent studies have shown that Arc protein is involved in an important aspect of late-phase synaptic plasticity that appears to be required for at least some forms of memory consolidation.

We begin with a brief overview of mRNA localization and local translation in dendrites. These topics have been considered in other recent reviews (Steward and Schuman, 2003; Pfeiffer and Huber, 2006; Hirokawa, 2006; Schuman et al., 2006) and so we will focus here on core concepts that set the stage for a detailed consideration of Arc. We will then discuss how and when Arc is expressed in response to synaptic activity and behavior, and how Arc mRNA is transported into dendrites and targeted to active synapses, and then consider the functions of Arc protein. Throughout, we will point out unresolved issues or assumptions for which definitive evidence is lacking. Key questions and future directions in Arc research will be discussed, with special emphasis on the consequences of an mRNA targeting mechanism for inducing, maintaining, and modulating synaptic plasticity. Finally, we will outline a hypothesis about how Arc expression may play a role in memory consolidation.

4.29.1.1 Activity-Dependent Synaptic Plasticity, Receptor Alterations, and the Process of Consolidation

Mechanisms of synaptic plasticity have been extensively studied and are discussed in other chapters of this volume. Here, we will mention only the core concepts that are of particular relevance for our consideration of dendritic protein synthesis and Arc.

There is an emerging consensus that changes in synaptic efficacy at excitatory glutamatergic synapses in the brain occur initially as a result of changes in the number and characteristics of glutamate receptor subtypes, especially changes in the number and subunit composition of alpha-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA) receptors (Malenka and Bear, 2004). For example, increasing evidence suggests that induction of long-term potentiation (LTP) is accompanied by, and presumably mediated by, insertion of additional AMPA receptors into the postsynaptic membrane, which increases postsynaptic currents and postsynaptic signaling responses within minutes after stimulation. Similar changes occur within neurons of the amygdala after fear conditioning (Rumpel et al., 2005).

Similarly, induction of certain forms of long-term depression (LTD) is accompanied by, and presumably mediated by, removal of AMPA receptors from the postsynaptic membrane by endocytosis, which leads to decrements in synaptic efficacy (for a review, see Malenka and Bear, 2004). Importantly, if synapses are modified on an individual basis, then experience-induced insertion and removal of AMPA receptors must occur selectively at synapses that are interspersed with others that are unmodified. Indeed, this can be shown for neurons in the lateral amygdala that receive projections from the auditory thalamus. Using viral delivery of glutamate receptor 1 (GluR1) subunits with altered rectification, it was shown that after auditory fear conditioning only some synapses on a given neuron showed GluR1 insertion (Rumpel et al., 2005).

Key questions remain, however, about the mechanisms that allow changes in synaptic weights to either persist or be converted into more stable forms. Several models have been proposed (Dudai, 2002; Malenka and Bear, 2004). One model incorporates the apparent constitutive recycling of heteromeric GluR2-GluR3 AMPA receptors in the postsynaptic membrane and

postulates that this receptor population incorporates and replaces the newly inserted heteromeric GluR1-GluR2 receptors that were inserted in association with a potentiation event (Shi et al., 2001). In this way, synaptic homeostasis is re-established at a new, enhanced level of AMPA receptors in the postsynaptic membrane. This mechanism would require some gauge that independently records or represents the number of AMPA receptors present in the postsynaptic membrane at a given time (that is, some way to determine the homeostatic set point). One possibility is that a component of the postsynaptic density identifies slots into which AMPA receptors can be reinserted after recycling (Schnell et al., 2002).

Another model is based on the fact that particular patterns of activity trigger changes in synapse shape; the idea is that changes in shape are propagated stably to perpetuate alterations in synaptic weighting. The postsynaptic membrane specializations of excitatory glutamatergic synapses are typically found on the tips of dendritic spines. Spines possess high levels of actin but not microtubules, and changes in spine shape require the action of the actin cytoskeleton (Matus, 2000). It has long been known that alterations in spine shape accompany alterations in synaptic efficacy (Desmond and Levy, 1983), and new aspects of the story continue to emerge (Engert and Bonhoeffer, 1999; Fischer et al., 2000; Matsuzaki et al., 2004; Nagerl et al., 2004). Increases in postsynaptic density size that accompany the enlargement of spines would provide a scaffold for the insertion of additional AMPA receptors. That is, the initial structural change would drive the functional modification and allow the modification to be maintained. But again, there must be some way to propagate this new homeostatic state, and so both models may be aspects of the same underlying mechanism.

There is one important disconnect, however, in the linkage between changes in receptor number and/or synapse size and shape and persistent changes in synaptic efficacy. The changes in molecular and structural properties as well as changes in synaptic efficacy occur essentially immediately (or at most within a few minutes) after the triggering event. Yet modifications in synaptic efficacy remain labile for hours, and then undergo some transition so that they become more stable. This conclusion is based primarily on the fact that blockade of protein synthesis (and sometimes RNA synthesis) causes both LTP and LTD to degrade over time. We will use the term consolidation to refer to this transition from a labile to a stable state, with the cautionary note that the term was applied originally to memory storage (see, for example, McGaugh and Alpern, 1966), which may involve different mechanisms than are involved in the consolidation of synaptic plasticity. The investigations that point to the requirement for protein synthesis form a separate line of inquiry into the basis for persistent changes in synaptic efficacy, and it remains to be established how protein synthesis-dependent processes relate to the early changes in receptor number and synapse size and shape that mediate the essentially immediate changes in synaptic efficacy after an inducing event.

It is noteworthy that the early events underlying LTP and LTD and also behavioral memory become stable (that is, undergo consolidation) in a way that is generally seamless in terms of the readout. Once induced, persistent LTP and LTD are reflected by a new level of synaptic strength. In a similar fashion, behavioral memory is generally manifested by stably enhanced behavioral performance. This fact has probably led us to favor models in which the initial changes (increases or decreases in receptor number for example) are somehow cemented during consolidation. It remains possible, however, that the later protein synthesis-dependent events cause different modifications than are responsible for early stage plasticity. As we will see in the next section, new information on the function of Arc protein has raised new possibilities in this regard.

4.29.1.2 Protein Synthesis at Synaptic Sites on Dendrites: A Possible Mechanism for the Selective Modification of Individual Synapses

4.29.1.2.1 Identification of polyribosomes at subsynaptic sites

The discovery that polyribosomes were selectively positioned beneath synaptic sites on dendrites (Steward and Levy, 1982) triggered a new line of thinking about how individual synapses could be modified in a protein synthesis-dependent fashion. This discovery suggested a mechanism whereby synaptic activation could induce the translation of mRNAs within dendrites, and the newly synthesized proteins could then act locally to alter properties of activated synapses. Many lines of evidence converge to support this model and help define its importance and scope in the stable modification of synaptic properties during synaptic plasticity and learning and memory. The discovery of Arc opened a new dimension in thinking because Arc mRNA was

induced by activity and then delivered into dendrites. Thus, synaptic activity could lead to the positioning of Arc mRNA near activated synapses and newly translated Arc protein could act locally to alter the properties of activated synapses. Much remains to be established about how this is coordinated. In considering what is known and what remains to be established, it is useful to begin by considering the nature of the protein synthetic machinery at synapses and the mRNAs that are potentially present.

4.29.1.2.2 Components for protein synthesis in dendrites, including mRNAs

Since the discovery of synapse-associated polyribosome complexes (SPRCs), significant effort has been directed toward demonstrating that components needed for local translation exist in dendrites and that dendrites are indeed capable of synthesizing proteins independently of the cell body (Steward and Schuman, 2001). Other components of the translation machinery (initiation and elongation factors) have also been shown to be present in dendrites, as have components of the endoplasmic reticulum and Golgi complex, which would allow posttranslational modifications of integral membrane proteins and proteins destined for release (for a review, see Steward and Schuman, 2001). It is interesting that recent studies have further confirmed the presence of Golgi outposts in dendrites, but also showed that the Golgi vesicles were mobile (Horton and Ehlers, 2003).

Some of the mRNAs that are present in dendrites of neurons in vivo have been identified (for a more comprehensive discussion of the localization and distribution of mRNAs other than Arc, see Steward, in press). Transcripts that are easily detectable by *in situ* hybridization in dendrites of neurons *in vivo* comprise a functionally diverse set of proteins, including protein components of the postsynaptic signaling complex such as Ca^{2+}/calmodulin-dependent protein kinase IIα (CaMKIIα) (first reported by Burgin et al., 1990), Shank (Bockers et al., 2004), and Arc. The mRNA for microtubule-associated protein-2 (MAP2) is present in the proximal third to half of dendrites of many neurons (first reported by Garner et al., 1988). The mRNA for the elongation factor EF1a is present in many dendrites (Huang et al. 2005), as is the mRNA for dendrin, a protein of unknown function (Herb et al., 1997). The mRNAs for some glutamate receptor subunits, including the *N*-methyl-D-aspartate (NMDA) receptor subunit NR1 (Benson et al., 1991) and the AMPA receptor subunit GluR2 (Grooms et al., 2006), appear to be present at lower levels. A noncoding pol-III transcript called BC1 is also present in many dendrites (Tiedge et al., 1991). A different subset of mRNAs are present constitutively in the dendrites of Purkinje cells of the cerebellum, including the mRNAs for L7 (Bian et al., 1996) and the inositol triphosphate (IP3) receptor (Furuichi et al., 1993). Finally, in addition to Arc, brain-derived neurotrophic factor (BDNF) mRNA is delivered into dendrites following seizures that lead to epileptic circuits (Tongiorgi et al., 2004). Amplification of mRNAs harvested from dendrites of young neurons growing in culture have identified other potential dendritic mRNAs (Miyashiro et al., 1994; Moccia et al., 2003), but definitive evidence for dendritic localization in the CNS by *in situ* hybridization is lacking for most of these. It is not known how the translation of the different mRNAs is regulated. This is likely to be crucial, because there are only a small number of ribosomes at individual synaptic sites in mature neurons and this is likely to be rate limiting for translation (for a consideration of this issue, see Schuman et al., 2006).

Importantly, no mRNAs other than Arc have been shown to rapidly redistribute in response to synaptic activation. Only a few have been tested, however (the mRNAs for CaMKII, MAP2, dendrin, and EF1α; Steward et al., 1998; Huang et al., 2005) and so it remains to be seen whether Arc is entirely unique in terms of its relocalization in response to synaptic activation.

4.29.1.3 mRNA Translation in Dendrites

Several studies have demonstrated that dendrites are capable of synthesizing proteins independently of the cell body, and in response to treatments that induce changes in synaptic efficacy (Kang and Schuman, 1996; Huber et al., 2000; Aakalu et al., 2001). In these experiments, dendrites of either single neurons in culture or neurons in brain slice preparations were mechanically separated from the cell body and new proteins were shown to arise from the isolated dendritic segments. In addition, very slowly diffusing fluorescent protein reporters have been used to demonstrate protein synthesis in the dendritic compartment of neurons in culture (Aakalu et al., 2001), as has a novel bi-arsenic-based fluorescent compound that allows the *in situ* labeling of proteins possessing a particular tetracysteine motif (Ju et al., 2004). Although important for demonstrating protein synthetic capacity within dendrites, studies of translation of exogenous mRNAs do not necessarily recapitulate mechanisms of translational regulation of native mRNAs *in vivo*.

There have also been a number of studies of protein synthesis in subcellular fractions containing pinched-off dendrites called either synaptodendrosomes or synaptoneurosomes depending on the method of isolation. Synaptodendrosomes are isolated by differential centrifugation in sucrose gradients (Rao and Steward, 1991), whereas synaptoneurosomes are prepared by filtration (Weiler and Greenough, 1991). Both types of fractions contain pinched-off nerve terminals that are connected to small pinched-off dendritic fragments and/or spines, which appear as snowman figures at the electron microscopic level. The fractions are similar, but can be contaminated by resealed fragments of neuronal and glial cell bodies (Rao and Steward, 1993). Nevertheless, when proper care is taken to ensure preparations are not contaminated (Bagni et al., 2000), the preparations can provide valuable information about the mRNAs that are present in dendrites (Rao and Steward, 1993) and the proteins that are locally synthesized (Rao and Steward, 1991).

Studies involving synaptodendrosomes and synaptoneurosomes have been especially useful for assessing how dendritic protein synthesis is influenced by depolarization (high potassium, for example) and bath application of neurotransmitters and growth factors (Weiler and Greenough, 1991, 1993). Measures include biochemical studies of metabolically labeled proteins (Villanueva and Steward, 2001a,b; Leski and Steward, 1996) and the degree of ribosome loading onto particular mRNAs (Zalfa et al., 2003).

An important caveat is that bath application of neurotransmitters may activate both synaptic and nonsynaptic receptors, whereas physiological release activates primarily the receptors present at synapses. This is important because recent studies have shown that activation of synaptic versus nonsynaptic receptors triggers quite different molecular cascades in the postsynaptic cell (Zhang et al., 2007).

4.29.1.3.1 Proteins associated with mRNA in dendrites

Both the delivery of mRNA into dendrites and the translation of mRNAs within dendrites are thought to be regulated by mRNA-binding proteins. The prevailing view is that mRNAs possess discrete sequences, usually in the 3′ untranslated region (3′UTR), that mediate dendritic transport (called dendritic transport sequences). Some RNA-binding proteins have been identified that bind to the dendritic transport sequences with some degree of selectivity (for a recent review, see Wells, 2006). This review focuses on the possible role of mRNA-binding proteins in translational repression, but some of these mRNA-binding proteins may play a role in dendritic transport. To our knowledge, however, there has been no compelling evidence that any particular mRNA-binding protein plays an obligatory role in the dendritic transport of any mRNA.

Clearly, a number of proteins associate with mRNAs during translation, including various initiation and elongation factors, and there is evidence for a role of these proteins in synaptic plasticity (Klann and Dever, 2004). Other mRNA-associated proteins may act as regulators of translation, for example cytoplasmic polyadenylation element binding factor (CPEB) (Wu et al., 1998) and fragile X mental retardation protein (FMRP) (Zalfa et al., 2003). A thorough consideration of how mRNA-binding proteins regulate dendritically localized mRNAs is beyond the scope of the present review, but it is noteworthy that several mRNA-binding proteins have been implicated in synaptic plasticity, especially FMRP (Valentine et al., 2000) and CPEB (Wells et al., 2001).

4.29.1.3.2 Evidence that local translation at synapses is important for synaptic function

One of the mRNAs that is abundant in dendrites is the mRNA for the alpha subunit of CaMKII, and several lines of evidence strongly suggest that CaMKIIα protein is actually synthesized from dendritically localized mRNA (Ouyang et al., 1999). But is local synthesis critical? In order to assess the function of dendritically synthesized CaMKIIα protein, Miller et al. (2002) created transgenic mice in which the dendritic targeting of CaMKIIα mRNA was abrogated by replacing the 3′UTR, which contains the dendritic targeting sequence, so that a fusion transcript would be produced that contains the 3′UTR of bovine growth hormone and the coding region and 5′UTR of CaMKIIα. As expected, the transcript encoded by the transgene remained in the cell body rather than being delivered into dendrites. While total levels of CaMKIIα protein were somewhat reduced in these mice, levels of CaMKIIα protein in the postsynaptic density (PSD) were reduced by about half. Tests of LTP revealed subtle but definite deficits in the late phase of LTP in hippocampal slices, and subtle deficits in spatial learning, associative fear conditioning, and object recognition learning. One caveat of this experiment, however, is that the decrease in steady-state levels of CaMKIIα at the synapse might

alter signal transduction competence (Steward, 2002). Thus, it remains unclear whether it is the absence of local synthesis of CaMKIIα or a change in CaMKIIα-mediated signal transduction at synapses that accounts for the deficits in LTP and memory.

4.29.1.3.3 Evidence that protein synthesis is required for late-phase long-term potentiation, long-term depression, and memory

One of the strengths of the local protein synthesis model has been the concordance of the many studies demonstrating requirements for dendritic protein synthesis in both long-term memory and synaptic plasticity (Steward and Schuman, 2003; Pfeiffer and Huber, 2006; Schuman et al., 2006). A thorough review of this extensive literature is beyond the scope of the present chapter, but there is general consensus that both memory and many forms of synaptic plasticity have a short-lived, protein synthesis-independent phase, and a longer phase that can be blocked by inhibiting protein synthesis (for a review, see Kandel, 2001).

One weak link in the logic chain, however, is that to block the late expression of synaptic plasticity or memory, protein synthesis inhibitors have to be present at the time of initial stimulation or very soon thereafter (Frey et al., 1988). Inhibition protein synthesis later on is without effect, even though it is during the later period that LTP and LTD decay in the presence of protein synthesis inhibitors. One possible interpretation of these findings is that the critical protein synthesis occurs at the time of stimulation, but that the newly synthesized proteins do not actually act until sometime after the triggering event. In this model, the essential products would act to maintain changes in synaptic efficacy and consolidate long-term memories, but would not play a role in the initial changes in synaptic efficacy, even though the proteins are synthesized near the time of stimulation.

Other recent experiments suggest that the persistence of LTP after treatment with protein synthesis inhibitors depends on the exact testing protocol (Fonseca et al., 2006). In this study, protein synthesis inhibitors were applied to acute brain slice preparations during tetanic stimulation of Schaffer collateral-cornu ammonis area 1 (CA1) synapses. The degree of blockade of LTP caused by these inhibitors was found to be dependent upon the frequency of test stimulation delivered subsequent to tetanization. Thus, the resulting level of stable potentiation depends upon events (test stimulation) that occur soon after tetanization (less than 1 h). These findings reveal that protein synthesis normally acts around the time of tetanization to prevent the decay of potentiation in the face of subsequent stimulation.

4.29.1.4 Are the Proteins Synthesized by Synapse-Associated Polyribosomes Targeted Selectively to an Individual Synapse or Are They Distributed More Widely?

The selective positioning of SPRCs beneath synaptic sites invites the speculation that locally synthesized proteins could be targeted selectively to an individual synapse (Steward and Levy, 1982). There is no compelling evidence one way or another about this hypothesis, however. Indeed, if all synapses are equally modifiable, then either protein synthetic machinery (polyribosomes) would have to move from synapse to synapse or the protein that is synthesized would have to be available to more than one synapse (see later discussion in the section 'Arc and synaptic homeostasis,' as well as Schuman et al., 2006).

There is evidence for a phenomenon called synaptic tagging, the properties of which suggest the possibility that patterns of activity at one synapse can create some sort of tag that enables the synapse to capture molecules that are produced as a result of activity of other, perhaps distant synapses (Frey and Morris, 1997; Sajikumar and Frey, 2004; reviewed in Martin and Kosik, 2002; Fonseca et al., 2004). In one approach, it was shown that weak patterns of synaptic stimulation that do not produce long-term enhancement of synaptic transmission can cause long-term enhancement when paired with strong stimulation of other synapses. The strong stimulation can either precede or follow the weak stimulation. The interpretation put forward for these results is that strong stimulation causes the production of plasticity factors, which are necessary and sufficient for converting transient changes in synaptic efficacy into stable ones. The idea is that these plasticity factors are distributed in the general vicinity of strongly stimulated synapses, and that synapses that have experienced a particular pattern of activity capture the plasticity factors through the use of a tag which is envisioned to act as a docking signal set up only at active synapses.

An interesting twist is that weak stimulation that would typically lead to transient depotentiation of

synaptic response can indeed cause stable depotentiation when paired with not only another strongly depressing stimulation pattern, but also a strongly enhancing stimulation pattern (Sajikumar and Frey, 2004). In other words, when they act, factors produced in response to strong stimulation preserve the direction of synaptic change at separate synaptic inputs at which a tag has been generated by weak activation. Thus, by implication, the sets of plasticity factors induced by LTP and LTD are the same, and this common pool contains factors that persistently enhance and persistently decrement synaptic efficacy.

In its purest form, the synaptic tagging hypothesis is agnostic as to where and how the putative plasticity factors are produced, or even what the factors are. The critical idea is that the factors are not tightly restricted to the synapses whose activity triggered the production of the factors; instead, the factors are available to other synapses and captured as a result of an activity-dependent tag. The conversion of short to long LTP at weakly activated inputs is blocked by protein synthesis inhibitors, however, suggesting that either the tag or the plasticity factors are newly synthesized proteins. If the factors are newly synthesized proteins, they could be synthesized either in the cell body or in dendrites. If the factors are the products of dendritic protein synthesis, then this would imply that proteins synthesized at one synapse would be available to other synapses. If so, then synapses within a particular dendritic domain may compete for locally synthesized proteins. At least some of the proponents of the synaptic tagging hypothesis consider it as an alternative hypothesis to the idea that critical molecules are synthesized locally at synapses. Also, it has been shown that antidromic stimulation is effective at rescuing LTP at weakly activated synapses on CA1 pyramidal cells in a manner similar to what occurs with strong synaptic activation (Dudek and Fields, 2002). This finding does not exclude the possibility that the critical factors are produced in dendrites (there could be back-propagating dendritic spikes, for example). In any case, there is not yet definitive evidence on the molecular nature of either the plasticity factors or the tag, and so the site of production of the factors remains open to debate.

There is in fact evidence for some form of competition for newly synthesized proteins during LTP induction that has come from experiments assessing synaptic plasticity after varying periods of protein synthesis inhibition (Fonseca et al., 2004). In these experiments, two independent pathways in the CA1 region of the hippocampus were tested. One was weakly tetanized and then reactivated, and the other was a strongly activated test pathway. As in the synaptic tagging experiments detailed previously, simultaneous delivery of the strong and weak stimulation resulted in LTP at both the reactivated pathway and test pathway. Subsequent weak stimulation of the reactivated pathway led to further potentiation of this pathway. However, when the preparation was treated with anisomycin while the reactivated pathway was stimulated again, the reactivated pathway was still potentiated but the test pathway was simultaneously decremented. The interpretation put forward for this result is that there is a limited supply of plasticity factor(s) that act, and redistribute, over the course of several hours, and that synapses compete for the factor(s).

4.29.2 Arc: A Rosetta Stone for Understanding Mechanisms of Synaptic Plasticity

With this general background, we can now consider how Arc fits into the story. Arc (also known as Arg 3.1) was initially discovered in two independent screens for IEGs, defined as genes whose transcription was induced by neural activity in a protein synthesis-independent manner (Link et al., 1995; Lyford et al., 1995). In both these experiments, electroconvulsive seizures were induced in animals that had been pretreated with protein synthesis inhibitors. Then mRNAs whose transcription was upregulated were identified through differential hybridization of mRNA from seizure-treated and control animals. A number of novel IEGs involved in synaptic structure and function were identified, including Arc, Homer, and NARP; these have been called effector IEGs because they have direct functions in contrast to IEG transcription factors such as c-fos and zif268 that induce transcription of other genes. Arc was unique, however, because its mRNA was rapidly delivered into dendrites, whereas the mRNAs for all other IEGs remained tightly localized in the neuronal cell body. Lyford et al. (1995) coined the name activity-regulated cytoskeleton-associated protein (Arc) based on biochemical studies that revealed that Arc protein remained in detergent-insoluble extracts in a manner similar to cytoskeletal proteins. Link et al. (1995) called the same gene activity-regulated gene 3.1 (Arg 3.1).

In addition to identifying Arc, these two studies provided the first evidence that Arc mRNA was delivered into dendrites, where it might be locally translated. A reasonable speculation is that translation occurred at synapse-associated polyribosome complexes, but this has not been shown definitively. Subsequent studies revealed that the Arc gene has an additional layer of regulation that at least so far has not been shown for other dendritically localized mRNAs in that Arc mRNA localizes selectively in dendritic domains contacted by recently activated synapses (Steward et al., 1998). The presence of at least two and possibly three independent mechanisms controlling the spatial domain of Arc protein expression (delivery into dendrites, localization at or near active synapses, and local translation) provides a compelling mechanism whereby Arc protein could be synthesized in local domains very near individual synapses that have recently experienced particular patterns of activation and ultimately targeted selectively to the active synapses. As will be discussed further, different parts of the mechanism have been demonstrated, but there is not yet direct evidence for selective targeting of either Arc mRNA or Arc protein to individual synapses or local regulation of Arc mRNA translation.

4.29.2.1 Activation of Arc Transcription by Synaptic Activity and Behavior

In the initial study by Lyford et al. (1995), it was shown that Arc transcription was strongly induced by strong synaptic activation (for example, the induction of LTP) and that levels of expression in the cerebral cortex are influenced by sensory experience (closure of one eye results in downregulation of Arc mRNA in the visual cortex). Subsequent studies have built upon these initial findings to show that Arc transcription is strongly induced in a number of behavioral settings, including ones that involve learning. Indeed, Arc mRNA and protein have been used to identify brain regions and neuron types that are activated during attentive brain states or experiences associated with learning. These experiments have informed us about where, when, and how Arc transcription in the brain links with experience of the external world.

A thorough review of these studies is beyond the scope of the present work, but selective consideration allows us to highlight general conclusions and unresolved issues. Studies of behavioral induction of Arc transcription also provide clues about what Arc does in the cells that express it and why and how Arc expression is induced. In the next two sections, we first consider what behavioral and neurophysiological induction tells us about the function of Arc and second, how patterns of induction of Arc expression inform us about neuronal subsets used to encode, and perhaps store, aspects of sensory–behavioral experience.

Initial experiments indicated that Arc transcriptional induction was rapid, in the manner of other IEGs. In these original studies, it was shown that a brief seizure or brief high-frequency stimulation of the perforant path *in vivo* led to Arc mRNA accumulation in the nucleus and cell body within 30 min (Link et al., 1995; Lyford et al., 1995). Subsequent studies using high-resolution fluorescent *in situ* hybridization (FISH) revealed that transcriptional induction began rapidly; 5 min after introducing a rat to a novel spatial environment, two discrete foci of Arc mRNA (the sites of Arc transcription) were evident in the nucleus of neurons in the CA1 region of the hippocampus, and newly synthesized Arc mRNA appeared in the cytoplasm within 20 min (Guzowski et al., 2005). The epochs of transcription induced by experience were brief, lasting as little as 16 min in CA1 neurons. This is in contrast to what is seen in the dentate gyrus following LTP or single seizures, where high levels of Arc mRNA persist for hours after a single episode of activity (Lyford et al., 1995; Wallace et al. 1998). It remains to be established whether the persistence of Arc mRNA in the dentate gyrus reflects continued transcription or delayed Arc mRNA degradation (the former is more likely).

4.29.2.2 Expression of Arc Following Stimulation Patterns that Lead to Long-Term Potentiation

In the studies that initially identified Arc, it was also shown that Arc transcription is strongly induced in dentate granule cells following induction of perforant path LTP *in vivo*. This has been confirmed in several subsequent reports. Other IEGs are also induced following induction of perforant path LTP (Abraham et al., 1993; Worley et al., 1993), although the threshold for induction for the different IEGs is somewhat different (Worley et al., 1993; see also Waltereit et al., 2001).

Although Arc transcription is typically induced in conjunction with perforant path LTP, the relationship between LTP induction and Arc expression is not completely established. For example, induction

of perforant path LTP in anesthetized rats by delivering thirty 8-pulse trains at 400 Hz reliably induces Arc transcription, whereas LTP can be induced by three 50-pulse trains at 250 Hz without accompanying induction of Arc expression in dentate granule cells (Steward et al., 2007). The LTP that is induced by 250-Hz stimulation persists for up to 5 h, but it is not known whether LTP that is induced in the absence of IEG expression persists for days and even months, as is true of the LTP induced by 400-Hz stimulation (for a review on LTP persistence, see Abraham, 2003).

It should be noted that Arc is induced if 250-Hz trains are delivered repeatedly for more prolonged periods than are necessary to induce perforant path LTP (Steward et al., 2007). It is noteworthy that a different study reported that induction of perforant path LTP with three trains of 50 pulses at 250 Hz led to increases in Arc protein in the stimulated dendritic lamina 2 and 4 h after inducing LTP, as assayed by electromagnetic immunocytochemistry (Rodriguez et al., 2005). There are concerns about these findings, however, because labeling was seen in both dendrites and glial processes, yet there is no evidence that glia express Arc mRNA. In any case, it seems that experimental details may determine whether or not Arc is induced in conjunction with perforant path LTP. Additional studies will be needed to resolve this question.

A relationship between LTP induction and Arc transcription is even less clear in other situations. For example, there have been no reports of induction of Arc transcription following induction of LTP in hippocampal slices. A technical complication may explain the lack of data, however. Arc and other IEGs can be strongly induced during preparation of the slice (Taubenfeld et al., 2002). In this case, high levels of Arc present as a result of preparing the slice might mask subsequent induction by synaptic stimulation. Indeed, one study (French et al., 2001) does report increases in Arc mRNA levels in hippocampal slices in the absence of LTP and no increase in Arc mRNA following LTP induction. Thus, a relationship between hippocampal LTP and Arc transcription remains to be established.

4.29.2.3 Behavioral Activation of Arc Expression

Although evidence linking Arc transcription and different forms of LTP is not compelling or extensive, there is ample evidence that Arc transcription is strongly induced by behavioral experience. Arc is strongly induced in cells throughout the forebrain by simple exposure to a novel environment (Temple and Steward, 2003), and in particular cell populations following training in any of a number of tasks (see following). In specific training paradigms, the neurons that express Arc differ to some extent. In general, however, one is impressed more by the similarities in expression patterns in different settings than the differences.

Can Arc induction serve as a marker of neurons that are engaged during particular behavioral experiences? This general question has been considered previously in terms of other IEGs. A comprehensive review of this area is beyond the scope of the present chapter (but for an example of the approach, see Gall et al., 1998). The benefits of using IEG transcription to monitor neural activation in the brain include: (1) the ability to assay the entire brain in a single experiment, (2) the ability to interrogate essentially every cell in a given section, (3) the presence of a defined and uniform readout of neural activation of an essentially digital nature, and (4) the possibility that IEG expression reflects not merely neural activity but neuronal activation that accompanies long-term changes in neural transmission. Indeed, many studies were probably motivated originally by the hope that Arc induction might mark neurons that were engaged in learning.

The first question is whether Arc expression, or the expression of any other IEG for that matter, reflects overall levels of neuronal activity (number of action potentials per time unit) or some other aspect of neuronal activation, for example, a sufficient level of synaptic activation to trigger a postsynaptic signal transduction cascade. The latter idea is embodied in the concept of a genomic action potential in which particular patterns of activity trigger signal transduction cascades that broadly activate gene expression (Clayton, 2000). The answer to this simple question is not entirely clear. Detailed studies comparing the number of neurons in which Arc transcription is activated versus the number of neurons activated by spatial environments have revealed that similar proportions of neurons in the CA1 region of the hippocampus respond transcriptionally and physiologically to exploration of a novel spatial environment (Guzowski et al., 1999). Indeed, this correspondence extends to cases in which two different environments are presented sequentially. In these experiments, similar fractions of neurons respond to the first environment, the second, or both using

either Arc transcription or place cell firing as a readout. At first pass, this might be interpreted as indicating that transcriptional activation of Arc is related to simple neuronal activity. But there is evidence against this interpretation. For example, repeated exposure to an environment over a single day reduces the percentage of hippocampal neurons in which Arc is induced, whereas the number of cells that respond physiologically is similar (Guzowski et al., 2006). Thus, Arc expression is not simply a readout of neural activity of place cells, implying that the coupling between neural activity and Arc expression is not direct.

So, what types of behavioral experience activate Arc transcription? The simplest experiment is one in which animals are taken out of their home cage and allowed to explore a novel environment (Temple and Steward, 2003). This experience strongly activates Arc transcription in neurons throughout the forebrain. Thus, one interpretation is that Arc is induced as a result of any novel experience.

Although Arc expression is strongly induced in neurons throughout the forebrain by exposure to a novel environment, the relationship between novelty and Arc induction may be complex. Indeed, the threshold for activating Arc appears to vary by task and the number and spacing of repetitions. Repeated exposure to the same environment, when separated by 1 day between exposures, activates the same portion of cells in the hippocampus as a single exposure (Guzowski et al., 2006). Thus, in this case novelty *per se* is not a prerequisite for Arc expression. At the same time, as noted above, repeated exposure to an environment over a single day reduces the percentage of hippocampal neurons in which Arc is induced (Guzowski et al., 2006). There are several interpretations of this finding, including that the animal recognizes that the environment is not novel when repeated exposures occur over the course of a single day, or that there is some refractory period for repeated genomic activation that prevents full transcriptional activation when repeated exposures are closely spaced in time.

Arc transcription is also activated following explicit training in a variety of different learning tasks. Arc is preferentially induced in newly trained verses overtrained animals in both an operant lever-pressing task (Kelly and Deadwyler, 2002) and the Morris water maze (Guzowski et al., 2001). Similarly, Arc is induced after two training sessions in an *in vitro* eyeblink conditioning preparation but not after five sessions (Mokin et al., 2006). These differences in induction may reflect the novelty of the task or the stage of learning (early learning vs. reinforcing a previously learned association).

Although Arc induction is useful for mapping cells activated by various patterns of neural activity, Arc is not unique in this regard. Many studies of this type were undertaken with other IEGs (for reviews, see Herrera and Robertson, 1996; Tischmeyer and Grimm, 1999). Nevertheless, the unique pattern of Arc mRNA localization within dendrites suggests that Arc may be particularly useful for monitoring brain states associated with long-term changes in synaptic properties. Moreover, Arc might be a better choice for monitoring learning-induced changes in the brain than other commonly used IEGs. Comparison of the expression levels between Arc, c-fos, and zif268 after spatial, cued, and reversal variants of the Morris water maze suggests that induction of Arc is more closely associated with learning tasks than induction of the other two IEGs (Guzowski et al., 2001). Also, comparisons of the expression of Arc versus that of other IEGs is useful because comparisons between IEG expression patterns can help define the nature of the genomic response to neural activity. For example, in most, perhaps all situations, there are multiple genomic responses to neural activity, and it remains to be established whether these are independent or form a related series (Clayton, 2000; Guzowski et al., 2001).

The approach of assessing behavioral induction of a single IEG suffers from some drawbacks, including a lack of within-subject comparisons (leading to a lack of precision in comparing control and experimental populations) and an inability to discern the degree of overlap in expression profiles induced by experiences that differ only in a few key details (Guzowski et al., 2005). These problems have been circumvented in part through the use of an *in situ* hybridization protocol that captures expression patterns from two distinct, though recent, time periods in the past. Because this protocol typically uses Arc expression as a readout, precise localization patterns and subtle aspects of neural encoding have been associated with the expression of Arc, but not other IEGs. This technique, termed catFISH (for cellular compartment analysis of temporal activity by FISH), relies upon the brief pulse of Arc transcription induced by discrete behavioral–sensory experiences together with high-resolution *in situ* hybridization to follow the temporal progression of Arc transcript maturation and nucleocytoplasmic transport at the subcellular level in tissue sections (Guzowski et al.,

1999). Newly synthesized Arc mRNA can be detected in the nucleus as paired transcriptional foci. Twenty minutes after induction, the newly synthesized mRNA begins to exit the nucleus to enter the cytoplasm. Labeling of nuclear foci and cytoplasmic structures by Arc mRNA can be easily distinguished by confocal microscopy. Thus, expression within individual cells can be associated with either recent activation (nuclear labeling only), activation at least 20–30 min previously (cytoplasmic labeling), or both.

This technique has not only identified brain regions differentially responsive to closely related behavioral–sensory experiences, but has also been used to extract general principles of neural processing in the active brain regions. Using Arc CatFISH, it was shown that different environments are encoded by overlapping, but not identical, subsets of hippocampal pyramidal neurons in CA1 (Guzowski et al., 1999). Similar studies demonstrate differences between CA3 and CA1 in encoding the spatial environment, with CA1 exhibiting a more graded transition in response to changes in the spatial environment and CA3 exhibiting a more discontinuous response (Vazdarjanova and Guzowski, 2004). CatFISH was also used in the experiments demonstrating that Arc expression can be decoupled in part from neural activity during spatial exploration if the environment is repeatedly presented to the rat on the same day (Guzowski et al., 2006). Arc CatFISH has also been used to reveal the extent to which cortical layers in the prefrontal and gustatory cortex are responsive to spatial context (Burke et al., 2005). In this case, the more superficial layers were preferentially activated during context discrimination.

Arc expression can be used not only to determine where aspects of behavioral tasks might be processed in the brain, but also when. After exploration of a novel environment, Arc protein is reinduced between 8 and 24 h after initial behavioral induction (Ramirez-Amaya et al., 2005). Reinduction was accompanied by close correlation of Arc expression between brain regions, suggesting that network reactivation underlies this late phase of Arc induction and enabling Arc to participate in network-driven memory consolidation processes. Others have identified two phases of Arc induction in mice in response to a one-trial novel gustatory stimulus (Montag-Sallaz et al., 1999) and a one-trial inhibitory avoidance task (Montag-Sallaz and Montag, 2003).

Other IEGs are induced together with Arc, so what do the patterns of co-expression tells us about the existence and nature of the genomic program(s) induced by activity? Early studies demonstrated that all IEGs are not induced to the same extent by neurons activated by LTP-inducing stimulation delivered *in vivo*, with zif268 requiring fewer stimulus trains than junB, c-jun, or c-fos (Worley et al., 1993; Abraham et al., 1993). Similarly, during induction of perforant path LTP, Arc is induced by fewer stimulus trains than c-fos (Waltereit et al., 2001). As mentioned above, Arc expression is dissociable from and may be more closely associated with learning-associated tasks than zif268 or c-fos expression (Guzowski et al., 2001). While expression of zif268, c-fos, and Arc after water maze training are correlated in the entorhinal and visual cortices, and expression of Arc and zif268 are correlated in the hippocampus as well, the degree to which each of these pairs of genes is correlated varies widely (Guzowski et al., 2001). In chick, Arc and zif268 are both induced by auditory imprinting, but in different regions (Bock et al., 2005). Together, these studies indicate that there is not a unitary program induced by activity and suggest a degree of independence and cell type specificity to programs that induce IEGs in the brain. In contrast, Arc is coexpressed with Homer 1a to a very high degree, strongly suggesting that a single genomic program induces both of these genes (Vazdarjanova et al., 2002).

4.29.2.4 Signal Transduction Pathways Responsible for Arc Induction by Synaptic Activation and Behavior

Having summarized the situations in which Arc transcription is induced, it is useful to briefly summarize what is known about the signal transduction cascade(s) that actually trigger the transcriptional activation. We focus here on experiments in intact animals where there is a greater probability that the signal transduction cascades that are identified are actually the ones that are engaged physiologically.

The foundation for the first and most definitive conclusion comes again from the initial study that identified Arc (Lyford et al., 1995), which showed that Arc induction following seizures is blocked by NMDA receptor antagonists (MK801). There is a confounding factor with the approach of delivering antagonists systemically, however, because the antagonist is likely to affect neural activity generally (for example, reducing the duration of a seizure). Thus it is important to explore the question in situations where the pattern of activity used to induce Arc transcription

is closely controlled. A critical role for NMDA receptor activation has been confirmed in subsequent studies involving activation of glutamatergic pathways. For example, activation of Arc transcription in perforant path LTP is completely blocked by NMDA receptor antagonists (Steward and Worley, 2001a). Signal transduction pathways downstream from the NMDA receptor remain to be identified.

It is unclear whether nonglutamatergic neurotransmitter systems activate Arc independently of the NMDA receptor and if so, what signal transduction pathways are involved. A comprehensive review of this question is beyond the scope of the present chapter, but it is clear that the issue may be hard to parse out in the intact brain. To give a single example, local injections of agonists for beta-adrenoreceptors into the amygdala activate Arc expression in the hippocampus (McIntyre et al., 2005). However, this is likely due to muscarinic activation of glutamatergic neurons in the amygdala, which then synaptically activate hippocampal neurons. In this case, Arc transcription could ultimately be activated via NMDA receptors on hippocampal neurons. Elucidating the role of different neurotransmitter and receptor subtypes will likely require studies *in vitro*, but the problem in this case will be translating the conclusions to physiological settings *in vivo*.

4.29.2.5 Dendritic Transport of Arc mRNA

Once induced, Arc mRNA is rapidly delivered into dendrites. Because Arc is expressed as an IEG, it is possible to roughly define the time course of transport of Arc mRNA *in vivo* by killing animals at different times after a stimulus and determining the distribution of newly synthesized mRNA over time. These studies have provided important information about the time course of changes in overall mRNA distribution *in vivo*. Recent studies using live cell imaging *in vitro* have provided more detailed information about the movement of individual packets of mRNA. As described in the next section, delivery is mediated by a dendritic transport mechanism that moves Arc mRNA at a variety of rates, some of which strongly suggest a microtubule-based transport mechanism. Whether the process of dendritic transport is regulated remains to be determined.

4.29.2.5.1 Delivery of Arc mRNA to dendrites in vivo

The original estimates of the rate of movement of Arc mRNA into dendrites were derived by inducing Arc expression with an electroconvulsive seizure (ECS) and assessing the distribution of Arc mRNA over times using *in situ* hybridization (Wallace et al., 1998). These experiments indicated that Arc mRNA moved from the nucleus to the distal tips of dendrites of dentate granule cells (approximately 300 μm) within 60 min, indicating a minimal rate of 300 μm/h. It was recognized that this was an underestimation, however, because of the time required for Arc mRNA to be transcribed and undergo nucleocytoplasmic transport (approximately 20–30 min; see earlier). This paper also revealed that Arc mRNA was delivered into dendrites in the presence of protein synthesis inhibitors, indicating that dendritic delivery did not require sequences within the nascent protein and that dendritic transport was determined by sequences within the Arc mRNA itself. As will be discussed further, signals that mark Arc mRNA for dendritic delivery are localized in the 3′UTR, as is also true for most other mRNAs that are localized in different cell types (for a review, see Steward and Singer, 1997).

4.29.2.5.2 Multiple rates of transport of Arc mRNA-containing granules

We have recently used an exogenous expression approach to directly visualize the transport of transcripts containing the Arc 3′UTR (Dynes and Steward, 2007). In these experiments, the localization of messages from transiently transfected constructs was visualized in living rat cortical neurons by way of coexpressed green fluorescent protein (GFP)-MS2 phage coat protein fusions, which bind to an MS2 binding site inserted into the transcribed sequences containing the Arc 3′UTR. These exogenously expressed transcripts assemble into particles, which we refer to as Arc/MS2 mRNA particles. By following the movement of Arc/MS2 mRNA particle movement in unstimulated cortical neurons *in vitro* using live cell imaging, it was shown that Arc/MS2 mRNA particles are transported bi-directionally at a variety of rates. If Arc mRNA particles were to move continuously at the most rapid rate seen in these experiments (almost 70 μm/min), they could move from the nucleus to a synapse hundreds of micrometers distant within minutes, which is much faster than previous estimates would suggest. This rapid movement could allow transcription and translation-dependent synaptic modifications to occur much faster than previously imagined.

Because of differences in experimental parameters (such as neuron type and maturity, image acquisition rate, incubation temperature), direct comparisons of

data from our study with data from other studies of dendritic mRNA transport are difficult. Moreover, because a continuous range of velocities was seen, measures of central tendency (either mean or median) may not be very meaningful. Nevertheless, some general qualitative comparisons are reasonable. The mean and maximum instantaneous velocities calculated for Arc/MS2 mRNA particles differ from those reported for CaMKIIα mRNA (four- to sixfold slower than Arc; Rook et al., 2000) and the actin mRNA-binding protein ZBP1 (four- to sixfold faster than Arc; Tiruchinapalli et al., 2003). However, transport rates for Arc are not markedly different from those for complexes containing the mRNA binding proteins Staufen (Kohrmann et al., 1999), FMRP (De Diego Otero et al., 2002), CPEB (Huang et al., 2003), and RNA particles of unknown type labeled with syto 14 (Knowles et al., 1996). It is noteworthy that complexes that contain KIF5A and Pur α also move more slowly (maximum of 7.4 μm/min) and for shorter distances (maximum of 8 μm) (Kanai et al., 2004) than the Arc mRNA containing particles. Possibly, the KIF5A motor may be used by Arc mRNA particles for the slow, local phase of movement (see following).

Analysis of Arc/MS2 mRNA particle movement revealed the existence of several phases of movement. Although transport velocities for orthograde and retrograde Arc/MS2 mRNA were similar, orthograde translocations were typically longer than retrograde translocations. This suggests the use of multiple types of motor proteins or multiple modes of motor regulation. Arc/MS2 mRNA particles also moved slowly and progressively on the submicron scale. The fast and slow movements occurred at rates that are consistent with microtubule and actin- or myosin-based transport, respectively, but there was also a continuous distribution of velocities that were intermediate between what is considered typical for microtubule-based and actin- or myosin-based mechanisms.

Our experiments using the MS2 system were done in the absence of stimulation, and so there was no explicit signal to cause Arc mRNA targeting and docking at active synapses (see following). Indeed, at the ages examined (9–16 days of age), synapses are just forming on the dendrites. Nevertheless, some Arc/MS2 particles stopped during the recording period, and the behavior of Arc/MS2 mRNA particles as they stopped was suggestive of some form of local targeting. Nearly half of the particles that stopped during the recording period exhibited stereotyped reversals in direction just before stopping. These movements were nonrandom in direction, slow, and progressive, and thus are difficult to explain as being due to random dissociations from the transport machinery. In addition, none of the particles exhibiting stereotyped reversals before stopping resumed rapid transport during the rest of the imaging session. While this behavior is consistent with some final targeting based on local cues, it may also result from asymmetries in the cytoskeleton in the vicinity of stopping mRNA particles.

It remains to be established whether the principles derived from studies of Arc/MS2 mRNA apply to native Arc mRNA. Using an induction protocol that involves the washout of previously added tetrodotoxin from rat cortical neurons in culture, Rao et al. (2006) reported that newly induced Arc mRNA is localized to granules in dendrites, but it remains to be established whether the granules formed by native Arc mRNA are the same as the ones in which Arc/MS2 localizes. It is important to recall that fusion transcripts expressed by transfected DNAs have not undergone the same nuclear processing as native mRNAs (for example, splicing). If proteins involved in splicing remain with the mRNA and are important for proper localization, then the movement and localization of fusion transcripts may fail to reflect what actually occurs with native mRNAs.

4.29.2.6 Targeting Arc mRNA to Active Synaptic Sites

One of the most striking features about Arc mRNA is that it localizes selectively in dendritic segments contacted by synapses that have been recently activated. So far, no other mRNA has demonstrated a similar response. Localization near recently activated synapses was demonstrated in studies in which high-frequency stimulation was delivered to the perforant path in anesthetized rats (Steward et al., 1998). The perforant path terminates in a topographically organized fashion along the dendrites of dentate granule cells; projections from the medial entorhinal cortex terminate in the middle molecular layer on mid proximal-distal dendrites, whereas projections from the lateral entorhinal cortex terminate in the outer molecular layer on distal dendrites (Steward, 1976). Hence, by stimulating different parts of the entorhinal cortex, it is possible to selectively activate sets of synapses that terminate selectivity on different dendritic segments. This selectivity allows one to define how mRNA becomes localized in response to synaptic activation.

Our studies revealed that delivery of eight pulse trains at 400 Hz at 0.1-s intervals for 1–2 h strongly induced Arc expression and caused the newly synthesized mRNA to localize in the activated dendritic lamina (Steward et al., 1998). Stimulation of the medial perforant path caused Arc mRNA to localize in the middle molecular layer; stimulation of the lateral perforant path caused Arc mRNA to localize in the outer molecular layer.

The minimal stimulation required for inducing localization has not been precisely defined. Simple induction of LTP, which can be achieved by delivering thirty 400-Hz trains at 0.1-s intervals, strongly induces Arc transcription, but when Arc mRNA reaches the dendrites 45 min to 1 h later, there was little evidence for selective localization in the activated lamina (Steward and Worley, 1998). Instead, the mRNA is distributed uniformly throughout dendrites. In contrast, when high-frequency stimulation was delivered at a rate of 0.1 s for 1–2 h, when Arc mRNA is being transported into dendrites, the mRNA localizes selectively in the activated lamina (Steward et al., 1998).

There are two possible explanations for the failure to localize after simple LTP induction: (1) localization may require repeated stimulation over long periods of time and (2) the signals generated by activity may be relatively short-lived. Evidence against the first possibility came from experiments in which *Arc* expression is induced by an ECS; time was allowed for the mRNA to migrate into dendrites, and then the newly synthesized mRNA was targeted to particular dendritic domains by subsequent synaptic activation. Rats received an ECS, and then high-frequency trains were delivered to the perforant path beginning 1.75 h after the ECS. Remarkably, 15 min of synaptic activation was sufficient to cause Arc mRNA to redistribute to the activated dendritic lamina (Steward and Worley, 2001a). Indeed, in this situation, delivering thirty 400-Hz trains was sufficient to generate a detectable band of Arc mRNA in the activated lamina. Hence, a signal sufficient for localization can be generated with short periods of stimulation, although stimulation for longer periods (30 min to 1 h) is required to cause a full redistribution of Arc mRNA to the activated lamina. Because 30 trains is sufficient to cause localization of Arc mRNA that is already present in dendrites, the likely explanation of the failure of Arc mRNA to localize in a simple LTP paradigm is that by the time the Arc mRNA that is induced by the stimulation reaches the dendrite, the signal for localization has waned.

What are the signals that mediate localization of Arc mRNA in the region of activated synapses? Again, the NMDA receptor plays an obligatory role. Using the localization assay described above, in which Arc is first induced by a generalized seizure and then targeted by synaptic stimulation, Steward and Worley (2001a) provided definitive evidence that activation of NMDA receptors was critical. NMDA receptor antagonists completely prevented localization despite continued strong synaptic activation via AMPA receptors. Instead of localizing at active synaptic sites, Arc mRNA remained diffusely distributed throughout the dendrite. The signal transduction pathways that are downstream of the NMDA receptor remain to be defined.

Studies of Arc mRNA localization using light microscopic *in situ* hybridization document that Arc mRNA is targeted to regions of dendrites contacted by active synapses, but it remains to be established whether the mRNA is actually selectively targeted to active synapses or spines. There is evidence that synaptic activation can cause mRNAs and ribosomes to move from the dendritic shaft into spines, which could represent the final stage of the localization process. For example, electron microscopic studies following tetanic stimulation of the Schaffer collateral system in hippocampal slices from 15-day-old rats revealed that an increased percentage of spines contained polyribosomes, and there was a corresponding decrease in polyribosomes in dendritic shafts (Ostroff et al., 2002). The likely explanation is a translocation of polyribosomes from the shaft into the spine proper. In another study, perforant path LTP was induced in unanesthetized adult rats, and subcellular fractionation techniques were then used to isolate synaptoneurosomes which contain pinched-off spines (Havik et al., 2003). Real-time polymerase chain reaction was then used to determine the levels of the mRNA for CaMKIIα in the synaptoneurosomes and also in total homogenates. It was found that levels of CaMKIIα mRNA increased approximately 2.5-fold in the synaptoneurosomes after inducing LTP, whereas there was no change in CaMKIIα mRNA levels in total homogenates. These findings suggest that CaMKII mRNA translocates into spines as a result of stimulation that induces perforant path LTP.

There were also increases in the levels of Arc mRNA in synaptoneurosomes, but these were accompanied by increases in Arc mRNA levels in the total homogenates. Thus, the increases in synaptoneurosomes could reflect the overall increase in Arc mRNA

levels and not necessarily translocation of Arc mRNA into spines. Thus, although these results are provocative, it remains to be established whether Arc mRNA localizes selectively at activated synapses or is instead targeted to the general region of the dendrite contacted by active synapses.

4.29.2.6.1 Arc mRNA-binding proteins and ribonucleoprotein transport complexes

In many cell types, mRNA transport and localization in particular subcellular domains are mediated by mRNA-binding proteins that recognize particular sequences on the mRNA (see, for example, Ainger et al., 1997). Nevertheless, no protein has yet been identified that selectively binds to particular domains in Arc mRNA, although there are few published reports of proteins that associate with Arc mRNA message (Zalfa et al., 2003; Kanai et al., 2004). Perhaps newly synthesized Arc mRNA is bound by precisely placed but sequence nonspecific factors, which are required for subsequent binding of additional proteins that bind selectively. These factors may not be assembled properly *in vitro*. Alternatively, association of Arc mRNA with the critical RNA-binding proteins may require Arc to fold into a tertiary structure that does not form *in vitro*.

Interestingly, Arc mRNA contains introns that are spliced out in the nucleus prior to export. Comparison of the cDNA with the genomic sequence indicates that while the Arc coding region and 5′UTR do not contain introns, the 3′UTR possesses two introns whose size and relative position are conserved between humans and rodents (NCBI Unigene; Rao et al., 2006). This is important because recent evidence suggests that components of the exon junction complex (EJC) might remain with spliced mRNAs and play a role in localization. For example, components of the EJC are essential for anterior localization of oscar mRNA during *Drosophila* oogenesis (Hachet and Ephrussi, 2004). Little is known of the early events of Arc mRNA maturation, except that they are completed within 20 min after being induced, by which time the mRNA has begun to leave the nucleus (Guzowski et al., 1999). High-resolution *in situ* hybridization reveals that intronic sequences are only found in the nucleus of hippocampal neurons after behavioral induction and thus must be spliced out of the mature mRNA (Guzowski et al., 2006).

It remains to be established whether components of the EJC remain associated with Arc mRNA. Because components of the EJC participate in nonsense-mediated decay of mRNA when present downstream of the stop codon, these proteins may contribute to the short half-life of Arc mRNA.

FMRP has also been reported to associate with Arc mRNA with the noncoding pol-III transcript BC1 RNA serving as a linker (Zalfa et al., 2003). FMRP also associates with a large number of other mRNAs that are not transported into dendrites, and so the significance of this association in the case of Arc is not clear.

Arc mRNA has also been shown to coprecipitate with huge, 1000S ribonucleoprotein complexes that contain the kinesin KIF5 (Kenai et al., 2004; Hirokawa, 2006). Forty-one other proteins that cosediment in these large complexes were identified, including Pur alpha and beta, Staufen, FMRPs, EF1 alpha, and other RNA-associated proteins. As noted earlier, Kanai et al. (2005) further showed that granules labeled with GFP fused to Pur alpha, which is tightly associated with KIF5, exhibited slow (maximum of 7.4 μm/min), short-distance (maximum of 8 μm) movements in dendrites of hippocampal neurons in culture. This behavior is unlike the movement of Arc/MS2 particles that move at rates approaching 70 μm/min over distances of at least tens of micrometers (Dynes and Steward, 2007). Many technical factors could account for these differences, and additional work will be required to identify and characterize the primary transport structures for Arc mRNA, especially *in vivo*.

It is important to recall that mRNA may be present in different types of structures at different times. For example, there are large numbers of Arc/MS2 granules that show no movement during recording sessions, which could represent a storage depot for Arc mRNA. Also, it remains to be established how Arc mRNA (or any other dendritic mRNA, for that matter) is degraded; some of the granules that contain Arc mRNA may represent sites of mRNA degradation rather than being structures involved in transport. It is also possible that Arc mRNA destined for degradation must be transported back to structures in the cell body (more on this in the following section). Clearly, there are a host of unresolved questions about the trafficking of Arc mRNA that will motivate many studies over the coming years.

4.29.2.6.2 Arc mRNA degradation

While docking is an attractive model for the mechanism underlying the localization of Arc mRNA to the vicinity of recently active synapses, local RNA

degradation may contribute to the selectivity of the localization. After a seizure, Arc mRNA is localized throughout the dendrites of dentate granule cells. If the medial entorhinal cortex is stimulated in rats that previously experienced a seizure, Arc mRNA is rapidly redistributed to the activated dendritic region (the middle molecular layer). However, the total amount of Arc mRNA summed throughout granule cell dendrites is reduced on the side of the synaptic activation in comparison to the contralateral side that received the ECS only. This result is consistent with the degradation of Arc mRNA present in adjacent lamina after local activation.

Although degradation in inactive dendritic laminae is possible, we favor an alternative interpretation that is based on processes that are plausible but not yet shown to occur in the case of Arc. First, it is likely that the Arc mRNA induced by a seizure is being transported back and forth in the absence of a localizing signal. Clearly, synaptic activation causes the mRNA to localize in the activated lamina, and if the mRNA is being transported back and forth in the dendrite, the capture of the moving mRNA in the active region would slowly deplete mRNA from other parts of the dendrite. How then can the progressive decreases in total mRNA levels be explained as stimulation continues? Our working hypothesis is based on two other processes that remain to be documented. The first is the idea that docking of the mRNA at active synapses activates translation (a speculation at this point). The second is that Arc mRNA is subject to translation-dependent mRNA degradation. If all of this actually does occur, then capture of the mRNA in the active lamina would deplete the mRNA from other parts of the dendrite, and the enhanced translation would then cause the mRNA to undergo degradation. Together, these processes would cause an activity-dependent depletion of mRNA from the dendrite. Although speculative, the model invokes processes that are biologically plausible and that have been shown to operate in other settings. Nevertheless, two key pieces of the model remain to be documented, specifically, that localization enhances translation and that translation enhances degradation.

4.29.2.7 Functions of Arc Protein

The biological functions of Arc protein remained a mystery for 11 years after Arc was first discovered. Nevertheless, very recent elegant studies have provided a compelling story about the role of Arc protein in GluR recycling. We begin by reviewing the basic biochemical characteristics of Arc protein and then the recent evidence regarding its physiological role at glutamatergic synapses.

4.29.2.7.1 Biochemistry of Arc protein

The original studies that identified Arc revealed that the Arc gene encodes a hydrophilic protein of 45 kDa with limited homology to other proteins in the genome (Link et al., 1995; Lyford et al., 1995). Apparent homologs of Arc are found in mammals, birds (chicken – by sequence homology; Bock et al., 2005), and reptiles (turtle – by antibody cross reactivity; Mokin et al., 2006) but not in fish or invertebrates. Arc has limited amino acid sequence similarity with the actin-binding protein alpha-spectrin. Arc also possesses a region of amino acid similarity with the retroviral gag protein, although the functional significance of this finding is unclear (NCBI Unigene).

As its name indicates, Arc protein associates with components of the cytoskeleton. Arc has been shown to associate with F-actin by cosedimentation, potentially through a separate actin-binding protein (Lyford et al., 1995). Arc protein is surprisingly insoluble, and its inclusion in the pellets of salt- and triton-extracted hippocampal lysates after seizure indicates that newly synthesized Arc protein also associates with the cytoskeleton. Arc protein has been shown to associate with microtubules by taxol precipitation from nerve growth factor-stimulated PC12 cells; attenuation of MAP2 antibody binding after Arc induction suggests that Arc associates with MAP2 as well (Fujimoto et al., 2004).

There is evidence of other protein–protein interactions involving Arc. Arc has been shown to associate with CaMKIIα *in vitro* and when jointly expressed in Nb2a neuroblastoma cells (Donai et al., 2003). Arc contains one consensus site for CaMKIIα phosphorylation, and CaMKIIα can phosphorylate Arc *in vitro*. Another protein, Amida, has been identified using a two-hybrid screen for proteins associated with Arc; in this case Arc appears to modulate the pro-apoptotic activity of Amida (Irie et al., 2000).

4.29.2.7.2 Localization of Arc protein in the postsynaptic density

Several lines of evidence indicate that Arc protein associates with the proteinaceous material comprising the postsynaptic membrane specialization. Arc protein is enriched in subcellular fractions of PSDs (Steward and Worley, 2001), and proteomic analyses have revealed its presence in the collection of proteins that coprecipitate with the NMDA receptor, which

are thought to be part of a large multimolecular signaling complex in the postsynaptic membrane (Husi et al., 2000). In addition, electron microscopic immunocytochemical studies have demonstrated that Arc protein is localized to the PSD of dendritic spines in the rat dentate gyrus (Moga et al., 2004) and caudate putamen (Wang and Pickel, 2004). Arc-GFP fusion proteins also localize to spines when expressed in neurons in culture, suggesting that Arc protein targets the fusion protein to the postsynaptic region (Fujimoto et al., 2004). Importantly, Arc protein that is synthesized in response to strong patterns of synaptic activation also localizes in or near the PSD (Moga et al., 2004). It remains to be seen whether the same is true of the Arc protein that is synthesized in response to behavior.

Arc protein synthesized in response to strong synaptic activation localizes in a pattern similar to that of the mRNA. When induced by a seizure, both mRNA and protein are localized in the cell body and throughout dendrites (Lyford et al., 1995). When induced by activating particular sets of synapses, both mRNA and protein are present in cell bodies, and within dendrites, the protein localizes predominantly in the region of the active synapses (Steward and Worley, 2001b). The similarities in the subcellular distribution of Arc mRNA and protein are consistent with the hypothesis that the selective localization of Arc protein is determined by the distribution of the mRNA, but this has not been shown definitively. It remains possible that Arc protein is targeted independently of the mRNA, although separate and redundant mechanisms seem unlikely. As noted, it remains to be shown whether or not the newly synthesized protein in the activated dendritic lamina associates with the PSD.

It is interesting that after strong synaptic activation, there is strong immunostaining for Arc protein in the nucleus in addition to the staining in the activated dendritic lamina (Steward and Worley, 2001b). What role, if any, Arc protein plays in the nucleus remains to be defined.

4.29.2.8 Evidence That Arc Is Critical for Long-Term Synaptic Changes and Long-Term Memory

Arc has been implicated in long-term synaptic changes and long-term memory by studies using antisense oligonucleotides to inhibit expression of Arc protein and, more recently, from studies of Arc knockout mice. Infusion of Arc antisense oligonucleotides into the hippocampus 1.5 h before delivery of LTP-inducing stimuli inhibited the maintenance but not the initial potentiation of the perforant path. Infusion of antisense oligonucleotides either 3 h before or immediately after training blocked spatial memory retention assayed 2 days later using the Morris water maze (Guzowski et al., 2000). These studies implicate Arc in late-phase LTP and memory.

Initial attempts to generate Arc-knockout mice were not successful because of embryonic lethality (Liu et al., 2000). In this experiment, a neomycin cassette remained and only part of the Arc coding region was deleted, so that lethality may have been caused by dominant negative effects or fusion peptides. More recent studies have been successful in generating Arc knockout mice that survive to maturity and appear healthy. One paper reported the generation of an insertion mutation in which a destabilized version of the GFP gene was inserted into the Arc gene (Wang et al., 2006). Homozygous mice with insertions of GFP into the Arc locus lived to adulthood. The presence of the GFP tag allowed imaging of Arc expression patterns in living mice. These experiments also revealed a potential role for Arc in visual cortical plasticity. The mice were repeatedly exposed to a patterned visual environment, and Arc/GFP expression patterns were monitored repeatedly in the primary visual cortex using two-photon microscopy. The pattern of expression indicated the expected orientation selectivity of visual cortical neurons in that a subset of neurons was activated by stimuli of a particular orientation. Daily presentation of the same stimulus led to reactivation of a progressively smaller population of cells, and this adaptation process was not disrupted in homozygous mice lacking Arc. At the same time, the number of cells with low orientation selectivity was greater and tuning curves to visual stimulation were broader in mice that were homozygous for the transgene. These findings suggest a role for Arc in promoting the development of orientation selectivity, which is an important form of activity-dependent plasticity.

A more recent study has extensively evaluated different forms of synaptic plasticity and learning ability in Arc/Arg 3.1-knockout mice (Plath et al., 2006). In the Morris water maze task, the knockout mice showed the same initial learning, as measured by decreases in the time required to navigate to a hidden platform, but failed to continue to improve their performance during the late acquisition phase (training blocks 6–9). The homozygous knockout

mice also exhibited substantial deficits in learning a new platform location. The knockout mice showed normal acquisition in a fear conditioning task (measured by the time spent freezing after a CS-US trial in which tone and context signaled delivery of shock) but exhibited retention deficits (less freezing in response to both tone and context) when tested 24 h after initial training. Knockout mice also were deficient in a conditioned taste aversion task and in long-term object recognition as measured by the time spent exploring a novel object versus objects seen 24 h previously. Taken together, the experiments provide a consistent picture of normal initial acquisition of different forms of memory but a deficit in the formation of long-term memories of various types.

Arc/Arg 3.1-knockout mice also displayed deficits in the late phase of perforant path LTP *in vivo* induced by 400-Hz trains and in Schaffer collateral LTP in CA1 neurons in hippocampal slices induced by pairing synaptic stimulation with depolarization. At the same time, there were no detectable differences in basal synaptic properties. Interestingly, early-phase LTP was actually greater in the knockout mice in comparison to controls, but response amplitude decayed back to baseline within about 2 h in the case of perforant path LTP and 70 min in the case of Schaffer collateral LTP. Taken together, the physiological results suggest a deficit in the conversion of early LTP to the form of LTP that is able to persist for hours or longer.

4.29.2.9 A Role for Arc Protein in AMPA Receptor Endocytosis

An extraordinary set of studies has recently established a physiological role for Arc protein in AMPA receptor endocytosis. First, biochemical assays revealed that Arc protein binds to two proteins (endophilin 3 and dynamin 2), which are involved in the endocytosis of membrane vesicles (Chowdhury et al., 2006). This binding occurs via separate nonoverlapping domains in Arc. When expressed alone in HeLa cells, Arc and endophilin 3 were distributed diffusely, but when coexpressed in HeLa cells, Arc and endophilin localized in vesicular structures near the plasma membrane that also contained transferrin receptors, which are considered to be a marker for early endosomes. When coexpressed in primary neurons in culture, Arc and endophilin 3 colocalized in puncta that were distributed along the length of dendrites. Interestingly, internalized GluR1 receptors were also present in some of the Arc/endophilin-positive structures, as was endocytosed transferrin. These results suggested that the Arc/endophilin-positive structures were receptor-trafficking endosomes.

Probably the most important finding was that transgenic expression of Arc caused a marked reduction in surface GluR1 receptors, suggesting that Arc protein actually regulates glutamate receptor endocytosis. GluR1 endocytosis was selectively affected because surface NMDA receptors were not significantly altered (and were perhaps even slightly increased). To further test this hypothesis, Chowdhury et al. took advantage of the Arc/Arg 3.1-knockout mice generated by Plath et al., and showed that hippocampal neurons from knockout mice exhibited twice as many surface GluR1 receptors in comparison to neurons from control mice. Also, the rate of endocytosis of GluR1 receptors was lower in neurons from the knockout mice. Thus, overexpression of Arc protein is associated with increases in GluR1 endocytosis and decreases in the number of surface receptors, whereas deletion of Arc protein decreases GluR1 endocytosis, causing increases in the number of surface GluR receptors.

Another paper in the series filled in other aspects of the story, showing that overexpression of recombinant Arc in neurons in hippocampal slices reduced the amplitude of AMPA-mediated synaptic currents (Rial Verde et al., 2006). This effect was blocked by knocking down Arc expression with RNAi and was not seen when the portion of recombinant Arc protein that interacts with endophilin 3 was deleted. This paper also confirmed that overexpression of Arc promoted GluR endocytosis. Another interesting finding was that overexpression of recombinant Arc occluded LTD induced by 1-Hz stimulation, suggesting that the mechanism for LTD involves the same type of GluR endocytosis that occurred in response to overexpression of Arc.

Still another paper in the series focused on the role of Arc in a phenomenon called homeostatic scaling (Shepherd et al., 2006). Previous studies have shown that chronic treatment of neurons in culture with tetrodotoxin (TTX), which blocks action potentials, leads to a compensatory increase in surface AMPA receptors, whereas increasing activity by chronically blocking gamma-aminobutyric acid-mediated inhibition with bicuculline leads to decreases in surface AMPA receptors (O'Brien et al., 1998; Turrigiano and Nelson, 2004). These responses are thought to be homeostatic adjustments that allow neurons to maintain a constant level of synaptic

drive. Using the same types of manipulations, Shepherd et al. demonstrated that Arc expression by primary neurons in culture is regulated by neuronal activity; Arc expression was reduced following long-term TTX blockade and increased following treatment with bicuculline, consistent with it playing a role in homeostatic scaling. Overexpression of Arc led to the expected cell-wide decreases in GluR1 surface receptor levels in the same way as chronic treatment with bicuculline. Critically, overexpression of Arc also blocked the homeostatic increases in Glu1Rs that were otherwise seen following chronic TTX treatment. Finally, using the Arc-knockout mice prepared by Plath et al., Shepherd et al. showed that neurons from knockout animals expressed high levels of surface GluRs and larger AMPA receptor-mediated excitatory postsynaptic currents. Most critically, neurons from Arc-knockout mice did not exhibit the homeostatic rescaling in response to chronic activity blockade with TTX or chronic blockade of inhibition with bicuculline. Taken together, these results suggest that Arc protein plays a critical role in the mechanisms underlying homeostatic rescaling of AMPA receptor number.

4.29.2.10 Arc and Synaptic Homeostasis

Taking all the information into consideration, a novel function for Arc protein was proposed that is consistent with the timing of its expression following LTP induction and its physiological role in regulating GluRs (Rial Verde et al., 2006). The basic idea is that induction of LTP increases efficacy at some synapses, causing an increase the total synaptic weight. If this was not adjusted, progressive increases in total synaptic strength could overactivate the neuron. Accordingly, the idea is that induction of LTP at some synapses triggers a later homeostatic adjustment to adjust overall synaptic weights to return total input strength to a preset level that the neuron can accommodate.

The model proposed by Rial-Verde et al. is illustrated in **Figure 1(a)** using a drawing that shows a realistic representation of the distribution of spine synapses along a dendrite and the distribution of protein synthetic machinery beneath the spines. As in the model proposed by Rial-Verde et al., synaptic activation triggers strong LTP at some synapses and weaker LTP at others, and leaves other synapses unaffected. Their model proposes that homeostatic adjustments would occur equivalently across synapses irrespective of the history of activity, which would preserve the differential in strength between the synapses while importing a late reduction in synaptic strength at synapses that initially did not experience LTP. An impartant feature of the model is that there would be heterosynaptic LTD at synapses that did not initially undergo LTP, which would develop slowly over time after a potentiating event. No such slowly developing LTD has been reported, but that may be because the appropriate studies have not been carried out.

Another important implication of the model is that if Arc is involved in the homeostatic reductions in synaptic strength, and if these occur at synapses that did not initially experience LTP, then Arc protein must be delivered to synapses that were 'not' activated by the initial stimulation. Putting this together with the fact that Arc protein is selectively localized to dendritic domains contacted by active synapses, the implication is that homeostatic adjustments in synaptic strength might occur locally in parts of the dendrite, not generally over the entire postsynaptic receptive surface. It will be interesting to see if the key phenomena predicted by this model can be demonstrated (slowly developing LTD and targeting of Arc protein to synapses that were not initially activated).

In considering the model, it is useful to consider limitations imposed by the nature and distribution of the machinery for protein synthesis. Several points are noteworthy. (1) Quantitative electron microscopic studies indicate that not all spines in adult neurons *in vivo* have underlying polyribosomes. For example, in the middle dendritic regions of dentate granule cells (the site of termination of the perforant path) only approximately 25% of the spines have polyribosomes (Steward and Levy, 1982). (2) There is no reason to think that inputs would selectively activate synapses with polyribosomes, and so activity-induced modifications (such as LTP) would presumably occur at synapses with and without polyribosomes. For example, **Figure 1** illustrates a biologically realistic dendrite with spines in which the distribution of polyribosomes is indicated. If Arc protein is targeted to synapses that have experienced a particular pattern of activity, either the protein that is made at one synapse must be available to other nearby synapses or the machinery for protein synthesis must move from one synapse to another. This

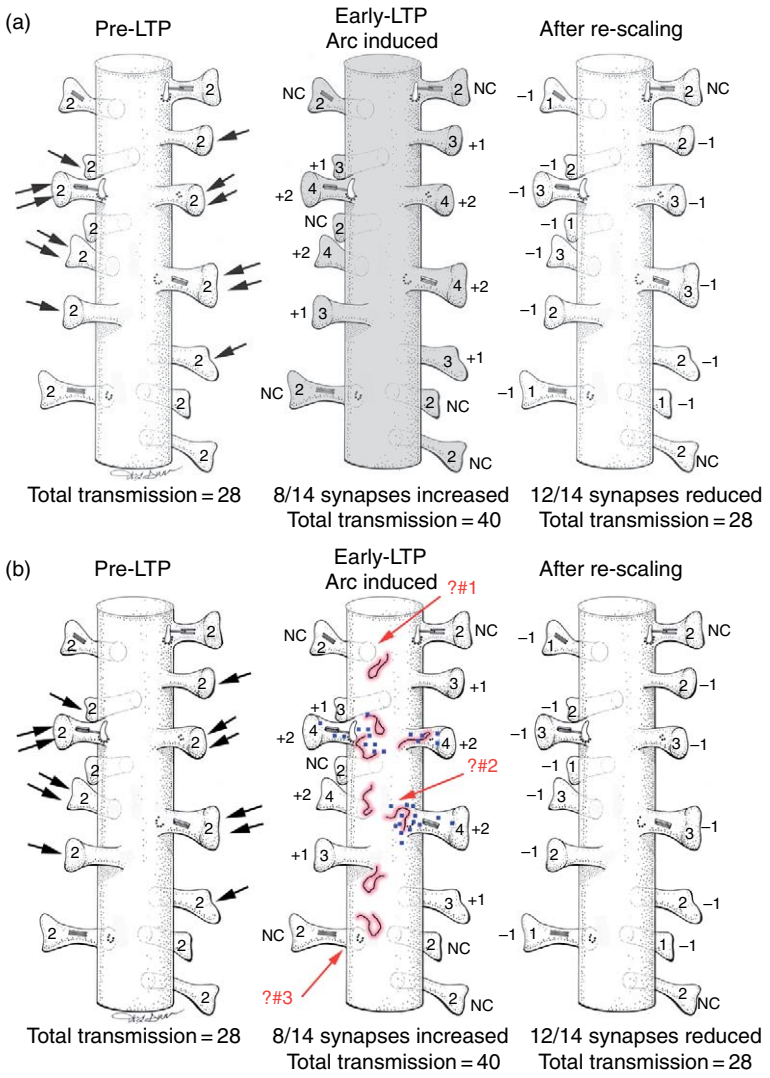

Figure 1 (a) illustrates the model proposed by Rial-Verde et al (2007) using a drawing that shows a realistic representation of the distribution of spine synapses along a dendrite and the distribution of protein synthetic machinery beneath the spines. The model assumes that synaptic activation triggers strong LTP at some synapses (double arrows), weak LTP at others (single arrows), and leaves other synapses unchanged (NC). The shaded dendrite (middle) illustrates the situation after induction of LTP and before homeostatic adjustments have occurred, and the dendrite on the right illustrates the situation after homeostatic rescaling. One difference between the model shown and the one proposed by Rial-Verde is that the proposed synaptic modifications occur selectively in a localized region of the dendrite. In the Rial-Verde model, rescaling occurs equivalently across synapses irrespective of the history of activity, which would preserve the differential in strength between the synapses while returning the total synaptic strength to its initial level. Thus, there would be heterosynaptic LTD at synapses that did not initially undergo LTP, which would develop slowly over time. (b) illustrates questions regarding the delivery of Arc mRNA and protein to the synapses undergoing rescaling. Arc mRNA is shown as a line with red halo. Arc protein is shown as blue squares. If Arc is involved in the homeostatic reductions in synaptic strength, and if these occur at synapses that did not initially experience LTP, then Arc protein must be delivered to synapses that were NOT activated by the initial stimulation, which raises several questions. Question #1 (?#1): Is Arc mRNA targeted selectively to synapses that were NOT activated, but that undergo rescaling? If the synapse does not already have ribosomes, where do the ribosomes come from to translate the mRNA? Question #2 (?#2): If Arc mRNA is targeted exclusively to synapses that were activated in a way as to induce LTP, can the Arc protein that is synthesized at one synapse be captured by nearby synapses that were NOT initially activated? Also, in the case of synapses that capture Arc mRNA but do not already have ribosomes (the synapse on the left side of the dendrite at the arrow), do ribosomes relocalize in order to translate captured Arc mRNA or do ribosomes come along with the mRNA when it is transported from the cell body? Question #3 (?#3). If a synapse that was not initially activated has polyribosomes, does the process of rescaling enable it to capture and translate Arc mRNA?

could involve movements of polyribosome assemblies or individual ribosomes.

The combined results on Arc protein function can also be accommodated by other models. For example, the proposed homeostatic adjustments in synaptic strength could occur at the same synapses that had undergone LTP in the first place. This model would predict a slow decrease in synaptic strength from the initial peak after induction of LTP during the period of Arc expression. An important feature of this model is that the homeostatic changes would involve the same synapses that had initially undergone LTP.

4.29.2.11 Speculations on the Role of Arc in Cellular and Molecular Mechanisms Underlying Memory Consolidation

The models for Arc function at synapses take on special interest in the context of the studies that implicate Arc in memory consolidation. If Arc's role is to promote receptor endocytosis and reduce synaptic strength, then the implication would be that this process plays a role in memory consolidation. One can easily imagine, for example, that the initial alterations in synaptic strength triggered by LTP create an inherently unstable synaptic circuit that becomes stabilized by the homeostatic adjustments mediated by Arc. In the absence of such homeostatic adjustments, the initial alterations in synaptic strength would thus decrement in a nonspecific fashion, erasing the synaptic representation of the memory trace. This novel concept opens up a new dimension in thinking about the cellular and molecular mechanisms of memory consolidation. It is likely that the new findings regarding the functions of Arc protein will trigger a host of follow-up studies testing predictions and seeking to demonstrate the predicted synaptic modifications.

In thinking about mechanisms of synaptic plasticity that require transcription and translation, it is relevant that mature Arc transcripts can be synthesized within minutes after a period of stimulation (Guzowski et al., 2005) and Arc mRNA can be transported within dendrites at rates in excess of 1 μm per second (Dynes and Steward, 2006). Thus it is possible to transcribe an mRNA and deliver it to any point of a dendrite within minutes of the inducing stimulus. Studies of Arc thus reveal a mechanism that was not previously anticipated.

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4.30 Glutamate Receptor Trafficking in LTP

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4.30.1 Introduction

For several decades now, long-term potentiation (LTP) has been studied as a putative mechanism for memory formation. Because of this potential link to memory (see, for example, Whitlock et al., 2006, but see also Shors and Matzel, 1997), there has been a tremendous amount of interest in understanding the molecular mechanisms of LTP. Two complimentary molecular mechanisms have been widely recognized as potentially underlying LTP. The first of these, protein phosphorylation, was shown to be correlated with LTP in the late 1970s and early 1980s (Williams and Rodnight, 1977; Browning et al., 1979a,b; Lovinger et al., 1985; Hu et al., 1987; Sweatt and Kandel, 1989). Subsequent studies using inhibitors of protein kinase C (PKC) (Hu et al., 1987; Malinow et al., 1988; Klann et al., 1992; Osten and Sacktor, 1992; Hvalby et al., 1994), Ca^{2+}/calmodulin kinase II (CaMKII) (Finn et al., 1980; Malenka et al., 1989; Malinow et al., 1989; Sweatt and Kandel, 1989), and Src-family tyrosine kinases (Lu et al., 1998; Huang and Hsu, 1999) have provided compelling evidence for a role of phosphorylation in the induction of LTP. Interestingly, in adult animals, cAMP-dependent protein kinase (PKA) appears to have little role in the induction of LTP, but this kinase is thought to be critical for the long-lasting maintenance of LTP (Frey et al., 1993; Matthes and Reymann, 1994; Nayak et al., 1998). In contrast, PKA appears to be required for the induction of LTP early in development (Esteban et al., 2003; Yasuda et al., 2003). The second mechanism, receptor trafficking,

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has more recently emerged as a leading candidate for a mechanism underlying LTP (Boehm and Malinow, 2005; Malinow and Malenka, 2002). The focus of this chapter is to examine the evidence for such receptor trafficking in LTP and to describe how protein phosphorylation may play a role in this trafficking. Any review of LTP is plagued by the huge number of publications in this field. Because of space limitations, one must be selective of one's coverage of the topic, and this necessarily requires that many outstanding studies go unrecognized. For this chapter, we focus primarily on studies using *in situ* hippocampal slices, *in vitro* organotypic hippocampal slice cultures, or dissociated neuronal cultures. The rationale for this focus is described next.

4.30.2 Basic LTP Experimentation Practices

4.30.2.1 Neuronal Preparations for Studies of LTP

LTP was initially discovered in the dentate region of the hippocampus in an *in vivo* (i.e., in a live animal) rabbit preparation (Bliss and Lomo, 1973). Subsequent work in the 1970s extended these studies using *in situ* hippocampal slices, with the CA1 region being the focus of most studies. The advent of the hippocampal slice offered many advantages for studies of LTP, including easy access to a stable preparation for intracellular and extracellular as well as whole-cell recordings. The *in situ* slice preparation also greatly facilitated pharmacological studies of LTP because of the ease with which one could control the extracellular milieu by simply changing the constituents of the buffer bathing the slice. However, there are also numerous disadvantages with the *in situ* preparation, including the long-lasting effects of sacrifice of the animal and the widespread loss of cells and synaptic proteins, as well as the fact that the slices do not survive well when maintained at 37 °C. A number of other preparations, including *in vitro* cultured neurons or organotypic slice cultures and nonneuronal preparations such as human embryonic kidney (HEK) cells, have been used to study LTP and trafficking. As with the *in situ* hippocampal slice, such reduced preparations have both advantages and limitations. The *in vitro* systems permit a variety of genetic manipulation, and it is also possible to visually monitor receptor trafficking. However, it is not possible to elicit LTP in neuronal cultures using the classical LTP induction protocol. Moreover, cultured neurons and organotypic slices are virtually always prepared from embryonic or neonatal tissue in which massive activity-dependent trafficking is ongoing because this postnatal period is when most synaptogenesis occurs. Nonneuronal systems (cell lines) using expression of recombinant receptors have provided some of the greatest insight into the role played by individual receptor subunits in trafficking. However, major limitations of such preparations are that they cannot exhibit LTP and they lack the plethora of synaptic proteins that are thought to participate in glutamate receptor trafficking in neurons. For these reasons, and because of the focus of this chapter, we emphasize studies that use neuronal systems, particularly those using *in situ* hippocampal slices prepared from neonatal or adult animals as well as *in vitro* cultured neurons and organotypic slice cultures. We attempt to identify exactly which type of preparation is used in each of the studies we discuss.

4.30.2.2 LTP Induction Paradigms

In addition to the myriad systems used to study LTP, there are several different electrical stimulation patterns used to induce LTP. Among these are high-frequency stimulation (HFS), theta burst stimulation (TBS), primed burst stimulation (PBS), and pairing (**Table 1**). The most common paradigms used when studying glutamate receptor trafficking are HFS and pairing. Stimuli such as theta burst and primed burst are sometimes used, as these appear to more closely mimic endogenous firing patterns of the hippocampus (Diamond et al., 1988; Larson and Lynch, 1989). HFS is typically used with extracellular recordings and entails from one to four trains of 100 stimuli at 100 Hz given at 10- to 30-s intervals. Although the hippocampus does fire *in vivo* at rates of 100 Hz, such firing rarely lasts more than 50 ms. Pairing stimulation involves delivering 100 stimuli at 2 Hz concomitant with depolarization of the neuron. It is important to note that the pairing protocol is typically used in whole-cell patch clamp experiments where the membrane potential of the postsynaptic neuron can be controlled. An important, and often overlooked, caveat with whole-cell experiments is that such recording results in dialysis of the cell with the electrode buffers and thus leads to dilution of the intracellular content of the neuron. Indeed, the ability to induce LTP using the whole-cell configuration declines rapidly after the first 30–45 min of recording. This issue may be particularly germane to controversies that develop concerning the relative contributions of intracellular signaling molecules to LTP.

Table 1 The range of stimuli used in the various LTP induction paradigms[a]

Paradigm	*Stimulus*	*Reference*
HFS	1 burst of 100 pulses at 100 Hz, 1–4 trains 10–30 s apart	Bliss and Lomo (1973) Shi et al. (1999) Grosshans et al. (2002)
TBS	3–10 bursts of 4–6 pulses at 100 Hz at 5 Hz (1 burst = 4–6 pulses at 100 Hz), 1–4 trains 10 s–5 min apart	Larson et al. (1986) Larson and Lynch (1989)
PBS	1 pulse followed 140 or 170 ms later by 2–10 pulses at 100 Hz	Diamond et al. (1988)
Pairing	10–200 pulses at 0.1–2 Hz, while depolarizing the postsynaptic neuron	Gustafsson et al. (1987) Liao et al. (1995) Durand et al. (1996)

[a]HFS, high-frequency stimulation; TBS, theta burst stimulation; PBS, primed burst stimulation.

4.30.2.3 Brain Regions and LTP

LTP or LTP-like potentiation has been described in a great many different brain regions. For example, in the CA3 region of the hippocampus, two apparently distinct forms of LTP have been described. One of these is classical *N*-methyl-D-aspartate (NMDA) receptor (NMDAR)–dependent LTP (Zalutsky and Nicoll, 1990). The other, exhibited by the mossy fiber input to this region, is a form of LTP not dependent on NMDAR activation but, rather, requires activation of PKA and is possibly presynaptic in nature (Weisskopf et al., 1994). However, in this chapter we focus primarily on NMDAR-dependent LTP in the CA1 region of the hippocampus.

Having established the ground rules for our discussion, we are now ready to discuss the role of glutamate receptor trafficking in LTP. We also discuss the molecular mechanisms that may underlie this trafficking. Given the different roles played by the alpha-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA) receptor (AMPAR) and NMDAR in LTP, and because their trafficking mechanisms appear to differ, we discuss trafficking of the AMPAR and the NMDAR separately.

4.30.3 AMPA Receptor Trafficking in LTP

Excitatory postsynaptic potentials (EPSPs) in the CA1 are composed largely of AMPAR currents, with smaller contributions made from NMDAR currents. LTP is principally expressed as an enhancement of the slope and amplitude of the EPSP and as such is largely a product of an increase of AMPAR current, although LTP can also be measured as an increase in pharmacologically isolated NMDAR responses (Bashir et al., 1991; Grosshans et al., 2002). The mechanism of enhanced AMPAR function following LTP has been a topic of intense debate for decades. One possibility is that LTP increases channel currents without an increase in the number of receptors at the synapse (Banke et al., 2000; Benke et al., 1998). However, an increasingly more popular idea is that LTP increases the number of glutamate receptors at synapses. In the case of AMPARs, the argument for LTP-induced trafficking of receptors to the synapse was fueled by the discovery of silent synapses, which lack functional AMPARs but contain a detectable number of NMDARs (Isaac et al., 1995; Kullmann, 1994; Liao et al., 1995). The idea of an increased number of synaptic glutamate receptors as a mechanism of LTP was first proposed in the 1980s (Baudry et al., 1986). For decades, this provocative hypothesis was obscured by the heated debate over pre- versus postsynaptic expression of LTP. More recently, studies of acute and cultured hippocampal slices have provided conclusive evidence that both spontaneous activity and LTP induction lead to trafficking of AMPARs to the synapse in these preparations from neonatal animals (Hayashi et al., 2000; Zhu et al., 2000; Oh et al., 2006).

The two potential mechanisms of LTP, conductance change and trafficking of AMPARs, are not mutually exclusive, as both seem likely to play a role in LTP. Moreover, both mechanisms may require protein phosphorylation (Benke et al., 1998; Shi et al., 1999; Grosshans et al., 2002). For example, CaMKII activation leads to increases in AMPAR conductance and may also contribute to AMPAR trafficking via phosphorylation of the AMPAR auxiliary protein stargazin (Derkach et al., 1999; Tomita et al., 2005). However, for the purposes of this chapter, we focus on AMPAR trafficking and LTP.

4.30.3.1 Molecular Biology of AMPARs

AMPARs gate Na^+ entry into neurons and mediate fast excitatory neurotransmission in response to glutamate release. Various combinations of four homologous subunits (GluR1 to GluR4) form tetrameric ion channels with unique characteristics (Lambolez et al., 1992; Hollmann and Heinemann, 1994). Each subunit is encoded by a separate gene with developmentally and regionally regulated expression patterns (review: Palmer et al., 2005). All four subunits exist as two alternatively spliced forms, flip and flop (Borges and Dingledine, 1998). The flop form predominates in adult CA1 and desensitizes less slowly than the flip form (Dingledine et al., 1999). Coimmunoprecipitation studies suggest that AMPARs are primarily GluR1/GluR2 or GluR2/GluR3 heterotetramers in the adult hippocampus (Wenthold et al., 1996). The presence of GluR2 renders the receptor impermeable to calcium (Hollmann et al., 1991; Verdoorn et al., 1991). Interestingly, a recent study suggests that GluR2-lacking receptors may be present at the synapse for a brief time period following plasticity events such as LTP (Plant et al., 2006). Expression of GluR4 is restricted to the immature hippocampus (Zhu et al., 2000). AMPAR subunit composition may also regulate receptor trafficking, as GluR2/3 receptors are thought to cycle continuously, whereas trafficking of GluR1/2 and GluR4/2 receptors can be regulated by synaptic activity (Hayashi et al., 2000; Zhu et al., 2000; Shi et al., 2001). These differences in trafficking correlate with C-terminal tail length, as GluR1 and GluR4 have long tails, whereas GluR2 and GluR3 have short tails. These intracellular C termini mediate AMPAR interactions with scaffolding proteins as well as components of the actin cytoskeleton and trafficking machinery (Dong et al., 1997; Leonard et al., 1998; Nishimune et al., 1998; Osten et al., 1998; Song et al., 1998; Dev et al., 1999; Xia et al., 1999; Chen et al., 2000; Shen et al., 2000).

4.30.3.1.1 Phosphorylation of AMPARs

The C-terminal tails also contain several sites of phosphorylation that may mediate protein–protein interactions and/or alter channel kinetics. Electrophysiological studies have demonstrated that CaMKII and PKA regulate AMPAR channel gating by increasing open channel conductance and probability, respectively (Derkach et al., 1999; Banke et al., 2000). Site-directed mutagenesis and phosphopeptide mapping studies suggest that these effects are mediated by direct phosphorylation of GluR1 subunits at serine 831 and serine 845 (Roche et al., 1996; Barria et al., 1997). Production of site-specific phosphoantibodies confirmed that serine 831 can be phosphorylated by CaMKII and PKC, whereas serines 845 is phosphorylated by PKA (Mammen et al., 1997). Knock-in mutations of both serines 831 and 845 suggest that at least one of these sites is important for AMPAR trafficking, LTP, and learning in the adult hippocampus (Lee et al., 2003). Interestingly, phosphorylation of serine 845, but not serine 831, is necessary for synaptic incorporation of GluR1 subunits in slices from neonates (Hayashi et al., 2000; Esteban et al., 2003). Furthermore, a recent report suggests that phosphorylation of serine 845 delivers GluR1 to the extrasynaptic membrane, and coincident synaptic activation induces lateral translocation to the synapse (Oh et al., 2006). PKA has also been shown to regulate surface expression of GluR4 subunits via direct phosphorylation of serine 842 (Carvalho et al., 1999; Esteban et al., 2003; Gomes et al., 2004).

In contrast to the consistently demonstrated potentiating effects of PKA and CaMKII, other kinases seem to have differential effects of AMPAR function and localization. The result of phosphorylation correlates with the location of the phosphorylated residue within the C terminus of AMPAR subunits. For example, PKC and Src-family tyrosine kinase phosphorylation of the distal C terminus of GluR2 can downregulate AMPAR transmission and surface expression (Seidenman et al., 2003; Ahmadian et al., 2004; Hayashi and Huganir, 2004). Conversely, recent evidence demonstrates that PKC phosphorylation of serine 818 in the membrane proximal region of the GluR1 C terminus correlates with increased AMPAR transmission and surface expression observed following LTP in juvenile hippocampus (Boehm et al., 2006).

Activated PKC is translocated to dendritic spines by PICK1, a protein that also interacts with GluR2 subunits. This translocation correlates with phosphorylation of serine 880 on GluR2 and reduced surface expression of exogenous GluR2 subunits (Perez et al., 2001). Loss of surface expression is consistent with phosphoserine 880– and phosphotyrosine 876–mediated disruption of GluR2 binding to the synaptic scaffolding proteins glutamate receptor-interacting protein (GRIP) and AMPA-binding protein (ABP) (Matsuda et al., 1999; Chung et al., 2000; Hayashi and Huganir, 2004). However, a recent

study suggests that tyrosine dephosphorylation, possibly at a different site in the C-terminal tail of GluR2, regulates internalization of GluR1 subunits during metabotropic glutamate receptor–mediated long-term depression (LTD) (Moult et al., 2006). Thus, both phosphorylation and dephosphorylation events are likely to mediate specific mechanisms of AMPAR trafficking in response to various stimuli.

Additional PKC phosphorylation sites include serine 863 on GluR2 and potentially threonine 830 on GluR4 (Carvalho et al., 1999; McDonald et al., 2001). The functional consequence of PKC phosphorylation at these sites has not been investigated in neurons.

4.30.3.1.2 Developmental profile of AMPAR subunit expression

Because *in situ* hippocampal slices and organotypic hippocampal slice cultures are commonly prepared from rodents at various stages of postnatal development, it is appropriate that we review the developmental profiles of AMPAR subunit expression. Immunoblot analysis of hippocampal homogenates reveals that expression of GluR1, GluR2, and GluR3 (as determined with anti-GluR2/3 antibody) subunits gradually increases from P0 to steady state around P15 (Zhu et al., 2000). An *in situ* hybridization study demonstrated that total brain expression of the long-tail splice variant of GluR2, GluR2L, peaks during the second postnatal week (P7–P14) of development (Kolleker et al., 2003). High levels of expression were observed in the hippocampus at these developmental stages. Conversely, expression of the GluR4 subunit peaks from P0 to P2 before rapidly declining to 20% of maximum expression level at P9. Expression of GluR4 is near undetectable by postnatal day 15 (Zhu et al., 2000). Interestingly, when GluR4 expression is highest (P2), the relative abundance of GluR1 and GluR2 subunits is similar to that of GluR4. The distinct developmental profiles of AMPAR subunit expression suggest that each protein may mediate unique mechanisms of synaptic plasticity at different stages of postnatal development.

4.30.3.2 Evidence for AMPAR Trafficking in LTP

4.30.3.2.1 Early postnatal development

The studies discussed in this section were conducted primarily in acute hippocampal slices prepared from neonatal (<P10) rodents or in organotypic hippocampal slice cultures prepared from postnatal day 5–8 rats. The cultured slices were generally infected with recombinant AMPAR proteins 0–2 days following preparation and maintained in culture for 2–3 days prior to experimental observation. These studies are therefore likely to reflect mechanisms that contribute to synaptic plasticity during the first ~7–11 days of life, hence 'early' postnatal development. Given that GluR4 subunits are highly expressed during the first few days of postnatal development, receptors containing these subunits may play a key role in mechanisms of plasticity during this time period.

4.30.3.2.1.(i) GluR4 Pyramidal neurons infected with recombinant GFP-tagged GluR4 subunits show enhanced AMPAR transmission when compared to uninfected control neurons (Zhu et al., 2000). To determine whether the enhanced transmission was due to synaptic incorporation of the recombinant subunits, this group used an electrophysiological assay developed in their laboratory (Hayashi et al., 2000). Coimmunoprecipitation and electrophysiological studies indicate that GFP-GluR4 subunits form homomeric channels that display inward rectification (Zhu et al., 2000). Because most endogenous hippocampal AMPARs exhibit a linear current–voltage relationship (Verdoorn et al., 1991; Wenthold et al., 1996), this characteristic of GFP-GluR4 homomers permitted electrophysiological monitoring of the functional incorporation of the recombinant channels (Hayashi et al., 2000). Indeed, AMPAR EPSCs recorded from GFP-GluR4-infected cells exhibit greater rectification than uninfected control cells (Zhu et al., 2000). Furthermore, the observed enhancement of AMPAR transmission and rectification was occluded by synaptic activity blockade or treatment with APV, suggesting that spontaneous activity delivers GluR4 to synapses in an NMDAR-dependent manner. Subsequent experiments revealed that PKA phosphorylation of GluR4 on serine 842 was necessary for activity-dependent delivery of GluR4 to synapses (Esteban et al., 2003). Additional work utilizing chimeric GluR1/GluR4 subunits indicates that the membrane proximal region of the GluR4 intracellular C-terminal tail is required for delivery of GluR4 by spontaneous activity (Boehm et al., 2006).

4.30.3.2.1.(ii) GluR1 Although expression of GluR4 peaks during the first few days of postnatal development, the relative abundance of GluR1 is

near equivalent to that of GluR4 at postnatal day 2 (Zhu et al., 2000). It is therefore possible that GluR1 subunits contribute to mechanisms of synaptic plasticity during this time period. Electron microscopy studies of recombinant GFP-tagged GluR1 expressed in organotypic hippocampal slice cultures suggest that these subunits are largely excluded from synapses (<1% localized to postsynaptic densities; Shi et al., 1999). Similar results (~3% localized to postsynaptic densities) were obtained for endogenous GluR1 subunit expression at postnatal day 10. Tetanic stimulation (two trains 100 Hz for 1 s separated by 20 s) induced NMDAR-dependent delivery of GluR1-GFP to spines and clustering of GluR1-GFP in dendrite shafts as assayed by two-photon laser scanning microscopy (Shi et al., 1999). Similar results have been obtained using acute hippocampal slices prepared from postnatal day 9 rats and biochemical measures of AMPAR trafficking (Grosshans et al., 2002). Studies of dissociated hippocampal neurons in culture have also provided evidence for NMDAR- and activity-dependent recruitment of AMPARs to previously silent synapses (Liao et al., 2001a; Lu et al., 2001; Pickard et al., 2001).

The immunofluorescence method utilized by Shi et al. (1999) does not discriminate between synaptic and extrasynaptic receptors. Subsequent coimmunoprecipitation and electrophysiology experiments demonstrated that the recombinant GluR1-GFP subunits form homomeric, rectifying channels (Hayashi et al., 2000). Thus, as described for GFP-GluR4 homomers, functional incorporation of these receptors into synapses can be assessed electrophysiologically. Expression of the GluR1-GFP had no effect on AMPAR transmission or rectification under basal conditions (Hayashi et al., 2000). This result supports the conclusion of the previous report that these subunits are largely excluded from synapses at this stage of development (Shi et al., 1999). Following induction of LTP via a pairing protocol, synaptic delivery of GluR1-GFP was observed as increased rectification of AMPAR responses relative to uninfected control cells (Hayashi et al., 2000). Mutation of a threonine residue within the PDZ ligand near the C terminus of GluR1 (T887A) led to short-lasting potentiation, which eventually decayed to a depression at 45 min post–pairing stimulation. Furthermore, at least two reports have indicated that the canonical PDZ protein PSD-95 is necessary and sufficient for LTP during various developmental stages (Stein et al., 2003; Ehrlich and Malinow, 2004). Taken together, these data suggest that GluR1 delivery following LTP stimulation likely involves an interaction between GluR1 and a PDZ-domain-containing protein. However, extensive studies of knock-in mice with the last seven amino acids of GluR1 deleted provide *in vivo* evidence that the GluR1 PDZ ligand is not required for NMDAR-dependent LTP in mature animals (aged 3 weeks to 7 months; Kim et al., 2005). Clearly, further study is necessary to determine the contribution of AMPAR-PDZ interactions to mechanisms of AMPAR trafficking and LTP. One possibility is the sequential involvement of multiple-PDZ-domain-containing proteins such as PSD-95 and stargazin-like TARPs (review: Malenka, 2003).

Although constitutively active CaMKII is sufficient for synaptic delivery of recombinant GluR1, CaMKII phosphorylation of GluR1 at serine 831 seems not to be required for this process, as mutation of this residue (S831A) had no effect on CaMKII-induced amplification of AMPAR transmission and rectification (Hayashi et al., 2000). Unfortunately, the effect of this mutation on LTP-induced delivery of recombinant GluR1 was not tested in this study. However, work from another group suggests that hippocampal LTP does not require CaMKII activity during early postnatal development (Yasuda et al., 2003). Taken together, these studies suggest that CaMKII may be sufficient – yet not necessary – for LTP during early postnatal development.

4.30.3.2.2 *Late postnatal development*

The studies discussed in this section were conducted primarily in acute hippocampal slices prepared from 2- to 3-week-old rodents or in organotypic hippocampal slices prepared from postnatal day 5–7 rats. The slices were then cultured *in vitro* for 5–14 days before introduction of recombinant AMPAR proteins and were maintained in culture for an additional 1–3 days prior to experimental observation. Thus, these slices correspond to an approximate developmental age of 11–24 days and are likely to suggest mechanisms that contribute to synaptic plasticity during 'late' postnatal development. This period is also characterized by peak levels of synaptogenesis.

4.30.3.2.2.(i) GluR1 The LTP-dependent delivery of recombinant GluR1-GFP to synapses observed by Hayashi et al. (2000) has been revisited more recently (Boehm et al., 2006; Esteban et al., 2003). In the more current studies, organotypic slice cultures were maintained *in vitro* for 7–10 days prior to

infection with GluR1 to more closely mimic the endogenous expression profile of GluR1 subunits. These experiments confirmed previous findings that constitutively active CaMKII drives delivery of recombinant GluR1 subunits to synapses. However, mutation of GluR1 (S818A) blocked CaMKII-induced potentiation (Boehm et al., 2006). Additional experiments demonstrated that serine 818 is phosphorylated by PKC both *in vitro* and in brain homogenates and that increased phosphorylation of this site correlates with synaptic incorporation of GluR1 following induction of LTP. These data suggest that both CaMKII and PKC, either independently or in concert, contribute to mechanisms of AMPAR trafficking following induction of LTP.

These and other lines of evidence suggest that various protein kinases may be linked to AMPAR trafficking and LTP via multiple signaling pathways. For example, CaMKII appears to activate Ras signaling as constitutively active Ras mimics CaMKII-induced delivery of GluR1 to synapses (Zhu et al., 2002). In addition, PKA phosphorylation of GluR1 on serine 845 is necessary, but not sufficient, for LTP and CaMKII-dependent delivery of GluR1 (Esteban et al., 2003). Furthermore, activation of PKA correlates with increased localization of GluR1 within extrasynaptic membrane fractions purified from acute hippocampal slices prepared from slightly older rats (4–6 weeks; Oh et al., 2006). Subsequent TBS resulted in stronger LTP, suggesting that PKA, perhaps via serine 845 phosphorylation, primes extrasynaptic membrane with AMPARs readily available for synaptic incorporation on synaptic activation. However, another group reported that PKA activity is not required for induction of LTP in hippocampal slices prepared from animals older than postnatal day 27 (Yasuda et al., 2003). Clearly there are numerous mechanisms of AMPAR trafficking mediated by an intricate mix of signaling pathways. It will be exciting to see how future work is able to tease out the contributions of various signaling molecules to mechanisms of synaptic plasticity during different stages of postnatal development.

Our discussion thus far has been restricted to studies carried out in the hippocampus. We diverge here briefly to discuss a study conducted in the amygdala (Rumpel et al., 2005). The authors used a GFP-tagged construct containing the last 81 amino acids of the GluR1 C terminus to evaluate the contribution of AMPAR trafficking to associative learning. The phrase 'plasticity block vector' was used to describe the construct, as it was previously shown to block LTP in organotypic hippocampal slices and neurons cultured from visual cortex (Shi et al., 2001; Watt et al., 2004). The more recent paper replicated these results in the amygdala and extended the findings by correlating associative learning with synaptic incorporation of recombinant GFP-GluR1. Importantly, the source of the newly incorporated subunits is unknown. It is therefore possible that these subunits are trafficked from the extrasynaptic membrane rather than an intracellular compartment. Additional *in vivo* experiments demonstrated that the plasticity block vector impairs fear acquisition (Rumpel et al., 2005). It will be exciting to see how similar studies in the hippocampus and other brain regions strengthen the ever-elusive correlation between molecular mechanisms of LTP and learning.

4.30.3.2.2.(ii) GluR2 The alternative splice variant of GluR2, GluR2L, is highly expressed in the hippocampus during the second and third weeks of postnatal development (Kolleker et al., 2003). Interestingly, GluR2L seems to function independently from GluR2, as neither endogenous nor recombinant GluR2L coassembles with GluR2. Conversely, endogenous GluR2L was found in complex with GluR1 and GluR3 in homogenates of neonatal hippocampus (Kolleker et al., 2003). Like GluR1 and GluR4, GluR2L subunits have long C-terminal tails and therefore may be subject to activity-dependent regulation of trafficking during this developmental period (Shi et al., 2001). To test this hypothesis, Kolleker et al. (2003) transfected organotypic hippocampal slice cultures with recombinant GFP-tagged GluR2L subunits containing the R586Q mutation (flop form). Much like the GluR1 and GluR4 constructs described earlier, these receptors form homomeric channels that display inward rectification. Indeed, infected cells exhibit increased amplitude and rectification of AMPAR excitatory postsynaptic currents (EPSCs) compared to uninfected cells, and these effects were occluded by synaptic activity blockade (Kolleker et al., 2003). These results suggest that spontaneous activity delivers GluR2L subunits to synapses during late postnatal development. A Ras signaling pathway, possibly linked to PI3-kinase, is likely involved in this process, as a previous study demonstrated that neurons coexpressing dominant negative Ras and GFP-GluR2L exhibit depressed AMPAR transmission and normal rectification (Zhu et al., 2002; Man et al., 2003).

In the absence of continued spontaneous activity, the newly incorporated GluR2L-containing

AMPARs are replaced by GluR2-containing receptors (Kolleker et al., 2003). This result is consistent with a recent study that reported transient synaptic incorporation of calcium-permeable, GluR2-lacking AMPARs during the first 10 min following LTP induced by a pairing protocol (Plant et al., 2006). Furthermore, activation of these calcium-permeable AMPARs may be required for subsequent stabilization of LTP via replacement of the GluR2-lacking AMPARs with GluR2-containing channels. Taken together, these findings suggest that activity-dependent delivery of GluR2L may mediate mechanisms of LTP. Supporting this idea, when synaptic activity is reduced to block delivery of GluR2L, subsequent induction of LTP delivers GluR2L to synapses, as determined by increased amplitude and rectification of AMPAR EPSCs (Kolleker et al., 2003). Furthermore, expression of the C-terminal tail of GluR2L completely abolishes LTP in GluR1 knock-out mice (Jensen et al., 2003; Kolleker et al., 2003). Therefore, activity-dependent delivery of GluR2L subunits may mediate GluR1-independent mechanisms of LTP during late postnatal development.

4.30.3.2.3 Adult

Very few studies of molecular mechanisms of receptor trafficking during LTP are conducted in preparations of adult hippocampus. One exception is work from our lab using acute hippocampal CA1 minislices prepared from 6- to 8-week-old rats (Grosshans et al., 2002). LTP of field EPSPs (fEPSPs) was induced by application of HFS. More specifically, four 1-s trains of 100-Hz stimulation were separated by 30 s. Importantly, a multirake electrode was used to stimulate 16 points per minislice. This method ensures saturation of LTP within each minislice and increases the ratio of stimulated to unstimulated tissue used for biochemical analyses (Clayton et al., 2002a; Nayak et al., 1996). Both stimulated and control minislices were harvested 30 min after LTP induction and subjected to three independent biochemical assays to evaluate the subcellular distribution of glutamate receptors. All three measures indicated that neither the intracellular pool nor the total surface expression of the AMPAR subunits GluR1 and GluR2 was altered by LTP in adult rat CA1 (Grosshans et al., 2002). Importantly, these data do not address the possibility that AMPAR may be trafficked from extracellular sites to the synapse. Rather, these studies indicate that LTP-related trafficking from an intracellular pool to the synapse, as seen during the first few weeks of postnatal development (Hayashi et al., 2000; Esteban et al., 2003; Boehm et al., 2006), does not appear to occur in the adult. This result is likely due to the significantly smaller intracellular pool of AMPAR subunits present in adult CA1 (12–18%) as compared to 30–35% intracellular AMPAR subunits in CA1 hippocampus at postnatal day 9 (Grosshans et al., 2002). These results are consistent with electron microscopy studies demonstrating that synaptic AMPAR expression increases during development (Petralia et al., 1999). In the absence of altered AMPAR surface expression during early-phase LTP in adult hippocampus, the enhancement of AMPAR transmission may be largely due to increased channel conductance (Benke et al., 1998; Luthi et al., 2004). Alternatively, as mentioned earlier, AMPARs may be trafficked within the surface membrane (i.e., from extrasynaptic to synaptic sites).

In our forgoing discussions, we have only examined evidence that implicates AMPAR trafficking in early-phase LTP. There is also evidence to suggest a role for AMPARs in late-phase LTP. In one study, adult rats (250–500 g) were anesthetized, and hippocampal CA1 fEPSPs were recorded *in vivo* (Heynen et al., 2000). Robust LTP was achieved by application of 10 trains of HFS. More specifically, two 1-s trains of 100 Hz separated by 30 s were applied every 15 min for 1 h. Approximately 5.5 h after stimulation, GluR1 and GluR2 levels increased ~20% in synaptosomal fractions purified from LTP stimulated hippocampus versus nonstimulated yoked controls. This result is consistent with another study from our laboratory that suggests that maintenance of late-phase LTP correlates with a 45–55% increase in *de novo* synthesis of GluR1 and GluR2/3 subunits (Nayak et al., 1998). This increase in AMPAR synthesis was dependent on both transcription and translation. Additional experiments performed by Heynen et al. (2000) included application of low-frequency stimulation (LFS) to induce LTD; LFS was applied alone and in combination with LTP stimulation. The results of these experiments revealed a strong correlation between changes in GluR2 and GluR1 protein levels and changes in synaptic strength during late-phase LTP (Heynen et al., 2000). A key issue that was not addressed in these studies was how the newly synthesized AMPARs could be trafficked specifically to potentiated synapses. Because LTP is confined to stimulated synapses, some sort of targeted trafficking would be required for specific synaptic delivery of

new AMPARs resulting from transcriptional and translational events. We discuss briefly how NMDARs could play a role in such targeted trafficking in a later section of this chapter.

4.30.3.3 Summary

It has become more widely accepted that multiple mechanisms of hippocampal LTP evolve throughout the course of postnatal development (Grosshans et al., 2002; Malenka, 2003; Malinow, 2003; Yoshimura et al., 2003; Palmer et al., 2004). It is primarily from this perspective that we have examined studies of AMPAR phosphorylation and trafficking as mechanisms of hippocampal LTP (**Table 2**). It is also quite interesting that recent work indicates that AMPAR trafficking can occur during learning (Rumpel et al., 2005). Thus, the various mechanisms that contribute to LTP and learning may be complimentary and even additive, such that more complex mechanisms become required as animals mature.

Early in postnatal development, homomeric GluR4 receptors are delivered to silent synapses by spontaneous activity (Zhu et al., 2000). This process requires the membrane proximal region of the GluR4 C terminus, NMDARs, and PKA phosphorylation but not CaMKII (Zhu et al., 2000; Esteban et al., 2003; Boehm et al., 2006). GluR1 subunits may also contribute to mechanisms of synaptic plasticity during early postnatal development. Studies conducted in preparations corresponding to postnatal day ~9 have reported surface or spine delivery of GluR1 following induction of LTP (Shi et al., 1999; Hayashi et al., 2000; Grosshans et al., 2002). The mechanism of GluR1 trafficking remains unclear, but it likely involves CaMKII activity and/or interaction with one or more PDZ domain–containing proteins (Hayashi et al., 2000; Malenka, 2003; Yasuda et al., 2003; Kim et al., 2005).

Trafficking of GluR1 has been studied more intensely in the juvenile hippocampus, where NMDARs, CaMKII, Ras, PKC, and PKA signaling pathways all seem to make significant contributions to synaptic delivery of AMPARs following induction of LTP (Zhu et al., 2002; Esteban et al., 2003; Boehm et al., 2006; Oh et al., 2006). Interestingly, robust LTP occurs in the juvenile hippocampus of GluR1 knockout mice (Jensen et al., 2003), and considerable evidence suggests that GluR2L subunits may mediate GluR1-independent mechanisms of LTP during this stage of postnatal development (Zhu et al., 2002; Kolleker et al., 2003). Although the source of these newly incorporated AMPARs has not been widely studied, it is likely that the large intracellular pool of receptors constitutes a stock of AMPARs available for synaptic incorporation following LTP stimulation in these preparations (**Figure 1**; Grosshans et al., 2002; Park et al., 2004).

Although studies of knock-out mice demonstrate that GluR1 subunits are required for LTP at mature synapses (Zamanillo et al., 1999; Mack et al., 2001), the contribution of AMPAR trafficking to mechanisms of LTP is less well characterized in adult animals. It is likely that the intracellular pool does not constitute an adequate source for synaptic incorporation of AMPARs during early-phase LTP

Table 2 Glutamate receptor subunits trafficked during hippocampal LTP at various developmental stages, and the phosphorylation sites involved[a]

Subunit trafficked	*Equivalent developmental stage*	*Phosphosite implicated*	*Experimental preparation*	*Reference*
GluR1	P8–11	pS831 (CaMKII) not required	Organotypic hippocampal slices	Shi et al. (1999) Hayashi et al. (2000)
GluR1 and GluR2	P9	None evaluated	Hippocampal slices	Grosshans et al. (2002)
GluR1	P13–15	pS845 (PKA)	Organotypic hippocampal slices	Esteban et al. (2003)
GluR1	P17–18	pS818 (PKC)	Organotypic hippocampal slices	Boehm et al. (2006)
GluR2-lacking AMPARs	P14–21	None evaluated	Hippocampal slices	Plant et al. (2006)
NR1, NR2A, and NR2B	6–8 weeks	Unspecified Tyr(s) pY1472 (Fyn) on NR2B	Hippocampal slices	Grosshans et al. (2002) Nakazawa et al. (2001)

[a]Studies of other brain regions, dissociated neurons in culture, LTP-independent AMPAR trafficking, and chemically induced LTP are not included.

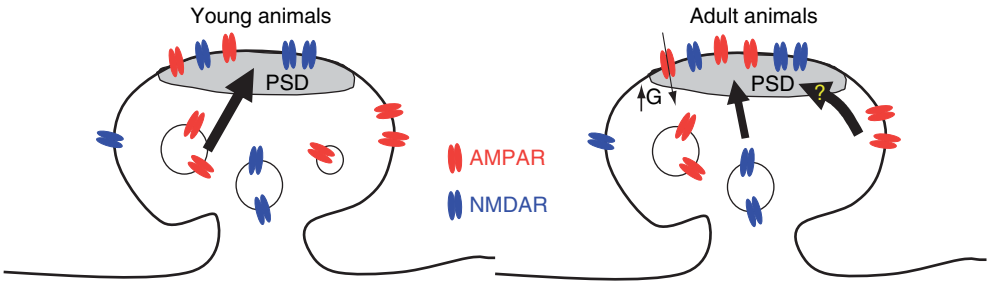

Figure 1 Proposed model for developmentally divergent mechanisms of AMPAR enhancement during LTP in the hippocampus. During postnatal development, hippocampal LTP leads to trafficking of AMPARs (red) from intracellular compartments to the postsynaptic density (PSD) of potentiated synapses (left). This mechanism does not occur in the hippocampus of mature animals, probably because the intracellular pool of AMPARs is much smaller than that in juvenile animals. Two alternative mechanisms, increased conductance (G) of existing AMPARs and lateral movement of AMPARs from extrasynaptic to synaptic sites, likely contribute to LTP of AMPARs in the adult hippocampus (right). Trafficking of NMDARs (blue) may contribute to one or both of these possibilities, as discussed in **Figure 2**.

(Grosshans et al., 2002). However, it remains possible that AMPARs are trafficked within the membrane (**Figure 1**). Alternatively, modulation of AMPAR channel conductance may be primarily responsible for enhanced transmission during the early phase of LTP in the adult hippocampus (Luthi et al., 2004).

4.30.3.4 Gaps in Our Knowledge

The precise mechanism of AMPAR potentiation in adult hippocampus remains one of the most intangible results in studies of LTP. As mentioned earlier, two possible mechanisms are (1) increased conductance of AMPARs already localized to the synapse and (2) lateral movement of extrasynaptic AMPARs to the postsynaptic density (**Figure 1**). There is clear evidence to suggest that the first mechanism occurs, at least in juvenile rats (Benke et al., 1998; Luthi et al., 2004). In support of the lateral trafficking mechanism, electrophysiological and single-molecule tracking studies indicate that AMPARs are highly mobile and move laterally within the plasma membrane (Choquet and Triller, 2003; Triller and Choquet, 2005). Additional studies suggest that synaptic and extrasynaptic glutamate receptors may be subject to differential regulation by synaptic activity and phosphorylation (Clark and Cull-Candy, 2002; Oh et al., 2006). Furthermore, detergent extraction studies indicate that distinct pools of glutamate receptors exist within the plasma membrane. Importantly, these pools exhibit differential association with the PSD (Alvestad et al., 2005). It is exciting to speculate that receptors that are loosely associated with the PSD represent a pool, possibly localized to extrasynaptic membrane, that may be subject to dynamic regulation in response to stimuli such as induction of LTP.

4.30.4 NMDA Receptor Trafficking in LTP

As discussed in the previous section, potentiation of AMPARs constitutes a majority of the enhanced postsynaptic responses seen after high-frequency stimulation of the hippocampal Schaffer collateral-CA1 synapse (Muller and Lynch, 1988). However, pharmacologically isolated NMDAR responses also undergo robust, synapse-specific LTP that can last for hours in many different LTP paradigms (Bashir et al., 1991; Xie et al., 1992; Grosshans et al., 2002; but also see Kauer et al., 1988). As mentioned previously, LTP induction requires NMDAR activation (Collingridge et al., 1983; Coan et al., 1987; Bashir et al., 1990) and tyrosine phosphorylation (O'Dell et al., 1991; Lu et al., 1998). In addition, NMDAR function is highly regulated by tyrosine phosphorylation (Wang and Salter, 1994; Chen and Leonard, 1996; Wang et al., 1996; Yu et al., 1997; Lu et al., 1999; Pelkey et al., 2002), and LTP of isolated NMDAR responses is blocked by tyrosine kinase inhibitors (Grosshans et al., 2002). Importantly, trafficking of NMDARs is also regulated by tyrosine phosphorylation (Dunah and Standaert, 2001; Roche et al., 2001; Vissel et al., 2001; Lavezzari et al., 2003, 2004; Goebel et al., 2005; Prybylowski et al., 2005; Snyder et al., 2005; Hallett et al., 2006; Nakazawa et al., 2006), and recent studies suggest that NMDAR surface expression is enhanced following LTP in an Src-family tyrosine kinase-dependent manner in area CA1 of the adult hippocampus (Grosshans et al., 2002). Therefore, it seems likely that potentiation and trafficking of NMDARs via tyrosine phosphorylation play key roles in LTP. We will now review evidence

concerning the mechanisms that underlie NMDAR trafficking and discuss the potential role of such trafficking in LTP in area CA1 of the hippocampus.

4.30.4.1 Molecular Biology of NMDARs

At depolarized membrane potentials, such as those achieved during high-frequency stimulation, NMDARs are permeable to Na^{2+}, K^{2+}, and Ca^{2+} (Ascher and Nowak, 1988). This influx of Ca^{2+} through the NMDAR is critical for LTP induction (Lynch et al., 1983) and is thought to lead to LTP via activation of downstream signaling cascades. To date, seven genes encoding the NMDAR subunits have been identified: NR1, NR2A-D, and NR3A-B (Moriyoshi et al., 1991; Meguro et al., 1992; Ishii et al., 1993; Ciabarra et al., 1995; Sucher et al., 1995; Chatterton et al., 2002). Functional NMDARs are thought to be tetrameric receptor complexes assembled as a pair of dimers formed by two NR1 subunits and two NR2 subunits (Ishii et al., 1993; Sheng et al., 1994; Laube et al., 1998; Schorge and Colquhoun, 2003), although the exact composition of synaptic NMDAR complexes remains unclear. (For a more comprehensive review of NMDAR molecular biology and structure, *See* Chapter 4.20.)

4.30.4.1.1 Developmental profile of NMDAR subunit expression

NR1, NR2A, and NR2B subunits are the most highly expressed NMDAR subunits in the adult hippocampus (Monyer et al., 1994; Nishi et al., 2001; Wong et al., 2002). NR1 mRNA (Monyer et al., 1994) and protein (Sheng et al., 1994; Sans et al., 2000) are abundant at birth and remain relatively constant throughout adulthood. Each of the three NR1 splice cassettes can be found in the hippocampus (Laurie and Seeburg, 1994; Coultrap et al., 2005); however, the C1 and C2 C-terminal cassettes are the predominant ones expressed in area CA1 of the adult hippocampus (Coultrap et al., 2005). Importantly, NR2 subunits have distinct patterns of developmental expression in the hippocampus. *In situ* hybridization studies show that NR2B is the primary NR2 mRNA expressed at birth in the hippocampus; however, in adults, NR2A and NR2B mRNA are both highly expressed, especially in areas CA1 and CA3 (Monyer et al., 1994). Interestingly, Sans et al. (2000) report that NR2A and NR2B proteins display opposing patterns of expression in the hippocampus. Specifically, this study reported that NR2A protein expression is undetectable at postnatal day 2 and substantially increases from postnatal day 10 to 6 months (Sans et al., 2000). Conversely, NR2B protein expression peaks early in development (postnatal days 2–10) and gradually decreases to 30% of maximum at 6 months (Sans et al., 2000). Importantly, NR2A and NR2B are still the most prevalent NR2 subunits expressed in the adult hippocampus (Monyer et al., 1994). NR2A and NR2B subunits also have distinct protein expression profiles within the different subregions of the hippocampus (Coultrap et al., 2005). For example, NR2A protein expression does not significantly differ among the three subregions; however, NR2B protein expression is significantly greater in area CA1 than the CA3 and dendate gyrus (Coultrap et al., 2005).

4.30.4.1.2 Tyrosine phosphorylation of NMDARs

It is well understood that NMDAR function can be modulated by tyrosine kinases and phosphatases. Src-family tyrosine kinases enhance NMDAR function (Wang and Salter, 1994; Lu et al., 1998), whereas tyrosine phosphatases decrease NMDAR function (Wang and Salter, 1994; Pelkey et al., 2002). Given that tyrosine kinases are required for induction of LTP (O'Dell et al., 1991; Lu et al., 1998), it is possible that tyrosine phosphorylation modulates NMDAR function during LTP induction. In fact, tyrosine phosphorylation of the NR2A (Grosshans et al., 2002) and NR2B (Rosenblum et al., 1996; Rostas et al., 1996; Nakazawa et al., 2001) subunits is enhanced following LTP. Moreover, Salter (1998) has proposed a model in which LTP induction leads to phosphorylation and enhancement of NMDARs via Src-family tyrosine kinase activation.

Although one group reported that NR1 surface expression is modulated by phosphorylation of a tyrosine residue (tyrosine 837) on NR1 in HEK293 cells (Vissel et al., 2001), NR1 does not appear to be tyrosine phosphorylated in brain (Lau and Huganir, 1995). Conversely, both NR2A and NR2B are tyrosine phosphorylated in brain homogenates (Lau and Huganir, 1995). Indeed, the intracellular C-terminal tails of NR2A and NR2B each contain 25 tyrosine residues that can be phosphorylated by nonreceptor tyrosine kinases, including Fyn and Src (Tezuka et al., 1999; Cheung and Gurd, 2001; Nakazawa et al., 2001; Yang and Leonard, 2001).

Studies in HEK293 cells report four potential sites of Src-mediated phosphorylation on the NR2A subunit: tyrosine 1105, tyrosine 1292, tyrosine 1325, and tyrosine 1387 (Zheng et al., 1998; Yang and Leonard,

2001). Two of these sites, tyrosine 1105 and tyrosine 1387, appear to modulate NR2A channel current by Src (Zheng et al., 1998); however, the role of tyrosine 1292 and tyrosine 1325 remains uncertain. Phosphorylation of another tyrosine residue on NR2A, tyrosine 1267, seems to play an important role in Src-induced potentiation of NR2A-containing receptors by reducing tonic inhibition of NMDARs by zinc (Zheng et al., 1998). Furthermore, phosphorylation of NR2A tyrosine 842 appears to inhibit internalization of NR1/NR2A recombinant receptors in HEK293 cells (Vissel et al., 2001). Although these studies demonstrate that the NR2A subunit can be tyrosine phosphorylated in heterologous cell systems, the exact sites of NR2A phosphorylation and their function in neurons remain incompletely understood. To the best of our knowledge, phosphorylation of only one of the aforementioned NR2A sites, tyrosine 1387, has been reported in brain (Besshoh et al., 2005). Thus, further work is required to determine the NR2A phosphorylation sites in neurons.

The NR2B subunit is reportedly the most tyrosine phosphorylated protein found within the PSD (Moon et al., 1994). *In vitro* kinase assays and work in HEK293 cells demonstrate that the NR2B subunit can be phosphorylated by Fyn at tyrosine 1252, tyrosine 1336, and tyrosine 1472 (Nakazawa et al., 2001). Importantly, tyrosine 1472 appears to be phosphorylated by both exogenous and endogenous PSD-associated Src-family tyrosine kinases (Cheung and Gurd, 2001). Phosphorylation of NR2B tyrosine 1472 has been proposed to regulate synaptic localization of NR2B-containing NMDARs (Roche et al., 2001; Lavezzari et al., 2003, 2004; Goebel et al., 2005; Prybylowski et al., 2005). It is important to note that phosphorylation of NR2B tyrosine 1472 increases after LTP induction in the hippocampus (Nakazawa et al., 2001). Phosphorylation of tyrosine 1252 and tyrosine 1336 has also been reported in neurons (Yaka et al., 2003); however, their role in LTP and NMDAR trafficking is incompletely understood. Because very little is known about the remaining 22 tyrosine residues located on the NR2B subunit, it is important for future studies to determine which additional NR2B residues are phosphorylated in neurons and to examine their role in regulating NMDARs.

4.30.4.1.3 Serine/threonine phosphorylation of NMDARs

Inhibitors of PKC block induction of LTP (Malinow et al., 1989). In addition, PKC activity and autophosphorylation are persistently elevated during LTP induction and maintenance (Klann et al., 1991, 1993; Sweatt et al., 1998). As such, PKC-mediated phosphorylation of its substrates appears to be an important part of LTP. We have shown that intracellular application of the constitutively active fragment of PKC potentiates NMDA-evoked currents by increasing the probability of NMDAR channel opening in cultured neurons and isolated CA1 hippocampal neurons (Xiong et al., 1998). This PKC-induced potentiation of NMDA-evoked currents in neurons is also dependent on the activation of Src-family tyrosine kinases (Lu et al., 1999). Notably, one study in oocytes suggests that PKC-induced potentiation of NMDARs is not due to direct phosphorylation of NR1 (Zheng et al., 1999) but is a result of phosphorylation of adaptor proteins, such as SNAP-25 (Lan et al., 2001).

Perhaps the most well characterized NMDAR PKC sites are found on the NR1 subunit. Tingley et al. (1997) reported that PKC activation increased phosphorylation of serine 890 and serine 896 in hippocampal slice homogenates. Phosphorylation of serine 890, but not serine 896, has been shown to increase NR1 surface expression in heterologous cell lines (Tingley et al., 1997). Two additional NR1 PKC sites have been identified *in vitro* (serine 889 and threonine 879); however, these sites have not been evaluated in neurons (Tingley et al., 1997). NR2A and NR2B can also be phosphorylated by PKC *in vitro* (Leonard and Hell, 1997). One study reported that NR2A can be phosphorylated by PKC at serine 1416 and suggested that this phosphorylation event inhibited CaMKII binding *in vitro* (Gardoni et al., 2001). Another group showed that PKC phosphorylation of NR2B at serine 1303 and serine 1323 enhances NR1/NR2B currents in oocytes (Liao et al., 2001b). However, the consequence of phosphorylating these sites remains incompletely understood in neurons.

PKA potentiation of NMDAR function has also been reported. In particular, PKA activation appears to overcome the constitutive activity of protein phosphatase 1, enabling PKA to enhance NMDAR currents in cultured neurons (Raman et al., 1996). A more recent report suggests that PKA facilitates LTP induction early in development by increasing Ca^{2+} entry through NMDARs (Skeberdis et al., 2006). Tingley et al. (1997) showed that the NR1 subunit is phosphorylated by PKA at serine 897 in brain homogenates. Notably, although both NR2A and NR2B can be phosphorylated by PKA *in vitro* (Leonard and Hell, 1997), the exact sites are unknown.

Other serine/threonine kinases are also capable of phosphorylating the NMDAR. NR2A appears to be phosphorylated by CaMKII at serine 1289 *in vitro* (Gardoni et al., 1999); however, it is unknown whether this residue is phosphorylated in neurons. NR2B serine 1303 is a major site of CaMKII phosphorylation in neurons (Omkumar et al., 1996), and this phosphorylation event appears to inhibit binding of CaMKII to NR2B in heterologous cells (Strack et al., 2000). Moreover, phosphorylation of NR2A by cyclin-dependent kinase 5 at serine 1232 has been proposed to be involved in LTP induction (Li et al., 2001). Furthermore, casein kinase II has been shown to phosphorylate serine 1480 on the NR2B subunit (Chung et al., 2004).

4.30.4.2 General Trafficking Mechanisms of NMDARs

Although NMDARs were previously thought of as a stable component of the PSD, mounting evidence in recent years has shown that NMDARs are actually a dynamic component of the PSD that can be trafficked between intracellular and plasma membrane compartments (reviews: Wenthold et al., 2003; Prybylowski and Wenthold, 2004), as well as between extrasynaptic and synaptic membrane compartments (Tovar and Westbrook, 2002). In addition, tyrosine phosphorylation has been proposed to modulate NMDAR function by regulating NMDAR trafficking. Roche et al. (2001) showed that endocytosis of NR2B-containing NMDARs in heterologous cells and dissociated neuronal cultures is dependent on the presence of a consensus tyrosine-specific internalization motif (YXXØ; Y = tyrosine, X = any amino acid, Ø = hydrophobic amino acid) located within the distal NR2B C-terminal tail. Binding of the clathrin-associated adaptor protein 2 (AP-2) $\mu 2$ chain to the NR2B subunit seems to require unphosphorylated tyrosine 1472, which is located within the YXXØ motif on the NR2B C-terminal tail (Lavezzari et al., 2003). Consistent with this hypothesis, recent evidence suggests that NR2B tyrosine 1472 phosphorylation and association with AP-2 are inversely correlated (Nakazawa et al., 2006). As a result, phosphorylated NR2B subunits remain bound to the cytoskeletal synapse-anchoring protein, α-actinin 2 (Nakazawa et al., 2006). Indeed, expression of a NR2B Y1472A mutant increases the number of synaptic NMDARs in dissociated neuronal cultures (Prybylowski et al., 2005). A similar YXXØ motif has been identified in the proximal C terminus of the NR2A subunit (Vissel et al., 2001). Mutation of the tyrosine residue within this motif (tyrosine 842) to phenylalanine enhances NR1/NR2A peak current in HEK293 cells, an effect that appears to be due to decreased clathrin-mediated endocytosis of NR1/NR2A receptors (Vissel et al., 2001).

Together, these studies provide evidence that phosphorylation of specific tyrosine residues regulates NMDAR trafficking in cultured systems. These mechanisms of NMDAR trafficking are also mirrored *in situ*. In particular, treatment of hippocampal slices with a tyrosine phosphatase inhibitor or Src-family tyrosine kinase inhibitor increases or decreases, respectively, NR2A and NR2B tyrosine phosphorylation, as well as the concentration of NMDARs in synaptosomal membrane fractions (Goebel et al., 2005). Notably, phosphorylation of tyrosine 1472 on the NR2B subunit is enhanced when hippocampal slices are treated with a tyrosine phosphatase inhibitor, suggesting a correlation between increased NR2B tyrosine 1472 phosphorylation and increased surface expression of the NMDAR (Goebel et al., 2005). Consistent with this hypothesis, Dunah and Standaert (2001) have established that dopamine D1 receptor activation leads to NMDAR movement to the membrane in striatal slices; this effect appears to be dependent on tyrosine phosphorylation. Moreover, in rat forebrain, ischemic challenge enhances NR2A and NR2B tyrosine phosphorylation and increases association of these subunits in the PSD (Besshoh et al., 2005).

We cannot exclude the possibility that phosphorylation of residues other than tyrosine 1472 regulates NMDAR trafficking and protein–protein binding interactions in the PSD. For example, phosphorylation of serine 1480 on the NR2B subunit negatively regulates binding of NR2B to the synaptic scaffolding proteins PSD-95/SAP90 and decreases NMDAR surface expression in cultured cortical neurons (Chung et al., 2004). Moreover, coordinated phosphorylation of specific PKC and PKA sites on the NR1 subunit (serine 896 and serine 897, respectively) has been shown to overcome ER retention signals and enhance surface delivery of NR1-containing receptors in heterologous cells and cultured neurons (Scott et al., 2003). It is also important to note that phosphorylation-mediated events are not likely the only means of modulating NMDAR trafficking. Indeed, recent evidence suggests that trafficking of the NR2A subunit is regulated by a dileucine motif in heterologous cells (Lavezzari et al., 2004).

4.30.4.3 Evidence for NMDAR Trafficking in LTP

The data just discussed have naturally led to the question of what is/are the physiological stimulations that trigger NMDAR trafficking to the synapse. Of particular importance to this review, Grosshans et al. (2002) reported that LTP stimulation leads to rapid redistribution of NMDARs from intracellular compartments to the synaptosomal membrane in area CA1 of the adult rat hippocampus (Grosshans et al., 2002). As described in the previous section, these authors saw no change in AMPAR surface expression in CA1 mini-slices obtained from adult animals following LTP. These data are in marked contrast to the selective increase in AMPAR surface expression that seems to occur early in postnatal development after LTP induction (Shi et al., 1999; Hayashi et al., 2000; Grosshans et al., 2002; Esteban et al., 2003; Boehm et al., 2006). In adults, up to 40% of total NR1, NR2A, and NR2B reside intracellularly in area CA1, providing a substantial pool of intracellular NMDARs that are available for recruitment to the synaptic membrane following LTP (Grosshans et al., 2002). Moreover, it is important to note that the increase in NMDAR surface expression seen following LTP in adults occurs in a PKC- and Src-family tyrosine kinase-dependent manner that persists for at least 3 h (Grosshans et al., 2002). As such, these findings highlight differential involvement of AMPAR and NMDAR trafficking in LTP induction between the neonatal and adult hippocampus. Because NMDARs do not significantly contribute to the enhancement of postsynaptic responses after LTP (Muller and Lynch, 1988), perhaps trafficking of NMDARs to the synaptic membrane in adults plays an indirect role in enhancing AMPAR function.

Consistent with the hypothesis that NMDARs can be trafficked as a result of synaptic activity, Quinlan et al. (1999) reported that NR2A-containing receptors are selectively trafficked to synaptoneurosomes in the adult primary visual cortex following light exposure, which is a form of sensory experience–dependent synaptic plasticity. Furthermore, recent work suggests that NMDAR-dependent LTD causes internalization of NMDARs via dynamin-dependent endocytosis and thus leads to a depression in isolated NMDAR currents (Montgomery et al., 2005; but see Morishita et al., 2005). Although the precise molecular mechanisms underlying NMDAR trafficking during these various types of synaptic plasticity are not well characterized, tyrosine phosphorylation likely plays an important role.

To date, relatively few studies have examined the correlation between NMDAR tyrosine phosphorylation, NMDAR surface expression, and LTP. As previously discussed, one exception is previous work from Nakazawa et al. (2001), which showed increased phosphorylation of NR2B tyrosine 1472 following LTP induction in the hippocampus. This study, along with work showing that nonphosphorylated tyrosine 1472 is important for endocytosis of NR2B-containing receptors (Roche et al., 2001; Lavezzari et al., 2004; Prybylowski et al., 2005), suggests the possibility that LTP increases tyrosine 1472 phosphorylation and blocks endocytosis of NR2B-containing receptors, thereby enhancing NMDAR function during LTP (Bashir et al., 1991; Xie et al., 1992; Grosshans et al., 2002). In agreement, our lab has reported that NMDAR surface expression is enhanced following LTP in the adult hippocampus (Grosshans et al., 2002). This study also provided evidence for the sequential activation of PKC and Src-family tyrosine kinases after LTP as a means to increase NMDAR tyrosine phosphorylation and suppress internalization of synaptic NMDARs (Grosshans and Browning, 2001; Grosshans et al., 2002). Moreover, a recent study by Nakazawa et al. (2006) showed that amygdaloid LTP is impaired in mice with a knock-in mutation of NR2B tyrosine 1472 to phenylalanine (Y1472F). Interestingly, localization of NR2B was found in more peripheral and perisynaptic regions of the PSD in the Y1472F mice (Nakazawa et al., 2006). These results suggest that phosphorylation of tyrosine 1472 and synaptically localized NR2B are essential for expression of LTP in the amygdala (Nakazawa et al., 2006). Although it has been shown that LTP increases NR2B tyrosine 1472 phosphorylation in the hippocampus (Nakazawa et al., 2001), hippocampal LTP was not affected in the Y1472F mice (Nakazawa et al., 2006). The authors suggest that some unknown compensatory mechanism may have developed in the Y1472F mice to suppress the hippocampal phenotype observed in the Y1472F mice (Nakazawa et al., 2006). Another possibility is that residues or subunits other than or in addition to tyrosine 1472 and NR2B, respectively, are responsible for hippocampal synaptic plasticity (Nakazawa et al., 2006).

4.30.4.4 NMDAR Subunit Requirements for Synaptic Plasticity

The contribution of the different NMDAR NR2 subunits to the expression of synaptic plasticity has been a

topic of intense debate in recent years. Clayton et al. (2002b) previously demonstrated that selective knock-down of the NR2B subunit using antisense oligonucleotides decreased isolated NMDAR fEPSPs and completely inhibited NMDAR-dependent LTP in the hippocampus. Furthermore, this study showed that NR2B expression and hippocampal-dependent spatial learning were significantly correlated, suggesting that the NR2B subunit has an important role in the expression of hippocampal LTP and behavioral learning (Clayton et al., 2002b). Notably, Tang et al. (1999) reported that overexpression of the NR2B subunit results in 'smart' mice, where LTP and spatial learning are enhanced.

More recent studies have utilized pharmacological antagonists to determine the role of NR2A and NR2B in LTP and LTD (Williams, 1993; Auberson et al., 2002). Using antagonists at doses purported to exhibit NR2A and NR2B selectivity, some studies concluded that NR2A-containing receptors are responsible for LTP induction, whereas NR2B-containing receptors are responsible for LTD induction (Liu et al., 2004; Massey et al., 2004). However, others provide compelling evidence that both the NR2A and NR2B subunits are capable of generating LTP and/or LTD (Berberich et al., 2005; Toyoda et al., 2005; Weitlauf et al., 2005; Zhao et al., 2005). Importantly, Neyton and Paoletti (2006) recently described the necessity of careful interpretation when analyzing data obtained using such NR2A and NR2B pharmacological antagonists. In particular, it seems as though the NR2A antagonist, NVP-AAM077, does not discriminate well between NR2A- and NR2B-containing receptors at the doses used by the studies cited above (Liu et al., 2004; Massey et al., 2004). In fact, one group suggested that this compound is not NR2A-selective at any concentration (Neyton and Paoletti, 2006). Furthermore, until recently, there has been no evidence to describe the selectivity of these pharmacological antagonists on triheteromeric NR1/NR2A/NR2B-containing receptors (Hatton and Paoletti, 2005), even though good evidence indicates that triheteromeric NMDARs exist in brain (Sheng et al., 1994; Cull-Candy and Leszkiewicz, 2004). One of the future challenges of the NMDAR field will be to reconcile these conflicting results with pharmacological tools that are able to take the complex, heteromeric nature of NMDARs into account.

4.30.4.5 Summary

Mounting evidence demonstrates that NMDARs can be trafficked between intracellular compartments and the plasma membrane, as well as between extrasynaptic and synaptic membrane compartments. In addition, phosphorylation of key tyrosine residues enhances the surface expression of NMDARs (Roche et al., 2001; Vissel et al., 2001; Lavezzari et al., 2004; Goebel et al., 2005; Prybylowski et al., 2005). Given that tyrosine phosphorylation of NR2A and NR2B subunits is increased following LTP induction (Rosenblum et al., 1996; Rostas et al., 1996; Nakazawa et al., 2001; Grosshans et al., 2002), it is reasonable to hypothesize that LTP also increases NMDAR surface expression. Indeed, work from our laboratory showed that the surface expression of NR1, NR2A, and NR2B is enhanced following LTP in area CA1 of the adult hippocampus (Grosshans et al., 2002). This process requires the sequential activation of PKC and Src-family tyrosine kinases, which is consistent with the hypothesis that tyrosine phosphorylation regulates NMDAR surface expression (Grosshans et al., 2002).

The notion that NMDARs are trafficked to the synapse following synaptic plasticity is not new. NR2A-containing receptors are selectively enriched in synaptoneurosomes prepared from the adult primary visual cortex following light exposure (Quinlan et al., 1999). NMDARs also appear to be internalized during LTD (Heynen et al., 2000; Montgomery et al., 2005). The mechanisms of NMDAR trafficking in these two forms of synaptic plasticity remain unclear, but they may involve tyrosine phosphorylation of the NMDAR.

The contribution of the different NR2 subunits in LTP is clearly still up for debate. Using pharmacological antagonists, some groups suggest that the NR2A subunit, but not the NR2B subunit, is required for LTP (Liu et al., 2004; Massey et al., 2004). However, other studies have shown that NR2B also plays an important role in LTP (Tang et al., 1999; Clayton et al., 2002b). Because recent reports cast doubt on the use of the NR2A pharmacological antagonist to determine the role of NR2A in LTP (Berberich et al., 2005; Weitlauf et al., 2005; Zhao et al., 2005), more sophisticated tools that are able to take the heteromeric nature of NMDARs into account are required to settle this problem.

4.30.4.6 Gaps in Our Knowledge

Key questions still remain regarding the role of NMDAR trafficking in LTP. Because the majority of LTP is expressed as an enhancement of AMPAR-mediated responses (Muller and Lynch, 1988), it remains unclear how increasing NMDAR surface expression contributes to LTP in the adult

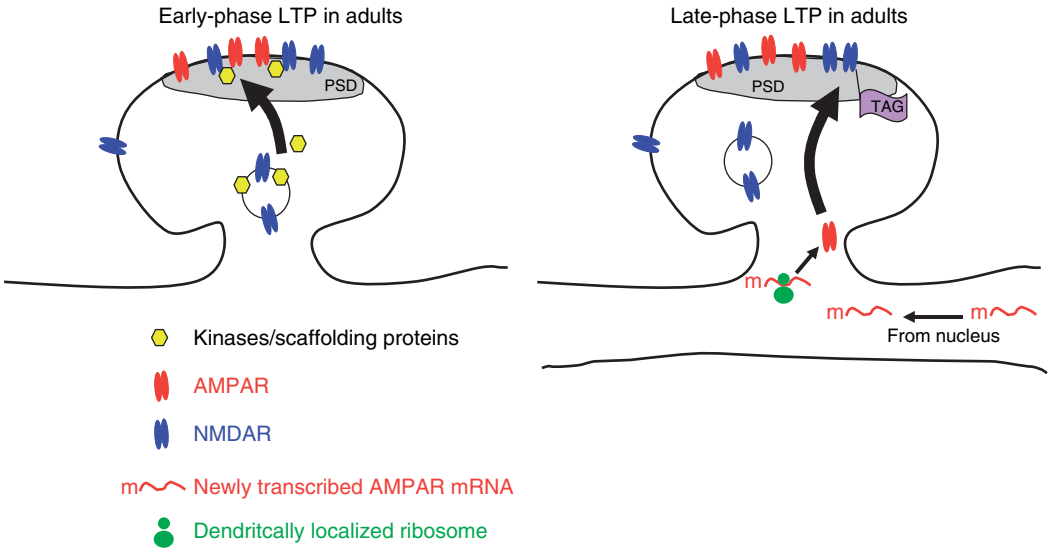

Figure 2 Proposed model for how increased *N*-methyl-D-aspartate receptor (NMDAR) surface expression during long-term potentiation (LTP) may enhance alpha-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA) receptor (AMPAR) function in the adult hippocampus. During early phase LTP in adults, NMDAR (blue) surface expression is increased. Because AMPARs (red) still constitute a majority of the enhanced excitatory postsynaptic potential after LTP, a mechanism other than increased AMPAR surface expression is required to potentiate the response. Certainly one possibility is lateral movement of AMPARs from extrasynaptic to synaptic sites, as proposed in **Figure 1**. It is also possible that NMDARs may act as chaperone molecules to transport kinases and/or scaffolding proteins (yellow) from intracellular compartments to the membrane during early-phase LTP (top). Translocation of these molecules to the postsynaptic density (PSD) may lead to increased conductance of AMPARs already present in the synapse. A third possibility is that increased NMDAR surface expression serves as a synaptic tag for targeted insertion of AMPARs during late-phase LTP (bottom). These receptors may be newly synthesized AMPARs shuttled from intracellular compartments or extrasynaptic membrane.

hippocampus. The data we reviewed here suggest that it may play an important role. In particular, inhibitors that decrease NMDAR surface expression also inhibit LTP (O'Dell et al., 1991; Lu et al., 1998; Dunah and Standaert, 2001; Goebel et al., 2005). Perhaps NMDARs are acting as chaperones and transporting kinases or scaffolding proteins to the synaptic membrane, where they can then enhance AMPAR responses (**Figure 2**). It is exciting to speculate that activated CaMKII may move with NMDARs to the synapse during LTP, where it can then phosphorylate serine 831 on GluR1-containing receptors (Barria et al., 1997) already localized in the synapse. CaMKII has been shown to bind tightly to NMDARs (Strack and Colbran, 1998; Leonard et al., 1999) and translocates to the PSD following synaptic stimulation (Shen and Meyer, 1999; Otmakhov et al., 2004). Given that phosphorylation of serine-831 by CaMKII potentiates GluR1 channel current (Barria et al., 1997), and AMPAR channel conductance is enhanced following LTP (Benke et al., 1998), it is conceivable that NMDARs chaperone CaMKII to the PSD and are responsible, at least in part, for mediating LTP in adults. Another intriguing possibility is that synapses with enhanced NMDAR surface expression serve as a tag for targeted insertion of newly synthesized AMPARs at potentiated synapses during the late phase of LTP (**Figure 2**; Nayak et al., 1998). Further investigation is required to rigorously address these questions and determine the role(s) of NMDAR trafficking following LTP in adults.

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4.31 AMPA Receptor Regulation and the Reversal of Synaptic Plasticity – LTP, LTD, Depotentiation, and Dedepression

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4.31.1 Introduction

Activity-dependent bidirectional changes in synaptic strength are recognized to be critical in mediating various functions of the brain, including learning and memory. Long-term potentiation (LTP) and long-term depression (LTD) are the two most studied forms of activity-dependent synaptic plasticity mechanisms, and both are implicated to play a role in memory formation. While LTP and LTD have been described in various synapses in the nervous system (reviewed in Malenka and Bear, 2004), molecular mechanisms of LTP and LTD are perhaps best understood at one particular set of synapses in the central nervous system: the Schaffer collateral inputs synapsing onto CA1 pyramidal neurons. The more commonly studied forms of LTP and LTD at this set of synapses are dependent on the activation of *N*-methyl-D-aspartate (NMDA) receptors, which act as coincident detectors of pre- and postsynaptic activity (reviewed in Malenka and Bear, 2004). A consequence of NMDA receptor activation is a rise in intracellular calcium, which acts on various signaling molecules, including several types of protein kinases and protein phosphatases, which can alter the biochemical makeup of the synapse. There is mounting evidence that one of the downstream molecules affected by NMDA receptor activation is alpha-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA) receptor. AMPA receptors are glutamate-gated ion channels, which mediate the majority of fast excitatory synaptic transmission in the central nervous system. They comprise four types of subunits, glutamate receptors (GluRs) 1–4 (or GluR A–D), which assemble in a combinatorial fashion (Nakanishi et al., 1990; Wenthold et al., 1992) into a tetrameric channel (Rosenmund et al., 1998; Safferling et al., 2001). Different subunits confer distinct properties to the receptor complex and show distinct developmental and spatial expression patterns (reviewed in Hollmann and Heinemann, 1994). For example, incorporation of GluR2 into the AMPA receptor complex makes the channel impermeable to calcium and contributes to the linear current-voltage relationship of the current flux (Hollmann et al., 1991; Verdoorn et al., 1991; Washburn et al., 1997). At least in the adult hippocampus, the majority of AMPA receptors comprise GluR1 and GluR2 subunits (Wenthold et al., 1996). As will be discussed further, regulation of AMPA receptor function has emerged as a critical endpoint for bidirectional synaptic plasticity (Malinow and Malenka, 2002; Lee, 2006a).

4.31.2 Reversibility of LTP and LTD

A key property of LTP and LTD is that they are readily reversible, which suggests that they are probably acting on common mechanisms for expression. Earlier evidence demonstrating reversibility came from electrophysiological studies using the fact that LTP and LTD can be saturated by multiple bouts of

high-frequency stimulation (HFS) and low-frequency stimulation (LFS), respectively. It was shown that after saturating LTP, LFS that elicits LTD 'unsaturates' LTP, so that a subsequent HFS can result in further potentiation (Dudek and Bear, 1993). Conversely, following LTD saturation, HFS can reverse LTD and increase the responses near the original baseline, and a further LFS can once again depress synaptic responses (Mulkey and Malenka, 1992). These results indicate that, when LTP increases synaptic weight through a cellular process, LTD can reverse this to decrease the synaptic strength, and that both synaptic changes are mediated by changing the same parameters but in opposite directions.

The observations that LTD and LTP mutually reverse each other led to the question as to whether LTP or LTD in a 'naive' pathway is the same as LTP or LTD in previously depressed or potentiation pathways. To distinguish these processes, the reversal of LTP is referred to as depotentiation (DeP) and reversal of LTD is called dedepression (DeD). Questions as to whether LTP or LTD *per se* is different from their reversal counterparts arose especially from earlier studies where several groups could not induce LTD in naive pathways, while still successfully inducing DeP (Barrionuevo et al., 1980; Staubli and Lynch, 1990; Fujii et al., 1991; Larson et al., 1993; O'Dell and Kandel, 1994). Although this may have come from differences in experimental preparations and/or stimulation paradigms, there is now accumulating evidence that the molecular underpinnings of LTD and DeP may indeed be different. Similarly, molecular mechanisms of LTP and dedepression seem to differ as well.

This review will focus on the regulation of AMPA receptors in LTP, LTD, DeD, and DeP. As will be discussed later, there is a form of DeP that can only occur within a critical time window after LTP induction, which is termed 'deconsolidation' (Bear and Abraham, 1996). We will compare the mechanisms of deconsolidation to DeP proper. In addition, mechanisms that lead to 'decay' of LTP (i.e., inability to maintain LTP) in terms of AMPA receptor regulation will also be discussed. A schematic comparison of all these processes is provided in **Figure 1**.

4.31.3 AMPA Receptor Regulation and LTP

Since the initial proposal of Lynch and Baudry (1984), regulation of AMPA receptors has emerged as one of the core mechanisms controlling synaptic strength. There is now a wealth of evidence that LTP is associated with an increase in synaptic AMPA receptor function. Activity-dependent changes in synaptic AMPA receptor function can be mediated by both alterations in the number of functional AMPA receptors or by changes in existing AMPA receptor function by mechanisms such as phosphorylation. There are data supporting both methods of AMPA receptor regulation during LTP, and these two mechanisms may be linked to each other.

An initial indication that LTP is associated with an increase in the number of functional AMPA receptors came from studies demonstrating the existence of 'silent synapses,' which lack functional AMPA receptors (Isaac et al., 1995; Liao et al., 1995). Importantly, these silent synapses could become functional after LTP induction (Isaac et al., 1995; Liao et al., 1995), which suggests that an LTP-inducing stimulus somehow allows functional AMPA receptors to be expressed at synapses. One of the first convincing demonstrations that LTP is indeed associated with synaptic incorporation of AMPA receptors came from Malinow's group, which visualized green fluorescent protein-tagged GluR1 subunits moving into spines following LTP using two-photon microscopy (Shi et al., 1999). Subsequent studies showed that these newly inserted AMPA receptors are functional and participate in synaptic transmission by measuring synaptic responses from electrophysiologically tagged GluR1 homomeric receptors (Hayashi et al., 2000; Shi et al., 2001). Synaptic insertion of AMPA receptors by LTP is not a universal feature of all AMPA receptor subunits, but only of GluR1 (Shi et al., 1999; Hayashi et al., 2000; Shi et al., 2001). On the other hand, exogenously transfected homomeric GluR2s are constitutively recycled into synapses independent of synaptic activity (Passafaro et al., 2001; Shi et al., 2001). The signal for the subunit-specific regulation was shown to be contained in the intracellular carboxy terminal regions of each subunit (Passafaro et al., 2001; Shi et al., 2001). The activity-dependent synaptic insertion of GluR1-containing AMPA receptors requires Ca^{2+}/calmodulin-dependent protein kinase II (CaMKII) activity (Hayashi et al., 2000; Shi et al., 2001) and depends on phosphorylation of GluR1-S818 (Boehm et al., 2006) and GluR1-S845 (Esteban et al., 2003) residues, but not GluR1-S831 (Hayashi et al., 2000). Proposed roles of these phosphorylation sites in LTP will be discussed in detail. An emerging view is that activity-dependent insertion of GluR1-containing AMPA receptors occurs at extrasynaptic sites followed by a lateral movement into synapses (Passafaro et al.,

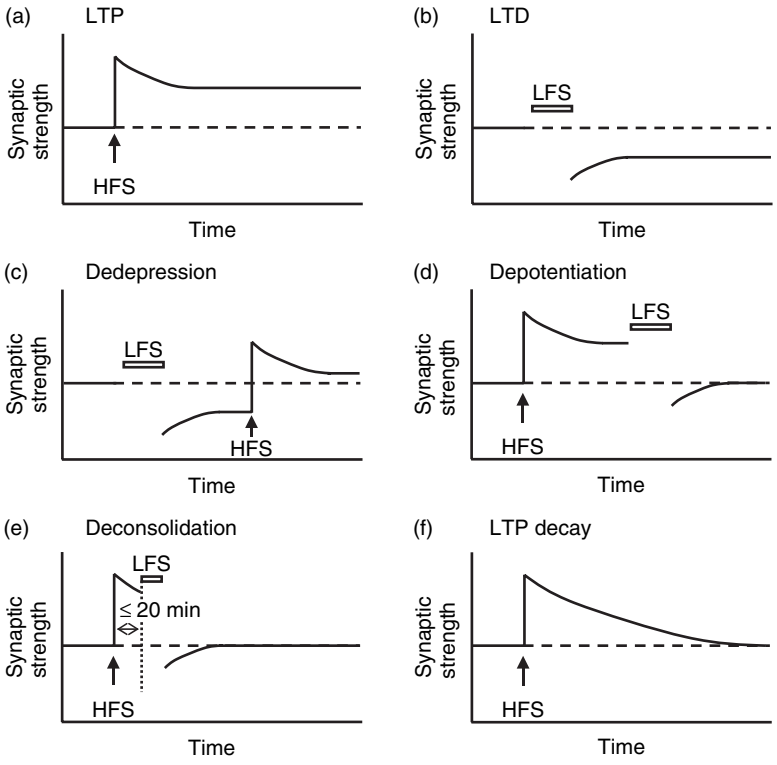

Figure 1 Different forms of synaptic plasticity. (a) Long-term potentiation (LTP). Brief trains of high-frequency stimulation (HFS) at a range of 100 Hz or theta burst pattern leads to a long-lasting increase in synaptic strength. (b) Long-term depression (LTD). A prolonged train of low-frequency stimulation (LFS) in the range of 1–3 Hz leads to a long-lasting decrease in synaptic strength. (c) Dedepression. LTD can be reversed by the same types of HFS used for inducing LTP. (d) Depotentiation. LTP can be reversed by a long train of LFS in the range of 1–5 Hz, often the same stimulation used for LTD induction. (e) Depotentiation that can only occur within a critical time window after LTP induction is sometimes referred to as deconsolidation. Stimulations that normally would not produce LTD (e.g., a shorter train of LFS) can result in deconsolidation. (f) LTP decay. Sometimes LTP fails to maintain stable expression and decays back to the original baseline.

2001; Andrasfalvy et al., 2003; Park et al., 2004). Recent data suggest that these newly inserted receptors containing GluR1, at least the ones lacking GluR2, are then replaced over time by GluR2-containing receptors (McCormack et al., 2006; Plant et al., 2006). Heteromeric GluR1s/GluR2s may also insert into synapses following LTP, since GluR1 acts dominantly over GluR2 (Shi et al., 2001).

The initial excitement over the role of AMPA receptor phosphorylation in LTP came from observations that phosphorylation of GluR1-S831 (Barria et al., 1997b; Lee et al., 2000), which may occur by CaMKII (Barria et al., 1997a; Mammen et al., 1997) and/or protein kinase C (PKC) (Roche et al., 1996), is increased following LTP. However, phosphorylation of this site increases single-channel conductance of only the homomeric GluR1s (Derkach et al., 1999; Oh and Derkach, 2005), while having no effect on heteromeric GluR1s/GluR2s (Oh and Derkach, 2005). This is interesting in light of recent data suggesting that there may be a transient insertion of homomeric GluR1s following LTP (McCormack et al., 2006; Plant et al., 2006). It is likely that GluR1-S831 phosphorylation may mediate the observed increase in AMPA receptor single-channel conductance following LTP induction (Benke et al., 1998; Poncer et al., 2002; Luthi et al., 2004). However, since the newly inserted homomeric GluR1s are replaced by GluR2-containing receptors over time (McCormack et al., 2006; Plant et al., 2006), this predicts that the increase in single-channel conductance would be transient. Whether this happens is unknown at present, but the time-dependent switch in AMPA receptor subunit composition may explain the cell-to-cell variability in detecting changes in AMPA receptor unitary conductance following LTP induction (Poncer et al., 2002; Luthi et al., 2004).

Phosphorylation of GluR1-S845 residue is also thought to participate in LTP by regulating CaMKII-dependent synaptic insertion of AMPA receptors (Esteban et al., 2003). This site is phosphorylated by protein kinase A (PKA) (Roche et al., 1996), which increases mean open probability of AMPA receptor channels (Banke et al., 2000). However, S845 phosphorylation is not significantly increased following LTP induction (Lee et al., 2000). A recent study suggested that phosphorylation of GluR1-S845 may 'prime' receptors for synaptic insertion by trafficking them to extrasynaptic sites (Oh et al., 2006). Collectively, these data support a model where basal phosphorylation of GluR1-S845 is permissive to LTP, but is not sufficient (Lee et al., 2000) (**Figure 2**).

In addition to their role in controlling AMPA receptor single-channel properties and synaptic trafficking, GluR1-S831 and S845 phosphorylation sites may participate in stabilizing LTP (Lee et al., 2003; Lee, 2006a). Recent characterization of a transgenic mouse line expressing GluR1 with both S831 and S845 sites mutated to alanine ('double phosphomutants') still expressed LTP, albeit in a lesser magnitude when compared to that of wild-type littermates (Lee et al., 2003). Specifically, LTP induced in double phosphomutants decayed faster than in wild type, which suggests that GluR1 phosphorylation may be important for stabilizing newly inserted AMPA receptors (Lee et al., 2003; Lee, 2006a). Based on the data from the double phosphomutants, we surmise that phosphorylation of GluR1 at S831 or

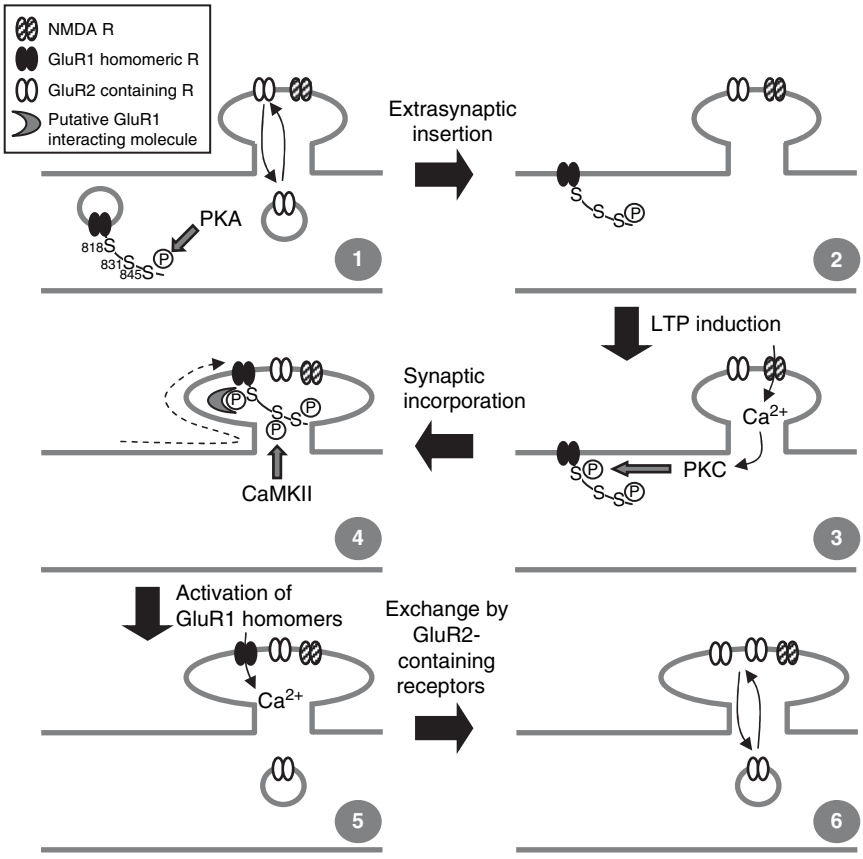

Figure 2 A model of AMPA receptor regulation during LTP. PKA phosphorylation of GluR1-S845 primes AMPA receptors for LTP by trafficking the receptors to extrasynaptic sites. Activation of NMDA receptors by LTP-inducing stimuli causes calcium influx and results in phosphorylation of GluR1-S818, which is likely mediated by PKC. Phosphorylation of GluR1-S818 is thought to bind a putative GluR1 binding protein, which ultimately incorporates AMPA receptors to synapses. GluR1 homomeric receptors inserted into synapses then may be activated to allow calcium influx that exchanges them with GluR2-containing receptors. This process may resemble calcium-permeable AMPA receptor plasticity (CARP) observed in cerebellar granule cells. Phosphorylation of synaptic GluR1 homomeric receptors at S831 by constitutively active CaMKII at synapses may facilitate the exchange process by perhaps allowing a larger influx of calcium.

S845 is not critical for synaptic insertion by LTP-inducing stimuli. However, this is does not exclude the proposed role of S831 in mediating the increase in AMPA receptor conductance following LTP or the priming role of S845. A possible role of GluR1-S831 and S845 phosphorylation in regulating LTP stability will be discussed in a later section.

A recent study highlighted another key phosphorylation site (S818) on the GluR1 subunit as being critical for LTP expression (Boehm et al., 2006). GluR1-S818 is phosphorylated by PKC and lies on the membrane-proximal region of the intracellular carboxy terminus (Boehm et al., 2006). While GluR1-S818 phosphorylation is increased following LTP, its function seems to depend also on phosphorylation of both S831 and S845 (Boehm et al., 2006). Mutating S818 to aspartate to mimic phosphorylation was itself insufficient for driving GluR1 into synapses, but adding aspartate mutations to S831 and S845 greatly increased synaptic incorporation of these receptors (Boehm et al., 2006). This suggests that the several phosphorylation sites on GluR1 may act synergistically in activity-dependent trafficking of the receptors. Boehm et al. (2006) further presented evidence that phosphorylation of S818 allows GluR1 to interact with a putative trafficking protein that allows synaptic incorporation of the receptors.

Collectively, the evidence so far suggests a model of LTP depicted in **Figure 2**. According to this model, the first step that happens before LTP induction is PKA phosphorylation of GluR1-S845, which primes the receptors for synaptic incorporation by inserting them at extrasynaptic sites (Oh et al., 2006). This is consistent with our previous model where GluR1-S845 phosphorylation is a prerequisite for LTP (Lee et al., 2000; Lee, 2006a). The next step is PKC phosphorylation of GluR1-S818, which allows the receptor to interact with a putative protein involved in delivering GluR1 to synapses (Boehm et al., 2006). This putative protein may be regulated by CaMKII, since activity of this enzyme is required for synaptic incorporation of GluR1 (Hayashi et al., 2000). In order for this model to work, both PKC and CaMKII activity should be coupled to NMDA receptor activation that results from LTP-inducing stimuli, while PKA activity may, in principle, precede NMDA receptor activation. There are reports that LTP-inducing stimuli do indeed activate both PKC and CaMKII (Akers et al., 1986; Klann et al., 1991; Fukunaga et al., 1993; Sacktor et al., 1993; Barria et al., 1997b; Ouyang et al., 1997) and both these enzymes are critical for LTP expression (Lovinger et al., 1987; Reymann et al., 1988; Malenka et al., 1989; Malinow et al., 1989; Silva et al., 1992; Abeliovich et al., 1993; Otmakhov et al., 1997; Giese et al., 1998). Activity of CaMKII and/or PKC may also phosphorylate GluR1-S831 (Barria et al., 1997b; Lee et al., 2000), which may increase the conductance of homomeric GluR1s (Oh and Derkach, 2005) that are newly anchored to synapses. Activation of these homomeric GluR1s may then drive their replacement by GluR2-containing receptors which are impermeable to calcium (Plant et al., 2006). This process may be similar to the calcium-dependent AMPA receptor plasticity (CARP) that has been described in cerebellar stellate neurons (Liu and Cull-Candy, 2000; Gardner et al., 2005). These GluR2-containing AMPA receptors then undergo constitutive recycling (Shi et al., 2001), which may allow for stable expression of LTP. While there is evidence that homomeric GluR1s are inserted following LTP induction (Plant et al., 2006), we cannot rule out synaptic insertion of heteromeric GluR1/GluR2 complexes after LTP. Indeed, exogenously expressed GluR1/GluR2 complexes are incorporated into synapses following LTP induction (Shi et al., 2001). Therefore, it is very likely that native heteromeric GluR1s/GluR2s may also insert into synapses following LTP. If this is the case, newly inserted heteromeric GluR1s/GluR2s will behave differently from homomeric GluR1s, since they are not calcium permeable (Hollmann et al., 1991; Washburn et al., 1997), and phosphorylation of GluR1-S831 does not affect their conductance (Oh and Derkach, 2005).

4.31.4 AMPA Receptor Regulation and LTD

Regulation of AMPA receptors is also critical for the expression of synaptic depression. LTD is known to involve endocytosis of synaptic AMPA receptors (reviewed in Carroll et al., 2001) and has been correlated with changes in phosphorylation of both GluR1 and GluR2 subunits (reviewed in Lee, 2006a). Specifically, LTD of 'naive' synapses in the CA1 region of hippocampus is associated with dephosphorylation of GluR1-S845 (Lee et al., 2000) and phosphorylation of GluR2-S880 (Kim et al., 2001). Furthermore, these changes in GluR1 and GluR2 phosphorylation sites accompany endocytosis of AMPA receptors (Chung et al., 2000; Ehlers, 2000; Perez et al., 2001; Lee et al., 2003). Indeed, LTD induction leads to synaptic loss of both GluR1 and GluR2 subunits (Heynen et al., 2000), and it is thought

that alterations in AMPA receptors phosphorylation may be responsible (reviewed in Lee, 2006a).

GluR1-S845 dephosphorylation by LTD was first demonstrated using a chemical method of LTD induction (chemLTD) that employs a brief bath application of NMDA (20 μmol l^{-1} for 3 min) (Lee et al., 1998). The dephosphorylation of GluR1-S845 was also observed following the induction of NMDA receptor–dependent LTD using a conventional LFS (1 Hz, 900 pulses) protocol (Lee et al., 2000). These findings suggest that the dephosphorylation of GluR1-S845 may be central to LTD expression. However, LTD induced by bath application of a metabotropic GluR (mGluR) agonist, (S)-3,5-dihydroxyphenylglycine (DHPG), resulted in an increase, not a decrease, in GluR1-S845 phosphorylation (Delgado and O'Dell, 2005). This is interesting in light of the observation that both NMDA- and DHPG-induced LTD cause a decrease in surface AMPA receptors (Nosyreva and Huber, 2005; Snyder et al., 2005; Holman et al., 2006). Together these results suggest that expression mechanisms of NMDA receptor–dependent and mGluR-dependent LTD may differ, one of the differences being the regulation of GluR1-S845 phosphorylation. The significance of the differential changes in GluR1-S845 during NMDA- and DHPG-induced LTD is unclear at this point. Nonetheless, at least for NMDA receptor–dependent LTD, dephosphorylation of GluR1-S845 (Lee et al., 1998, 2003; Ehlers, 2000; Vanhoose and Winder, 2003) and endocytosis of AMPA receptors (Ehlers, 2000; Lee et al., 2003; Nosyreva and Huber, 2005; Snyder et al., 2005; Holman et al., 2006) have been consistently observed. Whether these two processes are causal in nature or two independent parallel processes is currently undetermined. Nevertheless, the uncoupling of these two events during mGluR-dependent LTD clearly indicates that AMPA receptor endocytosis can occur independently from GluR1-S845 dephosphorylation.

One of the key properties of GluR1-S845 dephosphorylation following LTD is that it is long-lasting (Lee et al., 1998, 2000). This dephosphorylation is likely mediated by activity of a protein phosphatase, calcineurin (PP2B) (Lee et al., 1998). Since the phosphorylation level of GluR1-S845 is likely maintained by a balance of activity of PKA and PP2B (Lee and Huganir, 1999; Lee et al., 2000), the persistent dephosphorylation of GluR1-S845 may be achieved by either a persistent increase in calcineurin activity or a persistent decrease in PKA activity by LTD-inducing stimuli. It is recognized that both PP2B and PKA are placed in close proximity to GluR1 subunit by their association with A-kinase anchoring protein (AKAP), which in turn interacts with GluR1 through its binding protein SAP97 (Colledge et al., 2000). A ternary complex of GluR1-SAP97-AKAP has been observed in brain homogenates using a coimmunoprecipitation method, and the assembly of this complex seems to be required to increase phosphorylation of GluR1-S845 upon PKA activation (Colledge et al., 2000). A recent study showed that chemLTD induced by bath application of NMDA moves AKAP-PKA complexes away from synapses (Smith et al., 2006), which may be responsible for the persistent dephosphorylation of GluR1-S845 observed following LTD. The importance of AKAP-PKA complex in regulating AMPA receptor function can be further seen from studies that report a 'run-down' of fast excitatory synaptic transmission (Rosenmund et al., 1994; Snyder et al., 2005), and a reduction in surface GluR1 (Snyder et al., 2005) by distrupting PKA interaction with AKAP. Interestingly, synaptic depression caused by PKA dissociation from AKAP occluded further LTD (Snyder et al., 2005), suggesting that this mechanism is likely utilized for normal LTD expression. Collectively, these results suggest a model where LTD induction leads to a loss of PKA-AKAP complex from synapses, which then results in a persistent dephosphorylation of GluR1-S845 and a loss of surface AMPA receptors. The exact relationship between GluR1-S845 dephosphorylation and AMPA receptor endocytosis is unknown at present.

In addition to dephosphorylation of GluR1-S845, LTD increases phosphorylation of GluR2-S880 (Kim et al., 2001). S880 is part of a PDZ (PDZ: postsynaptic density-95, discs large, and ZO-1) ligand (-SVKI) on the extreme carboxy terminal tail of GluR2, which interacts with two types of PDZ domain–containing proteins, glutamate receptor interacting protein/AMPAR-binding protein (GRIP/ABP) and protein interacting with C-kinase 1 (PICK-1) (reviewed in Song and Huganir, 2002). Phosphorylation of S880 differentially affects GRIP and PICK-1 binding to GluR2 such that it prevents GRIP binding, but not PICK-1 (Matsuda et al., 1999; Chung et al., 2000). One of the current models suggests that activated PKC binds PICK-1, which in turn interacts with GRIP via its BAR (BAR: Bin1, amphyphysin, Rvs167) domain (Lu and Ziff, 2005). This allows PKC to locate in close proximity to GluR2 carboxy-terminal to phosphorylate S880 (Lu and Ziff, 2005). This dissociates GRIP from GluR2 and allows stable interaction with PICK-1 (Chung et al., 2000; Lu and

Ziff, 2005). PICK-1 is then thought to initiate endocytosis of GluR2-containing AMPA receptors by producing membrane curvature via its BAR domain (Lu and Ziff, 2005), which interacts with membrane lipids (Lu and Ziff, 2005; Jin et al., 2006). Recruitment of adaptor protein-2 (AP2) and clathrin coats is then thought to mediate endocytosis of the receptors (Carroll et al., 1999; Man et al., 2000; Lee et al., 2002). Although the role of PKC in cerebellar LTD is well established (Linden and Connor, 1991; De Zeeuw et al., 1998; Xia et al., 2000; Goossens et al., 2001; Chung et al., 2003), hippocampal LTD is not blocked by a PKC inhibitor (Kim et al., 2001). On the contrary, PKC activity is reported to decrease following LTD induction (Hrabetova and Sacktor, 1996; Thiels et al., 2000). Therefore, it is unlikely that PKC is responsible for GluR2-S880 phosphorylation following LTD in hippocampus.

Despite evidence supporting a role of GluR2-S880 in hippocampal LTD (Kim et al., 2001), GluR2 itself seems unnecessary for LTD expression. Knockout mice lacking GluR2 or double knockout of GluR2 and GluR3 still exhibit LTD (Meng et al., 2003), presumably via the remaining GluR1 subunit. The exact relationship between GluR1-dependent and GluR2-dependent LTD mechanisms are unclear at this point. They could be two independent mechanisms that can occur in parallel. If this is the case, while a GluR1-dependent mechanism may be able to support LTD in the absence of GluR2 (Meng et al., 2003), the opposite seems not to be the case when considering the observation that GluR1 phosphorylation site mutants lack LTD despite having GluR2 (Lee et al., 2003). There is some indication that GluR1- and GluR2-dependent mechanisms may occur in parallel. For example, NMDA receptor–dependent activation of Rab5 leads specifically to GluR1-S845 dephosphorylation and AMPA receptor endocytosis without changes in GluR2-S880 phosphorylation (Brown et al., 2005). An alternative possibility is that GluR1- and GluR2-dependent mechanisms of LTD occur in series, such that GluR1 dephosphorylation is required for GluR2-dependent endocytosis mechanisms to proceed, or GluR1 dephosphorylation stabilizes AMPA receptors that are endocytosed by GluR2-dependent machinery (for a more detailed discussion see Lee, 2006a). However, if this is the case, it is a bit more difficult to explain how a GluR1-dependent LTD mechanism can be sufficient in the absence of GluR2 subunits (Meng et al., 2003). **Figure 3** summarizes the proposed roles of both GluR1 and GluR2

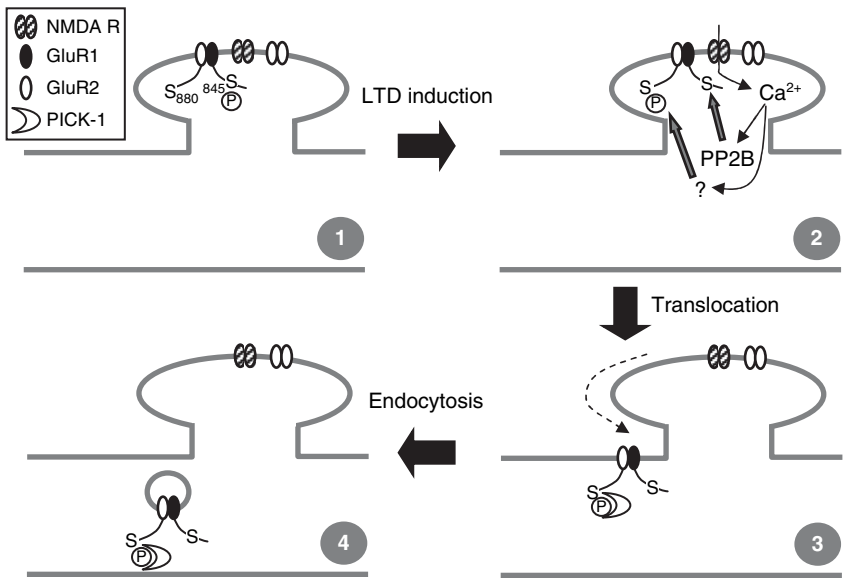

Figure 3 A model of AMPA receptor regulation during LTD. Activation of NMDA receptors by LTD-inducing stimuli activates calcineurin (PP2B), which dephosphorylates GluR1-S845. NMDA receptor activation also leads to phosphorylation of GluR2-S880 via an unknown protein kinase. Changes in phosphorylation of GluR1 or/and GluR2 lead to synaptic removal of the receptor, which occurs first by translocation to extrasynaptic sites followed by endocytosis. Binding of PICK-1 to the GluR2 subunit is thought to phosphorylate GluR2-S880 and initiate endocytosis. However, the exact interaction between GluR1 and GluR2 in mediating LTD is unclear. GluR1 dephosphorylation alone may be sufficient for LTD expression.

phosphorylation sites in LTD. How regulation of GluR1 and GluR2 phosphorylation coordinates AMPA receptor endocytosis during LTD awaits further investigation.

4.31.5 How Is LTP Different from LTD Reversal (Dedepression)?

HFS can result in synaptic potentiation whether it is from a naive state (as in the case of LTP) or from a previously depressed state (which is referred to as LTD reversal or DeD). There is emerging evidence that LTP and DeD may be expressed by different mechanisms (Abeliovich et al., 1993; Daw et al., 2000; Heynen et al., 2000; Lee et al., 2000) and can be affected differentially in some transgenic mouse lines (Abeliovich et al., 1993; Laird et al., 2005). This is quite interesting in light of the fact that both LTP and DeD are often induced by identical stimulation protocols, suggesting that prior history of synaptic activity can alter the mechanisms underlying subsequent synaptic potentiation.

While both LTP (Collingridge et al., 1983; Coan et al., 1987; Tsien et al., 1996) and DeD (Lee et al., 2000) in the CA1 region depend on NMDA receptor activation, distinct signaling cascades may be activated downstream. Although CaMKII is critical for LTP (Malenka et al., 1989; Malinow et al., 1989; Silva et al., 1992; Otmakhov et al., 1997; Hinds et al., 1998; Frankland et al., 2001; Elgersma et al., 2002), inhibition of CaMKII does not affect DeD (Daw et al., 2000; Lee et al., 2000). In addition, knockouts of PKCgamma display reduced LTP, but DeD was unchanged (Abeliovich et al., 1993). These results suggest that while CaMKII/PKC signaling is important for LTP, it is not critical for DeD. On the other hand, DeD is preferentially affected by inhibition of PKA (Lee et al., 2000). The observed differences in protein kinase requirement for LTP and DeD are consistent with distinct changes in GluR1 phosphorylation sites following the two forms of potentiation (Lee et al., 2000). Unlike LTP, which increases phosphorylation of GluR1-S831 (Barria et al., 1997b; Lee et al., 2000) and GluR1-S818 (Boehm et al., 2006), DeD is correlated with phosphorylation of GluR1-S845 (Lee et al., 2000). Collectively, these findings suggest that a CaMKII/PKC-dependent pathway in LTP, and a PKA-dependent signaling in DeD. One of the downstream events for LTP and DeD is distinct regulation of GluR1 phosphorylation sites (**Figure 4**).

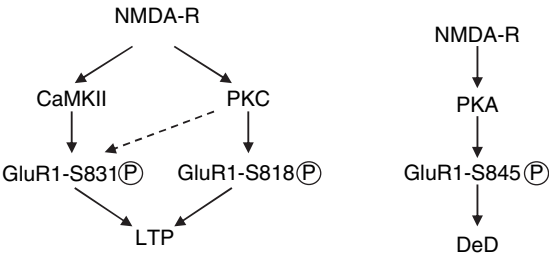

Figure 4 Comparison of AMPA receptor regulation underlying LTP and dedepression (DeD). While both LTP and DeD are induced by activation of NMDA receptors (R), they differ in their downstream signaling. LTP involves activation of CaMKII and PKC, which results in phosphorylation of GluR1 on S818 and S831. These two phosphorylation sites are involved in synaptic insertion of GluR1-containing AMPA receptors and increase in channel conductance of homomeric GluR1s as depicted in **Figure 1**. On the other hand, DeD is not dependent on activation of CaMKII or PKC, at least the gamma isoform of PKC, but requires PKA activity. PKA is then thought to increase GluR1-S845 phosphorylation to mediate DeD. Whether GluR1-S818 increases with DeD is currently unknown. How GluR1-S845 phosphorylation alone (if GluR1-S818 is not phosphorylated) leads to synaptic potentiation is unclear.

Despite the differences, there are also similarities between LTP and DeD, in that both can occur in the absence of GluR2 and GluR3 (Meng et al., 2003), which implies that GluR1 plays a dominant role in both forms of synaptic strengthening. In addition, DeD, like LTP, involves an increase in synaptic content of AMPA receptors without changes in total amount of these receptors (Heynen et al., 2000). (Differences with the findings of Nayak et al. (1998), who reported increases in newly synthesized AMPA receptors following LTP, will be discussed later.) These results suggest that, despite the differences in protein kinase requirement and regulation of distinct GluR1 phosphorylation sites, both LTP and DeD likely involve activity-dependent trafficking of existing AMPA receptors to synaptic loci mediated by GluR1. It is unclear at this point why LTP and DeD are mediated by regulating distinct GluR1 phosphorylation sites (**Figure 4**), if one of the downstream events is identical (i.e., more synaptic AMPA receptors). However, it may be that LTP and DeD have other downstream effectors that need differential regulation. In support of this view, while LTP does not increase synaptic content of an obligatory NMDA receptor subunit NR1, DeD does (Heynen et al., 2000).

4.31.6 How is LTD Different from LTP Reversal (Depotentiation)?

It is perhaps no surprise that LTD in naive synapses and DeP (i.e., synaptic depression following LTP) are also mediated by different molecular mechanisms. It has been recognized that LTD and DeP show distinct features (**Table 1**). One of the earliest indications that LTD and DeP may differ came from observations that stimulation protocols that fail to reliably induce LTD

Table 1 Comparison of long-term depression and depotentiation

	Long-term depression	*Depotentiation*
Induction protocols	1–3 Hz, 900 pulses (Dudek and Bear, 1992; Mulkey and Malenka, 1992).	1–5 Hz, 900–1200 pulses (Dudek and Bear, 1993; Bashir and Collingridge, 1994; O'Dell and Kandel 1994; Wagner and Alger, 1995; Huang et al., 1999; Heynen et al., 2000; Milner et al., 2004). TPS (theta pulse stimulation: 5 Hz, 1 min) (Larson et al., 1993; Staubli and Chun, 1996a,b).Strong intensity TBS (theta burst stimulation) (Barr et al., 1995). 1 Hz, 100 pulses (Staubli and Lynch, 1990).
Age dependence	Magnitude is reduced with age (Dudek and Bear, 1993; Wagner and Alger, 1995).	Reliably induced in adults (Staubli and Lynch, 1990; O'Dell and Kandel, 1994; Wagner and Alger, 1995; Heynen et al., 2000; Milner et al., 2004).
Time dependence	–	Depends on induction protocol. Only within 20 min post-LTP: TPS (Larson et al., 1993; Staubli and Chun, 1996b), 5 Hz (900 pulses) (O'Dell and Kandel, 1994), 2 Hz (1200 pulses) (Huang et al., 1999). Even after 30 min post-LTP: 1 Hz (900–1000 pulses) (Fujii et al., 1991; Dudek and Bear, 1993), 2 Hz (900 pulses) (Bashir and Collingridge, 1994), strong intensity TBS (Barr et al., 1995).
Induction mechanisms	NMDA-R dependent (Dudek and Bear, 1992; Mulkey and Malenka, 1992). Not blocked by group 1 mGluR antagonist, MCPG (Selig et al., 1995).	Depends on induction protocol. 1 or 5 Hz, 900 pulses: NMDA-R dependent (Fujii et al., 1991; O'Dell and Kandel, 1994; Wagner and Alger, 1995; Milner et al., 2004). TPS: Not dependent on NMDA-R (Staubli and Chun, 1996a), but on A1-adenosine R (Staubli and Chun, 1996a). 2 Hz, 900 pulses: Not dependent on NMDA-R, but on group 1 mGluR (Bashir and Collingridge, 1994). 2 Hz, 1200 pulses: Dependent on NMDA-R and A1-adenosine R, but not on group 1 mGluR (Huang et al., 2001). Strong intensity TBS: NMDA-R dependent (Barr et al., 1995).
Intracellular signaling	Blocked by inhibitors of PP1 and PP2A (Mulkey et al., 1993). Depend on calcineurin (Mulkey et al. 1994; Zeng et al., 2001). Reversed by PKA activators (Kameyama et al., 1998).	Blocked by PP1 inhibitors (O'Dell and Kandel, 1994; Huang et al., 2001). Does not require calcineurin (Lu et al., 1996; Zhuo et al., 1999; Zeng et al., 2001), except 5 Hz (900 pulses)-induced DeP (Zhuo et al., 1999). Dephosphorylation of CaMKII-T286 (Huang et al., 2001).
Regulation of AMPA receptors	Dephosphorylation of GluR1 S845 without changes in S831 (Lee et al., 2000). Increase in GluR2 S880 phosphorylation (Kim et al., 2001). Decrease in synaptic GluR1 and GluR2 (Heynen et al., 2000).	Dephosphorylation of GluR1 S831 without changes in S845 (Lee et al., 2000; Huang et al., 2001). Decrease in synaptic GluR1 and GluR2 (Heynen et al., 2000).

are still able to depress previously potentiated synapses (Staubli and Lynch, 1990; Larson et al., 1993; Bashir and Collingridge, 1994; O'Dell and Kandel, 1994; Barr et al., 1995; Staubli and Chun, 1996a; Huang et al., 1999). In addition, while the magnitude of LTD is diminished as animals mature (Dudek and Bear, 1993; Milner et al., 2004), several laboratories reported robust DeP in adults (Staubli and Lynch, 1990; O'Dell and Kandel, 1994; Wagner and Alger, 1995; Heynen et al., 2000; Milner et al., 2004).

Understanding the mechanisms of DeP has been complicated by the fact that the characteristics of DeP itself often seem to differ across laboratories (**Table 1**). For instance, there are contradictory reports as to whether DeP is NMDA receptor–dependent (Fujii et al., 1991; Bashir and Collingridge, 1994; O'Dell and Kandel, 1994; Barr et al., 1995; Wagner and Alger, 1995; Staubli and Chun, 1996a; Milner et al., 2004), or whether it can be elicited past a critical time window ($\leq$20 min) after LTP induction (Fujii et al., 1991; Dudek and Bear, 1993; Larson et al., 1993; Bashir and Collingridge, 1994; O'Dell and Kandel, 1994; Barr et al., 1995; Staubli and Chun, 1996b; Huang et al., 1999). Interestingly, the apparent contradiction seems to stem from differences in the induction protocols used for each study. If the induction protocol for DeP is also effective at producing LTD, it is likely NMDA receptor dependent (Fujii et al., 1991; Wagner and Alger, 1995; Staubli and Chun, 1996a; Milner et al., 2004) and it can be induced even 30–60 min after LTP induction (Fujii et al., 1991; Dudek and Bear, 1993; Wagner and Alger, 1995) (for exceptions refer to **Table 1**). However, if the induction protocol is ineffective at producing LTD, DeP is often induced in a manner independent of NMDA receptor activation (Larson et al., 1993; Bashir and Collingridge, 1994; O'Dell and Kandel, 1994; Staubli and Chun, 1996a; Huang et al., 1999) and is limited to a short critical time window ($\leq$20 min) after LTP induction (Larson et al., 1993; Staubli and Chun, 1996b; Huang et al., 1999) (for exceptions refer to **Table 1**). These differences in various DeP protocols led to a notion that they may not trigger the same process. Consequently, LTP reversal that can only happen in a narrow time window after LTP was coined as deconsolidation (Bear and Abraham, 1996), to distinguish it from DeP, which is not critically dependent on the time after LTP induction (**Figures 1(d)** and **1(e)**). As will be discussed, our knowledge on the molecular mechanisms of LTP reversal suggest that deconsolidation and depotentiation may be two distinct processes.

The observation that stimuli that do not produce LTD by themselves can still reverse LTP (i.e., deconsolidation) when delivered immediately after LTP induction raises an important point. This suggests that there is a critical window of time when LTP is vulnerable. The reason for this vulnerability is unclear at this point, but may have to do with some of the properties of LTP. As discussed in a previous section, LTP is thought to entail transient synaptic expression of GluR1 homomeric AMPA receptors, which are then replaced by GluR2-containing receptors over time (Plant et al., 2006). It will be of interest to know whether the vulnerable time window of LTP is due to the transient increase in calcium-permeable GluR1 homomeric AMPA receptors at synapses following LTP. If this is the case, it may be that once newly inserted calcium-permeable AMPA receptors are replaced by GluR2-containing receptors, LTP can only be reversed by stimuli that are more robust at inducing synaptic depression.

While there are data that DeP is blocked by inhibitors of protein phosphatase 1 (PP1) (O'Dell and Kandel, 1994; Huang et al., 2001), it is not affected by calcineurin (PP2B) inhibitors (Lu et al., 1996; Huang et al., 2001). This suggests that, unlike LTD, which is calcineurin dependent (Mulkey et al., 1994), DeP may be independent of this enzyme. This is further confirmed by the observation that DeP is normal, even though LTD is absent, in a forebrain-specific calcineurin knockout mouse (Zeng et al., 2001). However, the involvement of calcineurin in DeP seems to also depend on the induction protocol used. For instance, 5 Hz (900 pulses)–induced LTP reversal was absent in calcineurin A-alpha knockout mice, but 1 Hz (900 pulses)–induced DeP was intact (Zhuo et al., 1999). Since the 5 Hz (900 pulses) protocol does not reliably induce LTD in naive slices (Zhuo et al., 1999) and only occurs within a critical time window after LTP induction (O'Dell and Kandel, 1994), it may be considered deconsolidation rather than DeP. This again stresses the potential differences in mechanism between the two forms of LTP reversal.

Another difference between DeP and LTD is that they seem to involve dephosphorylation of distinct sites on the GluR1 subunit of AMPA receptors (**Table 1**, **Figure 5**). While LTD results in a dephosphorylation of GluR1-S845 (Lee et al., 2000), DeP correlates with a dephosphorylation on GluR1-S831 (Lee et al., 2000; Huang et al., 2001). These differences were interpreted on the basis that DeP is a reversal of LTP, which increases GluR1-S831 phosphorylation (Lee et al., 2000). The difference in

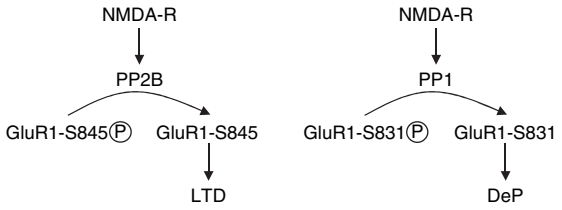

Figure 5 Differences in AMPA receptor regulation during LTD and depotentiation (DeP). NMDA receptor activation during LTD induction activates calcineurin (PP2B), which dephosphorylates GluR1-S845. This eventually leads to endocytosis of AMPA receptors to express LTD. On the other hand, DeP is independent of PP2B, but depends on protein phosphatase 1 (PP1). Activation of PP1 is thought to either directly or indirectly dephosphorylate GluR1-S831. How dephosphorylation of this residue leads to synaptic depression is currently unknown, but may involve changes in AMPA receptor conductance.

GluR1 phosphorylation sites affected by DeP could be due to two possibilities, which are not mutually exclusive. First, LTP induction may 'mask' the GluR1-S845 phosphorylation site; hence it is no longer accessible for dephosphorylation. Second, DeP may recruit a distinct signaling pathway, which may selectively target S831. For instance, if calcineurin selectively targets GluR1-S845, while PP1 targets S831, this may explain the differential dephosphorylation following LTD and DeP.

Interestingly, DeP not only reverses increases in GluR1 and GluR2 following LTP, but is also associated with a decrease in synaptic NMDA-type glutamate receptor subunit (NR1) (Heynen et al., 2000). This suggests that there may be an asymmetry between mechanisms of LTP and DeP. The decrease in NR1 observed following DeP was similar to what was observed following LTD (Heynen et al., 2000), suggesting that synaptic removal of AMPA receptors may be coupled to NMDA receptor downregulation. Functional relevance for the observed downregulation in NMDA receptor function following synaptic depression is unclear at this point. In addition, whether the decrease in synaptic AMPA receptors is due to dephosphorylation of GluR1-S831 is unknown.

4.31.7 Is There a Difference between LTP Decay and LTP Reversal (Depotentiation)?

In addition to activity-dependent reversal of LTP by DeP, it is known that LTP can decay over time (i.e., fail to stabilize) under certain conditions (**Figure 1(f)**). This raises a question as to whether LTP decay and DeP are mediated by the same molecular mechanisms. Another way to pose the question is whether LTP decay is an activity-dependent process that resembles DeP. There are conflicting data as to whether the magnitude of LTP is dependent on basal stimulation after LTP induction (Kauer et al., 1988; Volianskis and Jensen, 2003; Fonseca et al., 2006; Plant et al., 2006). Results from awake freely moving animals suggest that LTP decay is an active process requiring NMDA receptor activation (Villarreal et al., 2002). However, it is important to note that synaptic activity in freely moving animals may be high enough to trigger deconsolidation or DeP, which depends on NMDA receptors. Therefore, trying to tease apart LTP decay from LTP reversal is quite difficult. To start the discussion, it is prudent to define our use of the term 'LTP decay.' We will use this term to describe a reduction in synaptic strength by basal stimulation *in vitro* (which presumably has less endogenous synaptic activity compared to *in vivo* awake situations) after LTP without active stimulation at frequencies that are known to elicit DeP. Here we will discuss three types of manipulations that facilitate LTP decay to gain insights into the mechanism: (1) reagents that affect protein kinase or protein phosphatase activity, (2) pharmacological blockade of GluR2-lacking AMPA receptors, and (3) inhibition of protein transcription or translation. Keeping in line with the focus of this review, we will only discuss how these manipulations may affect AMPA receptor regulation. The evidence presented is consistent with a model of LTP decay as a failure to consolidate LTP.

It is known that application of various protein kinase inhibitors, especially PKC and PKA inhibitors, prevents stable maintenance of LTP (Lovinger et al., 1987; Reymann et al., 1988; Malinow et al., 1989; Klann et al., 1991; Matthies and Reymann, 1993; Huang and Kandel, 1994; Qi et al., 1996; Abel et al., 1997; Otmakhova et al., 2000; Ling et al., 2002; Duffy and Nguyen, 2003; Serrano et al., 2005). CaMKII inhibitors, on the other hand, do not affect LTP maintenance (Otmakhov et al., 1997). In addition, there is evidence that protein phosphatases, especially calcineurin, negatively regulate LTP maintenance (Winder et al., 1998; Malleret et al., 2001). These observations suggest that a balance between ongoing protein kinase (likely PKC and/or PKA) and protein phosphatase activity may be critical in determining the stability of LTP. The

critical substrates of the protein kinases required to stabilize LTP can be numerous, including molecules involved in controlling gene transcription (Impey et al., 1998; Pittenger and Kandel, 2003), translation (reviewed in Kelleher et al., 2004), and many of the synaptic proteins (reviewed in Lee, 2006b). In terms of AMPA receptor regulation, an interesting observation is made from mice lacking two of the GluR1 phosphorylation sites (S831 and S845). These GluR1 double phosphomutants show LTP, which then decays faster than that in wild-type littermates (Lee et al., 2003; Lee, 2006a). This suggests that one of the targets of the protein kinases implicated in LTP maintenance may be GluR1. How GluR1 phosphorylation stabilizes LTP is unclear at this point, but may have to do with either enhancing synaptic incorporation of AMPA receptors or promoting exchange with GluR2-containing receptors (refer to **Figure 2**).

Another manipulation that leads to faster LTP decay is application of a polyamine toxin, which selectively blocks GluR2-lacking AMPA receptors and prevents their exchange with GluR2-containing receptors (Plant et al., 2006). These results suggest that replacement of newly inserted AMPA receptors with GluR2-containing receptors may be critical in preventing LTP decay. Whether this process is dependent on ongoing protein kinase activity or is an independent mechanism for ensuring LTP stability is currently unclear. However, if the exchange of newly inserted GluR2-lacking receptors occurs via similar mechanisms as the activity-dependent AMPA receptor subunit exchange (referred to as CARP) described in cerebellar stellate cells (Liu and Cull-Candy, 2000, 2002; Gardner et al., 2005), it would require PICK-1 interaction with GluR2 (Gardner et al., 2005; Liu and Cull-Candy, 2005) and, hence, phosphorylation of GluR2-S880.

A third manipulation that leads to LTP decay is blockade of protein synthesis (Stanton and Sarvey, 1984; Frey et al., 1988; Huang and Kandel, 1994; Nguyen et al., 1994) or transcription (Nguyen et al., 1994; Frey et al., 1996; Vickers et al., 2005). Interestingly, a recent study found that LTP decay by protein synthesis inhibitor itself is dependent on activation of NMDA receptors following LTP induction (Fonseca et al., 2006). The interpretation of this finding was that synaptic stimulation following LTP may increase protein turnover rate via NMDA receptor activation and hence make LTP more susceptible to protein synthesis inhibitors (Fonseca et al., 2006). It is important to stress that inhibition of protein synthesis or transcription can affect a vast array of proteins (for a list of some of the genes regulated by LTP see Abraham and Williams, 2003), but we will restrict the discussion to regulation of AMPA receptor synthesis. Nayak et al. (1998) reported an increase in newly synthesized GluR1 and GluR2 measured 3 h following LTP induction. However, there is also a report that the total amount of GluR1 and GluR2 are not changed 3–6 h after LTP induction *in vivo* (Heynen et al., 2000). The discrepancy between the two studies may be due to differences in experimental methods. Nayak et al. (1998) measured newly synthesized AMPA receptors by ^{35}S-methionine labeling, while the Heynen et al. (2000) detected AMPA receptor subunits in total hippocampal homogenate, which will include not only newly synthesized receptors but also preexisting receptors. In any case, the former result suggests that increasing synthesis of AMPA receptor subunits may be one mechanism to stabilize LTP. Interestingly, inhibitors of PKA or transcription prevented the increase in newly synthesized AMPA receptors following LTP, suggesting that PKA signaling and transcriptional upregulation may be involved (Nayak et al., 1998).

From the standpoint of AMPA receptor regulation, it seems LTP decay and DeP differ only in how they affect LTP maintenance mechanisms. In the case of DeP, active stimulation of either NMDA receptors or other receptors (e.g., A1-adenosine receptors or mGluRs) results in a signaling cascade that ultimately downregulates synaptic AMPA receptors. As for LTP decay, whether it is an active (i.e., dependent on stimulation or NMDA receptor activation) or a passive (i.e., independent of synaptic activation) process, it ultimately seems to result from failure to maintain LTP-induced upregulation of AMPA receptor function.

4.31.8 Concluding Remarks

Reversible regulation of AMPA receptors has surfaced as one of the core mechanisms mediating bidirectional synaptic plasticity in the central nervous system. Studies on the mechanisms of LTP, LTD, and their reversal counterparts reveal that the molecular changes associated with synaptic potentiation and depression depend critically on the history of prior experience of the synapse. For example, synaptic potentiation in naive synapses recruits distinct signaling and downstream effectors when compared to synapses that have undergone prior LTD. These

results suggest that synapses have distinct states dependent on prior synaptic changes. Interpreting results of distinct GluR1 phosphorylation sites regulated by LTP, LTD, DeP, and DeD in hippocampal CA1 region, we have suggested that synaptic AMPA receptors may exist in at least three distinct states (Lee et al., 2000). As our understanding of the molecular events following bidirectional synaptic plasticity has expanded, we have come to gain more insights into the molecular mechanisms involved in transiting between different synaptic states by synaptic activity. Discrete synaptic states that depend on the history of synaptic activity have also been demonstrated in CA3-to-CA3 recurrent connections (Montgomery and Madison, 2002, 2004). While the details of the transition between distinct synaptic states at CA3-to-CA3 synapses differ from those observed in Schaffer collateral-to-CA1 synapses, these findings nonetheless indicate that this may be a common property of diverse sets of synapses.

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4.32 The Role of the Postsynaptic Density and the Spine Cytoskeleton in Synaptic Plasticity

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4.32.1 Introduction

The regulatory machinery that orchestrates induction, expression, and stabilization of long-term potentiation (LTP) and long-term depression (LTD) at excitatory synapses in the forebrain is located in specialized structures called synaptic spines. Spines are tiny structures, usually less than 1 μm wide and a few micrometers long (Harris, 1999). They have a specialized cytoskeletal structure that consists of an elaborate submembrane scaffold beneath synaptic receptors, termed postsynaptic density (PSD) (Kennedy, 2000), and an actin-based skeleton that maintains their mushroom-like shape (Matus, 2000). The PSD comprises an arrangement of scaffold proteins that organize signaling enzymes that respond to Ca^{2+} influx through activated *N*-methyl-D-aspartate (NMDA)-type glutamate receptors (NMDARS) or metabotropic glutamate receptors. The signaling machinery in the PSD, together with cytoskeletal regulatory proteins in the spine, work together to alter the structure of the spine to produce LTP or LTD.

To understand the intricate biology of the PSD and spine, it is important to understand (1) the protein components of the PSD, (2) the physical interactions among these proteins, and (3) the composition,

function, and regulation of the subdomains of the protein network formed by these interactions. Studies from our laboratory and many others over the past 15 years have identified the most prominent and ubiquitous PSD proteins (Kennedy et al., 1983; Cho et al., 1992; Kistner et al., 1993; Kornau et al., 1995; Muller et al., 1996; Niethammer et al., 1996; Kennedy, 1997; Kim et al., 1997, 1998; Chen et al., 1998; Boeckers et al., 1999a; Lim et al., 1999; Tu et al., 1999; Sheng and Kim, 2000). More recently, continuing proteomic analyses (Husi et al., 2000; Walikonis et al., 2000; Li et al., 2004; Peng et al., 2004) have resulted in a catalog of putative PSD-associated proteins that comprises a few hundred individual proteins. Although a few of these studies have begun to address the crucial question of the relative abundance of each protein and their stoichiometric ratios in the PSD fraction (Li et al., 2004, 2005; Peng et al., 2004), we are still in the early stages of understanding which core structures are common to most excitatory synapses, and which proteins are found in a subset of such synapses, perhaps conferring specialized properties.

One useful way to envision the molecular architecture of the PSD is to imagine it as a top-down hierarchy starting with the three major classes of glutamate receptors (NMDAR, α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid [AMPA]-type glutamate receptor [AMPAR], and mGluR) and continuing with scaffold proteins that bind directly to these receptors; for example, PSD-95, TARPs, and homer, respectively (**Figure 1**). These scaffolds, in turn, link to more distal scaffold proteins such as GKAP/SAPAP and shank/ProSAP, which form links between the protein complexes associated with each class of glutamate receptor. At every level (receptors, proximal scaffolds, and distal scaffolds), other effector proteins, regulatory enzymes, and adaptors are recruited into specific signal transduction modules that enable specialized adaptive functions.

4.32.2 Protein Complexes Associated With the NMDAR

The NMDAR (*See* Chapter 4.20) is a ligand-gated Ca^{2+}-permeable ion channel that plays a critical role in the initiation of several forms of synaptic plasticity, including long-term potentiation (LTP; *See* Chapters 4.16, 4.30) and long-term depression (*See* Chapters 4.17, 4.31). The predominant composition of the receptor is a tetramer containing a pair of NR1 subunits that contain a glycine-binding site and are essential for formation of the ion channel pore, and a pair of NR2 subunits that contain the glutamate-binding site and have long cytosolic tails of 500–600

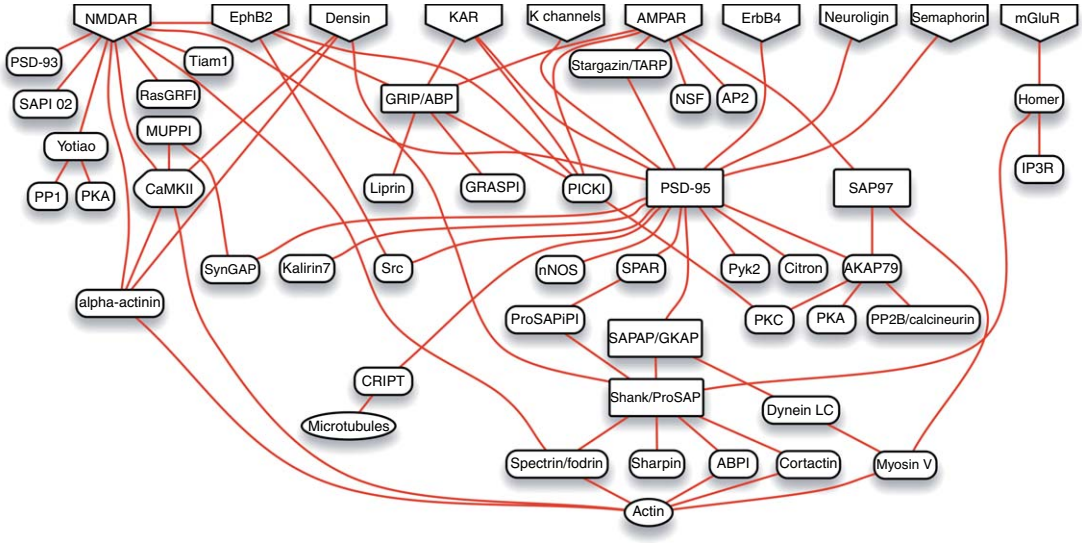

Figure 1 Protein–protein interaction map of postsynaptic density (PSD) and cytoskeletal proteins. The diagram represents protein–protein interactions documented in the literature and discussed in this chapter. The white boxes represent individual proteins, and the red lines represent binary physical interactions between two proteins. This representation does not imply that a series of interconnected proteins form an obligatory complex. PSD-95 is included as a representative of the related proteins PSD-93 and SAP-102, each of which interacts with many of the same proteins as PSD-95. As discussed in the text, these three proteins have partially redundant functions.

residues that extend into the cytosol and interact with scaffold proteins and signaling enzymes. Four different NR2 subunits are encoded on distinct genes, NR2A–D. NR2A and NR2B are the most abundant NR2 subunits in forebrain neurons. Individual receptors can contain two copies of either NR2A or NR2B, or they can contain one copy of each (Hollmann and Heinemann, 1994; Dingledine et al., 1999; Mayer and Armstrong, 2004; Mayer, 2005).

The most commonly studied forms of LTP (Bliss and Collingridge, 1993; Malenka and Bear, 2004) require activation of NMDARs (Morris et al., 1986), elevation of postsynaptic calcium concentration (Sabatini et al., 2001), and subsequent activation of Ca^{2+}/calmodulin-dependent protein kinase II (CaMKII) (Giese et al., 1998). Remarkably, a smaller increase in Ca^{2+} through these same receptors has been shown to trigger the opposite change in synaptic strength – long-term depression or LTD (Cummings et al., 1996). Thus, differences in the timing and extent of activation of NMDARs and the consequent influx of Ca^{2+} into the spine determine whether activation of a synapse will strengthen or weaken it (Zucker, 1999; Franks and Sejnowski, 2002). This delicate balance between distinct Ca^{2+}-activated processes is believed to depend on two characteristics of postsynaptic signaling proteins: their affinities for binding and activation by Ca^{2+} and their proximity to the source of Ca^{2+} influx, in this case the NMDAR pore. The spatial arrangement of signaling proteins is important because the influx of Ca^{2+} through the channel pores produces a transient rise and fall in Ca^{2+} concentration that is spatially restricted to the activated postsynaptic spine (Koester and Sakmann, 1998; Sabatini et al., 2002). Indeed, because Ca^{2+}-ATPases and exchangers rapidly pump Ca^{2+} back across the postsynaptic membrane, it is likely that Ca^{2+} influx produces a gradient with the highest Ca^{2+} concentrations reached near the mouth of receptor pores (Franks and Sejnowski, 2002). Thus, the precise location of individual Ca^{2+}-sensitive enzymes will help to determine how well they compete with others for binding and activation by Ca^{2+} moving through the NMDAR. A series of scaffold proteins and enzyme binding sites in the spine create highly organized signaling machinery near the NMDARs (Kennedy, 2000) (**Figure 2**). This machinery makes up a major portion of the PSD that is seen in the electron microscope.

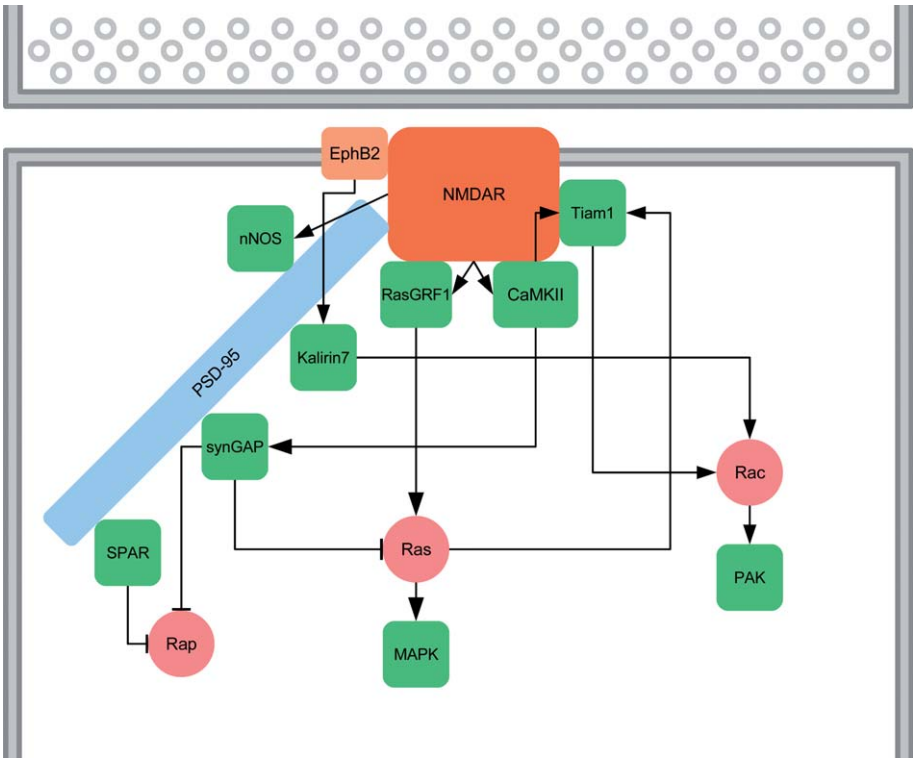

Figure 2 *N*-methyl-D-aspartate-receptor-associated complex. A schematic diagram of the major proteins of the NMDAR-associated complex. Binary physical interactions between two proteins are represented by their juxtaposition. Arrows represent regulatory actions (→ stimulatory, ⊣ inhibitory).

4.32.2.1 PSD-95

The first scaffold protein identified in the PSD was PSD-95 (also known as SAP90) (Cho et al., 1992; Kistner et al., 1993). Its sequence contains three PDZ domains, an SH3 domain, and a GUK (guanylate kinase-like) domain, all of which can act as specific protein-binding sites. Indeed, PDZ domains were first defined in PSD-95 (Cho et al., 1992; Kornau et al., 1995). The first and second PDZ domains of PSD-95 bind to the NMDAR primarily through association with an SXV motif at the C-terminus of the NR2A and NR2B subunits (Kornau et al., 1995). PSD-95 and its more recently identified homologs, including synaptic proteins SAP97 (Lue et al., 1994; Muller et al., 1995), PSD93/Chapsyn-110 (Kim et al., 1996; Brenman et al., 1996b), and SAP102 (Lau et al., 1996; Muller et al., 1996) have been termed MAGUKs (membrane-associated guanylate kinases).

The MAGUK proteins can induce clustering of NMDARs when both are overexpressed in heterologous cells (Kim et al., 1996). Studies on NMDARs expressed together with PSD-95 in *Xenopus* oocytes suggest that PSD-95 is capable of modulating their surface expression and biophysical properties (Yamada et al., 1999; Lin et al., 2006). Such findings were originally interpreted to mean that MAGUKs might be important for the surface expression and postsynaptic clustering of NMDARs. However, mutant mice with a mutation in the PSD-95 gene that deletes the first and second PDZ domains of PSD-95 have normal levels of synaptic NMDARs but display abnormal synaptic plasticity (Migaud et al., 1998). PSD-95, as well as other MAGUKs have been shown to play a role in targeting of AMPA receptors to the synapse in hippocampal neurons (Leonard et al., 1998; Chen et al., 2000; Schnell et al., 2002) (see also the section titled 'Stargazin binding to PSD-95'). Deletion from mice of both PSD-95 and PSD-93 alters AMPA-receptor trafficking in many synapses but does not reduce the number of NMDARs at synapses (Beique et al., 2006; Elias et al., 2006). Thus, the interaction between the NMDAR and MAGUK proteins is important for proper alignment and interaction of signaling proteins within the spine and for control of receptor trafficking rather than for receptor clustering at the synapse.

An important role that we emphasize in this chapter is the recruitment and organization of downstream signaling proteins that are regulated by Ca^{2+} flux through the NMDAR pore. For example, PSD-95 can bind to neuronal nitric oxide synthase (nNOS) through an atypical interaction with the first and second PDZ domains, linking it to the NMDAR (Brenman et al., 1996a; Christopherson et al., 1999). Similarly, synGAP, an abundant Ras-GTPase activating protein in the PSD fraction, binds to the third PDZ domain of PSD-95 (Chen et al., 1998; Kim et al., 1998). Both nNOS and synGAP are regulated by Ca^{2+} entry through the NMDAR (Christopherson et al., 1999; Oh et al., 2004). Thus, a principal role for PSD-95 may be to act as a scaffold for these proteins, placing them in proximity to a source of activating Ca^{2+} to form a signaling module whose activity is spatially and temporally restricted (Kennedy, 2000; Franks et al., 2006).

4.32.2.1.1 PSD-95 and regulation of small GTPases at the synapse

PSD-95 has been reported to bind to several regulators of small GTPase proteins, including synGAP (Chen et al., 1998; Kim et al., 1998), kalirin-7 (Penzes et al., 2000), SPAR (Pak et al., 2001), and citron (Zhang et al., 1999), suggesting that it may play a particularly important role in the orchestration of GTPase signaling in the postsynaptic spine.

Kalirin-7 is a guanine nucleotide exchange factor (GEF) for the Rho family of small GTPases that regulates the formation and maintenance of dendrites and spines (Penzes et al., 2000, 2001; Ma et al., 2003; Rabiner et al., 2005). When kalirin-7 is activated by binding of ephrin to the EphB2 receptor, it activates Rac and PAK1, leading to formation of mature dendritic spines (Penzes et al., 2003). We discuss the role of kalirin in actin dynamics in the section titled 'Kalirin.' Interestingly, activation of EphB2 promotes association between EphB2 itself and the NR1 subunit of the NMDAR (Dalva et al., 2000), as well as with postsynaptic PDZ domain-containing scaffold proteins such as GRIP/ABP (Irie et al., 2005) and PICK1 (Torres et al., 1998). In this way, activated EphB2 is recruited to the postsynaptic membrane. There it recruits and activates members of the Src family of nonreceptor tyrosine kinases, which phosphorylate NR2 subunits of the NMDAR, resulting in an increase in its ability to conduct Ca^{2+} upon glutamate stimulation (Wang and Salter, 1994; Yu et al., 1997; Takasu et al., 2002). By virtue of its association with kalirin-7, PSD-95 may help to coordinate regulation of NMDAR current and spine morphology by EphB2.

SynGAP is a Ras and Rap GTPase that is concentrated in the PSD, presumably by virtue of its interaction with the third PDZ domain of PSD-95 (Chen et al., 1998). Its GAP activity would be expected to increase the rate of inactivation of Ras that has been activated by RasGRF or by postsynaptic

TrkB receptors stimulated by BDNF. SynGAP's activity is enhanced by phosphorylation by CaMKII that is activated by stimulation of NMDARs (Oh et al., 2004). SynGAP appears to be a crucial synaptic regulatory protein because deletion of the synGAP gene is lethal in mice a few days after birth (Komiyama et al., 2002; Vazquez et al., 2004; Knuesel et al., 2005). Mice heterozygous for the synGAP deletion have learning deficits and defects in generation of LTP (Komiyama et al., 2002). Neurons cultured from mouse embryos homozygous for the synGAP deletion show derangements in cytoskeletal regulation (Vazquez et al., 2004) and in AMPA receptor trafficking (Krapivinsky et al., 2004; Rumbaugh et al., 2006).

The GUK domain of PSD-95 can bind directly to a spine-associated Rap-Gap, termed SPAR, and recruit it into the NMDAR complex. SPAR binds to actin and contributes to regulation of the actin cytoskeleton of the spine by downregulating the activity of Rap (Pak et al., 2001). SPAR also acts as a scaffold by associating with the master scaffold protein Shank/ProSAP (see the section titled 'Shank ProSAP scaffold of scaffolds') via an intermediate protein (ProSAPiP1) (Wendholt et al., 2006).

Finally, the third PDZ domain of PSD-95 associates with citron-N, a splice variant of citron kinase that lacks the kinase domain and is situated in neuronal cell bodies, as well as concentrated at glutamatergic synapses in inhibitory neurons in the hippocampus and cortex and excitatory neurons in the thalamus and basal ganglia (Furuyashiki et al., 1999; Zhang et al., 1999; Zhang and Benson, 2006). Citron-N is a target of activated Rho and participates in organization of the Golgi apparatus in neurons (Camera et al., 2003). It binds to the Golgi apparatus and controls actin filaments locally by assembling together the Rho effector ROCK-II and the neuron-specific actin-binding protein profilin IIa. The precise role of citron-N at synapses is still unknown. However, mutations in the gene encoding citron were recently implicated in genetic predisposition toward affective disorders (Lyons-Warren et al., 2005).

4.32.2.1.2 The GUK domain and GKAP

In addition to the PDZ domains, the C-terminal GUK domain of PSD-95 and other MAGUKs acts as a specific protein interaction site. A family of proteins called GK-associated proteins (GKAPs) bind to the GUK domain and are enriched in the PSD fraction (Kim et al., 1997; Satoh et al., 1997; Takeuchi et al., 1997). GKAPs in turn bind to members of the shank/ProSAP family (Naisbitt et al., 1999; Boeckers et al., 1999b). Shank proteins, like PSD-95, appear to function mainly as scaffold proteins because they contain a series of protein interaction motifs and lack identified catalytic domains. The single PDZ domain of shank binds to the C-terminus of GKAP. As we discuss in the section titled 'Shank ProSAP scaffold of scaffolds,' shank proteins interact with a series of other scaffold proteins, linking together protein complexes associated with the AMPA and metabotropic glutamate receptors.

4.32.2.1.3 Other membrane proteins that associate directly with PSD-95

A variety of other proteins have been reported to associate directly with PSD-95, including AKAP-79/150, which binds to the SH3 and GUK domains and acts as a scaffold for cAMP-dependent protein kinase (Colledge et al., 2000). The postsynaptic adhesion protein neuroligin binds to the third PDZ domain of PSD-95 (Irie et al., 1997; Song et al., 1999) and regulates synapse development (Prange et al., 2004) and retrograde signaling (Futai et al., 2007). PSD-95 – like EphB2 – can bind to Src kinases and thus may localize and/or regulate them in the PSD (Tezuka et al., 1999; Kalia and Salter, 2003; Kalia et al., 2006). The cytosolic C termini of both the receptor tyrosine kinase ErbB4 (Garcia et al., 2000; Huang et al., 2000) and semaphorin adhesion proteins (Schultze et al., 2001; Burkhardt et al., 2005) can bind to the first and second PDZ domains of PSD-95, as can the tails of shaker-type voltage-gated K^+ channels (Kim et al., 1995) and several inwardly rectifying potassium channels (Nehring et al., 2000). Finally, as we discuss below, PSD-95 and its homologs, including SAP-97, associate with complexes surrounding AMPA receptors. How and when interactions with each of these proteins occur at different synapses, how they are regulated, and how they alter synaptic function are subjects for future study.

4.32.2.1.4 Dynamics of association of PSD-95 with the PSD

Because PSD-95 and the other MAGUK proteins are capable of a large number of high-affinity associations with synaptic proteins (**Figure 1**), it is easy to imagine that individual PSD-95 proteins remain stably bound within a particular PSD once they arrive there. However, studies of tagged PSD-95 proteins *in vivo* reveal quite a different situation. Although the size of individual PSDs is generally stable, individual PSD-95 proteins exist in a highly dynamic equilibrium, moving rapidly among neighboring spines, with an average retention time in a single spine of 1–2 h (Gray et al.,

2006). This behavior suggests that individual spines compete for limiting amounts of PSD-95 and that larger PSDs contain more PSD-95 binding sites. This rapid dynamic equilibrium may permit correspondingly rapid remodeling of individual PSDs in response to biochemical changes in PSD-95-binding proteins triggered by synaptic activity.

Structural studies of PSD-95 itself hint at possible regulatory mechanisms that could control the availability of its binding sites. The SH3 domain of PSD-95 interacts intimately with the GK domain such that a portion of the GK domain is necessary to complete the SH3 fold (McGee and Bredt, 1999; McGee et al., 2001; Tavares et al., 2001). Point mutations that disrupt the interaction between the two domains interfere with the ability of PSD-95 to cluster potassium channels in heterologous cells, suggesting an alteration in availability of binding sites (Shin et al., 2000). Furthermore, patch analysis of the tertiary structure of PSD-95 suggests that it can exist in two distinct stable conformations: one that obscures the binding cleft of the third PDZ domain and another that exposes it (Korkin et al., 2006). Future studies of regulation of the conformation of PSD-95 may reveal new mechanisms that contribute to regulation of synaptic size.

4.32.2.2 Direct Association of Signaling Proteins with the Tails of NMDAR Subunits

Among several unusual specializations of the NMDAR are the extended C termini of the NR2 subunits. These tails comprise approximately 600 residues and extend into the cytosol, where they interact with PSD-95 (Kornau et al., 1995) but also bind directly to the structural protein; α-actinin (Wyszynski et al., 1997); the scaffold protein yotiao, which binds protein phosphatase 1 (PP1) and protein kinase A (PKA) (Westphal et al., 1999); and signaling enzymes CaMKII (Bayer et al., 2001) and RasGRFI (Krapivinsky et al., 2003).

4.32.2.2.1 CaMKII

Among the proteins that directly interact with NMDARs in the PSD, one of the best studied is Ca^{2+}/CaMKII (Rosenberg et al., 2005) (*See* Chapter 4.25). It is one of several critical determinants of synaptic plasticity that are activated by influx of Ca^{2+} through the NMDAR (Lisman et al., 2002; Kennedy et al., 2005). Its positioning near the NMDAR would place it in an ideal location to influence synaptic plasticity. Indeed, it appears that the level of CaMKII in the PSD correlates with the frequency threshold for induction of LTP (Elgersma et al., 2002).

To understand how CaMKII associates with the NMDAR (and other proteins in the PSD), it is necessary to understand a bit about its structure. CaMKII is activated by the Ca^{2+}-bound form of calmodulin (Ca^{2+}/CaM), which binds to a CaM-binding domain within the catalytic subunits and in so doing opens up the catalytic site so that it is able to interact with substrate proteins (Bennett et al., 1983; Bennett and Kennedy, 1987). The catalytic subunits are situated in a large holoenzyme made up of 12 individual catalytic units held together by interactions among association domains in the carboxyl third of their sequence (Bennett et al., 1983; Rosenberg et al., 2005, 2006). The holoenzymes are randomly assembled from two closely related catalytic subunits termed α and β. In the forebrain, α-subunits predominate over β-subunits by a ratio of 3 to 1. When all of the subunits are in an inactive state, the holoenzyme is actually composed of six dimers because individual pairs of catalytic subunits bind to each other by a coiled coil interaction between their calmodulin binding domains (Rosenberg et al., 2005). Activation of subunits is accompanied by autophosphorylation of a key residue in each subunit, threonine 286. Phosphorylation of this site prevents the catalytic site from closing when CaM dissociates; thus, it holds the subunit in an active conformation (Miller and Kennedy, 1986). In order for this autophosphorylation to occur, Ca^{2+}/CaM must be bound to two neighboring subunits, one that acts as the kinase and a second that acts as the substrate (Hanson et al., 1994). Based on their crystal structure, Rosenberg et al. (2005) proposed that binding of Ca^{2+}/CaM dissociates the members of an inactive dimer pair, exposing their active sites and permitting autophosphorylation to occur between them.

CaMKII is a prominent constituent of the subcellular fraction enriched in PSDs (Kennedy et al., 1983). Because the holoenzyme is so large ($\sim 20 \times 10$ nm), it contains many possible sites that could bind to docking proteins in the PSD. Furthermore, it is an abundant protein in the brain, constituting approximately 1% to 2% of the total protein content (Erondu and Kennedy, 1985), and is, therefore, likely to be present in excess of its individual binding sites in the PSD. At least three proteins in the PSD have been identified as potential docking sites for CaMKII in the PSD: the NR1 subunit of the NMDAR, the tails of the NR2 subunits of the NMDAR, and densin, a sialylated transmembrane protein that has a short cytosolic domain and is highly enriched in the PSD (Apperson et al., 1996). Three

other proteins located near the PSD also bind CaMKII. These are α-actinin, which forms a stable ternary complex with CaMKII and densin (Walikonis et al., 2001) and with CaMKII and NR2B (Leonard et al., 2002); F-actin, which binds the less abundant β-subunit of CaMKII, but not the more abundant α-subunit (Ohta et al., 1986; Shen et al., 1998); and the PDZ-domain-containing scaffold protein MUPP-1 (Krapivinsky et al., 2004).

The two most prominent NR2 subunits in the forebrain, NR2A and NR2B, both bind CaMKII at sites located in their long cytosolic C termini (Gardoni et al., 1998; Strack and Colbran, 1998; Leonard et al., 1999). Binding to either one requires activation of CaMKII by Ca^{2+}/CaM; however, the binding sites in NR2A and NR2B are not identical. The tail of NR2B binds CaMKII with high affinity at two distinct sites (Strack et al., 2000; Bayer et al., 2001): a distal site (residues 1259–1310) that requires binding of Ca^{2+}/CaM to CaMKII and a more proximal site (residues 829–1120) that only binds CaMKII after autophosphorylation at threonine 286 (Bayer et al., 2001; Schulman, 2004). Binding to the distal site stabilizes the activated form of CaMKII (Bayer et al., 2001). However, phosphorylation by CaMKII of serine 1303 on NR2B reduces the affinity of the distal site and leads to slow dissociation of the kinase from NR2B (Strack et al., 2000). The tail of NR2A binds CaMKII with a lower affinity than NR2B. Binding to a site in NR2A between residues 1244 and 1464 is enhanced by Ca^{2+}/CaM and by autophosphorylation (Gardoni et al., 1999). Phosphorylation by protein kinase C of serine 1416 in NR2A inhibits binding of CaMKII and promotes dissociation of the CaMKII/NR2A complex, providing a mechanism by which activation of mGluRs decreases binding of CaMKII to the NMDAR (Gardoni et al., 2001).

The short cytosolic tail of the NR1 subunit of the NMDAR can bind CaMKII in the 30-residue membrane proximal region termed C0 (Leonard et al., 2002). Like the binding to the membrane proximal region of NR2B, this binding requires autophosphorylation of CaMKII at threonine 286. Binding of CaMKII is competitive with binding of Ca^{2+}/CaM and α-actinin to NR1 (Leonard et al., 2002). Because binding of α-actinin increases the open probability of the NMDAR, the competitive binding of autophosphorylated CaMKII may contribute to Ca^{2+}-dependent inactivation of NMDARs (Krupp et al., 1999).

Differences in the requirements for the binding of CaMKII to different proteins in the spine and PSD suggest ways that translocation of CaMKII may be orchestrated in the spine under different conditions of stimulation. For example, binding of Ca^{2+}/CaM to CaMKII causes its release from F-actin (Ohta et al., 1986) and from the scaffold protein MUPP1 (Krapivinsky et al., 2004). In contrast, binding of Ca^{2+}/CaM and autophosphorylation dramatically increase its binding to all subunits of the NMDAR. Thus, Ca^{2+} influx into the spine would be expected to promote release of CaMKII from the spine cytoskeleton and subsequent binding to the NMDAR (Merrill et al., 2005; Ahmed et al., 2006). As another example, binding of CaMKII to both the NMDAR and to densin are facilitated by concomitant binding to α-actinin (Walikonis et al., 2001; Leonard et al., 2002). However, densin has a much greater affinity for the α-subunit than for the β-subunit (Walikonis et al., 2001), whereas the NMDAR does not show this selectivity. Since the α-subunit, but not the β-subunit, is synthesized from mRNA in dendrites in response to stimuli that induce potentiation (Burgin et al., 1990; Ouyang et al., 1999), densin may function to preferentially concentrate at the PSD holoenzymes that are newly synthesized in the dendrite.

Conditions that produce translocation of CaMKII into spines have been studied with the use of GFP-labeled CaMKII transfected into cultured neurons. Stimulation of NMDARs in cultured hippocampal neurons produced a rapid and transient redistribution of CaMKII from the actin cytoskeleton to synaptic sites (Shen and Meyer, 1999; Shen et al., 2000), whereas chemical induction of LTP in hippocampal slices by application of forskolin in medium containing no Mg^{2+} caused significant translocation of CaMKII into spines (Otmakhov et al., 2004). Because hypoxia can also cause accumulation of CaMKII in PSDs (Suzuki et al., 1994; Dosemeci et al., 2001), there has been some confusion about whether physiological stimulation *in vivo* produces translocation of CaMKII to the PSD. However, translocation under normal physiological circumstances *in vivo* was reported in living zebrafish (Gleason et al., 2003). Repeated sensory stimulation produced reproducible and reversible translocation of GFP-CaMKII to the PSD in an identified interneuron within the sensorimotor circuit.

The complex regulation of localization of CaMKII in the spine and PSD illustrates how scaffold proteins help to dynamically configure the location of regulatory proteins in the spine.

4.32.2.2.2 RasGRF1

Ras activation plays critical roles in synaptic plasticity both via regulation of insertion and removal of

AMPA receptors in the spine (Zhu et al., 2002) and via downstream activation of ERK1/2 (Sweatt, 2004). RasGRF1, a Ras-specific GDP/GTP exchange factor (GEF) mediates activation of Ras and the ERK1/2 pathway by NMDAR activation (Farnsworth et al., 1995; Hardingham et al., 2001; Krapivinsky et al., 2003). RasGRF1 is activated by Ca^{2+}/CaM (Farnsworth et al., 1995) and binds directly to the cytosolic tail of the NR2B subunit of the NMDAR (Krapivinsky et al., 2003). Location near the mouth of the NMDAR would render it able to efficiently sense a local rise in Ca^{2+} concentration around the activated receptor. As predicted, disruption of the interaction by introduction into neurons of a peptide blocker disrupts activation of the ERK1/2 pathway by NMDA (Krapivinsky et al., 2003). Moreover, learning and memory are impaired in RasGRF1 knockout mice (Giese et al., 2001).

The finding that RasGRF1 is associated directly with the NMDAR raises some interesting questions. The identified binding sites for RasGRF1 and CaMKII on NR2B overlap extensively; however, it is not yet known whether the two compete for binding to the NMDAR. The answer to this question will have implications for the orchestration of Ca^{2+} signaling in the spine. SynGAP, a Ras inactivating GTPase that binds to PSD-95, is stimulated by phosphorylation of CaMKII that is activated by Ca^{2+} flux through the NMDAR. Presumably the relative timing of activation of RasGRF and synGAP by Ca^{2+} flux would determine the time course of Ras activation. It will be interesting to learn how the PSD scaffold helps to regulate this timing.

4.32.3 Protein Complexes Associated with the AMPA Receptor

AMPARs are the principal mediators of rapid excitatory synaptic transmission in the brain. They are composed of four homologous subunits (GluR1–4 or GluRA–D) that assemble in combinations to form particular AMPAR subtypes (Hollmann and Heinemann, 1994; Dingledine et al., 1999; Mayer and Armstrong, 2004; Mayer, 2005). Regulation of trafficking, insertion, and removal of AMPARs to and from the postsynaptic membrane is a major mechanism underlying activity-dependent changes in synaptic strength such as LTP and LTD (Barry and Ziff, 2002; Malinow and Malenka, 2002; Song and Huganir, 2002; Bredt and Nicoll, 2003; Park et al., 2004). For example, activity-dependent recruitment of AMPARs to the postsynaptic membrane underlies some forms of LTP during development and in adult synapses (Isaac et al., 1995; Liao et al., 1995; Shi et al., 1999, 2001; Hayashi et al., 2000; Malinow, 2003; Matsuzaki et al., 2004; Bagal et al., 2005). In pyramidal neurons of the hippocampus, where LTP and LTD are most often studied, AMPARs are primarily heteroligomers of GluR1 and 2 subunits (GluR1/2) or of GluR2 and 3 subunits (GluR2/3). GluR2/3 AMPARs cycle constitutively in and out of the postsynaptic membrane, whereas GluR1/2 AMPARs are inserted into the postsynaptic membrane upon synaptic stimulation (Shi et al., 2001). AMPAR trafficking is thoroughly discussed in Chapter 4.30. Here we concentrate on interactions of GluR subunits with scaffold proteins that contribute to their localization at the postsynaptic membrane.

Five scaffold proteins interact directly with GluR subunits and participate in regulation of AMPAR trafficking: Stargazin, GRIP (GluR-interacting protein)/ABP (AMPAR-binding protein), PICK1 (protein interacting with C kinase 1), and NSF (N-ethylmaleimide-sensitive factor). In addition, PSD-95 binds directly to the GluR6 and KA2 subunits of kainate receptors (KARs; Garcia et al., 1998; Mehta et al., 2001).

4.32.3.1 Stargazin Binding to PSD-95

Stargazin/γ-2 and three structurally and functionally homologous proteins (γ-3, γ-4, and 8) are transmembrane auxiliary subunits of AMPARs and are collectively referred to as TARPs (Tomita et al., 2003; Nicoll et al., 2006; Osten and Stern-Bach, 2006). Stargazin/γ-2 is structurally related to the γ-1 subunit of the voltage-dependent calcium channel (VDCC) and was initially believed to be a calcium channel subunit (Letts et al., 1998). It was soon recognized that the TARPs are not part of the VDCC; instead, they bind to subunits of the AMPA receptor (Chen et al., 2000; Tomita et al., 2004) and influence their trafficking, targeting, and biophysical properties (Fukata et al., 2005; Nakagawa et al., 2005; Vandenberghe et al., 2005).

The C termini of the TARPs contain a consensus PDZ-domain-binding motif that binds selectively to the first two PDZ domains of PSD-95 (Fukata et al., 2005), and this interaction is required for activity-dependent translocation of AMPA receptors to postsynaptic sites (Schnell et al., 2002). Overexpression of PSD-95 increases the number of synaptic AMPARs (Schnell et al., 2002), whereas disruption of the synaptic localization of PSD-95 dramatically reduces it (El-Husseini Ael et al., 2002). The enhancement of AMPAR-mediated synaptic transmission that follows

PSD-95 overexpression occludes LTP and enhances LTD (Stein et al., 2003). Thus, the interaction of AMPAR/TARP complexes with PSD-95 may function to recruit extrasynaptic AMPAR/TARP complexes to postsynaptic sites in order to increase synaptic strength (Ehrlich and Malinow, 2004). Therefore, the scaffold protein PSD-95 appears to play a role in regulation of AMPAR location as well as in the formation of signaling complexes associated with the NMDAR.

4.32.3.2 GRIP/ABP, PICK1, and NSF

A distinct set of scaffold proteins bind selectively to GluR2/3 AMPARs and are believed to mediate regulation of their insertion and removal from synaptic sites (Lu and Ziff, 2005). The homologous PSD scaffold proteins termed GRIP (GRIP1) (Dong et al., 1997) and ABP (GRIP2) (Srivastava et al., 1998; Dong et al., 1999; Wyszynski et al., 1999) contain a total of six to seven PDZ domains and no obvious catalytic domain. The cytosolic tail of the GluR2 subunit of the AMPAR can bind to the fourth or fifth PDZ domain of either protein. GRIP/APBs exist in a palmitoylated form that is targeted to the postsynaptic membrane and a nonpalmitoylated form that is targeted to intracellular membranes (Yamazaki et al., 2001; DeSouza et al., 2002) and are thus thought to anchor AMPARs to either of these subcellular compartments in a regulated fashion to modulate AMPAR trafficking (Daw et al., 2000; Osten et al., 2000; Kim et al., 2001; Braithwaite et al., 2002; DeSouza et al., 2002; Fu et al., 2003; Hirbec et al., 2003; Seidenman et al., 2003). However, disruption of the interaction between the AMPAR and GRIP/ABP interferes most severely with the synaptic localization of the receptors (Dong et al., 1997).

PICK1 is a scaffold protein that contains a PDZ domain that binds selectively to protein kinase C-α (PKC-α) (Staudinger et al., 1997) and a BAR domain that that preferentially binds to curved membranes (Peter et al., 2004). GRIP/ABP also associates with the cytosolic tail of the GluR2 and GluR3 subunits of the AMPA receptor via the single PDZ domain (Dev et al., 1999; Xia et al., 1999). Because PICK1 forms homomultimers, even when the PDZ domain is occupied, PICK1 is believed to bring PKC-α in proximity to the tail of GluR2 (Chung et al., 2000; Perez et al., 2001), where it can promote phosphorylation of GluR2 on serine 880 by PKC-α (Matsuda et al., 1999; Perez et al., 2001). Phosphorylation of GluR2 on serine 880 releases GluR2 from GRIP/APB but does not alter its association with PICK1.

PICK1 and GRIP/ABP appear to work together to regulate trafficking and surface expression of GluR2/3 receptors. Since GRIP/ABP serves as an anchor for GluR2 subunit-containing AMPARs on both postsynaptic and intracellular membrane compartments, dissociation of GluR2 from GRIP/ABP is required for both insertion and removal of AMPARs at synaptic sites (Daw et al., 2000; Osten et al., 2000; Kim et al., 2001; Braithwaite et al., 2002; Fu et al., 2003; Hirbec et al., 2003; Seidenman et al., 2003). PICK1 appears to promote dissociation of GluR2 from GRIP/ABP when it binds to the GRIP/ABP/GluR2 complex and induces phosphorylation of GluR2 on serine 880 by PKC-α. As GRIP/ABP unbinds from the phosphorylated form of GluR2, PDZ domains on PICK1 multimers can bind to GluR2. The BAR domain on PICK1 then promotes GluR2 association with budding endo- and exocytotic vesicles that mediate the trafficking of AMPARs to and from the postsynaptic membrane. Thus, PICK1 paradoxically promotes both internalization and recycling of GluR2 (Lu and Ziff, 2005). As predicted from these findings, disruption of interaction of GluR2/3 with PICK1 or GRIP/ABP impairs expression of LTD (Kim et al., 2001; Seidenman et al., 2003).

NSF (*N*-ethylmaleimide-sensitive fusion protein) and the clathrin adaptor AP2 interact with the cytosolic tail of the GluR2 subunit of the AMPAR in a membrane proximal region distinct from the C-terminal PDZ-binding domain that interacts with GRIP/ABP and PICK1. NSF and AP2 are also required for correct regulation of AMPAR trafficking and surface expression and consequently play important roles in synaptic plasticity, particularly LTD (Nishimune et al., 1998; Osten et al., 1998; Song et al., 1998; Luscher et al., 1999; Lee et al., 2002).

In addition to the AMPAR, GRIP/ABP binds to other synaptic proteins including the receptor tyrosine kinase EphB2 (Hoogenraad et al., 2005), the scaffold protein liprin-α (Wyszynski et al., 2002), and the neuronal Ras-GEF GRASP-1 (Ye et al., 2000). Interactions with these proteins likely help to regulate other, less well understood, aspects of AMPAR function.

4.32.4 Protein Complexes Associated with the Metabotropic Glutamate Receptor

The excitatory neurotransmitter glutamate acts upon two major classes of postsynaptic receptors: ionotropic (NMDARs, AMPARs, and KARs) and metabotropic

(mGluRs). The G-protein-coupled mGluRs modulate neuronal excitability and synaptic strength by regulating the production of second messengers.

4.32.4.1 Homer

Group 1 mGluRs, which include mGluR1 and mGluR5, are linked to phospholipase C, which produces the second messengers IP3 and diacylglycerol. Group I mGluRs are present at the periphery of the PSD (Baude et al., 1993; Nusser et al., 1994; Lujan et al., 1997) and play an important role in synaptic plasticity (Bortolotto et al., 1999). Their linkage to the PSD is mediated by the scaffold protein homer (Brakeman et al., 1997; Xiao et al., 1998). Homer is actually a family of proteins comprising homer 1, 2, and 3, each of which is alternatively spliced into long and short isoforms (termed 'a' isoforms) (Kato et al., 1998; Xiao et al., 1998). All of the homers contain an N-terminal EVH1 domain, which binds to a characteristic proline-rich motif (PPXXF) in homer-interacting proteins (Beneken et al., 2000). In addition, the long homer isoforms contain a C-terminal coiled-coil (CC) domain, which promotes their self-association into tetramers (Tadokoro et al., 1999; Hayashi et al., 2006). The short isoforms do not contain the CC domain and thus are monomeric (Kato et al., 1998). Moreover, short homers, for example homer1a, were originally characterized as 'immediate early genes' whose expression is rapidly induced by synaptic activity. In contrast, long homers, for example, homer1b/c, are constitutively expressed (Xiao et al., 2000; Fagni et al., 2002).

4.32.4.2 Linkage to the IP3R

In addition to group 1 mGluRs, several other proteins bind to the EVH1 domain of Homer via the characteristic PPXXF motif (Xiao et al., 2000; Fagni et al., 2002). Two such proteins are the type 1 inositol trisphosphate receptor (IP3R) (Tu et al., 1998) and shank, a PSD scaffold protein (Tu et al., 1999). Because of their ability to self-associate, long homers function in the PSD as hubs that crosslink homer-interacting proteins (Tu et al., 1998, 1999). On the other hand, short monomeric homers antagonize this function and thus act as endogeneous 'dominant negatives' that are rapidly and transiently expressed upon synaptic stimulation (Brakeman et al., 1997; Kato et al., 1997).

An interesting example of the structural and functional coupling provided by long homers is the link between group 1 mGluRs and the IP3 receptor (IP3R) (Tu et al., 1998) (**Figure 3**). The IP3R is an IP3-sensitive Ca^{2+} channel localized to the smooth endoplasmic reticulum (SER). Activation of group 1 mGluRs by glutamate results in production of the second messenger IP3. IP3 then binds to the IP3R, causing it to open and release Ca^{2+} from the SER, which functions as an intracellular calcium store. The mGluR/homer/IP3R ternary complex thus couples synaptic activation of group 1 mGluRs with the release of calcium from SER compartments located in a subset of dendritic spines (Spacek and Harris, 1997; Sala et al., 2005). Remarkably, the SER of dendritic spines is juxtaposed with the postsynaptic membrane at the periphery of the PSD, exactly where group I mGluRs and homer are located (Xiao et al., 1998). Activity-induced expression of short isoforms of homer can apparently exert a dominant negative effect on the coupling between mGluRs and the IP3R, because overexpression of a short homer impairs release of intracellular calcium evoked by quisqualate, an mGluR agonist (Tu et al., 1998).

A second layer of regulation of mGluRs is provided by the ability of homer 1a to induce constitutive activation of mGluR1a and mGluR5 when it binds to their intracellular domains (Ango et al., 2001). The long homers can then disrupt this interaction and antagonize the constitutive activity conferred by homer 1a (Ango et al., 2001).

The regulatory properties of homer proteins may play a role in mechanisms of addiction (Szumlinski et al., 2004, 2005) and in susceptibility to schizophrenia (Szumlinski, 2005), as evidenced by the phenotypes of mice with deletions of homer 1 or homer 2.

4.32.5 Shank/ProSAP Scaffold of Scaffolds

A laminar organization of the PSD scaffold is suggested by the localization of PSD scaffolds as measured by immunoelectron microscopy (Valtschanoff and Weinberg, 2001; Petersen et al., 2003). Peak concentrations of PSD-95, and GKAP-shank were detected in the PSD at an average distance of 12 and 24–26 nm, respectively, from the plasma membrane. Cript and dynein, proteins that may be involved in linking PSD proteins to cytoskeletal elements, lay even farther away, 29–31 nm, from the plasma membrane. The shank/ProSAP scaffold proteins (Lim et al., 1999; Naisbitt et al., 1999; Tu et al., 1999; Yao et al., 1999;

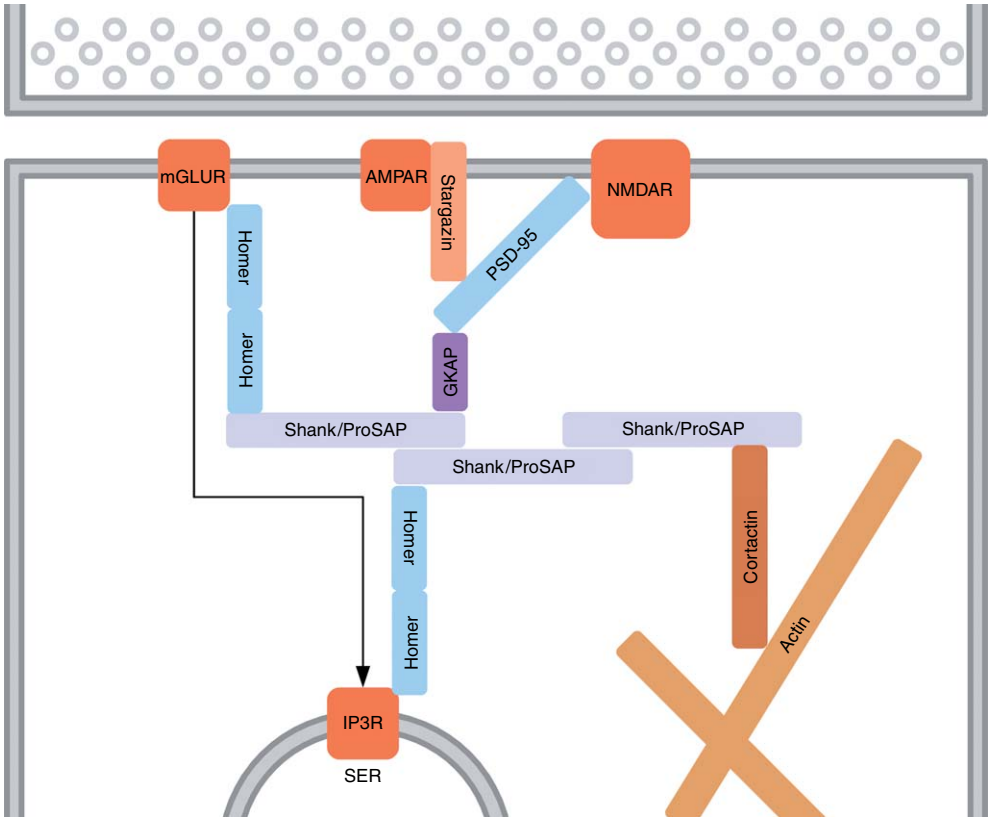

Figure 3 ProSAP/shank forms a master scaffold linking NMDA, AMPA, and metabotropic glutamate receptor-associated complexes in the postsynaplic density. Self-association of ProSAP/Shank scaffold proteins forms a lattice linking *N*-methyl-D-aspartate, AMPA, and metabotropic glutamate receptor-associated complexes together. The scaffold forms additional links with the actin cytoskeleton and the smooth endoplasmic reticulum (SER). Binary physical interactions between two proteins are represented by their juxtaposition. Arrows represent regulatory actions (→ stimulatory, ⊣ inhibitory).

Boeckers et al., 1999a, 1999b) act as master scaffolding proteins linking together PSD-95-associated proteins, homer-associated proteins, and the actin cytoskeleton (Sheng and Kim, 2000; Boeckers et al., 2002). Shank forms homomultimers through interactions of its SAM domains with each other and with sharpin (Naisbitt et al., 1999; Lim et al., 2001). Its single PDZ domain can also dimerize in an antiparallel configuration, contributing to multimerization (Im et al., 2003). These interactions suggest that shank can form a two-dimensional protein lattice that, by virtue of its rich collection of protein-interaction domains (6–7 ankyrin repeats, an SH3 domain, a PDZ domain, a proline-rich region), can accommodate several ligands including homer and GKAP/SAPAP (Baron et al., 2006). The shank complex can thus be thought of as a platform that cross-links the NMDA and metabotropic glutamate receptor complexes (**Figure 3**). It may also link to AMPAR complexes either via direct binding to the GluR1 subunit (Uchino et al., 2006) or through interactions between the TARP proteins and PSD-95 (Nicoll et al., 2006).

The physical interaction between group 1 mGluRs and the shank scaffold is mediated by the long isoforms of homer (Ehlers, 1999). This structural coupling may underlie functional coupling between group 1 mGluRs and the NMDAR (O'Connor et al., 1994), in which mGluR1 can upregulate NMDAR function through proline-rich tyrosine kinase 2 (Pyk2) and Src-family kinases that are part of the NMDAR complex (Heidinger et al., 2002). The long isoforms of homer and shank also appear to cooperate in morphogenesis of dendritic spines. Accumulation of shank in synapses depends on its ability to bind to homer (Sala et al., 2003) and also to GKAP/SAPAP (Naisbitt et al., 1999). When overexpressed alone or in combination, the long isoforms

of homer and shank synergistically increase the number and size of spines (Sala et al., 2001). As expected, overexpression of the short isoforms of homer have the opposite effect on spine morphogenesis, reducing synaptic targeting of shank and other PSD proteins and inhibiting synaptic transmission through their dominant-negative function (Sala et al., 2003).

Through its interactions with cortactin, Abp1, and spectrin/fodrin, the shank scaffold mediates binding of the PSD to the underlying spine actin cytoskeleton (Naisbitt et al., 1999; Bockers et al., 2001; Qualmann et al., 2004) and thus facilitates a functional connection between activation of postsynaptic receptors and changes in the spine cytoskeleton.

4.32.6 Attachment of the Cytoskeleton to the PSD by Actin-Associated Proteins

Dendritic spines contain a higher density of actin filaments than any other neuronal structure, and it is the arrangement of these filaments that gives spines their characteristic shape (Fifkova and Delay, 1982; Markham and Fifkova, 1986). The narrow neck contains longitudinally bundled filaments, whereas the head contains a lattice-like mesh that forms the bulbous head (Landis and Reese, 1983). The section titled 'Attachment of the cytoskeleton to the PSD by actin-associated proteins' discusses three actin-binding proteins that physically connect the cytoskeleton to the PSD: α-actinin, cortactin, and spectrin. Functional analysis of these proteins indicates that attachment of F-actin to the PSD immobilizes receptors at the synapse and regulates their function.

4.32.6.1 α-Actinin

α-Actinin is an actin-binding protein with filament crosslinking activity (Meyer and Aebi, 1990). The α-actinin family consists of four members (α-actinin1–4), of which isoforms 1, 2, and 4 are expressed in the brain (Wyszynski et al., 1998; Walikonis et al., 2000, 2001). The α-actinin family is characterized by an actin-binding domain at its N terminus, four spectrin repeats in the central region (Meyer and Aebi, 1990), and a Ca^{2+}-binding EF hand motif (Witke et al., 1993) and PDZ domain-binding sequence at the C terminus (Walikonis et al., 2001). α-Actinin self-associates in antiparallel dimers that can bind actin on both ends, giving it the ability to crosslink F-actin. Consistent with this function, overexpression of α-actinin-2 increases the length and density of dendritic protrusions in cultured hippocampal neurons (Nakagawa et al., 2004).

A number of studies indicate that α-actinin plays an important role in the localization and function of NMDARs. α-Actinin2 colocalizes with NMDARs in spines and binds directly to both the NR1 and NR2 subunits (Wyszynski et al., 1997; Dunah et al., 2000). This interaction is antagonized by both calcium-bound CaM and autophosphorylated CaMKII (Wyszynski et al., 1997; Leonard et al., 2002). Interestingly, dissociation of the NMDAR from F-actin causes rapid inactivation of the channel (Wyszynski et al., 1997; Zhang et al., 1998), while association with the cytoskeleton enhances NMDAR activity (Rosenmund and Westbrook, 1993). Thus, NMDARs are probably tethered to F-actin under resting conditions but dissociate and become inactivated in response to calcium influx through the channel.

Densin-180 is another point of attachment for F-actin within the PSD. The conserved C-terminal region of α-actinin-4 binds to the PDZ domain of densin. Densin also binds to CaMKII, which itself directly binds to α-actinin-4, forming a ternary complex. Since autophosphorylated CaMKII has an increased affinity for densin, this entire complex is probably dependent on intracellular calcium levels (Walikonis et al., 2001). Further studies will shed light on the functional significance of this complex.

4.32.6.2 Cortactin

Cortactin drives *de novo* actin nucleation and creates branch points on preexisting filaments by activating the ARP2/3 complex. In addition to promoting filament extension, cortactin binding also stabilizes F-actin. Structurally, cortactin contains an acidic domain at its N terminus similar to other actin nucleation-promoting factors such as WASP, N-WASP, and SCAR/WAVE; all proteins that interact with the ARP2/3 complex (Wu and Parsons, 1993; Weed et al., 2000; Uruno et al., 2001; Weaver et al., 2001). The central region contains 6.5 tandem copies of a 37–amino acid repeat that mediate its interaction with F-actin (Wu and Parsons, 1993). The C terminus contains a helical domain, a proline-rich domain, and a SH3 domain (Daly, 2004). The SH3 domain of cortactin interacts with shank, physically linking the PSD to the cytoskeleton (Du et al., 1998; Naisbitt et al., 1999).

Cortactin is highly enriched in spines where it colocalizes with F-actin (Hering and Sheng, 2003;

Racz and Weinberg, 2004). Electron microscopic studies of imunogold-labeled cortactin show that approximately 10% of cortactin in the spine is located in the PSD, whereas the bulk of the staining occurs within the spine core, 100–150 nm away from the PSD (Racz and Weinberg, 2004). Thus, two pools of cortactin exist in the spine, with only a small fraction interacting with the PSD. Interestingly, NMDAR activation induces translocation of cortactin from spines into the dendrite (Hering and Sheng, 2003), suggesting a role for cortactin in activity-dependent modification of the cytoskeleton.

As predicted by its ability to activate the Arp2/3 complex, cortactin is a strong determinant of spine morphology in cultured hippocampal neurons. Knockdown of endogenous cortactin in mature cultured hippocampal neurons by siRNA drastically reduces the total number of dendritic protrusions (Hering and Sheng, 2003). Thus, cortactin is required for the maintenance of mature spines, perhaps by stabilizing filaments or promoting growth of the actin network. Spines on cultured neurons overexpressing cortactin are abnormally long, similar to those observed in overexpression studies of α-actinin, and this elongation requires the N-terminal region of cortactin that interacts with F-actin and the Arp2/3 complex (Hering and Sheng, 2003).

4.32.6.3 Spectrin

Spectrin is the main component of the cell membrane skeleton. It was first identified in erythrocytes, where it forms a filamentous network required for red blood cells to maintain their shape and elasticity (Marchesi and Steers, 1968). The spectrin family of proteins includes α- and β-spectrin and α- and β-fodrin (also referred to as α- and β-spectrin II, or brain spectrin). In the following discussion, the term spectrin will refer to the brain form of spectrin. Several unique domains differentiate the α and β subunits: the α subunit contains 21 triple helical repeats with a segment containing an SH3 domain in the tenth repeat, and the β subunit has a shorter triple helical repeat region (17 repeat segments) and does not contain SH3 or EF hand domains. The β subunit also contains an actin-binding region at its N terminus, and some β subunits contain a PH domain. Within the membrane skeleton, the α and β subunits typically form heterotetramers by forming antiparallel heterodimers that interact head to head to form a tetramer. By interacting with transmembrane proteins such as ankryin, spectrin connects the actin cytoskeleton to cell membranes (Goodman et al., 1995; Czogalla and Sikorski, 2005).

In addition to interacting with the cell membrane, spectrin is also present in the PSD, where it binds directly to NR1, NR2A, and NR2B subunits of NMDARs in an activity-dependent manner (Carlin et al., 1983; LeVine and Sahyoun, 1986; Wechsler and Teichberg, 1998). CaM inhibits spectrin-NR1 interaction, and Ca^{2+} antagonizes the spectrin-NR2B binding (Wechsler and Teichberg, 1998). Spectrin binding is also inhibited by phosphorylation of the NR1 C-terminal domain by PKA and PKC and of the NR2A C-terminal domain by fyn (Wechsler and Teichberg, 1998). In the neuromuscular junction, removal of actin and spectrin from myotube membranes results in dispersal of AChRs (Bloch, 1986). By analogy, spectrin may be important for immobilizing receptors at the synapse (Adam and Matus, 1996). The calcium and kinase dependence of the spectrin-NMDAR interaction provides a potential mechanism by which NMDAR motility in the membrane may be regulated by synaptic activity.

In mature synapses, the actin cytoskeleton immobilizes some, but not all, components of the PSD. For example, treatment of cultured neurons with latrunculin A, a toxin that causes depolymerization of actin by sequestering G-actin, results in a decrease in AMPA and NMDARs, α-actinin-2, drebrin (an actin-binding protein that can cluster actin), and CaMKII from spines. However, PSD-95 remains in spines even after filament depolymerization (Allison et al., 1998, 2000), suggesting that PSD-95 may form a stable core scaffold that does not require the cytoskeleton for its aggregation.

4.32.7 Regulation of the Spine Actin Cytoskeleton by Signaling Complexes in the PSD

Real-time live imaging of fluorescently labeled spines of cultured neurons reveals that they are surprisingly motile structures (for reviews, see Yuste and Bonhoeffer, 2004; Oertner and Matus, 2005). When monitored continuously, one sees that spines exhibit a 'twitching' motion resulting from the continuous turnover of the actin cytosleteton even under resting conditions. Even more dramatic is the observation that certain patterns of synaptic activation cause spines to grow or shrink. Many investigators have documented that the volume of a spine head is proportional to its synaptic strength (Harris and Stevens,

1989; Nusser et al., 1998; Schikorski and Stevens, 1999). One likely mechanism proposed for LTP is the addition of new AMPA receptors at the postsynaptic site (Shi et al., 2001), with a corresponding enlargement of the spine head. This notion is supported by the observation that stable LTP cannot be induced if actin polymerization is inhibited pharmacologically (Kim and Lisman, 1999; Krucker et al., 2000). Likewise, there is evidence that smaller spines preferentially undergo LTP, whereas larger, mature spines are less plastic (Matsuzaki et al., 2004). A study that measured actin polymerization with a fluorescence resonance energy transfer (FRET) technique shows that tetanic stimulation that typically induces LTP increased formation of F-actin in spines. Conversely, low-frequency stimulation that typically induces LTD results in depolymerization of spine action (Okamoto et al., 2004). This dynamic property of spines, with its implications for cellular mechanisms of learning and memory, has provoked intense interest in identifying the signaling complexes that coordinate synaptic activity and regulation of the underlying actin cytoskeleton.

4.32.7.1 Mechanisms of Regulation of Actin Polymerization

Because actin monomers are continuously treadmilling on and off of filaments, regulation of the overall structure of the cytoskeleton in spines occurs at many different levels: growth by addition of monomers, attrition by actin depolymerization, capping of filaments to block addition of new monomers, sequestration of monomers to limit growth of filaments, severing capped filaments to initiate new growth, and filament elaboration by branching. The following is a brief description of some of the major regulators of actin polymerization in spines (for reviews, see Pollard and Borisy, 2003; Dillon and Goda, 2005).

Actin filaments are polar structures that appear to have a barbed end and a pointed end. Because actin-ATP has a greater affinity for the barbed end, the filament grows three times faster from this end than the pointed end (Pollard, 1976). Over time, ATP hydolyzes to ADP on the older end of the filament. ADF and cofilin are actin-severing proteins that bind to actin monomers with a higher affinity for actin-ADP than actin-ATP. Binding of ADF/cofilin to actin-ADP changes the twist in the actin helix and promotes severing (McGough et al., 1997; Maciver et al., 1998; Blanchoin and Pollard, 1999). In this manner they catalyze disassembly of the older, pointed ends of actin filaments. Phosphorylation of ADF/cofilin at serine 3 inhibits its ability to bind to actin (Agnew et al., 1995). Thus, depolymerization of actin can be regulated by cytosolic signaling pathways. Profilin, on the other hand, promotes the elongation of actin filaments by catalyzing exchange of ADP for ATP on actin monomers, maintaining a pool of actin-ATP monomers that can be added to the growing end of filaments (Wolven et al., 2000). The rate of filament growth is also controlled by capping protein and gelsolin, which bind to the barbed end of filaments, blocking addition of actin monomers (McGough et al., 2003; Wear et al., 2003). Last, *de novo* filament nucleation and branching are both achieved through activation of the Arp2/3 complex, which acts as a template for filament assembly. Thus, growth and maintenance of the actin cytoskeleton is a finely tuned process that has many points of regulation.

4.32.7.2 Regulation of Rac in the PSD

Identification of signaling pathways that link synaptic activity to cytoskeletal regulation in spines is an active field of research. In recent years, a clear role for Rho family GTPases, and Rac in particular, in spine morphogenesis has emerged. Neurons expressing a constitutively active form of Rac form ruffle-like protrusions, composed of many small spines (Luo et al., 1996; Nakayama et al., 2000; Tashiro et al., 2000; Pilpel and Segal, 2004). Conversely, expression of dominant negative Rac in neurons reduces spine density and causes significant lengthening of spines (Nakayama et al., 2000; Tashiro et al., 2000; Pilpel and Segal, 2004; Tashiro and Yuste, 2004). Blocking Rac1 activity in mature spines leads to reduction in spine head width, indicating that Rac1 plays a role in maintaining mature spine morphology (Tashiro and Yuste, 2004). Rac, like all members of the small GTPase superfamily, functions as a binary switch by cycling between the active GTP-bound and inactive GDP-bound states. To activate Rac-GDP, a Rac GEF must release GDP, allowing the more abundant GTP to bind. This section summarizes our current understanding of postsynaptic Rac activation by the GEFs, kalirin and Tiam1.

4.32.7.2.1 Kalirin

Activation of Rac signaling by kalirin is crucial for development and maintenance of dendritic spines. Kalirin is encoded by a single gene, but several isoforms are generated by alternative splicing

and different promoters (Johnson et al., 2000; McPherson et al., 2002, 2004). Kalarin-7, -9, and -12 are the most abundant isoforms in the CNS. Kalirin-9 and -12 are prevalent early in development, whereas kalirin-7 expression appears at the time of synapse formation and remains high throughout adulthood (Hansel et al., 2001; Ma et al., 2001, 2003). Of these three isoforms, only kalirin-7 contains a PDZ-binding motif that mediates its interaction with PSD-95. Indeed, kalirin-7 is enriched in PSD fractions of rat cerebral cortex and is labeled immunohistochemically in discrete puncta along dendritic processes of cultured cortical neurons (Penzes et al., 2000). When kalirin-7 is overexpressed in cortical neurons, the size and number of spines increases. Overexpression of a mutant lacking the PDZ-binding motif results in mislocalization of kalirin and formation of filopodia on the soma instead of on dendrites (Penzes et al., 2001). Thus, proper targeting of kalirin to the PSD is necessary for normal spinogenesis.

Kalirin-dependent spine formation is induced by EphB activation. EphB receptors are protein tyrosine kinase-linked receptors located on in the postsynaptic membrane (McPherson et al., 2004). Mutant mice with a triple knockout of EphB receptors (EphB1-3) fail to form dendritic spines in cultured hippocampal neurons (Henkemeyer et al., 2003). Ephrin B1 activation of EphB receptors induces rapid formation of mature spines along the dendrites of 10-day-old cultured hippocampal neurons by recruiting kalirin-7 into clusters along the dedritic shaft and in spines (Penzes et al., 2003). Furthermore, kalirin's effects are dependent on downstream Rac activation since expression of dominant negative Rac blocks ephrin-induced spine formation.

4.32.7.2.2 Tiam1

Tiam1 is a Rac GEF located just below the postsynaptic membrane and is expressed in the brain during development and throughout adulthood (Ehler et al., 1997; Tolias et al., 2005). RNAi knockdown of Tiam1 in cultured neurons results in a severe reduction in the number of dendritic spines, and the spines that remain are longer and more filopodial in shape, much like the spine phenotype induced by dominant negative Rac. Furthermore, knockdown of Tiam1 blocks NMDAR-mediated spine outgrowth, indicating that it plays a crucial role in NMDAR-dependent morphological plasticity (Tolias et al., 2005).

Tiam1 interacts with NR1 subunits and is activated in response to NMDAR stimulation, resulting in elevation of GTP-bound Rac (Tolias et al., 2005). Several signaling mechanisms might mediate NMDAR-dependent activation of Rac by Tiam1 (**Figure 2**). CaMKII can phosphorylate and activate Tiam1 (Fleming et al., 1999). Thus, CaMKII autophosphorylation in response to synaptic stimuli that activate NMDARs may in turn activate Rac through Tiam1. Tiam1 can also be activated by direct binding of Ras-GTP to its Ras binding domain (Lambert et al., 2002), raising the possibility that NMDAR-induced Ras activation through RasGRF1 may stimulate Rac signaling pathways. It is intriguing that knockout of synGAP, a suppressor of Ras activity, increases the number and size of spines in hippocampal cultures (Vazquez et al., 2004), suggesting that crosstalk between the Ras and Rac pathways in spines might coordinate synaptic strength and morphological plasticity.

4.32.7.3 Reshaping the Spine Cytoskeleton Through the PAK/LIMK/Cofilin Pathway

Spine morphogenesis by either kalirin- or Tiam1-mediated Rac activation is accompanied by increased PAK phosphorylation (Penzes et al., 2003; Tolias et al., 2005). PAKs are a family of closely related serine/threonine protein kinases that were discovered relatively recently in a screen for Rho-GTP binding partners (Manser et al., 1994; Bokoch, 2003). In the inactive state, PAKs form homodimers in which the regulatory domain of one PAK molecule inhibits the catalytic domain of another. Binding of GTP-bound Cdc42 or Rac1 to PAK causes conformational changes that destabilize folding of the inhibitory switch domain and facilitate catalytic activation (Bokoch, 2003). Phosphorylation of threonine 423 in the PAK catalytic domain is required to suppress autoinhibition and achieve full catalytic function.

It is clear that PAKs have a crucial role in regulation of spine formation. PAKs phosphorylated at threonine 423 (pPAKs), in particular pPAK1 and pPAK3, are concentrated in dendritic spines and enriched in the PSD fraction (Penzes et al., 2003; Hayashi et al., 2004). Treatment of cultured hippocampal neurons with an inhibitory peptide consisting of the PAK1 inhibitory domain fused to the cell-penetrating peptide TAT reduces the effect of ephrin-B ligands on spine formation (Penzes et al., 2003). Transgenic mice expressing the peptide inhibitor of PAK1-3 in forebrain neurons under the control of the α-CaMKII promoter show a significant reduction in spine density and increase in spine head size in cortical neurons by 4 weeks of age (Hayashi et al., 2004). When PAK3 is specifically inhibited by siRNA

in hippocampal organotypic cultures, spines become thin and elongated, and the total number of synapses decreases (Boda et al., 2004).

How do the PAKs influence spine morphology? PAKs have many potential downstream regulatory targets. One target likely to mediate effects on the actin cytoskeleton is the enzyme LIMK, which phosphorylates and inactivates ADF/cofilin. LIMK1 is a serine/threonine kinase that contains two LIM domains and a PDZ domain (Okano et al., 1995) and that is enriched in hippocampal pyramidal neurons (Foletta et al., 2004). Activated PAK forms a complex with LIMK1 and activates it by phosphorylating threonine 508 in the catalytic domain (Edwards et al., 1999). The activated LIMK1 then phosphorylates serine 3 of ADF/cofilin, inhibiting its activity (Arber et al., 1998; Yang et al., 1998). Golgi staining of neurons in brain sections from homozygous LIMK1 knockout mice reveals abnormal spine morphology compared with wild-type mice: spine head size is dramatically decreased, and spine necks are thicker. Furthermore, the actin-severing protein, cofilin, is significantly less phosphorylated in the mutant than in wild-type brains. In its dephosphorylated form, cofilin binds F-actin and promotes severing of the filament. This study supports a model in which LIMK1 inactivates cofilin, slowing the turnover of F-actin and permitting formation of larger spine heads.

Immunoelectron microscopy shows that cofilin is located in the PSD and the spine shell but is excluded from the core, providing evidence for the notion that F-actin in the core is relatively stable, whereas actin around the periphery and projecting into the PSD is more motile (Racz and Weinberg, 2006). *In vivo* LTP in the dentate gyrus is associated with an increase in the F-actin content in spines that is NMDAR-dependent and involves inactivation of cofilin (Fukazawa et al., 2003). These changes occur within 20 min of stimulation and last as long as 5 weeks. In acute hippocampal slices from neonatal rats, two-photon time-lapse imaging reveals that induction of LTD by low-frequency stimulation is associated with a shrinkage of spines that is mediated by cofilin activation (Zhou et al., 2004). Thus, cofilin appears to be a key player in the bidirectional morphological plasticity of spines.

4.32.7.4 Actin Dynamics and Mental Retardation

Mental retardation (MR) is defined as a significant impairment of cognitive and adaptive functioning, with the onset occurring before the age of 18 years. Although the underlying causes of MR are heterogeneous, several studies of golgi-impregnated postmortem brains from mentally retarded individuals reveal that dendritic spine abnormalities are a common feature of individuals with cognitive impairment (Huttenlocher, 1970, 1974; Marin-Padilla, 1972; Purpura, 1974). In recent years, a host of genetic forms of mental retardation have been mapped to gene mutations affecting Rho family GTPases and their downstream effectors that control actin dynamics (reviewed in Huttenlocher, 1974; Ramakers, 2002; Newey et al., 2005). Here we highlight recent research on one form of mental retardation, fragile X syndrome (FXS), which shows a compelling link between regulation of spine morphology and normal cognition in humans.

As early as the 1940s, clinicians identified families in which mental retardation segregated in a sex-linked pattern where more males than females inherited the condition (reviewed in Stevenson and Schwartz, 2002). Of the more than 1200 genes on the X chromosome, it is anticipated that mutations in approximately 200 genes give rise to different pedigrees of X-linked mental retardation (XLMR) (Stevenson, 2005). FXS is the most prevalent form of XLMR and is the second most common cause of mental disability, affecting 1 in 4000 males and 1 in 8000 females (Turner et al., 1996). In addition to cognitive deficits, FXS is also associated with other pathologies such as hyperactivity, attention deficit, and autistic-like behavior. Genetic analysis of affected families and studies of animal models have yielded tremendous advances in our understanding of the molecular mechanisms responsible for the cognitive deficits in FXS.

Postmortem analysis of human brain samples shows that pyramidal neurons from the cortex and hippocampus of FSX patients have an increased number of long, thin spines that resemble those normally seen early in development (Hinton et al., 1991; Irwin et al., 2001). In 1991, the gene mutated in FXS was cloned and named *Fmr1* (Verkerk et al., 1991). *Fmr1*-null mice have an increase in long, thin 'immature' spines similar to those observed in humans with FXS (Comery et al., 1997; Nimchinsky et al., 2001; Irwin et al., 2002; McKinney et al., 2005; Grossman et al., 2006). Since no gross anatomical brain abnormalities are associated with FXS, it is hypothesized that altered synaptic function or spine maturation is responsible for the observed cognitive deficits.

The mutation causing FXS is an unstable expansion of CGC repeats that results in transcriptional silencing of *Fmr1*, and consequently the absence of Fragile X mental retardation protein (FMRP) expression.

FMRP is an mRNA-binding protein that can act as a translational suppressor at synapses, suggesting that inappropriate regulation of target mRNAs contributes to the abnormal maturation of spines in FSX patients and FMRP knockout mice (reviewed in Bardoni et al., 2001). FMRP also interacts with two cytoplasmic proteins, CYFIP1 and CYFIP2, which specifically bind only to the GTP-bound form of Rac1 (Schenck et al., 2001). In *Drosophila*, Rac-GTP binding to CYFIP causes dissociation and activation of FMRP (Schenck et al., 2003), suggesting that Rac activation may suppress the translation of some mRNAs. Recent work in murine fibroblasts shows that Rac-induced actin remodeling is enhanced in the absence of FMRP (Castets et al., 2005). Comparison of proteomes of cells expressing FMRP compared with those without, revealed that expression of the catalytic domain of PP2A was enhanced in the absence of FMRP. Since FMRP is a known translational suppressor and binds to pp2acβ mRNA, it is likely that FMRP normally suppresses PP2Ac in these cells. The authors also found that phophorylation of the actin-severing protein, cofilin, was decreased in the FMRP-negative cells. Since PP2A can activate cofilin by dephosphorylating it, the authors propose that the increase in actin remodeling observed in the FMRP-negative fibroblasts is a result of the increased activity of cofilin induced by elevated PP2A expression. It remains to be determined whether FMRP suppresses PP2A translation in neurons. However, it is tempting to speculate that dysregulation of cofilin activity might account at least in part for the immature spine phenotype observed in FXS patients. Overactivation of cofilin could destabilize the F-actin network, causing a decrease in spine head size. Interestingly, Williams syndrome, which results from an autosomal mutation, is associated with a decrease in cofilin phosphorylation by a completely different mechanism. One of the genes deleted in Williams syndrome is LIM kinase. Knocking out LIM kinase in mice causes a decrease in cofilin phosphorylation and a corresponding decrease in spine head size. Furthermore, two forms of nonsyndromic X-linked mental retardation (so called because mental retardation is the only phenotype) are also caused by mutations in Rac signaling: ARHGEF6 (also called αPix or Cool-2), a Rac-GEF, and the downstream effector PAK3 (Kutsche et al., 2000) and (Allen et al., 1998), respectively. Such examples of deficits in human cognition caused by mutations in genes controlling Rac signaling add strength to the hypothesis that activity-dependent morphological plasticity in spines is important for encoding information at the cellular level.

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4.33 Translational Control Mechanisms in Synaptic Plasticity and Memory

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4.33.1 Introduction

It is well accepted that one of the critical events that is required for the conversion of short-term to long-term memory is the requirement for new gene expression. At the cellular level, the conversion of short-lasting to long-lasting synaptic plasticity, processes that in many instances require the same molecular mechanisms as their memory counterparts, also requires new gene expression. Many laboratories have contributed to our understanding of the molecular mechanisms that control gene expression during long-lasting synaptic plasticity and long-term memory. Initially these studies focused on transcriptional control (Kandel, 2001), although it had been known for many years that long-term memory requires protein synthesis (Flexner et al., 1963). Many of the early studies on the requirement of protein synthesis (commonly referred to as translation) for long-term memory relied nearly exclusively on the injection of general protein synthesis inhibitors into the brains of intact animals. These studies provided much information concerning the temporal (when) and spatial (where in the brain) requirements for protein synthesis during long-term memory formation for a particular behavioral task but provided virtually no information about the molecular mechanisms of translational control during memory. Fortunately, there has been an incredible increase in our knowledge of how translation, especially translation initiation, is regulated during long-lasting synaptic plasticity and long-term memory.

Hippocampal long-term potentiation (LTP) was first shown to require new protein synthesis *in vivo* more than 20 years ago (Krug et al., 1984). Later, it was shown that LTP has distinct temporal phases that can be separated on the basis of whether the LTP is transcription and/or translation dependent (Frey et al., 1988; Huang and Kandel, 1994; Nguyen et al., 1994; Nguyen and Kandel, 1997). More mild types of LTP-inducing stimulation induce short-lasting potentiation (1–2 h of potentiated synaptic transmission) that is dependent on neither transcription nor translation. This short-lasting LTP is now commonly referred to as early-phase LTP (E-LTP). In contrast, stronger types of LTP-inducing stimulation that result in long-lasting potentiation (3 or more h of potentiation) that is dependent on transcription and translation is now commonly referred to as

late-phase LTP (L-LTP). Distinct temporal phases similar to those in LTP have been described for long-term facilitation (LTF) in *Aplysia*, another form of long-lasting synaptic plasticity (Kandel, 2001).

Most forms of L-LTP require activation of the *N*-methyl-D-aspartate (NMDA) subtype of glutamate receptor, but protein synthesis–dependent potentiation of synaptic transmission is not exclusively glutamatergic. Translation-dependent synaptic plasticity in hippocampal slices can also be induced with neurotrophic factors (Kang and Schuman, 1996) and facilitated with agonists of dopamine (Huang and Kandel, 1995; Smith et al., 2005) and β-adrenergic receptors (Gelinas and Nguyen, 2005). Moreover, long-lasting protein synthesis–dependent forms of hippocampal long-term depression (LTD) also have been described. LTD, regardless of whether it is induced by NMDA receptors (Kauderer and Kandel, 2000; Sajikumar and Frey, 2004), group I metabotropic glutamate receptors (mGluR; Huber et al., 2000), or insulin receptors (Huang et al., 2004), is blocked by protein synthesis inhibitors. Thus, protein synthesis–dependent LTP and LTD can be induced via activation of multiple receptors in the hippocampus.

The basic temporal phases of synaptic plasticity that can be distinguished by transcription and translation inhibitors appear to have correlates in memory function. An excellent example is the gill-withdrawal reflex in *Aplysia*, which requires LTF. A single sensitizing stimulus (usually an electrical shock) results in a short-term memory that is insensitive to transcription and translation inhibitors. In contrast, multiple, spaced sensitizing electrical shocks result in long-term memory that is sensitive to both transcription and translation inhibitors (Castellucci et al., 1989). Similar observations have been made in rodents, where in general, new mRNA and protein synthesis is required for long-term but not short-term memory (McGaugh, 2000; Kandel, 2001).

4.33.2 Local Protein Synthesis and Synaptic Plasticity

One of the intriguing features of neurons is that dendrites and their spines contain all the components required for synthesizing proteins. Polyribosomes (Steward and Levy, 1982), translation factors (Tang et al., 2002), and mRNA that can be translated into protein are all present at synapses (Crino and Eberwine, 1996). Thus, neurons presumably are able to synthesize proteins without engaging transcription in the nucleus and without mRNA transport to their dendrites.

Does local protein synthesis occur in dendrites? Earlier studies addressing this question relied on reduced biochemical preparations of pre- and postsynaptic components termed synaptoneurosome preparations. Membrane depolarization (Bagni et al., 2000), brain-derived neurotrophic factor (BDNF) (Yin et al., 2002), and glutamate receptor agonists (Weiler et al., 1997; Bagni et al., 2000) all have been shown to translate new proteins in synaptoneurosomes. More direct evidence demonstrating that protein synthesis occurs in dendrites has been provided with imaging studies utilizing fluorescent reporters. Application of BDNF to neurons and manipulations that result in the blockade of neuronal activity have been shown to trigger protein synthesis in dendrites (Aakulu et al., 2001; Ju et al., 2004; Sutton et al., 2004, 2006). Is local protein synthesis required for long-lasting synaptic plasticity? Protein synthesis inhibitors block both BDNF-induced LTP (Kang and Schuman, 1996) and mGluR-LTD (Huber et al., 2000) when the cell bodies of hippocampal neurons are severed from their dendrites. Thus, these types of synaptic plasticity require local protein synthesis independent of a requirement for transcription. Local protein synthesis also is required for LTF in *Aplysia* sensory neurons and plasticity at neuromuscular synapses in *Xenopus* (Martin et al., 1997; Zhang and Poo, 2002). Thus, local protein synthesis is required for numerous types of synaptic plasticity.

An obvious question is whether local protein synthesis is required for hippocampal L-LTP. The answer to this question has been difficult to ascertain because L-LTP requires both transcription and translation. However, translation inhibitors block L-LTP at earlier times than the transcription inhibitors (Kelleher et al., 2004a; Banko et al., 2005), suggesting that L-LTP consists of an early translation–dependent phase that is independent of transcription, which is followed by a transcription- and translation-dependent phase. The translation-dependent early phase of L-LTP is presumably due to local protein synthesis. Consistent with this notion, local application of a protein synthesis inhibitor to dendrites has been shown to block L-LTP (Bradshaw et al., 2003). Furthermore, translation-dependent, transcription-independent LTP has been shown in isolated dendrites in hippocampal slices (Cracco et al., 2005; Vickers et al., 2005). Finally, β-adrenergic receptor-dependent facilitation of L-LTP also requires local dendritic translation independent of a requirement

for transcription (Gelinas and Nguyen, 2005). Thus, local protein synthesis appears to be required at fairly early temporal windows during L-LTP.

4.33.3 Translational Control of Gene Expression

As described earlier, translational control plays an important role in the regulation of gene expression, which leads to L-LTP and memory consolidation (Steward and Schuman, 2001; Kelleher et al., 2004b; Klann and Dever, 2004). Translational control provides the cell with a faster response to external stimuli than transcription, mRNA processing, and transport. In eukaryotes, translational control operates mainly at the rate-limiting initiation step, during which the small 40S ribosomal subunit is recruited to mRNA and is positioned at the initiation codon (Mathews et al., 2000). Translation initiation entails, first, the binding of the initiator Met – $tRNA_i^{Met}$ to the small 40S ribosomal subunit; second, location of the ribosome complex to the initiation codon; and third, joining of the large ribosomal subunit to generate a translation-competent ribosome (**Figure 1**). Translational control is generally modulated by changes in the phosphorylation status of initiation factors or their regulators. Two major mechanisms of regulation are (i) the phosphorylation of eIF2α, which prevents the exchange of GDP for GTP, and (ii) the modulation of eIF4F assembly.

4.33.4 Regulation by Phosphorylation of eIF2α

The eukaryotic initiator factor eIF2, which consists of three subunits (α, β, and γ), binds the initiator Met – $tRNA_i^{Met}$ and GTP to form the ternary complex. eIF2 then associates with the small ribosomal subunit in its GTP-bound form. GTP is hydrolyzed to release eIF2 from the ribosome in the GDP-bound state. Exchange of GDP for GTP on eIF2 is catalyzed by a five-subunit factor termed eIF2B and is required to reconstitute a functional ternary complex for a new round of translation initiation (Hinnebusch, 2000). By slowing the dissociation of eIF2 from eIF2B, phosphorylation of the α subunit at Ser51 blocks the GDP/GTP-exchange reaction and therefore causes a decrease in general translation initiation (Hinnebusch, 2000; Sonenberg and Dever, 2003) (**Figure 2**). Most cells contain more eIF2 than eIF2B, and consequently phosphorylation of a fraction of the eIF2α is sufficient to inhibit eIF2B and thus block translation.

While phosphorylation of eIF2α leads to a general inhibition of translation, it paradoxically results in translational upregulation of a subset of mRNAs that contain upstream open reading frames (uORFs) (Hinnebusch, 2000; Sonenberg and Dever, 2003) (**Figure 2**). The molecular mechanism underlying this selective translation was extensively studied in the general amino acid control response in the yeast *Saccharomyces cerevisiae* (Hinnebusch, 2000). Amino acid starvation of yeast leads to activation of the Gcn2p eIF2α kinase and inhibition of translation. However, translation of the GCN4 mRNA is stimulated because it contains in its 5′ untranslated region (5′UTR) four short ORFs. When amino acids are available, scanning ribosomes translate these short ORFs but dissociate from the mRNA before reaching the authentic GCN4 start codon. In contrast, when amino acids are scarce, eIF2α is phosphorylated, causing a fraction of the scanning 40S subunits to form active translational complexes only after they bypassed the upstream ORF and allowing initiation at the proper GCN4 start codon (Hinnebusch and Natarajan, 2002).

A similar mechanism was described in mammalian cells where the translation of the mRNA for the transcriptional modulator activating transcription factor 4 (ATF4) is enhanced by eIF2α phosphorylation (Harding et al., 2000; Vattem and Wek, 2004) (**Figure 2**). Therefore, eIF2α phosphorylation regulates both general and gene-specific translation. Importantly, ATF4 and its homologues play important roles as repressors of synaptic plasticity and memory formation in diverse phyla (Bartsch et al., 1995; Abel et al., 1998; Chen et al., 2003). An activity-dependent modulation of eIF2α phosphorylation in neurons is important for sustained alterations in synaptic transmission and learning and memory. In hippocampal slices, the gene expression-dependent L-LTP, induced by either tetanic stimulation or forskolin, is associated with decreased eIF2α phosphorylation (Costa-Mattioli et al., 2005). In hippocampal neurons treated with BDNF, which also induces L-LTP in hippocampal slices (Tang et al., 2002), phosphorylation of eIF2α is reduced (Takei et al., 2001). The evidence for the involvement of eIF2α in synaptic plasticity and memory formation was bolstered by a recent study on mice lacking the eIF2α kinase GCN2: in these mice, the

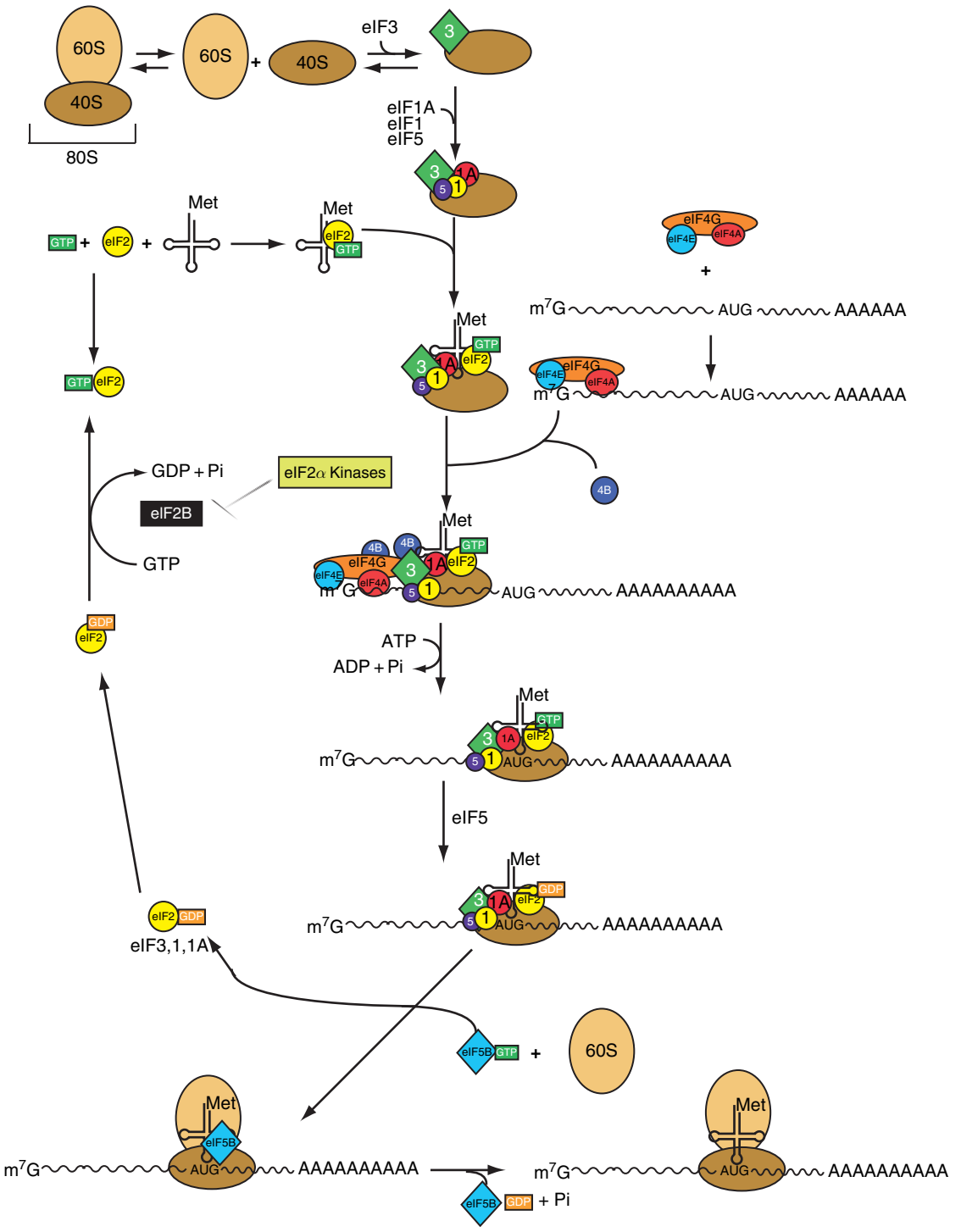

Figure 1 Translation initiation pathway in eukaryotes. Initiation factors are shown as color-coded symbols and appear when first implicated in the pathway.

reduction in phosphorylated eIF2α is associated with altered synaptic plasticity and memory (Costa-Mattioli et al., 2005; see following discussion).

In higher eukaryotes, the status of eIF2α phosphorylation is controlled by at least four known Ser/Thr protein kinases for which eIF2α is the

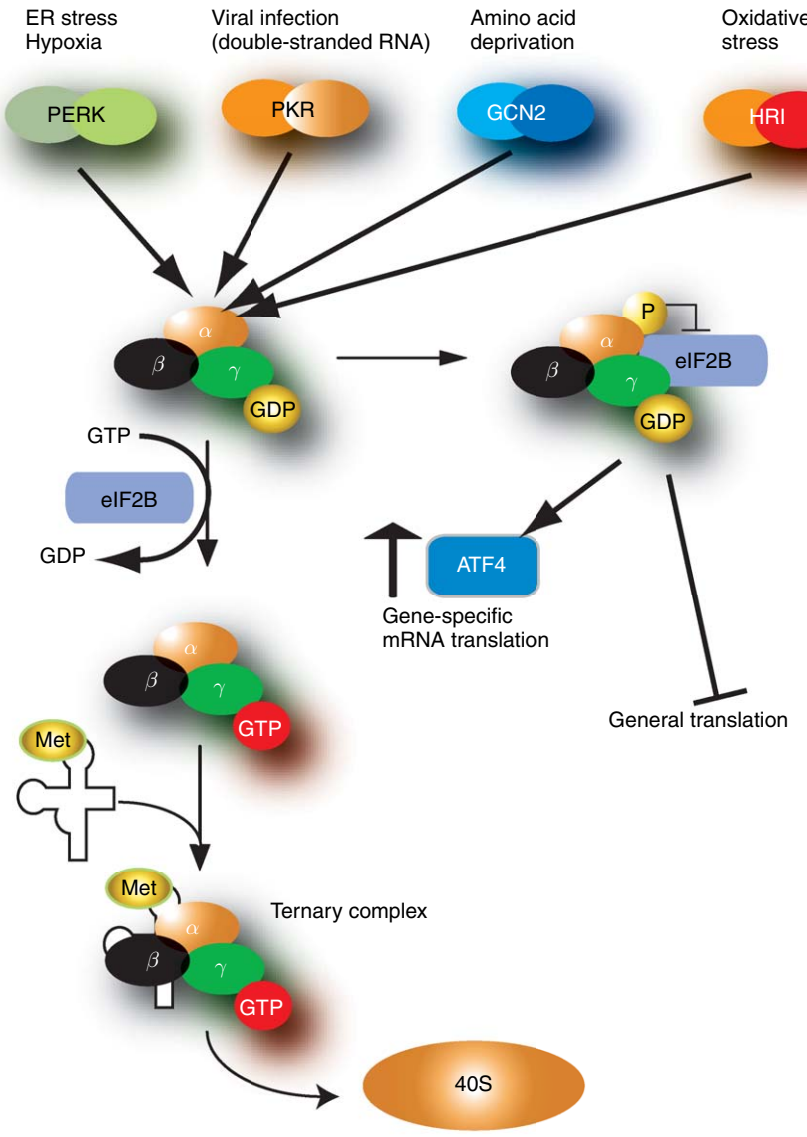

Figure 2 Translational control by the eIF2α kinases. Different stress conditions activate the eIF2α kinases, PERK (RNA-activated protein kinase (PKR)-endoplasmic reticulum–related kinase), PKR, HRI (hemin-regulated inhibitor kinase), and GCN2 (general control nonderepressible kinase), resulting in phosphorylation of α subunit of eIF2. Phosphorylation of eIF2α represses global rate of translation, by sequestering eIF2B, but stimulates translation of ATF4 mRNA. Studies from Costa-Mattioli et al. (2005) indicate that GCN2 and eIF2α phosphorylation are critical for long-lasting synaptic plasticity and memory.

major substrate (**Figure 2**). They include the double-stranded (ds) RNA-activated protein kinase (PKR), the hemin-regulated inhibitor kinase (HRI), the pancreatic eIF2α or the PKR-endoplasmic reticulum (ER)-related kinase (PEK/PERK), and the general control nonderepressible kinase (GCN2). The eIF2α kinases share extensive homology in the kinase catalytic domain (Meurs et al., 1990; Chen et al., 1991; Chong et al., 1992; Shi et al., 1998; Berlanga et al., 1999; Harding et al., 1999; Sood et al., 2000). They are activated in response to external stress, such as viral double-stranded RNA (dsRNA), heme deficiency, misfolded proteins in the ER, and amino acid deprivation, respectively. All four eIF2α kinases are expressed in the brain (but only a small amount of HRI) (Pal et al., 1991; Crosby et al., 1994; Mellor et al., 1994; Berlanga et al., 1999). GCN2 is the ancestral and the only eIF2α kinase that is evolutionarily conserved from yeast to mammals (Santoyo et al., 1997; Olsen et al., 1998; Sattlegger et al.,

1998; Berlanga et al., 1999; Hinnebusch, 2000; Sood et al., 2000). GCN2 is activated under conditions of amino acid deprivation via the accumulation of uncharged tRNA. However, it is also activated by other stimuli such as ultraviolet irradiation (Deng et al., 2002; Narasimhan et al., 2004). The finding that GCN2 mRNA is enriched in the brain of flies (Santoyo et al., 1997) and mammals (Berlanga et al., 1999; Sood et al., 2000), especially in the hippocampus (Costa-Mattioli et al., 2005), is consistent with a role for GCN2 in synaptic plasticity and memory storage.

4.33.5 Regulation of Synaptic Plasticity and Memory Consolidation by GCN2

GCN2 knockout mice exhibit a reduction in eIF2α phosphorylation in the hippocampus, which is accompanied by a decrease in the amount of the memory repressor ATF4 (Costa-Mattioli et al., 2005). Consistent with a decrease in ATF4 protein levels, the threshold for eliciting L-LTP was lower in hippocampal slices from GCN2 knockout mice. Specifically, a protocol that usually elicits a short-lasting E-LTP, which is independent of gene expression (translation and transcription), elicited a typical gene expression-dependent L-LTP (Costa-Mattioli et al., 2005). A comparable phenotype to that of GCN2 knockout mice was observed in mice expressing an inducible inhibitor of ATF4 and CCAAT/enhancer binding proteins (C/EBP) (EGFP-AZIP) (Chen et al., 2003). The *Aplysia* homolog of ATF4, ApCREB2 represses LTF (Bartsch et al., 1995). Upon injection of anti-ApCREB2 antibodies into *Aplysia* sensory neurons, a single pulse of 5-HT, which normally induces only short-term facilitation (lasting minutes), is sufficient to evoke a gene expression–dependent LTF that lasts longer than 1 day.

Surprisingly, in slices from GCN2 knockout mice, strong stimulation that normally induces L-LTP elicited only a short-lasting LTP. This effect of GCN2 deletion is specific to LTP because normal LTD could be induced in slices from the GCN2 knockout mice by low-frequency stimulation (LFS) or by incubation with DHPG, an agonist of group I mGluRs. Therefore, it is possible that either GCN2 does not play any role in protein synthesis–dependent LTD or the pathway that turns off GCN2 activity is not modified by LTD inducing protocols.

To determine whether the GCN2 deletion alters memory, GCN2 knockout mice were subjected to two distinct tasks: fear conditioning and the Morris water maze. Pairing tone presentations with foot shocks in a particular environmental context leads to both auditory and contextual fear conditioning. In the latter, the context, which is represented in the hippocampus and acts as the conditioned stimulus (CS), is associated with the foot shock, the unconditioned stimulus (US). Auditory fear conditioning, which associates a tone (CS) with the foot shock (US), depends on the amygdala but not the hippocampus (Fanselow and LeDoux, 1999; LeDoux, 2000). Both types of fear conditioning require new protein synthesis (Bourtchouladze et al., 1998; Schafe et al., 1999). When tested 24 h and 10 days after training, the GCN2 knockout mice showed deficits in contextual memory, which suggests that long-term memory is disrupted in these mice. Surprisingly, the lack of GCN2 selectively affects hippocampal-dependent memories but not amygdala-dependent auditory fear conditioning.

In agreement with the findings on contextual fear conditioning, the GCN2 knockout mice were deficient in long-term memory when tested in the Morris water maze. Specifically, in a conventional strong training protocol (three trials per day), spatial learning of GCN2 knockout mice was impaired, as indicated by longer escape latencies during training as well as by the increased time spent in the target quadrant and the number of platform crossings. In contrast, when the GCN2 knockout mice were given a weak training consisting of only one trial per day, they exhibited enhanced learning and memory (Costa-Mattioli et al., 2005). These behavioral findings correlate precisely with the electrophysiological findings in hippocampal slices from these animals, where weak stimulation elicited L-LTP instead of E-LTP, but strong stimulation failed to evoke the expected L-LTP. The findings reviewed here represent a significant advance in our understanding of the molecular mechanism underlying synaptic plasticity and memory formation. The levels of active GCN2 may be a determinant of the amount and schedule of training required for long-term memory. In *Aplysia*, *Drosophila*, mice, and rats, the balance between memory repressors and activators appears to be crucial for long-term synaptic plasticity and memory storage (Abel et al., 1998; Kandel, 2001). The GCN2 and 4E-BP2 knockout mice provide the first examples of memory repressor factors operating at the translation level.

4.33.6 Regulation by mTOR and the eIF4E-Binding Proteins

Ribosomal recruitment to the mRNA is facilitated by the 5′-cap structure (m7GpppX, where X is any nucleotide), which is present on all nuclear transcribed eukaryotic mRNAs (Shatkin, 1985). The cap structure is specifically recognized by eukaryotic initiation factor 4F (eIF4F) (Gingras et al., 1999), a complex comprised of three subunits: (1) eIF4E, the cap-binding subunit (Sonenberg et al., 1978); (2) eIF4A, a bidirectional RNA helicase; and (3) eIF4G, a large polypeptide that serves as a bridge between the ribosome and the mRNA (Imataka et al., 1998). eIF4E is limiting among all initiation factors and plays a critical role in translational regulation (Gingras et al., 1999). eIF4E is regulated at multiple levels (Raught and Gingras, 1999) via: (1) transcription, (2) phosphorylation, and (3) interaction with inhibitory proteins, the eIF4E-binding proteins (4E-BPs). In mammals, the 4E-BP family consists of three related small molecular-weight proteins, 4E-BP1, 4E-BP2, and 4E-BP3 (Pause et al., 1994; Poulin et al., 1998), which compete with eIF4G for eIF4E binding (Mader et al., 1995; Marcotrigiano et al., 1999). The 4E-BPs specifically inhibit cap-dependent translation initiation by preventing the assembly of the eIF4F complex and, consequently, ribosome binding to the mRNA (Haghighat et al., 1995; Pause et al., 1994) (**Figure 3**).

The mammalian target of rapamycin (mTOR) is a protein kinase that serves as a critical checkpoint for the integration of signals from the extracellular stimuli, growth factors, nutrient levels, and energy status (Hay and Sonenberg, 2004). mTOR triggers translation initiation by phosphorylating at least two translational modulators, p70 ribosomal S6 kinase 1 and 2 (S6K1/2), and the 4E-BPs (Gingras et al., 2001; Hay and Sonenberg, 2004) (**Figure 3**).

Extracellular stimuli such as growth factors, hormones (insulin), the G-conjugated protein receptor, and mitogens activate the phosphatidylinositol-3 kinase (PI3K) pathway. For instance, insulin activates the insulin receptor tyrosine kinase, which in turn phosphorylates the insulin receptor substrates (IRS) (**Figure 2**). PI3K then binds phosphorylated IRS via its SH2 regulatory domain. This interaction activates the PI3K catalytic subunit, which phosphorylates the membrane phospholipid phosphatidylinositol-4,5-bisphosphate (PIP2) to form PIP3, which then recruits Akt to the cell membrane, where it is phosphorylated and activated by PDK1 (Brazil and Hemmings, 2001). Akt activates mTOR through inhibition of the tuberous sclerosis complex (TSC). TSC is a heterodimer that contains TSC1 (hamartin) and TSC2 (tuberin), the latter serving as a GAP (GTPase activating protein) for Rheb, a *ras*-like GTPase. Activated Akt phosphorylates TSC2, decreasing its Rheb-GAP function (Garami et al., 2003; Inoki et al., 2005a,b). Subsequently, GTP-Rheb activates mTOR through a mechanism that is not well understood at this time.

One of the best characterized functions of mTOR is regulation of translation. mTOR exists in two distinct complexes (**Figure 3**). One complex contains raptor and is sensitive to the drug rapamycin (a macrolide that binds to the immunophilin FKBP12 to form a gain of function complex). The other complex contains rictor, is rapamycin insensitive, and phosphorylates Akt/PKB (Hara et al., 2002; Kim et al., 2002; Loewith et al., 2002; Hay and Sonenberg, 2004; Sarbassov et al., 2004). Raptor serves as an adaptor protein that recruits two downstream effectors: S6K1 and S6K2 and 4E-BPs (Beugnet et al., 2003; Choi et al., 2003; Schalm et al., 2003). Rapamycin disrupts the mTOR–raptor interaction, preventing mTOR from phosphorylating S6K1/2 and 4E-BP (Kim et al., 2002; Oshiro et al., 2004).

4E-BP1 (and the other 4E-BPs) binding to eIF4E is regulated by its phosphorylation status (Pause et al., 1994). Hypophosphorylated 4E-BP1 binds with high affinity to eIF4E, and hyperphosphorylation of 4E-BP1 prevents this interaction (Pause et al., 1994; Beretta et al., 1996). Upon cell stimulation with serum, growth factors, or hormones (e.g., insulin), 4E-BP1 becomes phosphorylated through an mTOR-dependent pathway and dissociates from eIF4E to relieve translational inhibition (Pause et al., 1994; Gingras et al., 2001) (**Figure 3**).

A large body of evidence implicates the mTOR signaling pathway in long-lasting synaptic plasticity and memory formation. First, rapamycin suppresses synaptic plasticity in invertebrates, including LTF in *Aplysia* sensory neurons (Casadio et al., 1999) and rodent hippocampal slices (Tang et al., 2002; Hou and Klann, 2004). It also blocks long-term spatial memory formation in mammals (Tischmeyer et al., 2003; Dash et al., 2006). Second, in hippocampal slices, several forms of synaptic plasticity are associated with the activation of mTOR and its downstream targets (Tang et al., 2002; Hou and Klann, 2004; Banko et al., 2005, 2006; Chen et al.,

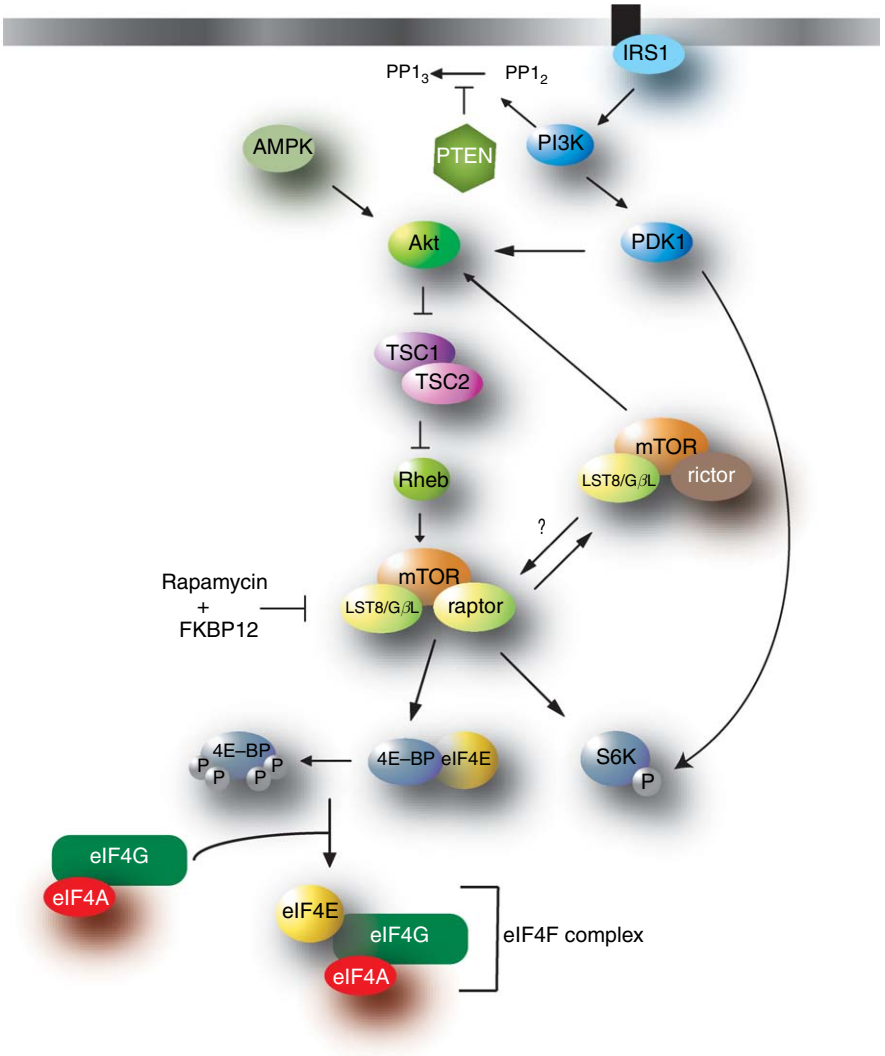

Figure 3 mTOR signaling to translation initiation. The regulation mTOR (mammalian target of rapamycin) activity is mediated by the PI3K/Akt signaling pathway leading to phosphorylation and inhibition of TSC2 by Akt and to the subsequent activation of Rheb, which activates mTOR by an as yet unknown mechanism. The raptor/GβL/mTOR complex is sensitive to rapamycin and mediates the phosphorylation of 4E-BP (eIF4E-binding proteins) and p70S6K. The rictor/GβL complex is rapamycin insensitive and phosphorylates Akt/PKB. The raptor/GβL/mTOR complex mediates phosphorylation of 4E-BPs and p70S6K. Hyperphosphorylation of 4E-BPs causes their release from eIF4E, allowing to eIF4E to form the ternary complex. A number of recent studies indicate that mTOR signaling and 4E-BP phosphorylation are critical for protein synthesis–dependent forms of long-lasting synaptic plasticity and memory.

2005; Tsokas et al., 2005). Third, the PI3K signaling pathway is also required for synaptic plasticity in different areas of the brain (Lin et al., 2001; Man et al., 2003; Opazo et al., 2003; Hou and Klann, 2004). In addition, PI3K inhibitors block memory consolidation in the hippocampus and amygdala (Lin et al., 2001; Chen et al., 2005). Fourth, the mTOR downstream target 4E-BP2 is critical for long-term synaptic plasticity and memory.

4.33.7 Regulation of Synaptic Plasticity and Memory Consolidation by 4E-BPs

Substantial evidence indicates that long-term synaptic plasticity and memory requires new mRNA translation. Significantly, in wild-type hippocampal slices, both LTD- and LTP-inducing stimulation are associated with an increase in the phosphorylation of

4E-BP2, the 4E-BP family member preferentially expressed in the mammalian hippocampus (Banko et al., 2005, 2006). The increase in 4E-BP2 phosphorylation during both LTP and mGluR-LTD is associated with an increase in eIF4F complex formation. To study the biological significance of these changes, 4E-BP2 knockout mice were recently used (Banko et al., 2005). The function of 4E-BP2 in synaptic plasticity has been most intensively studied at Schaffer collateral-CA1 pyramidal neuron synapses in the adult hippocampus. In accordance with an increase in general translation in mice lacking the translational repressor 4E-BP2, the amount of eIF4F complex was increased compared to wild-type mice (Banko et al., 2005). These data nicely correlate with an enhanced mGluR-LTD observed in these mice (Banko et al., 2006). Interestingly, rapamycin did not block the enhanced mGluR-LTD in slices from the 4E-BP2 knockout mice. Therefore, these data strongly argue that 4E-BP2 is the only mTOR downstream target involved in mGluR-dependent-LTD. In addition, as was the case for the GCN2 knockout mice in slices from the 4E-BP2 knockout mice, a standard stimulation paradigm that normally elicits protein synthesis–independent E-LTP in wild-type slices is converted to protein synthesis–dependent L-LTP (Banko et al., 2005). Taken together, these data indicate that the increase in eIF4F complex formation, a hallmark of ongoing translation, correlates with an enhanced LTP and LTD in slices from 4E-BP2 knockout mice. Strikingly, when LTP was elicited by a stronger stimulation protocol (four trains of HFS) that produced L-LTP in wild-type slices, the potentiation evoked in the slices from the 4E-BP2 knockout mice decayed to baseline in less than 2 h (Banko et al., 2005). Thus, there must be limits to the extent to which the threshold for LTP induction can be manipulated. Consequently, enhanced eIF4F complex formation and possibly excessive translation may be detrimental to L-LTP (Banko et al., 2005). 4E-BP2 knockout mice were studied in hippocampus-dependent learning and memory tasks. In correlation to what has been found with strong-LTP inducing protocols, 4E-BP2 knockout mice are impaired in spatial learning in the Morris water maze, as determined by both time spent in the target quadrant and the number of platform crossings (Banko et al., 2005). It would be interesting to determine whether this impairment can be rescued after intensive training, as was the case for other knockout mice implicated in learning and memory (Elgersma et al., 2002; Silva et al., 1992a,b). Consistent with these data, 4E-BP2 knockout mice are impaired in both auditory and contextual fear conditioning (Banko et al., 2005). These indicate that proper translational control is critical for long-term memory formation.

4.33.8 Translational Control by eIF4E Phosphorylation

In mammals, eIF4E is phosphorylated at a single site, Ser209 (Joshi et al., 1995; Whalen et al., 1996), by MAPK signal-integrating kinase/MAPK-interacting kinase 1 and 2 (Mnk1/2) (Fukunaga and Hunter, 1997; Pyronnet et al., 1999; Waskiewicz et al., 1999). Mnk1/2 physically interacts with the C-terminal region of eIF4G, which serves as a docking site to phosphorylate eIF4E. It is thought that eIF4E phosphorylation enhances general translation (Sonenberg and Dever, 2003). However, there has been debate as to the physiological function of phosphorylation of eIF4E at Ser209 (Scheper and Proud, 2002). In *Drosophila*, mutation at eIF4E–S251A, the equivalent Ser209 site, results in a delay in development and smaller flies (Lachance et al., 2002), indicating that eIF4E phosphorylation is important for normal development. In addition, *Drosophila* lacking Lk6, the functional homolog of the mammalian Mnk kinases, exhibit reduced viability, slower development, and reduced adult size under nutrient-deficient conditions (Arquier et al., 2005; Reiling et al., 2005). In apparent contrast to these findings, overexpression of the Mnks in mammalian cells and *Drosophila* results in a reduction in translation and/or growth rates (Knauf et al., 2001). These data are consistent with those in *Aplysia*, where overexpression of *Aplysia* Mnk resulted in a decrease in cap-dependent translation (Ross et al., 2006). Thus, it is possible that either too much eIF4E phosphorylation or other targets of Mnk1/2 are detrimental to cap-dependent translation. In mammals, Mnk1/2 double-knockout mice, in which eIF4E phosphorylation is abrogated, are fertile, develop normally, and have global translation rates that are unaltered (Ueda et al., 2004).

Multiple forms of neuronal activity and application of L-LTP induction protocols alter the phosphorylation status of eIF4E. For example, *Aplysia* neurons treated with serotonin, which elicits protein synthesis–dependent LTF, exhibited a p38-dependent increase in eIF4E phosphorylation (Dyer and Sossin, 2000). In mammals, NMDA receptor activation results in an extracellular signal-regulated kinase

(ERK)-dependent phosphorylation of eIF4E in hippocampal area CA1 (Banko et al., 2004), and BDNF treatment of cultured neurons elicits phosphorylation of eIF4E (Takei et al., 2001). In addition, forskolin treatment induces eIF4E phosphorylation, and NMDA-induced eIF4E phosphorylation requires PKA activity (Banko et al., 2004). Recently, Kelleher and collaborators demonstrated that both L-LTP and fear conditioning elicit an ERK-dependent increase in the phosphorylation of eIF4E (Kelleher et al., 2004a). In conclusion, several studies indicate that eIF4E phosphorylation correlates with protein synthesis–dependent synaptic plasticity and memory. Genetic evidence supporting the role of eIF4E phosphorylation in cap-dependent translation and long-term synaptic plasticity and memory remains to be provided.

4.33.9 Translational Control by CPEB

Cytoplasmic polyadenylation is another mechanism by which mRNA translation is regulated upon synaptic stimulation (Steward and Schuman, 2001). The molecular mechanisms underlying cytoplasmic polyadenylation have been extensively characterized in *Xenopus* oocytes, where a subset of dormant mRNAs have small poly(A) tails (usually ~20–40 nucleotides in length) (Mendez and Richter, 2001). In response to developmental cues, the poly(A) tails of the dormant mRNAs are lengthened, and translation ensues. Polyadenylation is controlled by two cis elements in the 3′ UTR of the mRNA: the cytoplasmic polyadenylation element (CPE, sequence of UUUUUAU) and the hexanucleotide AAUAAA, which is also important for nuclear pre-mRNA cleavage and polyadenylation (Fox et al., 1989; McGrew et al., 1989). Cytoplasmic polyadenylation element (CPE) binding factor (CPEB), which contains an RNA recognition motif (RRM) and zinc finger domain, binds with strong affinity the CPE (Hake and Richter, 1994; Hake et al., 1998) and interacts with a number of other regulatory factors. These factors include (i) symplekin, a scaffold-like protein; (ii) cleavage and polyadenylation specificity factor (CPSF), a group of four proteins that binds the hexanucleotide AAUAAA; and (iii) Gld2 (Barnard et al., 2004), an unusual poly(A) polymerase first discovered in yeast and *Caenorhabditis elegans* (Read et al., 2002; Saitoh et al., 2002; Wang et al., 2002). Polyadenylation is initiated when aurora (Eg2) phosphorylates CPEB (Mendez et al., 2000a), which induces CPEB to interact and possibly stabilize CPSF on the hexanucleotide AAUAAA (Mendez et al., 2000b). This process is likely necessary for the recruitment of poly(A) polymerase. One mechanism by which polyadenylation is believed to stimulate translation involves maskin, a protein that interacts with both CPEB and eIF4E (Stebbins-Boaz et al., 1999). The maskin-eIF4E interaction inhibits translation by precluding the eIF4E-eIF4G interaction and formation of the eF4F complex and ribosomal recruitment to the mRNA (Cao and Richter, 2002). Polyadenylation leads to the recruitment of the poly(A) binding protein (PABP), which binds to eIF4G (Wakiyama et al., 2000), a process that enhances the eIF4G-eIF4E association and promotes the dissociation of maskin from eIF4E (Cao and Richter, 2002).

4.33.10 Regulation of Synaptic Plasticity and Memory Consolidation by CPEB

Neuroguidin (Ngd), a maskin-like protein, was recently described in mammalian and *Drosophila* neurons (Jung et al., 2006). Ngd is similar to maskin in that it interacts with both eIF4E and CPEB. It is present as puncta in axons and dendrites and in growth cones and filopodia in mammalian neurons. In *Xenopus* embryos, Ngd is found both in the neural tube and neural crest cells and plays a role in neural development. The identity of the mRNAs whose translation is regulated by Ngd and its role in local translation remain to be established.

CPEB and several of its associated factors are localized postsynaptically, where local translation most likely occurs (Huang and Richter, 2004). Importantly, synaptic stimulation results in polyadenylation and translation of the α subunit of calcium-/calmodulin-dependent protein kinase II (αCaMKII) mRNA, which contains a CPE in the 3′UTR (Wu et al., 1998; Wells et al., 2001; Huang et al., 2002). Mutant mice with a deletion of the dendritic localization signal within the 3′ UTR of the αCaMKII mRNA exhibit a defect in dendritic protein synthesis of αCaMKII and an impairment in protein synthesis–dependent phase of LTP and memory (Miller et al., 2002).

Further evidence supports a role for CPEB in synaptic plasticity and memory. In CPEB knockout mice, LTP elicited by a single train of 100 Hz or one theta-burst stimulation was impaired. In contrast to GCN2 and 4E-BP2 knockout mice, gene-expression-dependent L-LTP or four trains of

theta-burst stimulation was not altered in slices from CPEB knockout mice. LTD evoked by 1 Hz stimulation appears to be moderately facilitated in CPEB knockout mice (Alarcon et al., 2004). These data indicate that CPEB is involved in specific forms of LTP.

A battery of behavioral tests was performed in CPEB knockout mice. Although no differences were found in the Morris water maze and fear conditioning, CPEB knockout mice exhibited a deficit in a form of memory known as extinction (Berger-Sweeney et al., 2006), an active and dynamic learning process that results in the formation of new memories. Extinction does not reflect forgetting but, rather, relearning, in which the new association of the CS with the absence of the original reinforcer comes to control behavior (Bouton and Nelson, 1994; Berman and Dudai, 2001).

Studies in *Aplysia* have provided additional evidence supporting a role for CPEB in synaptic plasticity and memory. In *Aplysia* sensory neurons, 5-HT treatment increased the content of CPEB four- to fivefold in a transcription-independent manner. Moreover, when CPEB mRNA levels were decreased by an antisense oligonucleotide, LTF was impaired (Si et al., 2003a), suggesting that CPEB-mediated translation could result in the establishment of a tag at synapses.

Curiously, the form of CPEB in *Aplysia* and other invertebrate neurons differs from that in vertebrate neurons in that it contains a long stretch of glutamines, which is often found in proteins that have characteristics of a prion (Si et al., 2003b). In fact, *in vitro* studies have established that *Aplysia* CPEB exhibits features of a prion protein. In these studies it was also demonstrated that CPEB could assume two forms; an aggregated form (i.e., prion-like) and a nonaggregated form (Si et al., 2003b). Only the aggregated form was able to bind RNA *in vitro*, and remarkably, the prion-like form of the protein could convert the nonaggregated form into the aggregated form. It was speculated by the authors of these studies that synaptic stimulation causes CPEB to assume a prion-like form that either stimulates the translation of some mRNAs, causing it to alter its substrate specificity, or releases some mRNAs from an inhibited state. These tantalizing findings support a dynamic model that would explain how memories are stored and maintained for long time periods. Whether polyglutamine-lacking CPEB in mammalian neurons plays a similar role to its *Aplysia* counterpart is a matter of current investigation.

4.33.11 Regulation of 5′TOP Translation

As mentioned earlier, several protein kinases and translation factors act as downstream effectors of mTOR and ERK to regulate cap-dependent translation initiation. Both of these kinases also may regulate translation via an additional downstream effector, p70S6 kinase (also known as S6K or p70S6K). Both mTOR and ERK, as well as phosphoinositide-dependent kinase 1 (PDK-1) and protein kinase Cζ, phosphorylate and regulate p70S6K activity (Dufner and Thomas, 1999). One substrate of p70S6K is ribosomal protein S6, a subunit of the 40S ribosome, whose phosphorylation usually coincides with the translation of mRNA species that contain a terminal 5′ oligopyrimidine tract (5′TOP). A unique feature of 5′TOP mRNAs is that they encode for translation factors and all vertebrate ribosomal protein subunits (Meyuhas, 2000). Because localization and the ongoing synthesis of functional ribosomes is energetically costly for neurons to maintain near synaptic sites, it might be advantageous to position polyribosomes at locations where they could be efficiently utilized and dynamically modulated to influence translational competence in response to the appropriate synaptic signals. Activation of S6 and concomitant 5′TOP synthesis represents one possible signal transduction pathway that may promote such events in neurons.

There is evidence that p70S6K and S6 are regulated during long-term synaptic plasticity and long-term memory. Protein synthesis–dependent forms of LTP are associated with increased phosphorylation, and presumably increased activation, of p70S6K (Cammalleri et al., 2003; Tsokas et al., 2005). Increased S6 phosphorylation has been reported in *Aplysia* synaptosome preparations treated with serotonin, a facsimile of protein synthesis–dependent LTF (Khan et al., 2001). Moreover, increased S6 phosphorylation also has been observed to be enhanced after the induction of LTP, following training in a hippocampus-dependent memory task (Kelleher et al., 2004a). Interestingly, the levels of S6, whose mRNA contains a 5′TOP, are increased by serotonin in *Aplysia* synaptosomes (Khan et al., 2001). These findings suggest that activation of p70S6K, and the subsequent phosphorylation and synthesis of ribosomal protein S6, may play a role in long-lasting synaptic plasticity and memory.

Another protein whose mRNA contains a 5′TOP sequence that may be crucial for protein synthesis–dependent synaptic plasticity is eEF1A, a critical translation factor that mediates peptide elongation by promoting GTP-dependent binding of aminoacyl tRNA to the ribosome. eEF1A synthesis has been reported to be elevated during the protein synthesis–dependent form of LTP (Tsokas et al., 2005), which was accompanied by an activation of p70S6K. Transport of EF1A mRNA from the soma to dendrites was first identified in *Aplysia*, where it was found to be synthesized during the maintenance phase of serotonin-induced LTF (Giustetto et al., 2003). The primary function of eEF1A is as an elongation factor, but noncanonical functions of EF1A are known to exist. One such function for eEF1A is to promote the assembly of actin monomers into F-actin (Liu et al., 2002). Intriguingly, eEF1A protein has been found to migrate toward activated synapses during *in vivo* LTP, an event that was blocked with the F-actin inhibitor lantruculin B (Huang et al., 2005). Regardless of its function, the involvement of eEF1A in multiple forms of synaptic plasticity strongly suggests that eEF1A is an essential molecule associated with the formation of long-term changes in synaptic strength.

It should be noted that in nonneuronal cells, there are reports that p70S6K is required for neither S6 phosphorylation nor synthesis of 5′TOP encoded proteins (Tang et al., 2001; Pende et al., 2004; Stolovich et al., 2005). Additional substrates and binding partners of p70S6K (for a review, see (Ruvinsky and Meyuhas, 2006) include neurabin (Oliver et al., 2002) and eukaryotic initiation factor 4B (Raught et al., 2004; Holz et al., 2004). Future studies on these potential effector molecules of p70S6K, as well as studies with p70S6K mutant mice, will be required for a fuller understanding of the role of this kinase in the expression of protein synthesis–dependent synaptic plasticity and memory.

Interestingly, at synapses, the protein synthetic machinery, which would largely consist of proteins that contain 5′TOP sequences in their mRNA, appears to be quite limited (for a review, see Schuman et al., 2006). The observation that there are relatively few polyribosomes at synapses is somewhat surprising, although there is evidence that polyribosomes move from dendritic shafts to dendritic spines following the induction of LTP (Ostroff et al., 2002). One way to increase the translational capacity would be make new ribosomes in response to synaptic stimulation. It has been reported that 5′TOP mRNAs that encode ribosomal proteins are present in *Aplysia* neurites (Moccia et al., 2003) and mammalian dendrites (Zhong et al., 2006). These findings suggest that ribosomal proteins can be synthesized and potentially assembled into ribosomes in neurons at locations quite distant from the nucleolus, where ribosomes are typically assembled in eukaryotic cells. Whether long-lasting synaptic plasticity and/or memory formation triggers the synthesis of ribosomal proteins and the subsequent assembly of these proteins into a translationally competent ribosome in dendrites remains to be determined.

4.33.12 Regulation of Eukaryotic Elongation Factor 2

Eukaryotic elongation factor 2 (eEF2) is a GTP-binding protein that mediates the translocation of peptidyl-tRNA from the A site to the P site on the ribosome (Moldave, 1985). eEF2 kinase, a calcium-/calmodulin-dependent kinase sometimes referred to as calcium-/calmodulin-dependent kinase III, specifically phosphorylates and inactivates eEF2 to decrease the translocation of the peptidyl-tRNA on the ribosome, thereby resulting in a general decrease in protein synthesis (Ryazanov and Davydova, 1989; Carlberg et al., 1990; Redpath et al., 1993). Interestingly, eEF2 and elongation also are regulated indirectly by mTOR. eEF2 kinase can be phosphorylated by mTOR near the calmodulin-binding site; this phosphorylation event decreases the ability of calmodulin to bind eEF2 and thus decreases eEF2 kinase activity (Browne and Proud, 2004). Thus, one might predict that mTOR activation associated with the various forms of protein synthesis–dependent synaptic plasticity and memory would result in increased translation initiation and increased translation elongation. The observation that serotonin stimulates an mTOR-dependent dephosphorylation of eEF2 in *Aplysia* synaptosomes is consistent with this notion (Carroll et al., 2004). However, NMDA receptor activation increases the phosphorylation of eEF2 in several types of reduced experimental preparations (Marin et al., 1997; Scheetz et al., 1997, 2000). Similar findings have been reported for chemically induced LTP (Chotiner et al., 2003). Although the increase in eEF2 phosphorylation was correlated with a decrease in overall protein synthesis, as would be expected, an increase in the synthesis of specific proteins such α-CaMKII

(calcium-calmodulin-dependent protein kinase II), Arc, and Fos was observed (Scheetz et al., 2000; Chotiner et al., 2003). These findings raise the intriguing possibility that the phosphorylation of eEF2, though decreasing overall protein synthesis, triggers the translation of specific mRNAs during protein synthesis–dependent synaptic plasticity.

A similar mechanism of regulation of translation elongation appears to occur during taste memory consolidation. Following novel taste learning, increased eEF2 phosphorylation was observed in synaptoneurosomes prepared from the insular cortex, but not the hippocampus (Belelovsky et al., 2005). Moreover, the learning-induced increase in eEF2 phosphorylation was associated with increased levels of αCaMKII in the same synaptoneurosomal fraction. These studies are remarkably consistent with the *in vitro* studies described in the previous paragraph and suggest that when coupled with increases in translation initiation, translation elongation is the rate-limiting step for new protein synthesis during memory consolidation (Belelovsky et al., 2005). Further investigations will be required to determine whether decreased elongation is a core mechanism required for protein synthesis–dependent forms of learning and memory.

4.33.13 Regulation of mRNA-Binding Proteins during Synaptic Plasticity and Memory

In addition to the regulatory mechanisms that regulate translation initiation and elongation during synaptic plasticity and memory, it is clear that proper protein synthesis also requires regulation of mRNA-binding proteins for long-lasting changes in synaptic strength. As discussed in a recent review (Wells, 2006), dendritic protein synthesis requires that mRNAs be identified after transcription, packaged in a way that keeps them from being translated, and transported to the appropriate dendritic location, where synaptic signals can initiate translation.

One mRNA-binding protein that is involved in synaptic plasticity and memory is CPEB. As discussed earlier, CPEB binds to specific sequences called CPEs in the 3′ UTR of mRNAs that suppresses their translation. Mice that lack CPEB-1, one mammalian isoform of CPEB, exhibit deficits in protein synthesis–dependent LTP induced by a theta-burst stimulation paradigm (Alarcon et al., 2004). The CPEB-1 knockout mice also exhibit a reduction in the extinction of two types of hippocampus-dependent memory (Berger-Sweeney et al., 2006). Similarly, elimination of CPEB results in impaired LTF in *Aplysia* (Si et al., 2003a). Taken together, these data suggest that CPEB might specifically regulate the synthesis of proteins encoded by mRNAs that bind to CPEB during synaptic plasticity and memory. One mRNA that is very likely to be regulated during these processes is that of α-CaMKII, which contains two CPEs in its 3′ UTR (Wu et al., 1998). The identities of additional CPEB-binding mRNAs, whose translation might be regulated during synaptic plasticity and memory in the hippocampus, has been provided in a microarray analysis of the CPEB-1 knockout mice (Berger-Sweeney et al., 2006). Whether these mRNAs are translated into protein during either long-lasting synaptic plasticity or memory formation remains to be determined.

Another mRNA-binding protein that is involved in synaptic plasticity and memory is fragile X mental retardation protein (FMRP). Genetic deletion of FMRP results in fragile X mental retardation, which is the most common inherited disease causing mental retardation, affecting approximately 1:4000 males and 1:8000 females (Turner et al., 1996). FMRP is an mRNA-binding protein thought to regulate translation of specific mRNAs, including its own mRNA (Feng et al., 1997; Brown et al., 2001; Darnell et al., 2001; Zhang et al., 2001; Miyashiro et al., 2003) via binding to KH domains (with homology to the RNA-binding region of hnRNP K) and an RGG (arginine-glycine-glycine) box. Previous studies have shown that FMRP is colocalized with polyribosomes in the neuronal soma as well as in dendritic spines, which suggests the possibility that FMRP is involved in local dendritic protein synthesis (Stefani et al., 2004). FMRP is critically involved in the biochemical regulation of translation during mGluR-LTD. For example, mGluR-LTD is augmented in mice that lack FMRP (Huber et al., 2002), and FMRP is translated in response to stimulation of group I mGluRs (Weiler et al., 1997; Todd et al., 2003a,b; Antar et al., 2004; Hou et al., 2006). In addition to FMRP, mGluR-LTD is associated with increased synthesis of proteins encoded by FMRP-binding mRNAs such as microtubule-associated protein-1B (MAP1B) and α-CaMKII (Hou et al., 2006). Interestingly, the augmented mGluR-LTD in mice that lack FMRP is not protein synthesis dependent (Nosyreva and Huber, 2006; Hou et al., 2006), consistent with studies demonstrating that protein synthesis is regulated improperly in the

FMRP-deficient mice (Weiler et al., 2004; Aschrafi et al., 2005; Qin et al., 2005; Hou et al., 2006). The FMRP-deficient mice also exhibit mild behavioral phenotypes that are consistent with fragile X mental retardation (Kooy, 2003). However, these phenotypes, including contextual fear conditioning, are exaggerated in a mouse model where both FMRP and a paralog, FXR2P, are absent (Spencer et al., 2006). The *Drosophila* model for fragile X mental retardation also exhibits cognitive deficits that can be reversed with mGluR antagonists (McBride et al., 2005). All together the findings described earlier indicate that FMRP plays a critical role in translational control during synaptic plasticity and memory, especially those that are dependent on mGluRs (Bear et al., 2004).

Other mRNA-binding proteins have been implicated in synaptic plasticity and memory. In *Drosophila*, the mRNA for the mRNA-binding proteins pumilio, staufen, and orb (the *Drosophila* homolog of CPEB) is upregulated during memory formation, and disruption of the pumilio and staufen genes disrupts long-term memory (Dubnau et al., 2003). Whether genetic disruption of the mammalian homologs of pumilio and staufen result in synaptic plasticity and memory abnormalities has yet to be determined. Genetic deletion of translin (also known as testis-brain RNA-binding protein), an mRNA-binding protein that has several characteristics similar to FMRP, results in impaired spatial learning in the Morris water maze (Stein et al., 2006). It seems quite likely that removal of any of a number of dendritically localized mRNA-binding proteins will alter protein synthesis–dependent forms of synaptic plasticity and memory.

4.33.14 Summary

Despite the enormous amount of progress that has been made in the last 5 years, a critical issue that remains for researchers studying translational control and how it contributes to synaptic plasticity and memory is identifying the proteins that are synthesized in response to either a particular pattern of synaptic stimulation or learning a memory task. Further complicating this issue, it appears that the signal transduction cascades that are required to initiate cap-dependent translation, at least with respect to the regulation of the joining of the mRNA to the ribosome, are similar, regardless of whether the plasticity induced results in either LTP or LTD. Several ideas have been recently advanced to address this quandary. One idea is that it is not the synthesis of specific proteins but, rather, the creation of specific synaptic tags, one for LTP and another for LTD, that permits the capture of distinct proteins from a large, homogeneous pool of newly synthesized plasticity-related proteins (Kelleher et al., 2004b; Govindarajan et al., 2006). If this idea is correct, one would predict that a similar set of proteins are synthesized after the induction of both LTP and LTD, and these plasticity-related proteins could enable bidirectional plasticity. For example, the induction of LTP would permit long-lasting LTD, and the induction of LTD would permit long-lasting LTP. Interestingly, this type of heterosynaptic associativity between LTP and LTD has been demonstrated (Sajikumar and Frey, 2004), suggesting that it is the synaptic tag, rather than the specific proteins that are made, that differ between LTP and LTD. However, there is evidence to the contrary. For example, protein kinase M ζ (PKMζ), a constitutive active isoform of protein kinase C, is required for the maintenance of L-LTP, but not L-LTD (Sajikumar et al., 2005), suggesting that PKMζ is an LTP-specific protein. Thus, the idea that LTP and LTD result in the synthesis of similar sets of proteins remains to be determined, perhaps with either microarray studies of polysome fractions or proteomic analysis. In regard to the latter, a recent technical advance that permits the identification of newly synthesized proteins in neurons bodes well for a proteomic approach to this critical question (Dieterich et al, 2006).

Does overall protein synthesis, particularly in dendrites, actually increase during either LTP or LTD? It has been reported that global protein synthesis is increased in response to LTP-inducing stimulation (Kelleher et al., 2004a), and treatment of synaptoneurosomes with metabotropic glutamate receptor agonists increased the number of polyribosomes (Weiler and Greenough, 1993) and the translation of a reporter mRNA (Job and Eberwine, 2001). On the other hand, *in vivo* application of the group I mGluR agonist DHPG to the hippocampus did not trigger a detectable increase in overall protein synthesis, though it did result in an increase in the synthesis of eEF1A (Huang et al., 2005). As mentioned earlier, glutamate receptor activation in cultured neurons and NMDA receptor activation in the tadpole tectum results in an overall decrease in protein synthesis that coincides with specific increases in α-CaMKII. Thus, the question of whether overall protein synthesis increases,

particularly in dendrites, or whether gene-specific translation via the specific regulation of mRNA-binding proteins is augmented during either LTP or LTD awaits further investigation.

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4.34 Activity-Dependent Structural Plasticity of Dendritic Spines

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4.34.1 Introduction

Neuroscientists have long been fascinated by the problem of how memory is formed, stored, and recalled, not only because learning and remembering are at the core of our human experience, but also for the evolutionary significance to adapt behaviorally by learning about and making predictions in response to our surroundings. As early as the later part of the nineteenth century and around the time when the cellular basis of brain structure was still hotly debated, Cajal and Tanzi speculated that the improvement of existing skills and the acquisition of new ones required structural changes in nerve cells. Current cellular and molecular models of learning and memory are deeply rooted in these pioneering ideas, whereby biochemical modifications and morphological remodeling of existing synaptic junctions, as well as the formation of new ones, lead to enduring functional changes in neuronal networks. This chapter will review the experimental evidence in support of the activity-dependent structural plasticity of dendritic spines, the small processes extending from the surface of dendrites where most excitatory synapses of the central nervous system (CNS) are formed. We will focus on the morphological consequences of environmental enrichment and behavioral learning of associative tasks, as well as *in vitro* manipulations of neuronal activity resulting in long-term synaptic plasticity in the hippocampus, a brain region critical for memory formation. We will review the actions of neurotrophins and hormones on dendritic spines in the context of hippocampal-dependent learning and memory. Since several developmental disorders associated with mental retardation present with abnormal dendritic spines, we will also discuss the potential implications of such structural differences in cognition as well as in learning and memory.

4.34.2 Brief Historical Perspective

The cellular and molecular mechanisms of memory acquisition, consolidation, and subsequent recall have been intensely studied by neuroscientists for more

than a century. The first ideas about the possibility that experience can modify the structure of the brain, including its comprising nerve cells and their junctions, can be traced back to the later part of the nineteenth century (Bain, 1872; James, 1890; Cajal, 1893; Tanzi, 1893; Foster and Sherringhton, 1897). Eugenio Tanzi and Santiago Ramón y Cajal postulated that the improvement of learned habits and skills by 'mental exercise' arises from the growth of existing synapses, whereas learning new ones requires the formation of new nerve cell connections. Despite the appeal of these remarkably intuitive ideas, it was later realized that the mature nervous system might not be as plastic as it is during development and that neuronal growth may be too slow to account for learning. Later, Donald Hebb proposed a 'dual trace mechanism' to address the discrepancy between the rapidity of learning and the time required for structural growth, whereby

> . . . a reverbatory trace might cooperate with the structural change, and carry the memory until the growth change is made . . . (Hebb, 1949).

Hebb's more famous contribution is a cellular mechanism for associative learning postulating that coincident activity at given synaptic junctions modifies the properties of those synapses, thereby increasing their efficiency (Hebb, 1949). Together with the pioneering ideas of Tanzi and Cajal, this 'Hebbian' principle dominates the current thinking of how use-dependent changes at synapses occur during learning.

Research into the mechanisms underlying learning has thus primarily focused on the computational unit of the nervous system, the synapse. It is now well known that synapses are not static: the cellular mechanisms of learning and memory, triggered by experience, involve molecular modifications and morphological remodeling of existing synapses, as well as genesis of new synapses, resulting in activity-dependent functional changes. The vast majority of the studies on the mechanisms underlying changes in synaptic strength as a consequence of behavioral experience have focused on one of the brain regions most relevant to learning and memory, the hippocampus. The study of synaptic plasticity in the hippocampus and its relationship to learning and memory has uncovered a labyrinth of molecular, cellular, and biophysical mechanisms that have been extensively reviewed (Martin et al., 2000; Abel and Lattal, 2001) (*See* Chapters 4.02, 4.16).

4.34.3 The Structure and Function of Dendritic Spines

The acquisition, consolidation, and storage of information are dynamic processes that involve constant modification of synapses. A dynamic structure that makes up one half of the synapse long thought to be critical for learning and memory is the dendritic spine (**Figure 1**). Spines were first described by Cajal as small protrusions extending from the surface of dendrites (Cajal, 1891), likely representing the points of reception of nerve impulses (Cajal, 1909). It is now well known that these micron-long dendritic projections are the main postsynaptic site of excitatory synapses in the CNS, whereby approximately 90% of spines are innervated by a single

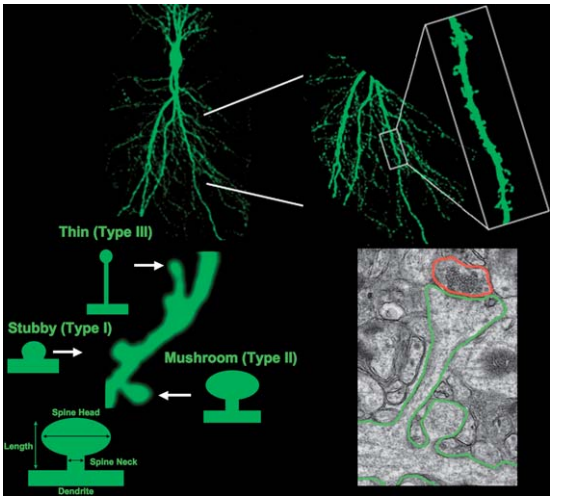

Figure 1 The structure of dendritic spines of hippocampal pyramidal neurons. Using particle-mediated gene transfer (a.k.a. gene gun), organotypic slice cultures were transfected with cDNA coding for enhanced yellow fluorescent protein. *Top panels:* Laser-scanning confocal microscopy images of a pyramidal neuron in area CA1 are shown at different magnifications to illustrate the complexity of their dendritic arbor and the abundance of dendritic spines in secondary and tertiary branches. *Bottom left panel:* A maximum-intensity projection of *z*-stacks shows a dendritic segment studded with the most common spine morphologies (i.e., stubby, mushroom, and thin). The cartoon illustrates the geometrical dimensions measured in individual spines to categorize them. *Bottom right panel:* A mushroom dendritic spine (outlined in green) forms an asymmetric synapse with a single presynaptic terminal (outlined in red) in *stratum radiatum* of area CA1 in organotypic slice culture. Bottom left panel adapted from Tyler WJ and Pozzo-Miller L (2003) Miniature synaptic transmission and BDNF modulate dendritic spine growth and form in rat CA1 neurones. *J. Physiol.* 553: 497–509, with permission.

presynaptic terminal (Gray, 1959b). Furthermore, 'naked' spines lacking a presynaptic partner are hardly observed in serial electron microscopy (EM) reconstructions of the hippocampus (Harris and Stevens, 1989). In some occasions, two or more spines from the same dendrite share the same presynaptic terminal, the so-called multiple synapse bouton. Structurally, a spine consists of a spherical head connected by a neck to its parent dendrite. In the hippocampus, the morphology of individual spines varies widely due to differences in their length, head shape, and neck diameter (Sorra and Harris, 2000). Simple spines can be characterized into three major types: (1) stubby, or Type-I spines, which lack obvious necks; (2) mushroom, Type-II spines, which have large heads and short narrow necks; and (3) thin, Type-III spines, which have small heads and long narrow necks (Peters and Kaiserman-Abramof, 1970). More complex morphological variations have also been observed, including bifurcated spines with multiple heads sharing the same neck (Harris, 1999). Time-lapse imaging of live spines has vividly demonstrated that dendritic spines are not static structures, but rather motile processes, especially during development (Matus, 2000; Dunaevsky and Mason, 2003). Thus, the aforementioned morphological types need to be considered as arbitrary snapshots of an underlying continuous distribution of possible geometrical forms, likely morphing from one another (Parnass et al., 2000). In the following sections, we will further discuss the intriguing possibility that spine geometry is modulated by synaptic activity, and how this structural modification may have functional consequences for synaptic transmission and plasticity.'

At the most distal region of the spine head facing the active zone (i.e., release site) of the apposing presynaptic terminal, a noticeable electron dense thickening is observed in EM micrographs, termed the postsynaptic density (PSD) (Kennedy, 1997; Ziff, 1997; Sheng and Sala, 2001). The PSD contains cytoskeletal components, scaffolding, and regulatory proteins, as well as neurotransmitter receptors. Simple dendritic spines do not contain mitochondria or microtubules, and the main cytoskeletal components are F-actin microfilaments (Peters et al., 1991). Notably, some spines will exhibit polyribosomes at the base of their necks (Spacek, 1985), suggesting that protein synthesis can occur locally near synapses. Approximately half of the spines of hippocampal pyramidal neurons contain one or several cisterns of smooth endoplasmic reticulum (SER) within their heads, a structure sometimes called the spine apparatus (Spacek and Harris, 1997; Cooney et al., 2002). Intriguingly, spine SER cisterns are not static but can dynamically interact with those within the parent dendrite (Toresson and Grant, 2005). Together with the role of SER as a Ca^{2+} source and sink (Berridge, 1998; Pozzo-Miller et al., 2000), the presence of Ca^{2+}-permeable ligand and voltage-gated channels in the spine membrane supports one of the most recognized functions of dendritic spines, i.e., a biochemical compartment for intracellular Ca^{2+} signaling (Muller and Connor, 1991; Petrozzino et al., 1995; Yuste and Denk, 1995; reviewed by Connor et al., 1994; Yuste et al., 2000; Sabatini et al., 2001).

It is becoming increasingly clear that the particular morphology of a dendritic spine may play an important role in determining its function, a concept that has been extensively reviewed (Shepherd, 1996; Yuste and Majewska, 2001; Nimchinsky et al., 2002). Two morphological features of dendritic spines seem to be critical for their role as biochemical compartments: the geometrical dimensions of the head and neck. If one formally considers a dendritic spine in terms of an electrical resistor and relates Ohm's law to diffusional resistance, the electrical coupling between the spine head and the dendritic shaft can be estimated from the diffusion of small fluorescence molecules from the spine to the parent dendrite. Using this approach, it was initially shown that diffusional coupling depends on the length and diameter of the neck (Svoboda et al., 1996). Furthermore, different levels of synaptic activity are able to regulate the diffusional isolation of individual spines (Bloodgood and Sabatini, 2005). Chronic blockade of excitatory synaptic transmission enhanced the diffusion of a photoactivatable green fluorescent protein (GFP) from the spine into the parent dendrite, while increasing excitatory transmission enhanced spine diffusional isolation. Notably, patterns of coincident synaptic activation and postsynaptic action potentials known to induce long-term potentiation (LTP) rapidly restricted diffusion across the spine neck. However, no correlation was found between spine geometry and diffusional isolation. These changes may be relevant for the induction and input specificity of Ca^{2+}-dependent forms of synaptic plasticity (i.e., long-term potentiation), because the diffusional coupling of individual spines affects the spatiotemporal profile of Ca^{2+} signals within spine heads (Korkotian and Segal, 2000; Majewska et al., 2000a). Indeed, a few experimental and modeling studies

have shown that the spine neck can function as a diffusional barrier for Ca^{2+} ions flowing between the spine head and the parent dendrite, whereby short spines facilitated the Ca^{2+} flux from the head to the dendrite and long spines prevented it (Volfovsky et al., 1999; Korkotian et al., 2004). Using two-photon uncaging of methoxy nitroindolino glutamate (MNI-glutamate) to activate *N*-methyl-D-aspartate-type glutamate receptors (NMDARs) in individual spines, it has been shown that larger spines permitted greater efflux of Ca^{2+} into the dendritic shaft, whereas smaller spines manifested a larger increase in Ca^{2+} within the spine compartment as a result of a smaller Ca^{2+} flux through the neck (Noguchi et al., 2005). However, this view has been challenged based on the observations that the spatiotemporal profile of intracellular Ca^{2+} signals within spine heads is primarily determined by sequestration and extrusion, likely within the head or neck (Nimchinsky et al., 2002; Sabatini et al., 2002). Additional confounding factors include the perturbation of the intrinsic Ca^{2+} buffering capacity by the Ca^{2+} indicator dyes, the apparent increased diffusion of Ca^{2+} ions bound to the highly mobile indicator dyes, and the temperature dependence of the sequestration and extrusion processes.

Despite the difficulty of determining the role of spine morphology in the coupling of spine Ca^{2+} signals with the parent dendrite (Majewska et al., 2000a; Nimchinsky et al., 2002), recent evidence supports an early suggestion that spine morphology and size correlate with synaptic strength (Pierce and Lewin, 1994). For example, the volume of the spine head is directly proportional to the number of docked vesicles at the active zone of presynaptic terminals (Harris and Sultan, 1995; Boyer et al., 1998; Schikorski and Stevens, 1999) and to the number of alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionate-type glutamate receptors (AMPARs) (Nusser et al., 1998). These observations indicate that larger spines bear larger synapses, which are more reliable and stronger in terms of probability of release and postsynaptic sensitivity, respectively (Murthy et al., 2001). Another measure of synaptic strength is the expression of AMPARs at individual excitatory spine synapses, as observed during brain development and after induction of LTP, the most studied cellular model of learning and memory (Malinow and Malenka, 2002). The expression of AMPARs within individual spines of different morphologies was mapped by two-photon uncaging of MNI-glutamate, revealing that mushroom (Type-II) spines show larger AMPAR-mediated responses than thin (Type-III) spines (Matsuzaki et al., 2001). These studies provide functional evidence that larger spines represent stronger synapses, as defined by their expression of AMPARs (Kasai et al., 2003). As we will discuss later, it has been shown that the induction of LTP leads to an increase in spine head size, while long-term depression (LTD) causes spine shrinkage and retraction.

Other functions beyond that of compartmentalization of Ca^{2+} signals have been proposed for dendritic spines (Shepherd, 1996). These include (1) providing additional neuronal surface area to increase synapse density, (2) isolation of cytoplasmic biochemical/molecular signals to activated synaptic inputs, (3) neuroprotection by isolating large Ca^{2+} elevations from parent dendrites, (4) amplification of synaptic potentials, and (5) enhancing the spatial spread of back-propagating action potentials. Because some of these functional properties have been observed in nonspiny neurons, it is still currently unclear what, if any, is the functional advantage of producing and maintaining dendritic spines. Besides the function/structure relationship of dendritic spines, a number of physiological, pathophysiological, and experimental conditions modify spine density and morphology, including behavioral learning, hormonal state, levels of excitatory synaptic activity, growth factors (including neurotrophins), aging, malnutrition, poisoning, and several neurological disorders, such as schizophrenia and mental retardation (Fiala et al., 2002). Before we consider each of the physiological factors that modify dendritic spine number and form, we begin discussing the development of dendritic spines.

4.34.4 The Development of Dendritic Spines

The dendrites of pyramidal neurons in neonatal mammals are initially smooth, devoid of dendritic spines (Cajal, 1909; Marin-Padilla, 1972a; Purpura, 1975b). Spine density in pyramidal neurons seems to follow a developmental pattern similar to synapse formation, where an initial overproduction is followed by an activity-dependent pruning of excess synapses. In addition, spine morphology also changes during brain development. The majority of spines in the developing hippocampus are stubby, although the most frequent dendritic processes are filopodia. Because dendritic spines represent the postsynaptic

compartment of the vast majority of excitatory synapses in the CNS, the study of their formation and evolution during brain development has been recently reviewed (Sorra and Harris, 2000; Yuste and Bonhoeffer, 2004).

Spine synapses were initially thought to originate from established synapses formed first on the dendritic shaft by the extension and narrowing of a neck and the appearance of a spine head (Miller and Peters, 1981). Alternatively, and due to their predominance and motility in the developing brain, dendritic filopodia have been considered as precursors of mature dendritic spines. Considering that approximately 90% of spines have a presynaptic partner (Gray, 1959a; Harris and Kater, 1994), it has been speculated that dendritic filopodia actively search for presynaptic axon terminals to form synapses, giving rise to spines with more mature morphologies after the initial contact (Jontes and Smith, 2000; Yuste and Bonhoeffer, 2004). This model was initially supported by studies showing that dendritic filopodia lacking presynaptic partners populate the dendrites of 1-week-old neurons, which later acquire shorter spines always associated with a presynaptic terminal (Papa et al., 1995). Time-lapse observations in organotypic cultures of hippocampal slices later demonstrated that filopodia are extremely dynamic, extending and retracting in short periods of time (Dailey and Smith, 1996). Furthermore, some filopodia of cultured neurons were shown to make contact with axons at their tips, and the number of filopodia decreased in parallel with an increase in the number of spines (Ziv and Smith, 1996). These observations led to the proposal that the initial contact with axons triggers the formation of spines from filopodia. Serial EM reconstructions in the developing hippocampus *in situ* also support the role of filopodia as the site of initial axon contact, but only after their complete retraction and morphing into a shaft synapse, which will then serve as the emerging site of a mature spine with a synapse on its head (Fiala et al., 1998). Lastly, longitudinal *in vivo* multiphoton imaging of dendritic spines in the neocortex of developing and even adult animals supports the view that spines constantly form by seeking out presynaptic partners in the surrounding neuropil and stabilizing into functional spines of 3-varied morphology, a process driven by sensory experience (Lendvai et al., 2000; Trachtenberg et al., 2002; Holtmaat et al., 2005; Knott et al., 2006).

Regardless of the specific model of spine synapse formation, the highly dynamic behavior of filopodia during synatogenesis and the continuous rapid motility of dendritic spines require a careful orchestration and modulation of the dendritic cytoskeleton (Matus, 2000). The mechanism responsible for filopodia and spine motility is based on actin polymerization (Fischer et al., 1998; Dunaevsky et al., 1999; Korkotian and Segal, 2001; Zito et al., 2004) and seems to involve the Rho family of small GTPases (GTP: guanosine triphosphate) (Nakayama et al., 2000; Tashiro et al., 2000; Hering and Sheng, 2001). Moreover, spine motility is not only modulated by intracellular Ca^{2+} levels (Oertner and Matus, 2005), but may also affect Ca^{2+} compartmentalization within individual spines (Majewska et al., 2000b). Actin-based rapid spine motility, whereby the spine head and neck are in a seemingly constant process of movement, is not only critical for spine formation and synaptogenesis during brain development, but has also been proposed to be fundamental throughout lifetime (Matus, 2005; Harms and Dunaevsky, 2006). As we shall discuss in the following sections, actin-based motility allows dendritic spines to be malleable structures sensitive to ongoing levels of neuronal activity, underlying activity-dependent structural plasticity during experience-driven behavioral learning.

4.34.5 Structural Plasticity of Dendritic Spines Induced by Synaptic Activity: Homeostatic Plasticity, LTP, and LTD

Despite speculation by Cajal and Tanzi more than 100 years ago that the improvement of learned skills by 'mental exercise' arises from the growth of existing synapses, whereas acquiring new ones requires the formation of new neuronal connections, it was not until the 1960s that such structural plasticity was observed in response to the amount of afferent synaptic activity. In these early studies, dendritic spines of pyramidal neurons in the visual cortex were lost when afferent input was deprived by either surgical lesions (Globus and Scheibel, 1966) or by rearing mice in total darkness (Valverde, 1967). Moreover, deafferentation-induced spine loss was reversible and not pathological, as spine density could recover after only a few days of exposure to normal lighting conditions (Valverde, 1971). On the other hand, rearing rats in continuous illumination led to an increase in spine density in visual cortical neurons (Parnavelas et al., 1973), suggesting that morphological changes induced by deafferentation were bidirectional. It was later shown that these effects were not exclusive of

the visual cortex. Surgical lesions of the perforant path – the afferent fibers from the entorhinal cortex into the dentate gyrus – caused an initial loss of dendritic spines of granule cells, which was followed by a recovery to prelesion levels due to subsequent axonal sprouting (Parnavelas et al., 1974). These early observations consolidated the concept that changes in the levels of afferent input evoked an adaptive response in terms of dendritic spine density in several brain regions.

4.34.5.1 Ongoing Synaptic Activity

Because dendritic spines represent the postsynaptic compartment of excitatory synapses, the aforementioned *in vivo* studies were followed by varied manipulations of glutamatergic synaptic transmission in brain slices and cultured neurons. Long-term exposure to gamma-aminobutyric acid$_A$ ($GABA_A$) receptor antagonists, which increases neuronal excitability and thus excitatory synaptic drive, caused a significant reduction in spine density in pyramidal neurons within organotypic cultures of hippocampal slices (Muller et al., 1993; Thompson et al., 1996). Due to the excessive recurrent innervation in long-term hippocampal slice cultures (Gutierrez and Heinemann, 1999), this manipulation may have evoked pathological levels of excitability, leading to spine loss as described in epilepsy patients (Scheibel et al., 1974). On the other hand, exposing cultured hippocampal neurons to $GABA_A$ receptor antagonists for shorter periods led to an increase in dendritic spine density (Papa and Segal, 1996). Additional studies in cultured neurons support the hypothesis that moderate levels of excitatory synaptic transmission promotes spine growth and formation while excessive levels cause spine shrinkage and loss, representing an adaptive response mediated by intracellular Ca^{2+} levels (Korkotian and Segal, 1999a,b; Segal et al., 2000). In fact, dendritic spines are rapidly formed and orient themselves toward a local source of glutamate (Richards et al., 2005). On the other side of the coin, weeklong pharmacological blockade of NMDARs or Na^{+}-dependent action potentials reduced spine density in CA1 pyramidal neurons in slice cultures (Collin et al., 1997). Furthermore, spontaneous quantal excitatory synaptic transmission, i.e., miniature synaptic currents or 'minis,' seemed to be sufficient for the maintenance of spines, as long-term blockade of AMPARs or inhibition of vesicular neurotransmitter release with *Botulinum* neurotoxins led to a decrease in spine density in hippocampal pyramidal neurons maintained in organotypic slice cultures (McKinney et al., 1999). The spine loss in these chronic blockade experiments may result from the prolonged absence of excitatory synaptic input (7 days *in vitro*), because shorter periods of inactivity (48 h) did not cause spine loss but rather a change in the proportion of morphological spine types (Tyler and Pozzo-Miller, 2003). In fact, even shorter inactivity (~8 h) induced filopodia and immature synapse formation in CA1 pyramidal neurons of acute hippocampal slices (Kirov and Harris, 1999; Petrak et al., 2005). Such brief periods of inactivity actually promote the active searching of presynaptic partners by filopodia and immature dendritic spines (Richards et al., 2005) that, if unsuccessful, would lead to the collapse and pruning of spines. It has been proposed that highly motile immature dendritic spines containing only NMDARs (i.e., silent synapses) (Isaac et al., 1995; Liao et al., 1995) may later stabilize by the acquisition of AMPARs (Shi et al., 1999). Taken together, these observations provide a physiological mechanism for deafferentation-induced spine loss and support the early notion that the levels of afferent synaptic input directly influence neuronal morphology, e.g., spine synapse density.

4.34.5.2 Homeostatic Plasticity

Due to the duration (days to weeks) and generalized extent of the experimental manipulations of synaptic activity, the aforementioned experiments may reflect cell-wide global adaptations as thought to occur during homeostatic synaptic plasticity. Homeostatic mechanisms maintain an 'optimal' level of input to a neuron by regulating the strength of afferent excitatory and inhibitory synapses, by modulating intrinsic neuronal excitability, and by altering synapse number (Turrigiano and Nelson, 2004; Davis, 2006). Despite the lack of changes in spine density in cultured developing neurons after activity blockade (Davis and Bezprozvanny, 2001; Burrone and Murthy, 2003), CA1 pyramidal neurons in acute slices exhibit more – and longer – spines after blockade of synaptic transmission (Kirov and Harris, 1999). In addition to the duration of the activity blockade (hours in acute slices vs. days in cultures), the developmental age of the neurons in these studies was different. The absence of synaptic activity during the critical period of synaptogenesis and pruning in the hippocampus (postnatal day 6 through postnatal day 16) did not evoke an homeostatic modulation in spine number (Kirov et al., 2004a), despite the

well-characterized changes in quantal scaling of glutamate receptors and neuronal excitability in developing cultured neurons (Turrigiano, 1999). Instead, homeostatic plasticity of spine density seems to appear once the adult-like dendritic complement of spines is achieved (Kirov et al., 2004a). Complementary studies focused on the morphological consequences of activity-dependent manipulations of glutamatergic synaptic transmission with 'Hebbian' features resembling associative learning (Hebb, 1949), such as LTP and LTD of excitatory synaptic transmission (Malenka and Bear, 2004). Since this topic has been reviewed extensively over the years (e.g., Wallace et al., 1991; Geinisman, 2000; Yuste and Bonhoeffer, 2001; Segal, 2005), we will briefly discuss the main classical findings in addition to the most recent observations employing state-of-the-art imaging technologies.

4.34.5.3 Long-Term Potentiation

Instead of reflecting an adaptive response to prolonged and widespread changes in synaptic input, LTP is induced and expressed by a small number of activated synapses. Due to several of its properties (e.g., input specificity, associativity, cooperativity), LTP has become the most recognized synaptic model of associative learning and memory (Bliss and Collingridge, 1993). As pointed out earlier, the need for a structural change to underlie the longevity of memories has been repeatedly speculated (Cajal, 1893; Tanzi, 1893; Hebb, 1949). In fact, this possibility was explicitly proposed as one of the potential mechanisms for the enhancement of excitatory transmission in the very first description of LTP (Bliss and Lomo, 1973). In this groundbreaking paper, Bliss and Lomo quoted Rall, stating that

> . . . a reduction in the resistance of the narrow stem by which spines are attached to the parent dendrite . . .

may change the relative weight of synapses and thus contribute to the observed potentiation of excitatory transmission (Rall, 1970, as cited in Bliss and Lomo, 1973; Rall and Segev, 1987; Diamond et al., 1970, as cited in Rall and Segev, 1987). Soon thereafter, Van Harreveld and Fifkova tested this hypothesis by using pioneering rapid freezing methods of tissue preparation for EM following *in vivo* stimulation protocols described in the first LTP papers. In these studies, dendritic spines within the outer two thirds of the dentate molecular layer – which received the stimulated perforant path fibers – had larger heads as well as wider and shorter necks, features observed as early as 2 min and lasting as long as 23 h from the stimulation (Van Harreveld and Fifkova, 1975; Fifkova and Van Harreveld, 1977; Fifkova and Anderson, 1981). The layer specificity and time course of these changes strongly suggest that they are directly correlated with the potentiation of synaptic responses, despite the lack of electrophysiological confirmation of LTP induction and the identification of activated synapses.

The pioneering ultrastructural studies by Van Harreveld and Fifkova were followed by a series of EM observations after LTP-inducing afferent stimulation both *in vivo* and in the more amenable *in vitro* brain slice preparation, which sometimes included contradicting observations:

1. Increase in the proportion of shaft synapses, without changes in spine number, size, or form in area CA1 *in vivo* and *in vitro*, although much of these observations might have come from relatively aspiny inhibitory interneurons (Lee et al., 1979, 1980).
2. Layer-specific increases in the proportion of spines with concave heads and larger PSDs, without net changes in the total number density of spine or shaft synapses on dentate gyrus granule cells (Desmond and Levy, 1983, 1986a,b, 1990).
3. Increase in the number of 'sessile' (likely stubby spines) and shaft synapses in area CA1 of *in vitro* hippocampal slices, without detectable changes in the number of spines or in the dimensions of their heads and necks, although typical cup-shaped spines seemed to have been replaced by more flat spine profiles (Chang and Greenough, 1984).
4. Decrease in the number of spine synapses and a parallel increase in shaft synapses on dentate granule cells after high-frequency stimulation of the perforant path in acute hippocampal slices (Gomez et al., 1990). Intriguingly, these changes were correlated with the magnitude and success rate of LTP induction observed in rats with different inborn learning capacity.
5. Increase in the ratio of perforated to nonperforated spines synapses in the dentate molecular layer in chronically implanted rats 1 h after perforant path stimulation, without changes in the total number of spine synapses (Geinisman et al., 1991). These studies were later followed by the observation of an increase in the number of spine synapses with multiple, completely partitioned transmission zones (Geinisman et al., 1993).
6. Increase in spine number in dentate gyrus after *in vivo* stimulation of perforant path fibers, as well as the proportion of 'bifurcated' spines (Trommald

et al., 1996), thought to originate from the splitting of spine synapses bearing perforated PSDs (Peters and Kaiserman-Abramof, 1969).

7. Increase in multiple-bouton spine synapses with perforated PSDs in area CA1 of slice cultures, which were known to have been activated during LTP-inducing afferent stimulation (Buchs and Muller, 1996; Toni et al., 1999, 2001). In these studies, synapses activated during the high-frequency conditioning stimulus – and thus presumably potentiated – were identified at the EM level using a Ca^{2+} precipitate protocol (Buchs et al., 1994).

8. No changes in the absolute number of spine synapses in area CA1 of acute slices (Sorra and Harris, 1998). However, these and other observations in acute hippocampal slices may be confounded by the fact that spine density is increased by temperature changes during slice preparation (Kirov et al., 1999, 2004b; Bourne et al., 2006).

9. Increase in spine synapses with perforated PSDs in dentate granule cells after successfully maintained LTP in aged rats, while spine branching was only observed after high-frequency stimulation without sustained potentiation (Dhanrajan et al., 2004).

10. Increase in the number of spines with perforated PSDs and of multiple-synapse boutons in area CA1 after chemically induced LTP in acute slices (Stewart et al., 2005).

Despite the increasing sophistication of the morphological studies at the EM level listed above, especially with regard to the confirmation of LTP induction and the identification of potentiatied synapses, it was clear from the beginning that time-lapse imaging of live neurons before, during, and after LTP induction would yield more consistent results. To this aim, repeated laser-scanning confocal microscopy was performed in live acute hippocampal slices to follow spine morphology during chemically induced LTP, a procedure that would ensure that most synapses in the slice are potentiatied (Hosokawa et al., 1995). The appearance of new dendritic spines in these DiI-labeled (DiI is a long-chain dialkylcarbocyanines dye) CA1 pyramidal neurons was a rare – and statistically insignificant – event. However, small spines were seen to extend, and others appeared to change their orientation with respect to the parent dendrite. Circumventing the optical limitations of confocal microscopy applied to acute slices, imaging live neurons within organotypic slice cultures (Gahwiler, 1981; Stoppini et al., 1991; Pozzo Miller et al., 1993) by multiphoton excitation microscopy – a.k.a. two-photon microscopy (Denk and Svoboda, 1997) – yielded a much clearer picture. High-frequency afferent stimulation was shown to induce the formation of protrusions that resembled dendritic filopodia in CA1 pyramidal neurons expressing enhanced GFP (eGFP) (Maletic-Savatic et al., 1999). Some of these synaptically induced filopodia persisted for the duration of the imaging session (up to 1 h) and later acquired a bulbous head – suggesting that they developed into spines – and their appearance required NMDAR activity, as LTP induction does. Similar spine formation associated with successful LTP induction was observed in CA1 pyramidal neurons injected with the fluorescent dye calcein (Engert and Bonhoeffer, 1999). These studies further showed that spine growth occurred selectively within a synaptically active region, which was restricted to a ~30-μm area, while some spines were seen to randomly disappear in distant regions where synaptic transmission was blocked, suggesting an homeostatic balance in spine number. Similar filopodia and spine formation was also shown to occur in CA1 pyramidal neurons after the intracellular application of autophosphorylated Ca^{2+}/calmodulin-dependent protein kinase II (CaMKII), an enzyme implicated in LTP induction (Jourdain et al., 2003).

In addition to spine number, time-lapse imaging by multiphoton excitation microscopy during and after LTP induction in hippocampal slices also revealed rapid changes in spine morphology, which resembled the spine head swelling initially described by Van Harreveld and Fifkova at the EM level (Van Harreveld and Fifkova, 1975). Induction of LTP by high-frequency stimulation in acute slices from eGFP-expressing mice caused a transient expansion of activated CA1 neuron spines, which were identified by synaptically mediated Ca^{2+} elevations (Lang et al., 2004). The predicted increase in spine volume observed in these studies was detected as early as 30 s after stimulation and lasted for 10–20 min. On the other hand, repetitive (1 min at 1 Hz) miniature excitatory postsynaptic current (mEPSC)-like two-photon uncaging of MNI-glutamate on individual CA1 neuron spines led to equally rapid (1–5 min) and selective enlargement of stimulated spines that was transient in large mushroom spines but persistent in small spines, lasting up to 100 min (Matsuzaki et al., 2004). Furthermore, the enduring spine enlargement was associated with an increase in AMPAR-mediated currents and required NMDAR activity, CaMKII, and actin polymerization. A similar photorelease approach – but using ultraviolet (UV) uncaging and wide-field fluorescence imaging in thinner slice

cultures – failed to reveal any morphological changes in spines during the potentiation of AMPAR-mediated currents (Bagal et al., 2005). However, the potentiation of uncaging currents in the latter studies was induced by a single UV flash paired with postsynaptic depolarization, suggesting that structural remodeling of dendritic spines may require repeated activation of NMDARs, as during LTP of synaptic responses. Further support to this idea comes from spine size changes after a global induction of LTP in slice cultures. CA1 pyramidal neurons were transfected with the fluorescent protein tDimer-dsRed and glutamate receptor subunits tagged with pH-sensitive GFP to monitor morphological changes and receptor trafficking simultaneously (Kopec et al., 2006). Generalized induction of 'chemical' LTP (Otmakhov et al., 2004) caused a significant increase in spine volume that was maximum by 2 min after the application of the chemical LTP-inducing cocktail. Furthermore, the observed spine enlargement preceded the membrane insertion of AMPA receptor subunits, suggesting that different mechanisms mediate structural and functional changes at individual spines after synaptic potentiation.

Regarding the molecular machinery responsible for the structural plasticity of dendritic spines, it was originally proposed that the actin cytoskeleton plays a critical role (Crick, 1982; Fifkova and Delay, 1982). Only recently it was shown that LTP-inducing stimulus enlarged spine heads in parallel with an increase in their content of filamentous actin relative to globular actin as imaged by fluorescence resonance energy transfer (FRET) (Okamoto et al., 2004). Furthermore, the latter studies in CA1 neurons in slice cultures showed that LTD-inducing stimulus led to the opposite results, namely a reduction in F-actin relative to G-actin and a reduction in spine size, suggesting that spine structural plasticity is bidirectional. In addition to multiple signaling pathways, in particular the one mediated by Rho and Rac small GTPases (Nakayama et al., 2000; Tashiro et al., 2000), several scaffold proteins and actin binding proteins converge on the actin cytoskeleton to regulate spine morphology and dynamics, an issue that has been recently reviewed (Tada and Sheng, 2006).

4.34.5.4 Long-Term Depression

Further evidence of the bidirectional nature of spine structural plasticity is beginning to emerge. GFP-expressing CA1 pyramidal neurons in slice cultures of Thy-1-GFP mice rapidly and persistently lost their dendritic spines after their afferent inputs were stimulated at low frequency (900 pulses at 1 Hz) (Nagerl et al., 2004), a protocol well known to induce NMDAR-dependent LTD (Dudek and Bear, 1992; Mulkey and Malenka, 1992). In addition, CA1 neurons individually filled with the fluorescent dye calcein showed spine shrinkage leading to spine retraction in response to low-frequency stimulation (Zhou et al., 2004). Similar to the requirements for LTD induction, spine retraction and pruning were also sensitive to NMDAR antagonists and calcineurin, an inhibitor of protein phosphatases (Nagerl et al., 2004; Zhou et al., 2004), strengthening the view that dendritic spines are formed and eliminated in an activity-dependent manner that correlates with bidirectional changes in synaptic efficacy.

4.34.6 Structural Plasticity of Dendritic Spines Induced by Experience and Behavioral Learning

The seminal ideas proposed by Hebb regarding associative learning sparked experimental research not only at the cellular level, but also on the structural consequences of behavioral training, spatial learning, or differential experience (Bailey and Kandel, 1993). Apparently inspired by Hebb's anecdotal report of the enhanced learning ability of laboratory rats when housed as home pets during their development (Hebb, 1949), Rosenzweig and colleagues showed that raising rodents in 'enriched' environments led to neurochemical and anatomical changes in the cerebral cortex (reviewed in Rosenzweig and Bennett, 1996). Among the anatomical consequences of rearing rodents in enriched environments was an increase in dendritic spine number in the visual cortex (Globus et al., 1973; Greenough and Volkmar, 1973), an observation in keeping with the concept that continuous use is critical for the maintenance and even the formation of new synaptic contacts on dendritic spines (e.g., Globus and Scheibel, 1966; Valverde, 1967).

Because these enriched complex environments were initially considered as visual and motor challenges, the majority of the studies focused in those brain regions thought to be engaged in such behaviors (e.g., somatosensory, motor, and visual cortices). It was later realized that a complex environment also represents a challenge for spatial navigation abilities, likely engaging the hippocampus, a region well known for the functional and structural plasticity of its excitatory spine synapses. Indeed, rats that were repeatedly trained in a complex environment (up to 5

floors connected by ladders, 4 h per day for 14–30 days) exhibit higher spine density in basal dendrites of CA1 pyramidal neurons, an observation that correlated with an improved performance in the Morris water maze compared to untrained littermates (Moser et al., 1994, 1997). Similar increases in spine density were also observed in the CA1 region of mice (Rampon et al., 2000) and marmosets, New World monkeys (Kozorovitskiy et al., 2005). However, an 'enriched' environment consists of a combination of enhanced social interactions, cognitive stimulation, and physical exercise in addition to requiring spatial navigation abilities. Not surprisingly then, environmental enrichment enhances several aspects of hippocampal function, including neurogenesis, LTP, and spatial learning (van Praag et al., 2000). It remains to be established which features of the enriched environment are more important for those lasting functional outcomes.

Learning-specific hippocampal-dependent associative tasks, such as trace eye blink conditioning or odor discrimination, were also correlated with increases in spine density on CA1 neurons, although on their apical dendrites (Leuner et al., 2003; Knafo et al., 2004). In contrast, unbiased stereological analyses failed to detect differences in spine synapse number on CA1 pyramidal neurons after Morris water maze training (Rusakov et al., 1997; Miranda et al., 2006) or trace eye blink conditioning (Geinisman et al., 2000). However, the latter study did reveal an increase in the area of the PSD of nonperforated spine synapses (Geinisman et al., 2000). These discrepancies may originate from 'snapshot' observations of likely transient changes made at different time points through a continuous distribution of morphological states. Indeed, learning-induced increases in spine density of dentate gyrus granule cells were transient, with maximum levels observed 6–9 h after passive avoidance (O'Malley et al., 1998) or spatial water maze training (O'Malley et al., 2000; Eyre et al., 2003), and returning to control levels after 24–72 h. Despite the attractiveness of the possibility that dendritic spines and memory formation mutually interact – i.e., "learning makes more spines" and "more spines promotes learning" – their causal relationship, if any, has eluded more than 100 years of intensive research and continues to raise alternative interpretations (Moser, 1999; Segal, 2005).

Although we have focused on the structural plasticity of dendritic spines in the hippocampus, it should be noted that recent advances in multiphoton excitation microscopy have allowed the study of dendritic spines in cortical regions of the intact brain *in vivo*. Due to the obvious limitation of accessibility, most of the work so far has focused in the cerebral cortex, where long-term imaging has demonstrated that subpopulations of spines appear and disappear while others remain for months (Grutzendler et al., 2002; Trachtenberg et al., 2002; Holtmaat et al., 2005). Furthermore, spines in the somatosensory barrel cortex are modulated by sensory experience (Trachtenberg et al., 2002; Zuo et al., 2005; Holtmaat et al., 2006), and such experience-driven spine formation precedes synapse formation (Knott et al., 2006). However, the only report of dendritic spine imaging in the hippocampus *in vivo* showed a remarkable stability, at least for up to 4 h, even after induction of epileptic seizures with the muscarinic agonist pilocarpine or the $GABA_A$ antagonist bicuculline (Mizrahi et al., 2004). However, the conditions used in this study for imaging spines in the intact hippocampus *in vivo* (e.g., halothane anesthesia and blunt resection of the overlaying cerebral cortex) may have affected their physiological behavior. Thus, it remains to be determined whether dendritic spines in the intact hippocampus *in vivo* display the types of rapid structural rearrangements they are capable of *in vitro* in response to varying levels of afferent synaptic activity. A further challenge will be to relate synaptic activity-dependent rapid spine plasticity with the morphological remodeling described after experience-driven hippocampal-dependent learning.

4.34.7 Structural Plasticity of Dendritic Spines Induced by Neuromodulators: Ovarian Hormones and Neurotrophins

In addition to the ongoing levels of excitatory afferent input, exposure to enriched environments and learning spatial navigation tasks, dendritic spines of hippocampal neurons are also sensitive to slow-acting neuromodulators. Due to their established role during brain development and throughout aging, some of the most attractive molecular candidates for modulating spine structural plasticity include the ovarian steroid hormones (i.e., estradiol and progesterone) and members of the family of neurotrophins, such as brain-derived neurotrophic factor (BDNF). As we shall discuss below, the interplay between estradiol and BDNF actions on hippocampal neurons is

reflected not only in the modulation of dendritic spine density, but also in the modulation of hippocampal synaptic plasticity thought to underlie associative learning and memory.

4.34.7.1 Estradiol

Traditionally, the effects of steroid ovarian hormones have been defined as 'organizational' and 'activational.' Organizational effects are permanent structural changes induced by hormones during a critical period of brain development, while activational effects represent the initiation or termination of previously established neuronal circuits (Arnold and Gorski, 1984). Initial studies of the effects of steroid hormones on neuronal and synaptic structure focused in sexually dimorphic brain regions that control reproductive behaviors, such as the arcuate and ventromedial nuclei of the hypothalamus (Matsumoto and Arai, 1979, 1986; Carrer and Aoki, 1982; Frankfurt et al., 1990; Pozzo Miller and Aoki, 1991, 1992). Relevant to learning and memory, several studies had shown that elevations in the circulating levels of estradiol induced experimentally, or those achieved during the proestrus in rodents, resulted in enhanced neuronal excitability in the hippocampus (Woolley and Timiras, 1962; Terasawa and Timiras, 1968; Kawakami et al., 1970). Inspired by the growing literature reporting that enhanced afferent synaptic activity promoted dendritic spine growth in several brain regions, McEwen and colleagues first demonstrated that ovariectomized female rats had reduced spine density in CA1 pyramidal neurons compared to sham-operated controls (Gould et al., 1990). Later they showed that this effect was reversible upon treatment with estradiol and progesterone (Gould et al., 1990; Woolley and McEwen, 1993), and that in fact spine density fluctuated during the estrous cycle in a manner that is consistent with the effects of estrogen and progesterone (Woolley et al., 1990). Similar effects of estradiol were observed in cultured hippocampal neurons (Murphy and Segal, 1996) and CA1 pyramidal neurons maintained in organotypic slice culture (Pozzo-Miller et al., 1999b). Furthermore, CA1 pyramidal neurons in the hippocampus of young and aged nonhuman primates (African green monkeys and Rhesus monkeys) also lose their spines after ovariectomy, an effect that is reversed by estrogen-replacement therapy (Leranth et al., 2002; Hao et al., 2003). Curiously, hippocampal CA1 neurons in ovariectomized mice of the C57BL/6J strain commonly used for genetic knockout and transgenic approaches did not exhibit changes in spine density after estrogen exposure, although the proportion of their mushroom Type-II spines was increased (Li et al., 2004). Since mushroom spines are thought to represent 'potentiated' spines (Kasai et al., 2003), these observations suggest a role of estrogen in dendritic spine structural plasticity during activity-dependent synaptic potentiation.

The changes in spine density induced experimentally by estradiol or during the estrous cycle were accompanied by increases in the binding sites for NMDARs (Weiland, 1992), enhanced immunofluorescence of the NR1 receptor subunit (Gazzaley et al., 1996), upregulation of NMDAR-mediated synaptic potentials (Woolley et al., 1997; Foy et al., 1999), and enhanced synaptic NMDAR-mediated Ca^{2+} elevations in spines of CA1 pyramidal neurons (Pozzo-Miller et al., 1999b). This enhancement of NMDAR-mediated synaptic transmission has consequences for long-term synaptic plasticity. Indeed, the likelihood for the induction of LTP is elevated at proestrous (when estradiol is highest) (Warren et al., 1995) and by estradiol treatment *in vivo* (Cordoba Montoya and Carrer, 1997), as well as in acute brain slices (Foy et al., 1999). The functional consequences of estradiol-replacement therapy in ovariectomized rats may also involve changes in the ratio of NMDAR-to AMPAR-mediated synaptic responses (Smith and McMahon, 2005). In these experiments, the magnitude of LTP after estradiol treatment was larger only when spine density increased simultaneously with an elevation in NMDAR transmission relative to AMPAR transmission, suggesting that the increase in functional synapse density alone is not sufficient to support heightened plasticity. Rather, estradiol may increase LTP via enhancing NMDAR transmission, likely through receptor insertion into newly formed or preexisting synapses. The mechanisms of estradiol action leading to spine formation resemble other forms of activity-dependent plasticity due to the requirement of NMDARs (Woolley and McEwen, 1994; Murphy and Segal, 1996), as well as activation of cyclic adenosine monophosphate (cAMP) response element binding protein (CREB) and CREB binding protein (CBP) (Murphy and Segal, 1997).

Parallel to these cellular and molecular studies, clinical studies in postmenopausal women receiving estrogen-replacement therapy initially reported improvements in mood and cognitive functions (Sherwin, 1996), although some lingering issues remain unanswered (Sherwin, 2005). The similarities in downstream signaling and cellular effects between ovarian hormones and neurotrophins such as BDNF

(Toran-Allerand, 1996) have suggested a potential neuroprotective role for estrogens in age-related memory loss and Alzheimer's disease dementia (Gibbs and Aggarwal, 1998; Lee and McEwen, 2001). BDNF is a polypeptide related to nerve growth factor (NGF) that has been shown to provide neurotrophic support for cholinergic (Knusel et al., 1992; Widmer et al., 1993), serotonergic (Mamounas et al., 1995), and dopaminergic (Hyman et al., 1994; Yurek et al., 1996) neurons. Thus, BDNF may influence hippocampal function by maintaining the innervation of one or more of these neurotransmitter systems. In addition, the expression of BDNF mRNA in the hippocampus fluctuates during the estrous cycle and is increased after estrogen replacement in ovariectomized rats (Gibbs, 1998). Hence, increases in BDNF expression following estrogen treatment may help to enhance and maintain proper hippocampal function. However, estradiol was shown to downregulate BDNF levels in cultured hippocampal neurons, leading to spine formation by reduced GABAergic inhibition and increased excitation (Murphy et al., 1998). BDNF itself blocked the effects of estradiol on spine formation, and BDNF depletion with selective antisense oligonucleotides or with anti-BDNF antibodies mimicked the effects of estradiol, increasing spine density (Murphy et al., 1998). As we shall see in the next section, the interplay between estrogen and BDNF may be more complex than initially proposed. We next discuss the actions of BDNF on the structure and function of hippocampal neurons, with particular attention to dendritic spines and their role in synaptic plasticity.

4.34.7.2 Brain-Derived Neurotrophic Factor

Neurotrophins are secretory proteins involved in neuronal survival and differentiation (Barde, 1989). Four neurotrophins have been identified in mammals, and all are widely expressed in the CNS: NGF, BDNF, neurotrophin-3 (NT3), and NT4/5 (Lewin and Barde, 1996). They exert their effects by binding to pan-neurotrophin $p75^{NTR}$ receptors and to specific tyrosine kinase receptors, members of the *trk* family of proto-oncogenes related to insulin and epidermal growth factor receptors (Barbacid, 1993). NGF binds selectively to tyrosine kinase A (TrkA), BDNF and NT4/5 to TrkB, and NT3 to TrkC and, with a lower affinity, to TrkB as well (Chao, 1992). Binding of a neurotrophin to a specific Trk receptor stimulates its kinase activity, leading to the activation of phosphatidylinositol 3-kinase (PI3K), mitogen-activated protein kinase (MAPK, also known as ERK), and phospholipase C-γ (PLC-γ) (Segal and Greenberg, 1996; Huang and Reichardt, 2003). Interaction of neurotrophins with $p75^{NTR}$ results in the activation of different signaling cascades mostly involved in the regulation of cell fate, such as nuclear factor kappa B (NF-κB) or c-*jun* N-terminal kinase (Kaplan and Miller, 2000). In addition to their well-established role in neuronal survival and differentiation during development, neurotrophins are strong candidates for providing the molecular signaling pathways that mediate the complex interactions between internal developmental programs and external environmental factors, ultimately leading to the appropriate development of dendrites and synapses (McAllister et al., 1999). Furthermore, BDNF has recently emerged as a fundamental modulator of hippocampal function, where its levels are amongst the highest in the brain (Murer et al., 2001). Together with the dendritic localization of GFP-tagged BDNF to dendritic process in cultured hippocampal neurons (Hartmann et al., 2001; Kojima et al., 2001; Brigadski et al., 2005), the significant release of native (i.e., endogenous) BDNF in response to stimulus patterns most amenable for LTP induction (Balkowiec and Katz, 2002; Gartner and Staiger, 2002; Aicardi et al., 2004) strongly supports the role of this prominent neurotrophin in activity-dependent functional and structural plasticity of hippocampal synapses. In the remaining paragraphs of this chapter we will focus on the morphological actions of BDNF, as its role in hippocampal synaptic transmission and plasticity in the context of learning and memory has been extensively reviewed (Lo, 1995; Thoenen, 1995, 2000; Black, 1999; Schinder and Poo, 2000; Poo, 2001; Tyler et al., 2002a,b; Vicario-Abejon et al., 2002; Lu, 2003; Bramham and Messaoudi, 2005).

Neurotrophins were shown to increase the length and complexity of dendrites of cortical pyramidal neurons when applied to slice cultures of ferret visual cortex (McAllister et al., 1995). Cortical pyramidal neurons in slice cultures treated with Trk 'receptorbodies' to scavenge endogenous neurotrophins showed limited dendritic growth and even dendritic retraction (McAllister et al., 1997), confirming that endogenous neurotrophins play a critical role in dendritic development. In addition, cortical pyramidal neurons that overexpress BDNF exhibit increased dendritic arborization, as well as enhanced rates of spine retraction and formation (Horch et al., 1999; Horch and Katz, 2002). More relevant to hippocampal function, BDNF increases spine density in CA1

pyramidal neurons, an effect blocked by the scavenger TrkB-immunoglobulin G, the receptor tyrosine kinase inhibitor k-252a, and inhibitors of the MAPK/ERK signaling cascade (Tyler and Pozzo-Miller, 2001; Alonso et al., 2004). Sustained Ca^{2+} elevations set off by BDNF-induced Ca^{2+} mobilization from inositol triphosphate (IP_3)-sensitive intracellular stores followed by transient receptor potential, canonical subfamily (TRPC)-mediated capacitative Ca^{2+} entry (Amaral and Pozzo-Miller, 2005) may play a critical role in BDNF-induced increase in spine density. Indeed, inhibition or siRNA-mediated knockdown of TRPC channels prevented not only the BDNF-induced sustained depolarization and dendritic Ca^{2+} elevation, but also the increase in spine density (Amaral and Pozzo-Miller, 2007). Moreover, the BDNF-induced structural remodeling of spiny dendrites of CA1 pyramidal neurons has consequences for Ca^{2+} signals evoked by coincident pre- and postsynaptic activity. Dendritic Ca^{2+} signals evoked by coincident excitatory postsynaptic potentials (EPSPs) and backpropagating action potentials (bAPs) were always larger than those triggered by bAPs in CA1 neurons exposed to BDNF (Pozzo-Miller, 2006), representing a potential consequence of neurotrophin-mediated dendritic remodeling of hippocampal neurons. BDNF promoted spine formation also in granule cells of the dentate gyrus (Danzer et al., 2002) and in cultured hippocampal neurons, where the effect was 'gated' by cAMP (Ji et al., 2005); but see earlier discussion of the opposite effect of BDNF in the context of estradiol-induced spine formation reported by Murphy et al. (1998).

In addition to increasing spine density *per se*, BDNF also increased the proportion of stubby spines (Type-I) under conditions of *both* action potential-dependent *and* independent synaptic transmission (Tyler and Pozzo-Miller, 2003). Even when SNARE (soluble N-ethylmaleimide-sensitive-factor attachment protein receptor)-dependent vesicular synaptic transmission was abolished with *Botulinum* neurotoxin C, BDNF was still capable of inducing spine formation. Under these conditions, however, BDNF selectively increased the proportion of thin spines (Type-III), while decreasing the proportion of stubby spines (Type-I). Consistent with an activity-dependent process of morphological differentiation of synapses, the effects of BDNF on hippocampal spines suggest that it cooperates with spontaneous miniature synaptic activity to sculpt the fine structure of CA1 pyramidal neurons in the postnatal hippocampus. Since BDNF also increases quantal transmitter release from presynaptic terminals (Tyler and Pozzo-Miller, 2001; Tyler et al., 2002b; Tyler et al., 2006), its role in spine growth and their morphological sculpting spans the synaptic cleft.

The activation of membrane-bound $p75^{NTR}$ and Trk receptors by neurotrophin binding – in addition to physical interactions between them – organizes precise signaling cascades that control their varied actions throughout development, such as cell survival, differentiation, neurite outgrowth, and synaptic function (Patapoutian and Reichardt, 2001; Hempstead, 2002; Roux and Barker, 2002; Chao, 2003; Huang and Reichardt, 2003; Nykjaer et al., 2005). Recent observations regarding the differential role of the two types of membrane receptors have uncovered an intriguing level of complexity in neurotrophin signaling, namely opposing functional actions of $p75^{NTR}$ and Trk receptors (Lu et al., 2005). For example, while Trk receptors are essential for neuronal survival, $p75^{NTR}$ has been implicated in neuronal death. Similarly, $p75^{NTR}$ signaling has been linked to the inhibition of axonal growth, an opposing effect to that of Trk receptors. Lastly, while TrkB receptors have been shown to be critical for the role of BDNF in hippocampal LTP (Minichiello et al., 1999, 2002; Xu et al., 2000), recent reports indicate that $p75^{NTR}$ is necessary for the induction and/or expression of hippocampal LTD (Rosch et al., 2005; Woo et al., 2005). With regard to dendritic organization, $p75^{NTR}$ and Trk receptors also seem to have functional antagonisms. TrkB activity has been shown to modulate dendritic growth (Yacoubian and Lo, 2000), while a recent report using $p75^{NTR}$ knockout and $p75^{NTR}$ overexpressing mice indicates that these receptors negatively modulate dendritic morphology in hippocampal pyramidal neurons (Zagrebelsky et al., 2005), further strengthening the 'yin and yang' model of functional antagonism between Trk and $p75^{NTR}$ signaling (Lu et al., 2005).

What is the contribution of these receptors to BDNF-induced changes in spine morphology and density? It is tempting to extend the 'yin and yang' model of functional antagonism also to the modulation of spine number and form. Indeed, dendritic spine density in hippocampal pyramidal neurons was higher in $p75^{NTR}$ knockout mice, which also showed a reduction in the proportion of stubby (Type-I) spines (Zagrebelsky et al., 2005). On the other hand, mice with a conditional deletion of the TrkB receptor have reduced spine density and a higher proportion of long spines (Luikart et al., 2005; von Bohlen und Halbach et al., 2006). In addition, the proportion of

stubby (Type-I) spines was reduced in CA1 pyramidal neurons of adult transgenic mice expressing a dominant-negative TrkB (Chakravarthy et al., 2006). Intriguingly, brief exposures to k-252a, an inhibitor of receptor tyrosine kinases such as TrkS, caused a significant increase in spine density in CA1 pyramidal neurons expressing enhanced yellow fluorescent protein (eYFP) (Chapleau and Pozzo-Miller, unpublished data). However, most of these spines are of the long and thin Type-III, known to be highly motile and unstable structures (Dailey and Smith, 1996; Dunaevsky et al., 1999) and characteristic of immature synapses (Sorra and Harris, 2000). The fact that longer exposures to k-252a by itself caused spine loss (Tyler and Pozzo-Miller, 2001; Alonso et al., 2004) suggests that an initial increase in long and thin spines may precede spine regression leading to spine pruning (Segal et al., 2000). Alternatively, these observations may reflect the functional antagonism between $p75^{NTR}$ and Trk receptor signaling for dendritic spine formation and maintenance (Lu et al., 2005). In this interpretation, preferential activation of $p75^{NTR}$ by spontaneously released BDNF under conditions of Trk receptor inhibition (i.e., in the presence of k252a) leads to an initial and transient increase in thin and unstable spines, followed by a persistent spine loss. Further complexity was uncovered by the outgrowth of dendritic filopodia through the interaction of the truncated TrkB receptor (TrkB.T1) with $p75^{NTR}$, intriguingly enough, in the absence of neurotrophin binding (Hartmann et al., 2004). The combination of all these structural and physiological effects in the hippocampus may underlie the role of BDNF in the consolidation of synaptic plasticity and hippocampal-dependent learning and memory (Tyler et al., 2002a; Bramham and Messaoudi, 2005).

4.34.8 BDNF, MeCP2, and Dendritic Spine Pathologies in Rett Syndrome

Neurological disorders associated with mental retardation (MR) are characterized by a prevalent deficit in cognitive function and adaptive behavior that range in phenotype severity and are often accompanied by specific symptoms. MR-associated disorders that have environmental, neurodegenerative, or genetic origins have long been associated with structural anomalies of dendritic spines that are a common feature of various neurological disorders (Fiala et al., 2002). The pioneering studies by Huttenlocher (1970, 1974), Marin-Padilla (1972b, 1976), and Purpura (1974, 1975a) described strikingly similar abnormalities in the dendritic organization of cortical neurons from human patients with unclassified MR-associated disorders. Pyramidal neurons of the cerebral cortex showed a reduction in the number and length of dendritic branches, a significant loss of dendritic spines, and a predominance of long 'tortuous' spines. Interestingly, no other neuropathologies were found in these patients. Since then, similar features of dendritic branching, as well as spine density and morphology, have been described in other MR-associated genetic developmental disorders, ranging from autosomal genetic forms of MR (e.g., Down syndrome, Angelman syndrome) to X chromosome–linked forms of MR (e.g., Rett syndrome, fragile X syndrome) (Kaufmann and Moser, 2000; Fiala et al., 2002; Newey et al., 2005). Thus, it seems that dendritic and spine anomalies are the major brain pathologies underlying the cognitive impairments observed in humans with MR, because such dendritic and spine changes will reduce postsynaptic surface area and the density of mature excitatory synapses.

One MR-associated disorder with an intriguing link to BDNF signaling is Rett syndrome (RTT) (*See also* Chapter 4.43). RTT is an X-linked neurodevelopmental disorder and the leading cause of severe mental retardation in females, affecting 1:10,000–20,000 births worldwide without predisposition to a particular racial or ethnic group (Percy, 2002; Neul and Zoghbi, 2004). Patients with RTT are born healthy and achieve standard developmental milestones until 6–18 months of age, when they begin a regression period associated with loss of acquired cognitive, social, and motor skills (Armstrong, 1997). Later symptoms and deficits include irregularities in motor activity, characterized by a stereotypic hand movement or useless hand movements, altered breathing patterns, gait and motor imbalance, and continued cognitive decline. As the period of regression concludes, the individuals are often left in a severely impaired condition, with a majority of children developing seizure activity, although seizure frequency diminishes with age. As the children get older, a period of stabilization occurs where they develop greater communication abilities with their eyes, yet motor function continues to regress gradually (Percy and Lane, 2005). Loss-of-function mutations in the gene encoding methyl-CpG-binding protein 2 (MeCP2) have been recently identified in RTT patients (Amir et al., 1999). MeCP2 binds specifically to CpG-methylated DNA and is thought to inhibit gene transcription by recruiting corepressor

and histone deacetylase complexes and altering the structure of genomic DNA (Nan et al., 1998). Because other neurodevelopmental disorders with autistic features have also been associated with MeCP2 dysfunction, the role of MeCP2 in neuronal function may extend beyond RTT (Percy, 2002). Intriguingly, one of the targets of MeCP2-mediated transcriptional repression is the gene encoding BDNF, whereby MeCP2 binds to and represses the transcription of the promoter region of the *BDNF* gene (Chen et al., 2003; Martinowich et al., 2003).

The neuropathology of RTT reveals several areas of abnormal brain development (Armstrong et al., 1995; Bauman et al., 1995a,b). Several studies reported abnormal dendritic structure in the frontal cortex (Jellinger et al., 1988), and pyramidal neuron dendritic area and growth are significantly decreased in the frontal and motor cortices, as well as in the *subiculum*, while dendritic length was not affected in the hippocampus (Armstrong et al., 1995). Another group reported a similar dendritic appearance; in addition, they observed a thickening of dendrites (Cornford et al., 1994). Finally, a reduction in the levels of microtubule-associated protein-2 (MAP-2), a dendritic protein involved in microtubule stabilization, was found throughout the neocortex of RTT brains (Kaufmann et al., 1995, 2000). Relevant to the role of neurotrophins in dendritic spine formation and maturation, RTT brains have reduced spine density in dendrites of the frontal cortex (Belichenko et al., 1994). In addition, the levels of cyclooxygenase-2, a protein enriched in dendritic spines, are reduced in the frontal cortex in RTT (Kaufmann et al., 1997). With regard to synapse density, reduced levels of the synaptic vesicle protein synaptophysin were detected in the motor, frontal, and temporal cortices by immunofluorescence (Belichenko et al., 1997), although another study reported unaltered synaptophysin levels in frontal cortex and cerebellum (Cornford et al., 1994).

To further the understanding of RTT, a number of different mouse models have been generated, either carrying a null deletion of *MECP2* (Chen et al., 2001; Guy et al., 2001) or expressing a truncated, nonfunctional form of the wild-type MeCP2 protein (*MECP2*^{308}) (Shahbazian et al., 2002). These mice have common phenotypes, including delayed onset of symptoms (at approximately 5 weeks of age) and motor impairment and abnormal gait, whereby the null *MECP2* mice have hindlimb irregularities, while the *MECP2*^{308} mice have forelimb impairment (Shahbazian and Zoghbi, 2002; Armstrong, 2005). In addition, null *MECP2* mice have altered synaptic transmission and plasticity, including impaired hippocampal LTP (Asaka et al., 2006), and reduced excitatory transmission in the hippocampus (Nelson et al., 2006) and neocortex (Dani et al., 2005). Deficits in hippocampal LTP and hippocampal-dependent learning and memory were also observed in the *MECP2*^{308} mice expressing the truncated protein (Moretti et al., 2006). On the other hand, transgenic mice that mildly overexpress wild-type MeCP2 (~2 times wild-type levels) display a higher rate of hippocampal-dependent learning and enhanced LTP, though they developed a neurological phenotype that included seizures after 20 weeks of age (Collins et al., 2004).

Intriguingly, observations regarding neuronal and synaptic morphology in these mouse models of RTT have produced varying results, sometimes inconsistent with the neuropathology found in RTT patients (Armstrong et al., 1995; Bauman et al., 1995a,b). Pyramidal neurons of layer II/III of the neocortex of null *MECP2* hemizygous mice (MECP2$^{-/y}$) are smaller and have reduced dendritic branching as observed in RTT brains, although no differences were found in dendritic spine density (Kishi and Macklis, 2004), a distinctive feature observed in one study of the cortex of RTT patients (Belichenko et al., 1994). Another study of the somatosensory cortex of null *MECP2* hemizygous mice (MECP2$^{-/y}$) reported that dendrites of layer II/III neurons were thinner and had fewer spines than those of wild-type littermates (Fukuda et al., 2005). On the other hand, transient overexpression of wildtype MeCP2 in cultured cortical neurons led to a significant increase in dendritic and axonal length and arborization, while neurons overexpressing a nonfunctional, truncated form of MeCP2 (*MECP2*^{293}) only showed increases in dendritic and axonal branching (Jugloff et al., 2005). Quantitative analyses of dendritic morphology in neurons from layers III and V of the frontal cortex of *MECP2*^{308} mice, which express a truncated *MECP2* allele, reported no major abnormalities in dendritic branching (Moretti et al., 2006). Further analyses at the EM level in area CA1 of the hippocampus yielded no differences in the density of asymmetric synapses and in the number of synaptic vesicles, either docked at the active zone or in the main vesicle cluster within the terminals. However, the mean length of the PSD was smaller in *MECP2*^{308} mice, driven by a concomitant increase in the smaller PSDs and a decrease in the larger ones (>150 nm) (Moretti

et al., 2006). Since the size of the active zone is tightly correlated with the size of the PSD across the synaptic cleft (Harris and Sultan, 1995; Boyer et al., 1998; Schikorski and Stevens, 1999), the observation of smaller PSDs and similar numbers of docked vesicles in the *MECP2*^{308} mice suggests that the density of docked vesicles is increased in the mutant active zone. These apparent discrepancies clearly warrant further investigations of the role of wild-type and mutant MeCP2 on dendritic and synaptic morphology in specific brain regions as a potential underlying mechanism for the functional impairments observed in animal models and RTT patients.

The molecular pathways contributing to the pathogenesis of Rett syndrome remain unclear. Considering that the best-characterized function of MeCP2 is as transcriptional repressor, numerous gene expression profiles have been conducted on RTT patients and in the available mouse models of RTT (Colantuoni et al., 2001; Tudor et al., 2002; Ballestar et al., 2005; Nuber et al., 2005; Delgado et al., 2006). However, these studies identified several genes that are either upregulated or downregulated, but their contribution to the disease remains unknown. A more targeted approach, such as chromatin immunoprecipitation, has identified the promoter region of the *BDNF* gene as a target of MeCP2 transcriptional control (Chen et al., 2003; Martinowich et al., 2003). These studies demonstrated that MeCP2 binds to and represses the transcription of mouse *BDNF* promoter IV, equivalent to the rat *BDNF* III promoter, which is well known to be activated by neuronal activity and the ensuing voltage-gated Ca^{2+} influx (Tao et al., 1998). As expected, the levels of *BDNF* exon IV transcript were low in cultured cortical neurons from wildtype mice in the absence of neuronal activity (i.e., in the presence of tetrodotoxin, TTX). On the other hand, neurons from *MECP2* null mice showed a twofold increase in their basal levels of *BDNF* exon IV transcript, as predicted from its transcriptional repressor function. Furthermore, *BDNF* exon IV transcript levels induced by a strong depolarizing stimulus (i.e., KCl) were similar between control and mutant cells, due to the large increase observed in wild-type neurons and the already elevated levels in *MECP2* null neurons, which were unresponsive to KCl depolarization (Chen et al., 2003). Considering that BDNF is a positive modulator of excitatory synaptic function (Thoenen, 2000; Poo, 2001; Tyler et al., 2002a,b; Lu, 2003; Bramham and Messaoudi, 2005), the observation of elevated BDNF levels (at least in terms of its activity-dependent mRNA transcripts) was unexpected and somewhat puzzling. However, BDNF protein levels measured by enzyme-linked immunosorbent assay (ELISA) were found to be lower in brain samples of *MECP2* null mice at 6–8 weeks of age compared to wild-type controls, a difference not observed at 2 weeks (Chang et al., 2006). In addition, conditional deletion of the *BDNF* gene in *MECP2* null mice exacerbated the onset of the RTT-associated phenotypes of the *MECP2* null animals, which included hypoactivity in the running wheel and reduced action potential frequency in pyramidal neurons of acute cortical slices *in vitro*. Surprisingly, overexpression of *BDNF* slowed down the disease progression in *MECP2* null mice, with increased wheel running behavior and augmented action potential firing in cortical neurons (Chang et al., 2006). Since BDNF mRNA and protein levels are tightly regulated by neuronal activity, the reduced firing frequency of neurons from *MECP2* null mice (Dani et al., 2005) may cause the reduced BDNF protein levels measured by ELISA (Chang et al., 2006). The discrepancy between the elevated basal levels of *BDNF* exon IV transcripts in cultured *MECP2* neurons (Chen et al., 2003) and the reduced BDNF protein levels in *MECP2* brain tissue (Chang et al., 2006) likely originates from different basal conditions (TTX in culture vs. naive fresh brain samples), developmental age (embryonic vs. postnatal), or the modulation of mRNA translation into protein. Whether direct or indirect, the relationship between MeCP2 function and BDNF-mediated signaling seems potentially relevant to the speculated impairments in Rett syndrome, especially with regard to synaptic function (Dani et al., 2005; Asaka et al., 2006; Moretti et al., 2006; Nelson et al., 2006).

Unfortunately, the few available studies of neurotrophin levels in RTT patients have not yielded results consistent with the 'BDNF hypothesis of Rett,' at least at a first approximation. First, reduced NGF levels were observed in cerebrospinal fluid (CSF) of RTT patients, a difference not found in blood serum levels, while BDNF was unaffected in CSF or blood serum RTT samples (Lappalainen et al., 1996; Vanhala et al., 1998; Riikonen and Vanhala, 1999; Riikonen, 2003). Second, the expression levels of NGF and its receptor TrkA were reduced in postmortem RTT brains, as assessed by immunohistochemistry (Lipani et al., 2000), while the number of basal forebrain neurons expressing $p75^{NTR}$ was unaffected in RTT brains (Wenk and Hauss-Wegrzyniak, 1999). Third, NGF serum levels

tend to decrease with age in RTT patients, opposite to the characteristic developmental increase in healthy individuals (Calamandrei et al., 2001). Taken together, these few reports reveal our incomplete understanding of the potential and intriguing role of neurotrophin signaling in the pathogenesis of Rett syndrome. Furthermore, they underscore the need for further cellular and molecular studies, perhaps at the single-cell level, of the consequences of mutant MeCP2 expression on neurotrophin expression, targeting, and/or release, as well as signaling through its $p75^{NTR}$ and Trk receptors.

4.34.9 Final Considerations

The observations reviewed in this chapter provide strong evidence that dendritic spines are highly specialized neuronal compartments that are exquisitely tuned to sense ongoing and fluctuating levels of afferent synaptic activity, which likely play a fundamental role in synaptic integration and plasticity. However, most of the experimental evidence is so far correlative: for example, while LTP or behavioral learning cause structural changes, those changes may not contribute to the synaptic potentiation or the formation of the engram. On the other hand, the new spines formed after LTP induction may not initially participate in synaptic transmission, but rather may be the sites of future synaptic plasticity (i.e., NMDA-only silent synapses). In any case, dendritic spines are prime examples that function affects structure and vice versa in a global and ongoing homeostatic balance (i.e., negative feedback), which can be locally modified by rapid 'Hebbian' positive-feedback changes. After more than a century of their discovery and the speculation that 'mental exercise' may promote their formation and growth, dendritic spines still represent a truly fascinating 'thorny' issue in our quest for the neurobiological basis of learning and memory.

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4.35 Integrins and Cadherins – Extracellular Matrix in Memory Formation

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4.35.1 Introduction

Certain types of molecules and biochemical processes have surfaced recurrently as important for learning and memory. For instance, one theme that frequently emerges is that signal transduction through the second messenger cyclic AMP is particularly important for many forms of learning. The MAP kinase pathway is also frequently implicated along with a host of other cellular molecules to include other protein kinases, transcription factors, metabolic enzymes for neurotransmitter synthesis and degradation, phosphatases, and so on. The widespread involvement of many cellular signaling pathways and many different classes of molecules is due to the fact that learning occurs through broad changes in the overall physiology of the relevant neurons.

One area of the cell that must play a major role in the modulation of cell–cell communication underlying learning is the surface – the interface for junctional communication. It is thus reasonable to believe that major classes of cell surface proteins, including the cell adhesion receptors, might be critical for learning to occur. Cell adhesion receptors serve two broad roles. First, they provide for the adhesive interactions necessary to maintain stable contacts between a neuron and the extracellular matrix or with other cells through ligands expressed on these adjacent cells. Second, they mediate cellular signaling processes and in some cases do this bidirectionally – from inside to out and from outside to in. Furthermore, the major classes of cell adhesion receptors reviewed here, the integrins and the cadherins, signal to the actin cytoskeleton to mediate changes in neuron and synapse structure. Since neuron and synapse structure are intimately related to function, these important attributes of the integrin and cadherin class of cell adhesion receptors position these molecules to be of central importance to various forms of synaptic and behavioral plasticity.

4.35.2 Structure of Integrins

Integrins are glycosylated transmembrane heterodimers composed of noncovalently bound α and β subunits. To date, 18 α and 8 β subunits have been characterized in mammals that together form at least 24 integrin heterodimers. Both subunits have (1) a large extracellular domain (ectodomain) from 778 to 1104 amino acids in length that together associate to form a globular, ligand-binding site, (2) a single-pass transmembrane (TM) domain, and (3) a short cytoplasmic domain from 30 to 70 amino acids in length, with the exception of the β4 subunit, which has a cytoplasmic domain of 1088 amino acids. The

cytoplasmic domain lacks enzymatic activity and interacts with a large number of molecules involved in signal transduction and cytoskeletal association. About half of the α subunits contain within the N terminus an additional I-domain in which a metal ion is coordinated at the ligand-binding interface through a conserved five–amino acid motif, the metal ion-dependent adhesion site (MIDAS). Variant sequences in the extracellular and/or cytoplasmic domain for several α and β subunits produced by alternative splicing have been reported (reviewed in Sastry and Horwitz, 1993; van der Flier and Sonnenberg, 2001), thus further expanding the structural and functional complexity of the family.

Several major advances have been made over the last few years in understanding the structural changes that take place during integrin activation (also referred as priming, i.e., the changes that promote ligand binding), with $\alpha_{IIb}\beta_3$ and $\alpha V\beta_3$ being used as prototypical molecules for these studies (**Figure 1(a)**). Although some differences exist in the details, a general conclusion is that global conformational changes underlie integrin activation. Integrin activation via inside-out signaling is triggered by the binding of the cytoskeletal protein talin to the cytoplasmic tail. This binding appears to be a final common step of intracellular signaling cascades (Tadokoro et al., 2003; Calderwood et al., 2004). Recent evidence has suggested that the cytoplasmic domains of the α and β subunits are clasped close to each other through a weak interaction at the membrane-proximal region in the resting state (Hughes et al., 1996; Vinogradova et al., 2002; Kim et al., 2003). Binding of talin to the β subunit (Vinogradova et al., 2002; Garcia-Alvarez et al., 2003; Ulmer et al, 2003) may disrupt this interaction between the two cytoplasmic tails, leading to a spatial separation of the subunit tails and an overall conformational change in the heterodimer.

The inside-out activation signal generated through the conformational change in the cytoplasmic tails must propagate to the ectodomain via the TM domain. It is not surprising that the TM domain has also been found to be critical in the conformational change that converts an integrin heterodimer from a low to a high ligand binding affinity state. Using disulfide bond scanning of the exofacial portions of α_{IIb} and β_3 TM domains, Luo et al. (2004) showed that restraining the two TM domains in the specific heterodimeric helical interface locks integrins in a low-affinity state. Conversely, mutations that disrupt interactions in the helical interface lead to the disassociation of the interface, most likely through a partial lateral separation of the two subunits, and an increase in ligand binding affinity (Takagi et al., 2002a; Li et al., 2005; Luo et al., 2005; Partridge et al., 2005).

The three-dimensional structure of the integrin ectodomain in both the resting and active states was recently clarified through X-ray crystallography, nuclear magnetic resonance, and electron microscopy. The first breakthrough came in 2001 when M. Amin Arnaout and colleagues successfully determined the crystal structure of the extracellular segment of $\alpha V\beta 3$ (Xiong et al., 2001). The studies revealed a structure with 12 domains assembled into an oval head connected to two legs that fold severely backward at an approximately 135° angle. A seven-bladed β-propeller domain from αV and a MIDAS-containing domain (βA) from $\beta 3$ formed the putative ligand-binding head. Subsequent studies from these and other investigators showed that binding of the cyclic peptide containing an RGD sequence, a motif found in many extracellular matrix (ECM) proteins such as fibronectin, laminin, vitronectin, and tenascin, indeed occurred in the groove between the β-propeller and the βA domain, accompanied with changes in both the tertiary and quaternary structure of the integrin subunits (Takagi et al., 2002; Xiong et al., 2002). However, it remains unclear whether activation and ligand binding are accompanied with the extension of the bent conformation, as evidence that supports both an extended (Nermut et al., 1988; Beglova et al., 2002; Takagi et al., 2002) and nonextended (Calzada et al., 2002; Butta et al., 2003; Adair et al., 2005) ligand-bounded ectodomain exists.

4.35.3 Expression of Integrins in the Adult Brain

The diversity of the integrin family makes it necessary to examine the regional, cellular, and subcellular expression patterns of individual members in order to understand how integrins are involved in various processes in the adult central nervous system. Using RNA *in situ* hybridization to adult rat brain sections, Pinkstaff et al. (1998, 1999) provided a comprehensive description of the regional and cellular localization of gene expression for nine α ($\alpha 1$–$\alpha 8$, αV) and five β ($\beta 1$–$\beta 5$) integrin subunits. A substantial amount of valuable information on the RNA expression in the brain can also be found from a number of online resources

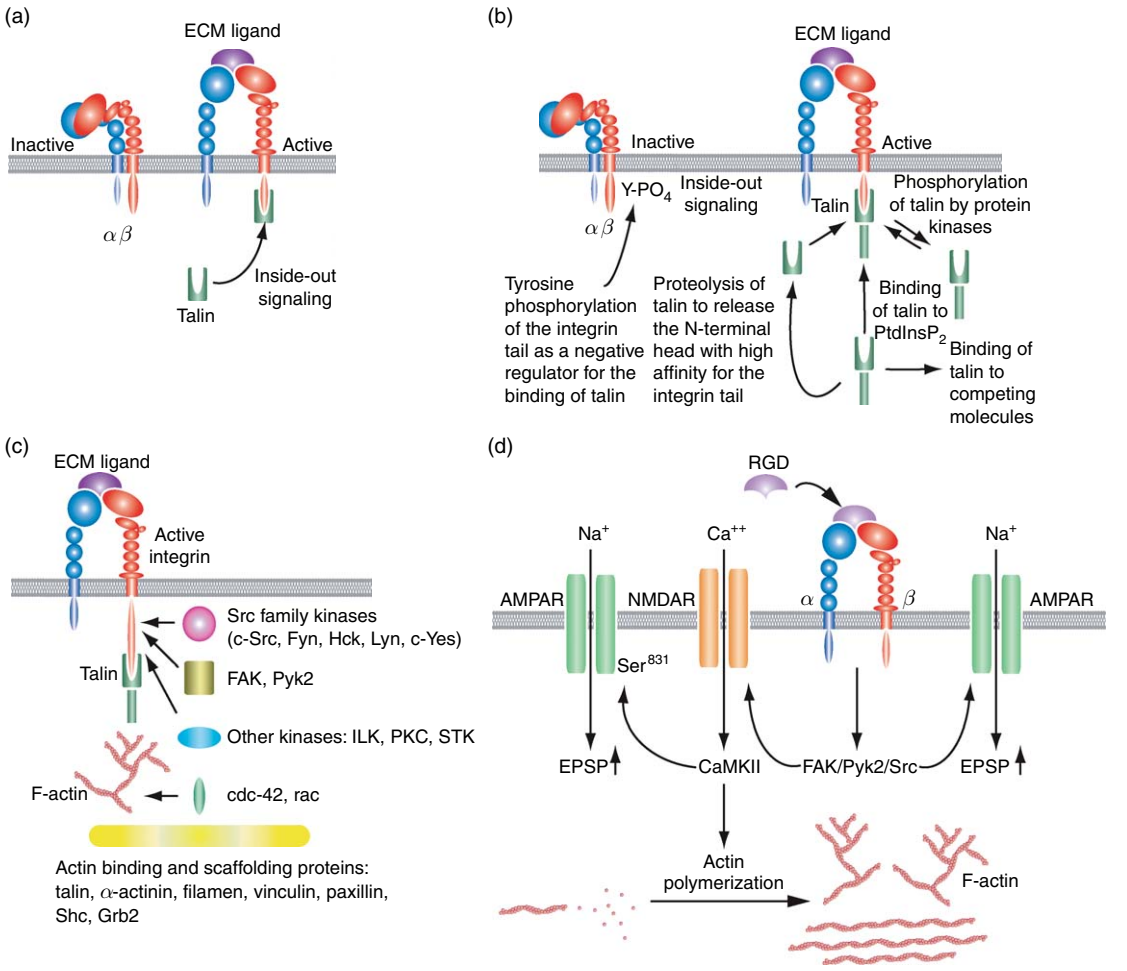


Figure 1 Integrin activation and signaling. (a) Inside-out signaling. In the resting state, the cytoplasmic domains and the transmembrane domains of the α and β subunits remain in close proximity to each other through weak interactions. Binding of talin to the cytoplasmic tail of the β subunit triggers separation of the two subunits, which leads to conformational changes in the extracellular domain and subsequently a higher affinity for the ligand. (b) Schematic diagram of some of the endpoints of cellular signaling that could lead to integrin activation through inside-out mechanisms. Tyrosine phosphorylation of integrin subunits alters the capacity to bind talin. Processing of talin by proteolysis creates a head domain of talin that has higher affinity to the β-integrin tail. Binding of $PtdInP_2$ by talin increases its affinity for β-integrin tails. Integrins compete for talin binding with other cellular molecules that have affinity for talin. Phosphorylation of talin may influence its ability to bind integrin tails. (c) Biochemical signaling pathways that are activated after ligand binding to integrins. Various members of the Src family of protein kinases are activated by integrin signaling. Members of the focal adhesion family of protein kinases and other types of protein kinases are activated after ligand binding to integrins. Small GTPases cdc42 and rac influence the actin cytoskeleton and are activated via integrin signaling. Other actin binding and scaffolding proteins are attached to focal complexes and participate in integrin signaling. (d) Schematic diagram of possible signaling cascades in integrin-induced potentiation of synaptic currents. Binding of RGD-containing peptides results in the activation of the Src and FAK/Pyk2 kinase pathways, which in turn promotes the phosphorylation of *N*-methyl-D-aspartate (NMDA) receptor subunits and the concomitant increase in the Ca^{++} influx. Activation of CaMKII caused by the increase in Ca^{++} leads to the phosphorylation of AMPA receptor subunits and the subsequent increase in synaptic currents. Activation of Src family kinases by integrins also leads to the increase in synaptic currents through a mechanism that is independent of CaMKII Thr^{286} and GluR1 Ser^{831} phosphorylation. Activation of CaMKII also induces structural reorganization at the activated synapses through actin polymerization and the formation of F-actin.

(St. Jude's Children's Research Hospital, 2004; Allen Institute for Brain Science, 2007; Max-Planck-Institute for Biophysical Chemistry, 2007). With the exception of $\alpha 2$, $\beta 2$, and $\beta 3$, RNA transcripts for all the surveyed integrin subunits are expressed in multiple brain regions. Extensive

overlapping patterns of expression were observed for a number of subunits. For example, $\alpha 3$, $\alpha 5$, $\alpha 7$, αV, and $\beta 1$ were detected in all of the neocortical layers, and $\alpha 1$, $\alpha 3$, $\alpha 5$, $\alpha 8$, and $\beta 1$ were detected in both the hippocampal CA1 and CA3 subfields. Since different integrin receptors can also overlap for their ligands and intracellular signaling pathways, neurons that express multiple integrins can potentially be stimulated by combinations of ligands and respond with the mobilization of multiple signaling pathways. The overlapping expression patterns, on the other hand, can provide a compensatory mechanism when one of the integrin receptors is missing. This potential redundancy needs to be accounted for when considering phenotypes from the integrin mutants.

Immunohistochemical studies, although available for only a limited number of integrin subunits, provide valuable additional information on the cellular localization of the receptors. At the level of light microscopy, immunoreactivity for $\alpha 1$, $\alpha 3$, $\alpha 5$, $\alpha 8$, $\beta 1$, and $\beta 8$ is generally observed broadly across the adult mammalian brains (Grooms et al., 1993; Einheber et al., 1996; Murase and Hayashi, 1998; Nishimura et al., 1998; Rodriguez et al., 2000; Bi et al., 2001). However, there are discrepancies between the reported mRNA and protein expression patterns from different studies for certain integrin subunits, probably due to technical limitations in the sensitivity of the probes used for RNA *in situ* hybridization experiments or the specificity of antibodies used for immunochemistry. For instance, although mRNA was undetectable in the cortical layers for $\alpha 1$ (except layer 5) and $\alpha 8$ (except layer 6) (Pinkstaff et al., 1999), both $\alpha 1$ and $\alpha 8$ immunoreactivity were observed in neurons throughout cortical layers 2–6 (Einheber et al., 1996; Murase and Hayashi, 1998). Some studies have also noted cellular compartmentalization of integrin immunoreactivity. Strong $\alpha 5$ immunoreactivity was observed within the primary apical dendrites of hippocampal CA3 pyramidal cells, with no detectable immunoreactivity in the basal dendrites (Bi et al., 2001), and $\alpha 8$ immunoreactivity has been reported as intensely punctate in the proximal portions of the apical dendrites (Einheber et al., 1996). Such compartmentalization and nonuniform distribution of immunoreactivity suggests a role of integrin receptors in the regional and/or synaptic specificity of neuronal properties such as synaptic plasticity.

In support of the possibility that integrins are involved in synaptic functions, biochemical and ultrastructural studies have demonstrated the presence of integrin receptors at the synaptic contacts. Integrins subunits $\alpha 3$, $\alpha 5$, αV, $\beta 1$, and $\beta 8$ have been localized in the synaptosomes isolated from hippocampus and cortex (Nishimura et al., 1998; Kramar et al., 2002; Chan et al., 2003). From immunoelectron microscopy experiments, strong immunoreactivity for $\alpha 3$ (Rodriguez et al., 2000), $\alpha 8$ (Einheber et al., 1996), $\beta 1$ (Schuster et al., 2001), and $\beta 8$ (Nishimura et al., 1998) has been detected in subsets of dendritic spines, most often close to or coincident with the postsynaptic density. In contrast, the examined presynaptic terminals were rarely labeled, and when they were, the immunoreactivity was weak. Interestingly, both immunopositive and -negative synapses can be detected from the same dendrite, and they can sometimes be found adjacent to each other, even making contacts with the same axonal terminal (Einheber et al., 1996; Schuster et al., 2001). The mechanism by which integrin receptors are precisely targeted to the specific synapses is unknown. Nor is it known whether multiple integrin receptors colocalize at the same synapse. Understanding these issues is critical for providing insights about the functional roles of integrins in the adult central nervous system.

4.35.4 Biochemical Signaling through Integrins

The accumulated evidence consistent with the idea that integrin activation is mediated through the binding of talin to the cytoplasmic tail of β-integrin has been a major step forward in conceptualizing the integrin-proximal steps involved in integrin signaling (Campbell and Ginsberg, 2004; Ginsberg et al., 2005). Talin is an integrin and actin-binding protein that colocalizes with integrins at sites of cell-substratum contact. Overexpression of the N terminus of talin activates integrins (Calderwood et al., 1999), and knockdown of talin expression blocks integrin activation (Tadokoro et al., 2003). Thus, the critical processes that lead to the activation of integrins via intracellular signaling potentially include the abundance of talin, its subcellular distribution, and the molecular modifications that might enhance or weaken its affinity for β-integrin tails.

The signaling pathways in neurons for which talin is a downstream target are largely unknown. However, there are several potential mechanisms (Cambell and

Ginsberg, 2004) that neurons could employ to alter talin binding to integrins (**Figure 1(b)**):

1. Modification of integrin tails by tyrosine phosphorylation inhibits the binding of talin in some cell types, providing a negative regulator for integrin activation.
2. The head domain of talin, which includes the integrin binding site, has a higher affinity for integrins than the complete molecule. The proteolytic cleavage of talin therefore offers a regulatory mechanism for integrin activation through talin binding.
3. The binding of phosphatidylinositol 4,5-bisphosphate ($PtdInsP_2$) to talin induces a conformational change that enhances talin's affinity for integrins. Cellular signaling processes that lead to an accumulation of $PtdInsP_2$ could therefore cause integrin activation.
4. Talin is known to bind other molecules besides integrins in the cell, such as the PtdInsP2 biosynthetic enzyme, phosphatidyl-(4)-phosphate 5-kinase type Iγ. Negative regulation of integrin activation could be effected by promoting talin's interaction with the cellular competitors for integrin binding (Calderwood et al., 2004).
5. Talin is the target for multiple protein kinases, likely including protein kinase C, protein kinase G, Akt, and the MAP Kinases. These phosphorylation events may have some role in regulating the binding of talin to integrins.

The activation of integrins is not a slow process but occurs within seconds and is transient, at least as revealed by cell-based adhesion assays using cells other than neurons (Constantin et al., 2000; Arnaout et al., 2005). The rapidity of these responses suggests that the adhesive-deadhesive responses of cellular subdomains, such as synapses, could occur within time frames of less than 1 s. Similarly, the formation of focal adhesion complexes containing integrins at the leading edge of motile cells grows to a size that can be detected by immunofluorescence in less than 1 min (Zaidel-Bar et al., 2004). Thus, many integrin-based signaling processes must be engaged by the time that these assemblies are visibly detectable.

These observations indicate that integrins can hypothetically be involved in processes that mediate short-term (seconds to minutes) neuronal processes as well as medium-term (minutes to hours) and long-term (hours to days) processes that may ensue.

The conformational change in integrin structure induced by the binding of talin alters the affinity of integrins for extracellular ligands. The ligands that interact with integrins are numerous and diverse (Plow et al., 2000). In general, ligands that participate in normal physiological responses fall into two classes: (1) ligands in the extracellular matrix that provide a basis for the integrins in adhesive functions to extracellular support scaffolds and (2) counter-receptors that are displayed for cell–cell interactions. Some of the better-characterized integrin ligands include collagen, fibrinogen, matrix metalloprotease-2, laminin, vitronectin, and thrombospondin (Plow et al., 2000; van der Flier and Sonnenberg, 2001). Each individual integrin heterodimer has the potential to interact with numerous ligands, and each ligand can interact with several different integrin heterodimers.

All of the integrin ligands listed above characterized from studies using tissues other than from the central nervous system have been reported to be expressed in neurons and/or glia. However, their specific central nervous system functions generally remain unexplored. Some surprising molecules that function as integrin ligands have been recently reported to be expressed in the central nervous system. Pasterkamp et al. (2003) reported that the molecule semaphorin 7A, a member of the semaphorin family of axon-repulsive guidance molecules, enhances axon outgrowth, and that this activity is dependent on β1-integrin receptors. Similarly, Dulabon et al. (2000) demonstrated that reelin, a large glycoprotein secreted principally by Cajal-Retzius cells in the marginal zone of the developing cerebral cortex, interacts with $\alpha3\beta1$ integrins to inhibit cortical neuron migration in the process of forming a normally layered cerebral cortex. Thus, semaphorin 7A and reelin, which have been shown to be expressed in the adult brain, must be added to the list of putative integrin receptors in processes underlying normal memory formation.

The binding of talin activates integrins, and activated integrins then bind to their endogenous ligands. Ligand binding to integrins then triggers the formation of different types of integrin signaling assemblies and diverse cell signaling events (Zaidel-Bar et al., 2004). These assemblies are likely to include dozens and perhaps hundreds of integrin and associated signaling molecules (Liu et al., 2000; Ginsberg et al., 2005) by the time that they can be visualized by immunofluorescence. This complexity has kept the details of such signaling complexes a secret. However, a few generalities have emerged regarding the major intermediates of integrin

signaling. Some of these are potentially relevant to memory formation.

One major theme that has emerged is that many different protein kinases are activated by binding directly or indirectly to integrins, providing a plausible mechanism for initiating intracellular signaling processes (**Figure 1(c)**). For instance, the Src family of protein kinases is frequently implicated in integrin signaling and are known to be required for integrin-mediated cellular responses (Ginsberg et al., 2005). The enzymes c-Src and Fyn bind directly to the tail of β3-integrin through a domain that is separate from the talin-binding region (Arias-Salgado et al., 2005), and other Src kinases including Hck, Lyn, and c-Yes bind to the tails of β1- and β2-integrins (Arias-Salgado et al., 2003). Thus, the activation of Src kinases by binding to the cytoplasmic domains of β-integrins appears to be an early and proximal event for integrin signaling.

Focal adhesion kinase (FAK) and proline-rich tyrosine kinase 2 (Pyk2) are the sole members of the FAK family of nonreceptor protein tyrosine kinases (Parsons, 2003). FAK colocalizes with integrins at cell-substratum contact sites and is thought to bind directly to β-integrin tails through sequences located at the N terminus of the kinase, although sequences at the C terminus known as the focal adhesion targeting (FAT) domain are necessary and sufficient for targeting the molecule to focal adhesions. Pyk2 is also a downstream effector of integrins with similar association and targeting properties (Litvak et al., 2000; Butler and Blystone, 2005).

The phosphorylation state and tyrosine kinase activity of FAK are regulated by integrin–ligand association, with the phosphorylation at Tyr-397 correlating with increased catalytic activity of FAK. This phosphorylation creates a high-affinity binding site for the SH2 domain of the Src family kinases and other enzymes such as phospholipase C-γ (PLC-γ) and phosphoinositide 3-kinase (Parsons, 2003).

Other kinases that potentially bind directly to the cytoplasmic tails of β-integrins include integrin-linked kinase (ILK), protein kinase C, and spleen tyrosine kinase (Syk) (Liu et al., 2000; Tadokoro et al., 2003; Ginsberg et al., 2005). In addition, integrins bind to numerous other proteins, some of which provide a platform for other signaling molecules. Four actin-binding proteins are among the best-characterized proteins that bind to β-integrin tails: talin, α-actinin, vinculin, and filamen (Liu et al., 2000; Hynes, 2002; Critchley, 2004). Some of the molecules that participate in integrin signaling complexes are illustrated in **Figure 1(c)**, although the specifics of many of the various molecular interactions are not yet known. However, some studies have revealed that antagonistic interactions occur: the binding site on the cytoplasmic tail of β-integrin overlaps with the binding site for talin, such that these proteins compete for binding to integrin tails (Kiema et al., 2006). Other interactions are likely to be cooperative. More complete lists of the molecules engaged in integrin signaling complexes can be found in many reviews on integrin signaling (Liu et al., 2000; van der Flier and Sonnenberg, 2001; Hynes, 2002)

The actin cytoskeleton is known to be one of the most important cellular targets of integrin signaling (**Figure 1(c)**; Wiesner et al., 2005). But how do the ligand-bound integrins signal for cytoskeletal rearrangements? As already mentioned, talin contains binding sites for the cytoplasmic tail of β-integrin as well as actin binding sites. Thus, the mere binding of talin and associated conformation changes is sufficient for the establishment of connections to and/or reorganization of the actin scaffold. Subsequent binding of talin to another actin-binding protein, vinculin, could establish secondary crosslinks to the actin cytoskeleton. In addition, some small GTPase molecules, such as rac and cdc-42, are activated by tyrosine phosphorylation by the src-family of protein tyrosine kinases. These GTPases are linked to the early phases of cell protrusion during cell migration and the remodeling of the actin cytoskeleton through actin-nucleating factors like Arp2/3 (Wiesner et al., 2006). Thus, these same molecules may therefore be involved in filopodia outgrowth and perhaps rapid modifications of synapse structure (DeMali et al., 2003).

4.35.5 Integrin-Dependent Synaptic Transmission and Plasticity

The possibility that integrins are involved in synaptic physiology was first reported 15 years ago when Xiao et al. (1991) observed that incubating hippocampal slices with RGD-containing peptides produced a reversible, dose-dependent decay of long-term potentiation (LTP) after stimulation of the Shaffer collaterals in the CA1 area over a period of 40 min but had no effect 1–2 min after the LTP-inducing high-frequency stimulation. This suggested that integrins are required for the stabilization rather than the induction of LTP. This idea was further substantiated in later studies showing that application of the RGD-containing peptides 10 min before to

10 min after LTP induction, but not 25 min or more after, caused a steady decay of the potentiation, suggesting that integrin activation and signaling occurring over several minutes after LTP induction was necessary for the stabilization (Stäubli et al., 1998). In addition to RGD peptides, function blocking antibodies against $\alpha 3$ and $\alpha 5$, but not αV and $\alpha 2$, as well as disintegrins, which are small, high-binding-affinity, RGD-containing integrin inhibitors found in various snake venoms, were also shown to block the stabilization of LTP in hippocampal slices (Chun et al., 2001; Kramár et al., 2002). More recently, Chan et al. (2003) have used a genetic approach as a more direct way of identifying and examining the functional roles of integrin subunits in synaptic plasticity. Consistent with the previous studies, animals heterozygous for an $\alpha 3$ null mutation exhibited a more rapidly decaying LTP compared to their wild-type littermates. The magnitude of LTP induced in the heterozygotes was not significantly different from control animals immediately after the tetanus, but it decayed rapidly so that a significant difference in magnitude was detected at 40 min. Surprisingly, when combined with the heterozygous $\alpha 8$ mutation, the double heterozygote $\alpha 3/+$; $\alpha 8/+$ produced a more severe deficiency immediately after LTP induction that has not been detected in previous studies using integrin antagonists. When a heterozygous $\alpha 5$ mutation was added to the double mutant, the triple heterozygotes showed a further reduction in the magnitude of potentiation. Thus, results obtained with mutants confirm the primary role of $\alpha 3$ in promoting the stability of LTP. They further suggest some redundancy and a role for $\alpha 8$ for the induction or stability of LTP within seconds or minutes after tetanus when $\alpha 3$ also is limiting, and for $\alpha 5$ on the magnitude of LTP that can be generated when both $\alpha 3$ and $\alpha 8$ are limiting. Furthermore, these results also suggest a different temporal requirement for integrins at different stages of LTP, with $\alpha 8$ more likely acting during and minutes after LTP induction and $\alpha 5$ during stabilization of LTP. A similar effect on LTP like that produced by the triple heterozygote $\alpha 3/+$; $\alpha 5/+$; $\alpha 8/+$ was obtained using conditional forebrain-specific knockouts of $\beta 1$-integrin, further confirming the adult physiological role of integrins in modulating LTP (Chan et al., 2006). In addition to LTP in the hippocampus, evidence indicating a function for integrins in other forms of synaptic plasticity has been obtained from studies focusing on different brain regions and from different animal models. In the cerebellum, a distinct type of long-lasting potentiation, named rebound potentiation (RP), is induced by postsynaptic depolarization at the GABAergic synapses between inhibitory interneurons and a Purkinje neuron (Kano et al., 1992). It was shown that activation of integrins by extracellular Mn^{2+} suppressed RP induction, and such suppression could be blocked by function blocking antibodies against the $\alpha 3$ or $\beta 1$ subunit. Overexpression of an $\alpha 3$ subunit in the Purkinje neurons also impaired RP induction (Kawaguchi and Hirano, 2006). These results thus suggest a critical role of $\alpha 3\beta 1$ integrin in negatively regulating the long-term plasticity at inhibitory synapses on cerebellar Purkinje neurons. In *Drosophila*, mutations in *volado*, the gene encoding the αPS3-integrin, result in significant defects in two forms of Ca^{2+}-dependent short-term facilitation, paired-pulse facilitation and frequency-dependent short-term facilitation, as well as in impaired posttetanic potentiation at the neuromuscular junction (Rohrbough et al., 2000).

How do integrins modulate synaptic plasticity? Given the properties of integrins and their ability to interact with a large number of molecules on both sides of the synaptic membrane, it is conceivable that they could modulate the adhesiveness of the cell membrane to the membrane of the synaptic partner, thus altering the physical structure of the synapse. An alternative is that they may modulate the properties of ion channels through intracellular signaling. Evidence supporting the latter possibility has been reported recently in which infusions of the RGD-containing peptides to hippocampal slices caused an approximately twofold increase in the amplitude and duration of *N*-methyl-D-aspartate (NMDA) receptor-mediated synaptic currents that was blocked by Src kinase inhibitor PP2 or function blocking anti-$\beta 1$ integrin antibodies (Lin et al., 2003; Bernard-Trifilo et al., 2005). Remarkably, a corresponding increase in the tyrosine phosphorylation of NMDA receptor subunits NR2A and NR2B was also observed in the treated hippocampal slices after the physiological testing (Bernard-Trifilo et al., 2005). When kinase activity was examined in the treated synaptoneurosomes isolated from adult rat forebrain, it was found that both the RGD-containing peptide and fibronectin induced a $\beta 1$ integrin-dependent, rapid increase in tyrosine phosphorylation of FAK, Pyk2, and other Src family kinases (Bernard-Trifilo et al., 2005). In addition to its influence in NMDA receptor-mediated currents, the RGD-containing peptide was also found to enhance the slope and amplitude of the excitatory postsynaptic responses through changes in

alpha-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA)-type glutamate receptors, and such enhancement was found to be blocked by a mixture of function-blocking antibodies against integrin $\alpha 3$, $\alpha 5$, and αV subunits; the NMDA receptor antagonist aminophosphonovaleric (APV); the Ca^{2+}/calmodulin-dependent protein kinase II (CaMKII) inhibitor KN-93 and, similar to that of NMDA receptor–mediated currents, by Src tyrosine kinase inhibitor PP2 (Kramár et al., 2003). Interestingly, the RGD-containing peptide also induced an increase in phosphorylation at CaMKII autophosphorylation site Thr^{286} and at GluR1 Ser^{831}, and both of these increases were markedly attenuated by APV but not by PP2, suggesting that the effect on RGD-induced AMPA-type currents by NMDA receptor is probably through these two phosphorylation events, whereas the effect by Src family kinases is through either other activation sites on these two molecules or other pathways. Chan et al. (2006) have also recently demonstrated that forebrain conditional $\beta 1$-integrin knockouts have impaired basal excitatory synaptic transmission through AMPA receptors at the same Shaffer collateral pathway. Taken together, a model emerges from these studies in which binding of some $\beta 1$ integrins results in the activation of the Src/FAK/Pyk2 kinase pathway, which in turn promotes the phosphorylation of NMDA receptor subunits and the concomitant increase in the Ca^{2+} influx (**Figure 1(d)**). Activation of CaMKII caused by the increase in Ca^{2+} concentration leads to the phosphorylation of AMPA receptor subunits and the subsequent increase in synaptic currents. Activation of Src family kinases by integrins also leads to the increase in synaptic currents through a mechanism that is independent of CaMKII Thr^{286} and GluR1 Ser^{831} phosphorylation. At the *Drosophila* larval neuromuscular junction (NMJ), integrins have also been shown to be involved in synaptic transmission. At reduced external Ca^{2+} concentration, Volado mutants showed a highly significant elevation in excitatory junctional currents (EJC) amplitude over the control animals (Rohrbough et al., 2000). Interestingly, since the mutant NJMs exhibit no changes in the postsynaptic miniature EJC amplitude or frequency sufficient to account for the large difference in the evoked EJC amplitude under the same recording conditions, it was concluded that Volado was involved specifically in the regulation of Ca^{2+}-dependent, evoked neurotransmitter release processes.

It has been well established that one of the key targets of the integrin-mediated signaling pathways is the actin cytoskeleton (Matus, 2000). This is important because it is widely believed that structural changes in synaptic morphology are necessary for memory (Lamprecht and LeDoux, 2004; Maviel et al., 2004) and synaptic plasticity. In neurons, actin filaments are highly concentrated in dendritic spines. Besides providing structural integrity, recent studies have shown that actin cytoskeleton is highly dynamic and its reorganization is involved in regulating the synaptic plasticity of the cells. For instance, it has been shown that stimuli that induce LTP spur actin polymerization resulting in a rapid and persistent increase in F-actin content within the dendritic spines (Fukazawa et al., 2003; Lin et al., 2005; Kramár et al., 2006), whereas manipulations that block the induction of LTP or reverse LTP within minutes after it is induced disrupt the increase (Lin et al., 2005; Kramár et al., 2006). Furthermore, inhibition of actin filament assembly in hippocampus by various agents such as latrunculin and cytochalasin impairs both HFS- and TBS-induced LTP, especially during consolidation at the late phase (Kim and Lisman, 1999; Krucker et al., 2000; Fukazawa et al., 2003). These effects on LTP produced by blocking actin cytoskeleton assembly are reminiscent of the effects produced by inhibiting integrin function, and together with the fact that there is a close interaction between integrins and the actin cytoskeleton, provide another possible mechanism by which integrins modulate LTP. Two recent studies support this hypothesis. Shi and Ethell (2006) have shown that treatment of cultured hippocampal neurons with RGD-containing peptides induced the elongation of existing dendritic spines and promoted the formation of new filopodia. In addition, immunofluorescent labeling of F-actin showed that these effects were accompanied by actin reorganization and synapse remodeling and were mediated by integrins, since they were partially blocked by function-blocking antibodies against $\beta 1$ and $\beta 3$ integrins. Furthermore, this RGD-induced actin reassembly was suppressed by the NMDA receptor antagonist MK801 and CaMKII inhibitor KN93 as in the integrin-mediated enhancement of AMPA-type currents described above, thus suggesting that integrins confer structural plasticity of spines through NMDA receptor- and CaMKII-dependent actin reorganization. Kramár et al. (2006) have shown that the actin reassembly caused by the LTP-inducing stimulation is blocked by function-blocking antibodies against $\beta 1$

integrins when they were applied shortly after the stimulation. Therefore, both the reorganization of the actin cytoskeleton and the expression of LTP require the functions of β1 (and possibly β3) integrins. It is unknown but particularly interesting how actin reassembly is mediated through NMDA receptor/CaMKII signaling, given that integrins are already known to physically link to the actin cytoskeleton through direct and indirect interactions with a large number of proteins that bind to the actin cytoskeleton.

However, there is no reason to exclude the possibility that shorter-lasting forms of memory may also employ actin-based structural plasticity of the synapse as a mechanism, albeit with changes that may be more modest than those observed after long-term memory. Indeed, changes in dendritic spine morphology have been observed in electron micrographs as early as 2 min after the induction of LTP (Lamprecht and LeDoux, 2004). Recent imaging studies of spine volume after repetitive or LTP-inducing stimulation revealed both rapid (Lang et al., 2004; Matsuzaki et al., 2004) and long-lasting spine enlargement (Matsuzaki et al., 2004), with increases in spine volume being detectable in less than 1 min after stimulation. Increases in spine volume were noticeable in both small and large spines, but persistent long-lasting changes in volume were detectable primarily in spines that were small prior to stimulation. Taken together, these studies indicate that integrin receptors can modulate neuronal synaptic plasticity through changes in the spine actin cytoskeleton and may provide a functional link between short- and long-term synaptic structural plasticity.

4.35.6 Integrins and Learning and Memory

The relatively large body of evidence showing that integrins are involved in the modulation of synaptic physiology and plasticity strongly suggests that they may function in memory formation and behavior. Indeed, the first direct evidence of integrin function in memory formation was obtained from the study of *Volado* mutants in *Drosophila* in which the mutants displayed impaired olfactory memories within 3 min of training, which could be rescued by conditional expression of a *Volado* transgene during adulthood, thus indicating that the integrin is required for the physiological processes underlying short-term memory formation (Grotewiel et al., 1998). The behavioral effect on short-term memory, although observed and reproduced in another laboratory (Tamura et al., 2003), has been difficult to observe routinely and reproducibly compared to other authentic learning mutants, suggesting that there are unknown and uncontrolled environmental factors that influence behavioral performance of the mutants. In vertebrates, mice with reduced expression of α3, α5, and α8 integrin subunits are defective in the hippocampus-dependent spatial reference memory in the Morris water maze but have normal fear conditioning (Chan et al., 2003). Furthermore, the forebrain and excitatory neuron-specific knockout of β1 integrin are impaired in a hippocampal-dependent, nonmatching-to-place T-maze working memory task (Chan et al., 2006). Additional support for integrin function in memory formation is provided indirectly from studies on integrin-associated protein (IAP). IAP is a member of the immunoglobulin (Ig) superfamily that has been shown to be physically and functionally associated with the several integrins, though they are also known to bind ligands other than integrins (Brown and Frazier, 2001). A reduction or elimination of IAP activity by injection of antisense oligonucleotides complementary to the IAP transcript, by gene-targeting or by function-blocking antibodies against IAP, significantly impaired memory retention in a one-way inhibitory avoidance paradigm using rats and also reduced the amplitude and slope of excitatory postsynaptic potential (EPSP) in hippocampal LTP (Huang et al., 1998; Chang et al., 1999, 2001).

4.35.7 Structure of Cadherins

A second major family of cell adhesion receptors that has been shown to be essential for synaptic function is the cadherins. Cadherins comprise a large superfamily of single-pass transmembrane glycoproteins that mediate the Ca^{2+}-dependent, adherin-based cell–cell interactions. In mammals, at least 80 members, approximately 20 of which belong to the classic cadherin subfamily and the remainder collectively belong to the protocadherin family (protocadherins and cadherins-related neuronal receptors), have been found in the nervous system with different adhesive and signaling properties, making them one of the most molecularly diversified groups of receptors found in the CNS (reviewed in Redies, 2000; Yagi and Takeichi, 2000). Owing to the complexity of the family, we focus our discussions on the classic

cadherins since they have been most extensively studied and should provide a general model of how cadherins function in synaptic physiology and plasticity, memory formation, and behavioral plasticity.

The structure of classic cadherins consists of an extracellular region with the five characteristic and tandemly arranged so-called EC domains (EC1–EC5) of approximately 110 amino acids, each with internal sequence homology, a transmembrane domain, and a highly conserved cytoplasmic domain that interacts with the actin cytoskeleton and mediates signaling through binding of catenins. Details of the three-dimensional structure of cadherins and their interactions have been elucidated through crystallographic and biochemical studies (Shapiro et al., 1995; Nagar et al., 1996; Tamura et al., 1998; Pertz et al., 1999; Tanaka et al., 2000; Boggon et al., 2002). In brief, the current view is that binding of Ca^{2+} to the binding pockets located between the EC domains stabilizes and rigidifies the elongated rod-like structure (**Figure 2(a)**). Monomeric cadherin molecules within the same membrane form *cis*-interaction pairs (strand-dimer) via lateral dimerization and clustering. Strong intercellular adhesion is achieved when the membrane-distal EC1 domain from the strand-dimers of one cell interact with the EC1 domain from the strand-dimers emanating from the opposite cell in the *trans* configuration, forming a zipper-like structure. However, in contrast to the integrins, it is unknown whether intracellular signaling can affect the adhesive properties of the molecules and the conformational coupling among the cytoplasmic, transmembrane, and extracellular domains.

Protocadherins were originally identified by PCR using degenerate primers for EC domains of classic cadherins to identify additional members of the cadherins family (Sano et al., 1993). Many more members of the protocadherin family have since been identified based on their similarity in the extracellular and transmembrane domains (Kohmura et al., 1998; Wu and Maniatis, 1999). One of the remarkable features of this family is its molecular diversity. In contrast to classic cadherins, protocadherins contain varying numbers of EC domains in their extracellular domain and vastly divergent cytoplasmic domains that suggest different intracellular interacting partners and functions, whereas CNR cadherins have six EC domains and a distinct cytoplasmic domain that interacts with Fyn. As with the classic cadherins, the EC domains in protocadherins have also been shown or implicated to be able to undergo homophilic interactions (Sano et al., 1993; Yamagata et al., 1999).

4.35.8 Expression of Cadherins in the Adult Brain

Owing to the diversity of the family, it is not surprising that the expression patterns in the adult brain for most of the cadherin family members have not been characterized. However, based on the more than 30 cadherins that have been studied in detail, it has become clear that most cadherins are expressed in a restricted and differential manner.

More importantly, a number of cadherins and their catenin-binding partners have been found by various approaches to be localized at the synaptic junctions as an integral part of the synaptic complex. For example, immunoprecipitation studies have identified N-cadherin as a major component of the rat forebrain postsynaptic density (PSD) preparations (Beesley et al., 1995). EM and confocal studies have revealed the synaptic presence of N-cadherin in the chick optic tectum (Yamagata et al., 1995; Uchida et al., 1996) and the hippocampus and cerebellum (Fannon and Colman, 1996; Benson and Tanaka, 1998; Bozdagi et al., 2000), cadherin-11 in the hippocampus (Manabe et al., 2000); CNR1 protein in the neocortex (Kohmura et al., 1998), and R-cadherin and T-cadherin in the developing chick retina (Miskevich et al., 1998). Several remarkable features, which may be generally true for other neural classic cadherins, have emerged from these studies:

1. The cadherin/catenin complex is localized at both the pre- and postsynaptic membranes of the same synaptic clefts, which is consistent with and reflective of their homophilic binding properties.
2. The cadherin/catenin complex is found adjacent to and often partially surrounding the active zones on the presynaptic membrane. The functional significance of this spatial localization appears to be related to synaptic vesicle trafficking and localization. By deleting β-catenin in hippocampal pyramidal neurons, Bamji et al. (2003) have shown that the β-catenin mutant brains have a selective reduction of undocked vesicles by 40% per synapse, with no detectable change for docked vesicles, suggesting that β-catenin is important for localizing the reserved pool of synaptic vesicles. Bamji et al. (2006) have further shown that

dissociation of the cadherin-β-catenin adhesion complex is required for the BDNF-mediated mobilization of synaptic vesicles at the synapses.

3. Different cadherins are localized at functionally distinct types of synapses. For example, Benson and Tanaka (1998) have demonstrated that N-cadherin is concentrated only at the PSD-95-positive excitatory synaptic sites in mature hippocampal neurons in culture or *in vivo* and is absent from glutamic acid decarboxylase (GAD)-positive inhibitory synaptic sites.

4. Each synapse appears to express only one member of the cadherin family, although multiple members can exist in the same neurons. For example, although the expression of both N-cadherin and E-cadherin are widespread and closely coincident, their expression patterns do not overlap but instead are distinct and mutually exclusive between synapses of the same cell (Fannon and Colman, 1996).

5. The expression domains of α- and β-catenin are broader than that of individual cadherins. For example, while N-cadherin is present only at the excitatory synapses in the mature hippocampal neurons, β-catenin is localized to both the excitatory and inhibitory synapses, suggesting that other cadherin proteins are expressed in the inhibitory synaptic sites (Benson and Tanaka, 1998).

Together, these characteristic features of cadherins support the general hypothesis that the cadherin/catenin complex could be involved not only in providing synaptic adhesion but also in mediating synapse target specificity, formation and/or stabilization of synapses during early brain development, and conceivably, some of the synaptic changes that occur in the adult due to activity.

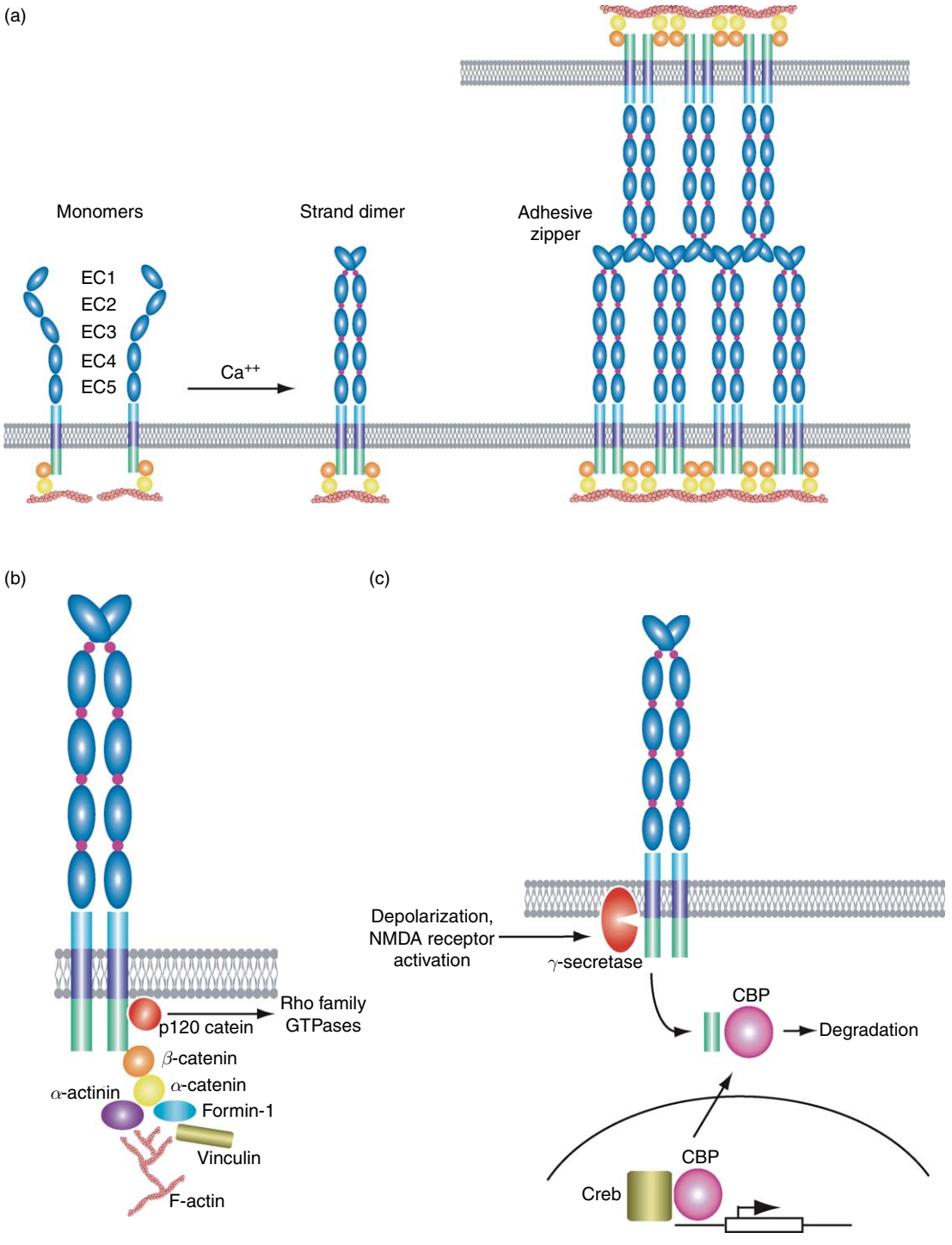
4.35.9 Biochemical Signaling through Cadherins

The accumulated data on cadherin function in the nervous system indicate that they are critical for axon targeting and synapse formation, maturation, stability, and plasticity through their roles as homophilic cell adhesion and signaling molecules (Junghans et al., 2005; Salinas and Price, 2005; Takeichi, 2007). Although cadherins may signal bidirectionally like the integrins, using both outside-inside and inside-outside signaling mechanisms, the evidence for inside-outside signaling is currently scant (Gumbiner, 2005). Furthermore, some of the mechanisms for outside-inside cellular signaling are only superficially understood. The complexity of the cadherin family of cell adhesion molecules suggests that their signaling mechanisms will prove to be diverse and perhaps cell-type specific. Therefore, the summary of cadherin signaling mechanisms provided below is limited to a few specific examples, which may or may not prove to be generally employed by different members of the cadherin family or even the same cadherin expressed in different cell types.

Like the integrins, neural activity is known to induce signaling through the cadherin pathway. Depolarization induces the mobilization of the cytoplasmic protein β-catenin from dendritic shafts into spines and leads to an increased association of β-catenin with cadherins and subsequent morphological and functional changes in synapses (Murase et al., 2002). This process is regulated by tyrosine phosphorylation of β-catenin. Dephosphorylation of tyrosine-654 of β-catenin is essential for the movement of β-catenin into spines.

Like the integrins, a major target for cadherin signaling is the actin cytoskeleton. Cadherins are thought to be able to induce actin filament formation in several different ways (**Figure 2(b)**). First, cadherin/catenin complexes recruit and activate proteins involved in actin polymerization, such as Arp2/3 and formin, thus establishing sites for the assembly of actin filaments (Bamji et al., 2005; Gumbiner, 2005). Second, after β-catenin binds to cadherins, it further associates with α-catenin, which either serves as a physical link between the cadherin/β-catenin complex or is involved in regulating the link of cadherins to the actin cytoskeleton through other molecular bridges (Bamji, 2005; Gates and Peifer, 2005). Third, cadherins interact with p120 cadherin via a segment close to the plasma membrane and can regulate the activity of the Rho family of GTPases (Bamji, 2005; Salinas and Price, 2005). This family of GTPases, as described earlier, are involved in regulating the state of the actin cytoskeleton. This signaling pathway may be involved in stabilizing the adhesive contacts established by the cadherins (Goodwin et al., 2003).

The cadherins may also be involved in modulating signaling through the transcription factor Creb (**Figure 2(c)**; Marambaud et al., 2003; Junghans et al., 2005; Salinase and Price, 2005). Cadherins are known to associate with presenilin 1, a γ-secretase also involved in the processing of amyloid precursor protein, resulting in the production of Aβ peptide. The stimulation of the γ-secretase activity of presenilin 1 by membrane depolarization or activation of the


(a)
Monomers
Strand dimer
Adhesive zipper
EC1
EC2
EC3
EC4
EC5
Ca++
(b)
Rho family GTPases
p120 catein
β-catenin
α-catenin
α-actinin
Formin-1
Vinculin
F-actin
(c)
Depolarization, NMDA receptor activation
γ-secretase
CBP
Degradation
CBP
Creb

NMDA receptor releases an intracellular peptide from cadherins. This peptide decreases cAMP response element binding protein (CREB)-mediated transcriptional activity by increasing the degradation of the CREB-binding protein, CBP. This is important because CREB activity has been widely implicated in the processes of long-term memory, suggesting that regulatory processes that influence the proximity of cadherins to γ-secretase and/or the activity of γ-secretase activity on cadherins are directly involved in long-term memory processes.

Other evidence has begun to emerge (see following) indicating that cadherins also participate in signaling through other molecules besides those mentioned above. It seems likely that future research will uncover many other new pathways for cadherin-based signaling that may underlie various forms of memory.

4.35.10 Cadherin-Dependent Synaptic Transmission and Plasticity

Accumulating evidence has made it clear that the functional roles for cadherins at synaptic sites provide more than just a static adhesiveness between the apposed pre- and postsynaptic membranes. For example, Tang et al. (1998) have shown that pretreatment of hippocampal slices with function-blocking antibodies raised against the extracellular domain of N- or E-cadherin significantly attenuated LTP at the Schaffer collateral-CA1 synapses. Infusion of peptides containing the His-Ala-Val (HAV) consensus sequence, which is known to be antagonistic for the formation of strand-dimers, also reduced LTP significantly. Notably, the inhibitory effect of the antibodies and the peptides is specific for the expression of early-phase LTP (E-LTP) since other physiological properties such as basal synaptic transmission, paired-pulse facilitation, posttetanic potentiation, and postsynaptic NMDA receptor–mediated currents were indistinguishable from the control slices. Similarly, Bozdagi et al. (2000) showed that LTP in area CA1 was disrupted by blocking N-cadherin adhesion functions with function-blocking antibodies. However, in contrast to the previous results, only late-phase LTP (L-LTP) was found to be defective. The reported difference in the time period in which N-cadherin functions are required during LTP between the two studies could be due to the differences in the protocol used for LTP induction, since a more intense stimulation protocol, such as the one used in the study by Tang et al., may have caused a more severe reduction in the extracellular Ca^{2+} and resulted in less stable N-cadherin complexes. An example for the importance of extracellular Ca^{2+} in affecting cadherin-dependent LTP induction was provided by a study on Arcadlin, a protocadherin-8-like molecule whose mRNA is dramatically induced in brain neurons after strong stimulation. It was shown that in the presence of Ca^{2+} in the medium, blocking antibodies against Arcadlin had no effect on LTP induction. However, by incubating the slices briefly with the antibodies in a Ca^{2+}-free medium before stimulation, the antibodies were able to block LTP even if Ca^{2+} was added back to the medium during the recordings (Yamagata et al., 1999). It is believed that, as in classic cadherins, Ca^{2+} is required for Arcadlin to exist at the synapses in a homophilic bound state that is resistant to the blocking antibodies. Depleting extracellular Ca^{2+}

Figure 2 Cadherin structure, adhesion, and signaling. (a) Model of structural rearrangements and adhesion of classic cadherins. Binding of Ca^{++} to the binding pockets located between the EC domains stabilizes and rigidifies the elongated rod-like structure. Monomeric cadherin molecules within the same membrane form a strand-dimer via lateral association and clustering. Strong intercellular adhesion is achieved when the membrane-distal EC1 domain from the strand-dimers of one cell interact with the EC1 domain from the strand-dimers emanating from the opposite cell in the *trans* configuration, forming an adhesive zipper. (b) Cytoplasmic signaling of cadherins. Cadherins signal to the Rho family of small GTPases through the association of p120 catenin. They also signal more directly through the actin cytoskeleton to effect changes in cell shape by binding β-catenin. β-catenin associates with α-catenin, which in turn binds to the actin cytoskeleton and/or regulates the association of cadherins to other molecular bridges, such as formin-1, α-actinin, or vinculin. (c) Nuclear signaling of cadherins. Cadherins signal to the nucleus to alter Creb signaling via the release of an intracellular fragment of cadherins. Depolarization or the activation of the NMDA receptor activates a γ-secretase, which cleaves and releases a peptide from the cytoplasmic tail of cadherins. This fragment is thought to sequester the co-activator protein, CBP, and target it to degradative pathways, thus inhibiting the signaling through CREB/CREB-binding protein (CBP) complexes. NMDA, *N*-methyl-D-aspartate.

destabilizes the intermolecular bond and renders the antibodies accessible for their function-blocking effect.

Molecular genetic approaches have also demonstrated the roles for cadherins/catenin complexes in synaptic plasticity. In the hippocampus, cadherin-11 is among one of the classic cadherins with strong expression (Suzuki et al., 1997; Manabe et al., 2000). Surprisingly, it was shown that LTP in the CA1 area was enhanced in the cadherin-11-deficient mice, in contrast to the strong reduction observed by blocking the functions of N-cadherin described above (Manabe et al., 2000). The mechanism underlying the observed LTP enhancement in these mutant animals remains unclear. Another mutant that was studied in detail physiologically was δ-catenin (δ-cat), a neuron-specific catenin that has been shown to colocalize with N-cadherin, PSD-95, and presenilin-1 (PS1) (Israely et al., 2004). The δ-cat$^{-/-}$ animals exhibit a significant deficit in PPF at multiple interpulse intervals. When tested for LTP, these mutants showed an abnormal enhancement of potentiation when a high-frequency stimulation was used for the induction, but a reduction when a low-frequency stimulation was used instead. Therefore, these results indicate that δ-cat is critically involved in both short-term and long-term synaptic plasticity. Interestingly, a significant reduction of N-cadherin and PSD-95 protein level was detected in the brain lysates from the δ-cat$^{-/-}$ animals when compared to the wild-type controls, while the level of PS1, E-cadherin, and synaptophysin remained unaffected. Since both N-cadherin and PSD-95 have been shown to be involved in LTP (Migaud et al., 1998; Tang et al., 1998; Ehrlich and Malinow, 2004), and PSD-95 is also linked to signaling cascades involved in the induction of LTP, the impaired synaptic plasticity and the nonlinear effect on LTP changes in the δ-cat$^{-/-}$ mice may be due to secondary effects caused by disrupting δ-catenin expression.

More recently, Jüngling et al. (2006) have generated N-cadherin knockout neurons in culture by *in vitro* differentiation of N-cadherin-deficient embryonic stem cells, thereby circumventing the early embryonic lethality of the N-cadherin null mutant animals. Physiological analysis on these N-cadherin knockout synapses revealed that during high activity there was a deficit in the readily releasable vesicle pool available for exocytosis, and consequently an impaired short-term plasticity, as evident by the increased paired-pulse depression. Interestingly, the same impaired short-term plasticity was still observed in synapses formed between wild-type neocortical neurons and ES-cell-derived N-cadherin-absent neurons in a heterogeneous culture with selective postsynaptic absence of N-cadherin. This result suggests the postsynaptic N-cadherin can regulate the release properties of the presynaptic sites in a retrograde manner, presumably through homophilic interaction with the apposed presynaptic partner. Taken together, the results from these different experimental approaches have demonstrated that different functional cadherin/catenin complexes are required for multiple forms of synaptic plasticity in the adult brain.

How do cadherins modulate synaptic plasticity? Since different mechanisms are known to underlie different forms of synaptic plasticity, it is likely that cadherins impart their effects by interacting, directly or indirectly, with different molecules and signaling cascades at specific synaptic sites. Both paired-pulse facilitation and paired-pulse depression are traditionally attributed to increased or decreased, respectively, neurotransmitter release from the presynaptic terminal (Zucker, 1999; O'Donovan and Rinzel, 1997). The impairment in these two forms of short-term plasticity observed in the δ-cat$^{-/-}$ animals and in the N-cadherin$^{-/-}$ cultured neurons is likely a consequence of the deficit in the transmitter vesicle pool as mentioned above (Bamji et al., 2003; Jüngling et al; 2006), although this deficit has not been described in the δ-cat$^{-/-}$ animals. The mechanism by which cadherin/catenin complexes regulate the vesicle pool at the active zone is unknown. However, by deleting specific domains of the β-catenin protein, Bamji et al. (2003) provided an additional important finding that the primary mechanism by which β-catenin regulates the reserve pool of synaptic vesicles is through recruitment of PDZ proteins to the cadherins, and not through the cadherin-actin cytoskeleton association. Consistent with this idea, β-catenin has been shown to bind directly through its carboxyl terminus to a PDZ domain of MALS/Velis, a component of the MALS/CASK/Mint-1 complex that regulates neurotransmitter release (Perego et al., 2000; Olsen et al., 2006).

Recent studies have also shed light on how cadherin/catenin complexes are involved in LTP. It has been long established that the NMDA receptor is one of the key factors in the induction of LTP. Remarkably, proteomic characterization of the NMDA receptor multiprotein complex has identified N-cadherin as being physically linked to the receptor

(Husi et al., 2000). The functional significance for such a close association between the two molecules might be tied by the studies by Tanaka et al. (2000), which showed that, in response to synaptic stimulation, N-cadherin dimerizes and becomes markedly resistant to degradation by proteases, indices that have been used to measure enhanced, strong intercellular adhesion. Importantly, the activity-induced dimerization and increase in protease resistance was attenuated by APV and was therefore required for NMDA receptor function. This activity-induced, NMDA receptor-dependent increase of adhesive interactions at the synapses might contribute to the potentiated response in LTP, although a direct demonstration of whether or how changes in synaptic adhesion affect synaptic plasticity is lacking. Nevertheless, L-LTP is accompanied by an increase in N-cadherin synthesis and dimer formation, implicating enhanced adhesion between the pre- and postsynaptic membranes in the process (Bozdagi et al., 2000). In addition to the enhanced adhesion at the synaptic junctions, LTP is also associated with the delivery of alpha-amino-3-hydroxyl-5-methyl-4-isoxazolepropionate receptors (AMPARs) to synapses (Heynen et al., 2000). Remarkably, recent evidence has shown that AMPARs associate with N-cadherin and β-catenin *in vivo* (Nuriya and Huganir, 2006). In primary cultures of hippocampal neurons, both GluR1 and GluR4 showed colocalization with N-cadherin clusters at synaptic sites by immunocytochemistry. When GluR1 was immunoprecipitated from the rat cortical membrane fraction, both N-cadherin and β-catenin were specifically coimmunoprecipitated. In addition, it was found that lowering the extracellular Ca^{2+} concentration increased the interaction between AMPAR receptors and N-cadherin, suggesting that extracellular Ca^{2+} negatively regulates this association. Interestingly, when N-cadherin was overexpressed in either heterologous cells or cortical neurons, the surface expression of GluR1 was specifically increased significantly. Conversely, overexpression of GluR1 also increased the surface expression of N-cadherin. Therefore, the association between the AMPA receptors and N-cadherin appears to regulate their surface expression in a mutual manner and provides an attractive mechanism for the potentiation of synaptic transmission that contributes to LTP.

LTP is also associated with structural remodeling and synapse formation, especially during the stabilization and maintenance phase (Engert and Bonhoeffer, 1999; Maletic-Savatic et al., 1999; Toni et al., 1999). Evidence from several studies indicates that cadherins are involved in activity-dependent changes in synaptic structure and function. Murase et al. (2002) showed that neural activity induced a persistent, NMDA receptor-dependent redistribution of β-catenin and an increase in its association with synaptic cadherins. In addition, a point mutation in β-catenin that increased its localization in dendritic spines resulted in expanded areas of postsynaptic PSD-95 protein clusters within the spines, as well as increased size and intensity of associated presynaptic synapsin-1 clusters, suggesting an overall increase in the synaptic contact area. Furthermore, these morphological changes were accompanied by a higher miniature excitatory postsynaptic current, indicating an increase in synaptic strength. In line with these results, αN-catenin, one of the linker molecules between cadherins and actin cytoskeleton through binding with β-catenin, was shown to be essential for dendritic spine stability in cultured hippocampal neurons (Abe et al., 2004). In αN-catenin-deficient cells, many spines showed unusually dynamic deformation of their heads, with abnormal, rapid protrusion and retraction of filopodia from most of these spines, indicating that the synaptic contacts in these αN-catenin deficient cells are unstable. Conversely, overexpression of αN-catenin led to an enhancement of spine stability, and consequently an increase in density as well. Notably, the expression level αN-catenin at the synapses is activity dependent, since its synaptic accumulation was suppressed by tetrodotoxin (TTX), a neural activity blocker, but was enhanced by bicuculline, a gamma-aminobutyric acid (GABA) antagonist. The synaptic accumulation of αN-catenin is functionally significant, since it was shown that excess αN-catenin could render spines resistant to the TTX treatment. Together, these studies suggest that the cadherin/catenin complexes contribute to enhanced synaptic size and density by conferring both strength and stability to synapses in an activity-dependent manner.

4.35.11 Cadherins and Learning and Memory

Compared to the extensive studies of the function of cadherin/catenin complexes at the molecular and cellular level, relatively little is known regarding their contributions to normal behavior. This is primarily due to the fact that most of the members in the cadherin and catenin families are essential during early development, and mutations are therefore

embryonic lethal, and the generation of conditional mutants has been lacking. Nevertheless, a few studies have been reported that did indicate that cadherin/catenin complexes are indeed involved in certain behavioral or learning and memory paradigms. For example, animals deficient in cadherin-11, which is not an essential cadherin, showed reduced fear- or anxiety-related responses as assayed by the elevated plus-maze behavioral test (Manabe et al., 2000). Deletion of δ-cat resulted in impairment in Pavlovian fear conditioning and in motor coordination, although their exploratory behavior and spatial learning remained intact (Israely et al., 2004). Surprisingly, mice expressing the dominant negative cadherin for both N- and E-cadherin in the adult forebrain showed only abnormal rearing behavior in the open field test, with no detectable defect in spatial learning and anxiety-related behaviors (Edsbagge et al., 2004). It may be that functional redundancy occurs in these animals that masks the *in vivo* functions, since multiple cadherins are expressed in the same neurons. Certainly, additional conditional knockouts, and perhaps in genetic combinations, will be needed to uncover the roles of cadherin/catenin complexes in learning and memory.

4.35.12 Cross-Talk among Cell Adhesion Receptors for Potential Synaptic and Behavioral Functions

It is clear from the above discussion that integrins and cadherins, the two major families of cell adhesion receptors, are critical for the regulation of synaptic functions. Both families provide a physical link between the extracellular environment and the actin cytoskeleton and the reorganization of the cytoskeleton in response to cell stimulation. Despite this commonality, studies of their signaling mechanisms have been performed separately. However, there now exists clear evidence for crosstalk between distinct members of these two families. In colon cancer cells, elevated Src activity disrupts the membrane localization of E-cadherin. This Src-induced redistribution of E-cadherin is dependent on functional αV- and β1-integrin subunits, along with FAK with intact Src-dependent phosphorylation acceptor sites (Avizienyte et al., 2002). Yano et al. (2004) have shown that siRNA-induced reduction in FAK and paxillin protein levels in HeLa cells inhibits the formation of N-cadherin-based cell–cell adhesions. In addition, signals involving FAK are implicated in downregulating the activity of Rac1, a Rho family GTPase stimulated by integrins (DeMali et al., 2003), at sites thought to be important for the formation of N-cadherin-based adhesions in motile cells. Therefore, FAK signaling pathways are linked to mechanisms that downregulate Rac1 activity, which is important for efficient formation and maintenance of cadherin-based cell–cell adhesions in motile cells. Rap 1, which is another small GTPase regulator of the inside-out signaling of integrins (Bos et al., 2003; Caron, 2003), has been shown to play a critical role in the integrin-cadherin crosstalk. In cultured epithelial cells, disassembly of adheren junctions triggered by internalization of E-cadherin led to Src-dependent Rap1 activation and the concomitant formation and localization of Rap1/E–cadherin–catenin complex at the recycling endosome compartment (Balzac et al., 2005). Remarkably, this E-cadherin endocytosis-dependent activation of Rap1 was associated with and was required for the assembly of integrin-dependent cell-matrix contacts, therefore suggesting a major role of Rap1 in relaying information from E-cadherin-based to integrin-based adhesive structures. Thus, an emerging theme is that the Src/FAK protein kinases and the ras/rho GTPases signaling pathways are pivotal links for the integrin–cadherin crosstalk, probably as effectors that directly influence actin polymerization. It will be interesting to determine whether similar crosstalk between these two protein families and the underlying mechanisms exist in the nervous system, and whether such crosstalk is essential for the regulation of synaptic plasticity and memory formation.

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4.36 Presynaptic Mechanisms in Plasticity and Memory

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4.36.1 Introduction

Presynaptic proteins and presynaptic plasticity have been implicated in learning and memory through decades of study in highly tractable, 'simpler' model systems, such as the marine mollusk *Aplysia* (Frost et al., 1988; Kandel, 2001; Roberts and Glanzman, 2003). In mammalian synaptic plasticity and learning and memory, however, the role of presynaptically mediated short- and long-term plasticity, presynaptic release machinery, and presynaptic signal transduction has received much less attention. This is likely due to a focus on proteins involved in long-term potentiation (LTP) in area CA1 of the hippocampus (*See* Chapters 4.16, 4.20, 4.29, 4.30, 4.32, and 4.39), which is expressed in part through postsynaptic molecular changes (Chen and Tonegawa, 1997; Silva et al., 1997; Martin et al., 2000). Given the overlap in molecular mechanisms between invertebrate and mammalian learning and memory, it is likely that presynaptic mechanisms will also play a prominent role in memory formation in the mammalian brain (Abbott and Regehr, 2004).

Presynaptic plasticity commonly refers to functional changes in synaptic strength that are due to alterations in the amount of transmitter release. Activity-dependent changes in transmitter release can be transient (up to a few minutes) or long-lasting (typically more than an hour). This chapter mainly discusses our current understanding of the molecular basis of presynaptic long-term plasticity and the potential role of this form of plasticity in learning and memory. It begins with an overview of molecular mechanisms of neurotransmitter release, followed by discussion of what we consider best examples of long-term, activity-dependent presynaptic plasticity that may be relevant to learning and memory in the mammalian brain. Finally, a review of recent studies using mammalian genetic models to link presynaptic proteins and presynaptic plasticity to learning and memory is presented. Portions of this chapter are based on or excerpts from previous reviews by the authors (Chevaleyre et al., 2006; Powell, 2006).

4.36.2 Neurotransmitter Release

Release of neurotransmitter from presynaptic terminals requires the highly regulated fusion of synaptic vesicle membrane with the plasma membrane in a specialized, electron-dense region known as the active zone. The details of this process and its regulation have been thoroughly reviewed (Sudhof, 2004), and a brief overview is provided here to orient the reader. Synaptic vesicles first dock at the active zone, and then an ATP-dependent priming reaction makes them competent for exocytosis when an action potential triggers Ca^{2+} influx into the presynaptic terminal via voltage-gated Ca^{2+} channels. Multiple studies have identified several important components of the molecular machinery responsible for synaptic vesicle fusion, its regulation by Ca^{2+}, and modulation of neurotransmitter release (**Figure 1**).

At the core of neurotransmitter release are the soluble N-ethylmaleimide-sensitive-factor attachment protein receptor (SNARE) proteins synaptobrevin/vesicle-associated membrane protein (VAMP), syntaxin, and SNAP-25. Synaptobrevin is a synaptic vesicle protein, whereas syntaxin and SNAP-25 are associated with the active zone. When docked vesicles are primed for release, SNARE complexes are formed that bring the synaptic vesicle into tight proximity to the active zone membrane (Sudhof, 2004).

The Ca^{2+} sensor for fast neurotransmitter release is synaptotagmin (Littleton et al., 1993; Geppert et al., 1994; Fernandez-Chacon et al., 2001). Synaptotagmin associates constitutively with SNARE complexes and, on binding Ca^{2+}, results in vesicle fusion and release of neurotransmitter (Sudhof, 2004).

As alluded to later, synaptic vesicle, cytoplasmic, plasma membrane, and active zone-associated proteins can regulate this process. These proteins can affect vesicle availability, docking, priming, Ca^{2+} triggering, and even Ca^{2+} entry, all of which can affect both the probability of neurotransmitter release and how neurotransmitter release changes as a function of patterns of presynaptic action potential firing.

Neurotransmitter release is a highly regulated process. Presynaptic ionotropic and metabotropic receptors can be activated in a homosynaptic (i.e., same presynaptic terminal) or heterosynaptic

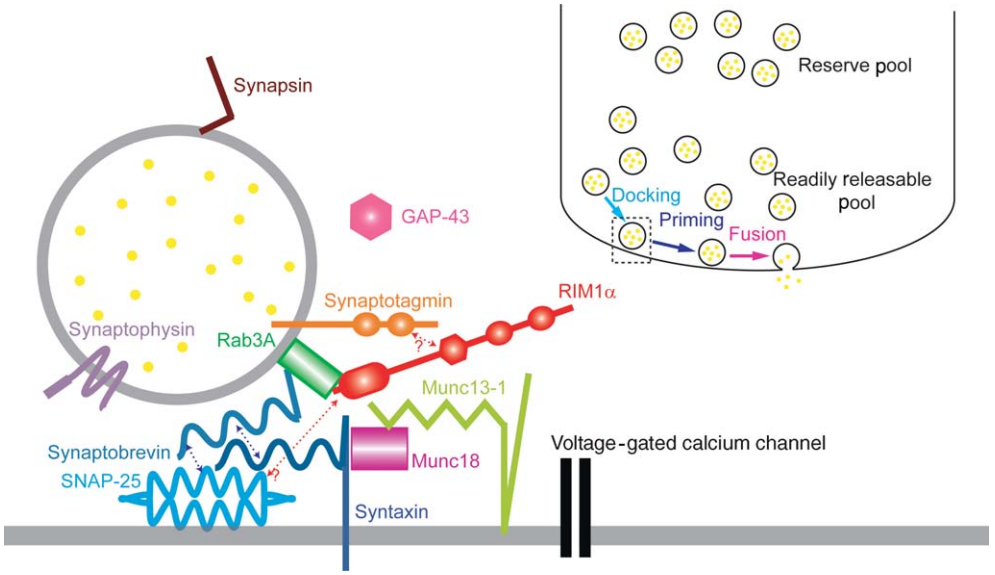

Figure 1 Schematic diagram of a presynaptic terminal and presynaptic proteins (Sudhof, 2004). Inset depicts a presynaptic terminal with reserve pool and readily releasable pool of synaptic vesicles. Also depicted are three final steps in neurotransmitter release: docking, priming, and fusion. The dashed box represents the region magnified in the main figure to the left. Main figure to the left depicts a single synaptic vesicle, synaptic vesicle–associated proteins, active zone proteins, and other proteins. The SNARE complex proteins, which interact to mediate fusion of the synaptic vesicle (synaptobrevin, SNAP-25, and syntaxin), are shown in shades of blue. Known interactions between proteins are depicted as bidirectional arrows. A question mark indicates that the interaction has not been completely validated. Additional possible interactions between the proteins depicted are described in the text. GAP-43, growth-associated protein-43. Figure modified from Powell CM (2006) Gene targeting of presynaptic proteins in synaptic plasticity and memory: Across the great divide. *Neurobiol. Learn. Mem.* 85: 2–15, with permission from Elsevier.

(i.e., neighboring presynaptic terminals) manner to modulate neurotransmitter release (Miller, 1998; MacDermott et al., 1999; Meir et al., 1999; Schoepp, 2001; Engelman and MacDermott, 2004). In addition, presynaptic receptors can be activated by retrograde signals generated from postsynaptic cells (Alger, 2002). The effects mediated by presynaptic receptors are typically short lasting. However, there is growing evidence demonstrating that transient activation of these receptors (i.e., presynaptic metabotropic receptors) can induce long-term changes in transmitter release.

4.36.3 Presynaptic Short-Term Plasticity

The probability of neurotransmitter release in response to a given action potential is robustly modulated by the pattern of presynaptic activation. In effect, the presynaptic terminal acts as a computational unit that can vary its output depending on the recent pattern of input. This type of presynaptic regulation of neurotransmitter release is generally referred to as short-term plasticity. For the purposes of this discussion, short-term plasticity refers to alterations in presynaptic release lasting for at most a few minutes. A brief overview of short-term plasticity is provided here, while more detailed accounts are available elsewhere (Fisher et al., 1997; Zucker and Regehr, 2002; Sudhof, 2004).

4.36.3.1 Facilitation/Depression

Paired-pulse facilitation (PPF) is a form of short-term plasticity lasting on the order of hundreds of milliseconds (**Figure 2(a)**). When two presynaptic action potentials occur within 25–400 ms, the postsynaptic response to the second action potential is increased dramatically relative to the first. This is widely held to be due to residual Ca^{2+} in the presynaptic terminal from the first action potential adding to the Ca^{2+} influx from the second pulse (Katz and Miledi, 1968; Zucker and Regehr, 2002). The larger presynaptic Ca^{2+} leads to facilitated or increased neurotransmitter release on the second 'pulse' or action potential. PPF is not limited to only the second of two presynaptic activations. In fact, responses to high-frequency stimulation beyond the second pulse can be facilitated even further; hence the more general term 'synaptic facilitation.' The magnitude of PPF varies depending on the initial probability of neurotransmitter release (Pr). At low baseline Pr, PPF is large (i.e., the response to the second 'pulse' is proportionately much larger than the first). At higher initial Pr, PPF can be smaller or can even reverse to become paired-pulse depression (PPD, with the response to the second pulse being smaller than the first). As a result, changes in PPF following an

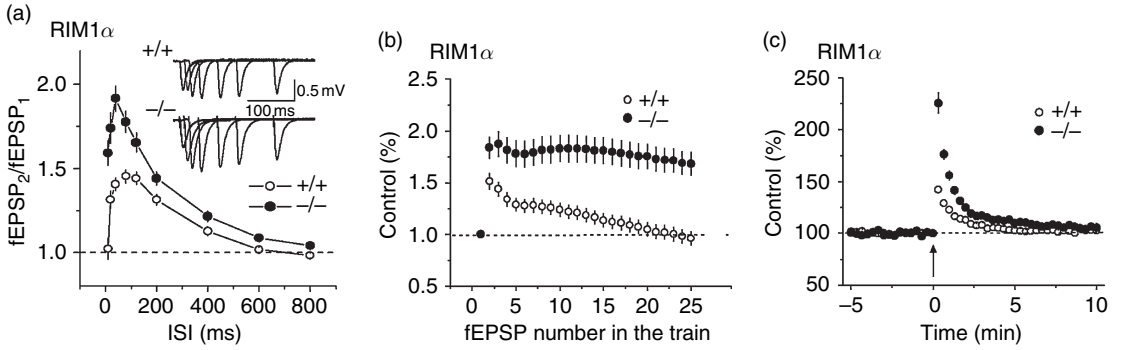

Figure 2 Examples of short and long-term presynaptic plasticity. (a) Example of paired-pulse facilitation (PPF) in wild-type (open circles, +/+) and Rab3A interacting molecule (RIM1α) knockout (filled circles, –/–) mice. The ratio of the first extracellularly recorded field excitatory postsynaptic potential (fEPSP) to that of the second fEPSP elicited at different interstimulus intervals (ISI) is plotted. RIM1α knockout mice show increased PPF compared to wild-type mice. Inset shows examples of fEPSPs elicited at various ISIs after the initial fEPSP. (b) Example of synaptic depression. In wild-type mice, stimulation at 14 Hz elicits an initial facilitation followed by synaptic depression. Depression in RIM1α–/– mice is markedly decreased. (c) Example of post-tetanic potentiation (PTP). In the presence of the *N*-methyl-D-aspartate inhibitor 4-aminophosphonovaleric acid to block long-term potentiation in area CA1 of the hippocampus, a high-frequency, tetanic stimulation (arrow) elicits immediate potentiation of the synaptic response lasting 2–3 min. PTP in RIM1α–/– mice is significantly enhanced. From Schoch S, Castillo PE, Jo T, et al. (2002) RIM1alpha forms a protein scaffold for regulating neurotransmitter release at the active zone. *Nature* 415: 321–326; used with permission from Nature Publishing Group.

experimental manipulation are often taken as an indication of changes in Pr, though alternative explanations can be offered, making PPF a useful tool in the study of synaptic plasticity mechanisms. The role of this robust integration of presynaptic activity in information processing remains a mystery, though initial studies of how PPF might play a role in behavior are detailed in the section titled 'Links between short-term presynaptic plasticity and learning and memory.'

Prolonged high-frequency presynaptic activation eventually leads to a decrease in neurotransmitter release known as synaptic depression (**Figure 2(b)**). This results in a lower steady-state level of neurotransmitter release that can take several minutes to recover. Synaptic depression is commonly taken to be the result of a decrease in the readily releasable pool (RRP) of synaptic vesicles, though the precise molecular mechanisms are poorly understood (Zucker and Regehr, 2002). The maximum number of synaptic vesicles released by a very strong, brief stimulus defines the RRP. Experimentally, the RRP can be defined by examining neurotransmitter release rates during sustained high-frequency stimulation, among other paradigms (Stevens and Tsujimoto, 1995; Rosenmund and Stevens, 1996; Goda and Stevens, 1998). Application of hypertonic sucrose is commonly used to measure the RRP. Hypertonic sucrose-induced release correlates well with that induced by high-frequency stimulation and with the number of vesicles visualized as 'docked' by electron microscopy (Zucker and Regehr, 2002). As with PPF, RRP measurements can be used to reveal alterations in presynaptic function following a given experimental manipulation.

4.36.3.2 Post-tetanic Potentiation

Post-tetanic potentiation (PTP) refers to an increase in neurotransmitter release after a brief, high-frequency train of action potentials (**Figure 2(c)**). This large enhancement may last on the order of several minutes and is measured after high-frequency stimulation. Although the precise mechanism of PTP remains to be determined, genetic manipulation of certain presynaptic proteins can clearly lead to altered PTP with or without changes in other forms of presynaptic plasticity (Zucker and Regehr, 2002). The time-course of PTP suggests that the relatively large presynaptic Ca^{2+} accumulation during high-frequency stimulation activates a Ca^{2+}-dependent biochemical process that leads to modification of presynaptic proteins. This modification may be slowly reversed over minutes following return of presynaptic Ca^{2+} signal to baseline. Although the exact purpose of PTP in the central nervous system is unclear, initial studies on the behavioral implications of altering PTP are discussed in the section 'Links between short-term presynaptic plasticity and learning and memory.'

4.36.4 Presynaptically Mediated Forms of Long-Term Plasticity

4.36.4.1 CA1 LTP: Evidence for Presynaptic Locus of Expression

The most-studied form of long-term synaptic plasticity is LTP at the Schaffer collateral-to-CA1 pyramidal neuron synapse in the hippocampus (Malenka and Bear, 2004). This form of LTP is induced postsynaptically via *N*-methyl-D-aspartate (NMDA) receptor activation and expressed, at least in part, postsynaptically via increased postsynaptic responsiveness to neurotransmitter release. The postsynaptic induction mechanisms for LTP, including Ca^{2+} influx through NMDA receptors and activation of postsynaptic Ca^{2+}-activated protein kinases, makes postsynaptic expression of CA1 LTP a parsimonious conclusion. Indeed, the belief that NMDA receptor-dependent LTP in area CA1 is expressed via postsynaptic mechanisms is so ingrained in current thought that several chapters in this work are focused largely on postsynaptic expression mechanisms (Chapters 4.16, 4.20, 4.21, 4.22, 4.23, 4.25, 4.27, 4.30). As discussed elsewhere, it seems likely that a portion of CA1 LTP expression is mediated by postsynaptic mechanisms.

A great deal of literature also provides evidence for a presynaptic component of CA1 LTP expression. Initial studies were based on metabolic labeling of glutamate and measuring release of labeled glutamate *in vivo* (Dolphin et al., 1982). These were followed by a flurry of studies applying quantal analysis to hippocampal synapses suggesting an increase in neurotransmitter release (Malinow and Tsien, 1990; Bekkers and Stevens, 1990; Malinow, 1991; Nicoll and Malenka, 1999). These studies led to important work identifying candidate retrograde messengers, molecules that could be generated postsynaptically, diffuse across post- and presynaptic membranes, and induce changes in the presynaptic neuron. Additional studies using sophisticated imaging of presynaptic vesicles suggest altered presynaptic function during CA1 LTP expression (Malgaroli et al., 1995; Zakharenko et al., 2001; Emptage et al., 2003). A more recent study finds

that the presynaptic proteins RIM1α and Rab3A may be required for successful expression of a protein kinase A (PKA)-dependent, late phase of LTP, implying that CA1 LTP lasting beyond 2–3 h may also be expressed in part via presynaptic mechanisms (Huang et al., 2005).

4.36.4.2 Mossy Fiber Long-Term Potentiation/Depression

Following the initial discovery of LTP at the perforant path to dentate granule neuron synapse by Bliss and Lomo (1973), it soon became apparent that LTP could also occur in area CA1 and at the dentate granule neuron-to-CA3 pyramidal neuron synapse (Alger and Teyler, 1976). The discovery of the role of NMDA receptors in CA1 LTP led to a focus on CA1 LTP due to the mechanistic implications of NMDA receptor involvement, though NMDA receptor-independent forms of LTP have also been reported at this synapse (Grover and Teyler, 1990; Powell et al., 1994).

Work by several laboratories has revealed a clearly different form of LTP in area CA3 of the hippocampus that is induced and expressed by mechanisms distinct from those of LTP in area CA1. Unlike CA1 LTP, this form of LTP does not require NMDA receptor activation (Harris and Cotman, 1986) and has been most studied at the mossy fiber-to-area CA3 pyramidal neuron synapse in the hippocampal formation (mossy fiber LTP or mfLTP) (Johnston et al., 1992; Nicoll and Schmitz, 2005). All available evidence suggests that mfLTP is expressed via an increase in presynaptic neurotransmitter release (Nicoll and Malenka, 1995; Weisskopf and Nicoll, 1995; Tong et al., 1996). Induction of mfLTP appears to require activation of PKA, whereas early phases of CA1 LTP do not (Hopkins and Johnston, 1988; Weisskopf et al., 1994; Huang and Kandel, 1994; Huang et al., 1995; Nguyen and Kandel, 1997; Villacres et al., 1998). The major events and molecules thought to be involved in mfLTP are depicted schematically in **Figure 3**.

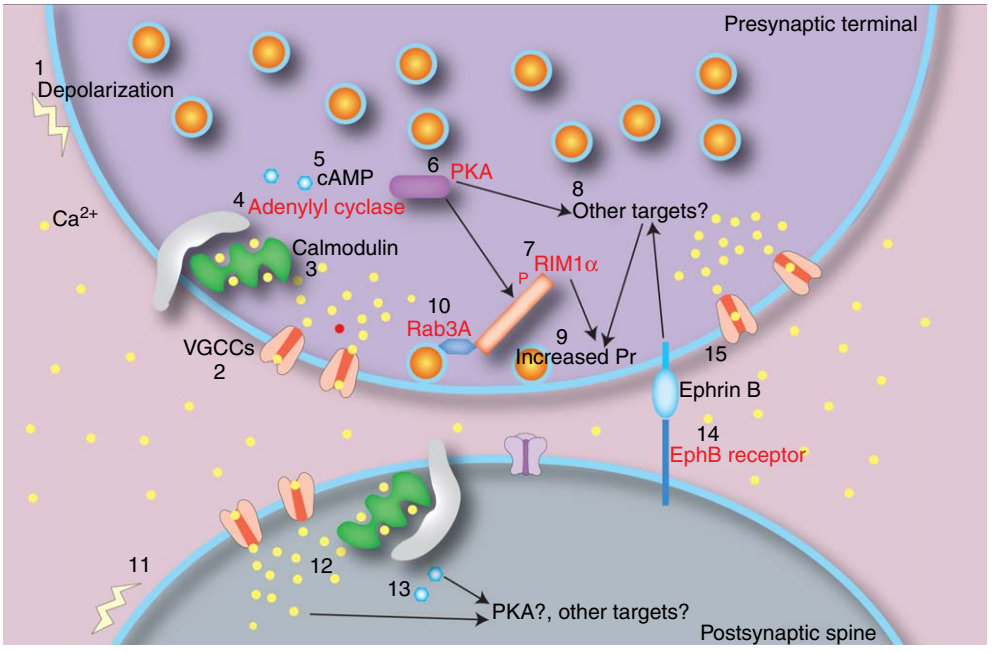

Figure 3 Schematic diagram of major processes thought to be involved in mossy fiber long-term potentiation (mfLTP). In one scenario, presynaptic depolarization (1) activates voltage-gated Ca^{2+} channels (VGCCs), (2) allowing significant Ca^{2+} entry. Ca^{2+} binds calmodulin (3), which activates Ca^{2+}/calmodulin-sensitive adenylyl cyclases (4) leading to increased cyclic adenosine monophosphate (cAMP) (5) and activation of protein kinase A (PKA) (6), which then phosphorylates RIM1α (7) or other targets (8), which leads directly or indirectly to increased probability of neurotransmitter release (Pr) (9). Rab3A (10), which interacts with RIM1α, is also required for induction/maintenance of mfLTP. Other reported contributors are postsynaptic depolarization (11), Ca^{2+} influx (12), and cAMP (13), as well as the postsynaptic signaling molecule EphB (14) and its presynaptic ligand Ephrin B (15). Genetic deletion of the molecules labeled in red is known to lead to decreased mfLTP.

Beyond the lack of NMDA receptor activation and a requirement for PKA activation, studies regarding induction mechanisms for mfLTP have produced different results. The mechanism and location of mfLTP induction are thus still unresolved. One series of studies suggests that that mfLTP can be induced purely by presynaptic activation (Castillo et al., 1994; Weisskopf et al., 1994; Nicoll and Malenka, 1995; Weisskopf and Nicoll, 1995; Tong et al., 1996; Mellor and Nicoll, 2001), requiring presynaptic Ca^{2+} influx via R-type voltage-dependent Ca^{2+} channels (Breustedt et al., 2003; Dietrich et al., 2003) and cAMP/PKA signaling (Weisskopf et al., 1994; Huang et al., 1995; Villacres et al., 1998; Wang et al., 2003). Another series of experiments indicates that mfLTP induction requires postsynaptic Ca^{2+} influx, depolarization, and cAMP (Williams and Johnston, 1989; Jaffe and Johnston, 1990, Urban et al., 1996; Yeckel et al., 1999; Alle et al., 2001; Contractor et al., 2002; Wang et al., 2004), necessitating the involvement of a retrograde messenger for presynaptic expression. Indeed, postsynaptic induction of mfLTP does not preclude presynaptic expression. In fact, transsynaptic interactions between postsynaptic EphB tyrosine kinase receptors and presynaptic B-ephrins have been implicated (Contractor et al., 2002; Armstrong et al., 2006). However, it is unclear how exactly mossy fiber repetitive stimulation triggers retrograde signaling or how presynaptic ephrins enhance transmitter release. Several pharmacological and mouse knockout studies (Contractor et al., 2001; Lauri et al., 2001; Bortolotto et al., 2003; Schmitz et al., 2003; Breustedt and Schmitz, 2004) support the notion that presynaptic kainate receptors are not essential for mfLTP but play a modulatory role in triggering this form of plasticity. Thus, activation of kainate autoreceptors by endogenous glutamate would increase presynaptic calcium entry during tetanization, thereby facilitating the induction of mfLTP (reviewed in Bortolotto et al., 2003; Nicoll and Schmitz, 2005). Long-term depression (LTD) at this synapse has been shown to be induced postsynaptically (Lei et al., 2003). It is worth noting that regardless of the induction mechanism, several studies have implicated increased presynaptic neurotransmitter release as the final common pathway for mfLTP expression (Nicoll and Malenka, 1995; Weisskopf and Nicoll, 1995; Tong et al., 1996). Thus, any remaining uncertainty is limited to the pre- versus postsynaptic requirement for mfLTP induction.

The molecular players involved in presynaptic mfLTP induction/expression are slowly being revealed. Presynaptic Ca^{2+}/calmodulin-sensitive adenylyl cyclases and presynaptic activation of PKA are critical for induction of mfLTP (Hopkins and Johnston, 1988; Huang et al., 1995; Villacres et al., 1998; Wang et al., 2003). In addition, the synaptic vesicle-associated small GTPase (GTP: guanosine triphosphate) Rab3A is required for mfLTP induction/expression (Castillo et al., 1997). Rab3A, however, is not a substrate for PKA *in vitro*, prompting a search for Rab3A interacting molecules with PKA phosphorylation sites. Rabphilin and the Rab3A interacting molecule (RIM1α) were then identified as PKA substrates that interacted with Rab3A. The PKA substrate RIM1α was found to be required not only for mfLTP but also for cerebellar parallel fiber to Purkinje cell LTP (pfLTP), highlighting that LTP expression at the hippocampal mossy fibers and cerebellar parallel fibers is mechanistically similar. However, at least for mfLTP, rabphilin was not required (Schluter et al., 1999). The discovery that the PKA substrate and active zone protein RIM1α and its vesicle-associated binding partner Rab3A were required for mfLTP induction/expression led to a specific, testable hypothesis that phosphorylation of RIM1α by PKA was required for mfLTP. The PKA site in RIM1α was subsequently identified, and RIM1α constructs with a serine to alanine substitution at this site were made (Lonart et al., 2003). Using a cerebellar culture system, this PKA/RIM hypothesis was tested at the parallel fiber-to-Purkinje neuron synapse. In RIM1α knockout mice, presynaptic cerebellar LTP was absent and could be rescued by wild-type RIM1α. This form of cerebellar LTP was not rescued by RIM1α containing a single serine-to-alanine point mutation at the PKA site, indicating that PKA phosphorylation of RIM1α may be necessary for induction/expression of cerebellar presynaptic LTP (Lonart et al., 2003). This negative finding has not yet been replicated, and a similar experiment has not been performed in mfLTP. Thus, although the presence of RIM1α is important for mfLTP induction/expression, it is not clear whether PKA phosphorylation of RIM1α is required for mfLTP. Even if PKA phosphorylation of RIM1α were required for mfLTP or cerebellar LTP, it would still not be clear whether PKA phosphorylation of RIM1α is the direct cause of increased neurotransmitter release or simply necessary for some other parallel mechanism to lead to increased neurotransmitter release. Interestingly, even in

RIM1α knockout mice, the adenylyl cyclase activator forskolin can still increase neurotransmitter release, suggesting that cyclic adenosine monophosphate (cAMP) or PKA activation can increase neurotransmitter release via mechanisms independent of RIM1α (Castillo et al., 2002; Schoch et al., 2002). This finding does not preclude RIM1α's involvement in mfLTP, but does complicate the interpretation that presynaptic cAMP activates PKA, leading to phosphorylation of RIM1α and the subsequent increase in neurotransmitter release.

The idea that mfLTP is due to a modification of the release machinery has been challenged by a study showing that hyperpolarization-activated cation channels (Ih) are necessary for mfLTP (Mellor et al., 2002). Activation of presynaptic Ih channels, by depolarizing mossy fibers and presumably increasing presynaptic Ca^{2+} influx, would facilitate transmitter release. Against this notion, another study showed normal mfLTP after pharmacological blockade of Ih channels (Chevaleyre and Castillo, 2002). Two independent groups showed that presynaptic Ca^{2+} entry remains unchanged during mfLTP (Regehr and Tank, 1991; Kamiya et al., 2002), supporting a model for mfLTP expression that involves persistent modification of presynaptic molecular targets residing downstream of Ca^{2+} entry. At the moment, the existence of Ih channels on mossy fiber terminals awaits discovery, and a role of Ih in mfLTP remains unproven.

Mossy fiber synapses also express presynaptic forms of LTD. At mossy fiber-to-CA3 pyramidal cell synapses, LTD is typically induced by moderate rates of stimulation ($\sim$1 Hz) for several minutes (Kobayashi et al., 1996). This form of LTD is induced by the activation of presynaptic metabotropic glutamate receptor 2 (mGluR2) (Yokoi et al., 1996) and is expressed as a reduction in glutamate release (Kobayashi et al., 1996). Tzounopoulos et al. (1998) reported that mGluR2 activation leads to a reduction in adenylyl cyclase activity followed by a decrease in PKA activity (Tzounopoulos et al., 1998). In addition, this study showed that mossy fiber LTD (mfLTD) requires Rab3A. Based on these observations, the authors suggested that mfLTD involves reversal of the presynaptic process involved in mfLTP. However, this model has been disputed by a more recent report showing that RIM1α, which is required for mfLTP, is not required for mfLTD (Castillo et al., 2002). Mossy fiber-to-interneuron synapses also express a presynaptic form of LTD (Lei and McBain, 2004). Interestingly, this form of plasticity is induced by the activation of postsynaptic Ca^{2+}-permeable alpha-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid receptors (AMPARs) but it is expressed presynaptically as a reduction in transmitter release, suggesting that some retrograde signal is required. How a postsynaptic rise in Ca^{2+} triggers presynaptic LTD remains unclear, but a recent study suggested that activation of presynaptic mGluR7 is necessary (Pelkey et al., 2005). Further studies will have to identify the molecular targets downstream from mGluRs mediating these forms of mfLTD.

4.36.4.3 Endocannabinoid Long-Term Depression

Endogenous cannabinoids (endocannabinoids, or eCBs) are signaling molecules that have emerged as key mediators of presynaptic plasticity in several brain areas, including those critically involved in learning and memory, such as the hippocampus, amygdala, striatum, and cerebellum (for a review see Chevaleyre et al., 2006) (*See* Chapter 4.37). In eCB-mediated plasticity, postsynaptic activation induces production of a lipid eCB messenger that generally moves in retrograde fashion across the synapse, binds to presynaptic cannabinoid receptors, and suppresses transmitter release either transiently or in a consolidated, long-term form.

Several endogenous ligands for the type 1 cannabinoid receptor (CB1R), the principal cannabinoid receptor in the brain, have been identified. Two of the best-characterized eCBs are 2-arachidonoylglycerol (2-AG) and anandamide (AEA). These eCBs are synthesized from membrane-derived lipid precursors. Because eCBs are lipid molecules that can pass through biological membranes, they cannot be stored in vesicles but are instead synthesized on demand by neuronal enzymatic activity (Piomelli, 2003). Notably, the CB1R is among the most highly expressed G protein–coupled receptors in the central nervous system (Herkenham et al., 1990), and it is responsible for most of the behavioral effects of Δ^9-tetrahydrocannabinol (Δ^9-THC), the principal psychoactive component of marijuana (for a more extensive discussion on endocannabinoid signaling, see the excellent recent reviews: Howlett et al., 2002; Freund et al., 2003; Piomelli, 2003; De Petrocellis et al., 2004; Howlett et al., 2004). There are many known types of eCB-mediated plasticity, each acting only at certain synapses and with a specific set of requirements for induction. In this section, we will focus on eCB-mediated long-term depression of transmitter release

(eCB-LTD), which probably constitutes the best-documented and most widely distributed form of activity-dependent long-term presynaptic plasticity in the brain (Gerdeman and Lovinger, 2003; Chevaleyre et al., 2006). Transient forms of eCB-mediated plasticity (Alger, 2002; Kreitzer et al., 2002; Wilson and Nicoll, 2002; Freund et al., 2003; Diana and Marty, 2004) will be briefly mentioned here to facilitate comparison with eCB-LTD.

Critical to our understanding of the induction of eCB-LTD are the mechanisms of eCB release and the signaling events downstream of CB1R activation. eCB production is a complex and tightly regulated, activity-dependent process that can occur as a result of two main mechanisms. The first eCB production mechanism is triggered by an influx of Ca^{2+} into the postsynaptic cell consequent to postsynaptic step depolarization or action potentials. This mechanism is typified by the transient (typically <1 min), eCB-mediated suppression of neurotransmitter release known as *D*epolarization-induced *S*uppression of *I*nhibition or *E*xcitation (DSI or DSE) (Alger, 2002; Kreitzer et al., 2002; Wilson and Nicoll, 2002; Diana and Marty, 2004), but it may also be involved in some forms of eCB-LTD (see later discussion). Postsynaptic Ca^{2+} rises may then be amplified by recruitment of Ca^{2+} release from intracellular stores. How Ca^{2+} promotes eCB release remains unknown. A second eCB production mechanism is triggered by repetitive activity of glutamatergic afferents and activation of group I mGluR, which couples to phospholipase C (PLC). This mechanism underlies most forms of eCB-LTD (Chevaleyre et al., 2006), as well as those short-term eCB-mediated forms of plasticity induced by synaptic activity (Maejima et al., 2001; Brown et al., 2003; Melis et al., 2004). The eCB 2-AG is likely involved because diacylglycerol lipase (DGL), the enzyme that synthesizes 2-AG, is required downstream of mGluR activation (Jung et al., 2005; Chevaleyre et al., 2006). Activation of AMPA receptors may also contribute to eCB-LTD mechanisms (Sjostrom et al., 2003; Soler-Llavina and Sabatini, 2006). Although each eCB production mechanism can be triggered independently of the other, some degree of cooperativity may exist; for example, increased intracellular Ca^{2+} greatly enhances mGluR-induced PLC activity (Hashimotodani et al., 2005; Maejima et al., 2005).

Unlike several common forms of long-term synaptic plasticity that critically depend on NMDA receptor–mediated Ca^{2+} influx for induction (Malenka and Bear, 2004), all known forms of eCB-LTD are independent of postsynaptic NMDA receptors. The newly synthesized eCB traverses the synaptic cleft and binds to presynaptic CB1Rs. The activation of CB1Rs coupled to $G_{i/o}$ proteins can affect many processes potentially involved in synaptic transmission, such as Ca^{2+} channel inhibition, a direct effect on the vesicle release machinery, and/or K^+ channel activation (Ameri, 1999; Mu et al., 2000; Schlicker and Kathmann, 2001; Howlett et al., 2002; McAllister and Glass, 2002; Mukhopadhyay et al., 2002). Although the transient reduction of transmitter release that occurs during DSI/DSE is most likely due to CB1R-dependent modulation of presynaptic voltage-dependent Ca^{2+} channels (Wilson et al., 2001; Varma et al., 2002), the mechanism by which CB1R activation leads to LTD of transmitter release has not yet been identified.

eCB-LTD has been reported in a number of brain structures including the dorsal striatum (Gerdeman et al., 2002; Kreitzer and Malenka, 2005), nucleus accumbens (Robbe et al., 2002), amygdala (Marsicano et al., 2002; Azad et al., 2004), hippocampus (Chevaleyre and Castillo, 2003), neocortex (Sjostrom et al., 2003; Bender et al., 2006), and cerebellum (Soler-Llavina and Sabatini, 2006). This prevalence suggests that eCB-LTD may be a fundamental mechanism for making long-term modifications to neural circuits and behavior. eCB-LTD is defined by certain common features. Induction of eCB-LTD requires the activation of presynaptic CB1Rs, and it is expressed presynaptically as a long-lasting reduction of transmitter release. The requirement for CB1Rs derives from results showing that eCB-LTD induction is blocked by CB1R antagonists and is abolished in CB1R knockout mice. eCB-LTD is likely to be presynaptically expressed because it is associated with enhanced paired-pulse ratio and failure rate. Importantly, once established, maintenance of eCB-LTD does not require continued CB1R activation (see later discussion). It is worth noting that the induction of cerebellar LTD has been recently reported to require eCB signaling (Safo and Regehr, 2005); but because cerebellar LTD is postsynaptically expressed, it constitutes a different class of eCB-mediated plasticity. Where eCB production is triggered by activation of glutamatergic synaptic inputs, eCBs can homosynaptically suppress these same glutamatergic inputs, forming an autoregulatory loop, or heterosynaptically suppress nearby GABAergic (GABA: gamma-aminobutyric acid) inputs (**Figure 4**).

Examples of homosynaptic eCB-LTD can be found at excitatory inputs to medium spiny neurons in the dorsal striatum (Gerdeman et al., 2002) and nucleus accumbens (Robbe et al., 2002) and also at the parallel fiber-to-stellate cell synapse in the

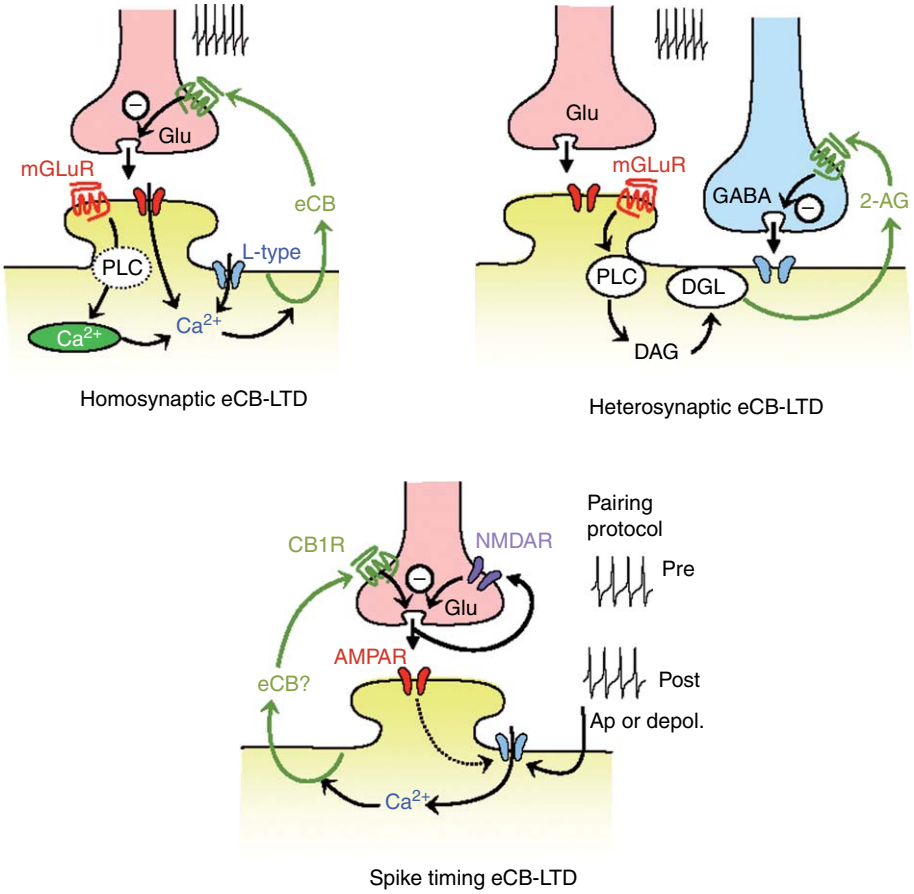

Figure 4 Schematic diagram of endocannabinoid-mediated LTD (eCB-LTD). *Homosynaptic eCB-LTD* has been reported at dorsal striatum and nucleus accumbens glutamatergic synapses. This form of plasticity is induced by repetitive activation of glutamatergic inputs and requires the activation of postsynaptic group I mGluRs (mGluR-I). Although postsynaptic [Ca^{2+}] increase is also required in both cases, the source may vary (intracellular stores in nucleus accumbens and L-type Ca^{2+} channels in the dorsal striatum). Heterosynaptic eCB-LTD occurs at GABAergic (GABA: gamma-aminobutyric acid) synapses in the hippocampus and basolateral amygdala. In both structures, LTD is initiated by glutamate release and group mGluR-I activation but results in a heterosynaptic decrease in GABA release. Unlike homosynaptic eCB-LTD at excitatory synapses, LTD induction at these inhibitory synapses does not require increased postsynaptic [Ca^{2+}]. The two structures differ in their eCB production pathways (see text); for clarity, only the hippocampal signaling pathway is shown. Timing-dependent eCB-LTD has been reported in the neocortex. In this case, LTD is triggered by pairing presynaptic stimulations with postsynaptic depolarizations or action potentials. Increased postsynaptic [Ca^{2+}] is required for eCB release. Interestingly, the induction of timing-dependent eCB-LTD requires activation of both presynaptic Type 1 cannabinoid receptors (CB1R) and presynaptic *N*-methyl-D-aspartate receptors (NMDARs). Glu, glutamate; mGluR, metabotropic glutamate receptor; PLC, phospholipase C; DGL, diacylglycerol lipase; DAG, diacylglycerol; 2-AG, 2-arachidonoylglycerol AMPAR, alpha-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid receptor.

cerebellum (Soler-Llavina and Sabatini, 2006). Homosynaptic eCB-LTD is triggered by repetitive stimulation (13 Hz) of prelimbic cortical glutamatergic inputs in the nucleus accumbens (Robbe et al., 2002), high-frequency stimulation (100 Hz) (Gerdeman et al., 2002), and moderate-frequency stimulation of excitatory inputs in dorsal striatum (10–25 Hz) (Ronesi et al., 2004; Kreitzer and Malenka, 2005), and bouts of stimulation (30 Hz) of parallel fibers in cerebellum (Soler-Llavina and Sabatini, 2006). In all cases, the induction of homosynaptic eCB-LTD requires group I mGluR activation and increased postsynaptic Ca^{2+}. Implicated in this [Ca^{2+}] increase are intracellular stores in nucleus accumbens (Robbe et al., 2002), L-type Ca^{2+} channels in the dorsal striatum (Calabresi et al., 1994; Choi and Lovinger, 1997), and postsynaptic calcium accumulation via Ca^{2+}-permeable AMPARs in the cerebellum (Soler-Llavina and

Sabatini, 2006). In the dorsal striatum, D2 receptor activation is also required for induction (Calabresi et al., 1997; Tang et al., 2001; Kreitzer and Malenka, 2005; Ronesi and Lovinger, 2005), and eCB release requires an uncharacterized transporter (Ronesi et al., 2004).

Heterosynaptic eCB-LTD has been reported at inhibitory synapses in the basolateral amygdala (BLA) (Marsicano et al., 2002) and the CA1 area of the hippocampus (Chevaleyre and Castillo, 2003). In both areas, repetitive activity of excitatory synaptic inputs onto principal neurons results in heterosynaptic LTD of GABA release from nearby inhibitory synapses (also known as I-LTD). This heterosynaptic LTD first requires the activation of postsynaptic group I mGluR on principal cells and then presynaptic CB1R on GABAergic terminals. In the hippocampus, eCB-LTD is triggered by stimulation protocols that commonly induce LTP at the Schaffer collateral to CA1 pyramidal cell synapses (i.e., high-frequency stimulation and theta-burst stimulation) and also by moderate-frequency stimulation of Schaffer collaterals (10 Hz) (Chevaleyre and Castillo, 2004). In contrast, BLA eCB-LTD is triggered by low-frequency stimulation (1 Hz) of excitatory inputs (Azad et al., 2004; Marsicano et al., 2002). The hippocampus and amygdala also differ in the nature of the eCB mediating LTD and their eCB production pathways. In the hippocampus, 2-AG release results from PLC-DGL activation (Chevaleyre and Castillo, 2003), whereas in the amygdala, AEA release requires the cAMP-protein kinase A (cAMP-PKA) pathway (Azad et al., 2004). It is unclear how PKA contributes to eCB release. Unlike eCB-LTD at excitatory synapses, LTD induction at inhibitory synapses does not require increased postsynaptic Ca^{2+}.

In addition to homosynaptic and heterosynaptic eCB-LTD, retrograde eCB signaling mediates a form of spike-timing-dependent synaptic plasticity (STDP), which is also known as timing LTD or tLTD. This form of plasticity was initially described at excitatory synapses between pairs of layer-5 pyramidal neurons in the visual cortex (Sjostrom et al., 2003) and more recently at excitatory inputs to layer 2/3 neurons in layer 4 of the somatosensory cortex (Bender et al., 2006). STDP refers to long-term changes in synaptic efficacy triggered by paired action potential firing in pre- and postsynaptic neurons (Abbott and Nelson, 2000; Bi and Poo, 2001; Dan and Poo, 2004). tLTD is triggered by pairing presynaptic stimulations with postsynaptic depolarizations or action potentials. Increased postsynaptic $[Ca^{2+}]$ is required for eCB release. Interestingly, LTD induction requires coactivation of both presynaptic CB1Rs and presynaptic NMDA receptors (see the section 'Presynaptic NMDA receptor-dependent forms of plasticity').

Activation of CB1Rs for several minutes is required for induction of eCB-LTD. In support of this idea, blockade of CB1Rs right after induction protocol (~1 min) abolished the induction of eCB-LTD in the hippocampus (Chevaleyre and Castillo, 2003) and in dorsal striatum (Ronesi et al., 2004). Although eCB-LTD is expressed as a reduction in evoked transmitter release, it does not reflect continued CB1R activation. Once established, eCB-LTD could not be reversed when CB1R antagonists were applied after a postinduction delay of 10 min in hippocampus (Chevaleyre and Castillo, 2003) and dorsal striatum (Ronesi et al., 2004), 15 min in visual cortex (Sjostrom et al., 2003), or 60 min in the nucleus accumbens (Robbe et al., 2002). Together, these observations suggest that eCB-LTD induction requires an additional molecular change at the presynaptic terminal that transduces the transient depression mediated by CB1R activation into a persistent depression that is CB1R independent. An important challenge is to understand what molecular processes occur in the presynaptic terminal during this time window that ultimately lead to a long-lasting change in transmitter release.

Spread of eCB signaling may affect the input specificity of eCB-LTD. Diffusion of eCBs could take place within the cytoplasm or plasma membrane or through the extracellular space, and its influence would be limited not only by dilution but also by transporter uptake and degradation (reviewed in Freund, 2003; Piomelli, 2003). Evidence that eCBs can diffuse between neighboring cells was reported in the hippocampus (Wilson and Nicoll, 2001) and cerebellum (Vincent and Marty, 1993; Kreitzer et al., 2002). In addition, spread of eCB signaling may also occur within or along a single cell. The dependence of eCB-LTD on retrograde signaling by diffusible messengers raises questions about the synapse specificity of eCB-LTD. However, studies in the cerebellum (Brown et al., 2003) and hippocampus (Chevaleyre and Castillo, 2004) suggest that synaptically driven release of eCBs may exert a subtle point-to-point regulation of synaptic inputs. Interestingly, in most synapses expressing eCB-LTD, depression of synaptic transmission induced by a CB1R agonist is fully reversed by CB1R antagonists, clearly suggesting that activation of CB1Rs is necessary but not

sufficient to trigger eCB-LTD. For example, coactivation of presynaptic NMDA receptor activation is also required for the induction of neocortical tLTD (Sjostrom et al., 2003; Bender et al., 2006). Although most forms of eCB-LTD are NMDA receptor independent, it is likely that along with CB1R activation, the induction of eCB-LTD also requires increased presynaptic Ca^{2+} (Chevaleyre et al., 2006), as occurs in the presynaptic terminal during repetitive activity. Thus, integration of these two signals by the presynaptic terminal may confer synapse specificity to this diffusible retrograde messenger–induced process.

What is the role of eCB-LTD in learning and memory? The functional relevance of this form of presynaptic plasticity obviously depends on the synapse type (i.e., excitatory or inhibitory) and brain structure where it is expressed. For example, by removing inhibition, eCBs may exert important effects in regulating the efficacy of excitatory inputs and in controlling the excitability of principal cells. Indeed, eCB-LTD at GABAergic inputs may increase postsynaptic excitability both in the hippocampus (Chevaleyre and Castillo, 2003; Chevaleyre and Castillo, 2004) and amygdala (Azad et al., 2004), two brain areas critically involved in the formation of new memories (LeDoux, 2000; Martin et al., 2000; Eichenbaum, 2004; Squire, 2004). In addition, disinhibition associated with heterosynaptic eCB-LTD facilitates the induction of LTP at hippocampal excitatory synapses (Chevaleyre and Castillo, 2004), a form of plasticity involved in spatial learning (Martin et al., 2000). This facilitation is a clear example of metaplasticity, a long-lasting change in the induction threshold for plasticity (Abraham and Bear, 1996). Thus, by regulating excitability and modifiability of LTP induction at excitatory synapses, eCB-LTD may contribute to experience-dependent changes of the neural circuits involved in memory formation.

In the amygdala, eCBs may play an important role in the extinction of learned fear (Marsicano et al., 2002). In fear conditioning learning, animals associate two stimuli (i.e., a tone and a foot shock) such that the repeated pairing of a tone with a foot shock leads to a freezing response when the tone is presented alone. Over the course of several repeated presentations of the tone without foot shock, animals learn to override this association (see reviews by Myers and Davis, 2002; Maren and Quirk, 2004; Kim and Jung, 2005) and stop freezing, an active process called extinction. Normal extinction of the conditioned fear response, not its acquisition or consolidation, requires intact eCB signaling (Marsicano et al., 2002). For example, in CB1R knockout mice, acquisition and consolidation of the conditioned response were normal, but the rate of extinction was much slower. In addition, when a CB1R antagonist was administered systemically to a trained mouse shortly before the first presentation of an unpaired tone, the degree of extinction that resulted was greatly reduced, an observation that has recently been confirmed by others in both mice (Suzuki et al., 2004) and rats (Chhatwal et al., 2005). eCB-LTD at GABAergic synapses may contribute to extinction of learned fear (Marsicano et al., 2002). However, the precise mechanism by which eCB-mediated disinhibition may lead to extinction learning is unclear. Interestingly, eliminating CB1R activity affects extinction memory in the Morris water maze, which suggests that the eCB system may play a role in facilitating extinction processes in the hippocampus as well (Varvel and Lichtman, 2002; Varvel et al., 2005).

It is commonly believed that long-term synaptic plasticity contributes to the enduring changes in brain reward circuits that follow exposure to drugs of abuse (Berke and Hyman, 2000; Hyman and Malenka, 2001; Nestler, 2001; Thomas and Malenka, 2003; Kauer, 2004; Jones and Bonci, 2005). The widespread nature of eCB-LTD suggests it may be involved in this process. Indeed, experimental evidence suggests that eCB signaling may influence the modifications of the brain reward circuits believed to underlie the rewarding properties of drugs of abuse (for reviews, see De Petrocellis et al., 2004; Lupica et al., 2004; Gardner, 2005). *In vivo* exposure to THC, presumably by inducing functional tolerance of the CB1R, abolishes eCB-LTD in nucleus accumbens (NAc) (Hoffman et al., 2003; Mato et al., 2004). This THC-induced modification of eCB-LTD could affect the development of drug addiction. Although eCB-LTD may contribute to reward-motivated, learned behaviors, direct evidence for this possibility still awaits discovery.

4.36.4.4 Presynaptic NMDA Receptor-Dependent Forms of Plasticity

Most forms of long-term plasticity commonly depend on postsynaptic NMDA receptors. For example, in the 'classic' form of synaptic plasticity at the Schaffer collateral-to-CA1 pyramidal cell synapse, depolarization produced by convergent synaptic inputs or postsynaptic firing removes the Mg^{2+} block of postsynaptic NMDA receptors, allowing

Ca^{2+} entry into target neurons. The NMDA receptor-mediated rise in postsynaptic Ca^{2+} activates a network of kinases and phosphatases that promote enduring changes in synaptic strength (Scannevin and Huganir, 2000; Winder and Sweatt, 2001). Growing evidence indicates that not all NMDA-dependent synaptic plasticity is induced postsynaptically. As discussed later, several studies have revealed presynaptic forms of plasticity that rely on the activation of presynaptic NMDA receptors and may play important roles in learning and memory.

The idea of presynaptic NMDA receptor-dependent forms of plasticity is supported by numerous studies showing a presynaptic location and function of these receptors. First, presynaptic NMDA receptors have been observed on several presynaptic fibers including primary afferent terminals in the spinal cord and brainstem (Liu et al., 1994; Lu et al., 2003); on GABAergic axon terminals in the basal forebrain, thalamus, hypothalamus, and some brainstem nuclei (Paquet and Smith, 2000); as well as on excitatory terminals in the neocortex (Aoki et al., 1994), hippocampus (Siegel et al., 1994), cerebellum (Petralia et al., 1994), and amygdala (Farb et al., 1995). Second, presynaptic NMDA receptors have been reported to enhance spontaneous transmitter release in the entorhinal cortex (Berretta and Jones, 1996; Woodhall et al., 2001), visual cortex (Sjostrom et al., 2003), and cerebellum (Glitsch and Marty, 1999) and to depress evoked transmitter release in the dorsal horn (Bardoni et al., 2004) and cerebellum (Glitsch and Marty, 1999). Together, these studies build a strong case for functional presynaptic NMDA receptors at both excitatory and inhibitory terminals. Furthermore, presynaptic NMDA receptors reportedly mediate enduring forms of transmitter release modulations (see later discussion). Thus, although most studies have examined postsynaptic roles for NMDA receptors, it has become clear that presynaptic NMDA receptors can transiently regulate transmitter release and also induce long-term changes in synaptic efficacy.

In the classic model of NMDA receptor operation, activation of these receptors requires simultaneous glutamate binding and sufficient depolarization to relieve the voltage-dependent Mg^{2+} block of the channel. Given a glutamatergic synapse, how then can presynaptic NMDA receptors be activated, if presynaptic depolarization is generally over by the time glutamate binds to these receptors? This question is particularly relevant when inducing protocols contain moderate (usually physiological) frequencies of presynaptic activity (<30 Hz). Two 'solutions' to this problem have been identified (**Figure 5**). First, glutamate may not arise from the presynaptic terminal but from neighboring afferents (e.g., heterosynaptic plasticity) or from the postsynaptic neuron. In these cases, presynaptic NMDA receptors operate as coincidence detectors of presynaptic depolarization and glutamate release (Duguid and Sjostrom, 2006), thereby conferring the properties of associativity and input specificity (i.e., only active synapses undergo plasticity) to this form of plasticity. Second, glutamate binding alone may be sufficient for activation of presynaptic NMDA receptors. It has been

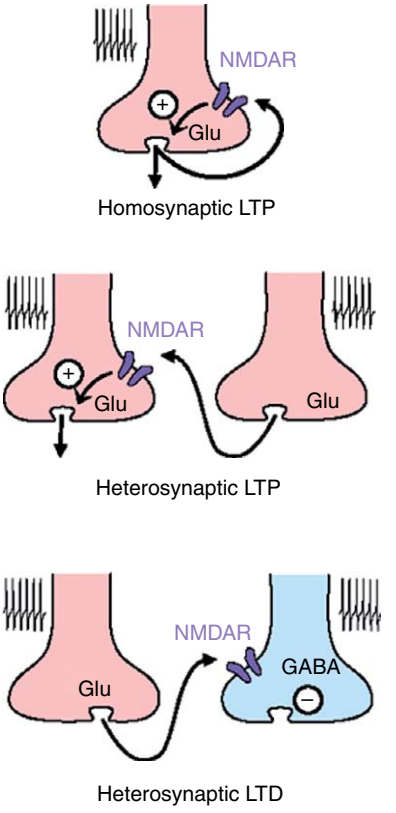

Figure 5 Schematic diagram of presynaptic *N*-methyl-D-aspartate receptor (NMDAR)-dependent forms of plasticity. Homosynaptic long-term potentiation (LTP), a nonassociative form of plasticity, has been observed at thalamic afferents in the central amygdala. Heterosynaptic LTP has been reported at excitatory cortical inputs to principal cells in the lateral amygdala. Glutamate (Glu) released from thalamic terminals following repetitive stimulations activates presynaptic NMDA receptors on cortical afferents, causing a long-lasting increase in transmitter release. Heterosynaptic long-term depression (LTP) occurs in the developing *Xenopus* retinotectal system, where repetitive activity of glutamatergic inputs (and presumably inhibitory inputs) reportedly induces long-term reduction of gamma-amino butyric acid (GABA) release from neighboring inhibitory terminals.

recently established that the subunit composition of NMDA receptors has important implications for the biophysical properties of the receptor. Indeed, NMDA receptors containing the NR2C and NR2D subunits have reduced sensitivity to Mg^{2+} (Kutsuwada et al., 1992; Monyer et al., 1992; Ishii et al., 1993; Monyer et al., 1994; Kuner and Schoepfer, 1996). A recent report provided functional evidence that these receptors may localize to presynaptic fibers (Mameli et al., 2005). As discussed later, there are good examples for both forms of presynaptic NMDA receptor-dependent plasticity. Although the precise mechanism by which a transient activation of presynaptic NMDA receptors leads to long-term changes in transmitter release remains to be identified, these forms of plasticity have already been shown to play an important role in memory formation and in sensory-dependent refinement of neuronal connections in the developing nervous system.

Good examples of presynaptic NMDA receptor-dependent plasticity can be found in the amygdala, a brain structure critically involved in the acquisition and possibly the storage of fear memories (LeDoux, 2000; Davis and Whalen, 2001; Maren, 2001; Maren and Quirk, 2004; Rodrigues et al., 2004). Importantly, some of the best evidence for the involvement of long-term synaptic plasticity in learning comes from fear conditioning (McKernan and Shinnick-Gallagher, 1997; Rogan et al., 1997; Rumpel et al., 2005). A key issue is what synaptic inputs are relevant in conditioning-related synaptic plasticity, and whether this plasticity is induced and expressed pre- or postsynaptically (for recent reviews, see Rodrigues et al., 2004; Maren, 2005). Several reports have demonstrated presynaptic forms of plasticity in the amygdala (McKernan and Shinnick-Gallagher, 1997; Huang and Kandel, 1998; Bauer et al., 2002; Tsvetkov et al., 2002; Humeau et al., 2003; Samson and Pare, 2005) that may contribute to fear learning (McKernan and Shinnick-Gallagher, 1997; Tsvetkov et al., 2002; Apergis-Schoute et al., 2005). LTP of thalamic or cortical inputs can be induced by pairing presynaptic and postsynaptic activation. This LTP is induced postsynaptically (involving NMDA receptors and L-type voltage-gated Ca^{2+} channels) but is expressed presynaptically as an increased probability of transmitter release (McKernan and Shinnick-Gallagher, 1997; Huang and Kandel, 1998; Bauer et al., 2002; Tsvetkov et al., 2002). The nature of the retrograde signal mediating this long-term increase in release remains to be identified.

Two recent studies in the lateral and central amygdala reported a form of LTP that is induced and expressed presynaptically and is NMDA receptor-dependent. In the lateral amygdala, repetitive activation of thalamic and cortical excitatory inputs to principal neurons produces LTP of cortical but not thalamic inputs (Humeau et al., 2003). Interestingly, activation of either pathway in isolation had no effect (except after pharmacological inhibition of glutamate uptake), indicating that this form of plasticity is associative. Although bath application of an NMDA receptor antagonist abolished LTP in the lateral amygdala, intracellular blockade of postsynaptic NMDA receptors as well as postsynaptic Ca^{2+} chelation or hyperpolarization did not prevent its induction. Importantly, this LTP was associated with a reduction in PPF, but no increase in quantal size, suggesting changes in synaptic release probability. In addition, LTP was occluded when release probability was artificially increased by raising the extracellular Ca^{2+} concentration. Taken together, these observations led the authors to conclude that glutamate released from thalamic terminals following repetitive stimulations activates presynaptic NMDA receptors on cortical afferents, causing a long-lasting increase in the probability of glutamate release. In the central nucleus of the amygdala, the primary output nucleus, high-frequency stimulation (HFS) of thalamic inputs led to LTP of thalamic responses. Induction of this form of LTP also requires presynaptic NMDA receptors (i.e., LTP was markedly reduced by global NMDA receptor antagonism, but blockade of postsynaptic NMDA receptors had no effect) (Samson and Pare, 2005). This form of LTP is also likely due to an increase in glutamate release as indicated by an associated reduction of PPF. Although changes in PPF generally indicate a presynaptic origin, a network effect cannot be completely discarded. Unlike LTP of cortical afferents in the lateral amygdala, LTP of thalamic afferents in central amygdala is homosynaptic and nonassociative. Recent studies have reported postsynaptically expressed forms of plasticity in the amygdala (Humeau et al., 2005; Rumpel et al., 2005), clearly highlighting the diversity of mechanisms underlying plasticity in this brain structure (Maren, 2005). Future studies will have to determine the precise functional role for the different forms of plasticity at thalamic and cortical inputs in the encoding, storage, and retrieval of fear memories.

Inhibitory synapses also exhibit NMDA receptor-dependent forms of presynaptic plasticity. Activation

of putative presynaptic NMDA receptors exerts complex effects at inhibitory synapses; namely, it increases miniature inhibitory postsynaptic current (mIPSC) frequency in Purkinje cells and interneurons (basket and stellate cells), and it decreases evoked IPSCs at interneuron–interneuron synapses (Glitsch and Marty, 1999) More recently, Duguid and Smart (2004) reported a novel form of plasticity at GABAergic inputs onto Purkinje cells that is caused by the activation of presynaptic NMDA receptors. These investigators showed that depolarization of Purkinje cells induces a potentiation of inhibitory synaptic transmission that lasts several minutes, a phenomenon they called 'depolarization-induced potentiation of inhibition' (DPI). This phenomenon is triggered by an increase in postsynaptic Ca^{2+} and can be evoked by repetitive activation of climbing fibers. DPI is blocked by the NMDA receptor antagonist D-APV and can be mimicked by brief applications of NMDA. Importantly, DPI induction affects mIPSC frequency and the paired-pulse ratio and coefficient of variation of evoked IPSCs, indicating that the site of action is presynaptic. Based on these observations, Duguid and Smart (2004) proposed that a rise in postsynaptic Ca^{2+} following depolarization of the Purkinje cell releases glutamate that diffuses to activate presynaptic NMDA receptors. They also presented experimental evidence that Ca^{2+} influx through NMDA receptors causes Ca^{2+} release from intracellular ryanodine-sensitive Ca^{2+} stores, which then causes an increase in GABA release probability. NMDA receptors are commonly blocked by external Mg^{2+} in a voltage-dependent manner. Interestingly, no prior depolarization of the interneuron was needed to induce DPI, suggesting that presynaptic NMDA receptors on GABAergic terminals have a low Mg^{2+} sensitivity. How glutamate is released from Purkinje cells is still unknown. Although a role of DPI in cerebellar-dependent learning awaits discovery, this potentiation of inhibition may provide a way to reduce Purkinje cell excitability following strong activity. It will be interesting to see if DPI is found in other brain structures as well.

A recent study has provided the first evidence of a presynaptic, NMDA receptor-dependent form of long-term plasticity that can occur *in vivo* (Lien et al., 2006). Lien et al. showed that light stimuli or theta-burst stimulation (TBS) of the optic nerve in the developing *Xenopus* retinotectal system induced LTP of glutamatergic synapses but LTD of GABAergic synapses onto the same tectal neuron. This LTD is likely due to a reduction of GABA release as indicated by an increase in both failure rate and coefficient of variation. Intriguingly, PPF was not changed after LTD induction. Other common manipulations known to decrease Pr in most synapses (i.e., by reducing extracellular Ca^{2+} concentration, blocking presynaptic Ca^{2+} influx with Cd^{2+}, or activating $GABA_B$ receptors) did not affect PPF either, suggesting that PPF may not be a sensitive method to assess Pr at these synapses. As for other presynaptic forms of plasticity that rely on the activation of presynaptic NMDA receptors, the induction of this LTD was blocked by D-APV application but was independent of postsynaptic activity in the tectal neuron, postsynaptic NMDA receptor activation, and postsynaptic Ca^{2+} influx. Although exogenous application of NMDA depressed the amplitude of IPSCs that were evoked at a low frequency (0.1 Hz), this effect was reversed on washout, suggesting that NMDA receptor activation is not sufficient to induce LTD. Interestingly, transient depression induced by exogenous NMDA became long lasting when NMDA application was paired with a weak TBS that by itself was insufficient to induce LTD. These observations strongly suggest that in addition to high-frequency bursts of excitatory fibers, which presumably release enough glutamate to activate presynaptic NMDA receptors on inhibitory fibers, the induction of LTD requires coincident high-level GABAergic activity. In this regard, the heterosynaptic LTD in the developing *Xenopus* retinotectal system constitutes a clear example of presynaptic NMDA receptors functioning as coincidence detectors for glutamatergic and GABAergic activities. Thus, coordinated glutamatergic and GABAergic presynaptic activity can lead to long-term synaptic modifications that may be important not only to experience-dependent changes of neural circuits during development but also to memory formation.

It is worth noting that the LTD reported by Lien et al. in the *Xenopus* retinotectal system resembles the LTD previously described at GABAergic synapses in the hippocampus (Chevaleyre and Castillo, 2003) and the amygdala (Azad et al., 2004). For example, these are all heterosynaptic forms of plasticity requiring repetitive activation of glutamatergic fibers resulting in a long-lasting reduction of GABA release. However, unlike LTD in the hippocampus and amygdala, tectal LTD is NMDA receptor-dependent but cannabinoid-receptor independent, clearly highlighting the variety of mechanisms underlying presynaptic plasticity at GABAergic synapses. Tectal LTD also resembles spike-timing dependent LTD in neocortex (Sjostrom

et al., 2003). In both cases, presynaptic NMDA receptors are necessary but not sufficient to induce LTD. Whereas simultaneous coactivation of presynaptic CB1 receptors is required for the induction of LTD in the visual cortex, GABAergic afferent activity seems to be the additional signal necessary for the induction of tectal LTD. Also, in both forms of plasticity, NMDA receptors appear to operate as coincidence detectors, presumably in a slightly different way than the more classic coincidence detection mediated by these receptors on the postsynaptic side (Duguid and Sjostrom, 2006). Future studies will determine how the presynaptic terminal integrates the diverse signals required for the induction of LTD and also which downstream signaling pathways and presynaptic targets mediate long-term reductions in transmitter release.

4.36.5 Links between Short-Term Presynaptic Plasticity and Learning and Memory

Short-term plasticity includes synaptic plasticity lasting on the order of a few minutes or less. The most commonly studied forms of short-term plasticity include PPF, PPD, synaptic depression, and PTP and augmentation. The physiologic characteristics and mechanisms of these forms of plasticity have recently been extensively reviewed (Zucker and Regehr, 2002). Very few studies have specifically addressed the question of the role of short-term plasticity in learning and memory (see **Table 1**).

Although progress has been made in understanding which presynaptic proteins might be involved in short-term forms of plasticity such as PPF and PTP (Castillo et al., 2002; Schoch et al., 2002; Zucker and Regehr, 2002; Sudhof, 2004), the role of short-term plasticity in information processing and storage remains to be determined (Abbott and Regehr, 2004). Is there a role for short-term plasticity in learning and memory? How could synaptic alterations lasting milliseconds to minutes play such a role? Does short-term plasticity play a role in rapid information processing such as sensorimotor gating or selective attention during learning? A complete picture of how the brain processes and stores information will depend on a fundamental understanding of these questions.

Initial studies suggested that a decrease in PPF may lead to impairments in hippocampus-dependent learning, whereas increased PPF has no effect. Silva and colleagues began with a systematic study of the role of PPF and PTP in learning and memory using mice that were heterozygous for Ca^{2+}/calmodulin-dependent protein kinase II (αCaMKII$^{+/-}$). αCaMKII-deficient mice exhibit increased PTP and decreased PPF in area CA1 of the hippocampus with normal CA1 LTP (Chapman et al., 1995) and were impaired in both fear conditioning and Morris water maze learning (Silva et al., 1996). Thus, increased PTP or decreased PPF was implicated in the learning deficits. In a study by Matilla et al., mice lacking ataxin-1 were found to have decreased PPF with normal PTP and LTP in area CA1 (Matilla et al., 1998). These mice with an apparently isolated decrease in PPF exhibited severe spatial learning deficits in the Morris water maze (Matilla et al., 1998), though baseline Pr was not measured in these mice. These studies suggest that decreased PPF can lead to impairments in hippocampus-dependent learning, whereas increasing PPF has no effect.

In addition to decreased PPF, decreased PTP alone appears sufficient to cause learning and memory deficits. In the same study by Silva et al., mice deficient in synapsin II or synapsins I and II showed decreased PTP, with normal PPF and LTP in CA1, and were impaired in fear conditioning (Silva et al., 1996).

Although decreases in PPF and PTP may lead to learning and memory deficits, it appears that increased PPF alone may not be sufficient to perturb learning and memory. A recent study by Powell et al. (2004) examined two presynaptic protein knockout mice that had previously been extensively characterized electrophysiologically and exhibited increased PPF in area CA1 of the hippocampus. Rab3A$^{-/-}$ mice had increased CA1 PPF with no change in baseline Pr (Castillo et al., 2002; Schoch et al., 2002) and exhibited completely normal water maze and fear conditioning learning (Powell et al., 2004). Two other studies confirmed this lack of learning and memory deficits in Rab3A$^{-/-}$ mice (Hensbroek et al., 2003; D'Adamo et al., 2004). One of these studies of Rab3A$^{-/-}$ mice demonstrated more subtle deficits in working memory and episodic-like memory, though these abnormalities were attributed to altered long-term plasticity at mossy fiber synapses in area CA3 of the hippocampus (D'Adamo et al., 2004). Similarly, synaptotagmin 1 point mutant knockin mice (Syt1R233Q$^{+/+}$) exhibited increased PPF along with an approximately 50% decrease in Pr in area CA1 (Fernandez-Chacon et al., 2001).

Table 1 Summary of short-term plasticity mutants and behavioral results

	PPF	*PTP*	*LTP*	*Fear conditioning*	*Water maze*	*Other*	*Reference(s)*
αCaMKII +/−	↓	↑	Normal	↓	↓		Silva et al., 1996
Ataxin I −/−	↓	Normal	Normal	–	↓		Matilla et al., 1998
Synapsin II −/−	Normal	↓	Normal	↓	–		Silva et al., 1996
Synapsin I/II −/−	Normal	↓	Normal	↓	–		Silva et al., 1996
Synapsin I −/−	↑	Normal	Normal	Normal	Normal		Silva et al., 1996
Rab3A −/−	↑	Normal	Normal	Normal	Normal	↓ mfLTP	Castillo et al., 2002; Powell et al., 2004; Schoch et al., 2002
				Normal	Normal	↓ mfLTP	Hensbroek et al., 2003
				Normal	↓ reversal learning		D'Adamo et al., 2004
				↓ Cue fear conditioning	Normal		Yang et al., 2006
Synaptotagmin 1 R233Q +/+	↑	–	–	Normal	Normal	↓ PrCA1	Powell et al., 2004
Synaptotagmin IV −/−	↑	↑	↑	↓ Passive avoidance (Normal object recognition)	–		Ferguson et al., 2000

PPF, paired-pulse facilitation; PTP, post-tetanic potentiation; Pr, probability of evoked neurotransmitter release; mfLTP, mossy fiber long-term potentiation in area CA3; —, not tested. All electrophysiological data are from area CA1 of the hippocampus unless otherwise specified.

Surprisingly, Syt1R233Q$^{+/+}$ mice showed normal water maze and fear conditioning learning (Powell et al., 2004) despite the significant alteration in Pr in CA1 and likely at other central synapses. Similarly, synapsin I deficient mice showed increased PPF but no deficits in fear conditioning or water maze learning (Silva et al., 1996). In synaptotagmin IV knockout mice, both PPF and PTP were increased, and the mice showed normal novel object recognition but impaired long-term passive avoidance memory (Ferguson et al., 2004). This difference could be due to the different behavioral paradigms compared to the other studies or due to an interaction between increased PPF and increased PTP. Thus, increased PPF alone did not appear to adversely affect learning and memory in the Morris water maze or fear conditioning paradigms, nor did it augment learning.

Although these initial studies are beginning to converge on the idea that PPF and perhaps PTP are necessary for or involved in normal learning and memory, additional confirmation is necessary. In particular, it will be important to examine additional mouse models in which short-term plasticity is affected in isolation of other synaptic physiological abnormalities. Mice lacking various modulators of presynaptic plasticity and release are excellent candidates for such genetic manipulation. In future studies, it will be helpful to restrict the knockout of such targets to specific presynaptic neurons, perhaps in subregions of the hippocampal formation using conditional knockout approaches. Similarly, it will be important to expand the number of learning and memory paradigms used to characterize these mice. Working memory is one such understudied form of memory in which short-term plasticity might play a critical role. In addition to learning and memory paradigms, it will also be important to assess other aspects of behavior, such as sensorimotor gating, selective attention, and impulsivity.

4.36.6 Links between Long-Term Presynaptic Plasticity and Learning and Memory

The mechanisms involved in presynaptically expressed forms of LTP are being elucidated (Weisskopf et al., 1994; Huang et al., 1995; Weisskopf and Nicoll, 1995; Salin et al., 1996a,b; Tong et al., 1996; Castillo et al., 1997; Kapur et al., 1998; Lonart et al., 1998; Villacres et al., 1998; Yeckel et al., 1999; Henze et al., 2000; Kumar et al., 2001; Mellor and Nicoll, 2001; Poser and Storm, 2001; Castillo et al., 2002; Lonart, 2002; Schmitz et al., 2003; Ferguson et al., 2004; Wang et al., 2004). Although present at cerebellar parallel fiber-Purkinje cell synapses and at corticothalamic synapses, presynaptically expressed LTP has been most extensively studied at the dentate granule cell mossy fiber axon-to-CA3 pyramidal neuron synapse in the hippocampus (mfLTP). Recent advances in our understanding of how the CA3 region of the hippocampus may be involved in certain types of episodic memory, pattern completion, and pattern separation make understanding the role of this form of presynaptic plasticity particularly relevant to understanding hippocampal function.

Several molecular manipulations in mice have led to absent or reduced mfLTP with or without additional synaptic plasticity deficits. Results of behavioral analyses in these mice are mixed, but a relatively clear picture is emerging (see **Table 2**). The convergent evidence from several studies suggests that a lack of mfLTP does not affect traditional water maze, delay fear conditioning, or trace fear conditioning, but may have a subtle effect on episodic-like memory versions of the water maze (delayed matching to place) and perhaps working memory. This conclusion, however, is subject to alternative interpretations due to the limitations of traditional knockout studies mentioned earlier.

mfLTP seems to be required for more complex forms of spatial memory requiring cognitive flexibility or pattern recognition, a function consistent with current ideas about the role of plasticity in recurrent-collateral synapses in area CA3 of the hippocampus (Nakazawa et al., 2002; Nakazawa et al., 2003; Kesner et al., 2004). The behavioral ramifications of altering mfLTP have been studied in several lines of mice lacking mfLTP. An early study from Eric Kandel's group demonstrated that absence of the regulatory (R1β) or catalytic (Cβ1) subunit of PKA prevents mfLTP while sparing several learning and memory tasks, including the Morris water maze, fear conditioning, and radial arm maze (Huang et al., 1995). Subsequently, Hensbroek and collaborators showed that the absence of mfLTP, in Rab3A$^{-/-}$ mice tested with two different genetic backgrounds, did not affect delay fear conditioning or the Morris water maze (Hensbroek et al., 2003). Our own data and those of D'Adamo et al. confirmed this finding (D'Adamo et al., 2004; Powell et al., 2004). A more thorough behavioral characterization of the Rab3A$^{-/-}$ mice,

Table 2 Summary of long-term presynaptic plasticity mutants and behavioral results

	mfLTP	*CA1 LTP*	*Fear conditioning*	*Spatial memory*	*Other*	*Reference(s)*
PKA Cβ1−/−	↓	Normal E-LTP ↓ L-LTP	Normal	Normal water maze and Barnes maze	↓ CA1 LTD	Huang et al., 1995; Qi et al., 1996
PKA R1β**−/−**	↓	Normal E-LTP Normal L-LTP	Normal	Normal water maze and Barnes maze	↓ CA1 LTD	Brandon et al., 1995; Huang et al., 1995
Rab3A−/−	↓	Normal E-LTP	Normal	Normal water maze	↑ CA1 PPF	Castillo et al., 1997; Castillo et al., 2002; Powell et al., 2004
	↓		Normal Normal delay and trace fear conditioning	Normal water maze Normal initial water maze ↓ Reversal learning		Hensbroek et al., 2003 D'Adamo et al., 2004
Type I adenylyl cyclase−/−	↓	↓ E-LTP	–	↓ Water maze		Villacres et al., 1998;Wu et al., 1995
MGluR1−/−	↓	Normal E-LTP			↓ Cerebellar LTP ↓ Corticostriatal plasticity Cerebellar synaptic abnormalities	Bordi et al., 1997; Conquet et al., 1994;Gubellini et al., 2001; Lapointe et al., 2004
PAC1−/−	↓	–	↓ Contextual normal cue	Normal water maze	↓ Anxiety ↑ Locomotor activity Normal PP-dentate LTP	Otto et al., 2001a; Otto et al., 2001b

PKA cβ1, catalytic subunit of protein kinase A; PKA R1β, regulatory subunit of protein kinase A; E-LTP, early phase of long-term potentiation (lasting 1–3 h); L-LTP, late protein synthesis-dependent phase of long-term potentiation; MGluR1, metabotropic glutamate receptor type 1; PAC1, pituitary adenylyl cyclase receptor type 1; PP-dentate LTP, LTP of the perforant path to dentate granule cell in the hippocampal formation.

however, indicated deficits in the delayed match to place version of the Morris water maze and in working memory (D'Adamo et al., 2004). A subsequent study of Rab3A$^{-/-}$ mice supported normal spatial learning and contextual fear conditioning, though the authors found a deficit in cue-mediated fear conditioning (Yang et al., 2007). Overall, these data indicate that mfLTP is only required for more complex forms of spatial memory but not for initial learning of fear conditioning or the Morris water maze.

Other reports have suggested a more direct role for mfLTP in learning tasks. Mice lacking the metabotropic glutamate receptor (mGluR1) and type I adenylyl cyclase (AC1) also show subtle deficits in the Morris water maze (Conquet et al., 1994; Wu et al., 1995; Villacres et al., 1998). AC1 knockout mice also exhibit abnormalities in area CA1 LTP, which could explain their learning and memory deficits (Wu et al., 1995). Similarly, mGluR1 knockouts had severe motor abnormalities (Conquet et al., 1994), cerebellar synaptic abnormalities (Conquet et al., 1994), and synaptic plasticity abnormalities at corticostriatal and other synapses (Bordi et al., 1997; Gubellini et al., 2001; Gubellini et al., 2003; Lapointe et al., 2004). Another line of mice, PACAP (pituitary adenylyl cyclase activating peptide) type I receptor knockout mice, have absent mfLTP and normal water maze learning but impaired fear conditioning (Otto et al., 2001a,b). However, PACAP knockout mice also exhibit impairments in other emotional behaviors (Otto et al., 2001b). Because these molecules are upstream of the likely final common effectors of mfLTP, there are many opportunities for molecular divergence and effects on neuronal functions unrelated to mfLTP, making direct interpretation difficult.

The three molecular manipulations closest to the presumed final effectors of mfLTP expression, Rab3A and R1β or Cβ1 subunits of PKA, all lead to absent mfLTP with intact learning and memory on multiple paradigms. Three separate groups, in more than two different genetic backgrounds, have confirmed the results in Rab3A$^{-/-}$ mice. Among the remaining studies, only one of two learning and memory paradigms, either fear conditioning or water maze, was affected where both were tested, and the affected paradigm varied across studies. Thus, although it is possible that specific molecular accommodations account for these differences, the simplest interpretation is that mfLTP is involved in more complex, episodic-like spatial memory tasks. mfLTP does not appear to be consistently involved in classic water maze or delay or trace fear conditioning. Still, additional studies of existing mice will be helpful in both confirming the involvement of mfLTP in episodic-like memory as well as its lack of involvement in fear conditioning and water maze learning.

4.36.7 Role for Additional Presynaptic Proteins in Learning and Memory Behavior

4.36.7.1 RIM1α

RIM1α is an active zone protein primarily expressed in the brain that is involved in several aspects of presynaptic function (Castillo et al., 2002; Schoch et al., 2002; Calakos et al., 2004). RIM1α was identified as a putative Rab3A effector protein and found to be highly localized to the active zone region of central synapses (Wang et al., 1997). RIM1α interacts with multiple presynaptic active zone and synaptic vesicle proteins *in vivo* and is thought to act as a molecular scaffolding protein involved in organizing and modulating synaptic release machinery (**Figure 1**) (Wang Y et al., 1997, 2000, 2002; Betz et al., 2001; Coppola et al., 2001; Ohtsuka et al., 2002; Schoch et al., 2002; Takao-Rikitsu et al., 2004). Interactions *in vitro* occur between RIM1α and active zone components Munc13-1 (Betz et al., 2001), SNAP25, voltage-gated Ca^{2+} channels (Coppola et al., 2001), and the cytomatrix at the active zone-associated structural protein (CAST) (Takao-Rikitsu et al., 2004) as well as the synaptic vesicle–associated proteins Rab3A (Wang et al., 1997) and synaptotagmin 1 (Coppola et al., 2001). Additionally, RIM1α interacts with 14-3-3 (Sun et al., 2003), α-liprins (Schoch et al., 2002), and a family of RIM binding proteins (Wang et al., 2002). Its selective localization to presynaptic active zones and its interactions with multiple presynaptic molecules place RIM1α in a key position to modulate presynaptic release and plasticity.

Available evidence from acute hippocampal slices suggests that RIM1α modulates neurotransmitter release and presynaptically mediated plasticity. Mice lacking RIM1α (RIM1$\alpha^{-/-}$) display an ~50% reduction in probability of evoked neurotransmitter release (Pr) at the Schaffer collateral to CA1 pyramidal neuron synapse in hippocampal slices (Schoch et al., 2002). This decrease in Pr is accompanied by an increase in PPF in the same synapses. Prolonged stimulation at 14 Hz reveals a lack of normal synaptic

depression (Schoch et al., 2002). In addition, PTP, another presynaptically expressed form of short-term plasticity, is augmented in RIM1$\alpha^{-/-}$ mice (Schoch et al., 2002). Analysis of hippocampal synapses in RIM1$\alpha^{-/-}$ mice at the electron microscopic level revealed no significant ultrastructural abnormalities. Additionally, Western blot data analyzing multiple other synaptic proteins revealed only a reduction in Munc13-1 levels. Reduction of Munc13-1 in Munc13-1 heterozygous mice, however, does not lead to any of the synaptic physiology abnormalities seen in RIM1$\alpha^{-/-}$ mice (Schoch et al., 2002), so decreased Munc13-1 is unlikely to contribute to the RIM1α knockout phenotype.

A subsequent study of RIM1α-deficient hippocampal autapses in culture revealed that the RIM1$\alpha^{-/-}$ mice exhibit decreased Pr via a decrease in the readily releasable pool of synaptic vesicles (Calakos et al., 2004). Curiously, the decreased Pr in RIM1$\alpha^{-/-}$ hippocampal cultures is observed only during the initial 4–5 stimuli during sustained, 14-Hz stimulation (Calakos et al., 2004). Wild-type synapses exhibit depression of Pr during 14-Hz stimulation, whereas RIM1$\alpha^{-/-}$ synapses do not. Thus, the relationship between Pr and frequency of synaptic activation in the RIM1$\alpha^{-/-}$ mice is complex. In autaptic hippocampal cultures, at low frequencies, RIM1$\alpha^{-/-}$ mice exhibit decreased Pr, whereas at 14 Hz, and presumably higher frequencies, the Pr of RIM1$\alpha^{-/-}$ mice becomes similar to that of wild-type mice after the first four to five responses (Schoch et al., 2002). Nevertheless, loss of RIM1α affects presynaptically mediated forms of synaptic plasticity including PPF, PTP, and synaptic depression at 14 Hz.

RIM1$\alpha^{-/-}$ mice were also deficient in presynaptically expressed PKA-dependent forms of LTP. At the mossy fiber to CA3 pyramidal neuron synapse in the hippocampus, mfLTP is absent in RIM1$\alpha^{-/-}$ mice (Castillo et al., 2002). Similarly, presynaptically expressed LTP at the parallel fiber/Purkinje cell synapse in the cerebellum is absent in these mice (Castillo et al., 2002; Lonart et al., 2003). RIM1α is a PKA substrate *in vivo* and is primarily phosphorylated by PKA at serine 413 (Lonart et al., 2003), and PKA is the only known kinase for this site (Lonart et al., 2003). Phosphorylation of RIM1α at serine 413 is also required for presynaptically expressed LTP in cerebellar neurons. RIM1$\alpha^{-/-}$ cerebellar cultures lack presynaptic LTP, which can be rescued by expression of wild-type RIM1α. Expression of PKA phosphorylation site mutant RIM1αS413A fails to rescue presynaptic LTP in the same cultures (Lonart et al., 2003). Thus, phosphorylation of RIM1α by PKA is thought to be responsible for synaptic potentiation via increased Pr, or at least to be permissive for some other mechanism of increased Pr.

RIM1α's interaction with 14-3-3 protein and the small, synaptic vesicle associated GTPase Rab3A is also required for presynaptic LTP at least in cerebellar cultured neurons (Simsek-Duran et al., 2004). Thus presynaptically expressed forms of LTP are thought to require presynaptic activation of PKA and phosphorylation of RIM1α, which leads, via required interactions with 14-3-3 and Rab3A, to increased Pr (or RIM1α phosphorylation, and its interactions are permissive for a parallel mechanism to increase Pr).

The abnormalities in both short- and long-term plasticity in RIM1$\alpha^{-/-}$ mice led to the question of whether RIM1α might play a critical role in learning and memory. A broad behavioral analysis of RIM1$\alpha^{-/-}$ mice revealed abnormalities in learning and memory in both Morris water maze and fear conditioning (Powell et al., 2004). Despite the various synaptic physiology abnormalities and the widespread expression of RIM1α throughout the brain, RIM1$\alpha^{-/-}$ mice revealed normal motor coordination and anxiety-like behaviors (Powell et al., 2004).

The abnormalities in Morris water maze behavior imply abnormal hippocampus-dependent learning in the RIM1$\alpha^{-/-}$ mice. Although RIM1$\alpha^{-/-}$ mice are deficient in mfLTP, Rab3A$^{-/-}$ mice with mfLTP deficits, increased PPF, and abnormal synaptic depression exhibit normal learning and memory (Powell et al., 2004). Because mfLTP, PPF, and depression alone are unlikely to play a role on the RIM1α learning and memory deficits, the decrease in Pr in area CA1 seemed a likely candidate.

A decrease in Pr in isolation, however, does not necessarily alter learning and memory behavior. Syt1R233Q$^{+/+}$ mice have a similar decrease in Pr in CA1 as the RIM1$\alpha^{-/-}$ mice, but they exhibit normal learning and memory using the same tasks (Powell et al., 2004). Regardless of any molecular accommodations in these mice, the decreased Pr remains, suggesting that Syt1R233Q$^{+/+}$ mice can somehow accommodate at the synaptic or circuit level to dramatic alterations in their Pr setpoint.

Other possibilities remain to explain the behavioral abnormalities in RIM1α-deficient mice. These include increased PTP at excitatory synapses and altered PPD at inhibitory synapses. Furthermore,

the decreased Pr in RIM1$\alpha^{-/-}$ mice may differ from that of Syt1R233Q$^{+/+}$ mice. For example, it is possible that the synaptotagmin 1 mutation affects inhibitory and excitatory synapses equally, whereas the loss of RIM1α may affect excitatory more than inhibitory synapses. Such differential regulation of neurotransmitter release between excitatory and inhibitory synapses has been described for other presynaptic proteins such as Munc13-1 and Munc13-2 (Varoqueaux et al., 2002). In addition, it may be that the multiple synaptic abnormalities in RIM1$\alpha^{-/-}$ mice interact to lead to the behavioral deficits, though this conclusion requires excluding individual synaptic abnormalities as a cause. Finally, although LTP in area CA1 of the hippocampus was reportedly normal in RIM1$\alpha^{-/-}$ mice, this experiment was performed in the presence of $GABA_A$ antagonists using a very strong, 3-s, 100-Hz, LTP induction paradigm. Huang et al. (2005) showed that RIM1$\alpha^{-/-}$ mice exhibited defects in late phases of LTP in area CA1, potentially implicating abnormal long-term plasticity in the RIM1$\alpha^{-/-}$ behavioral deficits.

In fear conditioning, RIM1$\alpha^{-/-}$ mice were abnormal in both context-dependent and cue-dependent fear conditioning. The abnormal cue-dependent fear conditioning implicates RIM1α in amygdala function. Thus, it will be of particular interest to examine LTP in the amygdala, where some forms of LTP are thought to be NMDA receptor-independent. This is a particularly interesting proposition in light of recent data suggesting that some forms of heterosynaptic LTP in amygdala are presynaptically induced and expressed.

Although the loss of RIM1α leads to learning and memory deficits, many questions remain. Is RIM1α modulated during learning *in vivo*? Could RIM1α be phosphorylated at its PKA site in the hippocampus during learning? Does phosphorylation of this PKA site play an active role in learning and memory *in vivo* or is some more basic function of RIM1α merely permissive for normal LTP? What RIM1α binding proteins might also be important for learning and memory? Does the loss of RIM1α in a particular brain region correlate with the learning and memory deficits? These are questions currently under active investigation.

4.36.7.2 Munc13-3

Munc13 proteins make up a family of three presynaptic proteins important for synaptic vesicle priming (Brose et al., 2000). The Munc13-3 isoform is strictly localized to cerebellar neurons, granule and Purkinje neurons in particular (Augustin et al., 1999). Augustin et al. examined Munc13-3 knockout mice and observed *increased PPF* at parallel fiber-Purkinje cell synapses with no alteration in synaptic response to sustained 14-Hz stimulation (other synaptic parameters were not reported) (Augustin et al., 2001). In addition, spontaneous miniature inhibitory postsynaptic responses were unchanged in Munc13-3$^{-/-}$ mice. Analysis of cerebellar synapses at the electron microscopic level revealed normal synaptic density and normal synaptic ultrastructure. Examination of multiple additional synaptic proteins showed no compensatory changes in other synaptic proteins. This regionally selective, synaptic perturbation at cerebellar synapses led to a marked deficit in motor learning with no change in baseline coordination using the rotating rod apparatus. These motor learning abnormalities were reminiscent of similar abnormalities in the mGluR4 subunit of metabotropic glutamate receptor knockout mice that showed decreased PPF at parallel fiber-Purkinje cell synapses. Thus, it seems that alterations in PPF at this synapse critically impair motor learning without affecting baseline coordination (Augustin et al., 2001). This conclusion is, as always, tempered by the lack of information from other synapses and additional forms of synaptic plasticity such as LTP and LTD at these synapses.

4.36.7.3 GAP-43

Elegant experiments over the past several years were among the first to implicate the presynaptic growth associated protein (GAP-43, F1, B50) in normal learning and memory. These studies by Routtenberg and colleagues (2000) identified GAP-43 as a presynaptic protein kinase C (PKC) substrate that is phosphorylated during LTP in the dentate gyrus. They showed that GAP-43 is required for normal learning and memory as assessed in GAP43 heterozygous knockdown mice (Rekart et al., 2005). Indeed, transgenic overexpression of GAP43, but not the PKC phosphorylation site mutant form of GAP43, has been shown to increase both dentate gyrus LTP and learning (Routtenberg et al., 2000). It seems that PKC phosphorylation of presynaptic GAP-43 is required for normal learning *in vivo* and that GAP-43 can bidirectionally modulate learning and memory *in vivo*.

Several possible mechanisms for GAP-43's involvement in learning and memory have been proposed,

including direct modulation of presynaptic neurotransmitter release (Routtenberg et al., 2000). GAP-43 has been shown to interact directly with components of the synaptic release machinery including the SNARE complex proteins (SNAP-25, syntaxin, and synaptobrevin) and synaptotagmin (depicted in **Figure 1**) (Haruta et al., 1997). Some of these interactions seem to be dependent on PKC phosphorylation of GAP-43 (Haruta et al., 1997), making this interaction intriguing for the dynamic regulation of plasticity and memory. In fact, GAP-43 phosphorylation may lead to increased neurotransmitter release (Dekker et al., 1990; Heemskerk et al., 1990), whereas decreasing GAP-43 may decrease evoked neurotransmitter release (Ivins et al., 1993; Hens et al., 1995). Whereas presynaptic effects of GAP-43 may serve to enhance plasticity and memory, another possible mechanism is GAP-43's involvement in synaptic and circuit development, which might function to regulate plasticity and memory via developmental effects.

4.36.7.4 SNAP-25

Reduction of the SNARE complex protein SNAP-25 expression via antisense oligonucleotide infusion into CA1 impaired contextual fear conditioning and spatial memory (Hou et al., 2004). SNAP-25 antisense also reduces CA1 LTP, apparently without affecting presynaptic release or short-term plasticity (Hou et al., 2004). The authors suggest that the reduction in SNAP-25 is acting postsynaptically to reduce LTP as well as learning and memory (Hou et al., 2004). The precise mechanism whereby this SNARE complex protein affects learning and memory remains to be determined.

4.36.7.5 H-ras

Transgenic mice overexpressing a constitutively active form of the small, GTP binding protein H-ras (H-ras^{G12V}) exhibit increased PPF, increased LTP, and increased learning and memory. In a very thorough study by Kushner et al., transgenic, H-ras^{G12V}–overexpressing mice showed increased activation of the extracellular regulated kinase (ERK), increased ERK-dependent phosphorylation of the presynaptic protein synapsin I, and increased docked vesicles (Kushner et al., 2005). These changes were also associated with increased numbers of docked synaptic vesicles, increased PPF, increased LTP, and increased miniature excitatory postsynaptic current frequency in area CA1 of the hippocampus. These changes were associated with enhanced water maze and contextual fear conditioning learning and memory in these mice. By crossing the H-ras^{G12V} transgenic mice with synapsin I deficient mice, Kushner et al. were able to show that most of these changes were dependent on synapsin I, providing a compelling link between activation of H-ras, activation of ERK, phosphorylation of synapsin I, and enhanced learning and memory via a presynaptic mechanism (Kushner et al., 2005). Although the physiologic relevance of this mechanism *in vivo* has yet to be determined, these studies provide a clear link between presynaptic functional manipulations, LTP, and memory.

4.36.8 The Future of Presynaptic Plasticity and Learning and Memory

The study of molecular mechanisms involved in presynaptic plasticity is in its infancy. One major obstacle in understanding presynaptic mechanisms in synaptic plasticity is the relative difficulty in accessing the intracellular presynaptic compartment both electrophysiologically and pharmacologically owing to its small size and spatial isolation from the presynaptic cell body. Fortunately, techniques for imaging presynaptic vesicle release, recycling, and trafficking are making the presynaptic compartment more accessible than ever before. Furthermore, our understanding of molecular mechanisms of presynaptic neurotransmitter release and modulation of neurotransmitter release is advancing rapidly through the use of genetic tools to dissect the functions of individual presynaptic proteins and protein components (Sudhof, 2004). The genetic tools deciphering presynaptic function hold great promise for our future understanding of how activity-dependent forms of plasticity are mediated molecularly in presynaptic terminals.

Even further behind are studies implicating presynaptic plasticity and individual presynaptic proteins in cognitive function. A complete picture of how presynaptic short-term plasticity, long-term plasticity, or presynaptic signaling is involved in learning and memory has not yet emerged. Ongoing studies will continue to examine existing presynaptic protein knockout mice that have been extensively characterized electrophysiologically. Future studies will focus on restricted, conditional knockout approaches, modulation of presynaptic proteins during learning, and examining how such dynamic regulation of presynaptic proteins is involved in learning and memory.

With some exceptions, the existing studies stop short of understanding how dynamic modulation of presynaptic proteins might play a role in both synaptic plasticity and learning. It will be important to understand how presynaptic proteins are modified during synaptic plasticity and during learning tasks *in vivo*. More precise molecular manipulations in mice including point mutations that alter targets of these modifications should also prove informative.

Also, it is likely that presynaptic proteins and presynaptic plasticity are critical for learning and memory in specific brain regions and indeed particular neuronal subtypes. Clearly, following screening studies on existing knockout mice, regionally targeted, conditional knockout approaches will be critical for understanding how specific molecules fit into various memory circuits. For example, the use of conditional knockout mice combined with focal, virally mediated Cre recombinase expression is one novel way to approach this problem (Hommel et al., 2003).

The use of genetic manipulations in combination with presynaptic electrophysiologic and imaging studies will be critical to understanding the molecular basis of presynaptic plasticity.

All of these issues must be approached in a multidisciplinary fashion combining targeted, conditional genetic manipulations in mice, broader behavioral screening, thorough electrophysiological characterization, biochemical studies, and correlation with effects on synaptic plasticity. We are now in an excellent position to identify molecular mechanisms of presynaptic plasticity and to link these mechanisms to cognition, thereby identifying potential new targets in the treatment of cognitive disorders. We must include presynaptic plasticity and presynaptic molecular mechanisms to gain a complete picture of the molecular, cellular, and physiologic basis of learning and memory.

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4.37 Regulation of Synaptic Function by Endocannabinoids

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4.37.1 Introduction

The synapse is the fundamental functional unit for intercellular communication between neurons in many nervous systems. Communication across synapses in the brain involves chemical messengers (neurotransmitters, neuromodulators, and growth factors among them) that traverse the synaptic cleft to interact with cognate receptors. It is now appreciated that this communication is bidirectional, as neurochemicals released from both presynaptic and postsynaptic neurons can produce transsynaptic

signals (Stanton et al., 2004). In addition, neurochemical release can activate receptors on the same cell in a homotypic fashion.

A variety of compounds act as neurotransmitters, neuromodulators, and growth factors, ranging from amino acids and other small molecules to large polypeptides. In addition to functioning in the regulation of synapse formation and synaptic transmission, these synaptic neurochemicals have also been implicated in synaptic plasticity of the types thought to underlie in learning and memory (see other articles in this volume as well as Stanton et al., 2004).

In the present article the focus is on synaptic modulation and plasticity involving endocannabinoids (ECs) that act as retrograde messengers linking postsynaptic signals to presynaptic mechanisms of plasticity expression. Recent studies have implicated this EC signaling in plasticity at a wide range of synapses, and thus it seems to be a widespread mechanism for synaptic regulation (Alger, 2002; Gerdeman et al., 2002; Chevaleyre et al., 2006). The mechanisms of EC production and release, as well as regulation of the duration of EC, signaling will be discussed. In addition, the mechanisms activated by EC interaction with receptors and the role of these mechanisms in synaptic physiology will be considered. Finally, some discussion of emerging evidence that EC-dependent synaptic plasticity participates in learning and memory will be presented.

4.37.2 What is an Endocannabinoid?

In addition to serving as barriers to separate and compartmentalize cells and subcellular domains, cell membranes are also rich reservoirs of biomolecules that can be used for intracellular and intercellular communication. Lipids are a prominent membrane source of precursors for such signaling molecules. Metabolism of membrane lipids by phospholipases is a well-known mechanism for generation of intracellular second messengers (Hokin and Hokin, 1953; Fain and Berridge, 1978). Roles for lipid metabolites in neural intercellular communication are also well established. For example, prostaglandins and related lipid metabolites that act as autocrine and paracrine agents throughout the body also have such signaling roles in the nervous system (see Bazan, 2005, for review).

Endogenous cannabinoids, or ECs, are a class of lipid metabolites that was discovered relatively recently. These fatty acid derivatives are synthesized by modification and/or metabolism of arachidonate-containing membrane lipids. The two most prominent ECs are arachidonoylethanolamide (AEA or anandamide) (Devane et al., 1992) and 2-arachidonoyl glycerol (2-AG) (Mechoulam et al., 1995; Sugiura et al., 1995). These hydrophobic messengers are now referred to as ECs because of their actions on the receptors for Δ9-tetrahydrocannabinol (THC), the major psychoactive component of cannabis-derived drugs such as marijuana. While AEA and 2-AG have actions at other molecular targets (van der Stelt and Di Marzo, 2005a; Oz, 2006), it is clear that cannabinoid (CB) receptors are among the major targets of these compounds.

4.37.2.1 Anandamide and 2-Arachidonoyl and Their Biosynthesis

Endocannabinoid synthesis involves a variety of biochemical pathways, some of which are well established, and others that are relatively novel. Production of AEA is thought to arise predominantly from phosphatidylethanolamine (PE) via a two-step process involving transacylation to produce an N-arachidonoyl-PE (NAPE) intermediate, followed by cleavage via phospholipase D (Schmid et al., 1990; Di Marzo et al., 1994). However, a new study suggests a novel AEA synthesis pathway involving phospholipase C (PLC) (Liu et al., 2006). Synthesis of 2-AG also involves well-known phospholipases. The predominant mechanism is PLC-mediated hydrolysis of an arachidonic acid–containing phospholipid to diacylglycerol (DAG) and subsequent deacylation at position 1 by DAG lipase (Mechoulam et al., 1995; Sugiura et al., 1995; Stella et al., 1997). Alternative synthesis pathways involving other phospholipases have also been proposed (Bisogno et al., 1999). Increased intracellular calcium can stimulate enzymes involved in the synthesis pathways for both AEA and 2-AG and appears to be an effective mechanism for 'on-demand' EC synthesis in neurons (Di Marzo et al., 1994; Bisogno et al., 1999).

4.37.2.2 Regulation of Synaptic Endocannabinoid Levels

Endocannabinoids are believed to be produced inside of cells, but the majority of their demonstrated actions involve signaling from extracellular sites. Thus, ECs are presumed to be released from cells, including

neurons, into the extracellular environment. There is good evidence for extracellular ECs in neural tissue, including neuronal cultures (Di Marzo et al., 1994) and brain dialyzate (Giuffrida et al., 1999). Increases in extracellular EC levels have been observed following stimulation of cell surface receptors and neuronal depolarization (Di Marzo et al., 1994; Giuffrida et al., 1999; Jung et al., 2005). The mechanisms by which hydrophobic ECs exit the cell and traverse the hydrophilic extracellular space are still not clear, with investigations focused on two potential mechanisms, simple diffusion or transporter/carrier-assisted movement through the membrane (Hillard and Jarrahian, 2000; Glaser et al., 2003; Fegley et al., 2004; McFarland and Barker, 2004; Ronesi et al., 2004; Moore et al., 2005). Extraction of these lipophilic messengers from the membrane may also require some sort of carrier protein in the extracellular space (Makriyannis et al., 2005a,b).

Termination of EC signaling may involve the proposed transmembrane transporter system mentioned above. Degradative enzymes also play key roles in creating a transmembrane EC gradient that favors uptake under most conditions. These enzymes also participate in termination of EC signaling. Anandamide can be degraded by fatty acid amide hydrolase (FAAH) (Cravatt et al., 1996) or by cyclooxygenase-2 (COX-2) (Kozak et al., 2002), although the FAAH pathway seems to predominate in neurons (Glaser et al., 2003). The degradation of 2-AG appears to be mediated by both COX-2 (Kozak et al., 2002) and monoglyceride lipase (MGL) (Dinh et al., 2002).

4.37.3 CB1 Receptors

4.37.3.1 Cannabinoid Receptors and Signaling

The actions of CB drugs and ECs on CB receptors account for many of their effects in the nervous system. Within the brain, the CB1 receptor appears to be the predominant receptor subtype (Matsuda et al., 1990; Freund et al., 2003; Mackie, 2005), although recent evidence indicates the presence of CB2 receptors in rodent brain (Van Sickle et al., 2005; Gong et al., 2006). The CB1 receptor is a member of the classical family of G-protein-coupled receptors that includes the muscarinic acetylcholine (ACh) and adrenergic receptors (Matsuda et al., 1990). As such, CB1 receptor activation produces modulatory effects via its ability to activate G-proteins. It appears that the Gi/o G-proteins are the major subtypes coupled to CB1 (Howlett et al., 1986; Matsuda et al., 1990). Activation of Gi/o is generally thought to be 'inhibitory,' because this G-protein inhibits adenylyl cyclase activity through its α subunit and produces inhibitory neuronal signals such as activation of K^+ channels and inhibition of Ca^{2+} channels via liberation of the associated β/γ subunits (Clapham and Neer, 1997). The majority of CB1 receptor–mediated responses observed in neurons to date appear to involve such inhibitory mechanisms (Freund et al., 2003), with notable exceptions (e.g., Glass and Felder, 1997). Through heterotrimeric G-protein signaling, CB1 receptor activation can impact acute electrophysiological properties of neurons and produce longer-lasting biochemical changes that could subtly influence neuronal function. Indeed, CB1 activation can stimulate signaling molecules, such as extracellular-signal-related kinase (ERK), that alter protein phosphorylation and gene expression (Wartmann et al., 1995; Davis et al., 2003). Thus, CB1 receptors are poised to produce transient and long-lasting effects on neurophysiology.

4.37.3.2 CB1 Receptor Pharmacology

In addition to Δ9-tetrahydrocannabinol (Δ9-THC) and ECs, a number of synthetic agonists for CB1 receptors have been created (Pertwee, 2005; Reggio, 2005). These compounds generally display high efficacy at the receptor. Synthetic CB1 antagonists have also been produced (Pertwee, 2005; Reggio, 2005). These compounds appear to have the capacity to act as inverse agonists, reducing intrinsic receptor activity under conditions where such activity is high (Pertwee, 2005). However, it is not yet clear if this action is relevant to their pharmacological effects, as ECs are likely to be present in tissue and perhaps even in single cells. In this case effects of these compounds in the absence of explicitly added agonist or tissue stimulation could reflect pure antagonism of EC actions.

4.37.3.3 Neuronal CB1 Receptors Are Mainly Presynaptic

Within the central nervous system (CNS), CB1 receptor immunoreactivity is found mainly at presynaptic terminals (Freund et al., 2003; Mackie, 2005). However, there is also immunological evidence for low levels of postsynaptic receptor expression in a few brain regions, such as the striatum (Tsou et al.,

1997). Sporadic reports of postsynaptic CB1 signaling have appeared. For example, CB1 receptors on neocortical low-threshold-spiking interneurons produce an inhibitory postsynaptic potential involving activation of a K^+ channel (Bacci et al., 2004). However, the bulk of physiological and neurochemical evidence also supports a predominantly presynaptic role for CB1 receptors (Alger, 2002; Mackie, 2006). These presynaptic receptors generally inhibit neurotransmitter release. Two mechanisms have been predominantly suggested to underlie this presynaptic inhibition, activation of K^+ channels and modulation of voltage-gated Ca^{2+} channels (Mackie and Hille, 1992; Henry and Chavkin, 1995; Mackie et al., 1995; Pan et al., 1996; Bacci et al., 2004; Guo and Ikeda, 2004). While it is not yet clear which of these mechanisms predominates in synaptic modulation at all synapses, evidence from direct studies of calcium dynamics at cerebellar synapses and the calyx of Held favors inhibition of calcium channels (Brown et al., 2004; Kushmerick et al., 2004). In addition, a recent paper indicates that CB1-mediated synaptic depression is itself modulated via frequency-dependent changes in the function of N-type calcium channels (Foldy et al., 2006). This issue will be discussed in more detail later in this article. Regardless of the mechanism, it is clear that CB1 activation inhibits neurotransmitter release on an acute timescale. This mechanism can account for the short-lasting forms of synaptic modulation that will be discussed below. However, it appears that additional mechanisms need to be invoked to explain CB1 participation in long-term synaptic plasticity.

4.37.4 Depolarization-Induced Suppression of Synaptic Transmission

4.37.4.1 Depolarization-Induced Suppression of Excitation and Inhibition

Transient synaptic depression involving EC activation of CB1 receptors underlies several forms of short-term synaptic plasticity at excitatory glutamatergic and inhibitory gamma-aminobutyric-acid (GABA)ergic synapses (see Alger, 2002; Chevaleyre et al., 2006, for review). The first of these to be discovered were depolarization-induced suppression of excitation (DSE) and inhibition (DSI) (Llano et al., 1991; Pitler and Alger, 1992). Postsynaptic depolarization of cerebellar Purkinje neurons or CA1 hippocampal pyramidal neurons leads to a decrease in glutamatergic (cerebellum, Llano et al., 1991) and GABAergic (hippocampus, Pitler and Alger, 1992) transmission onto the postsynaptic neuron. Several pieces of evidence in these studies indicated that this synaptic depression involved decreased glutamate or GABA release from presynaptic terminals. More recent findings indicate that CB1 receptors are expressed presynaptically, but not postsynaptically, at synapses that show DSI and DSE (see Freund et al., 2003; Mackie, 2005, for review).

Examination of DSE/I in hypothalamus is providing information that could prove relevant for understanding EC roles in feeding and bodily homeostasis. In particular, the role of the EC system in feeding behavior has led investigators to postulate actions of these neuromodulators in the hypothalamic feeding circuitry (Giuliani et al., 2000; Di Marzo et al., 2001). Recent papers from three laboratories have confirmed that retrograde EC signaling modulates neurotransmitter release in hypothalamus (Di et al., 2003; Hentges et al., 2005; Jo et al., 2005). The EC effects include DSE at synapses onto magnocellular neurons in the supraoptic nucleus (SON) (Di et al., 2003) and DSI at synapses onto lateral hypothalamic neurons that express melanin-concentrating hormone (Jo et al., 2005). In the arcuate nucleus GABAergic inhibitory inputs to proopiomelanocortin (POMC)-expressing neurons appear to be under tonic EC suppression (Hentges et al., 2005). This is not true of the neurons that do not express POMC. The tonic disinhibition appears to be due to postsynaptic EC production, as increases in inhibitory transmission produced by CB1 antagonists are blocked by postsynaptic loading of calcium chelators. Thus, like many other instances of tonic EC signaling in brain, the tone is due to endogenous agonist activation of CB1 receptors rather than autonomous CB1 receptor activity (cf. Zhu and Lovinger, 2005).

Various forms of EC-dependent DSE and DSI have now been observed in several other brain regions including basolateral amygdala (BLA) (Zhu and Lovinger, 2005), brainstem (Mukhtarov et al., 2005), dentate gyrus (Isokawa and Alger, 2005), neocortex (Trettel and Levine, 2003; Bodor et al., 2005), striatum (Narushima et al., 2006), substantia nigra (Yanovsky et al., 2003), and the ventral tegmental area (VTA) (Melis et al., 2004a), in addition to the cerebellum and CA1 region of the hippocampus. **Table 1** summarizes our current state of knowledge concerning synapses that exhibit DSE/I and the

Table 1 Characteristics of DSE/DSI and STD in different brain regions[a]

Brain region	*DSE/DSI receptors*	*Ion channels*	*Expression involved*	*Involved measure*
Basolateral amygdala (Zhu and Lovinger, 2005)	DSI	mGluR, CB1		sIPSC frequency
Brainstem, hypoglossal nucleus (Mukhtarov et al., 2005)	DSI (glycinergic synapses)		Postsynaptic VGCCs & NMDARs	eIPSCs, paired-pulse responses, synaptic failures, mIPSCs
Calyx of Held (Kushmerick et al., 2004)	STD	mGluR, CB1	Postsynaptic VGCCs, presynaptic VGCCs	eEPSCs, presynaptic calcium levels, presynaptic capacitance
Cerebellum climbing fibers (Maejima et al., 2005; van Beugen et al., 2006)	STD	mGluR, CB1	Postsynaptic VGCCs, presynaptic VGCCs	eEPSCs, presynaptic calcium levels, prevents PF-LTP
Cerebellum parallel fibers (Llano et al., 1991; Kreitzer and Regehr, 2001; Brown et al., 2003)	DSE, STD	mGluR, CB1	N, P/Q, R-type VGCCs	eIPSCs, sIPSCs, presynaptic calcium transients
Cerebellum parallel fiber-stellate synapses (Rancillac and Barbara, 2005)	STD	CB1		eEPSCs, synaptic failures
Cerebellum inhibitory inputs (Galante and Diana, 2004)	STD	mGluR, CB1		eIPSCs, sIPSCs
Hippocampus (see Alger, 2002, for review; Heinbockel et al., 2005)	DSI, STD	mAChR, mGluR, CB1	Postsynaptic VGCCs, presynaptic N-type VGCCs	eIPSCs, sIPSCs, anandamide uncaging
Hypothalamus, SON (Di et al., 2003)	DSE	Glucocorticoid receptor	Postsynaptic VGCCs	mEPSCs
Hypothalamus, arcuate nucleus (Hentges et al., 2005)	EC tone	CB1		eIPSCs, mIPSCs
Hypothalamus, lateral nucleus (Jo et al., 2005)	DSI		Postsynaptic VGCCs	eIPSCs, sIPSCs
Sensory cortex (Trettel and Levine, 2003)	STD	mAChR	Postsynaptic VGCCs	eISPCs, mIPSCs, paired-pulse responses
Striatum (Narushima et al., 2006)	DSE, STD	mGluR, mAChR, CB1	Postsynaptic VGCCs	eEPSCs, paired-pulse responses
Striatum (Yin and Lovinger, 2006)	STD	D2R, mGluR, CB1		eEPSCs, paired-pulse responses
Substantia nigra (Yanovsky et al., 2003)	DSI	mAChR		eIPSCs, sIPSCs
VTA (Melis et al., 2004a)	DSE	CB1	Postsynaptic VGCCs	eEPSPs, eEPSCs, mEPSCs
VTA (Melis et al., 2004b)	STD	mGluR, CB1		eESPCs
VTA (Riegel and Lupica, 2004)	STD	CB1	Postsynaptic VGCCs	$GABA_B$R-mediated eIPSCs

[a]Abbreviations: CB1, cannabinoid 1 receptor; D2R, D2 receptors; DSE/I, depolarization-induced suppression of excitation/inhibition; eEPSC/P, evoked excitatory postsynaptic current/potential; eIPSC, evoked inhibitory postsynaptic current; $GABA_B$R, gamma-aminobutyric acid type B receptor; LTD, long-term depression; mAChR, muscarinic acetylcholine receptor; mEPSC, miniature excitatory postsynaptic current; mGluR, metabotropic glutamate receptor; NMDAR, *N*-methyl-D-aspartate receptor; sIPSC, spontaneous inhibitory postsynaptic current; STD, short-term depression; VGCC, voltage-gated calcium channel; VTA, ventral tegmental area; mIPSC, miniature excitatory postsynaptic current.

mechanisms implicated in these forms of synaptic plasticity.

4.37.4.2 Mechanisms of Depolarization-Induced Suppression of Excitation and Inhibition Induction

Surprisingly little is known about the mechanisms linking depolarization to the postsynaptic EC release apparently needed for DSE/I, despite the fact that DSI and DSE were the first forms of synaptic plasticity shown to involve retrograde EC signaling. Increased postsynaptic intracellular calcium is implicated in both DSI and DSE, as postsynaptic infusion of calcium chelators blocks both forms of plasticity (Llano et al., 1991; Pitler and Alger, 1992), and uncaging-induced increases in postsynaptic calcium induce DSI (Wang and Zucker, 2001; Wilson and Nicoll, 2001). Voltage-gated calcium channels are almost certainly involved in this calcium increase, although the identities of the postsynaptic calcium channels involved in DSE/I have not been fully elucidated. The steps linking the calcium increase to production and release of the EC messenger are less clear. Calcium can activate the phosphotransferases and phospholipases implicated in AEA synthesis, as well as the lipases necessary for 2-AG production (Di Marzo et al., 1994; Bisogno et al., 1999). However, treatment with compounds that block PLC and DAG lipase do not alter DSI in hippocampus (Edwards et al., 2006), despite other evidence that 2-AG is involved in this form of plasticity (Dinh et al., 2002; Kim and Alger, 2004). In hippocampus, DSI is unaffected by gene-targeted knockout of PLCβ1 (Hashimotodani et al., 2005), and similar results were obtained using PLCβ4 knockout mice for cerebellar DSI (Maejima et al., 2005). Both the enzyme inhibitors and PLCβ isoform knockouts prevent other forms of EC-mediated synaptic depression in the same neurons in which they fail to affect DSI or DSE, as will be discussed later in this article. The inability of lipase inhibition to affect DSI/E has led to the suggestion that *de novo* synthesis of the EC retrograde signal is not necessary for plasticity induced by depolarization alone. One idea put forward by Alger and colleagues is that a 'mobilization' step is all that is needed to activate retrograde signaling by preformed ECs in DSE/I (Edwards et al., 2006). However, the biochemical or cell biological nature of such a step is a matter of speculation at this point. Perhaps a fatty acid carrier protein of some sort is involved.

4.37.4.3 Presynaptic Depression during Depolarization-Induced Suppression of Excitation and Inhibition

The postsynaptic release of the EC retrograde messenger during induction of DSE or DSI leads to activation of presynaptic CB1 receptors that inhibit neurotransmitter release (Kreitzer and Regehr, 2001; Maejima et al., 2001; Ohno-Shosaku et al., 2001; Wilson and Nicoll, 2001). The aforementioned mechanisms set into motion by activation of Gi/o-type heterotrimeric G-proteins, namely activation of K^+ channels and inhibition of Ca^{2+} channels, could both inhibit neurotransmitter release. Activation of K^+ channels can reduce the magnitude or duration of presynaptic depolarization, while inhibition of voltage-gated calcium channels directly reduces excitation/secretion coupling. In addition, some Gi/o-coupled receptors inhibit steps involved in vesicle fusion (reviewed in Dunwiddie and Lovinger, 1993). Which of these mechanisms contributes to presynaptic inhibition in DSE and DSI is a matter of debate that has not been resolved at most synapses. Experiments in which CB1 receptors are activated by synthetic agonists indicate that these receptors can activate K^+ channels and inhibit Ca^{2+} channels (Mackie and Hille, 1992; Mackie et al., 1995; Pan et al., 1996; Guo and Ikeda, 2004). Most of the existing evidence indicates that CB1 receptors do not reliably inhibit transmission at a site downstream of these channels (Hoffman and Lupica, 2001; Katona et al., 2001; Zhu and Lovinger, 2005), with notable exceptions (Gerdeman and Lovinger, 2001). Examination of DSE at the parallel fiber (PF) synapses onto cerebellar Purkinje cells has generated convincing evidence that Ca^{2+} channel modulation is the predominant mechanism of inhibition (Brown et al., 2004). Similar studies were also carried out at a specialized sensory synapse known as the calyx of Held (Kushmerick et al., 2004). This synapse consists of a single large presynaptic terminal that is wrapped around the majority of the postsynaptic cell body. The large presynaptic element affords electrophysiologists the opportunity to record ion current in a 'whole-terminal' configuration. Using this recording approach, Kushmerick and coworkers (2004) showed that CB1 activation produces a decrease in synaptic transmission that is accompanied by a decrease in the presynaptic calcium current in the calyx terminal. However, mechanisms of DSE and DSI at other synapses remain to be explored, and it has been suggested that activation of K^+ channels plays a

role in DSE and DSE expression in the cerebellum and hippocampus (Daniel and Crepel, 2001; Varma et al., 2002; Diana and Marty, 2003). **Figure 1(a)** presents a schematic diagram of the molecular events believed to be involved in DSE and DSI.

4.37.4.4 Regulation of the Timing and Duration of Depolarization-Induced Suppression of Excitation and Inhibition

Evidence collected to date suggests that the duration of DSE/I is determined by the length of time that the EC is present and activating CB1 receptors within the synapse. Early experiments with compounds that block cellular uptake of ECs indicated that enhancing EC levels in the slice preparation produced a depression of transmission that occluded DSI (Wilson and Nicoll, 2002). Furthermore, inhibiting the EC-degrading enzymes COX-2 and monoglycerol lipase (MGL) prolongs the duration of DSI, most likely through an increase in the duration of 2-AG presence in the synapse (Kim and Alger, 2004; Makara et al., 2005). Kreitzer and Regehr (2001) demonstrated that DSE at parallel-fiber-Purkinje neuron synapses is well correlated in time with a decrease in the presynaptic calcium transient. Furthermore, the decreased duration of DSE at higher temperatures (Kreitzer and Regehr, 2001) could be explained by increased uptake or enzymatic degradation of the EC and fits well with the idea that DSE/I time course is determined by the synaptic lifetime of the EC messenger.

An elegant series of experiments from Alger and colleagues also supports the idea that DSI follows the time course of EC release and synaptic actions (Heinbockel et al., 2005). These investigators used a photoactivatable form of AEA to precisely time the production of this EC and examine the effect on transmission. Suppression of transmission was rapidly induced with this paradigm, and the time course of this 'uncaging-induced' DSI suggested that it persisted as long as the free EC was present. These lines of evidence all indicate that DSE/I directly result from transient increases in synaptic levels of the EC messenger that activates CB1 receptors, leading to inhibition of presynaptic calcium transients and the resultant depression of neurotransmitter release.

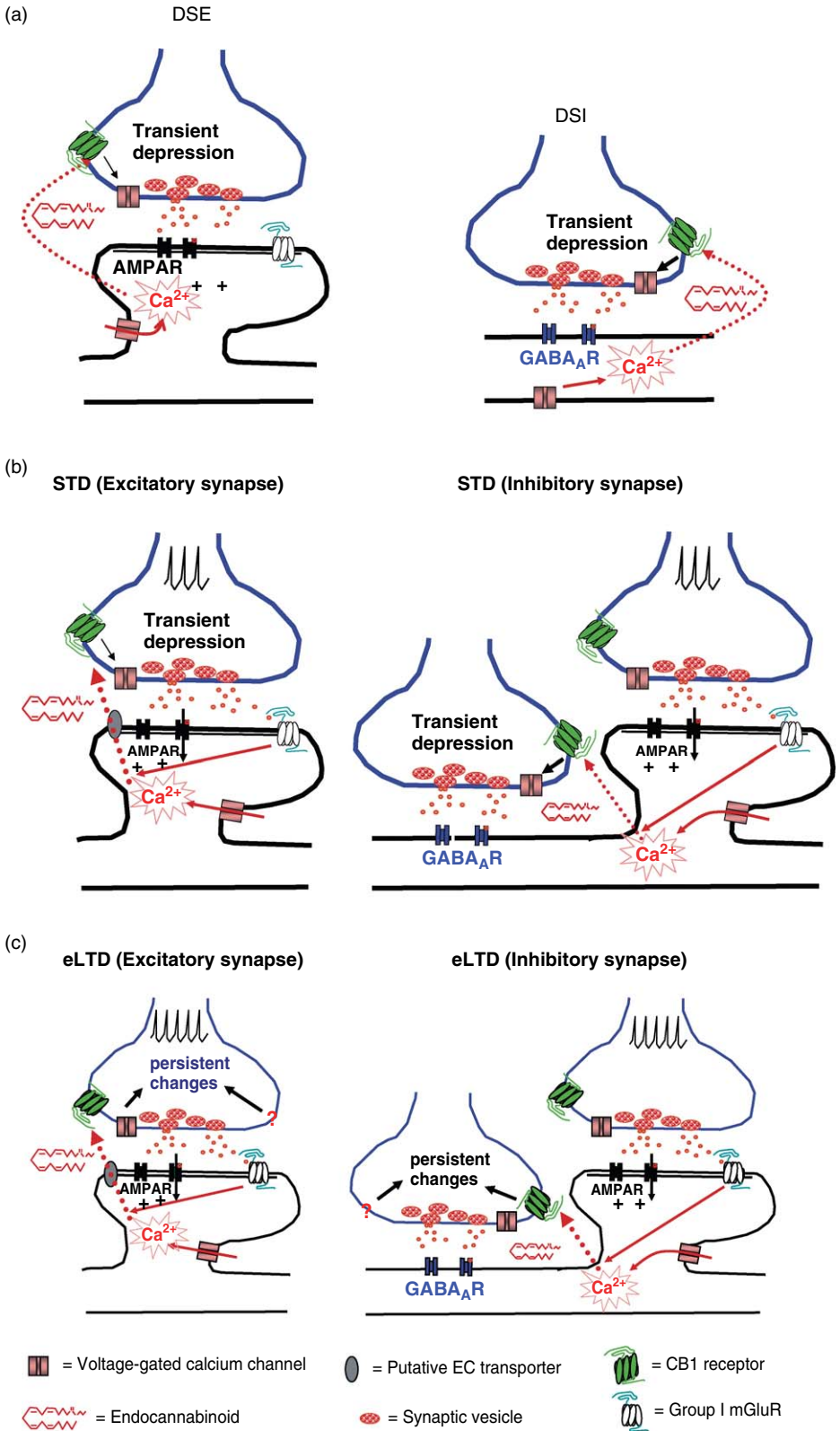
The photoactivation experiments, coupled with data from purely electrophysiological studies, also provide information about the rate of onset of EC-mediated synaptic depression. The time to onset of inhibition following AEA photoactivation is in the range of 50–175 ms, while the latency from depolarization to the onset of inhibition is about 400 ms. These findings indicate that steps between depolarization and EC release take somewhere between 225 and 350 ms, and the estimated time from the rise in postsynaptic calcium to EC release is somewhere between 75 and 190 ms (Heinbockel et al., 2005).

4.37.5 Synaptically Driven, Endocannabinoid-Mediated Short-Term Depression

4.37.5.1 Short-Term Depression Induced by Activation of Metabotropic Receptors

While retrograde EC signaling leading to presynaptic modulation was shown to be inducible by artificial, and often prolonged, postsynaptic depolarization, it was important to determine if DSI and DSE could be induced by physiological mechanisms such as synaptic stimulation. In the late 1990s Alger and colleagues demonstrated that activation of muscarinic ACh receptors and metabotropic glutamate receptors (mGluRs) enhanced and mimicked DSI, respectively, in the hippocampus (Morishita et al., 1998; Martin and Alger, 1999). This finding provided a powerful clue as to the type of molecular pathway that could stimulate EC signaling. Subsequently, it has been shown that this depression is stimulated by activation of group I mGluRs (Edwards et al., 2006). Similar group I mGluR-stimulated short-term depression (STD) has now been observed in cerebellum (Galante and Diana, 2004), and several other mechanisms involving G-protein-coupled, metabotropic receptors have been linked to EC retrograde signaling in various brain regions, as discussed below. With the discovery of the EC retrograde-signaling role in DSI/DSE, it became apparent that signals which would enhance postsynaptic intracellular calcium or stimulate phospholipases might be good stimuli for induction of retrograde messenger formation and action.

Synaptic depression produced by mGluR activation and mediated by EC retrograde signaling has also been observed at the calyx of Held (Kushmerick et al., 2004). This form of depression is blocked by a CB1 antagonist, and immunochemical analysis of this giant synapse indicated that CB1 receptors are localized on presynaptic but not postsynaptic elements of the calyx. Recordings from the presynaptic terminal showed inhibition of the presynaptic calcium current associated with mGluR-induced retrograde synaptic


(a)
DSE
Transient depression
AMPAR
Ca2+
DSI
Transient depression
GABAAR
Ca2+
(b)
STD (Excitatory synapse)
STD (Inhibitory synapse)
Transient depression
AMPAR
Ca2+
Transient depression
AMPAR
GABAAR
Ca2+
(c)
eLTD (Excitatory synapse)
eLTD (Inhibitory synapse)
persistent changes
?
AMPAR
Ca2+
persistent changes
?
AMPAR
GABAAR
Ca2+
= Voltage-gated calcium channel
= Putative EC transporter
= CB1 receptor
= Endocannabinoid
= Synaptic vesicle
= Group I mGluR

depression. Measurement of presynaptic terminal capacitance was used to estimate the magnitude of vesicle fusion/neurotransmitter secretion during synapse activation, and it was determined that CB1 activation reduced the capacitance increase. These findings constitute the most direct evidence to date that retrograde signaling to presynaptic CB1 receptors reduces calcium currents and vesicle fusion in a presynaptic terminal.

4.37.5.2 Short-Term Depression Induced by Synaptic Activation

Synaptic depression mediated by mGluRs can also be produced by synaptic activation. Stimulation of PF inputs to cerebellar Purkinje neurons produces a short-lasting depression of excitatory transmission that is blocked by both mGluR1 and CB1 antagonists (Brown et al., 2003; Maejima et al., 2005). This synaptically induced STD can synergize with DSI in a manner that is dependent on the relative timing of the two stimuli and the resultant increase in postsynaptic intracellular calcium (Brown et al., 2003). A similar form of STD can also be induced at climbing fiber–Purkinje cell synapses (Maejima et al., 2005). Brief high-frequency stimulation of PF inputs to stellate neurons reveals a CB1-dependent STD at this synapse as well (Rancillac and Barbara, 2005).

Two intriguing forms of EC-dependent STD are observed in the ventral tegmental area (VTA), a brain region with known roles in reward and addiction processes. Excitatory glutamatergic inputs to dopaminergic neurons in VTA display an activity-dependent form of STD that involves EC retrograde signaling (Melis et al., 2004b). This STD of excitatory transmission is blocked by mGluR1, but not mGluR5, antagonism. Conditions that promote enhanced excitation and bursting activity in VTA neurons lead to inhibition of both excitatory glutamatergic and inhibitory GABAergic transmission (Riegel and Lupica, 2004). These forms of STD are actually promoted by mGluR antagonists. The mGluRs in this case appear to be presynaptic group III receptors that are normally involved in inhibiting glutamatergic transmission. When these receptors are blocked, glutamatergic drive is enhanced and helps to stimulate postsynaptic EC production. The inhibitory synapses that are targeted by the retrograde EC action in this case are those which primarily activate $GABA_B$-type receptors. Thus, the net effect of the EC signal is to produce disinhibition of a slow inhibitory postsynaptic potential (IPSP) that would normally limit VTA neuronal excitability.

Within the sensory and auditory neocortices EC-dependent STD induced by synaptic activation has also been observed (Trettel and Levine, 2003; Trettel et al., 2004). This synaptic depression occurs at GABAergic inhibitory synapses between interneurons and 'right-arrow' pyramidal cells in superficial cortical layers. The interneuron subtype involved in this transmission is strongly stimulated by activation of muscarinic ACh receptors. Trains of as few as three action potentials could evoke this form of STD, indicating that EC signaling can be triggered by brief bursting events within the physiological range of cortical neuron firing.

Figure 1 Endocannabinoid-dependent depolarization-induced suppression of excitation (DSE) and inhibition (DSI), short-term depression (STD), and long-term depression. (a) Schematic diagram of mechanisms involved in DSE at excitatory (left) and DSI at inhibitory (right) synapses. Postsynaptic depolarization leading to activation of voltage-gated calcium channels stimulates endocannabinoid (EC) production and/or release. EC activation of CB1 (cannabinoid 1) receptors produces a transient decrease in neurotransmitter release, and inhibition of presynaptic voltage-gated calcium channels is one mechanism strongly implicated in this presynaptic depression. (b) Schematic diagram of mechanisms involved in homosynaptic STD at excitatory synapses (left) and heterosynaptic STD at inhibitory synapses (right). Activation of group I metabotropic glutamate receptors (mGluRs) is a common mechanism involved in postsynaptic EC production in STD. Some forms of STD require postsynaptic depolarization and calcium channel activation, while others do not. A postsynaptic membrane transport system has been implicated in EC release for STD at excitatory synapses in the ventral tegmental area (Melis et al., 2004b), but the role of this mechanism in other forms of STD has not been examined. ECs activate presynaptic CB1 receptors that inhibit neurotransmitter release, presumably via mechanisms similar to those involved in DSE/I. (c) Schematic diagram of mechanisms involved in homosynaptic EC-dependent LTD (eLTD) at excitatory synapses (left) and heterosynaptic eLTD at inhibitory synapses (right). Activation of postsynaptic mGluRs and depolarization-induced activation of postsynaptic voltage-gated calcium channels is a common theme in eLTD induction. Transporter-mediated EC release has been implicated in eLTD at synapses in striatum (Ronesi et al., 2004) and somatosensory cortex (Bender et al., 2006). Maintained expression of eLTD appears to require combined activation of presynaptic CB1 receptors and additional mechanisms that are not yet well understood (although, see Kreitzer and Malenka, 2005). For example, presynaptic, NMDA receptors appear to synergize with CB1 receptors in the induction of LTD at synapses in visual cortex (Sjostrom et al., 2003). $GABA_A$R, gamma-aminobutyric acid type A receptor; AMPAR, alpha-amino-3-hyroxyl-5-methyl-4-isoxazolepropionate receptor.

Recent studies suggest some evidence that the EC responsible for synaptically induced STD is 2-AG. Blockade of 2-AG synthesis prevents STD in hippocampus (Edwards et al., 2006), cerebellum (Galante and Diana, 2004), and VTA (Melis et al., 2004b). The molecular mechanisms linking mGluR activation to 2-AG production may involve activation of phospholipase C and DAG lipase (Jung et al., 2005). There is also evidence that EC release from postsynaptic VTA neurons involves a transmembrane transport system (Melis et al., 2004b). Similar evidence has been gathered for EC-dependent LTD (Ronesi et al., 2004; Bender et al., 2006), a type of plasticity that will also be discussed later in this article.

The duration of EC-dependent STD suggests that the ultimate expression mechanism is similar to that of DSE/I, namely presynaptic CB1 activation leading to inhibition of calcium channels and depression of neurotransmitter release. However, there is as yet little experimental evidence directly supporting this conclusion. Mechanisms involved in STD at excitatory and inhibitory synapses are schematized in **Figure 1(b)**.

4.37.5.3 Dopamine-Induced Short-Term Depression in Striatum

In the striatum, dopamine acts on D2 receptors to stimulate formation of AEA (Giuffrida et al., 1999). This D2 receptor activation of EC signaling has recently been implicated in depression of glutamatergic corticostriatal transmission (Kreitzer and Malenka, 2005; Yin and Lovinger, 2006). Activation of D2 receptors in concert with cortical input at frequencies above 20 Hz induces STD of glutamatergic synaptic input onto striatal medium spiny neurons (MSNs) (Yin and Lovinger, 2006). The mechanisms underlying this synaptic depression include synaptic activation of postsynaptic mGluR1, release of calcium from intracellular stores, and activation of postsynaptic PLC. Expression of D2-stimulated STD is associated with increased paired-pulse ratio, indicative of a presynaptic decrease in probability of neurotransmitter release.

As all of these mechanisms bring to mind EC retrograde signaling, examining the role of ECs and CB1 receptors in this form of STD seemed like a logical course of action. Indeed, STD produced by combined D2R activation and 20-Hz afferent stimulation is blocked by CB1 antagonism and is absent in CB1−/− mice (Yin and Lovinger, 2006). Pharmacological studies indicate that CB1 receptor activation takes place subsequent to D2 activation. The mechanism through which D2 receptors participate in EC production is not yet known. Given the similarity of the mechanisms involved in this corticostriatal STD to those involved in STD at other synapses (e.g., mGluR activation, PLC activation), it is tempting to speculate that D2 receptors have a modulatory role similar to their role in LTD (Kreitzer and Malenka, 2005; Wang et al., 2006, to be discussed in a subsequent section of this article). This may be one example of a mechanism through which other neurotransmitters can modulate the core mGluR–EC signaling process necessary for STD.

4.37.5.4 Physiological Roles of Depolarization-Induced Suppression of Excitation and Inhibition and Short-Term Depression

The roles of EC-dependent DSE/I and STD in circuit function and behavior are not yet fully known. The temporary pauses in synaptic transmission produced by these mechanisms could certainly influence circuit throughput. While DSE would be likely to decrease activity of postsynaptic neurons, DSI and STD at inhibitory synapses have the potential to produce disinhibition that would amplify the effect of excitatory transmission. Short-term depression of transmission could also play a role in biasing circuitry toward one set of outputs in preference to another. In this context, dopamine-mediated STD in striatum could affect the output of MSNs contributing to the two major basal ganglia pathways, the direct and indirect pathways (Gerfen, 1992). Patterns of alteration at different synapses have the potential to influence action patterns at the level of the whole organism. Disinhibition produced by DSI and STD at GABAergic synapses might participate in circuit timing and generation of patterned activity. As discussed later, these forms of disinhibition provide a time window for enhanced likelihood of long-term potentiation. Endocannabinoids may promote the storage of information through such a mechanism. Transient inhibition of excitatory transmission might also be neuroprotective, as excess glutamatergic transmission is often neurotoxic. Indeed, CB1 receptor antagonists have been shown to be neuroprotective (see Mechoulam, 2002; van der Stelt and Di Marzo, 2005b, for review).

Table 1 summarizes data on EC-dependent STD induced by agonist application and/or synaptic

activation in different brain regions. Information on common and divergent mechanisms and measures of STD expression are also provided in this table.

4.37.6 Endocannabinoid-Mediated Long-Term Synaptic Depression

In addition to producing transient depression of transmission, ECs also play key roles in certain long-lasting forms of synaptic plasticity. Retrograde EC signaling has generally been implicated in long-term synaptic depression (LTD), and this topic will be discussed in detail. **Figure 1(c)** shows schematic diagrams of the mechanisms that are common to most forms of EC-dependent LTD at excitatory and inhibitory synapses. It is worth noting that activation or inhibition of CB1 receptors was shown to influence long-term potentiation (LTP) induction prior to the discovery of EC-dependent LTD. For example, Stella et al. (1997) showed that 2-AG suppresses LTP induction, while Auclair et al. (2000) showed that CB1 activation suppresses and CB1 antagonism promotes LTP in rat prefrontal cortex (PFC). Working in the cerebellum, Levenes et al. (1998) also showed that CB agonists suppress LTD induction. Such modulatory roles for ECs are part of a growing pattern of findings indicating that ECs participate in determining the types of long-term plasticity evoked at synapses in several brain regions.

4.37.6.1 Homosynaptic Endocannabinoid-Dependent Long-Term Depression at Glutamatergic Synapses

While the earlier studies hinted that ECs and CB1 could modulate long-lasting synaptic plasticity, evidence that these molecules directly participate in the induction of LTD began to appear in 2002. The work began with investigators examining excitatory synaptic transmission onto MSNs in the dorsal and ventral striatum (the latter is also known as the nucleus accumbens). It was established in the early 1990s that high-frequency activation of excitatory glutamatergic inputs to MSNs in dorsal striatum produces a long-lasting decrease in synaptic efficacy termed striatal LTD (Calabresi et al., 1992a,b; Lovinger et al., 1993; Walsh, 1993). This form of LTD requires postsynaptic induction mechanisms, but expression appears to involve a presynaptic decrease in neurotransmitter release probability, indicating the involvement of a retrograde signal in the induction process (Choi and Lovinger, 1997a,b). Studies over the last decade have established that striatal LTD requires activation of group I mGluRs (Gubellini et al., 2001; Sung et al., 2001), the L-type voltage-gated calcium channel (most likely the CaV1.3 isoform, Calabresi et al., 1992b; Wang et al., 2006), and dopamine D2 receptors (Calabresi et al., 1992a,b; Tang et al., 2001; Kreitzer and Malenka, 2005; Wang et al., 2006). As we have already seen, these molecules are implicated in postsynaptic production of ECs.

Combined electrophysiological, pharmacological, and genetic approaches were used by Gerdeman et al. (2002) to establish a role for ECs and CB1 in striatal LTD. These investigators showed that LTD induction was blocked by a CB1 antagonist and was absent in gene-targeted CB1−/− mice. Experiments involving manipulation of postsynaptic calcium signals and filling the postsynaptic neuron with ECs provided evidence that the compounds are produced in the postsynaptic neuron and can signal in a retrograde manner to inhibit glutamate release (Gerdeman et al., 2002; Ronesi et al., 2004). Based on these findings, the authors suggested that ECs constitute the retrograde signal in striatal LTD, and that LTD induction involves presynaptic CB1R activation.

Shortly thereafter, a report indicating a similar role for ECs and CB1 in LTD of excitatory prelimbic cortex inputs to MSNs in the nucleus accumbens was published (Robbe et al., 2002). Manzoni and colleagues described LTD activated by moderate-frequency (13 Hz) activation of afferent fibers to accumbens MSNs. This LTD shared many of the properties already established for striatal LTD, namely, dependence on activation of an mGluR (mGluR5 in this case) and the necessity of an increase in postsynaptic intracellular calcium. A presynaptic mechanism of expression was also shared between the two forms of LTD. The similarity between LTD in the two striatal subregions that was the focus of the Robbe et al. (2002) paper was the involvement of ECs and the CB1 receptor. This was demonstrated using CB1 antagonists as well as CB1−/− mice. This study was the first to indicate that the long-lasting expression of LTD was independent of sustained CB1 receptor activation. This was demonstrated by applying the CB1 antagonist well after LTD was established, at which time it had no effect on the depressed synaptic response. Similar observations were subsequently made in hippocampus (Chevaleyre and Castillo, 2003) and dorsal

striatum (Ronesi et al., 2004). These important findings indicate that CB1 receptor activation is only important for the initiation of LTD.

There were, however, noticeable differences in the induction mechanisms of EC-dependent LTD (henceforth eLTD) in the two striatal subregions. Most notable was the absence of a role for dopamine and its receptors in the accumbens (Robbe et al., 2002). This finding was interesting in light of the observed role of dopamine in eLTD in the dorsal striatum (Calabresi et al., 1992a,b; Tang et al., 2001; Kreitzer and Malenka, 2005; Wang et al., 2006) and the fact that both striatal subregions contain abundant dopaminergic afferents. These findings also appear to have provided the first clue that dopamine modulates striatal LTD rather than being strictly necessary for this form of plasticity. Another interesting difference between eLTD in dorsal striatum and nucleus accumbens is the role of depolarization. The LTD induced by high-frequency stimulation in dorsal striatum depends on depolarization (Choi and Lovinger, 1997a), but it is unclear if depolarization is necessary for accumbens eLTD. Furthermore, the role of postsynaptic calcium channels, implicated in dorsal striatal LTD, is not yet clear in accumbens. These findings suggest that multiple mechanisms can participate in activation of EC release necessary for LTD induction, even at synapses onto very similar sets of neurons.

Subsequent to publication of the findings of Robbe et al. (2002), additional reports of eLTD induced by moderate-frequency stimulation in striatum appeared. Hoffman et al. (2003) essentially replicated the findings of Robbe et al. (2002) in nucleus accumbens, although 10-Hz stimulation was used. Ronesi and Lovinger (2005) working in dorsal striatum also found eLTD induced by 10-Hz stimulation, and this form of LTD had notable similarities and differences relative to accumbens eLTD. Like the eLTD described by Robbe et al. (2002), the synaptic depression induced by 10-Hz stimulation in dorsal striatum was independent of postsynaptic depolarization. Furthermore, the magnitude of 10 Hz–induced LTD was reduced only slightly by L-type calcium channel blockers. The similarities to LTD in accumbens do not extend to involvement of neurotransmitter receptors, however. No role was found for mGluRs in 10 Hz–induced dorsal striatal LTD, while D2 receptors appear to participate in LTD induction. At present the cellular source of ECs involved in 10 Hz–induced dorsal striatal LTD is unclear, given that postsynaptic manipulations do not influence LTD induction. This is a clear difference, relative to accumbens LTD, and signals the need for more research to determine the similarities and differences in the LTD mechanisms in these two related brain regions.

Recent work on PF inputs to cerebellar stellate neurons has revealed key elements of the postsynaptic signaling involved in a somewhat unusual form of eLTD (Soler-Llavina and Sabatini, 2006). This synapse is made by glutamatergic inputs impinging on dendritic shafts. Trains of 30-Hz stimuli produce an LTD that requires increased postsynaptic calcium arising from calcium influx through alpha-amino-3-hydroxyl-5-methyl-4-isoxazolepropionate receptors (AMPARs), presumably of the type that lack GluR2 and are therefore highly calcium permeable. Expression of this type of LTD also depends on activation of group I mGluRs and CB1 receptors, and the final expression mechanism appears to be presynaptic. High-resolution calcium-imaging techniques were used to show that the calcium signal necessary to initiate LTD is confined to a segment of dendrite no more than 10 μm along the axial dendritic length, accounting for the synapse specificity of eLTD. These findings provide a good mechanistic basis for the localized nature of mechanisms involved in eLTD induction. Alternative mechanisms by which EC production takes place during LTD initiation are also highlighted in this study. The source of calcium, AMPARs, is different from that involved in most forms of eLTD, and there is no need for a dendritic spine-like apparatus. This is not too surprising, as the inhibitory synapses that show eLTD in BLA and hippocampus are not localized to dendritic spines (Marsicano et al., 2002; Chevaleyre and Castillo, 2003). The supporting role of mGluRs in EC production, but not stimulation of the intracellular calcium transient, also links this form of LTD to STD found at other cerebellar synapses (Brenowitz and Regehr, 2005).

4.37.6.2 Heterosynaptic Endocannabinoid-Dependent Long-Term Depression at Gamma-Aminobutyric Acid–ergic Synapses

The characterization of eLTD in striatal subregions heralded a rapidly growing appreciation of the fact that EC-dependent long-term plasticity is a widespread mechanism for synaptic modification. Following the initial descriptions of eLTD in dorsal and ventral striata, a number of studies appeared in rapid succession which established eLTD at both inhibitory and excitatory synapses in several forebrain regions. At

GABAergic synapses in amygdala and hippocampus, LTD involving ECs has now been firmly established and is sometimes known as LTDi (the i refers to inhibitory synapses) (Marsicano et al., 2002; Chevaleyre and Castillo, 2003). Marsicano et al. (2002) described LTD at GABAergic inhibitory synapses onto principal neurons in the basolateral amygdala (BLA) as part of a study implicating ECs in extinction of fear conditioning. These investigators found that LTD induced by sustained low-frequency (1 Hz) stimulation was absent in BLA slices from CB1−/− mice and in wild-type mouse slices treated with a CB1 antagonist. This eLTD was accompanied by an increase in paired-pulse facilitation, indicative of a decrease in neurotransmitter release probability. It is worth noting that the original report of this form of eLTD did not present evidence for a retrograde signaling role of ECs. However, considerable evidence for such a role was obtained in a subsequent study (Azad et al., 2004).

Investigators examining GABAergic inhibitory transmission in hippocampus were also beginning to unearth evidence for eLTD. The discovery that the mechanism underlying DSI involved EC retrograde signaling onto terminals of GABAergic basket cells in the hippocampal CA1 region spurred Chevaleyre and Castillo to begin to investigate potential EC-dependent eLTD at these synapses (Chevaleyre and Castillo, 2003). The first report from this group indicated that eLTD could be induced by high-frequency stimulation and presented evidence that this long-lasting form of synaptic depression involved retrograde EC signaling. Like the eLTD observed at excitatory synapses, eLTD in hippocampus is dependent on activation of mGluRs, perhaps a combination of mGluR1 and mGluR5. A long-lasting depression similar to that induced by afferent stimulation could also be induced by application of the group I mGluR agonist, (S)-3,5-dihydroxyphenylglycine (DHPG). Chevaleyre and Castillo (2003) also presented evidence that mechanisms involved in induction of eLTD differed from those implicated in DSI. For example, blockade of enzymes implicated in EC production prevented eLTD without altering DSI. In this form of eLTD, CB1 receptors were necessary for induction but not sustained expression of plasticity, similar to what had been seen at excitatory synapses in nucleus accumbens (Robbe et al., 2002). Also similar to other forms of eLTD was the evidence for a presynaptic site of eLTD expression. The authors discussed the possibility that eLTD would secondarily enhance excitatory synaptic transmission, possibly enhancing LTP and promoting information storage (see discussion to follow).

4.37.6.3 Endocannabinoid-Dependent Long-Term Depression in Neocortex

In the sensory cortex, it was known that different forms of long-lasting synaptic plasticity could be induced by pairing pre- and postsynaptic activation with different timing protocols, phenomena called spike-timing-dependent plasticity (STDP). Activation of the postsynaptic neuron prior to presynaptic action potential firing was known to produce LTD at excitatory glutamatergic synapses between thick-tufted neurons in layer V of the visual cortex (Sjostrom et al., 2001). Elegant experiments involving paired recordings from synaptically connected pre- and postsynaptic neurons revealed that induction of STDP-LTD involved postsynaptic activation, while expression involved a decrease in neurotransmitter release probability (Sjostrom et al., 2001, 2003). These investigators found that this STDP-LTD was blocked by CB1 receptor antagonists, and that it could be induced by pairing application of AEA or the synthetic analog arachidonyl-2-chloroethylamide (ACEA) with afferent stimulation. Interestingly, coincident activation of presynaptic *N*-methyl-D-aspartate-type glutamate receptors (NMDARs) was required for STDP-LTD at these synapses. This led the investigators to propose a model in which synergistic effects of presynaptic CB1 and NMDAR activation lead to STDP-LTD at these excitatory synapses. It remains to be determined what signaling pathways are necessary for expression of this form of eLTD.

A subsequent series of studies have confirmed and extended the role of ECs in LTD at cortical synapses. Feldman and colleagues have examined synapses between layer 4 and layer 2/3 neurons in S1 somatosensory cortex that exhibit STDP-LTD which relies on EC-dependent mechanisms similar to those implicated in visual cortical eLTD (Bender et al., 2006). These investigators have implicated postsynaptic increases in calcium, most likely involving both T- and L-type voltage-gated calcium channels, in this LTD. Other postsynaptic mechanisms implicated in LTD induction at this synapse include mGluR5 activation, release of calcium from intracellular stores associated with IP3 receptors, and an AMT-like EC transporter. Blockade of CB1 receptors prevented eLTD at these S1 cortical synapses, and LTD was induced by combined afferent stimulation and

anandamide application. The major difference in LTD mechanisms in S1 versus visual cortex is the role of NMDARs. In S1, cortical blockade of NMDAR function does not appear to abolish LTD induction, whereas activation of presynaptic NMDARs is implicated in visual cortical LTD (Sjostrom et al., 2003; Bender et al., 2006). It is not yet clear if another presynaptic receptor or channel serves a role similar to presynaptic NMDARs in LTD within S1.

4.37.6.4 Endocannabinoids and Cerebellar Long-Term Depression

The LTD observed at PF synapses between granule cells and Purkinje neurons was the first form of long-lasting synaptic depression described (Ito and Kano, 1982) and remains one of the best characterized forms of synaptic plasticity (see Ito, 2001, for review). Years of research have revealed that both the induction and expression mechanisms of PF-LTD involve postsynaptic mechanisms. Induction requires postsynaptic activation of AMPA-type ionotropic receptors, as well as mGluR1. Postsynaptic increases in calcium, involving both voltage-gated calcium channels and release of calcium from intracellular stores, are also implicated in LTD induction at this synapse. There is strong evidence that expression of PF-LTD involves decreased surface expression of AMPARs, with no evidence for any change on the presynaptic side. Thus, it was surprising when Safo and Regehr (2005) reported that CB1 activation is required for induction of PF-LTD. This finding was doubly puzzling because CB1 receptors appear to be expressed solely on axon terminals at this synapse. This finding led to the hypothesis that cerebellar LTD involves a sequence of retrograde and anterograde signaling mechanisms. It was hypothesized that postsynaptic activation leads to production and release of an EC retrograde messenger that then triggers production and/or release of a chemical signal from the presynaptic terminal that acts on the postsynaptic cell to trigger the final steps in LTD expression. The identity of this anterograde signaling molecule is not yet clear, but nitric oxide (NO) has been implicated in PF-LTD and may thus serve such a role (Shibuki and Okada, 1991; Safo and Regehr, 2005).

However, there are a few elements of this mechanistic scenario that must be reconciled with previous findings regarding PF-LTD. Experiments conducted using acutely isolated Purkinje neurons or neurons grown in cell culture indicate that LTD can be induced in the absence of presynaptic inputs (Narasimhan and Linden, 1996). This suggests that LTD may rely on purely postsynaptic mechanisms that would not require a retrograde signal. However, it is possible that the mechanisms involved in the LTD observed in isolated neurons are different from those taking place during LTD induction in the slice preparation, even though the final expression mechanism appears to be the same in both situations. In addition, the proposed anterograde signal, NO, poses some complications. Recent data indicate that parallel fibers are not the source of the NO involved in cerebellar LTD (Shin and Linden, 2005). Thus, a scenario in which retrograde EC signaling leads to anterograde NO signaling at the same synapse is not likely to occur. Clearly, further studies are needed to clarify the EC role in cerebellar LTD.

4.37.6.5 Amphetamine-Induced Long-Term Depression in Amygdala

Working in the BLA in brain slices, Gean and colleagues found that application of amphetamine produces LTD at glutamatergic synapses that shares important mechanistic similarities with eLTD in other brain regions (Huang et al., 2003). Expression of this drug-induced LTD is associated with increased paired-pulse facilitation and decreased frequency of miniature excitatory postsynaptic currents (mEPSCs), consistent with a presynaptic role in expression. Neurochemical measurement of glutamate release also supported the conclusion that this form of LTD involves decreased neurotransmitter release. Importantly, amphetamine-induced LTD in BLA is blocked by CB1 receptor antagonists. Postsynaptic calcium chelation reduced LTD, indicating some role for postsynaptic activation on LTD induction. This study suggests that drugs of abuse are yet another trigger for eLTD.

4.37.7 Endocannabinoids and Long-Term Potentiation

The disinhibition produced by DSI enhances excitatory synaptic transmission. This has been demonstrated in the hippocampal CA1 region. In the absence of receptor antagonists, stimulation of Schaffer collateral/commissural afferents produces a fast-EPSP/IPSP sequence that reflects glutamatergic transmission followed by feedforward and feedback GABAergic inhibitory transmission. Reducing the inhibitory component is known to enhance the magnitude and duration of the excitatory drive, as

well as the probability of eliciting an action potential (Wagner and Alger, 1996). Carlson et al. (2002) have shown that a DSI-inducing depolarization enhances excitatory synaptic transmission elicited by a train of stimuli. This enhancement facilitates induction of LTP at the glutamatergic Schaffer collateral/commissural synapses via a mechanism that involves CB1 receptors (**Figure 2**).

Chevaleyre and Castillo have also gathered evidence that ECs participate in a 'metaplastic' enhancement of LTP (**Figure 2**) (Chevaleyre and Castillo, 2004). Metaplasticity refers to alterations in the threshold for induction of synaptic plasticity due to prior synaptic experience (Abraham and Bear, 1996). This can often take the form of modulation of one form of plasticity by another. At the basket cell–pyramidal cell synapses in hippocampus, Chevaleyre and Castillo (2004) found that the induction of EC-dependent LTD enhanced the induction of LTP at excitatory synapses onto the same pyramidal neurons. A weak theta-burst stimulus that was normally insufficient to induce LTP could do so after prior induction of eLTD using 10-Hz stimulation. Thus, EC signaling can promote LTP induction in either the short term, during DSI, or the long term, during expression of eLTD. This adds a level of modulation onto the basic plasticity of excitatory synapses in hippocampus. It is likely that similar mechanisms will also be found in other brain regions where plasticity at excitatory synapses is accompanied by EC-dependent synaptic depression at inhibitory synapses. Indeed, enhancement of LTP was observed in CB1−/− mice in the BLA that also show loss of eLTD at inhibitory synapses (Marsicano et al. 2002).

One line of evidence that appears to argue against EC facilitation of LTP comes from a recent study by Slanina et al. (2005). These authors found that CB1 antagonists enhanced LTP at excitatory synapses onto hippocampal CA1 pyramidal neurons, in contrast to the effects seen by others (Carlson et al., 2002; Chevaleyre and Castillo, 2004). The enhancement of LTP is attributed to relief from EC-mediated inhibition of excitatory transmission. The EC inhibition is most likely due to activation of CB receptors on glutamatergic presynaptic terminals in the CA1 region. While there has been much speculation about CB and EC actions at these excitatory synapses, and it has been suggested that a non-CB1 receptor mediates these effects (Hajos et al., 2001; Hoffman et al., 2005), recent evidence strongly suggests that CB1 receptors are indeed also present and active in this system (Domenici et al., 2006; Katona et al., 2006; Kawamura et al., 2006). Thus, the scenario suggested by Slanina et al. (2005) is certainly plausible.

Within the cerebellum, EC release and CB1 activation induced by climbing fiber (CF) activation also have an LTP-suppressing role (van Beugen et al., 2006). It appears that ECs released postsynaptically after CF activation of the Purkinje neuron act on PF terminals to decrease adenylyl cyclase activity that is necessary for LTP induction. The suppression of LTP persists even when PF-LTD is blocked, indicating that loss of potentiation is not simply a result of enhancement of competing LTD. This LTP suppression could, however, underlie promotion of PF-LTD by removing competing LTP. This remains to be determined.

The emerging evidence that ECs can both promote and reduce LTP suggests a complicated picture. The net effect of EC release and CB1 activation likely depends on the level and location of EC release as well as which synapses contain CB1 receptors within a given brain region. The factors that determine whether EC actions on inhibitory or excitatory transmission predominate, and the impact of these factors on LTP induction, need to be explored. The relationship between strength of afferent activation and subsequent EC release at postsynaptic elements associated with excitatory and inhibitory synapses also needs further characterization. It appears that the EC/CB1 system provides flexible neuromodulation that can produce differential mediation or modulation of synaptic plasticity in response to different patterns of neural activity, even within a single brain region. This flexibility in EC-dependent plasticity likely underlies the different roles of the neuromodulator in learning and memory, as will be discussed in the following section of this article.

4.37.8 Endocannabinoid and CB1 Receptor Roles in Learning and Memory

4.37.8.1 Exogenous Cannabinoid Agonists Impair Memory

Endocannabinoids and CB1 receptors have been implicated in a number of neural circuit functions and behaviors. These include feeding behavior, responses to stress, nociception, and several others. This discussion will focus on the roles of these molecules in learning and memory. Traditionally, evidence for a connection between the CB system and learning and memory came mainly from the actions of CB drugs and Δ9-THC itself (**Figure 2(a)**). It has long been

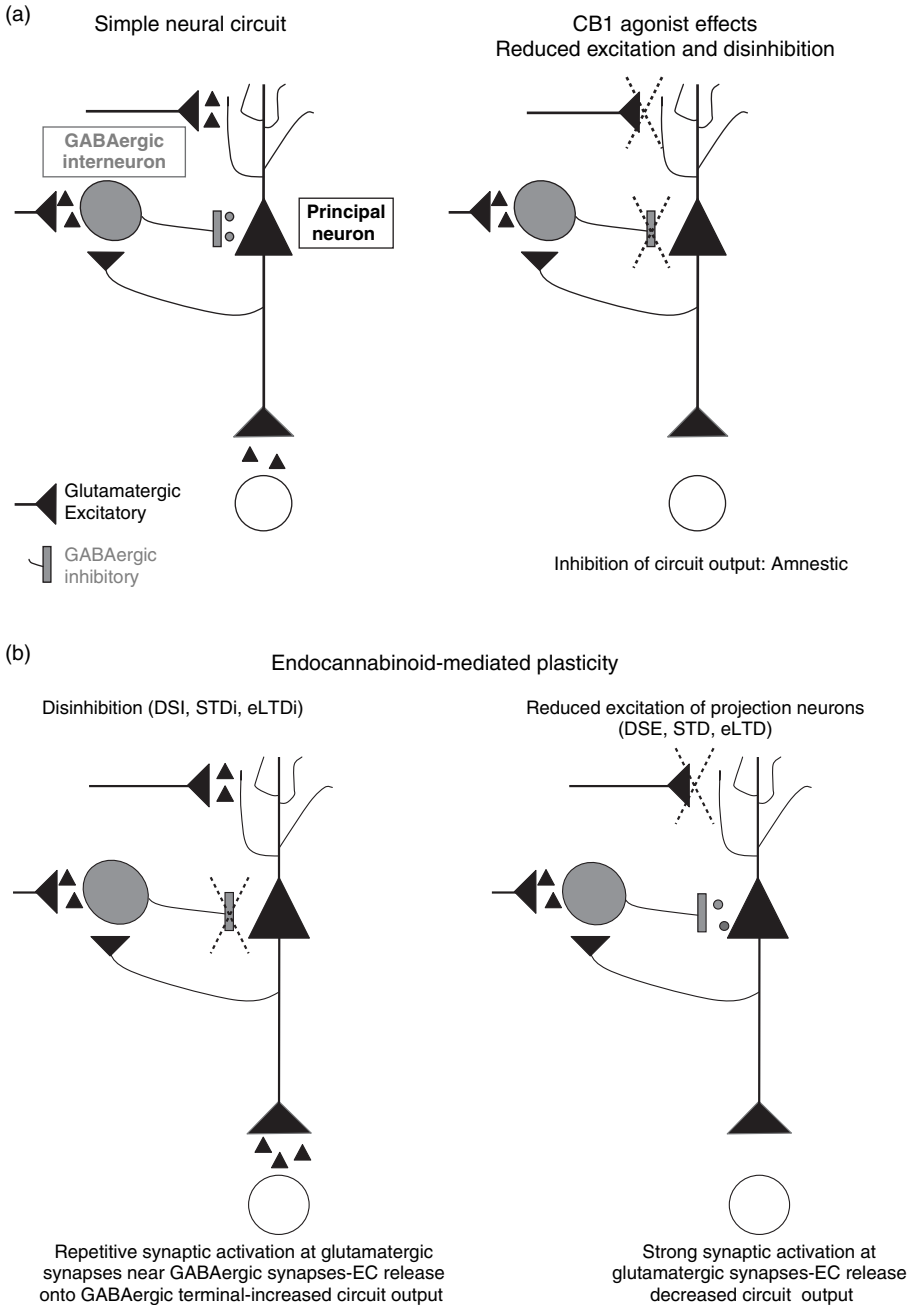


Figure 2 Effects of cannabinoid drugs and endocannabinoids on excitatory and inhibitory transmission and potential roles in information storage. (a) Schematic illustration of the arrangement of a principal neuron and an inhibitory interneuron that participates in both feedforward and feedback inhibition, similar to the arrangement of pyramidal and basket cells in the hippocampal CA1 region (left). Cannabinoid agonist drugs including Δ9-THC activate cannabinoid (CB) receptors on both excitatory and inhibitory terminals, reducing net circuit output and synaptic plasticity (right). This would account for impairment of learning and memory produced by these drugs. (b) Given the same circuit arrangement, postsynaptic activation that is sufficiently strong and localized to excitatory synapses located near inhibitory synapses will have a net disinhibitory effect. This would lead to increased circuit output and promote long-term potentiation. In this way endocannabinoid (EC) signaling could promote learning and memory. Sufficiently strong postsynaptic activation localized to excitatory synapses with CB1-containing presynaptic terminals would produce EC-dependent presynaptic depression that would tend to reduce circuit output. This might contribute to EC roles in impairment of information storage. GABA, gamma-aminobutyric acid; DSI, depolarization-induced suppression of inhibition; STDi, short-term depression (the i refers to inhibitory synapses); eLTDi, EC-dependent long-term depression (inhibitory synapses).

known that these drugs have a generally disruptive effect on learning and memory, and that some of the amnestic cannabis actions resemble effects of hippocampal lesions (Miller and Branconnier, 1983; Hampson and Deadwyler, 1998; Sullivan, 2000; Wilson and Nicoll, 2002).

4.37.8.2 Endocannabinoids and Memory Impairment

With the discovery of the CB1 receptor followed by the discovery of ECs, and finally the synthesis of CB1 antagonists, interest in examining the role of this receptor in learning and memory rapidly grew. The CB1 antagonist SR141716A (rimonabant) was found to reverse the amnestic effects of CB1 agonists or β-amyloid (Mallet and Beninger, 1998; Mazzola et al., 2003). The antagonist itself did not appear to have any effect on established memory in either the instrumental learning task used by Mallet and Beninger (1998) or the passive avoidance task used by Mazzola et al. (2003). However, effects on learning were not explicitly examined.

Additional evidence supports the idea that ECs can have memory-inhibiting function. For example, Shiflett et al. (2004) found that rimonabant given in the bird hippocampus improved memory for food storage locations but enhanced proactive interference for new location memories. This compound also improved rodent performance in a social recognition task (Terranova et al. 1996). Takahashi et al. (2005) have shown that the CB1 antagonist/partial agonist facilitates learning and reverses scopolamine-induced amnesia in rats using an elevated T-maze paradigm. The circuitry involved in this learning paradigm is not well defined. Using a radial arm maze task incorporating a significant training-retention delay, presumably a task that involves the hippocampus, Wolf and Leander (2003) found improved retention after rimonabant treatment. Improvement in the radial arm maze task was also observed by Lichtman (2000). Thus, there is ample evidence that EC activation of CB1 receptors impairs memory under certain conditions.

4.37.8.3 Endocannabinoid Roles in Promoting Learning and Memory

The development of gene-targeted mice lacking the CB1 receptor produced an opportunity to further examine the role of the EC system in brain information storage. Marsicano et al. (2002) used CB1−/− mice to implicate EC signaling in extinction of fear conditioning. A reduction in extinction similar to that observed in the CB1−/− mice was also seen upon injection of a CB1 antagonist, and evidence for increased EC levels in BLA during extinction-inducing trials was also observed. The eLTD observed at inhibitory BLA synapses was also lost in the CB1 knockout mouse. Studies in other laboratories have confirmed that loss of CB1 function reduces extinction in this aversively motivated task (Suzuki et al., 2004) and in the Morris water maze task, where CB1−/− mice show increased perseveration (Varvel and Lichtman, 2002; Varvel et al., 2005). However, a subsequent study by Marsicano, Lutz, and coworkers has added an interesting twist to the story (Kamprath et al., 2006). It appears that lack of CB1 receptor function also prevents habituation to the stimuli that produce fear conditioning. Thus, it is possible that the loss of extinction simply reflects an inability to habituate, perhaps reflecting an inability to adjust to sensory stimuli. It remains to be seen if there is a specific EC role in extinction.

A recent study has also implicated the EC system in aversive Pavlovian conditioning involving BLA inputs to the prefrontal cortex (Laviolette and Grace, 2006). These investigators have used a novel paradigm involving pairing of olfactory cues with a noxious stimulus in awake or lightly anesthetized rats. This pairing method produces conditioned freezing to the olfactory cue in unanesthetized rats and associated changes in the firing frequency and the incidence of bursting activity of medial PFC neurons observed during *in vivo* electrophysiological recordings in the anesthetized state. Treatment with a CB1 antagonist reduces conditioned freezing, while agonist treatment promotes learning to a normally subthreshold stimulus. Furthermore, these drugs are active when applied directly into the PFC, implicating this brain region as a site of important CB1 function. Much remains to be determined about the EC role in this form of learning, including the cellular localization of the CB1 receptors involved.

4.37.8.4 Possible Roles of Long-Term Depression and Long-Term Potentiation in Cannabinoid and Endocannabinoid Effects on Memory

Activation of CB1 receptors with exogenous agents also inhibits the induction of LTP in the hippocampal CA1 subregion (Nowicky et al., 1987; Terranova

et al., 1996; Stella et al., 1997; Misner and Sullivan, 1999), which could contribute to memory impairment produced by these compounds. Both synthetic and naturally occurring CB1 agonists are effective in this paradigm. The ability of CB1 activation to both promote and impair LTP may seem inconsistent at first glance. However, it must be kept in mind that the LTP-promoting effects are mainly observed during activation of EC signaling stimulated by physiological means, while LTP impairment has been observed primarily during application of exogenous agonists (**Figure 2**). Factors including the level of receptor occupancy and duration of receptor activation likely play a role in determining the net effect of CB1 activation.

Physiological activation of EC signaling primarily selectively depresses inhibitory synaptic transmission, presumably because these terminals are more sensitive to such depression (**Figure 2(b)**). The relatively high density of CB1 receptors on inhibitory terminals likely accounts for this increased sensitivity (Freund et al., 2003; Mackie, 2005). This disinhibitory effect primes LTP induction, as has been shown for many conditions that reduce GABAergic inhibition (see discussion above). In contrast, application of high concentrations of CB1 agonists depresses glutamatergic excitatory transmission in addition to synaptic inhibition. This reduced excitation suppresses the glutamatergic drive necessary for LTP induction and thereby prevents LTP induction (Misner and Sullivan, 1999). Such differences could explain the differential effects of EC signaling and CB drugs on learning and memory. Brief activation of CB1 receptors during physiological signaling localized on inhibitory synapses could produce disinhibition and enhance LTP. Strong and prolonged activation of receptors could depress excitatory transmission, leading to diminished LTP (**Figure 2(b)**). A potential role for disinhibitory effects of ECs/CB1 in memory is supported by de Oliveira Alvares et al. (2006), who demonstrated that CB1 blockade prevents LTP in the CA1 region *in vivo*. The antagonist also impairs memory on a single-trial step-down avoidance task. These data are intriguing, but the role of the hippocampus is not yet clear (although see Whitlock et al., 2006), and thus it is premature to conclude too much from this study.

The totality of evidence suggests that ECs have a complex involvement in learning and memory. The memory-promoting versus memory-impairing effects of this system may relate to the neural circuitry involved in a particular memory or neuronal locus of expression of the CB1 receptor. One idea is that EC-dependent DSI, STD, or LTD at inhibitory synapses made by interneurons promotes memory by enhancing LTP and increasing information flow through a circuit. In contrast, EC-dependent DSE, STD, or LTD at excitatory synapses onto projection neurons might impair memory by preventing or impairing LTP and reducing net circuit output (**Figure 2**). However, this is undoubtedly too simple a formulation, because one can think of instances in which depression of excitatory transmission might participate in memory formation (e.g., by inhibiting output from inhibitory projection neurons or sharpening signal/noise ratios for relevant stimuli). It is more likely that the net effect of all the ECs and CB1 receptors within the relevant circuitry is what determines the effect on information storage. Given that the EC/CB1 system is neuromodulatory, the extent of receptor activation may also be important. Subtle influences on the circuitry may push relevant synaptic and neuronal activity above or below thresholds necessary for plasticity and information storage.

Clearly more information is needed about which EC-releasing neurons and CB1 receptors are involved in the different types of information storage. More brain region- and neuronal-specific knockouts are needed, and these should be coupled with brain region–specific receptor activation and blockade. In addition, a wider range of behavioral tests needs to be employed to examine effects on learning, consolidation, extinction, relearning, and other relevant aspects of information storage that involve different neuronal circuitry. We are near the beginning of our quest to characterize EC roles in brain information storage, and much interesting work lies ahead.

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4.38 Transsynaptic Signaling by NO during Learning-Related Synaptic Plasticity

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4.38.1 Introduction

The relationship between pre- and postsynaptic mechanisms of plasticity has been one of the more controversial areas of neuroscience. On the one hand, studies from a variety of systems including hippocampus and *Aplysia* have suggested that protein and RNA synthesis–dependent long-term (hours to days) synaptic plasticity can involve changes in the number or structure of synapses, which by its nature requires coordinated pre- and postsynaptic alterations. On the other hand, short-term (up to about 1 h) plasticity and the early phases of long-term plasticity have been thought to involve a completely different type of mechanism, covalent modification of existing proteins in either postsynaptic (hippocampus) or presynaptic (*Aplysia*) structures (Kandel, 2001; Malinow and Malenka, 2002). However, this dichotomy was questioned by the discovery of intermediate (2–3 h) forms of plasticity in both hippocampus (Winder et al., 1998) and *Aplysia* (Ghirardi et al., 1995), and in recent years it has been challenged by additional evidence from electrophysiological and imaging studies.

At least under some conditions, pre- as well as postsynaptic physiological mechanisms have been found to contribute to early-phase long-term potentiation (LTP) in hippocampus (Arancio et al., 1995, 1996, 2001; Malgaroli et al., 1995; Ryan et al, 1996; Choi et al., 2003; Zakharenko et al., 2003; Ninan and Arancio, 2004; Wang et al., 2005; Lu and Hawkins, 2006; Ninan et al., 2006) and to a variety of types of short-term plasticity in *Aplysia* including homosynaptic potentiation (Bao et al., 1997; Jin and Hawkins, 2003), heterosynaptic facilitation by serotonin (5HT) (Chitwood et al., 2001; Jin et al., 2004; Li et al., 2005), activity-dependent facilitation (Bao et al., 1998), and the synaptic plasticity during behavioral sensitization (Antonov et al., 2006) and conditioning (Antonov et al., 2003, 2004). In many cases inhibitors injected into the pre- and postsynaptic neurons have more than additive effects (Bao et al., 1997, 1998; Arancio et al., 2001; Antonov et al., 2003, 2004, 2006; Jin and Hawkins, 2003; Wang et al., 2005; Lu and Hawkins, 2006), suggesting that the pre- and postsynaptic mechanisms are not independent but, rather, act synergistically. Furthermore, in some cases postsynaptic inhibitors have been shown to affect presynaptic properties, suggesting transynaptic signaling (Antonov et al., 2003).

One type of mechanism that is known to have these properties is the growth of new synapses or the structural modification of existing synapses (Cohen-Cory, 2002). Consistent with such a mechanism, imaging studies have shown that even the earliest phases of hippocampal LTP are accompanied by pre- and postsynaptic structural alterations. Tens of minutes after the induction of LTP there is an outgrowth of new pre- and postsynaptic processes (Engert and Bonhoeffer, 1999; Maletic-Savatic et al., 1999; Nikonenko et al., 2003), and even earlier (minutes), there are increases in spine size (Matsuzaki et al., 2004), clusters of postsynaptic glutamate receptors (Shi et al., 1999), and clusters of presynaptic

vesicle-associated proteins and sites where the pre- and postsynaptic clusters colocalize (Antonova et al., 2001). Moreover, the presynaptic alterations appear to depend on retrograde signaling from the postsynaptic cells (Nikonenko et al., 2003; Wang et al., 2005). Similarly, intermediate-term facilitation by 5HT in *Aplysia* is accompanied by increases in clusters of the postsynaptic protein GluR1 (glutamate receptor 1) and the presynaptic protein synaptophysin within hours (Kim et al., 2003; Li et al., 2004), and homosynaptic potentiation is accompanied by aggregation of synaptophysin into new clusters or puncta within minutes (Jin et al., 2003), as occurs during LTP in hippocampal neurons.

4.38.2 Transsynaptic Messengers

Collectively, these results suggest that the early phases of learning-related synaptic plasticity can engage a coordinated sequence of pre- and postsynaptic functional and structural changes that may be early steps in the formation of new synapses, as occurs during synaptic development (Sanes and Lichtman, 1999; Cohen-Cory, 2002). According to this view, early- and late-phase and pre- and postsynaptic mechanisms of plasticity may not be fundamentally different and independent, as previously thought, but, rather, may have features in common (structural alterations) that are involved in the transition from short- to long-term plasticity.

The growth of new synapses during development is thought to involve a variety of anterograde and retrograde messengers, which may serve different functional roles and act cooperatively (Scheiffele, 2003). Consistent with the idea that synaptic plasticity may engage similar mechanisms, LTP involves many of the same messenger molecules including adhesion molecules such as neural cell adhesion molecule (NCAM) (Luthi, 1994), integrins (Staubli et al., 1998; Chan et al., 2003, 2006; Kramar et al., 2006), cadherins (Tang et al., 1998; Bozdagi et al., 2000), and ephrins (Contractor et al., 2002; Armstrong et al., 2006); secreted molecules such as platelet activating factor (del Cerro et al., 1990; Clark et al., 1992; Wieraszko et al., 1993), tissue plasminogen activator (Baranes et al., 1998), brain-derived neurotrophic factor (BDNF) (Kang and Schuman, 1995; Thoenen, 1995; Korte et al., 1996; Zakharenko et al., 2003; Pang et al., 2004), and neuregulin (Kwon et al., 2005); and freely diffusible molecules such as arachidonic acid (Lynch et al., 1989; Williams and Bliss, 1989; Williams et al., 1989), carbon monoxide (Stevens and Wang, 1993; Zhuo et al., 1993), reactive oxygen species (Klann and Thiels, 1999), and nitric oxide.

4.38.3 Early Studies of the Role of Nitric Oxide in Long-Term Potentiation

In this chapter I focus on nitric oxide (NO), which plays a role in late-stage developmental processes and synaptogenesis in a variety of systems (Hindley et al., 1997; Van Wagenen and Rehder, 1999; Packer et al., 2003; Schwarte and Godfrey, 2004; Sunico et al., 2005; Zhang et al., 2005). A previous review (Hawkins et al., 1998) summarized the evidence to that date that NO also plays a role in LTP. Briefly, both neuronal NO synthase (nNOS) and endothelial NO synthase (eNOS) were thought to be present in hippocampal pyramidal neurons (Dinerman et al., 1994; O'Dell et al., 1994; Wendland et al., 1994). At least under some conditions, LTP in hippocampal slices is blocked by NO scavengers, NO synthase (NOS) inhibitors, or knockout of nNOS and eNOS (Bohme et al., 1991; O'Dell et al., 1991; Schuman and Madison, 1991; Haley et al., 1992; Son et al., 1996). LTP can also be mimicked by exogenous NO paired with weak presynaptic activity (in the presence of an *N*-methyl-D-aspartate (NMDA) receptor antagonist to prevent potentiation by the activity alone) (Zhuo et al., 1993). The requirement for pairing with presynaptic activity is thought to preserve the input specificity of LTP with a diffusible messenger such as NO, which might otherwise spread from active neurons and produce potentiation in inactive neighboring neurons.

Experiments on cultured hippocampal neurons indicated that NO acts specifically as a retrograde messenger during potentiation (Arancio et al., 1996). Long-lasting potentiation in culture is blocked by injection of a NOS inhibitor into the postsynaptic but not the presynaptic neuron, or by injection of an NO scavenger into either neuron. The potentiation can be mimicked by release of caged NO in either neuron, paired with weak presynaptic activity. Moreover, an extracellular NO scavenger blocks potentiation by release of caged NO in the postsynaptic neuron, but not in the presynaptic neuron. These results indicate that NO is produced in the postsynaptic neuron, diffuses across the extracellular space, and acts in the presynaptic neuron to produce potentiation.

Additional evidence suggested that NO acts through cyclic guanosine monophosphate (cGMP)

and cyclic GMP-dependent protein kinase (cGK), as it does in many other systems. Soluble guanylyl cyclase (sGC) and cyclic GMP-dependent protein kinase I (cGKI) are present in hippocampal pyramidal neurons (Matsuoka et al., 1992; Verma et al., 1993; Kingston et al., 1996). LTP in hippocampal slices is blocked by inhibitors of sGC or cGK and mimicked by membrane-permeable cGMP analogs paired with weak activity (Zhuo et al., 1994a; Boulton et al., 1995). Similarly, potentiation in cultured hippocampal neurons is blocked by injection of a peptide inhibitor of cGK into the presynaptic neuron and mimicked by injection of cGMP or cGKI into the presynaptic neuron paired with weak activity (Arancio et al., 1995, 2001).

These results made a strong case that the NO-cGMP-cGK pathway can play a role in retrograde signaling during hippocampal LTP. However, some of the results were not replicated in other labs, and the roles of NO, cGMP, and cGK remained controversial. Experiments since 1998 have addressed many of the areas of controversy and, in addition, have made progress in new directions.

4.38.4 Areas of Controversy

A likely reason for different results in different labs is that LTP is a complex phenomenon involving many signaling pathways, with the relative importance of the different pathways depending on a host of experimental variables such as species, strain, age, temperature, induction protocol, slice or culture preparation, saline composition, and so on. A number of studies have tried to define the conditions under which NO, cGMP, and cGK are more or less important (e.g., Son et al., 1998; Lu et al., 1999; Blackshaw et al., 2003; Kleppisch et al., 2003; for discussion, see Lu and Hawkins, 2002), but each of those studies can account for only some of the published data. Thus they support the general idea that experimental variables are important, but they have not provided a simple explanation that can account for all of the differences in published results. Recent studies have suggested that the dynamics of signaling may also be an important variable. For example, NO may play a tonic, as well as phasic signaling role in LTP (Bon and Garthwaite, 2003). In addtition, cGMP and cGK may activate phosphodiesterases that feed back to decrease cGMP levels *below* baseline (Montfort et al., 2002). That result may explain why brief application of cGMP analogs is more effective than prolonged application in producing potentiation (Son et al., 1998).

Another area of controversy has been whether NO, cGMP, and cGK have the correct cellular and subcellular localization to play a role in retrograde signaling during LTP. Recent imaging studies have shown that NO is released during the induction of LTP by tetanic stimulation in hippocampal slices (von Bohlen et al., 2002; Takata et al., 2005). Furthermore, nNOS is concentrated in postsynaptic densities, and sGC and cGKI are concentrated in presynaptic varicosities in hippocampal neurons, consistent with a role in retrograde signaling (Arancio et al., 2001; Burette et al., 2002; Wang et al., 2005). However, sGC and cGKI are also found in postsynaptic densities, suggesting that the NO-cGMP-cGK pathway may have postsynaptic actions as well. In addition, recent studies have shown that eNOS is *not* present in hippocampal neurons, as previously thought, but, rather, is restricted to endothelial cells (Blackshaw et al., 2003). However, NO generated by eNOS in the vascular endothelium produces tonic depolarization of axons (Garthwaite et al., 2006), which might explain both the role of eNOS in LTP (Kantor et al., 1996; Son et al., 1996) and the tonic role of NO (Bon and Garthwaite, 2003). Recent experiments on eNOS knockout animals have supported that idea (Hopper and Garthwaite, 2006).

4.38.5 Molecular and Functional Consequences of NO Signaling

There has also been progress in describing the molecular and functional consequences of NO signaling in hippocampal neurons. Postsynaptically generated NO produces increases in presynaptic cGMP and vesicle cycling, indicative of increased transmitter release, in cultured hippocampal neurons (Micheva et al., 2003). Long-lasting potentiation in culture is also accompanied by an increase in presynaptic vesicle cycling that is blocked by inhibitors of NOS or cGK and mimicked by application of an NO donor or cGMP analog paired with weak activity (Ninan and Arancio, 2004; Ninan et al., 2006). The effects of the NO donor or cGMP analog are blocked by an inhibitor of Ca^{2+}/calmodulin-dependent protein kinase II (CaMKII), suggesting that CamKII acts downstream of the NO-cGMP-cGK pathway.

Somewhat surprisingly, very similar results have been reported for long-term *depression* (LTD) in hippocampus, which is accompanied by a decrease in presynaptic vesicle cycling that is thought to involve

retrograde signaling by the NO-cGMP-cGK pathway (Stanton et al., 2001, 2003). In that case, cGK is thought to act by stimulating production of cADP ribose, the endogenous ligand for ryanodine receptors, leading to enhanced Ca^{2+}-induced Ca^{2+} release from intracellular stores and activation of CamKII in the presynaptic neurons (Reyes and Stanton, 1996; Stanton and Gage, 1996; Reyes-Harde et al., 1999a,b). Consistent with that idea, imaging studies have shown that NO produces a long-lasting increase in presynaptic Ca^{2+} transients (Martinez-Serrano et al., 1996). The idea that the NO-cGMP-cGK pathway acts in part by enhancing Ca^{2+} signaling may help to explain how that pathway can be involved in both potentiation and depression, because Ca^{2+} is also thought to be involved in both types of plasticity with different thresholds (for discussion, see Lu and Hawkins, 2002). Thus, although NO or cGMP analogs produce potentiation when paired with brief, high-frequency activity (50 Hz, 0.5 s), they produce long-term depression when paired with longer, low-frequency activity (0.25 Hz, 100 s) (Zhuo et al., 1994b). In both cases, the activity does not produce any plasticity by itself, and NO might act by increasing Ca^{2+} levels above the threshold for that form of plasticity. NO also contributes to both LTP and LTD induced by different patterns of stimulation in cerebellum (Shibuki and Okada, 1991; Lev-Ram et al., 2002).

In addition to acting indirectly by modulating Ca^{2+} levels, NO can regulate transmitter release directly by s-nitrosylation of N-ethylmaleimide sensitive factor (NSF), which is thought to disassemble soluble NSF attachment protein receptor (SNARE) complexes during exocytosis (Matsushita et al., 2003). Interestingly, NO-mediated s-nitrosylation of NSF has also been shown to contribute to a postsynaptic mechanism, membrane insertion of GluR2-containing alpha-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA) receptors during LTP in both hippocampus (Huang et al., 2005) and cerebellum (Kakegawa and Yuzaki, 2005).

4.38.6 Role of NO in Early Microstructural Alterations

The NO-cGMP-cGK pathway is also involved in microstructural alterations during LTP including the formation of new presynaptic filopodia, which begin to appear around 10 min (Nikonenko et al., 2003), and new puncta of synaptic proteins, which begin to appear in as little as 1 min (Wang et al., 2005). The increase in puncta is blocked by inhibitors of NOS or cGK and mimicked by exogenous NO or a cGMP analog. Furthermore, cGMP levels are elevated, and cGK is activated and phosphorylates an endogenous substrate, vasodilator-stimulated phosphoprotein (VASP), during potentiation. Somewhat surprisingly, these events occur on both sides of the synapse, and cGMP and cGK are involved in the increases in postsynaptic GluR1 puncta as well as presynaptic synaptophysin puncta and sites where the pre- and postsynaptic puncta colocalize. Consistent with that finding, the NO-cGMP-cGK pathway is also involved in clustering of postsynaptic Ach receptors at the neuromuscular junction (Jones and Werle, 2004; Schwarte and Godfrey, 2004). Furthermore, a few studies have suggested that NO, cGMP, and cGK may play physiological roles in the postsynaptic as well as presynaptic neuron during long-lasting potentiation in hippocampal neurons (Son et al., 1998; Lu et al., 1999; Arancio et al., 2001; Lu and Hawkins, 2002). Thus, NO may activate sGC and cGK simultaneously in both the pre- and postsynaptic neurons, perhaps engaging similar mechanisms and producing coordinate microstructural changes on the two sides of the synapse (**Figure 1**).

The early microstructural changes could in turn be some of the first steps in a sequence of events that leads to the growth of new synapses. That idea suggests a possible novel role for a diffusible messenger such as NO, which is to signal the *de novo* formation of synapses at new locations in response to activation of NMDA receptors at existing synapses. Potentiation is accompanied by a rapid increase in presynaptic puncta that colocalize with puncta of the postsynaptic proteins GluR1, NR1, or PSD95 and therefore might be triggered by very local retrograde signaling, for example, through adhesion molecules (Antonova et al., 2001). However, potentiation is also accompanied by an increase in presynaptic puncta that do not colocalize with any of these postsynaptic puncta but nevertheless require NMDA receptor activation, suggesting the involvement of a messenger that diffuses from the NMDA receptors to the location of the new puncta. Inhibitors of the NO-cGMP-cGK pathway block the increases in synaptophysin puncta that are either colocalized or not colocalized with postsynaptic puncta, suggesting that NO can act as such a messenger both very locally and at some distance (Wang et al., 2005). Furthermore, NMDA receptors and the NO-cGMP-cGK pathway also play roles in

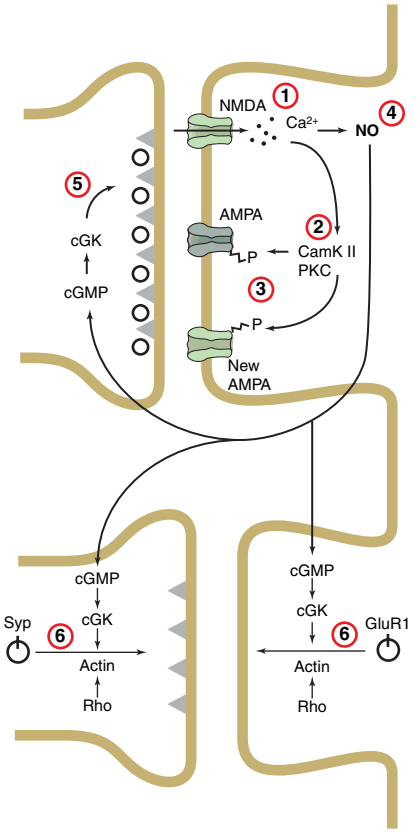

Figure 1 Role of NO in hippocampal long-term potentiation (LTP). Early-phase LTP in the CA1 region of hippocampus involves (1) Ca^{2+} influx through postsynaptic *N*-methyl-D-aspartate (NMDA) receptor channels, (2) activation of protein kinases including CamKII and PKC, and (3) increased conductance of existing AMPA receptor channels and membrane insertion of new alpha-amino-3-hydroxy-5-methylisoxazole-4-propionic acid (AMPA) receptors. Postsynaptic Ca^{2+} also (4) stimulates production of NO, which acts through cGMP and cGK to (5) enhance presynaptic transmitter release and (6) recruit pre- and postsynaptic molecules, including synaptophysin (Syp) and the GluR1 subunit of AMPA receptors, to new synaptic sites. See the text for details.

the formation of new GluR1 puncta that are either colocalized or not colocalized with synaptophysin puncta and, therefore, may form in new locations as well.

4.38.7 Role of NO in Late-Phase Long-Term Potentiation

In addition to playing roles in E-LTP, NO, cGMP, and cGK are also involved in late-phase LTP (L-LTP), which requires gene regulation and RNA and protein synthesis (Lu et al., 1999). NO can regulate genes directly by binding to a heme group in a nuclear receptor (Reinking et al., 2005). Alternatively, the NO-cGMP-cGK pathway can phosphorylate and activate the transcription factor CREB (cAMP response element binding protein) either directly (Gudi et al., 1996) or indirectly by activating other kinases such as protein kinase A (PKA), mitogen-activated protein kinase (MAPK), or CamK. CREB phosphorylation during L-LTP is blocked by inhibitors of either PKA or cGK and mimicked by application of either a membrane-permeable cAMP analog or a cGMP analog paired with weak activity (Lu et al., 1999). The cGMP-induced L-LTP and CREB phosphorylation are blocked by a high concentration of ryanodine, which blocks Ca^{2+}-induced Ca^{2+} release, suggesting that cGMP and cGK may act indirectly by enhancing postsynaptic Ca^{2+} release during the induction of L-LTP (Lu and Hawkins, 2002).

4.38.8 Roles of NO in Other Forms of Learning-Related Synaptic Plasticity

NO is also involved in LTP in a variety of other brain areas including amygdala (Schafe et al., 2005) and neocortex (Hardingham and Fox, 2006), where it may act as a retrograde messenger. In addition, NO contributes to both LTD and LTP in cerebellum, where it is thought to play a different role. Cerebellar LTD can be induced by prolonged low-frequency stimulation of presynaptic parallel fibers paired with either climbing fiber stimulation or direct depolarization of postsynaptic Purkinje neurons. In either case, the depression is blocked by an NO synthase inhibitor or extracellular NO scavenger and mimicked by substituting exogenous NO for the parallel fiber stimulation, suggesting that NO is involved and travels through the extracellular space (Shibuki and Okada, 1991; Lev-Ram et al., 1995). LTD can also be induced by uncaging NO in a Purkinje neuron paired with depolarization of that neuron (Lev-Ram et al., 1995). In that case, the depression is blocked by an intracellular but not by an extracellular NO scavenger, suggesting that NO acts directly in the postsynaptic Purkinje neuron to produce LTD.

Low-frequency stimulation of parallel fibers without postsynaptic depolarization can induce LTP, rather than LTD (Lev-Ram et al., 2002). Like LTD, the LTP is blocked by an NO synthase inhibitor or

extracellular NO scavenger and mimicked by exogenous NO. Uncaging NO in a Purkinje neuron without depolarization can also induce LTP, which (again like LTD) is blocked by an intracellular but not by an extracellular NO scavenger. These results suggest that NO can act directly in the postsynaptic neuron to produce either LTD or LTP, depending on whether the NO is paired with depolarization. In addition to this NO-dependent LTP, somewhat higher-frequency stimulation of parallel fibers can induce a different form of LTP that is thought to be entirely presynaptic and depend on cAMP rather than NO (Salin et al., 1996). However, NO also appears to be involved in the heterosynaptic spread of that form of LTP to neighboring presynaptic parallel fibers (Jacoby et al., 2001). For LTD and both forms of LTP, NO is generally thought to come from the presynaptic parallel fibers (Shibuki and Kimura, 1997), in which case it would act as either an anterograde or lateral messenger. Alternatively, NO may come from other cell types (Shin and Linden, 2005), in which case it would act as a paracrine messenger.

NO also plays a role in learning-related synaptic plasticity in *Aplysia*. NO synthase is present and active in the *Aplysia* central nervous system (Moroz et al., 1996; Bodnarova et al., 2005; Moroz, 2006), and NO is involved in a variety of cellular (Mothet et al., 1996; Koh and Jacklet, 1999; Sung et al., 2004) and behavioral effects, including conditioning of feeding behavior (Katzoff et al., 2002) and siphon withdrawal (Antonov et al., 2004). Classical conditioning of siphon withdrawal in a semi-intact preparation involves activity-dependent facilitation of monosynaptic excitatory postsynaptic potentials (EPSP) from siphon sensory neurons to siphon motor neurons in the abdominal ganglion, as well as enhanced membrane resistance and evoked firing of the sensory neurons (Antonov et al., 2001, 2003). The behavioral and cellular effects are blocked by bathing the ganglion in a membrane-impermeant NO scavenger, oxymyoglobin, suggesting that NO is involved and travels through the extracellular space. In addition, the facilitation and changes in sensory neuron membrane properties are blocked by injecting oxymyoglobin into the sensory neuron, suggesting that NO acts directly in that neuron. Facilitation of the EPSP is also reduced by injecting oxymyoglobin into the motor neuron, but not by injecting a NOS inhibitor into the motor neuron. Consistent with these physiological results, NADPH diaphorase staining suggests that NO synthase is not present in either the motor neurons or sensory neurons but is present in interneurons in the ganglion (Moroz, 2006). Thus, NO appears to play a paracrine role, coming from interneurons and acting in both the sensory and motor neurons to contribute to facilitation of the EPSP between them during conditioning.

4.38.9 Conclusions

Studies in different systems have suggested that NO can play a variety of different pre- and postsynaptic roles during learning-related synaptic plasticity, including retrograde signaling, anterograde signaling, lateral signaling, and paracrine signaling. In most of these cases, however, NO is thought to coordinate pre- and postsynaptic functional changes. In the case of hippocampal LTP, NO is also thought to contribute to coordinated pre- and postsynaptic structural alterations, which may be early steps in the formation of new synapses. Transsynaptic messengers like NO may be involved in similar structural alterations during other forms of synaptic plasticity as well. The idea that even the early phases of synaptic plasticity can recruit messengers that are also involved in the early stages of synapse formation might help to reconcile some of the seemingly conflicting evidence for pre- versus postsynaptic mechanisms of plasticity, because synapse formation would require both.

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4.39 Action Potentials in Dendrites and Spike-Timing-Dependent Plasticity

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4.39.1 Overview

Over the past 50 years it has become clear that individual neurons are sophisticated signal-processing devices rather than simple summing elements. The computational power of neurons originates from the morphological and physiological properties of their dendrites, which are the neuronal processes that receive most synaptic input. The electrical properties of these dendrites determine how inputs are integrated

and whether they lead to the generation of an action potential (AP) in the axon initial segment. Furthermore, dendritic excitability and synaptic potentials are themselves modified by neuronal output, an effect that is largely mediated by AP propagation or initiation in dendrites. This plasticity enables tuning of synaptic integration and continuous updating of the functions of individual neurons within the neuronal network. In this chapter we give an overview of the active properties of dendrites and discuss their influence on synaptic integration, circuit dynamics, and synaptic plasticity.

4.39.2 Introduction: Electrically Active Dendrites

Much of our understanding of dendritic properties and dendritic function originates from early theoretical work in which cable equations were used to describe the filtering and interaction of postsynaptic potentials (PSPs) in electrically passive dendrites (Rall, 1959, 1962, 1964, 1967; Rall et al., 1967; Rall and Rinzel, 1973; Jack et al., 1975). While passive models were building the framework for our current understanding of dendritic integration, evidence from both extracellular and intracellular recordings was accruing that dendrites are not merely passive but 'active' structures, i.e., that they contain voltage-gated conductances (extracellular: Cragg and Hamlyn, 1955; Fatt, 1957a,b; Andersen, 1960; Fujita and Sakata, 1962; Andersen and Lømo, 1966; Llinas et al., 1968, 1969; Rall and Shepherd, 1968; Wong and Prince, 1978; Llinas and Sugimori, 1979; Miyakawa and Kato, 1986; Turner et al., 1989; Herreras, 1990; intracellular: Spencer and Kandel, 1961; Fujita, 1968; Llinas and Hess, 1976; Wong et al., 1979; Llinas and Sugimori, 1980; Turner et al., 1991; Kim and Connors, 1993). The potential importance of active dendritic properties in shaping synaptic integration was recognized by early theoreticians (e.g., Jack et al., 1975), but it was not until infrared differential interference contrast (IR-DIC) optics were used to visualize dendrites in brain slices in combination with patch pipette recordings (Stuart et al., 1993; Stuart and Sakmann, 1994) that membrane potential recording from thick dendrites of the CNS became a routine procedure and active dendritic properties were studied in detail and their importance was firmly established (Johnston et al., 1996; Häusser et al., 2000; Magee, 2000; Reyes, 2001; Gulledge et al., 2005).

It is now clear that many neurons express a wide array of voltage-gated conductances in their dendrites, including Na^+, K^+, Ca^{2+} and nonselective cation channels (Johnston et al., 1996; Reyes, 2001; Migliore and Shepherd, 2002; Gulledge et al., 2005). The presence of these channels enables various forms of dendritic action potentials (**Figure 1**), which have several consequences for dendritic function. For instance, in hippocampal and neocortical pyramidal neurons, dendritic channels enable the local initiation of spikes within the dendritic tree ('dendritic spikes'). As a consequence, dendritic branches can act as independent (or semi-independent) integrative units (Gasparini et al., 2004; Gasparini and Magee, 2006), thereby expanding the computational power of the dendritic tree (Häusser and Mel, 2003; London and Häusser, 2005).

Voltage-gated channels also support retrograde propagation ('backpropagation') of action potentials (APs) into the dendritic trees of many types of neurons (Stuart and Sakmann, 1994; Stuart et al., 1997b; Waters et al., 2005). Since dendrites also contain voltage-gated Ca^{2+} channels, both backpropagation and dendritic spike initiation can evoke Ca^{2+} influx into dendrites and also into dendritic spine heads (dendrites: Jaffe et al., 1992; Markram et al., 1995; Schiller et al., 1995, 1997; Magee et al., 1995; dendritic spines: Jaffe et al., 1994; Yuste and Denk, 1995; Denk et al., 1996; Sabatini and Svoboda, 2000; Sabatini et al., 2002). Intracellular Ca^{2+} exerts a variety of effects on intracellular signaling pathways, depending on peak concentration, exact localization, and time course of the Ca^{2+} transient. In particular, Ca^{2+} signals that are associated with the propagation (or initiation) of APs in dendrites are essential for some forms of synaptic plasticity, including spike-timing-dependent plasticity (Magee and Johnston, 1997, 2005; Markram et al., 1997; Sjöström and Nelson, 2002; Frick et al., 2004; Nevian and Sakmann, 2004; Golding et al., 2002; Holthoff et al., 2004; Kampa et al., 2006). Action potential backpropagation and spike-timing-dependent plasticity (STDP) are two of the most intensely studied features of active dendrites and are the focus of this chapter.

4.39.3 Action Potentials in Dendrites

4.39.3.1 Variations on a Theme: Action Potential Backpropagation in Different Dendrites

In passive dendrites (i.e., those lacking voltage-dependent conductances) any perturbation of the membrane

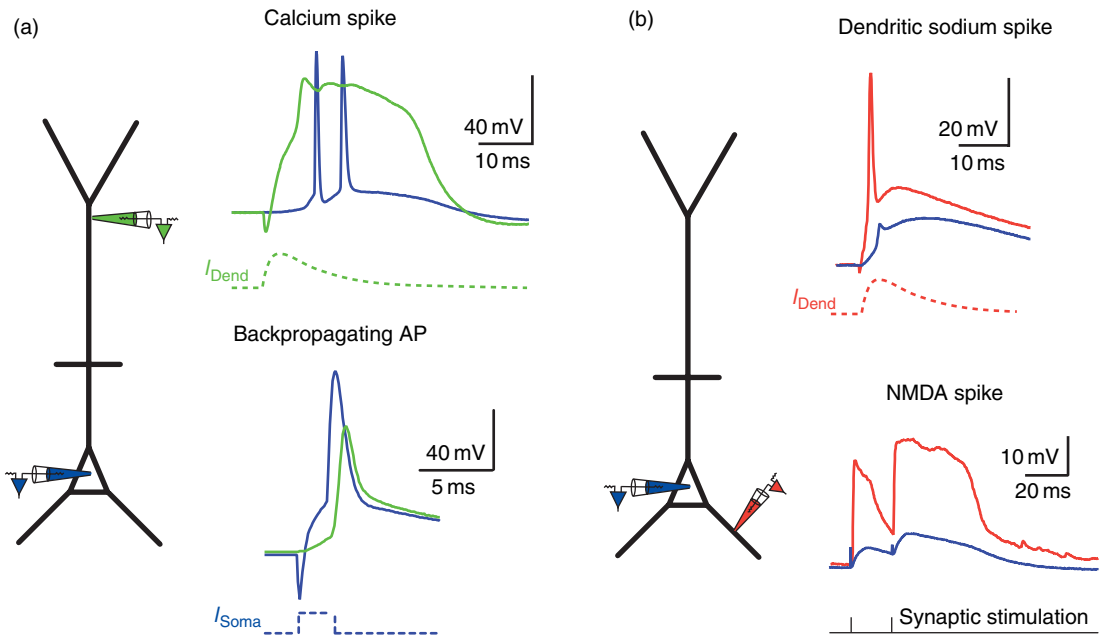

Figure 1 Diversity of dendritic excitability. Illustration of the different types of action potentials observed in dendrites of neocortical pyramidal neurons using multiple-electrode recordings. Electrodes schematically indicate the site of recordings from the soma (blue), distal apical dendrite (green), or basal dendrites (red). Membrane voltage recordings are shown in the corresponding colors. (a) Somatically evoked action potentials backpropagate actively and decrementally into the apical (and basal) dendritic tree (lower traces; example from a layer 2/3 pyramidal neuron). Apical dendrites of layer 5 pyramidal neurons can, in addition, generate calcium spikes mediated by voltage-dependent Ca^{2+} channels (upper traces; evoked in this case by local dendritic depolarization). (b) Sodium action potentials can be elicited locally in both apical and (as shown here) basal dendrites (upper part). Dendritic sodium spikes are greatly attenuated as they propagate toward the soma, thus not necessarily reaching threshold at the soma. Strong synaptic activation can result in the initiation of a *N*-methyl-D-aspartate (NMDA) spike (lower part). Adapted from Waters J, Larkum M, Sakmann B, and Helmchen F (2003) Supralinear Ca^{2+} influx into dendritic tufts of layer 2/3 neocortical pyramidal neurons *in vitro* and *in vivo*. *J. Neurosci.* 23: 8558–8567; Larkum ME, Zhu JJ, and Sakmann B (1999b) A new cellular mechanism for coupling inputs arriving at different cortical layers. *Nature* 398: 338–341; and Nevian T, Larkum ME, Polsky A, and Schiller J (2007) Properties of basal dendrites of layer 5 pyramidal neurons: A direct patch-clamp recording study. *Nat. Neurosci.* 10: 206–214.

potential will spread some measurable distance along the dendrite, but will be attenuated, i.e., cause a lesser perturbation in a neighboring region of the dendrite. The space constant of attenuation is typically on the order of a hundred micrometers or less. This is short compared to the length of most dendrites. In many types of neurons, dendritic ion conductances greatly extend the spatial spread of membrane potential changes, enabling propagation of voltage signals throughout much of the dendritic tree. In particular, dendritic Na^+ channels enable propagation of axonally initiated APs back into the dendritic tree.

4.39.3.1.1 Single action potentials

The extent of AP backpropagation varies in different cell types. In a few dendrites, such as those of dopaminergic substantia nigra neurons (Häusser et al., 1995), the apical dendrites of mitral cells in the olfactory bulb (Bischofberger and Jonas, 1997; Chen et al., 1997) and hippocampal interneurons (Martina et al., 2000), AP backpropagation is reminiscent of axonal propagation in that an AP propagates through the dendritic tree without a substantial decrease in amplitude. In contrast, backpropagation of axosomatically initiated APs approximates passive spread in the dendrites of cerebellar Purkinje neurons, even though local calcium spikes can occur in these dendrites (Llinas and Sugimori, 1980; Stuart and Häusser, 1994). However, in most dendrites examined to date, dendritic ion channels increase the spatial spread of an AP, but fail to maintain its full amplitude as it propagates through the dendritic arbor. Such 'decremental' backpropagation is observed in the apical dendrites of layer 5 and layer 2/3 pyramidal neurons (Kim and Connors, 1993; Stuart et al., 1993, 1997a; Stuart and Sakmann, 1994; Williams and Stuart, 2000b; Larkum et al., 2001; Waters et al., 2003), hippocampal CA1 pyramidal

cells (Andreasen and Lambert, 1995; Spruston et al., 1995), spinal motor neurons (Larkum et al., 1996), and thalamocortical neurons (Williams and Stuart, 2000a). In addition, decremental attenuation has recently been observed in the basal dendrites of layer 5 pyramidal neurons (Nevian et al., 2007) and oblique dendrites of hippocampal CA1 pyramidal neurons (Gasparini et al., 2007).

The inability of most dendrites to support full-amplitude AP propagation can be attributed primarily to a lower density of Na^+ channels in dendrites than in axons. Hence, compared to axons most dendrites can be considered weakly excitable, but even this weak excitability is sufficient to greatly facilitate the spread of APs into the dendritic tree.

Backpropagating APs are often described as 'global' retrograde signals, conveying information about the neuron's output to the sites of synaptic input in the dendritic tree. In light of the preceding discussion, the idea that AP backpropagation is a global signal obviously requires qualification. AP backpropagation generally is not global in that an AP typically does not propagate throughout the whole dendritic tree, but backpropagating APs are also not confined to small dendritic compartments. In this respect AP backpropagation contrasts with dendritically initiated spikes, which produce localized depolarizations that may be restricted to a subregion of the dendritic tree, such as a single branch (Golding and Spruston, 1998; Wei et al., 2001). Hence backpropagation of single APs is not truly global, but is widespread rather than localized.

In dendrites that support decremental backpropagation, the amplitude of a single backpropagating AP can become small enough in distal dendrites that it fails to evoke regenerative activation of Na^+ channels. AP backpropagation is passive distal to this point (Stuart and Häusser, 2001; Bernard and Johnston, 2003). In the part of the dendritic tree where propagation is passive, additional depolarization, such as that from an excitatory postsynaptic potential (EPSP), can restore active propagation, greatly enhancing the amplitude of the backpropagating AP and its conduction into the distal dendrite (Stuart and Häusser, 2001; Bernard and Johnston, 2003).

4.39.3.1.2 Trains of action potentials

Backpropagation of APs is affected differentially during trains of APs, depending on the frequency of the APs in the burst. At low frequencies (<50 Hz) the dendritic amplitudes of late APs in a train are reduced in hippocampal CA1 and neocortical layer 5 pyramidal neurons (Spruston et al., 1995; Stuart et al., 1997b). This accommodation of dendritic AP amplitude arises from a slow recovery of Na^+ channels from inactivation (Colbert et al., 1997). The effect seems to be cell-type specific, as accommodation is minimal in layer 2/3 pyramidal cells (Larkum et al., 2007). At higher AP frequencies, an opposing effect can be observed: bursts of APs can produce more distal depolarization than single APs in dendrites with decremental propagation (e.g., Spruston et al., 1995; Svoboda et al., 1999; Waters et al., 2003; Kampa and Stuart, 2006). With an increase in AP halfwidth during propagation, APs can merge in the dendrite. In effect, the APs act cooperatively to activate dendritic Na^+ and Ca^{2+} channels and depolarize the distal dendrite. Hence bursts of APs – rather than single APs – can provide a global retrograde signal. For example, in layer 5 pyramidal neurons a burst at sufficiently high frequency can evoke a calcium spike (see the section titled 'Calcium spikes'), so that the distal dendrite remains depolarized for tens of milliseconds. This depolarization then propagates forward to the soma and evokes further spiking (Larkum et al., 1999a).

4.39.3.2 Determinants of Action Potential Propagation

The degree of AP amplitude attenuation and the spatial extent of backpropagation are determined by several factors, including dendritic morphology, active dendritic conductances, neuromodulation, and coincident synaptic activity.

4.39.3.2.1 Morphology

Dendritic diameter, spine density and branching patterns affect the biophysical properties of a dendritic tree such as impedance and capacitance. These properties affect AP propagation, but distinguishing these effects from those on dendritic ion channels is difficult. The most informative studies on morphology to date have been compartmental simulations, based on histological reconstructions of single neurons. Compartmental modeling has revealed that dendrites can be interconverted between supporting no propagation, supporting decremental propagation, and supporting nondecremental propagation by changing solely their morphology (Vetter et al., 2001). Furthermore, some morphologies are more sensitive to changes in channel density and distribution than others (Vetter et al., 2001). In pyramidal neurons, the

location of oblique branches also influences backpropagation along the apical dendrite (Schaefer et al., 2003a). In short, dendritic morphology appears to determine to what extent a dendrite will be dependent on its complement of ion channels to support backpropagation. In those dendrites where gross morphology is not dominant, fine morphology and ion channels together fine-tune the degree of AP backpropagation.

Dendritic morphology also contributes to the difference in forward and backward propagation of spikes. Dendrites taper, becoming thinner with distance from the soma. As a result, backpropagating APs travel from regions with relatively low to relatively high impedance and relatively high to relatively low capacitance (Goldstein and Rall, 1974; Koch, 1999). These factors favor backward propagation as each successive patch of membrane in the AP's path along the dendrite depolarizes more readily than the previous one. Spikes traveling in the opposite direction (dendritically initiated spikes traveling toward the soma) suffer the reverse effect, with each successive patch of membrane depolarizing less readily than the previous one, and the AP is therefore heavily attenuated during forward propagation. As a result, AP backpropagation is typically stronger than forward propagation in the same dendrite (Vetter et al., 2001). This effect of morphology contributes to the frequent failure of dendritic spikes to propagate to the soma in dendrites with robust AP backpropagation (e.g., Golding and Spruston, 1998).

Impedance mismatch also makes backpropagating APs less prone to branch-point failure than forward propagating APs. Branch-point failure is normally the result of impedance mismatch between parent and daughter branches and occurs where the sum of the impedances of the daughter branches is lower than the impedance of the parent branch, giving a smaller depolarization in the daughter branches than in the parent dendrite (Koch, 1999). This situation arises in axons and also during the forward propagation of APs in dendrites, where the AP passes from a small-diameter distal dendritic branch into a larger-diameter proximal branch. In contrast, during backpropagation the AP travels from large- to small-diameter dendritic branches, i.e., from lower to higher input impedance branches. Branch-point failure is therefore unlikely during backpropagation of single APs. However, partial branch-point failure has been observed during the backpropagation of bursts of APs in hippocampal pyramidal neurons (Spruston et al., 1995). In this instance backpropagation was inferred from imaging an intracellular fluorescent calcium indicator. Throughout much of the apical dendritic tree the amplitude of the AP-evoked Ca^{2+} signal following a burst was substantially larger than that following a single AP, but in some branches the burst-evoked was comparable with the single AP-evoked Ca^{2+} signal (Spruston et al., 1995). Various potential explanations for this result have been suggested (Migliore, 1996), including failure of some APs in a burst to propagate through axonal branch points. This is likely attributable to the redistribution of ions during bursts of APs (Koch, 1999) and a higher density of A-type K^+ channels in the branch in which full propagation of bursts of APs fails to occur (see the following section titled 'Active conductances').

4.39.3.2.2 Active conductances

Dendritic channel densities are generally studied with whole-cell recordings and with cell-attached or excised patches. In addition, imaging of Ca^{2+} and Na^+ concentration changes using fluorescent indicator dyes can provide information about the distribution of ion channels. However, the influence of other factors such as surface-to-volume ratio and Ca^{2+} extrusion mechanisms largely precludes quantification of channel density using these imaging methods. Hence patch-clamp recordings have provided the most valuable quantitative information, although they also face a number of problems. Not only are dendritic recordings difficult to obtain, but formation of a GΩ seal can stretch the membrane in the patch, making estimates of the area of membrane in the patch approximate. This decreases the accuracy of estimates of channel density from patch clamp recordings. Seal formation can also affect channel properties (Clark et al., 1997; Fenwick et al., 1982). Nonetheless, cell-attached recordings probably provide the most accurate estimates of channel distributions, since they avoid the pronounced space-clamp problems inherent in whole-cell recordings from dendrites (but see Schaefer et al., 2003b).

A wealth of information on dendritic channel densities has been derived from these electrophysiological techniques (Reyes, 2001; Migliore and Shepherd, 2002). In most neurons the distributions of Na^+ channels, K^+ channels, and sometimes Ca^{2+} channels are the most important determinants of AP backpropagation. This is clearly illustrated in compartmental models. For example, the dendritic excitability of motor neurons can be reproduced with a dendritic

Na^+ current density approximately three orders of magnitude lower than the estimated axonal Na^+ channel density (Lüscher and Larkum, 1998). Estimates of dendritic Na^+ channel densities in various neurons correlate with the strength of propagation, from near zero in the dendrites of Purkinje neurons (that do not support AP backpropagation), around 40–60 $pS\,\mu m^{-2}$ in the apical dendrites of CA1 and neocortical pyramidal neurons (that support decremental AP backpropagation) to approximately 100 $pS\,\mu m^{-2}$ in the apical dendrites of olfactory mitral cells and hippocampal interneurons (that support nondecremental AP backpropagation; Migliore and Shepherd, 2002).

Potassium channel density and distribution have been investigated in detail only in pyramidal neurons of the CA1 region in the hippocampus and layer 5B pyramidal neurons in the neocortex (Hoffman et al., 1997; Bekkers, 2000a,b; Korngreen and Sakmann, 2000). In both cell types transient (A-type) K^+ currents are important regulators of AP backpropagation. In hippocampal, but not neocortical pyramidal neurons, A-type current density increases with distance from the soma (Hoffman et al., 1997). A-type current activates so rapidly upon depolarization by an AP that it can significantly limit the amplitudes of backpropagating APs, and suppression of dendritic A-type current substantially enhances AP backpropagation in hippocampal CA1 pyramidal neurons (Hoffman et al., 1997; Johnston et al., 2000).

Na^+ and A-type K^+ currents act synergistically in the apical dendrites of hippocampal pyramidal neurons to limit backpropagation during bursts of APs. During bursts the depolarization provided by each AP causes both inactivation of dendritic Na^+ channels and activation of dendritic A-type K^+ channels (Colbert et al., 1997). The resulting reduction in Na^+ current and shunting of the membrane together reduce the amplitudes of the subsequent APs. As a result, later APs in a burst propagate less readily into the dendritic tree than does the first AP, and this is seen as pronounced accommodation in the dendritic AP amplitude during a burst (Callaway and Ross, 1995). Similar accommodation occurs in the apical dendrites of some layer 5B neocortical pyramidal neurons (Stuart et al., 1997a; Larkum et al., 2001), but not in layer 2/3 neocortical pyramidal neurons (Larkum et al., 2007). Presumably these differences reflect differences in Na^+ or, more likely, K^+ channel expression patterns between these pyramidal neurons.

The distribution of Ca^{2+}channels has been described in less detail, but in hippocampal pyramidal neurons Ca^{2+} channel subtype expression changes along the apical dendrite, although total Ca^{2+} channel density remains constant (Magee and Johnston, 1995a). There is also indirect evidence for a dendritic 'hotspot' in Ca^{2+} channel density in the apical dendrites of layer 5 neocortical pyramidal neurons (Yuste et al., 1994; Schiller et al., 1997; Helmchen et al., 1999; Schaefer et al., 2003b).

4.39.3.2.3 Synaptic activity

In the preceding sections we have discussed the extent of AP backpropagation and the manner in which AP backpropagation is influenced by dendritic morphology and the presence of ion channels. These parameters are relatively static for any one neuron, changing only over relatively prolonged timescales. However, AP backpropagation will also be affected by excitatory and inhibitory synaptic activity, which can strongly alter dendritic properties on the millisecond timescale. In addition, neuromodulatory synapses can regulate AP backpropagation.

Both EPSPs and inhibitory postsynaptic potentials (IPSPs) can affect dendritic membrane potential and input resistance, and both can modulate AP backpropagation (Tsubokawa and Ross, 1996; Stuart and Häusser, 2001; Lowe, 2002; Xiong and Chen, 2002; Pérez-Garci et al., 2006). Since the chloride reversal potential is in most neurons close to the resting membrane potential (e.g., around −70 mV for layer 5 neocortical pyramidal neurons; Luhmann and Prince, 1991; Gulledge and Stuart, 2003), IPSPs exert their effects primarily by shunting the membrane. The time window over which this shunt is effective is the duration of the conductance change underlying the IPSP (approximately 10–20 ms; Destexhe et al., 2003; Koch, 1999). In contrast to IPSPs, the reversal potential for EPSPs is far from the resting membrane potential, and both shunting and depolarization will therefore shape the interaction of AP and EPSP. However, the conductance change of an EPSP is briefer than that of an IPSP (AMPAR-mediated EPSP lasts about 5 ms; Häusser and Roth, 1997; Koch, 1999; Magee and Cook, 2000). For both EPSPs and IPSPs the evoked voltage change is longer than the conductance change. Furthermore, conductance changes are more spatially localized in dendrites than are the resulting voltage changes (Rall, 1967; Koch et al., 1990; Williams, 2004). Hence the time window over which an EPSP can shunt a backpropagating AP is brief, whereas the time course over which the resulting depolarization can interact with the backpropagating AP is more prolonged.

The dominant effect of EPSPs is to enhance AP backpropagation, acting synergistically with a backpropagating AP to depolarize the dendritic membrane beyond threshold for Na^+ channel activation (Stuart and Häusser, 2001). EPSPs are therefore particularly effective in the distal dendrite, where AP amplitude is small and may not activate Na^+ channels (Stuart and Häusser, 2001; Bernard and Johnston, 2003). In addition, the combined depolarization of EPSP and backpropagating AP can initiate additional spiking in the dendrite (Magee and Johnston, 1997; Larkum et al., 1999a,b; Larkum and Zhu, 2002).

Backpropagation of APs is also influenced by neuromodulators, for example via muscarinic acetylcholine receptors (McCormick, 1992; McCormick et al., 1993). The effects of neuromodulators on AP backpropagation have been examined in most detail in hippocampal CA1 pyramidal neurons, where muscarinic agonists enhance the backpropagation by inhibiting K^+ channels (Tsubokawa and Ross, 1997; Hoffman and Johnston, 1999). Modulatory pathways may also effect dendritic spikes. For example, inhibitory inputs can cause a long-lasting blockade of the dendritic Ca^{2+} spike in the apical dendrite of layer 5 pyramidal neurons via metabotropic receptors of the $GABA_{B1b}$ isoform (Pérez-Garci et al., 2006). Most likely metabotropic action in this case involves inhibition of Ca^{2+} channels, although activation of K^+ channels may also contribute (Pérez-Garci et al., 2006).

4.39.3.3 Action Potential Backpropagation *in vivo*

Ongoing, background synaptic activity is higher *in vivo* than in brain slices (Paré et al., 1997, 1998). This has led to the suggestion that AP backpropagation may be different *in vivo* from that in slices (Steriade, 2001a,b). In principle, synaptic activity *in vivo* could enhance, suppress, or have little effect on AP backpropagation, depending on the spatiotemporal pattern of synaptic activity and the numbers of active excitatory, inhibitory, and neuromodulatory synapses. As already discussed, the dominant effects of EPSPs and IPSPs are enhancement and suppression of AP backpropagation, respectively. Hence one would expect the ratio of excitation to inhibition to be the principal factor in determining the effect of ongoing synaptic activity on AP backpropagation. The total amount of synaptic activity may also be important, since EPSPs and IPSPs both are generated by the opening of receptor-gated channels and therefore necessarily increase membrane conductance.

4.39.3.3.1 AP backpropagation in anaesthetized animals

There is now a substantial body of information available examining AP backpropagation in anaesthetized animals. Two early studies concluded that AP backpropagation was suppressed in the neocortex of the anaesthetized rat (Svoboda et al., 1997, 1999). However, the majority of studies concluded otherwise, indicating that AP backpropagation occurs in the hippocampus (Buzsáki et al., 1996), olfactory bulb (Charpak et al., 2001; Debarbieux et al., 2003), and neocortex (Waters et al., 2003; Waters and Helmchen, 2004) of anaesthetized animals.

A direct comparison of AP backpropagation in brain slices and in anaesthetized animals is available for only one cell type; the layer 2/3 pyramidal neuron from somatosensory neocortex (**Figure 2**). In layer 2/3 pyramidal neurons AP backpropagation is supported by Na^+ channels in the slice preparations and is similar in slices and during periods of little synaptic activity ('Down states') in anaesthetized rats, implying that AP backpropagation is also actively supported by dendritic Na^+ channels *in vivo* (Waters et al., 2003). Comparison of dendritic Ca^{2+} transients evoked by backpropagating APs during relatively quiet Down states and during periods with synaptic background activity ('Up states') revealed that ongoing synaptic activity slightly enhanced AP backpropagation (Waters and Helmchen, 2004).

The enhanced AP propagation during Up states in layer 2/3 neurons might suggest that, in these neurons, the ratio of excitatory to inhibitory synapses active during periods of pronounced background activity is greater than one. Consistent with this idea, the estimated numbers of synapses active during Up states was relatively low, producing only a small (<10 nS) conductance change at the soma (Waters and Helmchen, 2006). Furthermore, the ratio of active excitatory to inhibitory synapses was high (around one in ten synapses was inhibitory; Waters and Helmchen, 2006). These estimates were indirect, being derived from a combination of input resistance measurements and compartmental modeling. They indicate that ongoing, background synaptic activity in anesthetized rats has little effect on AP backpropagation principally because activity is sparse and mostly excitatory.

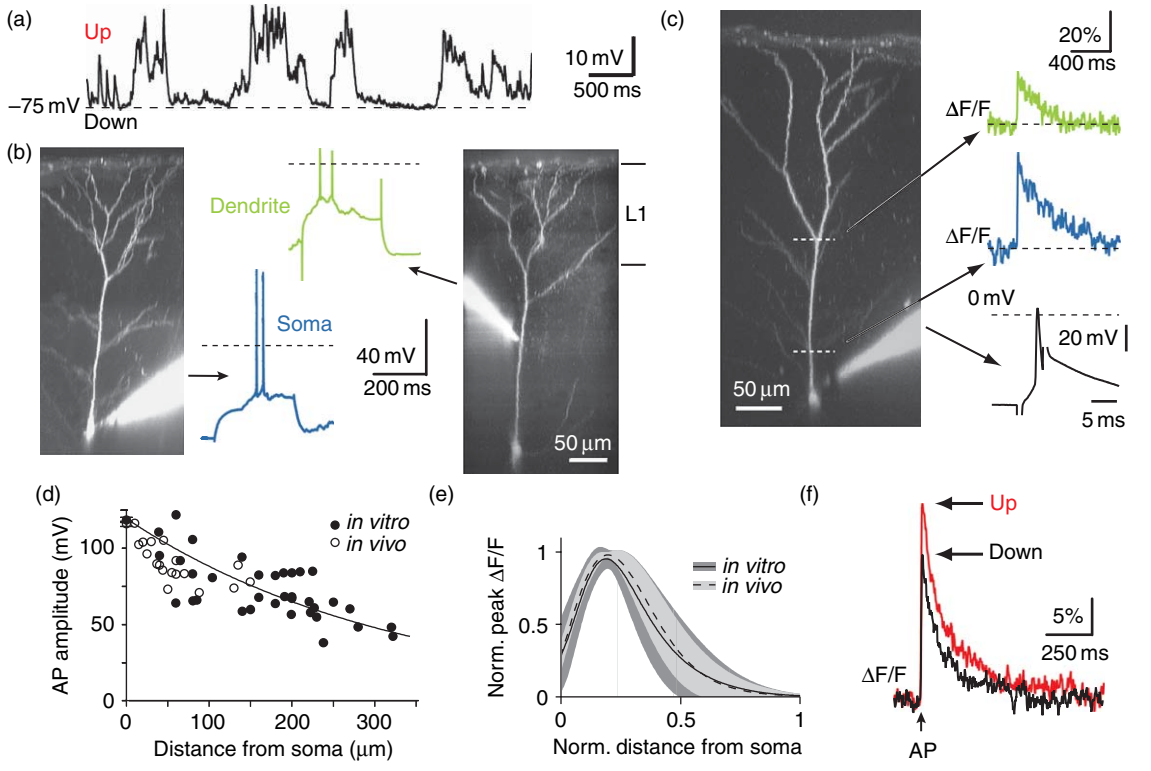

Figure 2 Action potential backpropagation. (a) Example of ongoing subthreshold membrane potential fluctuations *in vivo,* recorded with a whole-cell patch pipette from the apical dendrite of a layer 2/3 pyramidal neurons in a urethane-anesthetized rat. Spontaneously occurring synaptic inputs cause membrane potential fluctuations between so-called Up and Down states. (b) Fluorescence side-projections of two layer 2/3 pyramidal neurons in somatosensory cortex from anaesthetized rats. Each neuron was filled with a fluorescent indicator via the whole-cell recording pipette located on the soma (left) or the dendrite (right). Action potential recorded from the soma (blue) and dendrite (green) are also shown. (c) Calcium transients in the proximal (blue) and distal (green) apical dendrite evoked by single APs backpropagating into in the apical dendrite of a layer 2/3 neuron in a urethane-anesthetized rat. Capacitive artifacts on the AP trace are truncated. (d) Backpropagating AP amplitudes as a function of distance from the soma, determined with whole-cell recordings from the apical dendrites of layer 2/3 pyramidal neurons both in brain slices (*in vitro*) and in anesthetized rats (*in vivo*). (e) Spatial profile of single AP-evoked dendritic Ca^{2+} transients (normalized to maximum amplitude) along the apical dendrites of layer 2/3 pyramidal neurons both *in vitro* and *in vivo*. (f) Comparison of single AP-evoked Ca^{2+} transients in the distal apical dendritic trunk of a layer 2/3 pyramidal neuron during Up and Down states. Figure parts adapted from Waters J, Larkum M, Sakmann B, and Helmchen F (2003) Supralinear Ca^{2+} influx into dendritic tufts of layer 2/3 neocortical pyramidal neurons *in vitro* and *in vivo*. *J. Neurosci.* 23: 8558–8567; and Waters J and Helmchen F (2004) Boosting of action potential backpropagation by neocortical network activity *in vivo*. *J. Neurosci.* 24: 11127–11136.

Whether ongoing background synaptic activity is similar in other preparations and other types of neurons is unclear. Similar results have been reported for deep-layer pyramidal neurons in ferret visual cortex (McCormick et al., 2003; Shu et al., 2003; Haider et al., 2006). In contrast, results from deep-layer pyramidal neurons in cat neocortex are more consistent with the idea that the majority of active synapses during background activity are inhibitory (Destexhe et al., 2003). The reason(s) for this discrepancy are unclear. Anesthesia, species, and age differences and neocortical region studied are all possible contributory factors. However, one prominent difference is the rate of spiking in some studies, which may confound the interpretation of differences in input resistances between Up and Down states in terms of the numbers of active synapses (Waters and Helmchen, 2006).

4.39.3.3.2 AP backpropagation in awake animals: what should we expect?

High levels of ongoing synaptic activity also occur in awake animals (Steriade et al., 2001; Destexhe et al., 2003), and extracellular recordings suggest that AP

backpropagation is not completely suppressed in the hippocampus or neocortex in awake, behaving animals (Buzsáki and Kandel, 1998; Quirk et al., 2001). However, it is unclear how AP backpropagation in awake animals compares to that in brain slices and in anesthetized animals. Small changes in active properties, which might not be readily apparent in extracellular recordings, could have profound effects on AP waveform and spatial spread and, therefore, on time windows and the dendritic locations of synaptic plasticity.

The effect of synaptic input on both AP backpropagation and dendritic spike initiation depends strongly on the spatiotemporal pattern of synaptic activation (Stuart and Häusser, 2001; Gasparini and Magee, 2006; Pérez-Garci et al., 2006). Nonetheless, in light of the available extracellular data and data from anesthetized animals, showing robust AP backpropagation in the presence of synaptic activity, it is tempting to speculate that ongoing background excitatory and inhibitory synaptic activity have little effect on AP backpropagation in awake animals. However, AP backpropagation may be more strongly influenced by appropriately timed evoked activity, such as from sensory input, attentional processing, or motor activity. In addition, neuromodulatory input may be an important factor in awake animals. Many neuromodulators have strong effects on dendritic excitability in slice preparations. Neuromodulatory activity is relatively sparse in anesthetized animals, but neuromodulatory activity varies in many brain regions with behavior. In all probability, therefore, AP backpropagation will be modulated by synaptic activity in at least some behavioral states. Intracellular measurements describing the extent of AP backpropagation in awake (and ideally behaving) animals are required to resolve this issue (Lee et al., 2006).

4.39.3.4 Local Dendritic Action Potentials

Another consequence of active dendritic conductances is that regenerative potentials can be initiated in dendritic branches. These local APs propagate forward toward the soma and either remain subthreshold or trigger sodium APs at the soma. The conditions under which local dendritic APs are generated are still unclear, but it is presumed that they require synchronous and clustered synaptic inputs. Since they are typically associated with substantial dendritic Ca^{2+} influx, dendritic spikes may contribute to synaptic plasticity (Golding et al., 2002; Holthoff et al., 2006). Three types of local dendritic APs have been distinguished. They differ with respect to the shape of the voltage waveform and the underlying currents.

4.39.3.4.1 Sodium spikes

Due to the presence of voltage-dependent Na^+ channels in the dendrites of most cell types, fast APs can be initiated in the dendrites themselves. The idea of local dendritic APs, which then summate at the soma, is an old one (Spencer and Kandel, 1961), but a convincing demonstration of dendritic spikes had to await the development of direct dendritic recordings. In multiple cell types in brain slices, current injection into the dendrite can produce fast APs that are sensitive to tetrodotoxin (TTX) (Stuart et al., 1997a; Golding and Spruston, 1998; Larkum et al., 2001; Wei et al., 2001). These fast dendritic APs can also be triggered by synaptic stimulation (Turner et al., 1991; Golding and Spruston, 1998; Gasparini et al., 2004) and attenuate while propagating forward toward the soma. If they remain subthreshold at the soma they are often referred to as 'isolated' dendritic spikes.

Sodium spikes were first described in the apical dendrites of hippocampal and neocortical pyramidal neurons (Regehr, 1993; Stuart et al., 1997) and for mitral cell dendrites in the olfactory bulb (Chen et al., 1997). More recently evidence has accumulated that they can also occur in thinner dendrites such as the basal and oblique dendrites of pyramidal cells (Milojkovic et al., 2005; Losonczy and Magee, 2006; Nevian et al., 2007). It is likely that many thin dendrites are capable of generating APs, although it is unclear under what conditions they occur.

4.39.3.4.2 Calcium spikes

The activation of other channels, in particular Ca^{2+} channels, can cause more complex dendritic spikes that are typically characterized by a longer-lasting plateau phase. A clear example is the large Ca^{2+} spike in the apical dendrite in layer 5 neocortical pyramidal neurons, which can last 20–30 ms (Schiller et al., 1997; Larkum and Zhu, 2002). Calcium spikes in layer 5 apical dendrites in most cases are suprathreshold at the soma, meaning that they initiate one or more somatic Na^+ APs. In turn, Ca^{2+} spikes can be generated by applying high-frequency stimuli above a critical frequency of around 100 Hz to the soma (Larkum et al., 1999a). In this case depolarizations caused by the individual stimuli summate in the dendrite until sufficient numbers of Ca^{2+}

channels are activated for the depolarization to become regenerative.

A key signature of Ca^{2+} spikes is the large concomitant Ca^{2+} influx (Yuste et al., 1994; Schiller et al., 1997; Helmchen et al., 1999). Calcium imaging therefore can help to identify dendritic zones in which dendritic Ca^{2+} spikes might occur. For example, *in vivo* calcium imaging demonstrated the occurrence of nonlinear Ca^{2+} influx in distal dendrites of layer 2/3 neurons in anesthetized animals (Waters et al., 2003). A similar nonlinear behavior has been reported for basal dendrites of layer 5 pyramidal neurons (Kampa et al., 2006). It remains to be determined, however, whether these dendritic Ca^{2+} signals are caused by pure Ca^{2+} spikes or by more complex, mixed depolarizing currents.

4.39.3.4.3 NMDA spikes

Another mode of dendritic regenerativity involves the voltage dependence of the *N*-methyl-D-aspartate receptor (NMDAR). Schiller et al. (2000) noticed that glutamate uncaging at dendritic sites can elicit plateau potentials at the soma that depend nonlinearly on stimulus intensity. Similar results were obtained with focal synaptic stimulation, and these potentials depend on NMDAR activation (Schiller and Schiller, 2001). NMDA spikes result in plateau potentials with large dendritic depolarizations (around 30 mV). These spikes are less attenuated *en route* to the soma than subthreshold EPSPs and therefore represent a mechanism by which dendrite-to-soma coupling is greatly enhanced (Nevian et al., 2007).

4.39.3.5 Potential Physiological Functions

Why are dendrites excitable? What are potential functions of APs in dendrites? Are they essential for proper circuit function *in vivo?* Several functions can be attributed to APs in dendrites. These functions can be divided into two classes according to whether they predominantly act in a 'forward' manner, shaping the integration of synaptic inputs, or whether they provide a 'feedback' signal to the dendrites. In addition, one has to differentiate between effects occurring on disparate timescales. For example, APs in dendrites largely determine the spread of membrane potential changes on a millisecond time scale but they also can lead to short-term and long-term changes of dendritic excitability and of synaptic inputs.

First, voltage-dependent currents in the dendrites can help distal synaptic currents to contribute to membrane potential changes at the soma. For example, dendritic Na^{+} and Ca^{2+} channels can amplify synaptic potentials (Magee and Johnston, 1995b; Schwindt and Crill, 1995; Lipowsky et al., 1996; Gillessen and Alzheimer, 1997; Oviedo and Reyes, 2002). In contrast, transient A-type K^{+} currents in dendrites can counteract rapid and strong dendritic excitation, acting as a brake on dendritic excitability (Hoffman et al., 1997). Hence dendritic channels can modulate the summation of synaptic potentials in both directions.

Dendritic integration is transformed once local dendritic APs are initiated. As has been demonstrated for several neuronal cell types, dendrites can act as local, nonlinear thresholding devices (Polsky et al., 2004; London and Häusser, 2005). Parts of the dendritic tree or even single dendritic branches thus may form independent or semi-independent integration zones (Häusser and Mel, 2003; Poirazi et al., 2003). Conceptually, this finding is significant because it means that dendritic branches, rather than single neurons, may constitute the functional building blocks of synaptic integration. In network models each individual neuron may have to be treated as a small, but complicated 'neural network'. In addition, local spikes may contribute, via concomitant Ca^{2+} influx, to synaptic plasticity (as discussed below). Despite the demonstration of local dendritic spikes in many neurons in brain slice preparations, a confirmation of their existence *in vivo* is still lacking. A possible explanation for this lack of evidence is that the initiation of local spikes has stringent requirements for clustered and synchronized synaptic inputs (Gasparini and Magee, 2004). It remains unclear under what conditions and during which specific behavioral states these requirements might be fulfilled. Likewise, the function of dendritic spikes in circuit computations and behavior remains unknown.

Complex interactions are expected to occur between backpropagating APs, synaptic input, and dendritic spikes. Backpropagating APs, for example, can lower the threshold current required to generate a dendritic Ca^{2+} spike in neocortical layer 5 pyramidal neurons (Larkum et al., 1999a). Furthermore, dendritic APs can increase the probability of initiating an additional AP at the axon/soma. As a result, the ability of a neuron to initiate spikes in several locations can influence the neuron's spiking output. Hence synaptic input onto the distal dendrites of a layer 5 neocortical pyramidal neuron can act

synergistically with perisomatic input to generate a burst of APs (Schiller et al., 1997; Larkum et al., 1999b; Williams and Stuart, 1999; Schaefer et al., 2003b). The basal/oblique and the apical/tuft dendritic trees of pyramidal cells receive synaptic input from different sources. For example in somatosensory cortex, basal/oblique and apical/tuft dendrites receive ascending thalamic input from different nuclei, corresponding to specific sensory (lemniscal) and more diffuse (paralemniscal) pathways, respectively. As a result of the ability of layer 5B neurons to generate dendritic Ca^{2+} spikes, excitation might be particularly effective in those pyramidal neurons that receive coincident activation of their tufts located in layer 1 and of their basal dendrites in layer 5 during sensory stimuli. Hence it has been suggested that dendritic spiking enables pyramidal neurons to act as cellular coincidence detectors, associating input from different projections which arrive in different layers of the cortex.

Like individual APs, trains of APs are reliably conveyed via the axonal arbor toward the cell's target neurons. At axonal boutons, short-term facilitation of release can render the transmission of AP bursts more effective than the transmission of single APs (Lisman, 1997; Williams and Stuart, 1999). At synapses with short-term depression, this effect will be less pronounced (Markram et al., 1998a,b; Reyes et al., 1998; Thomson, 2003). Individual pyramidal neurons are connected to different classes of postsynaptic cells via synapses that are either facilitating or depressing, depending on the target cell type (e.g., Reyes et al., 1998; Rozov et al., 2001). Thus by inducing axosomatic AP bursts, backpropagating APs in neocortical pyramidal neurons contribute to short-term changes in synaptic transmission. Thus they dynamically regulate the direction of signal flow through neuronal circuits.

Backpropagating APs can also release transmitter in dendrites that form dendro-dendritic synapses. Here, backpropagating APs have a similar function to axonally propagating APs, triggering dendritic Ca^{2+} influx, which then leads to the release of fast-acting transmitter. For example, in the olfactory bulb, dendritic GABA release from granule cells, dendritic glutamate release from mitral cells onto granule cells, and mitral cell self-excitation are influenced by the extent of backpropagation (Isaacson, 1999; Margrie et al., 2001; Salin et al., 2001; Lowe, 2002; Xiong and Chen, 2002; Christie and Westbrook, 2003; Egger et al., 2003). As a consequence, lateral inhibition, which is a potential cellular correlate of contrast enhancement for odor discrimination (Yokoi et al., 1995; Shepherd and Greer, 1998; Urban, 2002; Margrie and Schaefer, 2003), relies on the spread of APs in these dendrites. Similarly, AP backpropagation in some neocortical interneurons (Kaiser et al., 2001, 2004) may evoke dendritic release of GABA onto axonal boutons of pyramidal cells (Zilberter et al., 1999), and backpropagating APs in pyramidal neurons may control the release of glutamate or cannabinoid from the dendrite (Zilberter, 2000; Kreitzer and Regehr, 2002; Sjöström et al., 2003; Trettel and Levine, 2003). These two local feedback loops could regulate the AP activity of both interneurons and pyramidal cells to maintain stable patterns of APs in local cortical circuits.

Another function of backpropagating APs could be the stabilization of nascent synapses. During postnatal development, neuronal circuits in many CNS regions are still in the process of shaping their longer-term connectivity, often in an experience-dependent manner. With elevated levels of spontaneous activity in young animals, backpropagating APs could not only contribute to synchronizing a developing network via dendro-dendritic gap junctions (Peinado, 2001; Peinado et al., 1993), but might also evoke dendritic release of trophic factors which could serve as attractors for axonal growth cones or as a signal for axon collateralization (McAllister, 1999; Poo, 2001). The dependence of synapse growth and stabilization on backpropagating APs, which transmit a modified copy of the axonal AP pattern to the dendrites, could help establish cell assemblies that fire together. NMDA-type glutamate receptors are probably key elements in this process, as they are abundant in postsynaptic structures early in development (Crair and Malenka, 1995; Washbourne et al., 2002). Backpropagating APs could lead to enhanced, highly localized Ca^{2+} influx into dendrites via postsynaptic NMDA receptors through a transient relief of the Mg^{2+} block of NMDARs (Mayer et al., 1984). This putative function of AP backpropagation in refining the specificity of neuronal circuits by coincident pre- and postsynaptic APs in neuronal assemblies during early postnatal development has yet to be studied in detail.

Finally, a backpropagating AP, as a widespread signal to the dendritic arbor, reports output activity to sites of synaptic input so that localized long-term changes in the efficacy of excitatory synapses may be induced. Such changes in synaptic strength depend on precise timing relationships between pre- and postsynaptic AP activity during a relatively narrow

time window on the order of about 100 ms (for review see Linden, 1999; Dan and Poo, 2006). This spike-timing-dependent plasticity (STDP) will be addressed in detail in the second part of this chapter.

4.39.4 Spike-Timing-Dependent Plasticity

4.39.4.1 Plasticity of Synapses and Memory Formation

The cellular basis of memory formation and information storage is thought to depend on long-term changes in synaptic efficacy. These changes depend on activity patterns in the connected neurons (Bliss and Collingridge, 1993; Moser et al., 1998; Morris, 2006; Whitlock et al., 2006). As proposed by Donald Hebb, the repeated activation of one neuron by other connected neurons results in the strengthening of the connections between them (Hebb, 1949). This form of synaptic plasticity, called long-term potentiation (LTP), was first demonstrated in the hippocampus using tetanic stimulation (Bliss and Lomo, 1973). The inverse process, weakening of a synaptic connection, occurs under different conditions, as observed in the form of long-term depression (LTD) following repeated low-frequency synaptic stimulation (Dudek and Bear, 1992; Mulkey and Malenka, 1992). Similar to some forms of behavioral learning and memory formation *in vivo*, the activity-dependent modification in synaptic strength is associative and input specific (Stanton and Sejnowski, 1989).

Changes in synaptic strength can also be accompanied by cellular and subcellular structural modifications. The structure of a synapse is dynamic, and new synaptic connections may be established and old ones degraded. On a more subtle level, the expression of LTP and LTD may be correlated with dendritic spine growth and shrinkage, respectively. The morphological changes associated with synaptic plasticity are summarized elsewhere (Harris, 1999; Lüscher et al., 2000; Muller et al., 2000; Yuste and Bonhoeffer, 2001; Kasai et al., 2003; Nimchinsky et al., 2002; Oertner and Matus, 2005). Here we focus on the role of active dendrites in the induction of changes in synaptic efficacy.

4.39.4.1.1 Timing matters: spike-timing-dependent plasticity

The rule for modifying synaptic weights proposed by Hebb (1949) suggested that coincident activity in two neurons (Hebb states: "cell A is near enough to excite cell B") is a prerequisite for the modification in synaptic strength. More recently it was found for excitatory synaptic connections that the precise timing and the order of pre- and postsynaptic APs can determine the magnitude and the direction of changes in synaptic weight (Markram et al., 1997; Bi and Poo, 1998; Feldman, 2000). For example, a postsynaptic AP that follows a presynaptic AP within a critical time window of 5–40 ms can result in LTP, whereas a postsynaptic AP preceding a presynaptic AP within a time window of 1–100 ms produces LTD, with a sharp transition from maximal LTP to maximal LTD at around zero time delay (**Figure 3**). This form of synaptic modification has been termed spike-timing-dependent plasticity (STDP). STDP is considered one plausible model for the induction of local synaptic modifications, which could account for experience-driven changes of connectivity in neuronal circuitry (Song and Abbott, 2001; Senn, 2002; Dan and Poo, 2006). In the following sections we will discuss the mechanisms that underlie the spike timing-dependence of synaptic plasticity, the shape of the curve describing the time dependence of STDP, the variability between cell types, and the dependence on the location of the synapse within the dendritic tree. First, we focus on the induction phase of STDP, during which the electrical activity pattern is translated into a biochemical signal that controls the subsequent expression of changes in synaptic efficacy (Derkach et al., 2007).

4.39.4.1.2 Induction protocols

The parameter space for pairing protocols that lead to changes in synaptic efficacy by near coincident pre- and postsynaptic APs is large. One can vary not only the timing interval between pre- and postsynaptic APs but also the number of spikes, their frequency, the number of pairings, and their repetition rate. In most cases a repeated number of pairings on the order of 30 to 100 is required for STDP, although single pairing–induced plasticity has been described recently (Holthoff et al., 2004). The specific requirements for STDP induction might reflect a need for a repeated dendritic depolarization and a rise in intracellular Ca^{2+} concentration. Downstream signaling cascades are activated and integrate the biochemical signals generated by individual pairings (Lisman and Spruston, 2005). One prominent example is the activation of calcium/calmodulin-dependent protein kinase II (CamKII) (Lisman, 1989; Lisman et al., 2002). CamKII is required for the induction of LTP.

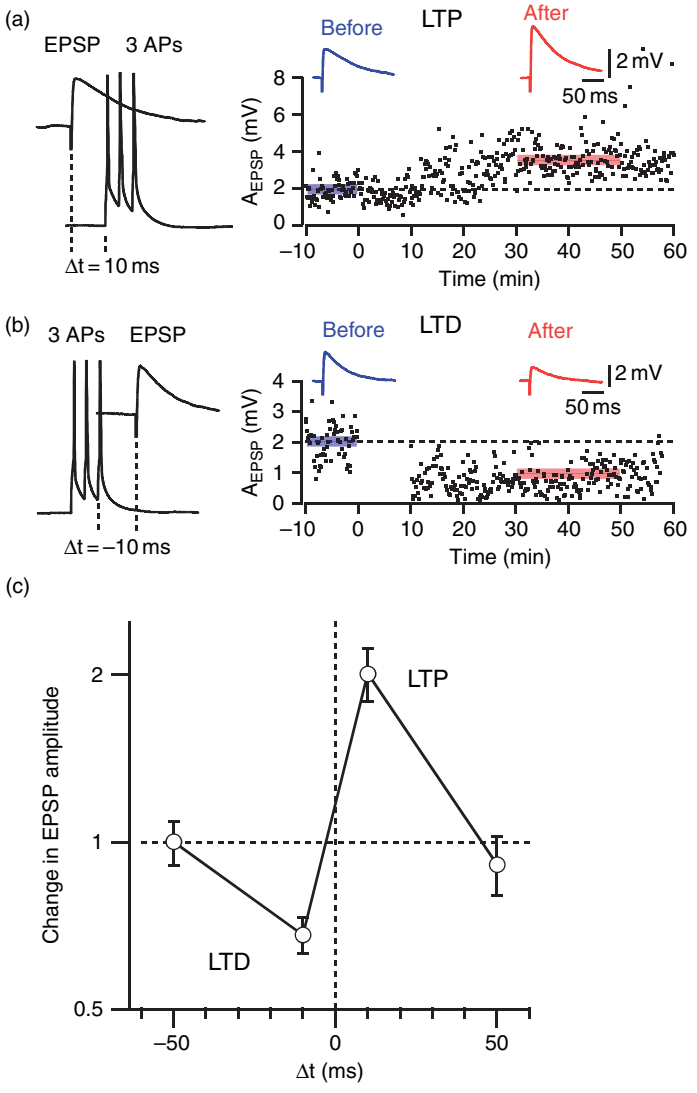

Figure 3 Spike-timing-dependent plasticity. Illustration of the influence of spike-timing on synaptic plasticity. (a) Pairing a burst of three backpropagating APs (50 Hz) following an EPSP by 10 ms results in the induction of LTP. The pairing protocol is depicted on the left. The right graph shows the peak EPSP amplitude over time. Mean EPSPs before (blue) and after (red) pairing are shown on the top. Mean amplitudes before and after are indicated by blue and red bars in the graph. (b) A burst of three backpropagating APs preceding an EPSP by 10 ms results in the induction of LTD. (c) STDP curve for pairing a burst of three backpropagating APs with an EPSP. Vertical line indicates onset of the EPSP (note definition of Δt in A and B). Horizontal line corresponds to no change in synaptic strength. Adapted from Nevian T and Sakmann B (2006) Spine Ca^{2+} signaling in spike-timing-dependent plasticity. *J. Neurosci.* 26: 11001–11013.

It becomes constitutively activated by autophosphorylation after binding several calmodulin molecules. Since Ca^{2+} transients evoked by a single pairing are brief, CamKII can integrate repeated pairings, provided they occur with an appropriate temporal separation (Lisman and Spruston, 2005). This may be one reason why the interval between pairings of individual pre- and postsynaptic spikes is critical (Sjöström et al., 2001). Pairing a presynaptic AP with a subsequent postsynaptic AP at repetition rates below 10 Hz failed to induce LTP in pairs of layer 5 pyramidal neurons, but low-frequency stimulation with reversed temporal order induced LTD (Markram et al., 1997; Sjöström et al., 2001). At repetition frequencies between 10 and 20 Hz LTP or LTD were observed, depending on the relative timing between APs and EPSPs. At a repetition frequency of 40 Hz, only LTP was induced, independent of the

relative timing. The increased frequency of spike pairs results in increased temporal summation of postsynaptic depolarization, which enhances synaptic Ca^{2+} influx for each spike and results in temporal integration of the Ca^{2+} transients. These findings demonstrate the sensitivity of STDP to additional factors beyond AP timing (Wittenberg and Wang, 2006).

The observation that LTP is not induced by repeated low-frequency pairing of single EPSPs followed by single APs has been supported by a number of studies (Sjöström et al., 2001; Wittenberg and Wang, 2006; Nevian and Sakmann, 2006). Hippocampal CA1 pyramidal neurons show a developmental switch between P14 and P23 from single-AP-induced LTP to a requirement of postsynaptic burst-firing to induce LTP (Meredith et al., 2003). In contrast, in other preparations repeated low-frequency pairing of single APs was shown to be sufficient to induce STDP (Bi and Poo, 1998; Feldman, 2000; Froemke and Dan, 2002). These contrasting effects of low-frequency pairing may reflect different degrees of Ca^{2+} accumulation in these neurons. The differences could result from variations in the local depolarization caused by an EPSP, the efficiency of AP backpropagation, or the interaction of EPSP and AP. Such variations could result in different Ca^{2+} transients and may render some synapses more sensitive to single-spike pairing. Nevertheless, bursts of postsynaptic APs following synaptic activation result in a robust induction of LTP.

An important question is how effectively spike-timing-dependent changes in synaptic efficacy can be driven by 'natural' spike patterns observed *in vivo*. Dual to quadruple AP pairings have been used to derive rules for how trains of spikes interact (Wang et al., 2005). Furthermore, variation of the pairing frequency and number of pairings have pronounced effects on the resulting STDP curves ranging from LTP-only to bidirectional and LTD-only learning rules (Wittenberg and Wang, 2006). Various interpretations have been suggested, including the views that the timing of the first AP in each burst is dominant in synaptic modification (Froemke and Dan, 2002), that synaptic weight changes are linearly summated for all pre- and postsynaptic spike pairs (van Rossum et al., 2000), and that potentiation and depression compete for the net change in synaptic strength (Wang et al., 2005). Whether a universal 'set of rules' can be derived from such experiments is still unclear because the interactions between EPSPs and backpropagating APs are variable and strongly nonlinear.

4.39.4.1.3 Diversity of spike-timing-dependent changes in synaptic efficacy

STDP occurs in many neuronal cell types in different regions of the brain. The first reports showed the importance of the timing of backpropagating APs in increasing or decreasing synaptic efficacy in connected pairs of layer 5 pyramidal neurons in somatosensory cortex (Markram et al., 1997), in pairs of hippocampal CA1 pyramidal neurons (Magee and Johnston, 1997), and in CA3 to CA1 pyramidal connections in hippocampal slice cultures (Debanne et al., 1998). In cultures of dissociated hippocampal neurons, changes in synaptic strength depend on the relative timing of single presynaptic APs and single postsynaptic APs, with an effective time window for the induction of LTP and LTD of 20 ms (Bi and Poo, 1998). An asymmetric time window for STDP was also found in layer 4 to layer 2/3 connections between excitatory neurons in somatosensory cortex with a longer time window for the induction of LTD (40–100 ms) as compared to LTP (20 ms) (Feldman, 2000; Celikel et al., 2004; Bender et al., 2006). Presumed layer 2/3 pyramidal-to-pyramidal connections in visual (Froemke and Dan, 2002), somatosensory (Nevian and Sakmann, 2006), and entorhinal cortices can also be modified bidirectionally. Interestingly, layer 4 spiny stellate neurons in somatosensory cortex show only LTD, independent of the timing sequence of pre- and postsynaptic spikes (Egger et al., 1999). STDP has been demonstrated also in perforant path synapses to granule cells in the dentate gyrus (Lin et al., 2006) and thalamic and cortical inputs to the amygdala (Shin et al., 2006). Different STDP rules were found at cortical versus thalamic projections to the same postsynaptic neuron in the lateral amygdala (Humeau et al., 2005). Whereas thalamic afferents showed bidirectional STDP, the cortical afferents showed no changes in synaptic strength.

In the dorsal cochlear nucleus the coincident activation of parallel fibers and either fusiform principal neurons or cartwheel inhibitory interneurons results in different STDP curves. Whereas the fusiform principal neurons exhibit LTD for post-pre pairing and LTP for pre-post pairing, cartwheel interneurons show LTD for the latter sequence (Tzounopoulos et al., 2004). A 'reversed' STDP curve with LTP for post-pre pairing and LTD for pre-post pairing has been described for Purkinje-like cells in the electrosensory lobe of mormyrid electric fish (Bell et al., 1997) and at the corticostriatal synapse (Fino et al., 2005). Such STDP curves are

described as 'anti-Hebbian,' since Hebbian learning is considered to result in LTP with pre- before post-synaptic spike pairing. In the spinal cord, substantia gelatinosa neurons show Hebbian spike-timing-dependent LTP that can be switched to LTD by blocking group I metabotropic glutamate receptors (mGluRs) or by release of Ca^{2+} from internal stores (Jung et al., 2006). These results indicate that the expression of LTP or LTD by a spiking pattern depends on additional factors, like the presence of mGluRs or neuromodulators.

All the examples of STDP described above were related to changes in glutamatergic synaptic transmission. STDP of inhibitory postsynaptic potentials results from correlated spiking of a cortical layer 5 pyramidal neuron and an inhibitory interneuron (Holmgren and Zilberter, 2001). Inhibitory synaptic transmission of interneurons in entorhinal cortex exhibits an asymmetric STDP curve with maximal depression for post- before presynaptic AP at $\Delta t = -10$ ms and maximal potentiation for pre- before postsynaptic AP at $\Delta t = +10$ ms, without any change in synaptic strength for shorter time intervals (Haas et al., 2006).

The multitude of synapses which can be modified bidirectionally by the precise timing of pre- and postsynaptic activity suggests that STDP could be a widely occurring mechanism for the regulation of synaptic strength in an activity-dependent manner. The variety of time windows and effective induction protocols for LTP and LTD suggests, however, that the characteristics of STDP depend critically also on other factors.

4.39.4.2 Effects of Action Potentials and Calcium Transients at the Synapse

A backpropagating AP paired with synaptic activation by a presynaptic spike can result in LTP or LTD. Therefore the backpropagating AP can be regarded as a retrograde signal to the dendritic arbor that reports the output AP activity back to the sites of synaptic input.

4.39.4.2.1 Long-term potentiation

Long-term changes in synaptic efficacy are often dependent on increased dendritic Ca^{2+} influx through NMDA receptors and voltage-dependent Ca^{2+} channels (VDCCs), suggesting that backpropagating APs are part of a mechanism that controls the change in synaptic strength through metabolic cascades that are driven by a brief rise in intracellular Ca^{2+} concentration (Lisman, 1989; Lisman et al., 2002). A backpropagating AP depolarizes the dendritic tree, including the activated synapse, and results in Ca^{2+} influx in most parts of the dendritic tree through VDCCs. In addition, the backpropagating AP briefly relieves magnesium block of NMDARs, thereby further enhancing Ca^{2+} influx, specifically at activated synapses (**Figure 4**). The resulting Ca^{2+} transient in an active spine is larger than the sum of the Ca^{2+} transients evoked by backpropagating AP and synaptic stimulus alone (Yuste and Denk, 1995; Nevian and Sakmann, 2006). This supralinear Ca^{2+} transient occurs when a backpropagating AP follows an EPSP within a time window of 50 ms, similar to the time window for the induction of spike-timing-dependent LTP. Therefore the NMDAR detects coincidence of glutamate release and of membrane depolarization, evoked by pre-before-postsynaptic APs. The spine-restricted supralinear Ca^{2+} influx triggers input-specific modifications in synaptic strength. Supralinear increases in Ca^{2+} concentration evoked by coincident EPSPs and backpropagating APs mediated by the NMDAR have been demonstrated in hippocampal CA1 neurons (Yuste and Denk, 1995), cortical layer 2/3 and layer 5 pyramidal neurons (Köster and Sakmann, 1998; Nevian and Sakmann, 2006), spiny stellate neurons in neocortical layer 4 (Nevian and Sakmann, 2004), cerebellar Purkinje cells (Wang et al., 2000), striatal medium spiny neurons (Carter and Sabatini, 2004), and a variety of interneurons in neocortical layer 2/3 (Goldberg et al., 2003; Kaiser et al., 2004). In dendritic spines of layer 4 spiny stellate neurons the time course of the supralinear summation of Ca^{2+} signals matches the deactivation time course of NMDAR channels (Nevian and Sakmann, 2004). Therefore the NMDAR kinetics probably determines the LTP timing window.

Induction of STDP requires coincident occurrence of pre- and post-synaptic spiking. Neither pre- nor post-synaptic spiking alone is sufficient when repeated a similar number of times as during pairing protocols. Backpropagating APs alone, as well as EPSPs alone, cause a postsynaptic elevation in Ca^{2+} concentration, but with different time courses and amplitudes from Ca^{2+} transients during coincident activity (Sabatini et al., 2002). As a result of these differences, APs and EPSPs alone presumably do not result in the activation of the biochemical signaling cascades that eventually cause changes in synaptic strength (Rubin et al., 2005). A possible explanation is that the Ca^{2+} binding proteins triggering LTP are

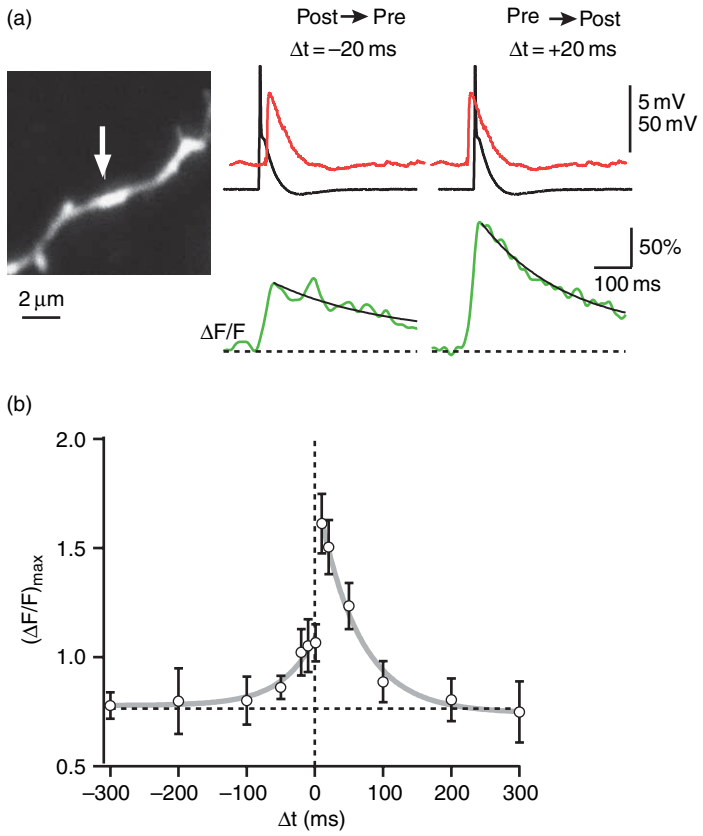

Figure 4 Dendritic spine Ca^{2+} signaling. (a) Two-photon image of a dendrite with spines. The arrow indicates the active synaptic contact in which the Ca^{2+} transients to the right were measured. The traces to the right illustrate the pairing protocols for the induction of STDP and the corresponding Ca^{2+} transients. The activity pattern of a backpropagating AP preceding an EPSP (post–pre, $\Delta t = -20$ ms) results in a smaller Ca^{2+} transient than the reverse order of a backpropagating AP following an EPSP (pre–post, $\Delta t = 20$ ms). (b) Spike-timing-dependent peak Ca^{2+} transient amplitude as a function of the timing interval between EPSP and backpropagating AP. Negative intervals correspond to the order post–pre and positive intervals to the order pre–post. The timing curve is asymmetric with larger calcium transients for positive time intervals. Adapted from Nevian T and Sakmann B (2004) Single spine Ca^{2+} signals evoked by coincident EPSPs and backpropagating action potentials in spiny stellate cells of layer 4 in the juvenile rat somatosensory barrel cortex. *J. Neurosci.* 24: 1689–1699.

located in close proximity to the NMDAR channel and sense the elevation of Ca^{2+} concentration only in the immediate proximity of the site of influx (Franks and Sejnowski, 2002). Such microdomains of Ca^{2+} elevation would result in a Ca^{2+} source-specific activation of biochemical cascades. However, this view was recently challenged for STDP-inducing protocols by the finding that the magnitude of both LTP and LTD was correlated the volume-averaged elevation of Ca^{2+} in a synaptic spine (Nevian and Sakmann, 2006).

4.39.4.2.2 Long-term depression

The LTP-part of the STDP-curve can be explained by activation of the NMDAR, but the mechanisms underlying the induction of LTD are probably more diverse. Two broad forms of spike-timing-dependent LTD have been observed: postsynaptically expressed LTD (Froemke et al., 2005) and presynaptically expressed LTD (Sjöström et al., 2003). Both types of LTD require an increase in postsynaptic Ca^{2+} concentration (Sjöström et al., 2003). LTD is induced at most synaptic contacts when the postsynaptic spike precedes the presynaptic spike, and the resulting postsynaptic Ca^{2+} signals either add linearly or sublinearly (Köster and Sakmann, 1998; Nevian and Sakmann, 2004). This result suggests that moderate elevations of intracellular Ca^{2+} are the trigger for the induction of LTD (Bear et al., 1987; Artola and Singer, 1993; Hansel et al., 1997). In agreement, the mechanism for the induction of postsynaptically expressed LTD depends on shunted Ca^{2+} influx

through the NMDAR. It has been suggested that backpropagating AP-evoked Ca^{2+} influx through VDCCs inhibits subsequent Ca^{2+} influx through the NMDAR (Froemke et al., 2005), which is prone to inactivation by intracellular Ca^{2+} (Legendre et al., 1993; Umemiya et al., 2001). The resulting expression of LTD is postsynaptic and due to modification of postsynaptic AMPA receptors (Lee et al., 2000). Thus, the waveform of the backpropagating AP can influence LTD induction, since it determines the size and time course of dendritic depolarization. In particular, a postspike after-depolarization could facilitate current flow through the NMDAR by relieving magnesium block while an after-hyperpolarization could have the reverse effect (Sjöström et al., 2001; Kampa et al., 2006).

By varying the number, frequency, and timing of a burst of APs preceding an EPSP, one can establish pairing protocols that induce LTD and have similar peak Ca^{2+} transient amplitudes to pairing protocols that induce LTP (Nevian and Sakmann, 2006). This result suggests that the peak amplitude of the Ca^{2+} transient alone is not sufficient to determine the direction of the change in synaptic efficacy. This finding hints to presynaptically expressed LTD, which depends on the release of retrograde messengers from the postsynaptic site that modify the presynaptic release probability (Egger et al., 1999; Sjöström et al., 2003, 2004). In cortical layer 5 and layer 2/3 pyramidal neurons the retrograde messenger is an endocannabinoid (Sjöström et al., 2003; Bender et al., 2006; Nevian and Sakmann, 2006). This form of LTD is independent of postsynaptic NMDARs but requires the activation of VDCCs, mGluRs, and presynaptic NMDARs (Anwyl, 2006; Duguid and Sjostrom, 2006). The results suggest that the sequence of post-before-presynaptic APs activates a putative coincidence detector other than the NMDAR (Karmarkar and Buonomano, 2002; Bender et al., 2006; Nevian and Sakmann, 2006). This coincidence detector senses Ca^{2+} influx through VDCCs before activation of an mGluR and a G-protein-coupled signaling cascade. It triggers the synthesis and release of endocannabinoids. Phospholipase C, which is involved in the Ca^{2+}-dependent synthesis of endocannabinoids (Hashimotodani et al., 2005; Maejima et al., 2005), might be this putative coincidence detector (Nevian and Sakmann, 2006). The sequence for the induction of LTD is qualitatively different from the sequence of activation for the induction of LTP. In the case of LTP, synaptic activation and thus mGluR activation precede (or are coincident with) Ca^{2+} influx, rendering the LTD pathway silent.

Modeling studies also indicate that the relationship between synaptic Ca^{2+} concentration increase and the magnitude and direction of the change in synaptic strength is more complex. Simulating the AP-evoked Ca^{2+} transients and correlating the concentration level of Ca^{2+} to a change in synaptic strength can predict the STDP curve for single spike pairs when a second coincidence detector is added to the model (Karmarkar and Buonomano, 2002) or if the stochastic opening of the NMDAR is taken into account (Shouval and Kalantzis, 2005). More complex spike patterns require differential binding kinetics of Ca^{2+} binding proteins to decode the Ca^{2+} transients (Rubin et al., 2005).

4.39.4.3 Additional Factors Affecting Spike-Timing-Dependent Plasticity

Synapses distributed throughout an elaborate dendritic tree are not equally influenced by backpropagating APs. This can have implications for the STDP learning rules at different synapses. Furthermore, the active dendritic properties of dendrites can result in the generation of local dendritic spikes, which change the timing dependence for STDP. Finally, modulation of dendritic channels and excitability can influence synaptic plasticity.

4.39.4.3.1 Synapse location

Since backpropagating APs attenuate as they invade the dendritic tree, the retrograde signal they provide depends on synapse location. For example, in the apical dendrites of layer 2/3 pyramidal neurons of the visual cortex, the time window for the induction of LTD depends on the distance of the synaptic input from the soma (Froemke et al., 2005). Similarly, in the apical dendrites of cortical layer 5 pyramidal neurons, synapse location determines the sign of the change in synaptic efficacy, with the same STDP induction protocol resulting in LTP at proximal and LTD at distal synapses (Letzkus et al., 2006; Sjöström and Häusser, 2006). Furthermore, when the distal dendrite is depolarized, by EPSPs for example, the amplitudes of backpropagating APs may be enhanced, converting distal LTD to LTP (Sjöström and Häusser, 2006). Hence the sign and magnitude of changes in synaptic efficacy depend upon the location of the synapse and the electrical state of the dendrite during the induction of changes in synaptic strength.

4.39.4.3.2 Local dendritic spikes

In the forms of STDP that we have described, axosomatically initiated APs, which backpropagate into the dendritic tree, are supported by Na^+ conductances and result in brief depolarizations of the dendritic membrane. Dendritic Ca^{2+} channels are activated, but contribute relatively little to the voltage waveform of the AP. This AP waveform interacts with synaptic conductances to evoke a large Ca^{2+} signal that is mainly restricted to dendritic spines. Local dendritic spikes, including sodium, calcium, and NMDA spikes (see the section titled 'Local dendritic action potentials'), have also been implicated in the induction of STDP (Holthoff et al., 2006). In the apical dendrites of hippocampal CA1 pyramidal neurons, for example, local dendritic spikes in the distal apical dendrite can substitute for backpropagating APs in the induction of LTP (Golding et al., 2002). This demonstrates that in these cells, at least, backpropagating APs are not necessary for the induction of LTP, since the depolarization required for Ca^{2+} influx can also be provided by local regenerative events.

In layer 5B neocortical pyramidal neurons a calcium spike can be triggered in the distal apical dendrite by strong synaptic activation (Schiller et al., 1997) or by pairing a backpropagating AP with a distal EPSP (Larkum et al., 1999b). Similarly, a train of backpropagating APs above a critical frequency (Larkum et al., 1999a) can result in the regenerative activation of voltage-dependent Ca^{2+} channels at a distal dendritic site. Pairing EPSPs with high-frequency bursts of APs above the critical frequency, which evokes a large dendritic depolarization and Ca^{2+} transient, reverses the timing rule for the induction of STDP at the distal apical dendrite in layer 5B pyramidal neurons. Here calcium spikes precede the EPSPs and result in LTP, whereas the reverse order results in LTD (Letzkus et al., 2006). A similar shift in the STDP curve occurs when pairing a presumed calcium spike in the basal dendrites of layer 5 pyramidal neurons with an EPSP (Kampa et al., 2006; Kampa and Stuart, 2006). The induction of LTP for this sequence of local dendritic spike followed by EPSP may result from the prolonged depolarization and maximal NMDAR activation, even for negative time intervals.

NMDA spikes were first described in the distal basal dendrites of layer 5 pyramidal neurons. Their initiation requires correlated and clustered synaptic input. They are generated by strong local synaptic stimulation (Schiller et al., 2000). Once the threshold for regenerative currents through NMDARs is passed, strong depolarization and large concomitant Ca^{2+} influx occur for several tens of micrometers along the dendritic branch. Interestingly, NMDA spikes paired with EPSPs do not induce LTP in distal basal dendrites. However, when NMDA spikes and EPSPs are paired with locally applied brain-derived neurotrophic factor (BDNF), LTP is induced at these locations within a time window of 200 ms between NMDA spike and EPSP and preferentially when the NMDA spike precedes the EPSP (Gordon et al., 2006). This is another example of an anti-Hebbian learning rule. This form of synaptic plasticity associates inputs that cause an NMDA spike with subsequent EPSPs arriving at synaptic sites in close spatial proximity.

Finally, dendritic spikes contribute to STDP mechanisms even in cell types that do not support the backpropagation of APs. For example in cerebellar Purkinje cells the coincident activation of parallel and climbing fibers results in a timing-dependent spineous and branchlet Ca^{2+} transients and parallel fiber LTD (Wang et al., 2000).

4.39.4.3.3 Neuromodulation

The mechanisms that result in changes in synaptic strength can be influenced by neuromodulators. For example, the induction of LTD can be converted to LTP by the presence of dopamine in the prefrontal cortex (Matsuda et al., 2006). In other instances dopamine can facilitate the induction of LTD (Otani et al., 2003). The mechanisms by which neuromodulators affect STDP are currently unknown. However, neuromodulators can influence AP backpropagation, including the shape of the AP waveform in the dendrite, and the shape and size of the synaptic potential, all of which can influence STDP. Furthermore, activation or inactivation of the biochemical signaling cascades that are required for synaptic modifications could alter the direction of plastic changes for the same pre- and postsynaptic spiking pattern. For example, blocking the activation of mGluRs can result in the spike-timing-dependent induction of LTP with a stimulation pattern that would otherwise result in LTD (Nevian and Sakmann, 2006). The influence of BDNF on the induction of LTP during an NMDA spike (Gordon et al., 2006) is another example of neuromodulatory effects on biochemical cascades in STDP. Therefore STDP at a given synapse might not be a static and fixed learning rule. It might rather be modifiable by the actual

state of the neuronal network, which is represented by the ambient concentration of neuromodulators.

Modifications in synaptic strength due to repeated correlated pre- and post-synaptic activity can also be accompanied by changes in dendritic excitability (Desai et al., 1999; Daoudal et al., 2002; Daoudal and Debanne, 2003; Frick et al., 2004). Induction of STDP that results in a bidirectional modification of synaptic strength was shown to also induce a bidirectional modification of the excitability in the presynaptic neuron (Li et al., 2004). Therefore STDP is a mechanism not only by which changes in synaptic strength are regulated but also by which also changes in dendritic excitability of both the pre- and postsynaptic neuron are controlled.

4.39.4.4 Spike-Timing-Dependent Plasticity *in vivo* and Circuit-Level Effects

STDP has been most extensively studied in brain slice preparations, but several groups have provided evidence that STDP is of importance *in vivo*. Convergent retinotectal synapses from developing *Xenopus* tectal neurons undergo LTP when synaptic inputs to the tectal neuron are stimulated 20 ms before spiking of the neurons, whereas the reverse order results in LTD (Zhang et al., 1998). The measured STDP curve for the retinotectal synapse was similar to recordings made *in vitro* (Bi and Poo, 1998). Nevertheless, the expression of long-lasting potentiation can be prevented by spontaneous spiking in the tectal neuron or random visual input (Zhou et al., 2003). Developing tectal neurons can be trained to become direction sensitive by repeated presentation of a moving bar to the retina (Engert et al., 2002). The asymmetric modification of the receptive field can be explained by spike-timing-dependent LTP and LTD (Mu and Poo, 2006). Subregions of receptive fields can be modified or even erased by STDP (Vislay-Meltzer et al., 2006). All these findings support the idea that STDP induces experience-driven refinement of developing neural circuits.

In the rat visual cortex visually evoked synaptic responses can be modified bidirectionally depending on the temporal order of synaptic responses evoked by visual stimulation and postsynaptic spikes (Meliza and Dan, 2006). Pairing of visual stimuli presented at two orientations results in a shift in the orientation tuning in the visual cortex of cats, and this shift depends on the temporal order of the visual stimulus pair (Yao and Dan, 2001). Asynchronous visual stimuli presented to two adjacent regions on the retina control the relative spiking of two groups of cortical neurons with high temporal precision (Fu et al., 2002). Repeated pairing of these asynchronous stimuli resulted in modifications of intracortical connections and shifts in receptive fields depending on the temporal order and timing interval between the pair of visual stimuli. Similarly, in kitten visual cortex, cortical map plasticity depends on the timing of visual stimuli and electrical cortical stimulation (Schuett et al., 2001). A shift in orientation preference toward the paired orientation occurs when the electrical stimulation follows the visual stimulus, consistent with STDP. Experience-dependent plasticity of somatosensory responses also depends on dendritic excitability (Komai et al., 2006), and whisker deprivation results in cortical map plasticity based on spike-timing-dependent mechanisms (Celikel et al., 2004).

Hence there is growing evidence that STDP is a fundamental synaptic mechanism by which experience forms memory traces and neural circuits are refined in an activity-dependent manner. This hypothesis could explain the remarkable capability of the nervous system to constantly adapt to changes in the environment and to modify its behavioral responses to sensory inputs. The notion that APs in the dendrites are at the heart of this mechanism highlights the importance of dendritic structure and ion channel distribution for our capability to learn and think.

4.39.5 Conclusions

We have provided an overview of the current understanding of dendritic excitability and its relationship to synaptic plasticity. While a number of key mechanisms have been identified, such as backpropagating APs, local dendritic spikes, and timing-based rules for changes in synaptic efficacy, we are still just beginning to understand their functional importance for signal processing in neural circuits in awake animals. Likewise we need to further understand the molecular bases for the marked variations in these mechanisms, i.e., the underlying distributions of ion channels and the activation of particular signaling pathways. Future research will doubtless focus on both the molecular basis of dendritic signaling and also the functional implications for circuit function. Although many mysteries about dendritic function remain, it is now evident that dendrites are sophisticated and powerful computational devices.

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4.40 Plasticity of Intrinsic Excitability as a Mechanism for Memory Storage

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4.40.1 Introduction

During the past several decades, the analysis of the cellular and molecular mechanisms underlying learning and memory revealed two major targets for learning-dependent neuronal modulation: synaptic efficacy and intrinsic excitability (for review see Byrne, 1987).

The efficacy of a synapse is highly plastic and can be modified by neuronal activity in different ways. Indeed, different patterns of neuronal activity can lead to distinct and enduring changes in synaptic strength associated with phenomena such as long-term potentiation (LTP) and long-term depression (LTD). In addition, several behavioral training tasks, capable of inducing nonassociative and associative forms of learning, alter synaptic efficacy, thus supporting a role for synaptic plasticity in the storage of memory (e.g., Martin and Morris, 2002). Because of the extremely large number of individual synaptic contacts that neurons can form with other neurons as well as because of some computational properties that synapses exhibit such as associativity and input specificity, a memory storage system based on changes in synaptic strength has a potentially massive storage capacity (Poirazi and Mel, 2001). Consequently, theories based on persistent experience-driven changes in synaptic function have been extensively used to explain the storage of information (e.g., Fusi et al., 2005).

Nevertheless, an accumulating body of work indicates that learning also leads to persistent changes in intrinsic neuronal excitability (e.g., Brons and Woody, 1980; Crow and Alkon, 1980; Moyer et al., 1996; Cleary et al., 1998; Antonov et al., 2001; Brembs et al., 2002; Lorenzetti et al., 2006). These changes are due to modifications of membrane conductances and can affect electrophysiological properties including the resting potential, the input resistance, the shape and the threshold of the action potential, and the discharge frequency (for reviews see Daoudal and Debanne, 2003; Debanne et al., 2003; Zhang and Linden, 2003). What is the functional relevance of changes in intrinsic neuronal excitability to memory storage?

In this chapter, we will review some of the earlier work illustrating plasticity of intrinsic excitability produced by experience together with some more recent findings. In addition, we will discuss the implications of these results and the extent to which changes in synaptic efficacy and changes in intrinsic excitability can both contribute to memory storage.

4.40.2 Changes in Intrinsic Excitability Produced by Learning and Experience

Research over the past three decades has discovered modifications in the intrinsic membrane properties occurring in invertebrates and vertebrates following different forms of nonassociative and associative learning.

4.40.2.1 Invertebrate Models

Crow and Alkon (1980) were the first to report that classical conditioning (i.e., the learned ability to

associate a predictive stimulus with a subsequent salient event) altered the excitability of the photoreceptors in the nudibranch mollusk *Hermissenda crassicornis*. The positive phototaxis that the animal normally exhibits in response to illumination was reduced when the animal was trained with multiple paired presentations of light (conditioned stimulus, CS) and rotation (unconditioned stimulus, US), which activates the vestibular system (Crow and Alkon, 1978). This form of learning was associative, and the suppression of phototaxis, the conditioned response, persisted for several days (Crow and Alkon, 1978). Classical conditioning produced changes in several membrane properties of the type B photoreceptors, which are the primary sensory neurons of the CS pathway. The type B photoreceptors are excited by light and also by synaptic drive from the statocyst cells, which are part of the vestibular system activated by rotation. The changes produced by classical conditioning included increases in input resistance, spontaneous firing and spike activity in response to light presentation or current injection, and generator potentials evoked by the CS (Crow and Alkon, 1980). Importantly, these changes persisted when the synaptic input to the photoreceptors was removed. Voltage-clamp analysis of the type B photoreceptors revealed that pairing-specific changes in several ionic currents were the biophysical substrate of the aforementioned altered membrane properties. Classical conditioning decreased two K^+ currents, a rapidly activating and inactivating K^+ current (I_A) and a Ca^{2+}-activated K^+ current ($I_{K,Ca}$) (Alkon et al., 1982, 1985; Farley, 1988), and also altered a voltage-dependent Ca^{2+} current (Collin et al., 1988). These biophysical changes were consistent with the increase in excitability observed in the type B photoreceptors following classical conditioning because a reduction of I_A and $I_{K,Ca}$ would tend to produce an enhanced depolarization and increased spike activity in the photoreceptors in response to the presentation of light. The pairing-specific increase in intrinsic excitability was observed for several days following conditioning. Also, the magnitude of the increase in excitability was positively correlated with the degree of phototactic suppression, thus suggesting a causal relationship between the biophysical changes in the type B photoreceptors and the expression of the conditioned response (Crow and Alkon, 1980).

Following the results observed in *Hermissenda*, other learning-related changes in intrinsic excitability were identified in other invertebrates including annelids and mollusks. In the leech *Hirudo medicinalis*, habituation and sensitization, two forms of nonassociative learning, altered in opposite directions the intrinsic excitability of an identified neuron. *Hirudo* exhibits a defensive withdrawal reflex that consists of whole-body shortening in response to light touch of the skin (Sahley, 1995). This reflex can be habituated, when it is repetitively evoked, or sensitized, when a strong, noxious stimulus is delivered (Sahley, 1995). Sensitization, which is mediated by serotonin (5-HT), requires the activity of a group of electrically coupled interneurons called the S-cells (Sahley et al., 1994). Sensitization increased the input resistance of the S-cells as well as their spike threshold and the number of action potentials evoked by intracellular depolarization (Burrell et al., 2001). These effects were mimicked by exogenous application of 5-HT (Burrell and Sahley, 2005). Conversely, habituation produced a decrease in intrinsic excitability (Burrell et al., 2001). These results indicate that in the leech bidirectional changes in intrinsic excitability represent cellular correlates of two distinct forms of nonassociative learning.

The marine mollusk *Aplysia californica* has been probably the most extensively used invertebrate model to study the cellular and molecular mechanisms of learning and memory. Several examples of experience-dependent changes in intrinsic excitability have been reported in *Aplysia* following both nonassociative and associative learning of simple defensive reflexes as well as associative learning of more complex behaviors such as feeding. Long-term (24-h) sensitization was associated with facilitation of the sensorimotor neuron synapse (Cleary et al., 1998) as well as changes in the biophysical properties of the tail sensory neurons (Cleary et al., 1998). The intrinsic changes included increases in the excitability and in the afterdepolarization following either a single spike or a burst of action potentials (Cleary et al., 1998). Voltage-clamp analysis revealed that long-term sensitization reduced the net outward current in the tail sensory neurons, which is consistent with the increased excitability observed in these neurons following learning (Scholz and Byrne, 1987). Interestingly, long-term sensitization training also altered the biophysical properties of tail motor neurons. These changes included a more hyperpolarized resting membrane potential and a decrease in the threshold for spike initiation (Cleary et al., 1998). The changes produced by long-term sensitization in the sensory neurons resembled those induced by short-term sensitization, thus suggesting that the phenotype of the expression mechanism for the

short-term memory is preserved for the long-term memory. However, this is not always the case. Indeed, a more robust sensitization training protocol, which produced morphological changes in the sensory neurons and long-term synaptic facilitation of the sensorimotor neuron synapse (Wainwright et al., 2002), did not change the intrinsic excitability of the sensory neurons (Wainwright et al., 2004), but instead it induced narrowing of their action potentials (Antzoulatos and Byrne, 2007).

In *Aplysia*, classical conditioning also produced changes in the intrinsic excitability of sensory neurons. In a simplified preparation of the *Aplysia* siphon-withdrawal reflex, classical conditioning led to an increased input resistance and excitability of the siphon sensory neurons (Antonov et al., 2001, 2003). The changes in intrinsic excitability, as well as the pairing-specific strengthening of the sensorimotor neuron synapses, were restricted to the sensory neurons that were activated by mechanical stimulation of the siphon (i.e., on-field neurons), thus providing CS specificity to the expression of the conditioned response (Antonov et al., 2001, 2003). The presence of biophysical as well as synaptic changes following both nonassociative and associative learning suggested the intriguing hypothesis that the same cellular phenotype underlying nonassociative learning may also serve as the physiological substrate for associative memory (Byrne, 1987; Lechner and Byrne, 1998).

In *Aplysia*, the cellular mechanisms of associative learning were also studied using a more complex behavior (for a review see Baxter and Byrne, 2006). Appetitive forms of classical and operant conditioning (i.e., the ability to learn the consequences of a behavior) both increased the frequency of feeding behavior after training (Lechner et al., 2000a,b; Brembs et al., 2002; Baxter and Byrne, 2006; Lorenzetti et al., 2006). Also, both operant and classical conditioning changed the biophysical properties of neuron B51, a key element of the feeding neural circuitry, but the changes were remarkably different. Operant conditioning induced changes in B51 membrane properties (i.e., increased input resistance and decreased burst threshold), suggesting an increase in intrinsic excitability, which can contribute to the increased activity in B51 observed after training (Nargeot et al., 1999a,b; Brembs et al., 2002; Mozzachiodi et al., 2006). In contrast to operant conditioning, classical conditioning did not alter the input resistance, but increased the burst threshold of B51, resulting in a decreased intrinsic excitability (Lorenzetti et al., 2006).

These findings provided a biophysical substrate for the hypothesis that at the cellular level operant and classical conditioning are mediated by fundamentally different mechanisms (for a review see Baxter and Byrne, 2006). The decreased excitability in B51 was counterintuitive because classical conditioning enhances feeding in response to the CS and also strengthens the CS-evoked excitatory drive to B51 (Lorenzetti et al., 2006). However, the analysis of the responsiveness of B51 to the CS after training revealed that classical conditioning indeed facilitated the recruitment of B51, indicating that the factors that enhanced the recruitment of B51 overpowered the diminished excitability (Lorenzetti et al., 2006). Although experimental evidence is needed, the pairing-specific decrease in the excitability of B51 might represent an adaptive mechanism to help shape the CS specificity produced by classical conditioning.

Although most of the effects of learning on the membrane properties involved changes in input resistance and/or intrinsic excitability, other forms of intrinsic modifications were described as a result of experience in mollusks. For example, as mentioned above, long-term sensitization in *Aplysia* was associated with an increase in the resting potential of the motor neurons (Cleary et al., 1998). In the pond snail *Lymnaea stagnalis*, long-term memory for appetitive classical conditioning of feeding, induced by repetitive paired presentations of mechanical stimulation of the lips (CS) and application of sucrose as a feeding stimulant (US; for a review see Benjamin et al., 2000), was associated with a persistent depolarization in the modulatory neuron CV1a involved in the initiation of feeding movements (Jones et al., 2003). The pairing-specific depolarization of CV1a was positively correlated with the fictive feeding response to the CS in reduced preparations from conditioned animals, but it was not associated with any change in other membrane properties such as input resistance, spike threshold, or spike frequency (Jones et al., 2003). The time course of the persistent depolarization also mirrored the duration of retention of long-term memory (Jones et al., 2003). This pairing-specific depolarization would render CV1a more likely to fire in response to the CS and allow the CS to more effectively generate feeding responses.

4.40.2.2 Vertebrate Models

The first evidence of experience-dependent changes in neuronal excitability in a vertebrate model system was reported by Brons and Woody in 1980.

These authors used a conditioning paradigm in the cat in which repetitive paired presentations of an auditory click (CS) and a glabella tap (US), which evoked an eyeblink response, led to the appearance of the conditioned response consisting of a combined eyeblink and nose twitch (Brons and Woody, 1980). Cellular analysis conducted in the sensorymotor cortical areas and in the facial nucleus of awake cats revealed that classical conditioning was associated with an increased excitability as measured by the minimum amount of injected current necessary to evoke an action potential (Brons and Woody, 1980). An increase in input resistance was also measured in neurons of the facial nucleus. The persistence of the increased excitability, once the conditioned response was extinguished, indicated that this mechanism was not directly implicated in the formation and retention of the conditioned response (Brons and Woody, 1980). A detailed analysis of the biophysical substrate of the increased excitability has not been pursued.

Several other examples of experience-dependent changes in intrinsic excitability were identified in vertebrate models. In the rabbit, delay eyelid conditioning, which is performed using a tone as CS and a co-terminating air puff as US, depends on the activation of both the cerebellar cortex and cerebellar deep nuclei (for reviews see Kim and Thompson, 1997; Christian and Thompson, 2003). Recordings from Purkinje cells revealed a reduced spike threshold and a reduced afterhyperpolarization (AHP) evoked by bursts of spikes in slices from conditioned animals when compared to control animals (Schreurs et al., 1998). The AHP that follows action potentials is an important determinant for the firing activity of neurons, and a pairing-specific reduction of its amplitude is consistent with an increased excitability observed following classical conditioning. Importantly, these changes were long-lasting (up to 30 days), restricted to defined microzones of the cerebellar lobule HVI and the degree of reduction in spike threshold positively correlated with the conditioned response in the paired animals (Schreurs et al., 1997, 1998). Another form of classical conditioning is trace eyelid conditioning, which requires the hippocampus and occurs when a trace interval is imposed between the CS offset and the US onset. Cellular analysis of trace eyelid conditioning revealed that hippocampal pyramidal neurons in the CA1 and CA3 areas from conditioned animals exhibited a greater number of spikes evoked by depolarizing current injections (Moyer et al., 1996; Thompson et al., 1996) and a decreased AHP due to a reduction in the Ca^{2+}-dependent K^+ current underlying the AHP as compared to control animals (Coulter et al., 1989; Moyer et al., 1996; Thompson et al., 1996). These changes were consistent with a pairing-specific increase in excitability. Although the increased excitability was similar to that found following delay conditioning, it was not restricted to a specific area, but it was instead widespread through the dorsal hippocampus and also could not be detected 7 days after training when the memory for trace eyelid conditioning was still retained (Moyer et al., 1996; Thompson et al., 1996). These findings indicate that it is unlikely that this transient increased excitability in hippocampal neurons constitutes a part of the memory trace itself, but it could represent a mechanism through which the hippocampal circuit is set in a more permissive state for input-specific synaptic modification to occur during memory formation. A similar learning-dependent reduction in AHP also occurred in CA1 pyramidal neurons in the dorsal hippocampus following a spatial learning task that depends on the hippocampus (Oh et al., 2003).

In rats, pairing-specific enhancements in both CS-evoked synaptic drive and neuronal excitability (i.e., increased input resistance and a reduced amount of injected current necessary to elicit an action potential) were measured *in vivo* in neurons from the lateral nucleus of the amygdala of anesthetized rats trained with a conditioning paradigm during which an odor (CS) was repetitively paired with a foot shock (US; Rosenkranz and Grace, 2002). The synaptic and the intrinsic changes were both prevented when dopamine signaling, which is relevant for amygdala-dependent forms of learning, was pharmacologically blocked in the lateral nucleus of the amygdala (Rosenkranz and Grace, 2002). These results are consistent with the aforementioned studies of *Aplysia* withdrawal reflexes in which both synaptic and intrinsic plasticity occur together during memory formation.

Changes in intrinsic excitability have also been found in vertebrates following operant conditioning tasks. In monkeys, operant conditioning of the spinal stretch reflex is associated with changes in the intrinsic properties of the motor neurons. The spinal stretch reflex is among the simplest vertebrate reflexes and is largely mediated by a monosynaptic pathway between Ia afferent neurons and alpha motor neurons (Wolpaw, 1997). Because this reflex is influenced by descending activity from supraspinal structures, it can be operantly conditioned and

animals can learn to gradually increase or decrease this reflex or its electrical analog, the H-reflex (Wolpaw, 1997). In particular, operantly conditioned decrease in the H-reflex in monkeys was associated with an increase in the depolarization needed for the spinal motor neuron to fire and a decrease in its conduction velocity (Carp and Wolpaw, 1994). These apparently intrinsic changes were consistent with the hypothesis that a positive shift in motor neuron firing threshold and a consequent increase in the depolarization needed to reach that threshold could contribute to weaken the magnitude of the H-reflex following operant conditioning. Modeling studies proposed that a positive shift in the activation of voltage dependence of Na^+ channels might represent the biophysical substrate for the operantly conditioned decrease in the H-reflex (Halter et al., 1995).

Changes in excitability were also reported in rats following an olfactory discrimination task (for a review see Saar and Barkai, 2003). Rats were trained to discriminate pairs of odors for a water reward. Several consecutive days of training were required for a rat to successfully discriminate between a first pair of odors, but once the rat reached good performance, its ability to learn to discriminate between a new pair of odors improved dramatically and only 1 day was needed to achieve good performance (Saar and Barkai, 2003). Cellular analysis revealed that both spike frequency adaptation and AHP amplitude were reduced in pyramidal neurons of the rat olfactory cortex following olfactory discrimination training when compared to naive or pseudo-trained animals (Saar et al., 1998, 2001). These changes were detected 1–3 days after training, but they decayed to baseline values by 5–7 days after training (Saar et al., 1998, 2001). It has been speculated that this example of increased excitability is not *per se* a mechanism of memory storage, but it might be involved in rule learning, setting the neural circuits in the piriform cortex in an excitable, more permissive state for activity-dependent synaptic modifications to occur (Saar and Barkai, 2003).

4.40.3 Activity-Dependent Modulation of Intrinsic Excitability

In addition to learning tasks *in vivo*, intrinsic excitability can be modulated by patterns of electrical stimulation of neurons and neural pathways. Depending on the protocol of stimulation, some of these patterns of neuronal activity may induce either an enhancement or a reduction of synaptic strength, or LTP (for a review see Lynch, 2004) and LTD (for a review see Ito, 2001), respectively. LTP, which is probably the most studied example of activity-dependent modulation of synaptic efficacy, can be induced in several areas of the vertebrate brain, including the hippocampus, the amygdala, the neocortex, and the cerebellar cortex, and is commonly considered to be a mechanism underlying aspects of learning and memory (for reviews see Bliss and Collingridge, 1993; Martin and Morris, 2002). Because several forms of LTP and LTD can be induced in isolated brain slices, the biochemical cascades underlying these forms of neuronal plasticity can be analyzed.

Although most of the work on LTP has focused on the mechanisms underlying the persistent augmentation of synaptic efficacy, several lines of evidence indicate that, at least in some cases, LTP is also accompanied by modifications in intrinsic excitability. Bliss and Lømo, who first described LTP in the dentate area of the rabbit hippocampus in 1973, also reported an increase in the population spike (i.e., the extracellularly recorded signal representing the summation of the evoked action potentials in postsynaptic neurons) accompanying LTP, which could not be entirely explained by the LTP-evoked increase in the population excitatory postsynaptic potential (EPSP). This nonsynaptic component of LTP was termed E-S potentiation, or E-S potentiation (Bliss and Lømo, 1973). The mechanisms underlying E-S potentiation are still subject to some debate. Two hypotheses have been developed to explain this phenomenon: an LTP-induced decrease in the ratio of inhibitory to excitatory drive (e.g., Abraham et al., 1987; Chavez-Noriega et al., 1989) and an LTP-induced increase in the intrinsic excitability of the postsynaptic neurons through modulation of voltage-dependent channels (e.g., Taube and Schwartzkroin, 1988; Bernard and Wheal, 1995). Several lines of evidence indicate that a reduction in the ratio of inhibitory to excitatory drive is not sufficient to account for the E-S potentiation, thus pointing to a role for LTP-induced intrinsic plasticity, the consequence of which would lead to a higher efficiency of E-S coupling (e.g., Wathey et al., 1992). One example, which suggests the modulation of voltage-dependent channels as a mechanism underlying the increased excitability accompanying synaptic LTP, has been reported in hippocampal CA1 pyramidal neurons. In these neurons, a pattern of synaptic inputs paired with postsynaptic spikes induced a long-term increase of

intrinsic excitability concurring with synaptic LTP (Xu et al., 2005). The increased excitability was manifested as a decrease in the action potential threshold that was attributable to a shift in the activation curve of voltage-gated Na^+ channels (Xu et al., 2005). This form of enduring increase of intrinsic excitability shared a similar signaling pathway with the late phase of synaptic LTP, which included the requirement of activation of glutamate receptors of the *N*-methyl-D-aspartate (NMDA) type, the influx of Ca^{2+}, the activity of Ca^{2+}/calmodulin-dependent protein kinase II (CaMKII), and changes in protein synthesis (Xu et al., 2005).

The modulation of the E-S coupling appears to be bidirectional. In the CA1 region of the hippocampus, LTD, normally induced by low-frequency patterns of electrical stimulation, was accompanied by a reduction of the E-S coupling (Daoudal et al., 2002). This reduction of the E-S coupling was largely due to a decrease in the intrinsic excitability of hippocampal cells (Daoudal et al., 2002). Therefore, opposite modulation of the synaptic strength is associated with bidirectional changes in E-S coupling, which reflect bidirectional modifications of intrinsic excitability.

Other examples of activity-dependent changes in intrinsic excitability produced by synaptic activation have been reported in different brain areas. For example, in the rat entorhinal cortex, persistent graded increases in firing frequency were induced in pyramidal neurons by repetitive excitatory synaptic activation (Egorov et al., 2002). These sustained levels of firing could be either increased or decreased in an input-specific manner and relied on activity-dependent changes of Ca^{2+}-dependent cationic current (Egorov et al., 2002). This intrinsic ability of the neurons in the entorhinal cortex to generate graded persistent activity has been proposed as a cellular mechanism for working memory (Egorov et al., 2002). Another example of modulation of intrinsic excitability induced by synaptic activation has been reported in the cerebellar cortex. High-frequency stimulation of the mossy fiber-to-granule cells pathway led to LTP and was also accompanied by a persistent enhancement of intrinsic excitability of granule cells, which was associated with increased input resistance and decreased spike threshold (Armano et al., 2000). Neurons of the cerebellar deep nuclei also exhibited a rapid, synaptically driven, increase in their intrinsic excitability, which required Ca^{2+} influx through activation of NMDA-type glutamate receptors (Aizemann and Linden, 2000). However, in this case, the pattern of synaptic stimulation used to activate these neurons did not produce any facilitation of the synaptic input (Aizemann and Linden, 2000). This last example indicates that modulation of intrinsic excitability can be expressed without the occurrence of changes in synaptic function, thus providing a contribution to the information storage independent from synaptic plasticity.

Recent findings indicate that changes in intrinsic excitability may not require synaptic activation. For example, a train of high-frequency intracellular depolarizations delivered to pyramidal neurons in layer V of the primary visual cortex produced an enduring increase in intrinsic excitability (Cudmore and Turrigiano, 2004). This change in excitability required Ca^{2+} entry during neuronal activity and did not affect the passive membrane properties, thus suggesting the involvement of voltage-dependent channels (Cudmore and Turrigiano, 2004). Another example of synaptic-independent modulation of intrinsic excitability has been reported in hippocampal CA1 pyramidal neurons (Fan et al., 2005). A pattern of suprathreshold repeated intracellular stimulation (i.e., theta-burst firing) produced a decrease in somatic excitability, which was due to an upregulation of the hyperpolarization-activated cationic current I_h (Fan et al., 2005). This decrease in excitability was prevented by Ca^{2+} chelators; by low concentrations of tetrodotoxin, which blocked back propagation of action potentials from the soma to the dendrites; and by NMDA receptor antagonists even in the absence of synaptic activation (Fan et al., 2005). These findings suggest that during theta-burst firing, back-propagating action potentials would lead to Ca^{2+} influx through the NMDA receptor, which, in turn, would increase the I_h via a mechanism mediated by CaM kinase II.

Recent findings indicate that the synaptic drive does not need to be excitatory to induce an increase in intrinsic excitability. In the medial vestibular nucleus, brief periods of inhibitory synaptic input, or direct membrane hyperpolarization, triggered a long-lasting increase in both the spontaneous firing rate and firing responses to intracellular depolarization (Nelson et al., 2003). This increase in excitability, termed firing rate potentiation, was due to a decrease in cytosolic Ca^{2+}, which reduced CaM kinase II activity and, in turn, downregulated BK-type $I_{K,Ca}$ (Nelson et al., 2005). This novel form of neuronal plasticity might contribute to motor learning in the vestibulo-ocular reflex.

The intrinsic changes produced by patterns of neuronal activity are not restricted to ion channels and can

include modulation of the activity of membrane transporters. For example, interneurons of the rat dentate gyrus exhibited a long-term depolarization of their resting membrane potential after high-frequency stimulation of the perforant path that was attributed to an activity-dependent change in the rate of the electrogenic Na^+ pump (Ross and Soltesz, 2001). This activity-dependent depolarization, which occurred in the absence of any potentiation of the excitatory synaptic input, required the rise of intracellular Ca^{2+} and the activation of alpha-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA), but not NMDA receptors (Ross and Soltesz, 2001). As a result of the depolarization, the interneurons of the dentate gyrus responded with action potential discharge to previously subthreshold EPSPs even in the absence of synaptic potentiation, thus indicating an increase in the E-S coupling (Ross and Soltesz, 2001). Activity-dependent changes in the function of the electrogenic Na^+ pump have been found also in invertebrate neurons. In the leech, low-frequency repetitive stimulation of touch (T) sensory neurons led to a lasting increase in the amplitude of the AHP produced by the firing discharge, which is due to an increase in the activity of the electrogenic Na^+ pump (Scuri et al., 2002). The modulation of the Na^+ pump activity was regulated by both the influx of Ca^{2+} during neural activity and the release of Ca^{2+} from intracellular stores. In addition, phospholipase A2 and the downstream activation of arachidonic acid metabolites, derived from the 5-lipoxygenase pathway, were necessary for the increase of the AHP amplitude (Scuri et al., 2005). The increase of the AHP amplitude was associated with a persistent depression of the synaptic connection between T cells and their follower neurons (Scuri et al., 2002). This synaptic depression may be a cellular mechanism contributing to short-term habituation. Since 5-HT mediates sensitization in the leech (e.g., Catarsi et al., 1990; Sahley et al., 1994; Zaccardi et al., 2004), it has effects on the amplitude of the AHP in T neurons opposite those produced by repetitive stimulation. Indeed, 5-HT reduced the AHP amplitude by inhibiting the Na^+ pump (Catarsi and Brunelli, 1991) in a cyclic adenosine monophosphate (cAMP)-dependent manner (Catarsi et al., 1993). Injection of cAMP into a T neuron enhanced the synaptic connection between the T cell and its follower neurons (Scuri et al., 2007). Together these findings indicate that bidirectional changes in the AHP amplitude that alter synaptic efficacy may represent cellular mechanisms underlying habituation and sensitization, two simple forms of learning.

4.40.4 Plasticity of Intrinsic Excitability as a Mechanism for Memory Storage: Hypotheses and Lines of Evidence

The general motif that emerges from the examples above is that several different learning tasks, nonassociative and associative, as well as activation of neurons and neural pathways, induce changes in the biophysical properties of neurons in both invertebrates and vertebrates. These neurophysiological correlates have been identified at the level of sensory neurons, interneurons, and motor neurons.

What is the functional relevance of these changes? In some cases, a direct role for the intrinsic plasticity in memory storage appears straightforward. For example, in *Aplysia* and *Hermissenda*, because the loci of plasticity induced by classical conditioning were found at the level of the first central relay in the circuits controlling the responses, neuron-wide changes in intrinsic excitability in response to the conditioned stimuli appear to be an appropriate memory mechanism to express and retain the conditioned response. However, in other cases the changes in intrinsic excitability failed to correlate, or correlated poorly, with the learned behavioral changes, thus making it difficult to assess their contribution to the storage of memory (e.g., Cleary et al., 1998). Although additional results are required, one hypothesis is that some of the intrinsic changes may not function as part of the engram itself, but they may represent either adaptive mechanisms to shape the stimulus specificity of the learned response (as in the case of classical conditioning of feeding in *Aplysia* (Baxter and Byrne, 2006; Lorenzetti et al., 2006), or mechanisms through which a neural circuit is set to a permissive state to facilitate the occurrence of the synaptic modifications necessary for memory formation (as in the case of trace eyelid conditioning in rabbits (Moyer et al., 1996; Thompson et al., 1996) or olfactory operant conditioning in rats (Saar and Barkai, 2003).

Another issue that requires more investigation is the extent to which the changes in intrinsic excitability are complementary to experience-dependent modifications in synaptic efficacy. The majority of theoretical models of memory storage have been largely based on persistent experience-driven changes in synaptic function (Poirazi and Mel, 2001; Fusi et al., 2005). Certain unique properties of the synapses such as associativity and input specificity cause

systems of memory storage based on changes in synaptic strength to have a potentially massive storage capacity (Poirazi and Mel, 2001; Fusi et al., 2005). In contrast, global changes in intrinsic excitability would theoretically alter the throughput of all the synaptic inputs impinging on a given neuron. If this is the case, memory mechanisms based on neuron-wide altered intrinsic excitability would have a lower storage capacity and would be less versatile than systems of memory storage based on changes in synaptic strength.

Some recent results may help to reconcile differences between models of memory storage based on synaptic plasticity and models based on intrinsic plasticity. Patch-clamp techniques and simultaneous Ca^{2+} imaging that allow for recordings from individual dendrites (for a review see Magee and Johnston, 2005) have revealed that the induction of LTP was accompanied by a local increase in dendritic excitability that favored back propagation of action potentials into that dendritic region with a subsequent boost in the Ca^{2+} influx (Frick et al., 2004). A shift in the inactivation curve of a transient A-type K^+ current was found to account for the enhanced excitability (Frick et al., 2004). Importantly, the activity-dependent increase in dendritic excitability was localized at, or in the vicinity of, the synaptic site, which was potentiated in an input-specific manner following electrical stimulation (Frick et al., 2004). A local increase in dendritic excitability could facilitate enhanced propagation of synaptic potentials toward the site of action potential initiation (for a review see Magee and Johnston, 2005). Therefore, enhancement of local excitability could contribute to the higher efficiency of coupling between synaptic potentials and spike as observed following LTP induction.

Although validation in *in vivo* preparations is required, these findings indicate that changes in intrinsic excitability can be induced in restricted membrane compartments and can be co-expressed with changes in synaptic efficacy in a manner that affects signal integration locally and can preserve input specificity.

4.40.5 Summary

Data collected over the past decades in both vertebrate and invertebrate model systems indicate that learning and memory as well as patterns of electrical stimulation of neurons and neural pathways not only alter synaptic function but also produce changes in intrinsic excitability. These changes in intrinsic excitability can be neuron-wide or restricted to specific membrane compartments such as the dendrites, thus affecting neuronal function and signal integration either globally or locally. Although the functional relevance of certain changes in intrinsic excitability in the context of a given form of learning has not been fully elucidated, it is becoming clear that experience-dependent changes in intrinsic excitability may function as part of the engram itself, as adaptive mechanisms to shape the stimulus specificity of the learned response or also as mechanisms through which a neural circuit is set to a permissive state to facilitate the occurrence of the synaptic modifications necessary for memory formation and retrieval.

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4.41 Neurogenesis

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4.41.1 Introduction

During the development of the central nervous system (CNS), neural stem cells give rise to neurons, oligodendrocytes, and astrocytes, the three major lineages that constitute the brain. Although a fundamental dogma of neuroscience predicted that neurogenesis ceases with the end of development, as early as 40 years ago studies by Altman and Das showed that dividing cells persist throughout life in the mammalian

CNS (Altman and Das, 1965). Indeed, Altman and colleagues' data suggested that not only glial cells but also new neurons are continuously added into the adult brain circuitry. Despite confirming reports by other groups (Hinds, 1968; Kaplan and Hinds, 1977), the finding that neural stem cells persist and can give rise to new neurons even in the adult brain remained a subject of great controversy. Nevertheless, improved techniques to identify newborn cells and their respective neural phenotype within the adult tissue finally led to the acceptance of the fact that (1) neural stem cells persist in the adult brain and (2) new neurons are continuously generated in the adult CNS in two restricted areas: the hippocampal dentate gyrus (DG) and the subventricular zone (SVZ) of the lateral ventricle (Ming and Song, 2005). In the meantime, adult neurogenesis was found to exist in all mammals, including humans (Eriksson et al., 1998). In this chapter, we mainly focus on adult neurogenesis in the hippocampal dentate gyrus and present recent advances in the understanding of the biology of adult neural stem cells, the maturation of new neurons into the preexisting circuitry, and the potential significance of adult neurogenesis in hippocampal function.

4.41.2 Stem Cells in the Adult Brain

4.41.2.1 Neural Stem Cells *in vitro*

Despite the early reports by Altman and colleagues in the 1960s, it took almost another three decades to successfully grow neural stem cells that were isolated from the adult brain in the culture dish (Reynolds and Weiss, 1992). The *in vitro* characterization of proliferative cells that were isolated from adult brain tissue was crucial to identifying a subpopulation of them as stem cells. Even though the consensus definition of a stem cell has been modified several times (Smith, 2006), two characteristics are generally required for a cell to be identified as a true stem cell: (1) the capacity for theoretically unlimited self-renewal and (2) the ability to generate cells different from themselves through asymmetric cell division. In general, stem cells can be divided into several levels of potency: (1) the 'totipotent' zygote capable of generating a complete organism when implanted into the uterus of a living animal; (2) the 'pluripotent' embryonic stem cell, which can give rise to all body tissues except the trophoblasts of the placenta and, perhaps, the gonads; and (3) the 'multipotent' stem cell that self-renews but gives rise to only tissue-specific cell types. Even though there is recent evidence that neural stem cells (NSCs) can give rise to tissue types other than CNS tissue under *in vitro* conditions (Wurmser et al., 2004), they are still mainly classified as multipotent stem cells (Gage, 2000).

4.41.2.1.1 Culturing of neural stem cells

The neurosphere assay is the most commonly used technique to analyze the stem cell capacity of isolated brain cells [**Figure 1(a)**] (Reynolds and Weiss, 1992; Lie et al., 2004). In this preparation, the brain area of interest is dissected and plated as a single-cell suspension that is propagated as floating aggregates. The addition of

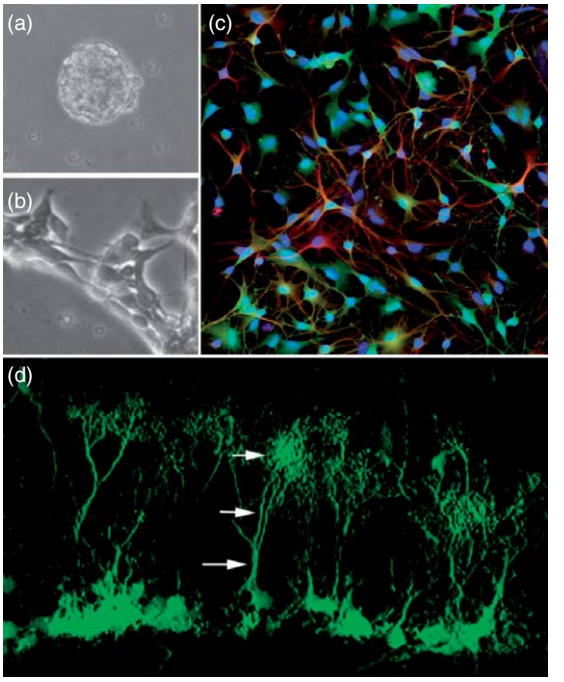

Figure 1 Neural stem cells (NSCs) *in vitro* and *in vivo*. NSCs can be grown as free-floating cell aggregates, so called neurospheres (a), or under adherent culture conditions forming monolayers (b). In both conditions strong mitogens such as EGF and/or FGF-2 are added (for details see Ray and Gage, 2006). *In vitro* propagated stem cells retain the capacity to differentiate into all three neural lineages. (c) Rat NSCs (expressing green fluorescent protein, green) that were differentiated into neuronal cells expressing MAP2ab (red) with the addition of retinoic acid and forskolin in the culture medium for 4 days. Nuclei were counterstained with 4′,6-diamidino-2-phenylindole (DAPI) (blue). (d) A population of NSCs residing in the adult brain is expressing nestin. Shown is the dentate area of an adult transgenic mouse expressing green fluorescent protein under the control of the nestin promoter. Note the typical morphology of type 1 nestin cells with a tree-like process that branches in the inner molecular layer (arrow). In contrast to this morphology, type 2 cells are small, rounded cells that show a higher proliferative activity *in vivo* than type 1 cells (for details see Kempermann et al., 2004).

strong mitogens such as epidermal growth factor (EGF) and fibroblast growth factor 2 (FGF-2) allows the propagation of endogenous proliferative cells (the putative NSCs). The forming spheres, referred to as neurospheres, can be propagated over many passages and have the potential to differentiate into all three neural lineages after the withdrawal of mitogens and/or addition of differentiating factors. Even though it appears that neurospheres do not grow clonally (Fessberger et al., 2006; Singec et al., 2006), self-renewal can be tested by the formation of secondary or tertiary spheres; a single cell is grown in a miniwell until the sphere reaches a certain size, after which the sphere is again dissociated into single cells that can give rise to a new, and thus secondary, multipotent neurosphere. An alternative method to grow adult NSCs is the so-called monolayer assay, where multipotent cells grow adherent to the dish surface [**Figure 1(b)**] (Ray et al., 1993; Gage et al., 1995; Palmer et al., 1995). Using these culturing techniques, NSCs can be isolated and propagated from many species, including humans (Palmer et al., 2001). Using the neurosphere assay, several laboratories have tried to identify differences in the potency of NSCs derived from different regions of the adult brain. Due to conflicting *in vitro* results, which might be due to differences in dissection and culturing methods, there is an ongoing controversy about whether the hippocampus contains true stem cells with theoreticallyunlimited self-renewal, or whether hippocampus-derived neurosphere cultures have only limited proliferative and differentiating capacities (Seaberg and van der Kooy, 2002).

4.41.2.1.2 Proliferation and differentiation of NSCs

The *in vitro* propagation of adult NSCs allowed the extensive biochemical and molecular characterization of fate choice, proliferative capacity, and cellular potency. *In vitro* studies identified a variety of factors and mechanisms that regulate cell proliferation and instruct NSCs to adopt a neuronal or glial fate [**Figure 1(c)**] (Lie et al., 2004; Ming and Song, 2005). Subsequent studies showed an *in vivo* role for most of those factors as well, thus confirming the *in vitro* NSC model system. New mechanisms that affect proliferation and/or differentiation of NSCs are constantly being discovered; the regulation of proliferation and differentiation clearly occurs on multiple molecular levels. We and others have shown that epigenetic mechanisms, growth factors such as brain-derived neurotrophic factor (BDNF) and vascular endothelial growth factor (VEGF), sonic hedgehog signaling, WNT signaling, small double-stranded RNAs, retrotransposition, transcription factor expression such as E2F, cyclin-D2 expression, and cell-cycle inhibitors such as p27kip1 (Lie et al., 2004; Ming and Song, 2005; Cao et al., 2006) are among important regulators of proliferation and/or neuronal differentiation. Considerably less is known regarding differentiation toward glial cells, though several important signaling pathways have been identified that induce NSCs to differentiate into oligodendrocytes, such as insulin-like growth factor (IGF) signaling (Hsieh et al., 2004), or astrocytes, such as bone morphogenetic protein (BMP) and leukemia inhibitory factor (LIF) signaling (Bonaguidi et al., 2005).

Despite the great utility of *in vitro* assays in the biochemical and molecular characterization of NSCs, there are several important caveats about using them to study NSCs. Isolated cells must be exposed to high levels of mitogens that potentially induce cellular changes different from those seen in an *in situ* stem cell. Furthermore, NSCs in culture are 'naked' and may not receive the same factors as those that are embedded in their respective cellular niche. Importantly, cells with stem cell capacity can be easily isolated from almost all areas of the adult brain and the spinal cord, even though only two areas in the adult brain are capable of generating new neuronal cells.

4.41.2.2 Neural Stem Cells *in vivo*

The adult DG continuously produces new neuronal cells. Neurons born in the adult hippocampus are glutamatergic granule cells, the principal cell type of the DG. Granule cells reside in the densely packed granule cell layer (GCL), receive their main excitatory input from layer II of the entorhinal cortex (EC), and largely project toward area 3 of the cornu ammonis (CA3) via the mossy fiber tract. The DG is a very densely packed structure: a single mouse DG consists of approximately 300,000 granule cells, rat DG about 1 million, and human DG between 15 and 20 million (Simic et al., 1997). These cell numbers are very large relative to the DG's input (EC) and output (CA3) structures, both of which have an order of magnitude fewer neurons.

4.41.2.2.1 The hippocampal neurogenic niche

Proliferative activity within the dentate area occurs largely in the zone just below the GCL and the hilus. This area, called the subgranular zone (SGZ), does not have strict boundaries and is commonly defined as an intermediate region about two cell layers deep into the hilus below the GCL (corresponding to

approximately 20–30 μm). Significant research has been devoted to determining how to identify dividing cells in the SGZ morphologically and whether they can be classified using differential gene expression. Acutely dividing cells can be visualized by the detection of cell cycle–associated genes such as Ki-67, PCNA, and phospho-histone H3.

Whether or not those proliferating cells in the DG are true NSCs *in vivo* is still unknown. This categorization requires the observation of self-renewal and multipotency, and neither has been definitively shown due to the technical difficulties in following single cells and their lineages over time *in vivo*. Several labeling techniques have been used to label proliferating populations of cells in the DG and track their progeny, including bromo-deoxyuridine (BrdU) and tritiated (^{3}H)-thymidine (nucleotide analogues) and marker-expressing retroviruses [**Figure 2(a)**].

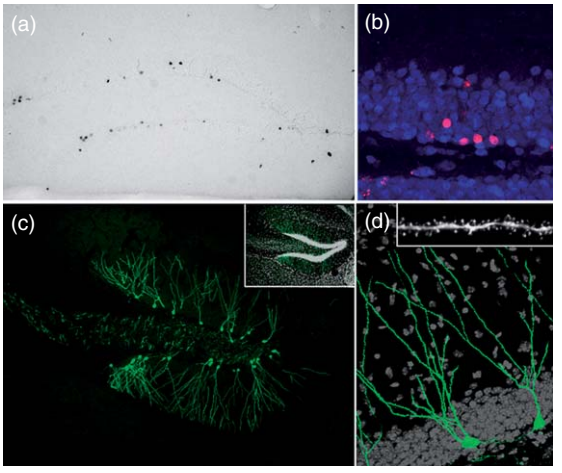

Figure 2 Labeling of newborn cells *in vivo*. The thymidine analogue bromo-deoxyuridine (BrdU) is integrated into the DNA of proliferating cells during S-phase. (a) BrdU-labeled cells can be visualized in the adult dentate gyrus with specific antibodies. The example shows a section of an adult mouse that received five consecutive BrdU injections and was sacrificed 4 weeks after the last injection. (b) The phenotype of the BrdU-positive cells (red) can be reliably analyzed by confocal microscopy using, for example, neuronal markers such as NeuN (blue), again 4 weeks after BrdU injection. Dividing cells and their progeny can also be visualized with retroviruses expressing green fluorescent protein (or any other label that can be later visualized), allowing the direct detection of newborn cells without the need for post hoc detection methods. (c) A 40-μm section of an adult mouse that was injected 4 weeks earlier with a retrovirus expressing green fluorescent protein under the control of the CAG promoter. (d) Retroviral labeling visualizes the whole cell (including dendritic and axonal processes) and even allows the analysis of neuronal fine structures such as dendritic spines (inset in (d); for details see Zhao et al., 2006).

The most commonly used label is BrdU, a thymidine analogue that becomes integrated into the DNA during the S-phase of the cell cycle. BrdU-labeled cells can be visualized with a specific antibody and then analyzed histologically to determine their neural phenotype [**Figure 2(b)**]. The major advantage of BrdU labeling is the easy and reliable quantification of new cells. There are several drawbacks to BrdU, however. Because BrdU incorporates into newly formed DNA, several successive rounds of DNA replication after BrdU administration will dilute the signal. Furthermore, the BrdU signal is restricted to the nucleus and thus does not show the morphology of newborn cells. Given these limitations, the use of retroviral vectors expressing green fluorescent (GFP) or red fluorescent protein (RFP) to visualize the whole cell morphology opened up a completely new level of analysis [**Figures 2(c) and 2(d)**] (van Praag et al., 2002). Because retroviruses require the breakdown of the nuclear membrane for genomic integration, only dividing cells are transduced. Retrovirus infection and delivery are not as ubiquitous as BrdU uptake, limiting their value as quantification tools; however, the transduction of progenitors results in a stable and robust expression of a marker protein that is detectable in both fixed and live tissue.

Using these markers, it has been shown that proliferating cells that can generate all three lineages exist in the DG (Kempermann et al., 2004). As it is unclear that these cells are individually multipotent and self-renewing, they are referred to as neural progenitor cells (NPCs). Accumulating evidence suggests that there are actually several types of dividing cells within the adult hippocampus, and their relationship to one another remains somewhat elusive (Kempermann et al., 2004). One type of NSC within the hippocampal DG appears to be very similar to a mature astrocyte [**Figure 1(d)**]. These cells express glial fibrillary acidic protein (GFAP) (Seri et al., 2001), have the electrophysiological properties of astrocytes, and possess astrocytic vascular end feet (Filippov et al., 2003). Depending on the nomenclature used, these cells are referred to as type 1 or B-cells. Type 1 cells also express the intermediate filament protein nestin, and a subpopulation of type 1 cells also colabels with the HMG-transcription factor Sox-2. The low proliferative activity of type 1 cells may indicate that these cells represent a largely quiescent, true stem cell within the hippocampus, but this hypothesis remains to be proven. In contrast to the slowly dividing type 1 cells, type 2 cells exhibit a much higher proliferative activity. Type

2 cells are negative for GFAP but still express nestin and Sox-2, and a fraction of type 2 cells expresses early neuronal markers such as doublecortin (DCX) and the bHLH transcription factor Prox-1. One theory is that the expression of these neuronal markers in a subset of dividing cells indicates that type 2 cells (and type 3 cells that divide but are negative for nestin and Sox-2 and positive for DCX) represent committed neuroblasts that have lost their multipotentiality (Kempermann et al., 2004).

The rate at which neurons are born in the adult hippocampus is not fixed but is instead dynamically regulated by a variety of factors. Despite increasing knowledge about how progenitor cells 'translate' signals such as local network activity into altered cell division or differentiation, the exact mechanisms remain unclear. Recent reports have shown that depolarizing events trigger NSCs to initiate neuronal differentiation (Deisseroth et al., 2004; Tozuka et al., 2005), providing an explanation for how network activity within the dentate gyrus may shape the cellular composition of the area itself. Furthermore, elegant *in vivo* studies have shown the synaptic integration of nestin-expressing cells in the SGZ and their functional GABAergic input (Tozuka et al., 2005). Those reports have led to the hypothesis that dentate NSCs may 'sense' the activity of surrounding mature, neuronal networks and respond with a distinct action, such as increased or decreased cell proliferation and/or differentiation. The regulation of adult neurogenesis on a systems level will be extensively discussed in the section titled "Systems regulation of adult neurogenesis."

As stated earlier, NSCs can be isolated and grown *in vitro* from almost all brain areas. Furthermore, Sox-2, nestin, and dividing GFAP-positive cells are scattered throughout the adult brain. One major question is what allows the DG and SVZ to be unique in permitting the generation of new neurons in the adult. Most likely, there is something in the microenvironmental niche that surrounds NSC *in vivo* that permits and/or supports neuronal differentiation and maturation. A detailed study by Seri and colleagues (2004) showed a close association of hippocampal NSCs with the vasculature, a proximity that might be a critical factor in the distribution of trophic support for not only NSCs but also immature neurons (Palmer et al., 2000). Importantly, hippocampal astrocytes also play a pivotal role in making the DG a neurogenic area (Song et al., 2002). While astrocytes from nonneurogenic regions such as the spinal cord appear to inhibit neurogenesis, hippocampal astrocytes appear to be the source of important differentiation and/or stem cell maintenance factors, such as WNT proteins (Lie et al., 2005).

4.41.2.3 Neurogenesis in Nonneurogenic Areas Following Manipulation

Although the DG and SVZ are the only brain regions that undergo continual neurogenesis in the healthy adult, it appears that endogenous NSCs throughout the nervous system maintain the capability to become neurons. When grown *in vitro,* NSCs from nonneurogenic areas can be induced to differentiate into neurons, and when transplanted into a neurogenic area they will differentiate into neurons. The exciting possibility also exists that nonneurogenic brain areas can be manipulated by traumatic insults or other means to induce the production of new neurons. A recent report showed that the production of new neurons in the hypothalamus could be induced by injection of ciliary neurotrophic factor (CTNF), which is critically involved in feeding behavior (Kokoeva et al., 2005). Thus, new neurons in the hypothalamus feeding centers may be involved in regulating the energy balance in rodents. The potential to produce new neurons apparently also holds true for the striatum and cornu ammonis area 1 (CA1) following ischemic stroke and for the cortex following targeted cellular ablation (Abrous et al., 2005). However, the numbers of new neurons produced following strokes, for example, are extremely low, and it is not clear what their functional impact may be. Nevertheless the potential for endogenous repair has sparked high levels of excitement, and future studies will have to determine the feasibility of neuronal repair from endogenous NSCs following brain injury.

4.41.3 Maturation of Adult-Born Granule Cells

4.41.3.1 Molecular Maturation and Identification of Adult-Bom Granule Cells

As described earlier, the availability of robust and reliable methods to label cells and/or their progeny enabled the identification of genes expressed during the maturation of adult-born granule cells (AGCs). Importantly, and in striking contrast to neuronal maturation during embryonic development, all stages of maturation coexist in the adult DG at any given time point. Therefore, every observation has to focus

on a single cell and is a mere snapshot of neuronal development in the adult brain. This heterogeneity of adult neurogenesis has complicated large-scale approaches to identifying specific gene expression patterns of AGCs at a certain stage of maturation. Moreover, the development of new neurons is accompanied by gliogenesis (Kempermann et al., 2004), and if the current assumption of a common precursor for all three neural lineages holds true, the decisive branching point between neuronal and glial development has not yet been identified. Finally, only a subset of the AGCs that are born and are clearly classifiable as neuronal cells eventually becomes stably integrated into the dentate circuitry (Kempermann et al., 2004). However, the work of several laboratories has generated an astoundingly clear picture of the molecular maturation of AGCs (Brandt et al., 2003; Fukuda et al., 2003; Kempermann et al., 2004; Gleiberman et al., 2005). Much of this information has been generated by characterizing the molecular makeup of BrdU-positive cells at different time points after the injection of the thymidine analogue.

The molecular characteristics of adult progenitors were described in the earlier section covering NSCs. **Figure 3** shows examples of several immature neuron–specific markers. The first gene considered to be neuronal is expressed in dividing cells: DCX. DCX is a microtubule-associated protein that is critically involved in neuronal migration during development; mutations in the DCX gene cause lissencephalic malformations in humans. Even though DCX is expressed in acutely dividing cells, expression persists for approximately 3 weeks after BrdU injection. Thus, the DCX-expressing cell population consists of a very heterogeneous population of newborn cells. The functional importance of DCX in AGC maturation remains unclear. Comparable to the timing of DCX expression is the expression of the polysialylated form of the neural cell adhesion molecule (PSA-NCAM) – a protein that appears to be involved in cell migration, axonal fasciculation, and neurite outgrowth. The proneural basic helix-loop-helix transcription factor NeuroD1 is expressed early after cell division and tapers off approximately 7 days after BrdU. NeuroD1 has been found to be critically involved in neuronal fate instruction, particularly for granule cells, as *NeuroD1* −/− mice fail to ever develop a DG. Expression of Prox-1, the mammalian homologue of the transcription factor prospero that is critically involved in cell cycle regulation, appears together with DCX and PSA-NCAM in newborn

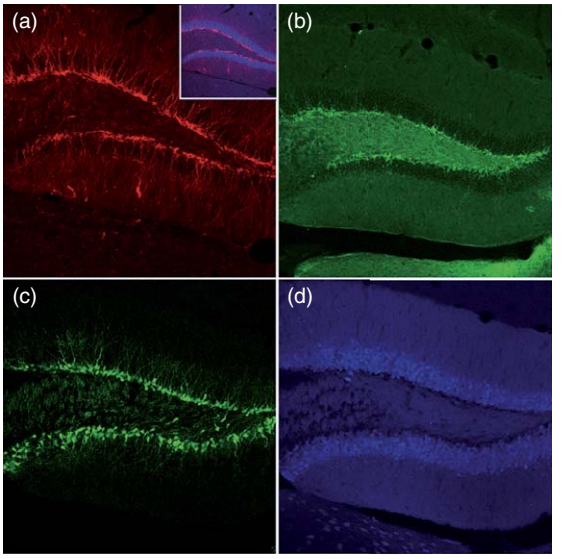

Figure 3 Endogenous and transgenic markers of adult neurogenesis. There are numerous endogenous proteins that are expressed during specific stages of neuronal maturation in the adult dentate gyrus. (a) Doublecortin (DCX) labeling of immature neurons lining the inner granule cell layer (visualized by prox-1 staining, blue in inset). (b) polysialylated neural cell adhesion molecule labeling of immature neurons, which includes staining of axons. (c) The dentate gyrus from a *POMC*-driven green fluorescent protein transgenic mouse (green) that was generated by Overstreet-Wadiche and colleagues. (4) Calbindin, the mature granule cell Ca^{2+}-binding protein, is only expressed in granule cells after 3–4 weeks of maturation.

cells. In contrast to DCX and PSA-NCAM, prox-1 remains expressed throughout the life of a granule cell. This expression pattern is similar to the pan-neuronal protein NeuN, which can be detected as early as 3 days after BrdU injection but remains expressed later on. The expression of the Ca^{2+}-binding protein Calretinin marks the definite exit from cell cycle. Calretinin is replaced approximately 3 weeks later by calbindin, the Ca^{2+}-binding protein of mature granule cells (Kempermann et al., 2004). As described later, the regulation of the survival and integration of AGCs seemingly occurs during many, if not all, stages of neuronal maturation.

4.41.3.2 Electrophysiology of Maturing AGCs

Critical to our understanding of the role adult neurogenesis has in cognition is the functional maturation of these neurons. Increasingly, it appears that these new neurons become functional parts of the DG circuit, passing through several maturation

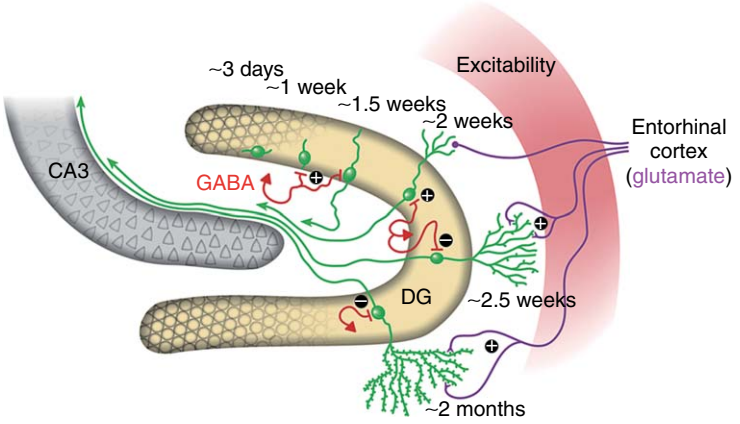

Figure 4 Maturation of newborn neurons. Adult-born granule cells progress through several states before they reach full maturity. Early in maturation, the neurons have limited processes and receive only GABA inputs from local interneurons. By 2 weeks, the neuron's dendrite protrudes into the molecular layer. Soon thereafter, spine formation begins, indicating the onset of glutamatergic input. After several months of maturation, the newborn neuron becomes indistinguishable from other granule cells. The bar labeled "excitability" indicates a period in which immature neurons may be more responsive to the network due to depolarizing GABA, depolarized resting potentials, and increased LTP. Figure from Aimone JB, Wiles J, and Gage FH (2006) Potential role for adult neurogenesis in the encoding of time in new memories. *Nat. Neurosci.* 9: 723–727, with permission.

stages and ultimately developing a cellular physiology indistinguishable from that of embryonic-born neurons (**Figure 4**). This section reviews the maturation process of newborn granule cells.

4.41.3.2.1 Techniques used in characterizing maturation stages of AGCs

Following the characterization of the critical steps leading to the molecular maturation of AGCs, several laboratories have made significant strides in characterizing the physiological maturation of AGCs using several different approaches to labeling and recording from specific ages of new neurons. One approach has been to segregate cells based on location and passive membrane properties (i.e., membrane resistance) and to use post hoc histological and morphological observations to confirm maturation state. While this approach has proven effective at distinguishing between mature and immature neurons, it is difficult to *a priori* identify neurons of a specific age or maturation state. Because early cell division markers such as ^{3}H-Thy and BrdU only label nuclei, studies investigating the maturation of AGCs were limited until the development of more sophisticated labeling techniques using fluorescent genetic markers. Two approaches in particular have boosted research in this area: GFP retrovirus and GFP driven by immature granule cell–specific promoters. By using a retroviral vector that only incorporates itself into dividing neurons to deliver GFP, researchers can identify and observe the morphology of cells that divide at a known time. This process allows the determination of the actual 'age' of the neuron. On the other hand, GFP driven by promoters that specifically label immature neurons or NPCs, such as *Pomc,* allows the identification of a group of cells at a similar maturation state (Overstreet et al., 2004). Importantly, since not all AGCs mature at the same rate, the retrovirus-GFP and cell-specific GFP labeling approaches are not immediately comparable, and care must be taken when interpreting data across experiments.

4.41.3.2.2 Depolarizing GABA input

Using retrovirus labeling, neurons – as defined by the ability to fire an action potential – can be observed as 1 day postinjection (dpi) in slice (Esposito et al., 2005). These early neurons have little distinguishing morphology beyond possibly a few small nonoriented projections. These young neurons lack synaptic inputs, though it appears that even at this young age there is a tonic GABA input, suggesting the presence of nonsynaptic somatic receptors that are sensitive to levels of local interneuron activity.

By around 1 week of age, these neurons begin to take on a neuronal morphology. Although at this age most neurons still lack a robust dendritic arborization, oriented processes extending to the molecular layer can be seen. It is at this stage in development that these

young neurons begin to receive synaptic GABAergic inputs from the local interneuron population. The fact that GABA-releasing axons are the first to contact the young neurons is important for several reasons. First, having GABA synapses preceding glutamate synapses is similar to the order seen during embryonic development. Like in development, this early GABA has a depolarizing impact on the neuron. Second, this depolarizing GABA appears to have long-lasting implications for the neurons' development, suggesting the onset of activity-dependent maturation.

By 2 weeks postinjection, there appears to be a substantial amount of depolarizing GABA input. A similar group of neurons has also been identified by Overstreet-Wadiche and colleagues (2005) by using GFP driven by the *Pomc* promoter. These GABA-only neurons have identifiable dendrites, but they barely extend into the molecular layer with limited branching. Despite not receiving glutamatergic inputs, they can be depolarized by perforant path stimulation, presumably via polysynaptic activation of GABAergic basket cells.

Although 1- to 2-week-old neurons receive depolarizing synaptic inputs, it is not clear at what point they begin to communicate with other neurons. While these GABA-excited neurons are capable of exhibiting action potentials, their spiking is not typical of mature granule cells' bursting patterns. The extent to which GABA can induce action potentials *in vivo* remains to be seen. GFP-labeled axonal processes do not appear in the CA3 until about 11 or 12 dpi and do not reach the CA3/CA2 boundary until 16 dpi.

4.41.3.2.3 Spine formation and the onset of glutamatergic inputs

According to several studies (Esposito et al., 2005; Zhao et al., 2006), spine formation begins just after 2 weeks and continues beyond 1 month of age. This time frame is consistent with electrophysiology studies, which show the onset of weak glutamatergic inputs at around 2 weeks of age (Esposito et al., 2005; Ge et al., 2006). The earliest stages of glutamate inputs are characterized by a high *N*-methyl-D-aspartate (NMDA) dependence; the blockade of NR1 receptors has been shown to kill most immature neurons between 2 and 3 weeks of age (Tashiro et al., 2006). Spine formation is rapid, increasing from about 0.43 spines/μm to 1.95 spines/μm between 21 and 28 dpi.

It is at these early glutamate stages that synaptic plasticity has been best characterized. Numerous studies have observed a difference in long-term potentiation (LTP) between immature and mature DG granule cells. Schmidt-Hieber and colleagues (2004) showed that high-resistance, PSA-NCAM+ neurons (corresponding to between 1 and 3 weeks of age) experienced associative LTP more readily than mature neurons (Schmidt-Hieber et al., 2004). Similarly, Wang et al. (2000) showed, using several paradigms, that medial perforant path stimulation did not induce LTP in mature neurons unless GABA was blocked with bicucilline, whereas immature neurons had robust LTP regardless of GABA blockade (Wang et al., 2000). Consistent with this increased propensity for LTP, Zhao et al. (2006) showed that immature neurons had higher levels of spine motility and a lower proportion of large mushroom spines.

By around 1 month of age, the neurons are similar to fully mature neurons, but not yet identical. Electrophysiologically, there are few significant differences at this stage, though it appears that some differences in plasticity remain when compared to fully mature neurons (van Praag et al., 2002). Morphologically, the dendritic arborizations appear roughly similar, but there are still morphological differences. Overall spine density continues to increase until about 2 months of age, after which the density remains relatively constant. Likewise, spine motility at 28 dpi is still higher than that seen in fully mature neurons. Furthermore, Toni et al. (in press) show that spines on new neurons preferentially form connections on already existing synaptic sites.

4.41.3.2.4 Timeline of projections to CA3

All indications suggest that AGCs project axons to the same neurons to which embryonic and postnatal granule cells project, specifically the CA3 and interneurons in the hilus, although these studies are limited. Markakis and Gage (1999) and Hastings and Gould (1999) both showed that fluorescent retrograde tracers injected into the CA3 colocalized with BrdU cells in the DG (Hastings and Gould, 1999; Markakis and Gage, 1999). Colabeling can be seen in some cells as early as 10 days after BrdU was first administered. This finding is consistent with observations using GFP retrovirus. Zhao et al. (2006) showed that GFP driven by the CAG promoter is present in the axons of immature neurons (Zhao et al., 2006). At 10 dpi, axons are restricted to the hilus, but they reach the CA3/CA2 boundary by 16 dpi.

Although the inputs to AGCs have been well characterized electrophysiologically, the physiology of newborn axonal projections to the CA3 has not

been studied. The mossy fiber projection of dentate granule cells has several unique characteristics, including its sparse topography – one granule cell connects to only about a dozen CA3 pyramidal neurons – and the ability of a single active mossy terminal to fire its downstream target. It will be interesting to see whether the unusual properties of DG mossy fibers are also found in adult-born neurons.

4.41.3.2.5 Regulation of maturation process

Although the phases of AGC maturation mimic those of the embryonic and early postnatal granule cells, the network into which they are maturing is unique in that it is a real-time functioning network. Not surprisingly, the maturation of AGCs is a tightly regulated process at each stage. Increasingly more is being learned about what regulates the way in which these new neurons integrate into the network.

Experience-dependent regulation of maturation appears to begin very soon after differentiation. Ge and colleagues (2006) have demonstrated that 3-dpi neurons receive tonic GABA depolarization several days before the appearance of synapses, and the depolarizing nature of this input is important for further development (Ge et al., 2006). Their data suggest that the depolarizing effects of GABA in very immature neurons are regulated by a tight balance between NKCCl and KCC2 transporters, and the disruption of this balance results in dramatically underdeveloped dendritic arborizations by 2 weeks.

After this GABA-dependent phase of maturation, the neuron appears to enter an NMDA-dependent maturation phase. A study by Tashiro et al. (2006) demonstrated that immature neurons deficient in the NR1 subunit of the NMDA receptor develop normally for the first 2 weeks, but by 3 weeks most are dead. Furthermore, this required NMDA activation is relative to the local network; if NMDA is globally blocked using AP5, the *NR1*−/− neurons survive more successfully.

In summary, the maturation of new neurons appears to take a course similar to that seen during development, but over a more extended time scale. Importantly, at each stage of development, the efficacy of integration appears to be critical for further maturation and ultimate survival. Consistent with this dependence on activity, these neurons appear to be much more responsive to the network, in terms of both synaptic plasticity (spinogenesis, LTP) and basic physiological states (more depolarized resting potentials, higher input resistances, longer membrane time constant, higher E_{GABA}). Their excitable state stands out in contrast to mature granule cells, which are characterized by very low activity due to high levels of tonic inhibition.

4.41.4 Systems Regulation of Adult Neurogenesis

4.41.4.1 Physiological Regulators of Adult Neurogenesis

The amount of progenitor cell division and the subsequent numbers of new neurons that are born in the adult DG are dynamically regulated by a variety of both physiological and pathological factors. The finding that adult neurogenesis is a highly dynamic feature of adult brain plasticity challenged our understanding of how the mature brain responds to its environment and showed that the cellular composition in the adult dentate area is subject to constant change. How adult neurogenesis is regulated and what factors influence the number of adult-generated neurons have been important questions in the field.

The number of new granule cells can be changed by two mechanisms: (1) an increase in progenitor cell proliferation and (2) an increase in newborn neuron survival. Regulators of adult neurogenesis often have an effect on both proliferation and survival, and currently there is no easy way to completely separate these two mechanisms. However, some regulators act more strongly on proliferation than on survival and vice versa. The regulators discussed here and in the next section represent only the major factors that influence adult hippocampal neurogenesis, and the list of manipulations that enhance or abate adult neurogenesis is growing constantly (Ming and Song, 2005). These results are summarized in **Table 1**.

4.41.4.1.1 Natural variation in adult neurogenesis

Without a doubt there is a very strong genetic impact on the number of adult-born neurons. Inbred mice strains can differ 10-fold or more in the number of new granule cells, either by dramatically decreased/elevated levels of cell proliferation or by low/high levels of cell survival, respectively. Using quantitative trait loci analyses of inbred mice strains, several genetic loci have been identified that appear to be critically involved in the regulation of adult neurogenesis (Kempermann et al., 2006). Further studies are designed to narrow the identified loci down to single genes.

Table 1 Regulation of adult neurogenesis

Regulator	*Proliferation*	*Survival*	*Neuronal differentiation*
Physiological Regulators of Adult Neurogenesis			
Genetic background	+/–	+/–	+/–
Enriched environment	?	+	+
Physical exercise	+	no change	+
Learning	no change	+	?
Aging	–	?	–
Dietary restriction	no change	+	?
Neurotransmitters	see text		
Pathological Regulators of Adult Neurogenesis			
Stress	–	no change	?
Seizure activity	+	+ (?)	+
Ischemia	+	+ (?)	+
Irradiation	–	–	–
Neurodegenerative Diseases			
Alzheimer's disease	+/–	?	?/–
Huntington's disease	–	no change	no change
Parkinson's disease	–	?	?
Drugs			
Opiates	–	?	?
Antidepressants	+	+	+
Ethanol	no change/–	–	?

4.41.4.1.2 Environmental enrichment

Genetic background is not alone in having a powerful influence on adult hippocampal neurogenesis. It has been known for a long time that housing laboratory animals in an enriched environment exerts positive effects on the animals' behavior (van Praag et al., 2000), even though the structural correlate of this improvement remained unclear. Kempermann and colleagues found that housing adult mice for several weeks together in a large cage with toys and a changing environment had strong effects on their performance in hippocampus-dependent learning tasks (Kempermann et al., 1997). In addition to improving learning, environmental enrichment doubled the number of new neurons in the DG. This dramatic effect on hippocampal neurogenesis appeared to be mainly due to an increased survival of the new neurons, preventing the apoptotic death of immature neurons. Later studies showed that the effects of environmental enrichment on adult neurogenesis are mediated by VEGF signaling in the dentate area (Cao et al., 2004). Recent work has shown that 2- to 3-week-old neurons appear to be the most affected by an enriched environment, as cells labeled that far ahead of enrichment are substantially more likely to survive and respond to that environment at a later date (Tashiro et al., 2007).

4.41.4.1.3 Physical exercise

After the robust effects of environmental enrichment on adult neurogenesis were discovered, it remained unclear which of the multimodal effects of enrichment on the animals' life was responsible for the enrichment-induced increase in neurogenesis: larger cages may result in more physical activity, and bigger housing groups increase social interaction (to prevent the strong formation of social dominance, all initial experiments were performed using female mice). van Praag and colleagues tried to dissect out those factors and found that the physical activity itself – in contrast to the survival-promoting effect of enrichment – had a very potent effect on progenitor cell division, subsequently leading to an increased net number of newborn neurons (van Praag et al., 1999b). The exercise-induced increase in newborn neurons in the dentate area also correlated with improved performance in learning tasks and facilitated the induction of LTP on perforant path/granule cell layer synapses (van Praag et al., 1999a).

4.41.4.1.4 Learning

Hippocampus-dependent learning itself can increase the number of surviving neurons, even though the survival-increasing effect of learning on immature neurons appears to be restricted to a limited stage of neuronal development (Gould et al., 1999).

Supporting the finding of learning-enhanced neurogenesis are reports that the *in vivo* induction of LTP elevates the number of newborn neurons (Bruel-Jungerman et al., 2006). Recent experiments suggest that this effect on the hippocampal structure may also occur in humans (Draganski et al., 2006). However, it remains unclear whether the observed changes in hippocampal volume are indeed causally related to increased neurogenesis.

4.41.4.1.5 Aging

The age of the animal also has a dramatic influence on the number of adult-generated neurons. According to van Praag et al. (2005), the number of AGCs surviving for a month is estimated to be between 500 and 1000 per week in young adult mice (between 2 and 6 months of age) but drops to about 100 per week in aged animals. The reasons for this strong decrease are both low levels of progenitor cell proliferation and low levels of neuronal differentiation compared to young animals. An elegant study by Cameron and colleagues indicated that corticosteroids play a pivotal role in the age-related decline of adult neurogenesis (Cameron and McKay, 1999). However, it is important to notice that even in old age, both environmental enrichment and physical activity are still powerful tools to increase the number of newborn neurons (Kempermann et al., 2002; van Praag et al., 2005). To date it is still unclear whether decreased neurogenesis in aged animals is caused by an intrinsic progenitor cell failure or by changes in the neurogenic environment, but studies are under way that will address this critical question.

4.41.4.1.6 Neurotransmitters

Most neurotransmitters that have been studied appear to have an influence on the number of new neurons formed, although in most cases it has been technically very difficult to distinguish whether their effects on NPCs are direct or occur through the transmitters' effects on the surrounding network. For example, the direct roles of glutamate and GABA are still unclear. The principal input to the DG is glutamate from the EC. Disruption of the perforant path in the rat results in an increase in cell proliferation, suggesting that excitatory input might inhibit neurogenesis. Similarly, systemic NMDA receptor blockade enhances proliferative activity (Nacher and McEwen, 2006). However, as discussed in the following text, the activation of kainic acid glutamate receptors dramatically increases cell proliferation, and depolarization of progenitor cells appears to facilitate neurogenesis. Given these findings, the simple assumption that glutamatergic excitation generally downregulates neurogenesis might be too simple, especially considering that glutamate is rarely released in the DG without simultaneous release of GABA from interneurons. GABA signaling is not only involved in maturation – as outlined in earlier sections – but is also critically involved in proliferation (Tozuka et al., 2005; Ge et al., 2006). Therefore, it is probable that a delicate balance between excitation and inhibition is responsible for modulating the levels of neurogenesis (Deisseroth et al., 2004).

In addition to the glutamatergic EC input and internal circuitry, the DG receives inputs from a variety of different brain regions carrying a wide range of neurotransmitters. Cholinergic (as well as GABAergic and glutamatergic) fibers from the septum and nucleus basalis terminate throughout the DG, and particularly at the SGZ. Selective lesions of cholinergic neurons in the forebrain revealed a survival-promoting effect of acetylcholine, as lesions increased apoptotic cells' deaths and lowered neurogenesis levels (Cooper-Kuhn et al., 2004). Likewise, there is a strong serotonergic innervation of the SGZ from the raphe nuclei, and gain- and loss-of-function studies showed that release of serotonin strongly increased the numbers of newborn neurons (Gould, 1999). It has also been shown that drugs increasing serotonin levels such as fluoxetine upregulate adult neurogenesis (see following section for more details).

The DG also receives norepinephrine from the locus coereleus and dopamine from the ventral tegmental area. Less is known regarding the overall role of these neurotransmitters in the adult DG, but there are indications that both transmitters influence the levels of adult neurogenesis (Abrous et al., 2005). Furthermore, a wide range of neuropeptides and other molecules are released by axons in the DG, and several of these, including endocannabinoids, NPY, and endogenous opiates, appear to have effects on the proliferation of NPCs.

4.41.4.1.7 Additional regulators of adult neurogenesis

Another strong modulator of adult neurogenesis is dietary restriction, which increases the number of newborn granule cells by increased proliferation (Prolla and Mattson, 2001). Dietary intake may strongly influence the hormonal state, and several hormones have been shown to have a strong effect on the number of newborn neurons in the dentate area. Both positive (e.g., dehydroepiandrosterone,

DHEA) and negative (e.g., corticosterone) hormonal regulators could be identified (Abrous et al., 2005). Another modulator of adult neurogenesis is sleep deprivation. Interestingly, a single night of sleep deprivation appears to increase adult neurogenesis, whereas more chronic forms of sleep restriction had the opposite effect (Guzman-Marin et al., 2005; Grassi Zucconi et al., 2006). It is important to note that the number of physiological modulators is constantly growing, and the list described here is not complete (Abrous et al., 2005).

4.41.4.2 Pathological Regulators of Adult Neurogenesis

The previous section indicated the enormous degree of regulation that adult neurogenesis undergoes under normal conditions. Thus, it is not surprising that a long list of pathological conditions also have an effect on the number of newborn neurons in the DG.

4.41.4.2.1 Stress and depression

One very prominent negative regulator of adult neurogenesis is stress. Pioneering work by Gould and colleagues showed that psychosocial stress leads to a strong decrease in progenitor cell proliferation and new neuron numbers (Abrous et al., 2005). Mechanistically, the negative effects of stress on neurogenesis appear to be largely mediated by corticosteroids; adrenalectomy has been shown to prevent the stress-induced decline in new neuron production in rodents. Importantly, very similar results were obtained using primates (Abrous et al., 2005). The finding that stress has a strong, negative effect on adult neurogenesis lead to the hypothesis that hippocampal neurogenesis may also be critically involved in the disease process of depressive disorders. Interestingly, many human patients suffering from major depression have elevated serum levels of corticosteroids as well as reduced hippocampal volume, as measured by MRI (Sapolsky, 2000). Furthermore, it has been shown that several antidepressive drugs, such as fluoxetine, which belongs to the class of selective serotonin reuptake inhibitors (SSRIs), have a positive impact on adult neurogenesis. In addition, electroconvulsive seizures, which are a powerful clinical tool used to treat certain forms of major depression, increase the number of newborn neurons (Warner-Schmidt and Duman, 2006; and see following). The delay in the effectiveness of a variety of antidepressants for several weeks nicely fits with the idea that new neurons may be partially responsible for the drug's efficacy. In fact, Santarelli and colleagues showed that the inhibition of adult neurogenesis abolished the behavioral effects of fluoxetine on certain aspects of anxiety and stress (Santarelli et al., 2003). The link between adult neurogenesis and emotions will be discussed in more detail in the next section.

4.41.4.2.2 Seizures

Compared to stress and depression, seizure activity has the opposite effect on adult neurogenesis within the hippocampal circuitry. Epileptic discharges lead to a massive increase in cell proliferation and subsequent integration of new neurons into the dentate area (Parent, 2002). The exact mechanisms of how seizures result in increased numbers of new neurons is not fully understood, but it seems that cell death following seizure activity is not a critical factor. Even though the number of new neurons is dramatically upregulated within the first several days following seizures, recent data suggest that, after the initial insult, numbers drop below controls (Hattiangady et al., 2004). Whether this decrease is due to an 'exhaustion' of the progenitor population or to environmental changes in the neurogenic niche remains unclear.

Notably, a significant fraction of seizure-generated neurons either has aberrant basal dendrites (Shapiro and Ribak, 2006) or is ectopically located at the hilar/CA3 border (Parent et al., 1997; Scharfman et al., 2000). These basal dendrites reach deep into the hilus and, as observed with DCX analyses, have immature synapses that appear to integrate into the circuitry. However, little is known about the stability of these aberrant connections and what their functional impact on synaptic transmission through the dentate circuitry might be. Besides having abnormal dendritic processes, many seizure-generated granule cells ectopically migrate into the hilus toward area CA3. Studies by Scharfman provided evidence that these aberrant granule cells show synchronous firing patterns with CA3 pyramidal cells and may initiate recurrent excitation onto granule cells (Scharfman et al., 2000). These abnormal features of seizure-generated granule cells have led to the hypothesis that seizure-induced neurogenesis might be responsible for certain aspects of the epileptogenic disease process (Parent, 2002). However, it may also be true that seizure-induced neurogenesis is instead an attempt by the injured brain to replace lost cells and to rebuild damaged hippocampal structure (Bjorklund and Lindvall, 2000).

4.41.4.2.3 Ischemia

Similar to seizure activity, though less dramatic, ischemic insults to the adult brain increase the number of new neurons (Zhang et al., 2005). Unlike ischemia-induced neurogenesis in the SVZ, where newborn cells appear to migrate toward the lesion and possibly participate in repair function, the cause or value for increased neurogenesis within the hippocampus following ischemia remains unclear. Importantly, short ischemic episodes that do not result in measurable cell death still affect neurogenesis (Zhang et al., 2005). There are certain types of ischemic stroke induction, such as the four-vessel occlusion, that specifically target the hippocampal area in rodents. Experiments from the Nakafuku laboratory showed that poststroke infusion of the growth factors EGF and FGF-2 led to a robust repopulation of the damaged CA1 area with functional pyramidal neurons (Nakatomi et al., 2002), suggesting that endogenous progenitors are capable of repopulating substantial lesions in the adult brain. Further studies will have to determine the feasibility of this approach and what the actual benefit of endogenous neuronal replacement in injured subjects is.

4.41.4.2.4 Irradiation and inflammation

Brain irradiation is often used clinically to control tumorous growth within the CNS, especially in children. Palmer and colleagues showed that brain irradiation almost completely abolished cell proliferation within the SGZ, resulting in a lasting decrease in neurogenesis (Monje et al., 2002). Interestingly, the depletion of neurogenesis following irradiation was not entirely due to the radiation effect alone. Rather, the inflammatory response that accompanies irradiation strongly decreases the number of new neurons, and anti-inflammatory treatment augmented neurogenesis in radiated animals (Monje et al., 2003). The detrimental role of inflammation regarding neurogenesis was confirmed by studies showing that the decrease in neurogenesis following endotoxin-induced neuroinflammation was blocked by anti-inflammatory treatment (Hagberg and Mallard, 2005).

4.41.4.2.5 Neurodegenerative diseases and drugs

Clearly, the above-mentioned pathological regulators are not a complete list of disease states that influence the number of newborn neurons. If anything, people have sought unsuccessfully for a disease or pathological state that does not affect adult neurogenesis. There are reports that neurogenesis appears to be disturbed in animal models of Alzheimer's disease and in specimens from human Alzheimer's patients (Jin et al., 2004a,b). However, these findings remain controversial (Donovan et al., 2006). In addition, several other neurodegenerative disorders, such as Huntington's and Parkinson's diseases, have been shown to affect the number of newborn neurons in the dentate area (Abrous et al., 2005). The number of new neurons is also greatly influenced by abuse of substances such as ethanol, opioids, cannabionoids, barbiturates, nicotine, and benzodiazepines (Abrous et al., 2005).

Adult hippocampal neurogenesis is highly responsive and vulnerable to a variety of pathologies affecting the CNS. In most cases, however, the underlying cause or consequence of altered neurogenesis levels in the disease process of many neurological disorders remains unclear and may simply be epiphenomenological. Nevertheless, the finding that neurogenesis in the adult hippocampus is misregulated in many diseases has opened up several new approaches to understanding and ultimately curing human neurological disease.

4.41.5 Function of Neurogenesis

Two theories of hippocampal function have developed in parallel over the past 50 years, and both have been considered in the search for possible roles for neurogenesis. The first, originating in the 1950s from human amnesia studies, is that the hippocampus plays a critical role in the formation of episodic memories. Medial temporal lobe patients – the most famous being H.M. – display a robust, temporally graded retrograde amnesia (Milner et al., 1998). Hippocampal lesion patients, as well as monkeys and rodents, display difficulties in the recall of declarative memories (Squire et al., 2004). The second theory of hippocampal function originated in the 1970s from studies showing that hippocampal neurons in rats have strong place fields (O'Keefe and Conway, 1978; Jung and McNaughton, 1993; Best et al., 2001). This spatial map theory has been very well supported by the use of *in vivo* recordings of hippocampal and entorhinal cortex neurons. Despite these distinct theories, there is increasingly a consensus that the hippocampus has roles in both types of processing.

Although the precise function of granule cell neurogenesis in cognition is still not clear, computational and behavioral studies have led to more sophisticated ideas of what the addition of new neurons would mean to this well-studied circuit. This section describes the progress of both computational approaches and knock-down behavioral experiments.

4.41.5.1 Hippocampal Circuit Function and the Role of the DG

One reason that the hippocampus has been one of the more well-studied regions of the mammalian brain over the past century has been its seemingly simple neural circuitry. Although an oversimplification, the idea of a trisynaptic excitatory circuit (DG → CA3 → CA1) has proven very accessible to both electrophysiology and theoretical work. In particular, the recurrent connectivity of the CA3 region and the presence of strong place fields in CA1 have led to numerous theoretical ideas about the different hippocampal subregions.

Despite the lack of a consensus on the function of the hippocampus as a whole and for its subregions, a general agreement exists that the DG is most probably responsible for separating inputs presented to the hippocampus. This agreement has been based on the anatomy of the DG (Patton and McNaughton, 1995) and on *in vivo* electrophysiology studies (Jung and McNaughton, 1993), behavioral lesion studies (Kesner et al., 2004), and computational models (Rolls and Kesner, 2006). The region receives a very divergent projection from the EC (~200,000 cells project to >1 million granule cells in the rat) while having a very sparse projection onto the CA3. Within the network, granule cells receive strong tonic inhibition from local interneurons, and they are rarely active during behavior. However, when they are active, they have a potent effect on downstream CA3 neurons (Henze et al., 2002). These properties were used, and in some cases even predicted, by computational models describing the computational need for the DG in the production of sparse codes to facilitate memory formation (Rolls and Kesner, 2006).

4.41.5.2 Theoretical Functions of Adult Neurogenesis

Like most theories of hippocampal computation, most neurogenesis theories and network models have focused on a role in memory formation. However, as mentioned before, the DG's presumed role in pattern separation is similar in both mnemonic and spatial processing, suggesting that several of these conclusions are relevant for both general hippocampal functions.

Early theoretical and computational considerations of adult neurogenesis focused on the idea that neurogenesis might be a real-time regulator of memory capacity (Schinder and Gage, 2004). Indeed, several neural network studies demonstrated exactly this. Chambers et al. (2004) and Deisseroth et al. (2004) showed that a simple, three-layer neural network experiencing cell death and neurogenesis is capable of learning more information than a fixed-layer network. Although these models were only conceptual models of hippocampal circuitry and did not explicitly investigate pattern separation, their conclusions are still relevant to this theory of DG function. By replacing neurons in an input layer, they showed that the number of possible states of the output layer increases considerably, indicating increased memory capacity regardless of where the storage occurs.

Becker (2005) was one of the first to consider the effects of neurogenesis in a full hippocampal model and to limit the role of the DG to the encoding phase of learning, a hypothesis that has been the developing consensus in the hippocampal literature (Becker, 2005). The key result of this model was that DG size directly affects the storage capacity of the hippocampus, supporting the idea that neurogenesis facilitates the formation of new memories by permitting more network states. However, contrary to earlier models that involved the DG in the retrieval phase, Becker's model suggests that neural turnover actually reduces possible interference between memory traces in the CA3. The idea that neurogenesis keeps old and new memories separated was examined in detail by the network model of Wiskott et al. (2006). The Wiskott model suggests that interference between memories in the hippocampus is reduced by only permitting new neurons to adapt to new memories, consistent with physiological data showing increased LTP in immature neurons (Wiskott et al., 2006).

Most of these computational theories of neurogenesis have sought a role for new neurons in the framework of functions already considered for the hippocampus. Another view proposes that neurogenesis may have a function that is distinct from those traditionally assigned to the DG. The rationale behind this view is that most current hippocampal theories were derived without regard for adult neurogenesis.

For example, Aimone et al. (2006) described how increased excitability in immature neurons may contribute to the formation of temporal linkages between memories, a function that had not previously been attributed to the DG.

It is likely that any additional functions that neurogenesis contributes (beyond simply improving the DG's function) would be hippocampus dependent as well, just as linking memories in time would still be categorized as a type of declarative memory. However, without the benefit of the existing paradigms used to test memory, such novel functions will likely be difficult to investigate and will present additional challenges to behavioral scientists seeking evidence of a neurogenesis function in rodents. Despite the potential difficulties, it would not be surprising for neurogenesis to have several effects on cognition, as it increasingly appears that the hippocampus serves several functions in the brain.

4.41.5.3 Experimental Evidence for Functional Significance of Adult Neurogenesis

As outlined above, the hippocampus is critically involved in a variety of processes underlying certain forms of learning and memory. New neurons in the dentate area represent only a very small part of the hippocampal structure. As a result, a conclusive answer regarding the function of adult-born neurons is not easy to obtain experimentally. The difficulty in analyzing the functional significance of adult neurogenesis begins in choosing the right test. As mentioned above, the exact function of new neurons might be best appreciated in tests specifically designed to challenge new neurons. Furthermore, there might be species-dependent differences in the use for neurogenesis. Nevertheless, it appears to be mandatory to characterize a potential contribution of new neurons in standard hippocampus-dependent tasks to reach the goal of designing adult neurogenesis–specific tests.

The gold standard of testing hippocampal function remains the Morris water maze (MWM), which is highly sensitive to hippocampal lesions. Many of the behavioral data discussed below are indeed derived from using this test. Recent studies have provided evidence that newborn neurons functionally respond to the acquisition and recall phase during water maze testing (Jessberger and Kempermann, 2003; Kee et al., 2007). However, it is crucial to keep in mind that even the very standardized MWM tasks have an endless list of variations that may utilize newborn neurons differently. The following section will discuss correlational evidence and emerging causal evidence for a role for adult neurogenesis in hippocampal function.

4.41.5.3.1 Correlational evidence

Because the number of neurons born in the adult DG is highly dynamic, an extensive number of studies have been conducted correlating adult neurogenesis with hippocampus-dependent behavior. Kempermann and colleagues used a large set of recombinant inbred (RI) mice and found that the number of new neurons correlates with certain aspects of the MWM (Kempermann and Gage, 2002). Even though the findings using RI mice are still correlational, they are especially valuable because the number of strains analyzed was very large. Reduced latencies for the RI mice to reach the hidden platform in the water maze compared to standard housed control animals were also found when the net number of newborn granule cells was increased with environmental enrichment or physical exercise in animals with identical genetic backgrounds (Kempermann et al., 1997; van Praag et al., 1999a). The same was true when aged animals that show an age-dependent decline in adult neurogenesis were housed in the enriched environment or had access to a running wheel: both numbers of newborn neurons and performance in the Morris water maze were enhanced (Kempermann et al., 2002; van Praag et al., 2005). However, it is obvious that alterations induced by enrichment or physical exercise may not be exclusive to adult neurogenesis. Thus, additional changes within the hippocampal formation that are different from increased neurogenesis may also be partially responsible for the observed behavioral effects.

In addition to these positive correlations, there are also several studies that showed a relationship between low levels of neurogenesis and poor performance in hippocampus-dependent learning tasks. The low levels of neurogenesis in aged animals showing an age-related memory decline correlated well with the performance in the MWM (Zyzak et al., 1995). Furthermore, the age-dependent decline in neurogenesis was also associated with high levels of serum corticosteroids, confirming a potential mechanism explaining how aging may affect neurogenesis (Montaron et al., 2006).

Correlations between neurogenesis and performance in hippocampus-dependent memory tasks were also found in a variety of transgenic mice. The genetic deletion of methyl-CpG binding protein results in low levels of neurogenesis and is also associated with impaired MWM performance (Zhao

et al., 2003). The same is true for NT-3 mutant mice and neuropeptide Y mutant mice, both showing reduced numbers of new neurons in the adult hippocampus and impaired learning and memory (Howell et al., 2005). Using a conditional knock-out of the *presenelin* gene, Tsien and his laboratory found decreased numbers of newborn neurons, whereas the acquisition of hippocampus-dependent learning was normal (Feng et al., 2001). However, the mutant animals showed apparent deficits in the clearance of older memories, suggesting that new neurons might be important not only for the acquisition but also for the clearance of memory traces (Feng et al., 2001).

4.41.5.3.2 'Causal' evidence

The correlative nature of the above-mentioned studies implies several caveats regarding the specificity of the manipulation of neurogenesis or genetic mutations. Therefore, current endeavors in the field aim to specifically knock down hippocampal neurogenesis without affecting other neural structures. To date, there is no widely accepted technical approach that is devoid of any unwanted side effects. This section discusses current strategies as well as the advantages and potential disadvantages of three experimental approaches: cytostatic drugs, irradiation, and molecular knock-downs.

An early study that was designed to reduce neurogenesis and analyze its impact on hippocampus-dependent learning was a report by Shors and colleagues (Shors et al., 2001). In this study, the cytostatic drug toxin methylazoxymethanol acetate (MAM) was used to block cell division and subsequent neurogenesis in the adult rat DG. Rats treated with MAM were found to be impaired in a trace eyeblink-conditioning paradigm that was associated with a decrease in dentate synaptic plasticity. When the treatment was discontinued and neurogenesis was allowed to recover, performance in the trace eyeblink-conditioning was normal. Similar results were obtained when testing the animals in a trace fear-conditioning test, whereas other hippocampal memory tasks, such as the MWM and contextual fear conditioning, appeared to be unaffected by MAM treatment (Shors et al., 2002). MAM was also found to block the beneficial effects of environmental enrichment in an object recognition task (Bruel-Jungerman et al., 2005). Notably, in each of these experiments MAM was injected systemically and potentially resulted in secondary effects on the animals' health that might account for some of the observed behavioral deficits (Dupret et al., 2005). Furthermore, MAM has been shown to interfere with protein synthesis, a crucial requirement for long-term memory (Grab et al., 1979).

Another approach to knock-down cell proliferation has been to use X-ray irradiation, which results in a robust and lasting reduction of neurogenesis in the adult brain (Parent et al., 1999; Monje et al., 2002). Whole-brain irradiation of adult rats left the acquisition phase of the MWM unaffected but impaired the long-term retention (over 14 days after the last training trial) of the platform location, which is suggestive of a functional role of new neurons in spatial memory (Snyder et al., 2005). Furthermore, the performance in a non-matching-to-sample (NMTS) task was impaired when the delays between sample and test trials were relatively long (Winocur et al., 2006). In contrast to MAM-treated animals (Shors et al., 2002), irradiation also led to memory deficits in contextual fear conditioning (Winocur et al., 2006). Studies by the Hen laboratory have selectively targeted the hippocampus instead of using whole-brain irradiation to determine the contribution of newborn neurons in cognitive improvement following environmental enrichment. Irradiated mice showed no impairment in the water maze, and enriched housing was effective at improving the acquisition of the hidden platform location, even in the absence of new neurons (Meshi et al., 2006). These findings indicate that certain aspects of the beneficial effects of enrichment may not depend on adult neurogenesis.

As outlined in an earlier section, adult neurogenesis might be also associated with anxiety and depression, and targeted irradiation of the hippocampus was used to address the relationship between new neurons and antidepressant treatment. Indeed, intact hippocampal neurogenesis appears to be required for the effectiveness of the antidepressant fluoxetine (Santarelli et al., 2003). However, it seems that neurogenesis is not a critical component *per se* in emotional control, because the anxiolytic effects of environmental enrichment were completely unaffected by the ablation of new neurons (Meshi et al., 2006). Despite the robust and complete ablation of new neurons in the adult hippocampus following irradiation, this approach also implies some unwanted side effects, such as ablation of all dividing cells (including glial progenitors outside the dentate gyrus), inflammation, and potential radiation-induced damage of mature neurons.

The existing causal evidence regarding a functional role for new neurons in hippocampal function allows one to speculate that newborn neurons are

required for certain aspects of learning and memory and might also be involved in emotional behavior. However, the available data remain inconclusive. The hope for identifying the functional significance of adult neurogenesis unambiguously lies in the development of specific behavioral tests and new strategies to knock down neurogenesis as specifically and gently as possible. These new strategies will likely include the use of neural progenitor and immature neuron-specific promoters to drive the expression of suicide genes and the virus-mediated inhibition (or enhancement) of adult neurogenesis.

4.41.6 Conclusions

The discovery of ongoing neurogenesis throughout adulthood has undoubtedly challenged our understanding of neuronal development and adult hippocampal function. Even though our understanding of fate instruction, neuronal maturation, and integration is quickly growing, several key questions remain unanswered. From a cellular and molecular standpoint, it will be very important to understand the *in vivo* potency of NSCs and why neurogenesis only occurs in two restricted areas of the adult brain under normal conditions. Furthermore, little is known about which signaling pathways are involved in the extension and pathfinding of axonal and dendritic processes arising from newborn neurons.

Finally, the ultimate challenge will be to truly decode the functional role of adult neurogenesis. Defining that role might not only fundamentally change current concepts regarding hippocampal function but could also help us to understand and eventually improve treatment of human neurological disease.

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4.42 Epigenetics – Chromatin Structure and Rett Syndrome

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4.42.1 Introduction

Much scientific effort over the last three decades has focused on understanding the molecular basis of memory formation. A great deal of progress has been made, and we have a very comprehensive understanding of the molecules and signaling pathways engaged during the early stages of memory formation. Despite the wealth of knowledge that exists about the molecular basis for memory formation, we have an incipient understanding of how a memory persists in the brain for the lifetime of an organism. For instance, what molecules are used to actually store the memory of your first kiss, or your first car accident?

A major question that will drive the next three decades of research into the molecular mechanisms of information storage in the brain is what are the molecular substrates for ‘lifetime’ memory storage? This question is particularly intriguing when one considers that nearly every molecule in the brain has a lifetime that is measured in minutes to hours. It has been estimated that the molecular composition of the brain is renewed every 2 months. How can information persist in the face of constant molecular renewal?

The answer to the question of how information is stored in the nervous system for the lifetime of an organism might not lie in the classic mechanisms identified as important for information storage in the brain thus far. Every cell in a metazoan must ‘remember’ at least one complex piece of information for the lifetime of the organism: cellular phenotype.

Early in development, stem cells are induced to differentiate into phenotypically distinct tissues. Once differentiated, the memory for cellular phenotype must endure in the face of molecular turnover and even mitosis. Is it possible that the nervous system has co-opted this ancient and evolutionarily conserved form of cellular memory for use in lifelong storage of memory?

Cell phenotype is due, in large part, to epigenetic marking of the genome. Recent studies have demonstrated that epigenetic gene regulation has a critical role in neuronal cell fate specification, synaptic development, and synaptic function (reviewed in Hsieh and Gage, 2004, 2005). These epigenetic marks induce lifelong changes in gene expression that reside in the cell and determine its phenotype. Epigenetics and its associated terminology have several different connotations, and specific terms need to be defined before we can discuss them in detail. We define the genome as a complete set of haploid DNA and the functional units that it encodes. In the nucleus, DNA exists as a highly compressed structure known as chromatin. The epigenome is the sum of both chromatin structure and the pattern of DNA methylation, which is the result of an interaction between the genome and the environment. There are three commonly used definitions for the term 'epigenetic' in the literature currently.

The broadest definition of epigenetics includes the transmission and perpetuation of information through meiosis or mitosis that is not based on the sequence of DNA. This process is not restricted to DNA-based transmission and can also be protein-based. This definition is broadly used in the yeast literature, wherein phenotypes that can be inherited by daughter cells are perpetuated past cell division using protein-based mechanisms (Si et al., 2003a,b; Pray, 2004). A second definition of epigenetics has arisen from the field of developmental biology that posits some meiotically and mitotically heritable changes in *gene expression* are not coded in the DNA sequence itself. The altered patterns of gene expression can occur through several mechanisms that are based on DNA, RNA, or proteins (see below) (Egger et al., 2004). A third definition of epigenetics is the mechanism for stable maintenance of gene expression that involves physically 'marking' DNA or its associated proteins, which allow genotypically identical cells to be phenotypically distinct. The molecular and physical basis for this type of change in DNA or chromatin structure is the focus of this review (Rakyan et al., 2001). By this definition, the regulation of chromatin structure is equivalent to epigenetics.

Epigenetics is an attractive candidate molecular mechanism for lifetime storage of memory. DNA and chromatin are relatively constant molecular substrates in neurons and most other cell types. DNA and chromatin are not continually degraded and resynthesized as all other molecular constituents of a neuron are. Thus, the half-life of epigenetic marks is not affected by the lifetime of the molecule. Therefore, epigenetic marks are a more direct reflection of the ongoing state of the neuron, or any other cell (**Figure 1**(**a**)). This positions chromatin as an ideal molecular substrate for long-term signal integration and information storage (**Figure 1**(**a**)).

4.42.2 Mechanisms of Epigenetic Marking

There are several mechanisms whereby epigenetic marks can be placed on chromatin. Each mark has been associated with distinct effects on transcription and chromatin structure. We will briefly review several of the most commonly studied epigenetic marks.

4.42.2.1 Epigenetic Marking of Histones

Histones are highly basic proteins that comprise the major protein component of chromatin. Histones are some of the most evolutionarily conserved proteins but among the most variable with respect to posttranslational modifications, which include acetylation, phosphorylation, methylation, ubiquitination, and sumoylation (reviewed in Thiagalingam et al., 2003). One function of histones is structural; DNA is tightly packaged into chromatin through interactions with histone octamers, referred to as nucleosomes. Interaction between histones and DNA is mediated in part by the N-terminal tail of histone proteins. The primary interface between histone and DNA is believed to be an electrostatic interaction between positively charged lysine residues within the N-terminal tails of histones and the negatively charged sugar–phosphate backbone of DNA. Interestingly, structural studies indicate that the N-terminal tails of histones protrude beyond the chromosomes (Luger et al., 1997), where they could potentially serve as signal integration platforms. Posttranslational modification of

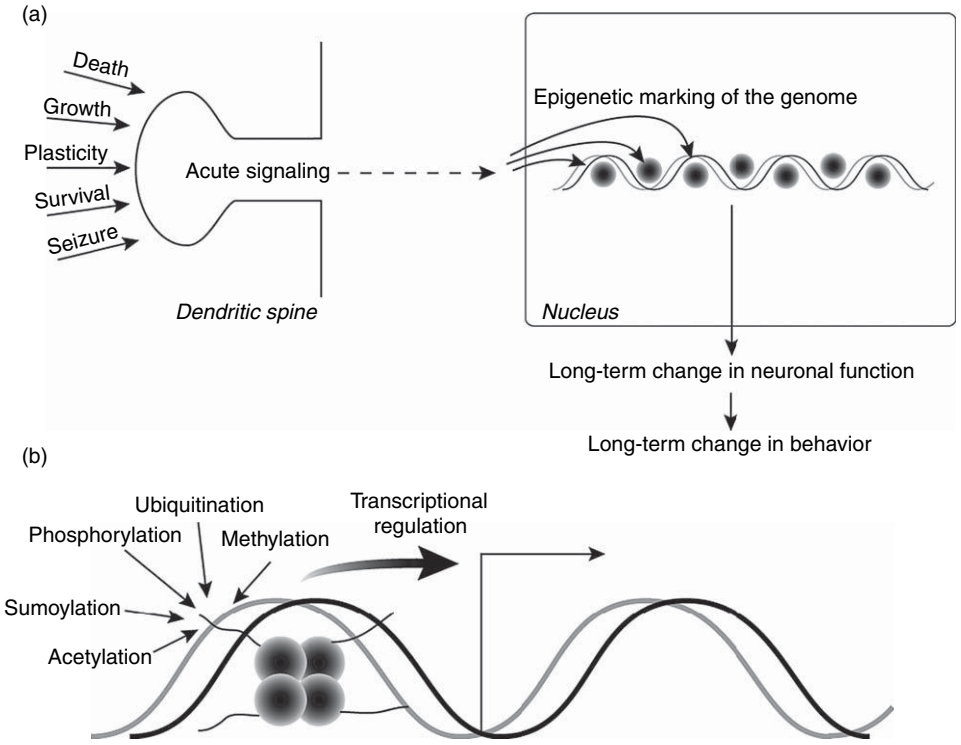

Figure 1 Chromatin as a substrate for signal integration. We propose that epigenetic marking of the genome represents a form of cellular memory utilized by neurons to subserve information storage. (a) Dendritic spines receive several distinct extracellular signals. These signals activate a variety of intracellular signaling cascades that ultimately lead to epigenetic marking of the genome. The half-life of these epigenetic marks is much longer than traditional posttranslational modifications, and so their effects on gene expression persist for much longer periods of time. Therefore, as a consequence of epigenetically modifying the genome, neuronal function and ultimately behavior of the animal are modified for very long periods of time. (b) Histone tails and cytosine residues of DNA can receive a variety of distinct epigenetic marks. Each mark has a different effect on transcription. The functional consequence of epigenetically marking the genome is ultimately precise modulation of transcription.

histones, either on their N-terminal tail or within the globular domain (van Leeuwen et al., 2002), represents one mechanism for epigenetic marking of the genome. Current hypotheses posit that post-translational modifications along the N-terminal tails are combined in a 'histone code' that ultimately directs the activity of numerous transcription factors, cofactors, and the transcriptional machinery in general (Strahl and Allis, 2000). The histone code is the specific pattern of posttranslational modifications of a given histone octamer in chromatin (**Figure 1(b)**). This code, or pattern, is read out as an influence on the specific level of expression of the associated gene(s). We have chosen to focus on N-terminal–directed posttranslation modifications because of the rapidly growing literature describing these different modifications, their associated effects on transcription, and their interdependence.

4.42.2.2 Histone Acetylation

Several specific sites of posttranslational modification exist within the N-terminal tails of histone proteins, and modifications of these sites can modulate the overall structure of chromatin. Currently, five post-translational modifications of histone tails have been characterized: acetylation, methylation, ubiquitination, sumoylation, and phosphorylation. Acetylation is the best characterized of the posttranslational modifications on histones to date. Acetylation of histones occurs on lysine residues, effectively neutralizing their positive charge and interfering with the electrostatic interaction between histone and nucleotide. Acetylation is catalyzed by histone acetyltransferases (HATs), which transfer an acetyl group from acetyl-CoA to the ε-NH^+ group of a lysine residue within a histone (Tanner et al., 1999, 2000a,b; Lau et al., 2000). Histone acetylation is reversed via the action of histone deacetylases (HDACs).

4.42.2.3 Histone Methylation

Histone methylation is another histone-directed epigenetic tag. Like acetylation, methylation of histones occurs on ε-NH^+ groups of lysine residues, and it is mediated by histone methyltransferases (HMTs). Unlike acetylation, lysines can accept up to three methyl groups, and methylation does not affect the positive charge of lysine residues. Thus, methylation of histones on lysine residues does not interfere with the electrostatic histone–DNA interaction. Methylation of histones can also occur on arginine residues, catalyzed by protein arginine methyltransferases (PRMTs). Arginines can be either mono- or dimethylated on their guanidine nitrogen.

4.42.2.4 Histone Ubiquitination

Ubiquitination of histones has only recently begun to be characterized in detail. Ubiquitin, a protein with 76 amino acids that is named for its ubiquitous distribution in all cell types and high degree of conservation across species, is a posttranslational modification usually used as a signal for degradation by the proteasome when substrates are polyubiquitinated (Pickart, 2001). Interestingly, most histones appear to be monoubiquitinated (reviewed in Hicke, 2001). Monoubiquitination has been shown to be involved in a range of cellular functions distinct from proteasome-dependent proteolysis (reviewed in Pickart, 2001; Murphey and Godenschwege, 2002). Histones, like other proteins, are ubiquitinated through attachment of a ubiquitin to the ε-NH^+ group of a lysine (Nickel and Davie, 1989). Ubiquitination of histones H2A, H2B, H3, and H1 has been observed (Goldknopf et al., 1975; West and Bonner, 1980; Chen et al., 1998; Pham and Sauer, 2000). Although sites of ubiquitination have been identified on histones, the function of histone ubiquitination remains unclear.

4.42.2.5 Histone Sumoylation

There are several ubiquitin-like posttranslational modifications that proteins can undergo. One example is the small ubiquitin-related modifier (SUMO) (Melchior, 2000; Hay, 2001; Johnson and Gupta, 2001). SUMO modifications are attached to proteins in a manner similar to the mechanisms involved in ubiquitination. Although SUMO is related to ubiquitination, sumoylation of proteins does not result in their degradation, suggesting that sumoylation of proteins has relevance in other signaling cascades. Initial studies suggested that histone H4 can be sumoylated, and that sumoylation of histone H4 is associated with transcriptional repression (Shiio and Eisenman, 2003). In yeast, the SUMO-conjugating enzyme Hus5 is highly enriched in heterochromatic regions, and knockout of SUMO results in loss of heterochromatic silencing, providing direct evidence that, at least in simple eukaryotes, SUMO modification of chromatin results in transcriptional silencing (Shin et al., 2005).

4.42.2.6 Histone Phosphorylation

Phosphorylation of histones H1 and H3 was first discovered in the context of chromosome condensation during mitosis (Bradbury et al., 1973; Gurley et al., 1974). Phosphorylation of histone H3 has since been associated with activation of mitogenic signaling pathways (Mahadevan et al., 1991). Phosphorylation of serine 10 on H3 is mediated by Rsk2, Msk1, and the aurora kinase family member Ipl1 (Sassone-Corsi et al., 1999; Thomson et al., 1999; Hsu et al., 2000; Di Agostino et al., 2002). Recent evidence also implicates aurora kinases in the phosphorylation of serine 28 in histone H3 (Goto et al., 2002). As with other proteins, phosphatases are responsible for catalyzing the removal of phosphate groups from histones (Mahadevan et al., 1991; Ajiro et al., 1996). To date, the phosphatases PP1 and PP2A have been shown to regulate levels of phosphorylation on H3 (Hsu et al., 2000; Nowak et al., 2003).

4.42.2.7 Other Histone Modifications

While a great deal of attention has been given to the N-termini of the histones, there is increasing evidence suggesting that other histone regions are targeted for modulation. For example, dot1p has been shown to methylate histone H3 on lysine 79, a residue that lies within the globular domain (van Leeuwen et al., 2002). Additionally, higher-order folding of chromatin is also undoubtedly involved in the regulation of gene expression. In this regard, there is increasing evidence that the linker histone H1 plays a role in modulation of chromatin structure (Brown, 2003).

4.42.2.8 DNA (Cytosine-5) Methylation

In addition to posttranslational modification of histones, DNA itself can be directly modified via methylation. Methylation of DNA is catalyzed by a

class of enzymes known as DNA (cytosine-5) methyltransferases (DNMTs) (Bestor et al., 1988; Yen et al., 1992; Okano et al., 1998; Lyko et al., 1999; Okano et al., 1999). DNMTs transfer methyl groups to cytosine (C) residues within a continuous stretch of DNA, specifically at the 5-position of the pyrimidine ring (Chen et al., 1991). Not all cytosines can be methylated; usually cytosines must be immediately followed by a guanine (G) to be methylated (Bird, 1978; Cedar et al., 1979). These 'CpG' dinucleotide sequences are highly underrepresented in the genome relative to what would be predicted by random chance; however, about 70% of the existing CpG dinucleotides are methylated (Cooper and Krawczak, 1989). The rest of the normally unmethylated CpG dinucleotides occur in small clusters, known as 'CpG islands' (Bird, 1986).

DNA methylation leads to marked changes in the structure of chromatin that ultimately result in significant downregulation of transcription. It can directly interfere with the ability of transcription factors to bind to regulatory elements. The transcription factor Ets-1 and the boundary element CCCTC binding factor (CTCF) can efficiently bind to nonmethylated, but not methylated DNA (Bell and Felsenfeld, 2000; Maier et al., 2003). Moreover, several proteins recognize and bind to methylated CpG residues independent of DNA sequence. The five proteins that are known to bind to methylated CpGs are MeCP2, MBD1, MBD2, MBD4, and Kaiso (Hendrich and Bird, 1998; Prokhortchouk et al., 2001). These proteins mediate transcriptional repression by recruiting chromatin remodeling enzymes. For example, MeCP2 directly associates with the transcriptional corepressor Sin3A and HDAC (Jones et al., 1998; Nan et al., 1998).

4.42.2.9 Epigenetic Modulation of Transcription

Several hypotheses have been put forth to explain how the many modifications made on histone proteins are interpreted to extend the information potential of the genetic (DNA) code. The most popular of these hypotheses is the histone code initially described by B. M. Turner (1993) and more recently elaborated and popularized by C. D. Allis and colleagues (Strahl and Allis, 2000; Jenuwein and Allis, 2001). Distinct histone posttranslational modifications can generate synergistic or antagonistic interaction sites for transcription factors and associated chromatin remodeling enzyme complexes. The combinatorial nature of these histone modifications may be thought of as a histone code that extends the information potential of the genetic code (Jenuwein and Allis, 2001). However, a very different hypothesis suggests that it is not combinatorial complexity, but rather a simple net charge effect. Genetic experiments, microarray analysis, and hierarchical cluster analysis were used to demonstrate that the interchangeability of acetylation sites on histone H4 provides support for a charge neutralization model (Dion et al., 2005; reviewed in Henikoff, 2005). Lastly, histone modifications have been related to signal-transducing modifications, and histone modifications provide switch-like properties and ensure robustness of the signal (Schreiber and Bernstein, 2002). This idea has been termed the signaling network model. These three hypotheses for how histone modifications direct transcriptional activation are not mutually exclusive, and thus, it will be interesting to see how future studies into chromatin regulation and information storage regulate cell fate, cellular memory, and as we propose below, long-term memory storage at the behavioral level.

4.42.3 Epigenetic Mechanisms in Synaptic Plasticity

Synaptic plasticity, defined as the activity-dependent change in synaptic strength, is currently viewed as a widely accepted hypothesis to explain the underlying cellular mechanisms of memory processes. The discovery of two forms of synaptic plasticity, long-term facilitation in *Aplysia,* where brief exposure to the neuromodulator serotonin induces a persistent enhancement of neurotransmitter release (Kandel and Tauc, 1964; Brunelli et al., 1976), and long-term potentiation (LTP), a form of synaptic plasticity whereby high-frequency stimulation of a neuronal pathway induces long-lasting increases in synaptic efficacy (*See* Chapter 4.16) (Bliss and Lomo, 1973), sparked decades of research into the role of synaptic plasticity in learning and memory. A recent review by Martin and Morris (2002) elegantly discusses the validity of synaptic plasticity as a cellular mechanism for memory formation. In the previous section of this article, we introduced the intriguing idea that epigenetic regulation of transcription may provide a mechanism for cellular memory in a neuron. This cellular memory may be involved in maintaining the long-lasting neuronal changes required for long-term synaptic plasticity changes. In this next section, we

discuss the recent evidence that epigenetic regulation of transcription may be required for certain forms of long-lasting synaptic plasticity.

4.42.3.1 Transcription and Chromatin Structure

Transcriptional activation is required for certain long-lasting forms of synaptic plasticity and memory storage (Nguyen et al., 1994; Korzus, 2003). Activation of transcription factors in neurons can occur via several mechanisms. In relation to activity-dependent synaptic plasticity and memory formation, it is generally accepted that increased neuronal activity engages a wide variety of intracellular signaling cascades including PKC, PKA, Ca^{++}, PI3-K, and ERK (*See* Chapters 4.23, 4.25) (Adams and Sweatt, 2002). Once engaged, these signaling pathways activate several distinct transcription factors, ultimately leading to the expression of gene products required for maintenance of synaptic plasticity and consolidation of long-term memory. For many actively transcribed genes, it is necessary to overcome the repressive chromatin structure surrounding the gene, especially in the promoter region. Genomic DNA is tightly packaged into chromatin, the major protein component of which is composed of nucleosomes that are octamers of core histone proteins. DNA is wrapped around the nucleosomes (~146 bp) and is then subjected to higher-order folding, resulting in a chromatin structure that is highly inhibitory to transcription. As described earlier, histone acetylation has been shown to alter chromatin structure (Norton et al., 1989), which can result in increased accessibility for transcriptional regulatory proteins to chromatin templates (Vettese-Dadey et al., 1996). The identification of transcriptional cofactors with HAT and HDAC activity has strengthened the relationship between histone acetylation and gene expression. Recently, several studies examining the role of HAT and HDAC enzymes in long-term synaptic plasticity demonstrated that there is a correlation between changes in synaptic plasticity and corresponding modifications of chromatin structure.

4.42.3.2 Chromatin Remodeling Enzymes and Synaptic Plasticity

One of the first demonstrations that chromatin remodeling enzymes are involved in synaptic plasticity came from a study in *Aplysia*. Several different forms of transcription-dependent synaptic plasticity have been identified in *Aplysia*, including long-term facilitation (LTF, characterized by enhanced synaptic transmission) and long-term depression (LTD, characterized by decreased synaptic transmission) (Montarolo et al., 1986, 1988; Hammer et al., 1989; Dale and Kandel, 1990). To understand the changes in chromatin structure that affect transcription required for LTF and LTD, Guan et al. (2002) examined the chromatin surrounding the promoter of ApC/EBP. ApC/EBP is an immediate early response gene critical for induction of LTF (Alberini et al., 1994) and is a downstream target of the transcription factor ApCREB1. Guan et al. (2002) showed that induction of LTF by treatment with serotonin activates ApCREB1, which recruits the transcriptional coactivator CREB binding protein (CBP, a potent HAT) to the ApC/EBP promoter (i.e., **Figure 2(a)**). Recruitment of cAMP response element binding protein (CREB) and CBP correlates with increased histone H4 acetylation and ApC/EBP expression (Guan et al., 2002). In contrast, induction of LTD by treatment with neuropeptide FMRFamide resulted in the recruitment of ApCREB2, an inhibitory form ApCREB that lacks a transcription activation domain, and an HDAC5-like molecule to the ApC/EBP promoter decreasing histone H4 acetylation and ApC/EBP expression (Guan et al., 2002). These two results suggest that, in *Aplysia* sensory neurons, the genome serves as a substrate for signal integration upon which long-term neuronal state is encoded. In support of this hypothesis, increasing levels of histone acetylation with the HDAC inhibitor trichostatin A (TSA) and mimicking the epigenetic state of the genome normally seen after induction of LTF transforms short-term synaptic facilitation into LTF (Guan et al., 2002). These results demonstrate that enzymes such as HATs and HDACs are actively involved in regulating chromatin structure at specific gene promoters relevant for induction of long-term synaptic plasticity.

Recently, several studies have demonstrated that CBP is involved in specific forms of hippocampal synaptic plasticity and hippocampus-dependent long-term memory formation (**Figure 2(a)**) (Oike et al., 1999; Bourtchouladze et al., 2003; Alarcon et al., 2004; Korzus et al., 2004; reviewed in Josselyn, 2005; Wood et al., 2005, 2006). These six studies utilized five different *cbp* genetically modified mice in which the activity of CBP was specifically impaired to investigate the role of CBP in synaptic plasticity and memory storage. With respect to synaptic

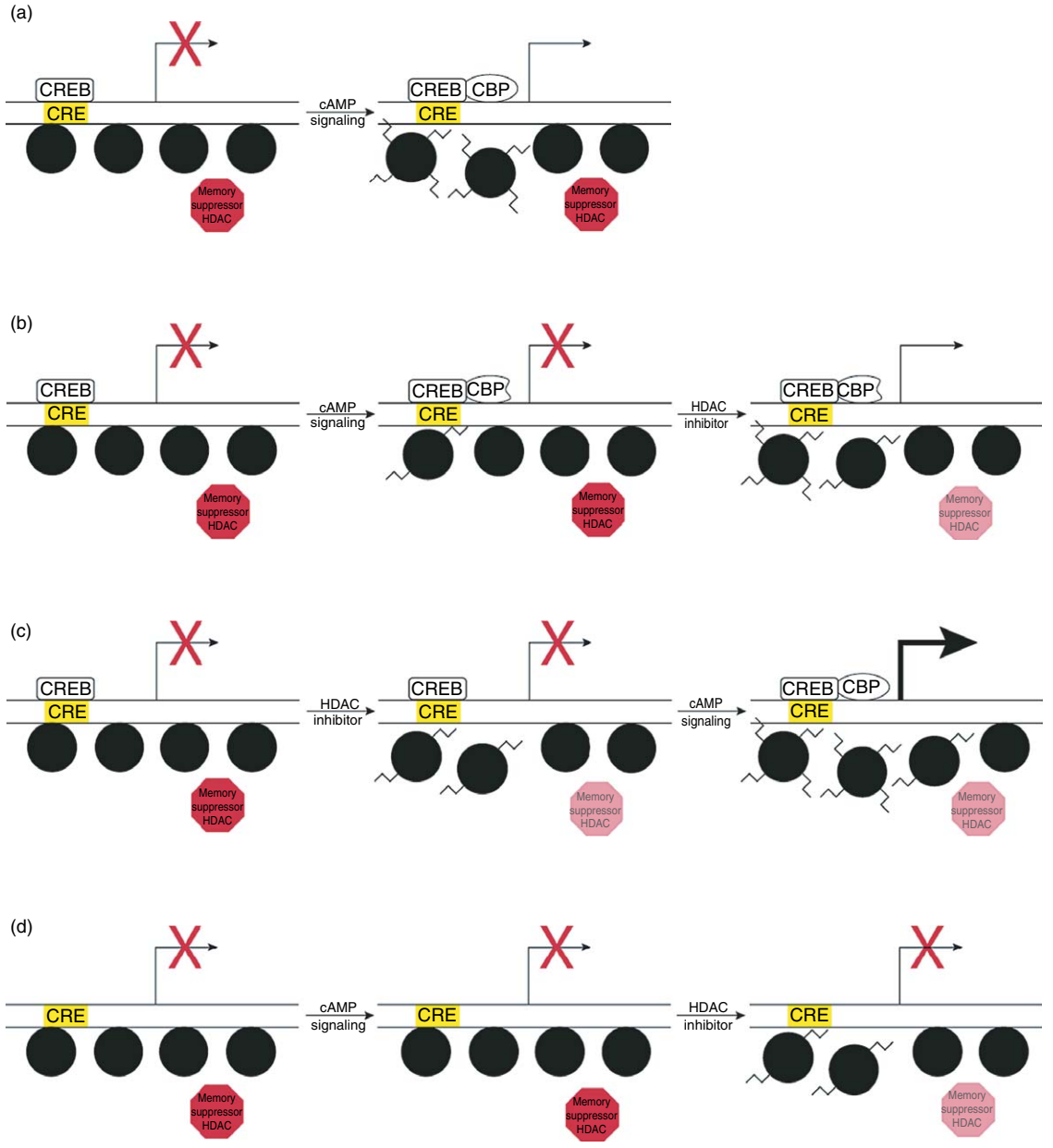

Figure 2 Epigenetics in synaptic plasticity and memory formation. Long-term forms of synaptic plasticity and memory require transcription for consolidation and maintenance. The diagrams indicate many aspects where epigenetics are involved in synaptic plasticity and long-term memory formation. (a) Induction of synaptic plasticity or formation of long-term memory requires the transcription factor CREB and the coactivator CBP. CBP is a HAT that acetylates histones and facilitates transcription of consolidation-associated genes. (b) Loss of CBP function, either through truncation mutations that eliminate the HAT domain or through haploinsufficiency, prevents CREB-mediated transcription of consolidation-associated genes. Addition of an HDAC inhibitor ameliorates the inability to induce long-term synaptic plasticity or form long-term memory. (c) Administration of HDAC inhibitors alone does not affect the expression of memory-associated genes. However, HDAC inhibitor preadministration facilitates the induction of synaptic plasticity and long-term memory. (d) Loss of CREB function, such as in CREB $\alpha\Delta$ mice, prevents induction of synaptic plasticity or formation of long-term memory. Administration of HDAC inhibitors does not restore plasticity or memory formation in these mice.

plasticity, $cbp^{+/-}$ heterozygous mice that lack one allele of *cbp* were found to have impairments in long-lasting LTP (L-LTP, a form of synaptic plasticity requiring transcription and translation), but normal early-phase LTP (E-LTP, a form of synaptic plasticity that is independent of transcription; *See* Chapter 4.16) (Alarcon et al., 2004). Similarly, transgenic mice expressing an inhibitory truncation mutant of CBP in forebrain neurons also exhibited deficits in L-LTP, with normal E-LTP (**Figure 2(b)**) (Wood et al., 2005). To overcome the deficits in histone acetylation and synaptic plasticity, mutant *cbp* mice were treated with HDAC inhibitors to induce a hyperacetylated genomic state. HDAC inhibition by suberoylanilide hydroxamic acid (SAHA) ameliorated impairments in hippocampal LTP in $cbp^{+/-}$ heterozygous mice (**Figure 2(b)**) (Alarcon et al., 2004). These results demonstrate that synaptic plasticity deficits in *cbp* mutant mice may be overcome by increasing histone acetylation via HDAC inhibition. However, the effect of modifying chromatin structure on synaptic plasticity and memory storage was not specifically addressed by these studies.

4.42.3.3 Histone Acetylation and Synaptic Plasticity

To directly examine the effect of increasing histone acetylation on synaptic plasticity, a recent study showed that the HDAC inhibitors TSA and sodium butyrate enhance LTP at Schaffer-collateral synapses in area CA1 of the hippocampus (**Figure 2(c)**) (Levenson et al., 2004b). Further, the authors demonstrated that the HDAC inhibitor–enhanced LTP is dependent on transcription. A similar result was observed in a study examining the effects of HDAC inhibition on E-LTP in the CA1 region of hippocampal slices from C57BL/6 mice. In this study, synaptic efficacy at both the collateral and commissural pathways was monitored simultaneously. Both pathways received baseline stimulation, but LTP was only induced in the collateral pathway, with the commissural fibers serving as a control. TSA had no effect on baseline responses in the commissural pathway as compared to vehicle, similar to other observations in TSA-treated slices (Alarcon et al., 2004; Levenson et al., 2004b). However, in the collateral pathways where E-LTP was induced, treatment with TSA converted transcription-independent E-LTP to L-LTP (Vecsey et al., in press). Other studies in the amygdala have shown that TSA can enhance forskolin-induced LTP at the sensory input synapses (**Figure 2(c)**) (Yeh et al., 2004). Together, these studies demonstrate that increasing histone acetylation via HDAC inhibitors can enhance synaptic plasticity in the hippocampus and amygdala.

What is the molecular mechanism whereby HDAC inhibitors enhance synaptic plasticity? A clue to answering this question came from examining the molecular mechanism underlying TSA-dependent enhancement of E-LTP. This form of E-LTP is independent of transcription, translation, protein kinase A (PKA), and CREB (reviewed in Nguyen and Woo, 2003; Pittenger and Kandel, 2003). This allowed Vecsey et al. (in press) to specifically examine whether the TSA-dependent enhancements in E-LTP were dependent on CREB function. CREB is thought to activate transcription through the coactivator CBP and its associated HAT activity. In addition, the significant phenotypic overlap between memory and synaptic plasticity deficits exhibited by transgenic mice expressing dominant negative inhibitors of CREB or CBP suggests that they function together during memory processes (Pittenger et al., 2002; Wood et al., 2005). Mice with targeted deletions of two *creb* isoforms (α and Δ; CREB $\alpha\Delta$ mice) on a defined F1 hybrid genetic background of C57BL/6 and 129/SvEvTac strains were used to investigate the role of CREB in TSA-dependent enhancement of E-LTP (Walters and Blendy, 2001; Graves et al., 2002). Hippocampal slices from CREB $\alpha\Delta$ mice did not exhibit the TSA-dependent enhancement of E-LTP observed in wild-type littermates, suggesting that a CREB-dependent process is involved in regulating the transcription of genes sensitive to histone hyperacetylation required for TSA-dependent enhancement of E-LTP. Further, genetically modified *cbp* mutant mice carrying a triple point mutation in the CREB-binding (KIX) domain of CBP ($CBP^{KIX/KIX}$ knockin mice) failed to exhibit TSA-dependent enhancement of E-LTP as well, specifically implicating the CREB:CBP complex in enhancement of LTP by HDAC inhibitors (Vecsey et al., in press). Together with the observations made concerning the effects of HDAC inhibitors on CBP-deficient and normal animals (Korzus et al., 2004; Levenson et al., 2004b; Yeh et al., 2004; Vecsey et al., in press), these results also suggest that HDAC inhibitors reduce the signaling threshold required for engagement of CREB-dependent maintenance of plasticity, but that these treatments by themselves do not induce expression of CRE-containing genes.

4.42.3.4 Histone Acetylation and Seizure

As reviewed at the beginning of this section, different patterns of synaptic activity can lead to differential

regulation of gene expression and ultimately a change in synaptic efficacy. A particularly dramatic example is seizure, which is a widespread burst of abnormal excitatory synaptic activity in the central nervous system (CNS). Seizure induces many changes in gene expression in the nervous system, which can lead to the development of chronic epilepsy and/or neurodegeneration. Early studies revealed that transcription of the immediate early gene *c-Fos* was upregulated in many regions of the brain after seizure (Morgan et al., 1987). Electron microscopy studies showed that c-Fos protein is preferentially localized to the euchromatic regions of chromosomes, which suggests that part of the transcriptional response to seizure involves changes in chromatin structure (Mugnaini et al., 1989).

Other studies have shown that expression of the glutamate receptor 2 (GluR2) alpha-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA) receptor subunit and brain-derived neurotrophic factor (BDNF) are also regulated by seizure (Ernfors et al., 1991; Isackson et al., 1991; Timmusk et al., 1993; Kokaia et al., 1995; Nibuya et al., 1995; Binder et al., 1999; Grooms et al., 2000; Sanchez et al., 2001). Expression of GluR2 mRNA is downregulated by seizure, whereas expression of BDNF mRNA is upregulated (Ernfors et al., 1991; Timmusk et al., 1993; Nibuya et al., 1995; Grooms et al., 2000). If seizure-induced changes in gene expression are due to changes in chromatin structure, then changes in chromatin structure should occur around the genes that are regulated by seizure. Recent studies have used the chromatin immunoprecipitation (ChIP) approach to monitor posttranslational modifications of histones that are located in the promoters of the BDNF and GluR2 genes. The first study to characterize the epigenetic regulation of GluR2 and BDNF demonstrated that pilocarpine-induced seizure significantly decreased acetylation of histone H4 in the GluR2 promoter, whereas acetylation of H4 in the P2 promoter of BDNF was significantly increased (Huang et al., 2002). The GluR2 promoter contains a repressor element-1 (RE1)-like silencer element in its promoter, and a recent study demonstrated that treatment of cultured neurons with kainic acid, an *in vitro* model for induction of seizures, resulted in increased binding of the transcriptional corepressor REST (RE1 silencer transcription factor), suggesting one possible mechanism for the downregulation of histone H4 acetylation (Jia et al., 2006). In another line of experiments, Tsankova et al. (2004) investigated changes in chromatin structure after electroconvulsive seizures (ECS), a form of human antidepressant therapy. ECS increased the acetylation of H4 at the P2 promoter of BDNF, and acetylation and phosphoacetylation of H3 were regulated within the P2 and P3 promoters of BDNF (Tsankova et al., 2004). These results indicate that ECS induces complex regulation of the epigenetic state of the BDNF promoters. ECS also had significant effects on the acetylation of H4 and phosphoacetylation of H3 within the *c-Fos* promoter and on the acetylation of H3 and H4 in the CREB promoter (Tsankova et al., 2004). Together, these data indicate that the synaptic activity and/or action potential firing that occurs during seizure results in complex regulation of the epigenetic state of chromatin.

4.42.3.5 Epigenetics in Plasticity and Seizure – Conclusions

Observations made regarding the role of epigenetics in plasticity and seizure strongly support a role for chromatin remodeling enzymes and histone acetylation in activity-dependent changes in synaptic and neuronal function. Moreover, all of these results support the hypothesis that epigenetic changes in chromatin structure are the basis of a cellular form of memory that influences neuronal state. Further examination of additional chromatin remodeling enzymes, as well as the analysis of additional histone modifications and their coregulation, will undoubtedly expand our understanding of the transcriptional profiles regulated by chromatin structure that are involved in long-term memory processes.

4.42.4 Epigenetics in Memory Formation

There are numerous studies indicating that many of the molecules necessary for induction of LTP are also required for normal long-term memory storage (reviewed by Malenka, 2003; Lynch, 2004). In light of the relationship between LTP and long-term memory formation, we next review the evidence associating epigenetic changes in chromatin structure with long-term memory storage. We will focus on the chromatin remodeling enzymes that appear to be involved, the specific chromatin modifications that correlate with memory storage, and what chromatin remodeling may provide in terms of mechanistic advantage for long-term memory storage processes.

4.42.4.1 Chromatin Remodeling Enzymes and Memory Storage

Rubinstein-Taybi syndrome (RTS), a human disease characterized by developmental abnormalities and mental retardation, is caused by translocations, deletions, and point mutations of the *cbp* gene (Petrij et al., 1995; Coupry et al., 2004). As discussed above, CREB and CBP are critical for successful induction of synaptic plasticity in species ranging from flies and slugs to rodents. Considering the role of CREB in learning, memory, and synaptic plasticity that has emerged from studies in *Aplysia, Drosophila,* and mice (Silva et al., 1998), it was reasonable to hypothesize that the cognitive impairments observed in RTS patients may be due directly to alterations in CBP activity in the brain. In a screen designed to identify genes involved in developmental regulation in mice, Oike et al. (1999) isolated a truncation mutation of CBP that lacks the HAT domain. These mice, which have one wild-type allele of *cbp* and one truncated allele, phenocopied many aspects of RTS quite well, with mice even exhibiting memory impairments (Oike et al., 1999). More recently, these animals were used in a study by Bourtchouladze et al. (2003) in which mice possessing the truncated CBP exhibited long-term memory deficits but normal learning and short-term memory in an object-recognition task (Bourtchouladze et al., 2003). Interestingly, inhibitors of phosphodiesterase 4, which enhance CREB-dependent gene expression, ameliorate memory deficits for object recognition in a dose-dependent manner in the mice containing the truncated form of CBP (Bourtchouladze et al., 2003). This suggests that pharmacologically enhancing cAMP-mediated signaling can compensate for the disruptions caused by the inhibitory truncated-CBP mutant expressed in these mice. Together, these results suggest that CBP, a potent HAT and chromatin remodeling enzyme, is involved in formation of long-term memory. However, this interpretation must be viewed with the caveat that the mice generated by Oike et al. (1999) have severe growth and developmental abnormalities, suggesting the alternative hypothesis that the memory impairments in these CBP-deficient mice are secondary to a neurodevelopmental abnormality.

To more directly study the effect of altering CBP activity on memory storage, two recent studies generated mice carrying spatially and temporally restricted expression of CBP mutants. Korzus et al. (2004) generated conditional transgenic mice expressing a HAT-deficient CBP transgene. The CaMKIIα promoter was used to spatially restrict transgene expression to forebrain neurons (Mayford et al., 1996) and the tetracycline system to temporally restrict transgene expression. These mice exhibited impaired memory for spatial and object recognition tasks that were ameliorated by intraperitoneal administration of the HDAC inhibitor TSA (Korzus et al., 2004). Thus, inducing a hyperacetylated histone state was able to compensate for the lack of HAT activity in these HAT-deficient CBP transgenic mice (i.e., **Figure 2(b)**). In a second study, Wood et al. (2005) generated transgenic mice expressing an inhibitory truncation mutant of CBP that contains the CREB-binding domain but lacks the HAT domain. The CaMKIIα promoter was used to spatially restrict transgene expression to forebrain neurons (Mayford et al., 1996), and this promoter does not activate transcription until 10–21 days postpartum (Kojima et al., 1997). These mice exhibited long-term memory deficits when assessed with hippocampus-dependent tasks such as contextual fear conditioning and the spatial water maze, but not in amygdala-dependent cued-fear conditioning (Wood et al., 2005). Together, these results demonstrate that more spatially restricted and temporally restricted alterations of CBP activity affect long-term memory storage (**Figure 2(b)**). Further, Korzus et al. (2004) were able to ameliorate long-term memory deficits by increasing histone acetylation using HDAC inhibitors. Similarly, HDAC inhibitors ameliorate long-term memory deficits for contextual fear in CBP heterozygous mice (non-regionally restricted deletion of one endogenous *cbp* allele) that exhibit long-term memory deficits for contextual fear and object recognition (Alarcon et al., 2004). Together, these studies demonstrate that brain-specific reduction of CBP activity results in long-term memory impairments and that these impairments may be ameliorated by increasing histone acetylation in general via administration of HDAC inhibitors (**Figure 2(b)**).

4.42.4.2 Histone Acetylation and Memory Storage

If a reduction of CBP activity results in memory deficits, and increasing histone acetylation via HDAC inhibitors can overcome these memory deficits, then an intriguing hypothesis arises suggesting that increasing histone acetylation might be a mechanism to enhance cognitive ability in general. Even more fundamental is the question: how do

specific forms of memory formation affect histone acetylation? And lastly, what signaling cascades are involved in the transduction of information from the synapse to chromatin? Levenson et al. (2004b) addressed these questions in a set of experiments that were the first to demonstrate that chromatin structure modifications are involved in long-term memory formation. First, the authors examined histone acetylation in hippocampal area CA1 of rats subjected to contextual fear conditioning or latent inhibition (when an animal is preexposed to a novel context before receiving the shock in that context). They found that contextual fear conditioning correlated with increased acetylation of histone H3, whereas latent inhibition correlated with increased acetylation of histone H4 (Levenson et al., 2004b). These results demonstrate that differential regulation of hippocampal histone acetylation may be induced by specific forms of memory formation. Second, after demonstrating that fear conditioning correlates with increases in histone acetylation, the authors continued by examining the signaling cascades involved in histone acetylation changes. Using acute hippocampal slice preps, they found that *N*-methyl-D-aspartate receptor (NMDAR)-dependent synaptic transmission and signaling via the ERK-MAPK pathway were essential for acetylation of histone H3, but not acetylation of histone H4 (Levenson et al., 2004b). These results suggest that signaling pathways involved in the acetylation of H3 are different from those required for the acetylation of H4. Third, the authors showed that rats treated with HDAC inhibitors via intraperitoneal injection exhibited enhanced long-term memory for contextual fear conditioning when tested 24 h postconditioning, but normal short-term memory when tested 1 h postconditioning (**Figure 2**(**c**)) (Levenson et al., 2004b). Importantly, control experiments demonstrated that the HDAC inhibitors did not have an indirect effect on performance (Levenson et al., 2004b). Together, the study by Levenson et al. (2004b) demonstrated that changes in chromatin structure correlated with long-term memory formation and activation of second messenger signaling pathways that are known to be involved in memory processes.

In a similar study, Vecsey et al. (in press) found that delivering HDAC inhibitors directly to the hippocampus in mice via intrahippocampal cannulae enhanced long-term memory for contextual fear conditioning (**Figure 2**(**c**)). Interestingly, the authors found that delivery of the HDAC inhibitors during memory consolidation, but not during retrieval, enhanced memory. These results suggest that increasing histone acetylation during the consolidation stage of memory is involved in memory enhancement, a stage of memory formation that has been shown to be dependent on transcription (Abel and Lattal, 2001). The authors investigated the underlying molecular mechanism for HDAC inhibitor–dependent enhanced memory formation by examining the effect of HDAC inhibitors in CREB $\alpha\Delta$ knockout mice. These mice carry a targeted deletion of the α and Δ isoforms of CREB, the two most abundant isoforms of CREB (Hummler et al., 1994). CREB $\alpha\Delta$ knockout mice failed to exhibit enhanced memory for contextual fear after intrahippocampal administration of an HDAC inhibitor. These results suggest that the TSA-enhanced memory for contextual fear conditioning requires the transcription factor CREB (**Figure 2**(**d**)). As mentioned earlier in the synaptic plasticity section, the authors also demonstrated that HDAC inhibitor–dependent enhancement of LTP required CREB as well. Finally, the authors showed using quantitative real-time reverse transcriptase polymerase chain reaction that only a subset of CREB-target genes is affected by HDAC inhibition following contextual fear conditioning. Thus, CREB:CBP-mediated transcription appears to be essential for HDAC inhibitor enhanced memory and synaptic plasticity, which seems reasonable considering that CREB is a transcription factor that recruits CBP, a potent HAT, for transcriptional activation of memory-associated target genes.

4.42.4.3 Factor Acetylation and Memory Storage

Acetylation is a posttranslational modification that can occur on lysine residues in general. Therefore, acetylation can occur on non-histone (factor) proteins. For example, the histone acetyltransferase CBP acetylates several factors including the transcription factors p53, CREB, and NF-κB (Gu and Roeder, 1997; Furia et al., 2002; Kiernan et al., 2003; Yeh et al., 2004; Hassa et al., 2005). Thus, although HDAC inhibitor–dependent enhancement of memory and synaptic plasticity most likely occurs through histone acetylation that directly facilitates transcriptional activation, it is possible that this process may also be regulated by factor acetylation. For example, p65 (an NF-κB subunit also known as Rel-A) acetylation increases in the amygdala of rats subject to a fear-potentiated startle paradigm (Yeh et al., 2004). Increases in p65 acetylation correlate with increased

CBP interaction, whereas decreases in p65 acetylation correlate with increased HDAC3 interaction (Yeh et al., 2004). Interestingly, the HDAC inhibitor TSA blocks the interaction between HDAC3 and p65, suggesting that TSA may be able to increase NF-κB-mediated transcription in a manner that does not involve histone acetylation. Finally, the authors demonstrated that TSA injections directly to the amygdala enhanced fear-potentiated startle (Yeh et al., 2004). These results open the possibility that factor acetylation may also be involved in the mechanism by which HDAC inhibitors enhance memory storage. This idea is further supported by studies showing that the HDAC inhibitor TSA disrupts interactions between CREB and HDAC1/PP1, and Akt and HDAC1&6/PP1 (PP1 is a protein phosphatase that regulates CREB and other factors), thus increasing phosphorylation of CREB and Akt, directly regulating their function (Canettieri et al., 2003; Brush et al., 2004; Chen et al., 2005). An important distinction to make here is that acetylation of a factor will be transient. In contrast, histone acetylation establishes a pattern of histone modifications that may be much more stable, resulting in a long-term transcriptional profile required to maintain a cellular memory for memory storage. This idea is discussed in detail in the first section of this article.

4.42.4.4 DNA Methylation and 'Lifetime' Memory Storage

All of the studies discussed thus far have investigated the role of epigenetic marking of the genome in an early and acute phase of memory formation. A recent study published from the Meaney laboratory (Weaver et al., 2004) suggests that DNA methylation patterns of specific genes in the brain are used as part of the mechanisms for storing early childhood experiences. Mother rats exhibit strong nurturing behaviors toward their pups, most notably in the form of licking and grooming their offspring. Patterns of DNA methylation in the glucocorticoid receptor gene are directly correlated with the quality of maternal care, and these patterns of DNA methylation persist into adulthood (Weaver et al., 2004). Moreover, these changes in DNA methylation patterns result in decreased anxiety and a strong maternal nurturing instinct in the adult offspring (Weaver et al., 2004). Therefore, alterations in DNA methylation result in a 'learned' and persistent change in adult behavior. Interestingly, maternally induced patterns of DNA methylation can be altered by central infusion of either an HDAC inhibitor or L-methionine, a precursor in the synthesis of the methyl donor S-adenosyl-methionine, suggesting that learning-induced changes in patterns of DNA methylation are dynamic and can exhibit plasticity during the lifetime of the animal (Meaney and Szyf, 2005; Szyf et al., 2005; Weaver et al., 2005). It is important to note that disruption of the epigenetic state of neurons did not result in a loss of neuronal phenotype, as might be expected if the same kind of cellular memory were used to store long-term memory of phenotype and childhood experience. Moreover, persistence of neonatally acquired patterns of DNA methylation in the mature CNS is consistent with our hypothesis that epigenetic mechanisms contribute to persistent changes in neural function. Interestingly, this study suggests a unique mechanism whereby the status of at least one component of the epigenome is transmitted across generations using the basic mechanisms in place for information storage in the nervous system.

4.42.4.5 Epigenetics in Memory Formation – Conclusions

Formation of several different types of memory appears to be dependent upon some of the molecular machinery involved in chromatin structure remodeling; specifically HATs. Formation of some types of long-term memory is correlated with epigenetic tagging of the genome. Finally, treatment with HDAC inhibitors can compensate for loss of HAT function and even enhance normal long-term memory formation. All of these observations indicate that the processes involved in induction of long-term memory formation utilize epigenetic mechanisms. Future experiments should begin to investigate the role of epigenetics in other phases of long-term memory formation, including consolidation and lifetime or remote (see Frankland et al., 2004) storage.

4.42.5 Epigenetics in Cognition: Rett Syndrome

As reviewed, there is considerable evidence implicating epigenetic mechanisms in neural function and memory formation. Moreover, even though epigenetic regulation of chromatin structure is a vital step in cellular differentiation, the studies published

to date indicate an active and ongoing role for epigenetic regulation of chromatin structure in the nervous system as it relates to plasticity and memory formation. Thus, postdevelopmental regulation of chromatin structure in the nervous system appears to be an important component of cognition. This suggests that derangement of the mechanisms responsible for regulation of chromatin structure would lead to severe cognitive impairment. Rett syndrome (RS) represents one disease of cognition where a specific molecule important for regulation of chromatin structure is mutated, resulting in severe cognitive impairments.

RS, first described by Austrian pediatrician Andreas Rett (1966), is an inherited, X-linked disease that afflicts about 1 in 15 000 females by 2 to 18 years of age and is estimated to be the second leading cause of mental retardation in women (Ellaway and Christodoulou, 2001). Development during the first 3 to 6 months of life is normal in RS patients; symptoms of RS first appear between 3 months and 3 years of age. The trademark of RS is a display of continuous, stereotypical hand movements, such as wringing, washing, clapping, and/or patting, which appear after the loss of purposeful hand movement. Other signs of RS include decreased growth (including microcephaly), abnormal respiration, gait ataxia, autism, seizures, and other neurological dysfunctions. Mapping studies identified a putative RS locus at Xq28 (Sirianni et al., 1998). Recent studies indicate that, in a percentage of patients, a mutation of the methyl CpG binding protein 2 (MeCP2) located in Xq28 is correlated with Rett syndrome (Sirianni et al., 1998; Amir et al., 1999; Ellaway and Christodoulou, 2001).

MeCP2 is a member of a family of methyl CpG binding proteins that function to link DNA (cytosine-5) methylation with gene silencing. As discussed earlier, DNMTs catalyze the methylation of cytosines at the 5-position of the pyrimidine ring. Once methylated, 5mCpG is bound by MeCP2, which then recruits a complex of proteins including histone deacetylases and transcriptional corepressors such as Sin3A (**Figure 3**(**a**)) (Roopra et al., 2000). The histones associated with the 5mCpG become hypoacetylated, promoting tight association between DNA and histones, ultimately resulting in formation of a transcriptionally repressive heterochromatin complex (**Figure 3**(**a**)).

The role that MeCP2 might play in the memory deficits observed in RS is still unclear. DNA methylation is thought to be involved in genomic imprinting and dosage compensation. Therefore, MeCP2 could play a prominent role during development. Indeed, MeCP2 appears to be critically involved in neuronal maturation, and not surprisingly, early attempts to create mouse models lacking MeCP2 resulted in embryonic lethality (Tate et al., 1996; Cohen et al., 2003; Matarazzo et al., 2004; Matarazzo and Ronnett, 2004; Fukuda et al., 2005). However, RS is a progressive disease that does not result in symptoms until early childhood. More recent attempts to create MeCP2-deficient mice have succeeded (Chen et al., 2001; Guy et al., 2001). These mice display several of the characteristics of human RS; however, no studies of cognitive performance in these mouse models have been published to date (Chen et al., 2001; Guy et al., 2001). To more closely approximate the mutations commonly found in RS patients, another strain of mouse was developed where the last one-third of MeCP2 was removed ($MeCP2^{308/y}$) (Shahbazian et al., 2002). $MeCP2^{308/y}$ mice share phenotypic similarities with human RS, including stereotypy, spontaneous seizures, increased anxiety, altered diurnal activity levels, and abnormal social interaction (Shahbazian et al., 2002; Moretti et al., 2005). Initial studies of $MeCP2^{308/y}$ animals suggested that long-term memory formation was normal (Shahbazian et al., 2002); however, further studies of these mice have revealed significant deficits in formation of hippocampus-dependent long-term memory and induction of synaptic plasticity in the sensory-motor cortex and hippocampus (**Figure 3**(**b**)) (Moretti et al., 2006). The derangements observed in $MeCP2^{308/y}$ animals do not appear to be due to aberrant development, as mice where MeCP2 was removed from forebrain neurons postnatally display many of the same social and memory impairments (Gemelli et al., 2006).

Several recent studies have begun to explore the function of MeCP2 in neurons and have identified a few mechanisms by which loss of MeCP2 could result in the cognitive derangements observed in RS model mice and human patients. One study demonstrated that a truncated form of MeCP2 protein, which mimics the most common mutation observed in human RS patients, tightly associates with methylated DNA in *Xenopus* embryos, suggesting that mutated MeCP2 protein can have profound effects on early developmental processes and possibly also interfere with normal regulation of chromatin structure and gene expression in the adult (Stancheva et al., 2003). Morphological studies have revealed that MeCP2 is expressed in excitatory cortical

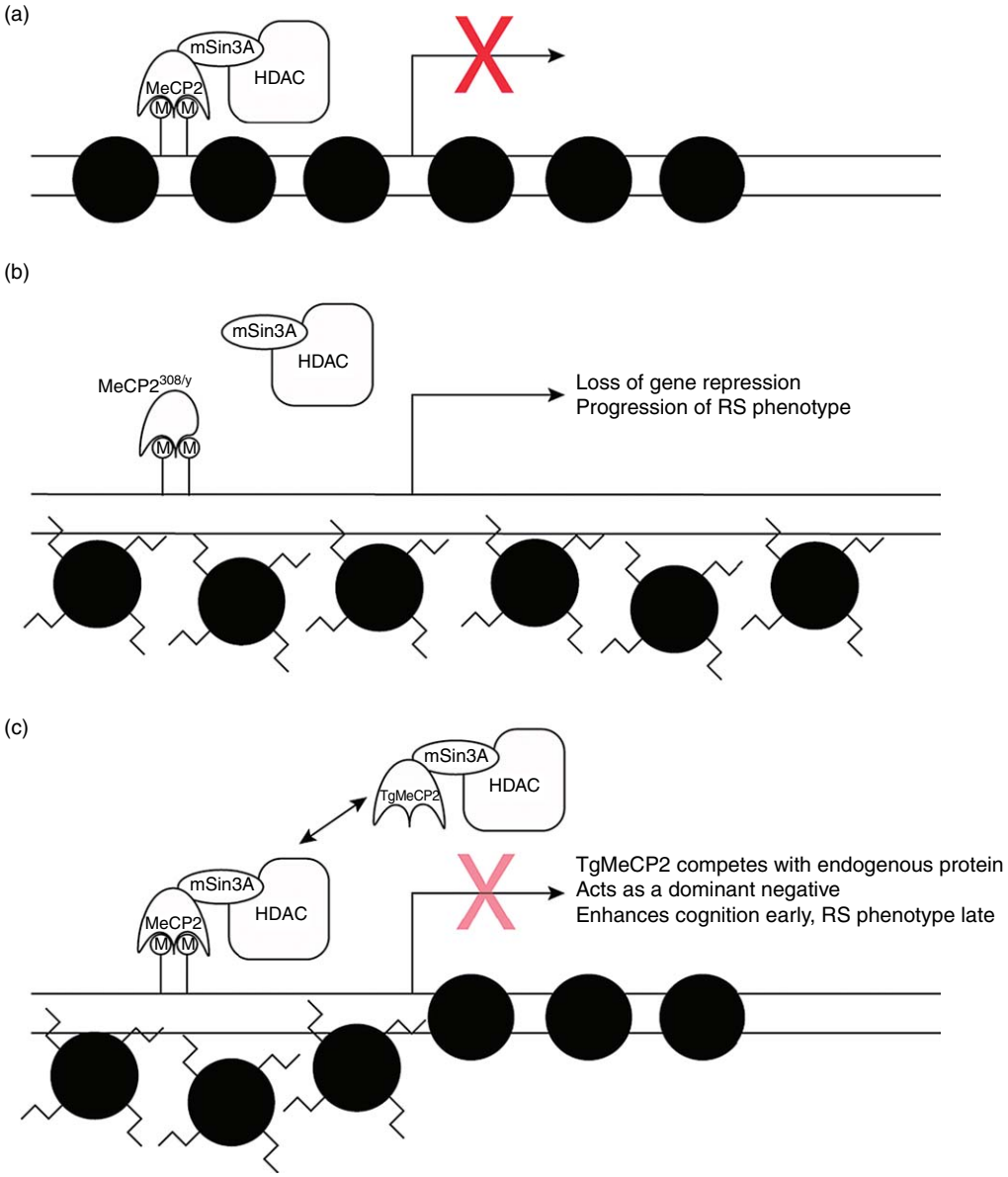

Figure 3 Role of MeCP2 in Rett syndrome, synaptic plasticity, and memory formation. MeCP2 is a member of the family of methyl CpG binding proteins. (a) MeCP2 binds to methylated cytosines and recruits the adapter protein mSin3A and HDAC to repress gene expression. (b) Loss of MeCP2 function through truncation mutations is thought to underlie RS. Truncation of MeCP2 results in loss of gene silencing, aberrant gene expression, and progression of the RS phenotype. (c) Overexpression of MeCP2 represents one possible therapeutic approach for treatment of RS. MeCP2 overexpression appears to have a dominant negative effect on normal MeCP2 function as determined through measures of synaptic plasticity and cognitive performance. Excess MeCP2 may sequester mSin3A and HDAC, limiting the ability of MeCP2 to repress gene expression. The early effects of MeCP2 overexpression lead to enhancement of synaptic plasticity and cognition. Continuous overexpression of MeCP2 eventually results in progression of a RS-like phenotype and death.

neurons and GABAergic interneurons, with little to no expression in glial cells (Akbarian et al., 2001; LaSalle et al., 2001; Adachi et al., 2005; Pelka et al., 2005). Restriction of MeCP2 expression to excitatory and inhibitory neurons in the cortex is consistent with the observations that expression of *dlx5*, a gene responsible for the regulation of GABA-synthesizing enzymes, is increased due to a loss of MeCP2-mediated imprinting in human fibroblasts (Horike et al., 2005), and cortical inhibitory tone is enhanced

in one strain of MeCP2-null mice (Dani et al., 2005). Investigating possible causes of the aberrant social behavior, several studies indicate that expression of stress-related genes is increased and levels of norepinephrine, dopamine, and serotonin were decreased in MeCP2-null mice (Ide et al., 2005; Nuber et al., 2005). Additionally, loss of MeCP2 leads to aberrations in chromatin structure around the Prader Willi and Angelman loci, two regions of the genome implicated in autism (Makedonski et al., 2005; Samaco et al., 2005). It is interesting to note that all of these aberrations observed in the context of loss of MeCP2 function are due to dysfunction of chromatin structure and gene expression (Tudor et al., 2002).

As detailed earlier, several studies indicate that loss of MeCP2 function leads to severe alterations in chromatin structure, gene expression, and neuronal function. Several labs have begun to explore the efficacy of overexpression of normal MeCP2 as a potential therapy to treat RS patients. In one study, overexpression of MeCP2 was shown to enhance long-term memory formation and the induction of hippocampal LTP in young mice (**Figure 3(c)**) (Collins et al., 2004). However, as the mice aged, overexpression of MeCP2 led to impairments in motor function and a decrease in longevity (Collins et al., 2004; Luikenhuis et al., 2004). Interestingly, overexpression of MeCP2 in mice that lack MeCP2 rescues the RS phenotype, suggesting that genetic replacement of MeCP2 expression is sufficient to rescue some of the RS-associated phenotypes (Luikenhuis et al., 2004). Moreover, these results suggest that levels of MeCP2 are coupled to cognitive performance, and suggest the broader implication that methyl-DNA binding protein function, and possibly DNA (cytosine-5) methylation itself, plays a significant role in induction of plasticity and memory formation in the adult CNS.

In support of an active role for DNA (cytosine-5) methylation in plasticity, a recent study by Levenson et al. (2006) demonstrated that inhibition of DNMT activity blocks induction of LTP in the hippocampus. Moreover, activation of PKC through the use of phorbol esters significantly increased expression of DNMT3A, suggesting that DNMT expression and activity is regulated by a signaling pathway that is crucial for the induction of synaptic plasticity and memory formation (*See* Chapter 4.25) (Lovinger et al., 1987; Abeliovich et al., 1993; Weeber et al., 2000). Collectively, these results are consistent with the observations made in MeCP2 model mice and suggest an active role for DNMT activity and DNA (cytosine-5) methylation in synaptic plasticity and long-term memory formation.

4.42.6 Conclusions

Epigenetic cellular memory is an ancient and evolutionarily conserved form of lifetime memory. Every cell in a metazoan relies on cellular memory to exist and function normally in its environment. A common theme emerging in the field of learning and memory is that the nervous system has co-opted several evolutionarily conserved processes to subserve long-term information storage. Some examples include molecules relevant for immune system function, such as class I major histocompatibility complex proteins and the NF-κB family of transcription factors (Meberg et al., 1996; Corriveau et al., 1998; Albensi and Mattson, 2000; Huh et al., 2000; Kassed et al., 2002; Yeh et al., 2002; Meffert et al., 2003; Levenson et al., 2004a; Oliveira et al., 2004) and signaling pathways involved in early development, such as the ras-MEK-ERK MAPK signaling pathway (English and Sweatt, 1996, 1997; Atkins et al., 1998; Silva et al., 1998; Selcher et al., 1999; Schafe et al., 2000; Giese et al., 2001). The realization that the processes involved in forming epigenetic cellular memory have been co-opted by the nervous system for induction of long-term memory and storage of some forms of lifetime memory has brought the field of neuroscience to an exciting juncture. For example, therapies based on modulation of epigenetic states could be used to treat a host of neurological conditions potentially including Huntington's disease, Alzheimer's disease, schizophrenia, and Rubinstein-Taybi syndrome (Kimberly et al., 2001; Steffan et al., 2001; Ferrante et al., 2003; Hockly et al., 2003; Kim et al., 2004; Numachi et al., 2004; Rouaux et al., 2004; Von Rotz et al., 2004; Grayson et al., 2005; Tremolizzo et al., 2005). Perhaps most relevant for the general population is the exciting possibility that, as we gain a deeper understanding of how epigenetics factors into cognition, novel drugs could be developed to enhance memory formation in otherwise normal individuals (Levenson et al., 2004b).

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Cumulative Index

Arrangement:, This index is in word-by-word order, whereby hyphens are treated as spaces (e.g. 'multi-trial' precedes 'multiple'). Prefixes and terms in parentheses are excluded from the initial alphabetization.

Cross-reference terms in italics are general cross-references, or refer to subentry terms within the main entry (the main entry is not repeated to save space). Readers are also advised to refer to the end of each article for additional cross-references - not all of these cross-references have been included in the index cross-references.

Major discussion of a subject is indicated by bold page numbers. Page numbers suffixed by T and F refer to Tables and Figures respectively. *vs.* indicates a comparison.

Subentries listed under a main entry with the same page numbers were included to illustrate the breadth of the coverage of the topic.

To save space in the index, the following abbreviations have been used:

BrdU - bromo-deoxyuridine
CREB - cAMP response element binding protein
CS - conditioned stimulus (stimuli)
ECT - electroconvulsive shock treatment
ERPs - event-related potentials
fMRI - functional magnetic resonance imagery
LTD - long-term depression
LTM - long-term memory
LTP - long-term potentiation
MEG - magnetoencephalography
MS/VDB - medial septum/vertical limb of the diagonal band
MTL - medial temporal lobe
NBM/SI - nucleus basalis magnocellularis/substantia innominata
PSC - primary sensory cortex
SD - semantic dementia
S–R - stimulus–response
S–Rf - stimulus–reinforcer
SRTT - serial reaction time task
S–S - stimulus–stimulus
STD - short-term depression
STM - short-term memory
US - unconditioned stimulus (stimuli)
VBM - voxel-based morphometry
VOR - vestibulo-ocular reflex

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